PLATELET-RICH PLASMA (PRP) STANDARDIZATION & CELL THERAPIES

Antoine Turzi & REGEN LAB team
BIOBRIDGE FOUNDATION EDITIONS

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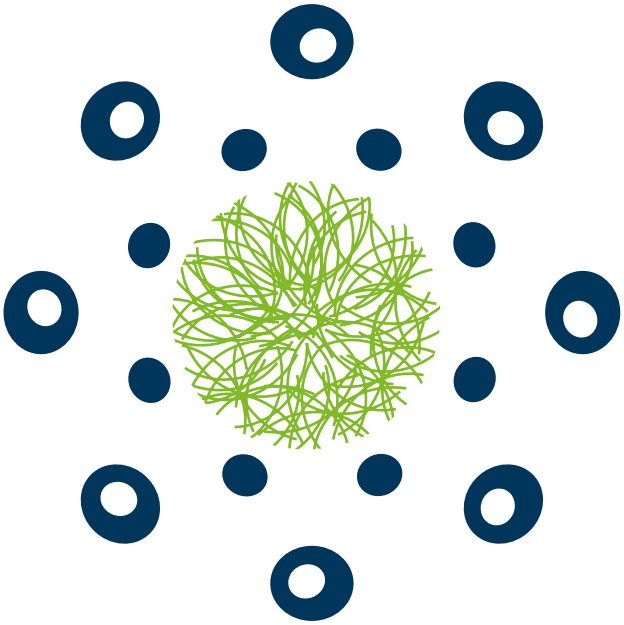

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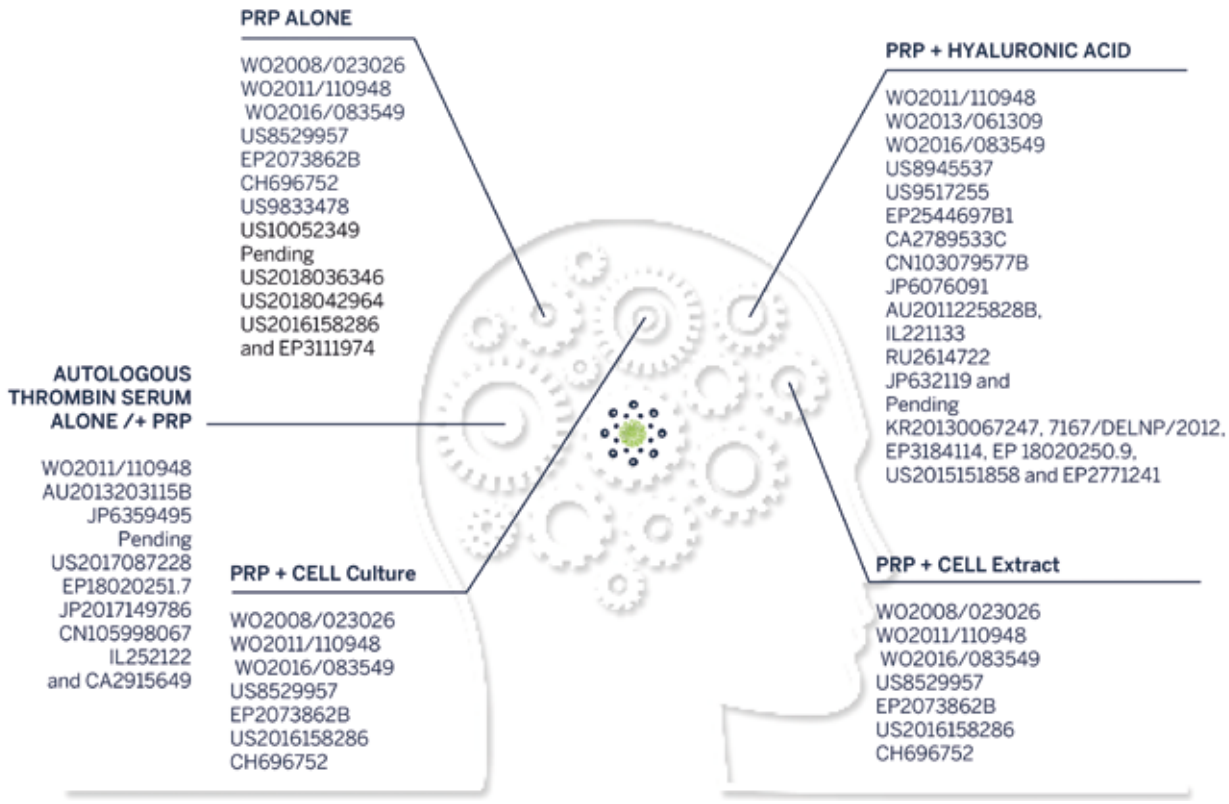
INTELLECTUAL PROPERTY

As a leading innovator of medical products in the PRP&HA regenerative field for more than 15 years, which it markets under the well-recognized REGENLAB® and RegenKit® brands, Regen Lab and its founder Mr. Antoine Turzi have developed a substantial patent portfolio.

Innovation represents one of the strongest pillars of the company with more than 60 in-house scientists and a unique network of talented and renowned medical doctors contributing to Regen Lab's Research & Development.

The patent portfolio relates to the protection of tubes & methods for the preparation of Platelet Rich Plasma, biological glue/gel, Autologous Thrombin Serum (ATS), combination of PRP with cell extracts like Bone Marrow Concentrate (BMC) and fat tissue as well as the major/ breakthrough technological innovation CellularMatrix® which combines PRP and hyaluronic acid (HA).

Additional information about Regen Lab's patents can be found at https://www.regenlab.com/patents



INTRODUCTION

In the 1960's, a scientist named Dr. Leonard Hayflick (Wistar Institute, Philadelphia) carefully characterized the serial cultivation of primary human fibroblast cells by demonstrating that primary human cell strains have a limited replicative lifespan or «doubling potential», restricted to 40-50 divisions. This finding led to the hypothesis that oxidative damage could be partially responsible for limiting in vitro lifespan, consistent with the free radical hypothesis of aging. We now know that cell senescence in vitro occurs because of critical shortening of the telomeres. The Hayflick theory has been greatly reconsidered by the observations from the Institut National de la Santé et de la Recherche Médicale (INSERM/Paris), group of Dr. Barlovatz-Meimon (Culture de cellules animales, 3° éd. Barlovatz-Meimon Georgia, Ronot Xavier) published in 2003 in Paris regarding in vivo (i.e. on the patient himself) cell growth. These studies hypothesized that the fibroblasts may reach a 2 to 3 log growth, meaning that they could potentially undergo up to 500 to 5'000 cell divisions.

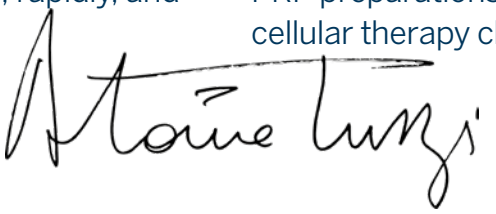
In this scientific context, since 2003, Regen Lab has been committed to the development of a unique expertise for the design, the manufacturing and the validation of high-quality medical devices, intended for cellular therapies and for the preparation of fresh and autologous platelet-rich plasma (PRP) from the patient's own blood. Our cellular therapies allow medical professionals to prepare PRP and freshly collected cells, safely, rapidly, and efficiently for multiple medical disciplines.

At the core of Regen Lab's business model lies our R&D department which has enabled the development of advanced technologies such as preparation of 100% autologous fibrin glue (RegenKit® Surgery), isolation of bone marrow mononuclear cells (MNC) (Regen Extracell BMC), and combination of PRP with adipose tissue (RegenKit Extracell Adipocyte) that can be used in clinical settings.

Furthermore, CellularMatrix™, a unique and innovative combination of PRP and Hyaluronic Acid (HA) within the same device has demonstrated to have huge clinical benefits superior to PRP alone. All Regen Lab's innovations are supported by a multitude of worldwide patents.

Our sterile and non-pyrogenic class II and III medical devices fully guarantee the patient safety thanks to the patented close-circuit system. They fully comply with the international applicable regulatory requirements and our manufacturing process is in full compliance with current Good Manufacturing Practice (GMP). The main quality of RegenLab is a devoted team composed of 60% with scientific profile collaborators. The team has contributed actively to the redaction and compiling of the datas of this book.

For all these reasons, RegenLab team believe that we bring to the medical community a powerful and reliable tool to standardize PRP preparations, and thus benefit future cellular therapy clinical outcomes.



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ABBREVIATIONS

3-D	Tridimensional
ANSM	Agence nationale de sécurité du médicament et des produits de santé
AT-MSC	Adipose tissue-derived mesenchymal stem cells, See
ATS	Autologous thrombin serum
BBR	Bromobutyl rubber
bFGF	basic fibroblast growth factor
BMP	Bone morphogenetic proteins
BRONJ	Biphosphonate-related osteonecrosis of the jaw
CAGR	Compound annual growth rate
CJD	Creutzfeldt-Jakob disease
CM-PRP-HA	PRP/HA combination prepared with CellularMatrix
CRP	C-reactive protein
ECM	ExtraCellularMatrix
EGF	Epithelial growth factor
FBS	Foetal Bovine Serum
FDA	Food and Drug Administration
FGF	Fibroblast growth factors
GMP	Good Manufacturing Practice
GSM	Genitourinary syndrome of menopause
HA	Hyaluronic acid
HGF	Hepatocyte growth factor
HHS	Harris Hip Score
HIV	Human immunodeficiency virus
HRT	Hormone Replacement Therapy
IGF-1	Insulin-like growth factors
IFU	Instructions for Use
INSERM	Institut National de la Santé et de la Recherche Médicale
ISO	International Organization for Standardization
KGF	Keratinocyte growth factor
KOA	Knee osteoarthritis
LAL	Limulus amebocyte lysate
LASIK	Laser in situ Keratomileusis
MDD	Medical Device Directive
MDR	Medical Device Regulation
MSC	Mesenchymal stem cell
MSK	Musculoskeletal
NHDF	Normal Human Dermal Fibroblast
NICE	National Institute for Health and Care Excellence
NSAID	Non-steroidal anti-inflammatory drug
OA	Osteoarthritis
OARSI	Osteoarthritis Society International
PDGF	Platelet-derived growth factor
PETG	Polyethylene terephthalate glycol
PPP	Platelet-poor plasma
PRP	Platelet-rich plasma
QA	Quality Assurance
RBC	Red blood cell
ROS	Reactive Oxygen Species
SAE	Severe adverse event
TGA	Therapeutic Goods Administration
TGF	Transforming growth factor
TGF-β	Transforming growth factor beta
UADE	Unexpected adverse device event
UV	Ultraviolet
VAS	Visual Analog Scale
VEGF	Vascular endothelial growth factor
w/v	Weight/Volume
WBC	White blood cells
WHO	World Health Organization
WOMAC	Western Ontario and McMaster Universities Arthritis Index

1. PRP SCIENCE AND CLINICAL APPLICATIONS

1.1 BASICS OF WOUND HEALING AND THE ROLE OF BLOOD COMPONENTS IN PRP

In general, wound healing can be divided into four partially overlapping phases: hemostasis (coagulation), inflammation, proliferation and remodeling. The initial phases are characterized by hemostasis, with platelets inducing clot formation and the release of growth factors that aids in activating and attracting inflammatory cells such as neutrophils and macrophages to the site of injury to prevent infection and removed damaged cells. The proliferation phase is characterized by the deposition of a new extracellular matrix associated with granulation tissue formation, new vessels formation, contraction and epithelialization. Finally, the remodeling phase is associated with reorganization of collagen and reduction of scar tissue (Figure 1). The physiologic progression through these phases of wound healing is orchestrated by growth factors and cytokines, many of which are released and modulated by blood components in PRP (Wu et al., 2016).

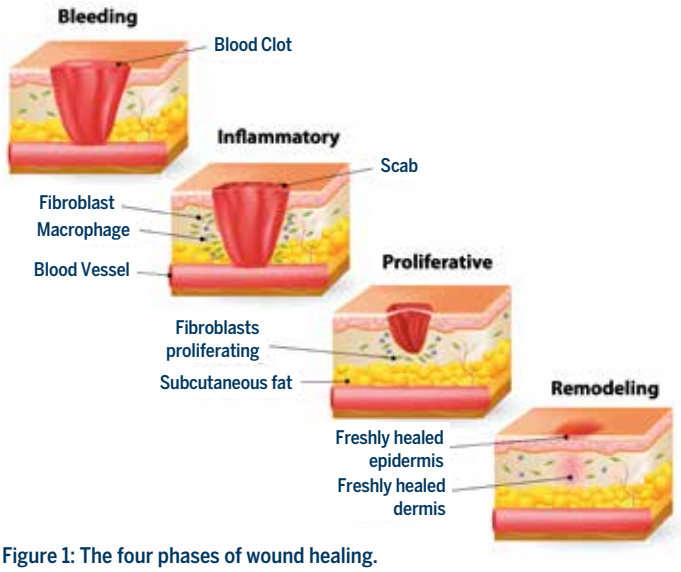


Figure 1: The four phases of wound healing.

1.2 BASICS SCIENCE OF PRP

1.2.1 Blood components

Blood contains plasma, red blood cells, (RBC, also known as erythrocytes), white blood cells (WBC, also known as leukocytes) and platelets (also known as thrombocytes, See Figures 2 and 4).

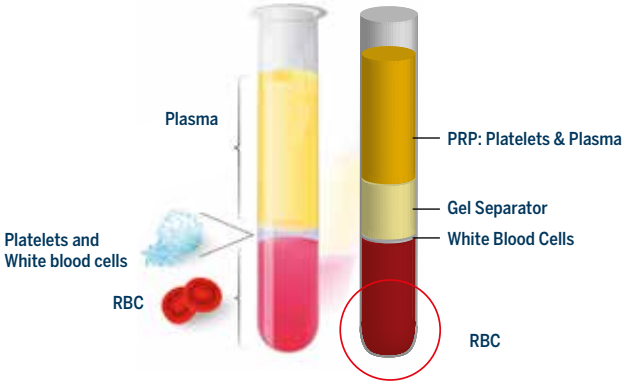


Figure 2: The blood components (left). In humans, plasma represents approximately 55% of the volume of blood, red blood cells about 44%, white blood cells <1% and platelets 0.2%. Regen Lab medical device (right) allows a perfect separation of blood components thanks to its specific separator gel.

Plasma is the yellow-colored liquid component of blood in which blood cells are suspended. Plasma contains nutrients, vitamins, hormones, electrolytes and many proteins, such as albumin, immunoglobulin and clotting factors, including fibrinogen. Fibrinogen is a soluble molecule that is converted into an insoluble fibrin network upon activation by thrombin (the last step of the coagulation cascade, see Figure 3). The fibrin network forms the clot and traps platelets at a wound site (Boswell et al., 2012; Sampson et al., 2008).

Blood cells, illustrated in figure 4 are subdivided in two main populations RBC and WBC.

• The primary function of RBC is to carry and deliver oxygen and other gases, such as carbon dioxide and nitric oxide using hemoglobin. RBC degradation, generates cytotoxic oxygen free radicals and oxidative stress that induce apoptosis of surrounding cells, suggesting that RBC concentrations should be reduced or eliminated in PRP preparations (Wu et al., 2016).

• WBC are essential mediators of the inflammatory response, host defense against infectious agents, and wound healing (Wu et al., 2016). They are classified as mononuclear cells (monocytes and lymphocytes) or granulocytes (neutrophils, eosinophils and basophils). Granulocytes, due to their multilobed nucleus, are also referred to as polymorphonuclear leukocytes (Figure 4).

Monocytes (5.3% of the WBC) are found in peripheral blood and differentiate into macrophages when they

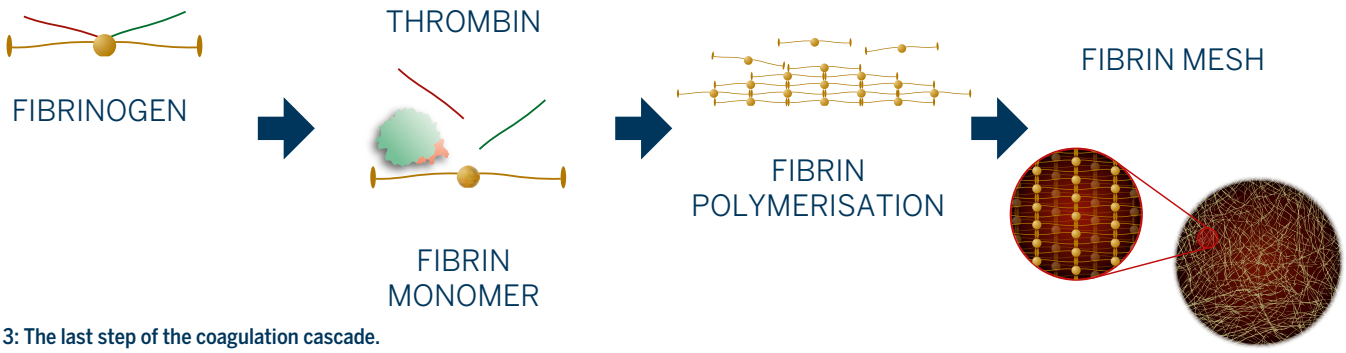


Figure 3: The last step of the coagulation cascade.

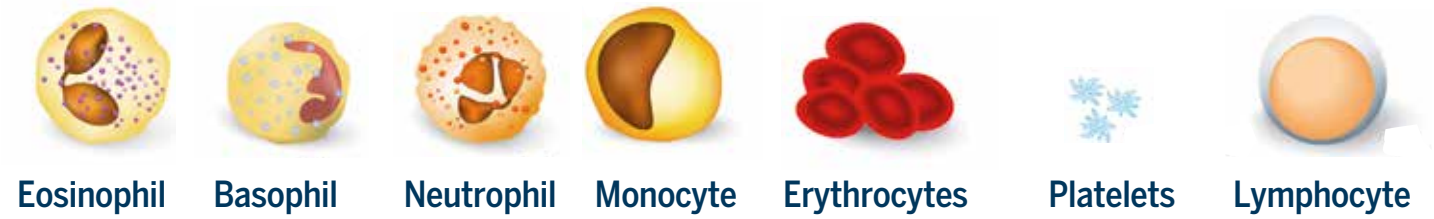


Figure 4: Blood cell types.

migrate into tissues. Macrophages are necessary for the regenerative process through active phagocytosis of necrotic tissues and debris.

Lymphocytes (30% of the WBC) are not required for the initiation of wound healing, but an intact cellular immune response is essential for a normal outcome of tissue repair. Lymphocytes play a modulatory role in wound healing, mediated in part by the release of soluble factors (lymphokines). It is also known that lymphokines exert both stimulatory and inhibitory signals on all aspects of fibroblast activity, and that there seems to be a balance between these effects (Schaffer et al., 1998).

The primary function of neutrophils (62% of the WBC) is to destroy infectious agents through phagocytic activity and the release of enzymes. Although these molecules (hydrolytic enzymes and reactive oxygen species (ROS), are intended to destroy microbes, they also incite local tissue damages (Boswell et al., 2012). For these reasons, the presence of neutrophils in PRP preparations is undesirable.

Eosinophils (2.3% of the WBC) are typically considered in context of host defense and are important mediators of allergic reactions. However, they are also known to produce a variety of growth factors and interleukins and have been found to participate in tissue remodeling by influencing fibroblasts and extracellular matrix (ECM). They also promote epithelial cell proliferation, angiogenesis, and organization of the wound site (Lana et al., 2014).

Basophils (0.4% of the WBC) have a long-recognized role in host-parasite interactions and in allergic reactions. Little is known about their role in wound healing, though it has been recognized that they migrate into wound sites upon injury and secrete proinflammatory mediators (Lana et al., 2014).

Platelets play a key role in the healing process, responsible for hemostasis, and through their controlled release of growth factors, they induce construction of new connective tissue, and revascularization (Sampson et al., 2008). They are small, discoid cells formed in the bone marrow by fragmentation of megakaryocytes. They have no nucleus and thus cannot replicate. As senescent platelets are destroyed by the spleen, their lifespan ranges from five to nine days in blood. After tissue injury and/or surgical stimuli that damage blood vessels, platelets are exposed to various extracellular matrix proteins (particularly collagen). This interaction causes the activation of platelets, changing their shape with the development of pseudopods to promote platelet aggregation and subsequent release of the contents of their granules, (Figure 5). Platelet alpha-granules contain more than 300 bioactive substances (Golebiewska et al., 2015). These substances play a fundamental role in hemostasis and/or tissue healing by stimulating cellular chemotaxis, proliferation and

differentiation, removal of tissue debris, angiogenesis, and the deposition of extracellular matrix (Mehta et al., 2008). Among them are notable growth factors involved in the healing process such as: transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epithelial growth factor (EGF), basic fibroblast growth factor (bFGF) keratinocyte growth factor (KGF), connective tissue growth factor (CTGF) and insulin-like growth factors (IGF) (Figure 6). Some of them, like PDGF, have a paracrine effect on different cell types like mesenchymal stem cells recruitment (Wu et al., 2016).

Platelets also release histamine and serotonin, which increases local capillary permeability to improve access for additional inflammatory cells to start the reparative process (Degen, 2017).

1.2.2 PRP definition

PRP is defined as a volume of autologous plasma that has a platelet concentration above baseline (i.e. above value in whole blood), (Marx et al., 2001). As such, PRP contains not only a high concentration of platelets but also the full content of plasma clotting factors, the latter

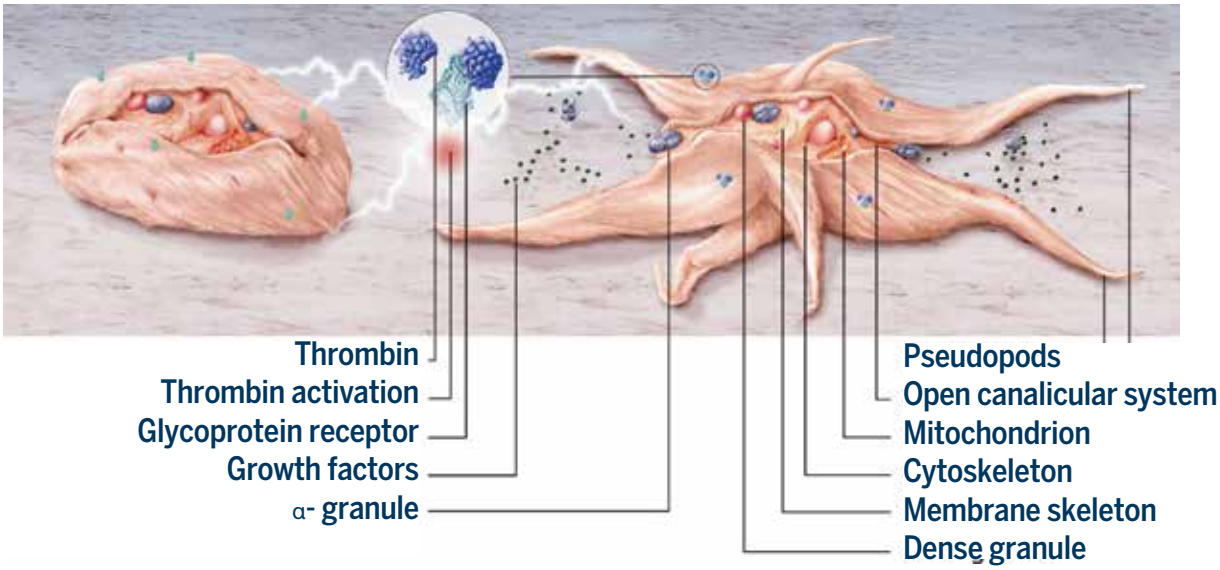


Figure 5: Platelet activation is a multiple step process. Initiated by a dramatic shape change, platelets being converted from a disc shape into enlarged cells with protruding pseudopods. From Everts et al. JCET. 2006;38:174-187.

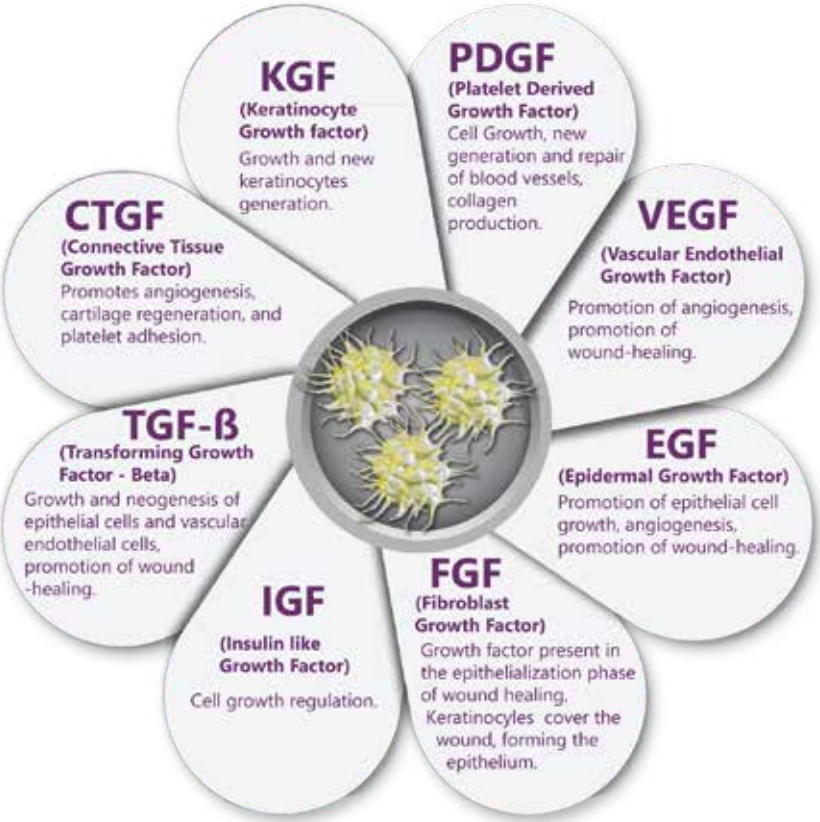


Figure 6: The most important platelet-released growth factors and their biological activities. Note that the plasma itself is also a source of IGF, which is synthesized in the liver.

of which typically remain at their physiologic levels (Eppley et al., 2006).

The rationale of PRP use for therapeutic applications is to mimic the biological healing process that normally occurs in the human body after injury (Mehta et al., 2010). PRP preparation consists in removing red and white blood cells, which delay the healing and concentrating platelets, thereby increasing factors that are useful in healing (Ahmad et al., 2012).

Because there are numerous PRP preparation protocols, differing by preparation devices, centrifugation conditions and operator dexterity, PRP is used to qualify biological products that greatly vary in their platelet concentration, quality and content in growth factors, and level of contamination with red blood cells and pro-inflammatory white blood cells (Harmon et al., 2011).

This large variability in PRP preparations creates a challenge when trying to accurately draw conclusions from the literature to guide PRP production and determine indications for use. This in turn has prompted the development of PRP classification systems to facilitate reporting of clinical investigations (Wu et al., 2016).

To fulfill the need of a standardized PRP preparation, RegenLab has developed the polymer-gel separation system, that allows to efficiently recover platelets and deplete red and white blood cells in an automated close circuit system.

1.2.3 Different forms of PRP

To fit specific therapeutic applications' needs, Regen Lab developed kits allowing for the preparation of PRP in different forms, ranging from liquid PRP (Figure 7A) to coagulated PRP or membranes with different consistencies (Figure 7B-F).

With RegenKit Extracell Membrane and RegenFibrin Polymer, liquid PRP is mixed with calcium gluconate and re-centrifuged, producing a suturable fibrin clot or membrane (Figures 7B and 7C, respectively). Due to the second centrifugation step, platelets entrapped in these products are concentrated at the bottom of the membrane or clot.

The RegenKits containing a RegenATS device in addition to the PRP devices allow to prepare platelet gel and autologous fibrin glue in which platelets are evenly dispersed. Autologous Thrombin Serum (ATS) prepared with the blood of the patient in the RegenATS device contains the coagulation enzymes, such as thrombin, in their active form. This serum is used as a natural and physiological activator of the PRP coagulation process.

Liquid PRP is mixed with autologous thrombin to produce a platelet gel in which platelets are uniformly dispersed. PRP and ATS once mixed in a double chamber applicator applies a powerful matrix gel at the targeted area where platelets release growth factors over time. With RegenKit-Surgery, PRP and ATS are mixed together and applied onto areas to be treated using the RegenSpray Applicator. The resulting autologous fibrin

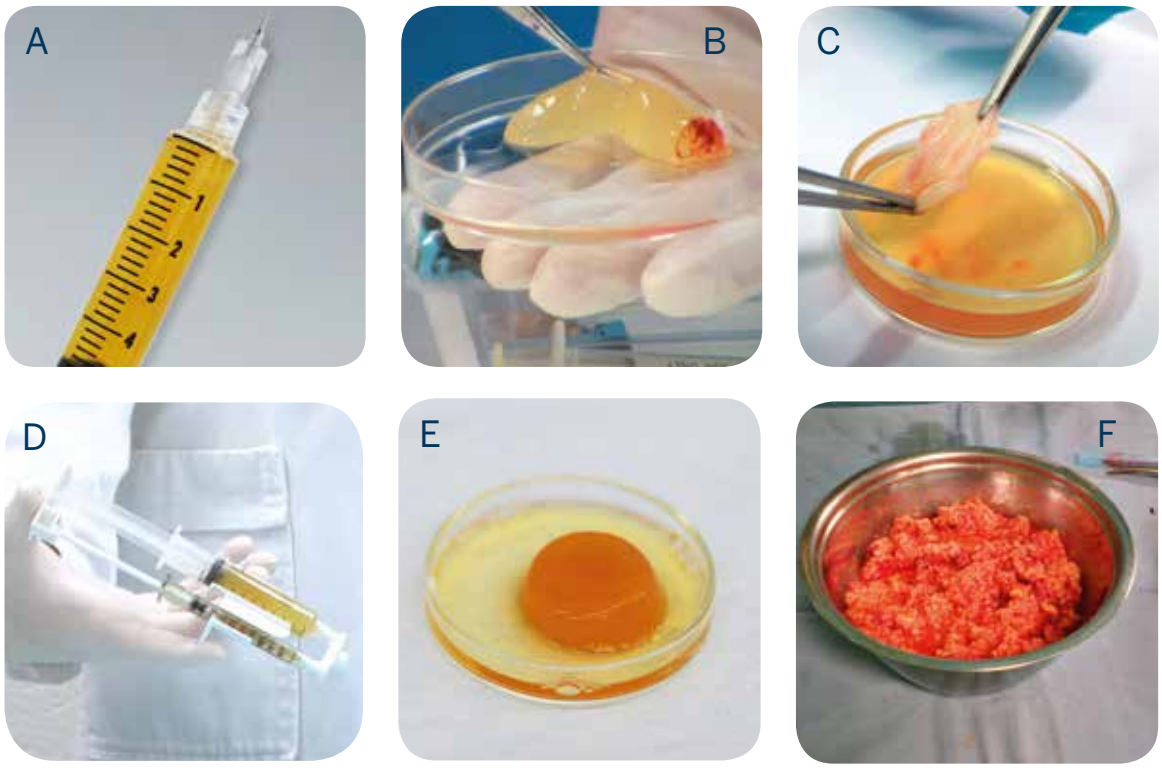


Figure 7: Different forms of PRP obtained with RegenKits.
A. Liquid PRP without additional manipulation. B-C. Suturable products obtained by addition of calcium gluconate 10% solution and performing a second centrifugation. B. Fibrin clot obtained with RegenFibrin Polymer, following a 10-minutes second centrifugation step. C. Fibrin membrane obtained with RegenKit Extracell Membrane, following a 20-30 minutes second centrifugation step. D-F. Combination with autologous thrombin, with or without addition of calcium gluconate 10% solution D. Autologous glue obtained with RegenKit-Surgery E. Biological dressing F. Bone putty

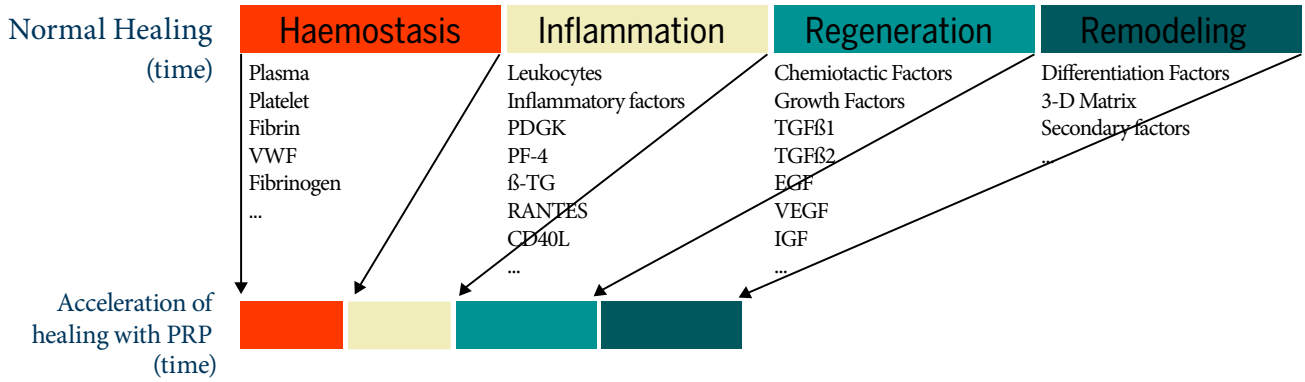


Figure 8: Illustration of A-PRP benefits in the healing cascade.

glue can be used to control bleeding, to glue tissue or to fill tissue defects for a wide range of surgical procedures and to cover wounds (Figure 7D). Optionally, a 10% injectable solution of calcium gluconate may be added to the serum to fasten the coagulation process. It is also possible to prepare a clot that is used as a biological wound dressing by mixing PRP with ATS and calcium gluconate solution in a sterile cupule, (Figure 7E). For orthopedic applications, bone marrow concentrates prepared with RegenKit Extracell Glue can be mixed with PRP, ATS and bone substitute to produce a bone putty enriched in stem cells (Figure 7F).

1.2.4 Advantages of PRP

PRP shortens the different phases of the natural healing process (Figure 8). Because PRP is prepared from autologous blood, it represents an inherently safe option to heal soft and hard tissue, thereby eliminating the risk of immune reactions or transmission of infectious diseases (Marx, 2001). PRP acts not only as a source of multiple growth factors, which stimulate cell proliferation, but also as a tridimensional (3-D) bioactive scaffold (fibrin gel), which enhances cell migration and new extracellular matrix deposition. Another presumed advantage of PRP is that unlike individually isolated growth factors that are used in arbitrary concentrations during treatment, platelets in PRP release multiple growth factors in a controlled manner, thus in physiological proportions and with a natural balance of proliferative and inhibitory agents (Yuan et al., 2013). Finally, several in vitro studies have also suggested that PRP may have an antimicrobial effect against pathogens such as Staphylococcus aureus and Escherichia coli (Bielecki et al., 2007).

1.2.5 Risks related to the use of PRP

General risks associated with the preparation of PRP have been defined as those associated with the phlebotomy and include the following as defined by the World Health Organization (WHO):

- Hematoma formation (the most common complication of venipuncture)
- Infection
- Nerve damage
- Vasovagal reaction, syncope and fainting
- Extensive bleeding
- Pain at blood sampling site

Even though the therapeutic use of PRP in the past 20 years has demonstrated to be a safe and effective treatment, there are some contraindications (Hall, 2009). A detailed list of the absolute and relative contraindications for the use of PRP is available under section (see Contraindications 2.2.9).

1.3 HISTORY OF PRP PREPARATION

PRP therapy has gained popularity in regenerative medicine and other specialties since the earliest reports of its clinical use in the 1980s and 1990s (Wu et al., 2016). David R. Knighton described the first use of “locally acting growth factors obtained from human platelets and applied topically”, highlighting how interesting it may be to isolate growth factor-secreting plasma cells from whole blood to induce tissue regeneration, particularly in chronic wound healing. At that time, Knighton used laboratory tubes without any polymer-based separating gel to prepare his PRP (Knighton et al., 1982; Knighton et al., 1986).

In maxillofacial surgery, Robert E. Marx evaluated the effect of PRP on bone maturation rates and bone density in bone graft reconstructions of mandibular continuity defects, demonstrating that the addition of PRP to grafts resulted in increased bone formation (Marx et al., 1998).

Since these pioneering applications, PRP use has spread to various applications around the world, including sports medicine, orthopedics, cardiovascular surgery, esthetic medicine, maxillofacial surgery, plastic surgery, ophthalmology and gynecology (Figure 9).

Henceforth, PRP use has spread to various applications around the world, including sports medicine, orthopedics, cardiovascular surgery, cosmetics, maxillofacial surgery, plastic surgery and ophthalmology (Figure 9).

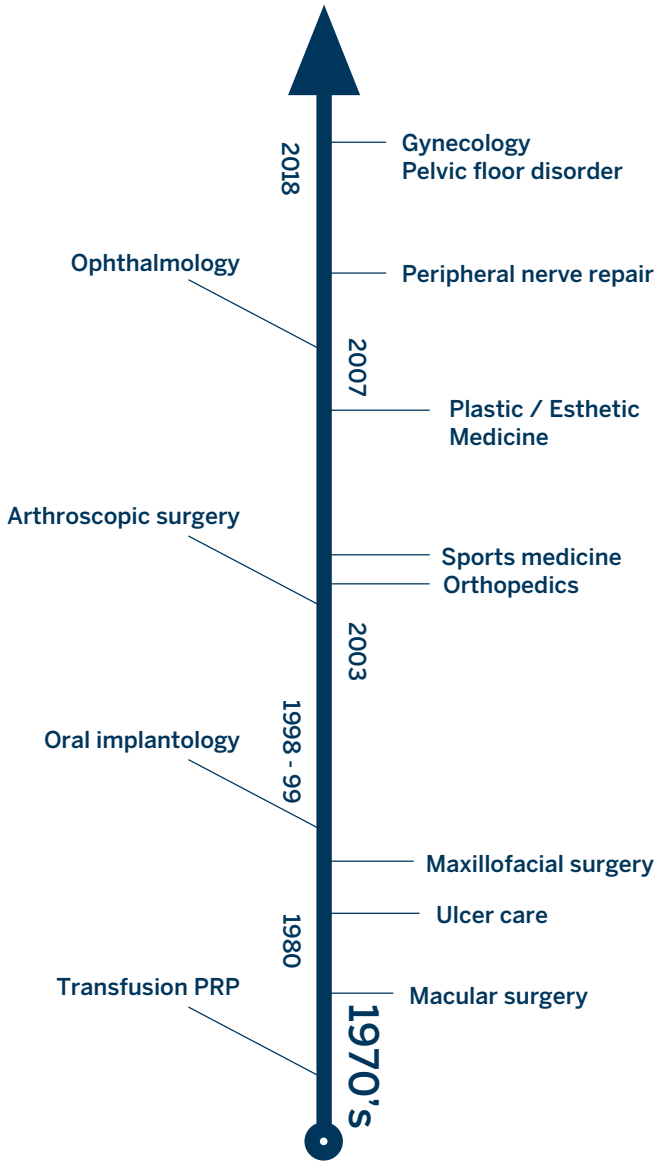


Figure 9: History of PRP preparations in different therapeutic fields.

The relatively low cost, ease of use, as well as major commercial involvement has facilitated PRP's rapid expansion within medical practices (Labusca et al., 2016). Nowadays, different technologies are available for PRP preparation, including small volume tube centrifugation and larger apheresis machines (Cohn et al., 2015). PRP preparation has been greatly simplified in recent years, thanks to the development of commercial PRP preparation devices. These technological advances have allowed PRP treatments to move from operating rooms to outpatient clinics and offices, and PRP can now be produced easily and safely in 15-30 minutes at the patient's bedside (Yuan et al., 2013). Regen Lab is the pioneer of the simplification and standardization of PRP preparation by designing devices using thixotropic separating gel that's acts as a physical barrier between blood components for precise isolation of platelets and plasma without undesired contamination with blood cells.

The current growing interest for PRP use is illustrated by the fact that PRP has been the subject of more than ten thousand publications in recent years as evidenced by a recent PubMed search (Figure 10). Moreover, there is currently more than one hundred open clinical trials involving PRP therapies to treat various pathological conditions with a vast majority focusing on musculoskeletal (MSK) conditions (https://www.clinicaltrials.gov/).

According to "Platelet Rich Plasma (PRP) - Global Market Outlook (2017-2023)", the global market for PRP was estimated at $158.23 million in 2016 and is expected to reach $383.56 million by 2023 growing at a compound annual growth rate (CAGR) of 13.4% from 2016 to 2023.
(https://www.reportlinker.com/p05088980/Platelet-Rich-Plasma-PRP-Global-Market-Outlook.html).

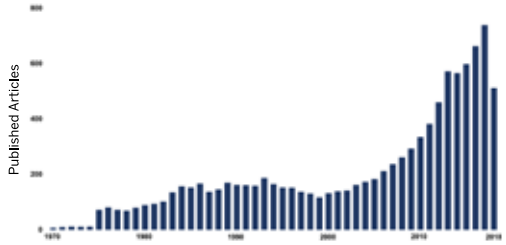


Figure 10: PubMed Results by year. Search Term: Platelet Rich Plasma. Search date: August 14, 2018.

1.4 TECHNOLOGICAL CONTEXT

Currently, there are several CE-Marked and/or US Food and Drug Administration (FDA)-approved devices available for the preparation of PRP from a relatively small volume of blood (8 to 60 ml) that are based on a centrifugation system. A systematic literature search aiming at identifying those devices and comparing their performance highlighted the great variety of PRP preparations produced by these different commercial devices (See examples in Table 1).

In all evaluated devices, blood is collected and mixed with an anticoagulant to avoid blood premature coagulation, then immediately processed by centrifugation. While their common purpose is to fractionate whole blood into its composite parts to allow isolation of plasma with elevated levels of platelets, each device functions differently. Specifically, these devices and their protocols differ in their method of isolation (with or without mechanical barrier between blood components), type and operation of the collection device, number and duration of centrifugation, relative centrifugal force required, and other processes of production. These variances result in PRP preparations with variable volumes of plasma, platelet concentration, quantities of growth factors, and residual white and red blood cells concentrations (Mazzocca et al., 2012). As described in Dr. Jane Fitzpatrick's publication (Fitzpatrick et al., 2017), PRP preparations can be classically divided into two types: "buffy coat" or Leukocyte Rich PRP and plasma oe Leukocyte Poor PRP.

Buffy-coat systems use centrifugation protocols that concentrate platelets at the level of white blood cells (the buffy-coat layer). These kits typically produce PRP with platelet concentrations between 3 to 8 times over the baseline level, or even higher. However, the resulting PRP is highly contaminated with WBC (2 to 7 time over the baseline values) and with variable amounts of RBC, as seen in Table 1. Buffy coat PRPs are pink to reddish due to the important level of RBC contamination.

On the contrary, plasma-based PRP aim to capture only plasma and platelets and exclude red and white blood cells. The resulting PRPs are golden yellow and with platelet concentrations around 1.5 to 3 times over baseline value in blood (Filardo et al., 2012). Most plasma PRP devices use a soft spin centrifugation with no physical separation between platelet-rich plasma and other blood components. Consequently the cellular composition of plasma-based PRP is strongly dependent on the operator's dexterity and thus with no reproducible composition. In addition, with such devices the larger and denser platelets that are at the level of the buffy coat line are not recovered.

Regen Lab's innovative technology allows to produce a PRP which combines the advantages from both preparation methods. The separating gel intercalates itself precisely inside the buffy coat line, forming a physical barrier that isolates the platelets and the plasma in the upper part of the device, while the undesired red and white blood cells are sequestrated in the lower part (see Figure 11). The resulting Regen PRP is a plasma PRP with a high platelet recovery (>80%), a low level of WBC (specific depletion of the pro-inflammatory neutrophils) and virtually no RBC.

It is important to note that too high platelet concentrations (more than 5 times over the baseline

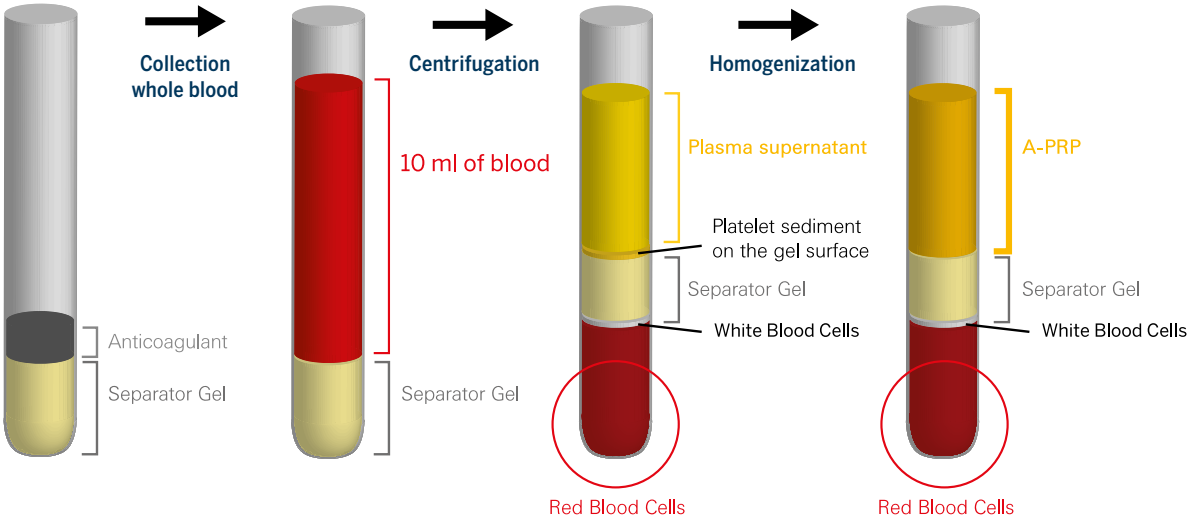


Figure 11: Principle of operation of Regen Lab technology for PRP preparation.

Table 1: Conforming commercial medical devices for the preparation of PRP and their performance characteristics

Technology	Device name		Blood vol.	PRP vol.	Platelet concentration factor	Platelet recovery	PRP Product
Regen Lab devices with separating gel	**RegenTHT**		**8 ml**	**4-5 ml**	1.7 X Can be increased up to 5X by discarding some PPP	**> 95* %**	**Fully automated blood component separation** **Very low contamination of RB**C in RegenTHT PRP (HCT < 1%) Virtually no RBC in Regen BCT & A-CP PRP (HCT < 0.2%) **Specific depletion of pro-inflammatory WBC** 85% granulocyte depletion in RegenTHT PRP 96% granulocyte depletion in Regen BCT & A-CP PRP
	Regen BCT **A-CP**		**10 ml**	**5-6 ml**	1.6 X Can be increased up to 5X by discarding some PPP	**> 80* %**	
Soft centrifugation and manual plasma aspiration	Arthrex ACP		15 ml	4-6 ml	1.6-2 X	≤ 60[1] %	**Plasma based PRP** **Operator dependent** results, no physical separation between plasma and blood cells. Virtually no red and white blood cells if plasma collected without getting near the buffy coat line
	BTI PRGF/Endoret		8 ml	1-2 ml	2-3 X	≤ 50[2] %	
Floating buoy	Biomet GPS III		27 ml 54 ml	3 ml 6 ml	4 to 6 X	43[3] to 73[4] %	**Buffy coat PRP** Highly concentrated platelets (> 4X) concentrated **white** blood cells (> 4X) concentrated **Granulocytes** (≥ 3X) **High red** blood cell contamination (HCT from 5% to > 30%)
Floating shelf	Harvest SmartPrep2		27 ml 54 ml	3 ml 7 ml	4 to 8 X	65[3] to 85[3] %	
Computer aided, light sensor systems	Arthrex Angel	HCT 2%	35 to 52 ml	3 ml 7 ml	6 to 10 X	40[5] to < 60[4] %	**Buffy coat PRP** **Setting dependent composition. Reducing cellular contaminant strongly reduces platelet recovery** Highly concentrated platelets (> 5X) Variable concentration of **white** blood cells Variable concentration of **Granulocytes** (> 3X) **High red** blood cell contamination (HCT ≥ 2% to 15%)
		HCT 7%				47[5] to 75[4] %	
	Arteriocyte Magellan	Low HCT	26 to 52 ml	3 to 7 ml	3-10 X	21[5] %	
		standard		3 to 7 ml		≤ 65[5] %	

*Data on file, 1. Arthrex Research and Development, 2016. Plasma-based Autologous Blood Systems: Arthrex ACP®, MTF Cascade®, and Orthovita® CellPaker®/Vitagel™ LA1-0809-EN_C

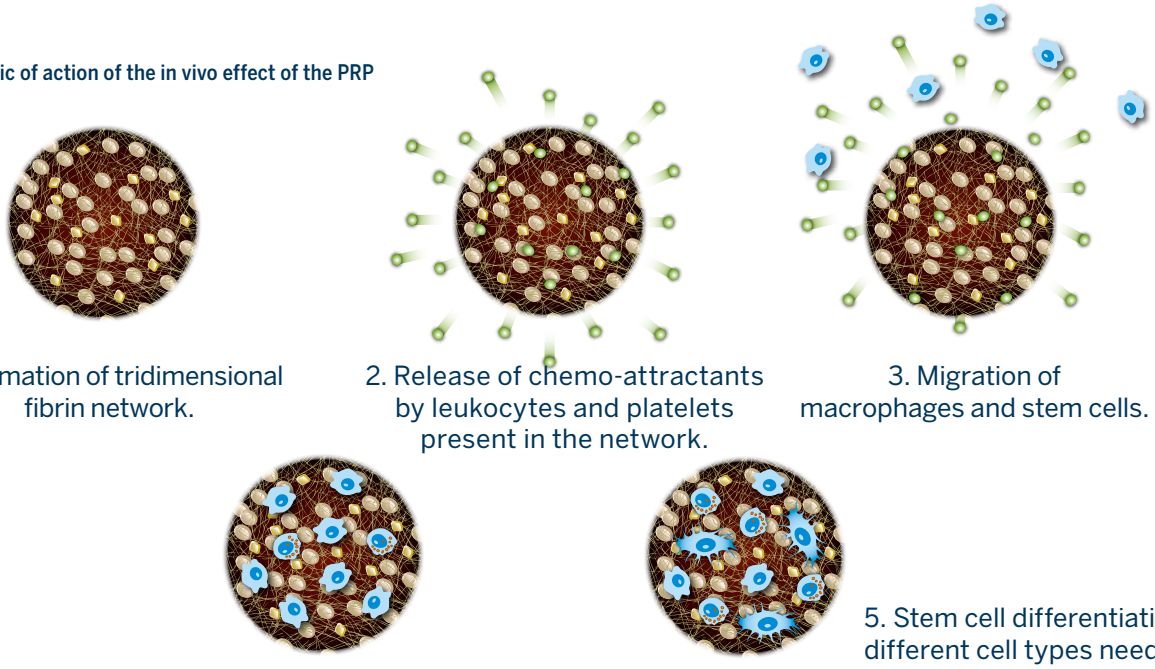
2. Anitua E, Pelacho B, Prado R, et al. Infiltration of plasma rich in growth factors enhances *in vivo* angiogenesis and improves reperfusion and tissue remodeling after severe hind limb ischemia. J Control Release 2015;202:31-9.
3. Kevy SV, Jacobson MS. Comparison of methods for point of care preparation of autologous platelet gel. J Extra Corpor Technol 2004;36:28-35.
4. Degen RM, Bernard JA, Oliver KS, Dines JS. Commercial Separation Systems Designed for Preparation of Platelet-Rich Plasma Yield Differences in Cellular Composition. HSS journal : the musculoskeletal journal of Hospital for Special Surgery 2017;13:75-80.
5. Arthrex Research and Development, 2015. Angel® vs Magellan®: A Comparative Study on Platelet Concentration and Activation. LA1-00028-EN_A;

values) have been shown to produce suboptimal outcomes or cytotoxic effects (Yoshida et al., 2014; Graziani et al., 2005). Comparative clinical studies always favor lower concentrated PRP (Fleming et al, 2014; Filardo et al., 2012; Rappl et al., 2011). The so-called therapeutic platelet concentration of 1 billion per ml (4 to 5 times over the baseline values) (Marx et al., 2001) is only valid for leukocyte-rich PRP, as a higher platelet concentration is needed to compensate for the negative effects of pro-inflammatory WBC (neutrophils). Leukocyte-poor PRPs with a platelet concentration 1 to 3 times over the baseline values (0.2 to 0.6 billion per ml), such as the RegenPRP, have shown their efficacy in a wide variety of therapeutic areas.

1.5 RATIONALE FOR PRP USE & CLINICAL RELEVANCE

The rationale behind the clinical use of PRP is based on the ability of platelets to release in an orchestrated manner supra-physiological levels of essential growth factors and cytokines from their alpha-granules (α-granules).

Figure 12: Kinetic of action of the in vivo effect of the PRP



1. Formation of tridimensional fibrin network.

2. Release of chemo-attractants by leukocytes and platelets present in the network.

3. Migration of macrophages and stem cells.

4. Stem cell proliferation.

5. Stem cell differentiation into different cell types needed for tissue restoration.

This provides regenerative stimuli that accelerates and promotes tissue repair (Wu et al, 2016), by increasing the recruitment, proliferation and differentiation of the cells involved in tissue regeneration (Figure 12).

The modulation of the inflammatory response is also an important mechanism of action mediated by PRP. In the context of inflammatory lesions, PRP control the secretion of pro- and anti-inflammatory factors which reduce and shorten the inflammatory response. In this sense, PRP is capable of modulating secretion and recruitment of key inflammatory cells, such as monocytes and leukocytes, to the injury site (Marques et al., 2014).

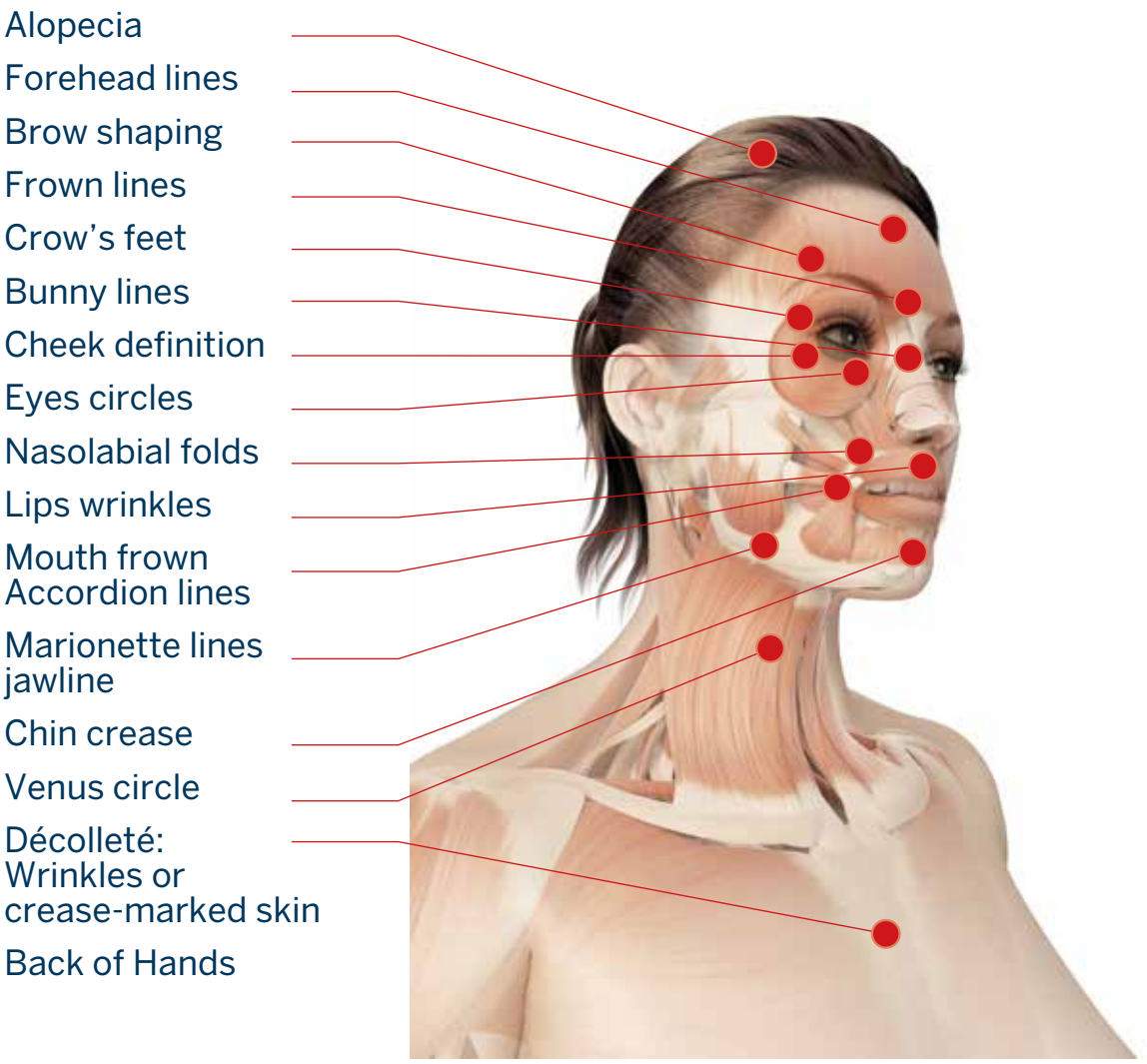
The application of PRP has been documented in many fields, ranging from hard tissue regeneration to soft tissues management. We present below a summary of the main findings regarding the most common PRP applications.

1.5.1 Skin care & plastic surgery

Due to its ability to induce collagen synthesis by dermal fibroblasts, PRP has been proposed as a promising treatment option in the field of skin care (Abuaf et al. 2016, Cho et al., 2010; Cho et al., 2012; Kim et al., 2011). For a few years, it is successfully used in numerous skin care applications, including alopecia, scar revision (keloids or traumatic scars), acne scars, stretch marks, skin rejuvenation (overall improvement in skin texture and firmness) and dermal augmentation (e.g. deep wrinkles like nasolabial folds) (Figure 13). For some indications, PRP may be used in association with other treatment modalities such as laser, radiofrequency or fat grafting to improve clinical outcomes.

In plastic surgery, PRP can be used as an autologous glue in surgical procedures involving the creation of skin flaps, such as face lift, breast reduction, limb lifting or abdominoplasty to reduce seroma and/or hematoma formation. As PRP is an autologous product, it can't induce any serious adverse effects. However, when injected intradermally, some minor side effects may occur, such as local injection site reactions like pain, erythema, itching or burning that spontaneously disappear after a few days.

Figure 13: Skin care and plastic surgery. Disclaimer: These applications have not been cleared or approved by US-FDA for the PRP injections



SKIN CARE APPLICATIONS:
- Fine lines
- Deep wrinkles
- Dark circles
- Alopecia
- In combination with laser or chemical peel treatments

SURGICAL APPLICATIONS
- Combined with Autologous Thrombin Serum (ATS) to obtain an Autologous Biological Glue
- Combined with fat tissue for volume correction

1.5.2 Orthopedic and pain treatment

PRP has become increasingly popular over the past few years in the field of sport medicine, as it represents an innovative way to manage musculoskeletal injuries (Figure 14).

The localized injection of PRP has been clinically evaluated for various musculoskeletal disorders, ranging from osteoarthritis (OA), tendinopathies (rotator cuff, patellar, Achilles, and elbow tendons), chondropathies, muscle tears and ligament tears (anterior cruciate ligament). Studies assessing its safety and effectiveness are plethoric but their outcomes greatly vary because of a lack of standardization of PRP preparation, injection technique, and outcome assessment. Nevertheless, it can generally be concluded that PRP, and more specifically leukocyte poor PRP such as Regen Lab PRP, represents an attractive therapeutic option for most of the above-mentioned indications based on the evidence which suggests it can effectively reduce/relieve pain and improve function.. Side-effects are rare. Most commonly encountered complications following the use of PRP occur following intra-articular injections and are limited to local inflammatory reactions like swelling and local pain.

ORTHOPAEDICS & SPORT MEDICINE
Injection of Autologous PRP from RegenKit®
- Promotes and accelerates the healing process providing significant improvement in symptoms
- Significantly reduces pain
- Reduces the need for alternative treatments such as analgesics, anti-inflammatories, cortisone injections and surgery
- Minimizes the risk of immunological cross-reactions
- Eliminates risk of cross-contamination with communicable diseases such as Human immunodeficiency virus (HIV), Creutzfeldt-Jakob disease (CJD) and hepatitis.

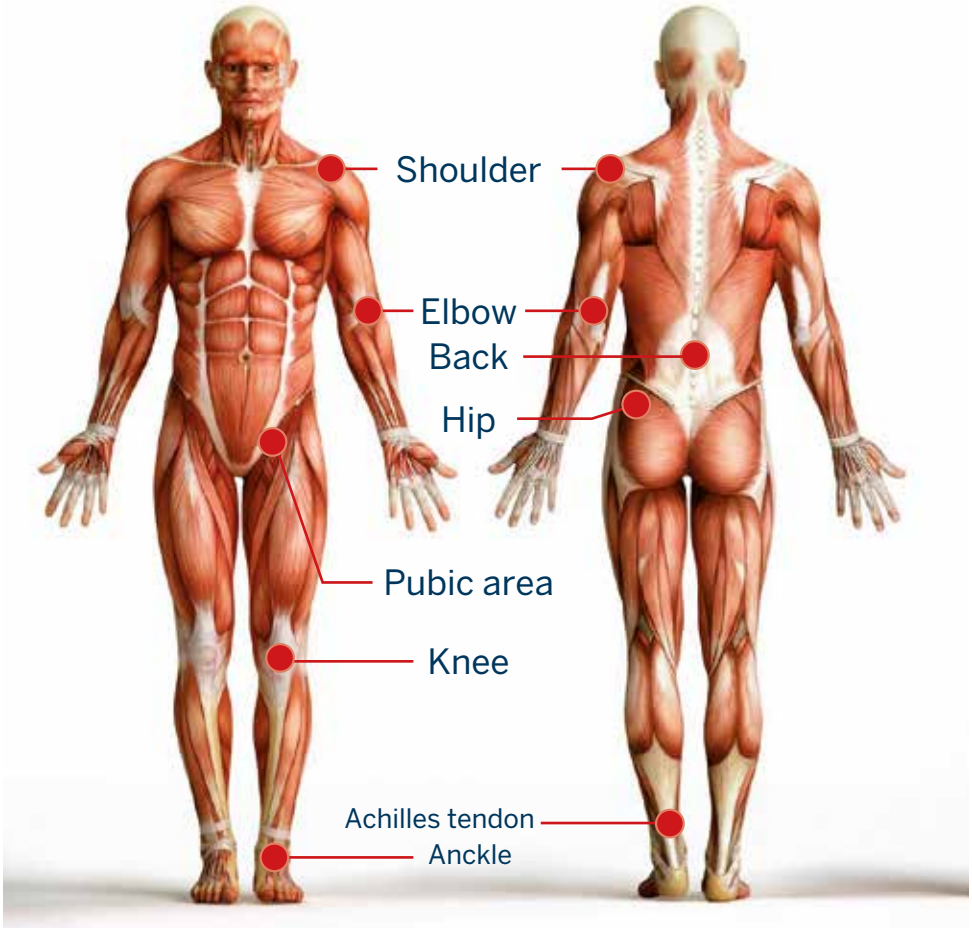


Figure 14: Orthopaedic and sports medicine.

APPLICATIONS:
- Tendon, ligament & muscular lesions
- Plantar fasciitis
- Cartilage lesions (potentially in combination with a synthetic 3-D matrix)

Combined with Autologous Thrombin Serum (ATS) to form autologous biological glue for different applications such as:
- Prosthetic implant biointegration
- Bone reconstruction (potentially in combination with bone substitute and / or bone marrow stem cells)

1.5.3 Wound care

The use of PRP represents a promising approach to treat hard-to-heal or non-healing wounds. Indeed, besides its ability to support the hemostasis and the healing process via the secretion of growth factors in a biologically determined ratio and in an orchestrated manner, it has been shown to have anti-microbial and pain-relieving properties. From a clinical point of view, the beneficial effect of PRP for the healing of chronic wounds such as legs ulcers, diabetic ulcers, pressures sores (Monami et al., 2017; Kontopodis et al., 2016) or acute wounds such as split-thickness skin graft, surgical wounds, fistulas, burns (Guerid et al., 2013; Filomia et al., 2013; Nicoli et al., 2015; Teodoreanu et al., 2014) has been evidenced in a high number of clinical investigations.

In chronic wounds, PRP treatment generally shows positive outcomes with no complication during the procedure or the post-treatment period. Interestingly, PRP is also able to provide a beneficial effect when there is no other therapeutic option for the treatment of a refractory wound. In general, complete closure can be obtained after several weekly topical applications and/ or injections in the wound bed (Cervelli et al., 2010).

For acute wounds, PRP treatment has also shown to be effective for a variety of wounds, especially when this cannot be achieved with conventional therapies. In addition, it was shown to reduce post-operative pain, healing time, and the occurrence of infections compared to alternative treatments.
In cardio-vascular surgery, PRP is able to significantly reduce the incidence of sternal wound infections (Serraino et al., 2013) or treat driveline infections (Jirritano et al., 2013).

No severe adverse effect has been reported following the use of PRP as a treatment for hard-to-heal wounds.

1.5.4 Dentistry

The use of PRP in dentistry has demonstrated to reduce bleeding and enhance soft tissue healing and bone regeneration. Human clinical studies have yielded promising results regarding the application of PRP to many dental and oral surgical procedures (i.e. tooth extractions, periodontal surgery, implant surgery). The

use of PRP in the alveolar socket after tooth extractions is capable of improving soft tissue healing and positively influencing bone regeneration (Traini et al., 2018, Forni et al., 2013). PRP has produced better results in periodontal therapy in association with other materials than when it was used alone. Promising results have also been obtained in implant surgery, when PRP was used as a coating material.

PRP is also useful in the management of biphosphonate-related osteonecrosis of the jaw (BRONJ) with the aim of enhancing wound healing and bone maturation. The combination of necrotic bone curettage and PRP application seem to be encouraging for the treatment of refractory BRONJ, as it has proven successful outcomes with minimal invasiveness.

Since PRP is safe for patients and is not difficult to prepare and use, it can be employed as a valid adjunct in many procedures in oral and dental surgery (Albanese et al, 2013).

1.5.5 Ophthalmology

PRP has been used more recently with successful outcomes in the treatment of refractory corneal ulcers (epithelial defects of the cornea that fail to heal), moderate to severe dry eye syndrome (Garcia-Conca et al., 2017), neurotrophic keratitis, ocular surface syndrome post Laser in situ Keratomileusis (LASIK), and for surface reconstruction after corneal perforation associated with amniotic membrane transplantation. Autologous plasma has number of epitheliotrophic factors including EGF, TGF-α, fibronectin, and vitamin A. These factors are essential for the proliferation and maintenance of corneal epithelial cells and are also useful in treating ocular surface disorders by triggering faster healing (Alio et al. 2012). Preparation of PRP is achieved through two available formulations, eyedrops and clot. No serious adverse effects have been described so far with the use of these products and it is generally well tolerated.

1.5.6 Gynecology and urology

According to WHO, up to 22% of women suffer from dyspareunia. Treatment involves topical Hormone Replacement Therapy (HRT) which requires compliance and is not suitable for all women, especially with a past or family history of breast cancer. Furthermore, many women are hesitant to use and express concerns toward this treatment modality.

A majority of women (70%) experience stress incontinence and prolapse in their lifetime, of whom 30-40% are symptomatic and will require treatment to improve their quality of life. Pelvic floor surgery is currently the most common treatment; however, it affects women physically, economically and socially due to possible complications, failures, and prolonged post-op recovery. Pelvic floor surgery has become more complex in recent times due to various factors. Additionally, recent mesh controversies and class actions have resulted in the US FDA and Australian Therapeutic Goods Administration (TGA) removal of many transvaginal implants for prolapse and incontinence and vaginal biological xenografts. To meet the need of better informed women that desire brief down-time gynecologists are seeking a more holistic and comprehensive approach to minimally invasive management of these pathologies.

PRP in addition to its regenerative properties, produce anti-inflammatory and immunomodulatory effects which lead to a resolution of many gynecological symptoms. Positive results were observed in patients resistant to conventional treatment, such as topical steroids for lichen sclerosus and topical estrogens for vaginal atrophy.

PRP is a promising, minimally invasive treatment option with no major complications whereby women have shown improvement in their quality of life and sexual function (Aguilar et al., 2016), as well as clinical improvement of symptoms in lichen sclerosus (Behnia-Willison et al., 2016), vaginal atrophy, recently recognized as genitourinary syndrome of menopause, (Hersant, 2018 and Behnia-Willison et al., 2017) and dyspareunia (Runels et al., 2014).

PRP injection into the vulvo-vaginal area was associated with vulvo-vaginal revitalization, dermal and skin regeneration, and lesion resolutions. PRP or PRP gel could be used as an innovative approach to treat vaginal mesh exposure, after mesh resection, with complete healing (Castellani et al., 2016 and Behnia-Willison et al., 2018).

PRP alone or as a multimodal therapy with vaginal laser is an alternative non-surgical management for stress urinary incontinence, urinary urge incontinence, and coital incontinence, with minimal side effects, good safety, and efficacy (Behnia-Willison et al., 2018).

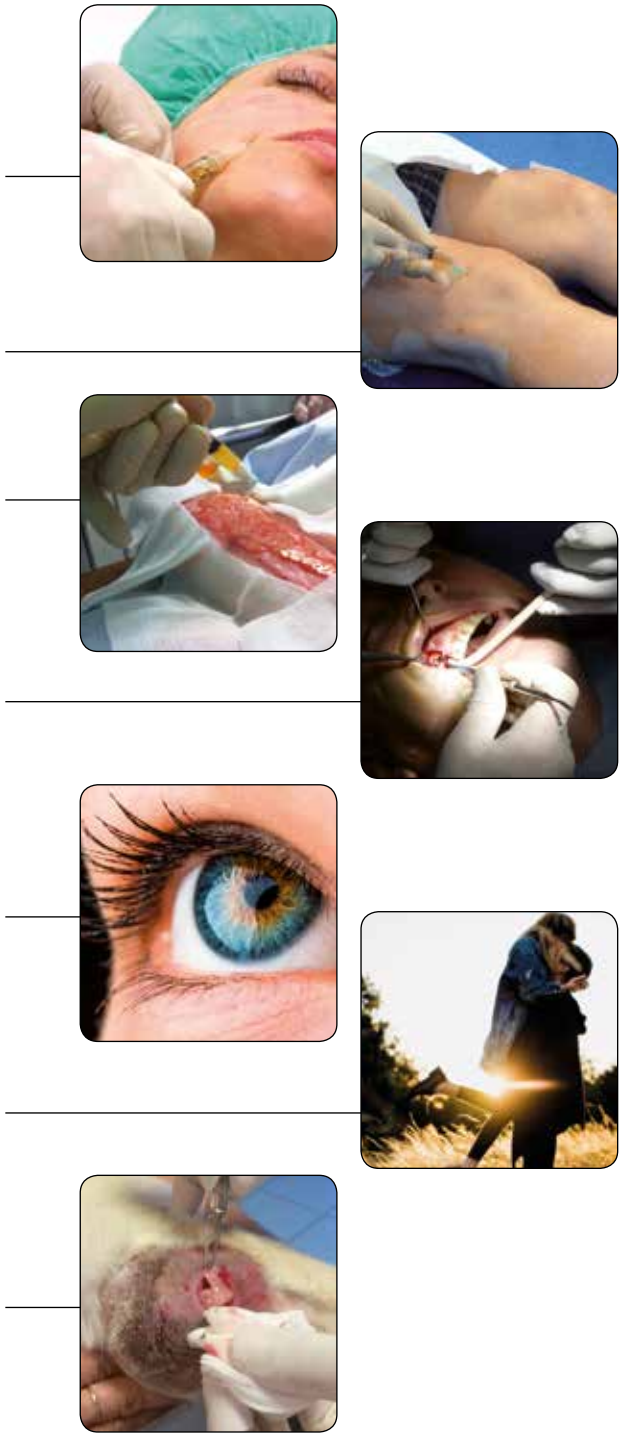
As PRP plays a significant role in vascular remodeling and tissue repair it is also a promising therapeutic option for varied male urologic pathologies such as erectile dysfunction and Peyronie's disease.

1.5.7 Veterinary

Veterinary physicians are currently using PRP to speed up the healing properties in specific animal injuries. PRP treatments used for many veterinary medical conditions across many different animal types: from dogs and cats to horses. A non-exhaustive list of the pathologies treated are for small animals: corneal ulcers, skin wounds, feline leukemia virus, hematological disorders, PRP transfusion for inherited and acquired coagulopathies, canine osteoarthritis and pain management. For horses: suspensory ligament injuries, damaged cartilage, non-healing wounds, corneal ulcers, tendon injuries, hock degenerative joint disease, tendon avulsions, fractures, periostitis. The autologous nature of PRP preparations makes them safer than other allogenic cell-based regenerative therapies and less expensive.

Figure 16: PRP Applications.

Skin Care & Plastic surgery	Hair loss management, Skin rejuvenation, Wrinkles, Acne scar, Fat grafting
Orthopedic & Pain Treatment	Osteoarthritis, Cartilage repair, Ligament repair, Tendonitis, Pain after surgery, Inflammation reduction, Chronic pain management
Wound Care	Diabetic ulcer, Venous ulcer, Pressure sore, Leg ulcer, Burn wound, Cyst
Dentistry	Bone graft including onlay & inlay, Sinus lift, Bone defect created by teeth removal or small cyst, Coser or cleft
Ophthalmology	Dry eye syndrome, Corneal ulcer, Epithelial defects of cornea that fail to heal, Ocular surface Syndrome post Laser surgery
Gynecology & Urology	Gynecology: Lichen sclerosus, Genitourinary syndrome of menopause Male erectile dysfunction: Peyronie's disease
Veterinary	Tendon and ligament injuries, Wound healing, Osteoarthritis treatment



1.6 BONE MARROW CELL CONCENTRATE

1.6.1 Rationale for bone marrow cell consentrate use & clinical relevance

Bone tissue regeneration occurs most predictably when compared with other tissues. Although most bone injuries heal without problems, there are several conditions under which enhancement of the repair process would be of great benefit to ensure the rapid restoration of skeletal function (Homma et al., 2013). For instance, non-union fractures and aseptic bone necrosis are two pathological conditions associated with an impairment of the cellular part of the repair, defined as a reduction in mesenchymal stem cells (MSCs) and of osteoblastic activation. In both conditions, bone lesions are not repaired in the normal timeframe nor in the normal manner. Both are therefore good candidates for cell-based therapies using bone marrow stem cells (Hauzeur et al., 2010).

Bone marrow host two main family of stem cells: Hematopoietic stem cells and MSCs. (Figure 17). Hematopoietic stem cells give rise to blood cells and platelets. MSCs differentiate into lineages of mesenchymal tissues, including bone, cartilage, fat, tendon, muscle, and marrow stroma. This potential of MSCs has created huge interest in traumatology and orthopedic surgery (Holton et al., 2016).

For bone healing, MSCs are recruited to the pathological area, where they are able to differentiate into osteoblasts under the influence of growth factors such as bone morphogenetic proteins (BMP), PDGF, TGF-α, IGF, and bFGF (Hauzeur et al., 2010).

MSCs are typically found in bone marrow, but are also present in fat tissue, synovium, periosteum, skeletal muscle, and the umbilical cord. Some data suggests that

the osteogenic differentiation capability of MSCs from bone marrow and from periosteum is higher than MSCs from adipose tissues (Hauzeur et al., 2010). This may be due to the fact that the potential of a multipotent cell may be considered not only an intrinsic capability of the cell alone but also its interaction with its physiological niche that provides a signaling network (i.e. the ECM, adhesion molecules, growth factors, cytokines, and chemokines secreted by the resident cells). Autologous bone marrow contains not only stem cells and precursor cells as a source of regeneration tissue, but also accessory cells that support angiogenesis and vasculogenesis by producing several growth factors (Giannini et al., 2009).

It should be considered, however, that bone marrow aspirates contain relatively low percentages of MSCs (0.001% to 0.01% of nucleated cells). Indeed, in clinical practice, it has been shown that efficacy of autologous bone marrow grafting is related to the number of progenitor cells in the graft. The number of progenitors available in bone marrow aspirate is greatly dependent of the site of collection and the aspiration technique used. (Hernigou et al., 2005). The iliac crest is the recommended site for bone marrow collection.

Newly developed systems, such as the RegenExtracell BMC kit, permit intra-operative processing of bone marrow aspirate to produce a bone marrow cell concentrate that is re-implanted during the same surgical procedure, without the need for a laboratory cell selection and expansion ("one-step therapy") (Holton et al., 2016).

RegenExtracell BMC kit are designed for bone marrow cell preparation, using the separating gel technology for optimal stem cell isolation.
By transferring the entire regenerative potential from the bone marrow to a site of lesion, bone marrow cell concentrate represents an interesting therapeutic option for the treatment of bone and osteochondral lesions.
Currently, bone or cartilage lesions that can be safely and successfully treated using RegenExtracell bone marrow cell concentrate one-step therapy range from osteochondral lesions, delayed unions, non-unions (pseudarthrosis), osteonecrosis of the femoral head, or cervical fusion.
Dr. Pietro De Biase and Prof. Rodolfo Capanna and their team introduced the "in vivo cell factory" concept to describe the use of autologous bone marrow cell concentrate combined with osteo-inductive scaffolds (demineralized bone matrix, autograft, allograft or synthetic scaffold combined with Regen PRP) for the repair of pseudo-tumoral cavity lesions. Results of their study conducted on 131 patients showed that healing could be achieved without recurrence of the lesion in 78.76% of patients (De Biase et al., 2016).

Dr. Roberto Civinini (Civinini et al., 2012) also described how he could achieve pain relief and prevent the progression of the disease in the majority of his patients suffering from early stage osteonecrosis of the femoral head by performing core decompression together with the injection of autologous bone marrow cell concentrate and the use of a new composite injectable

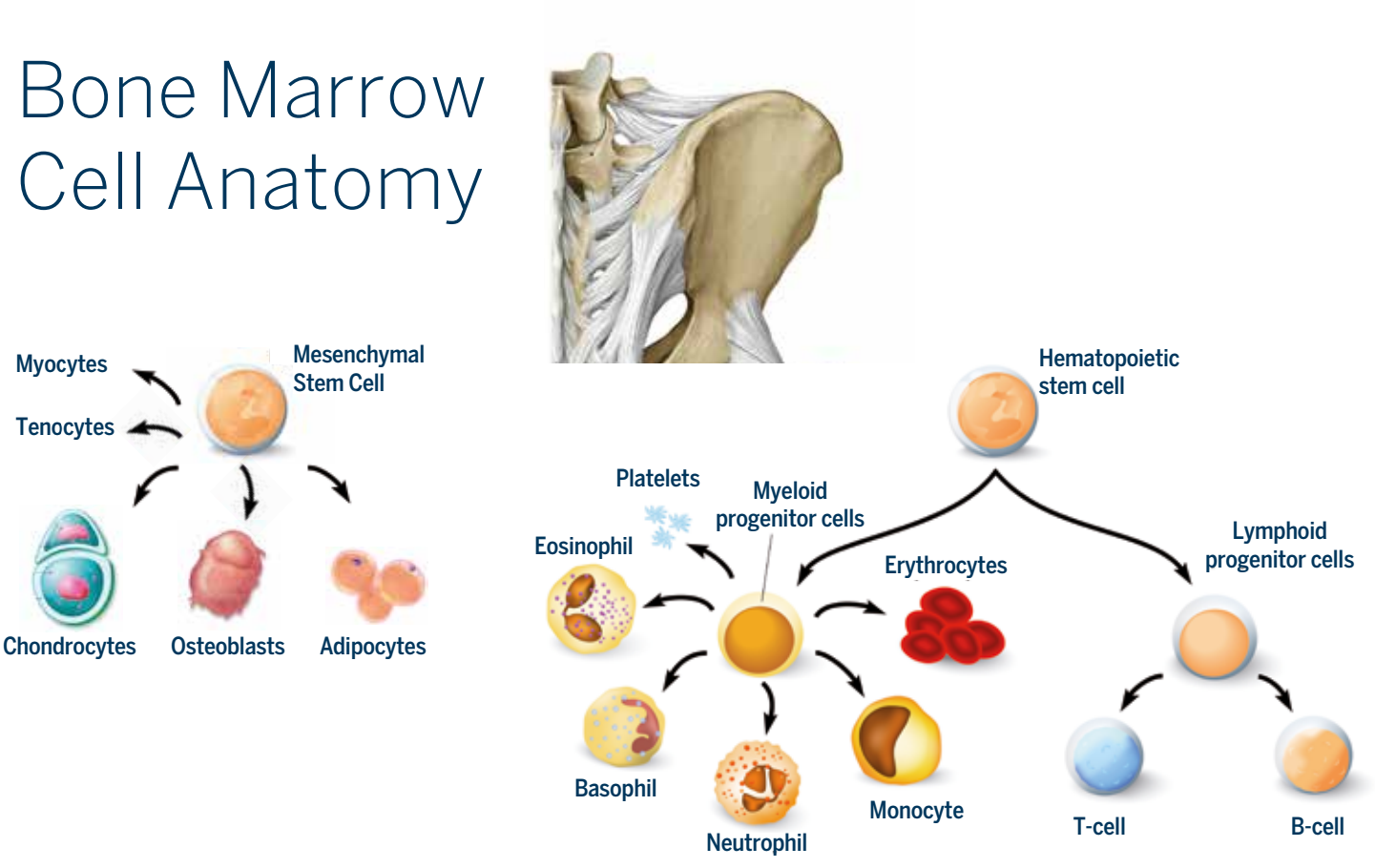


Figure 17: Cellular components of bone marrow aspirates concentrates.

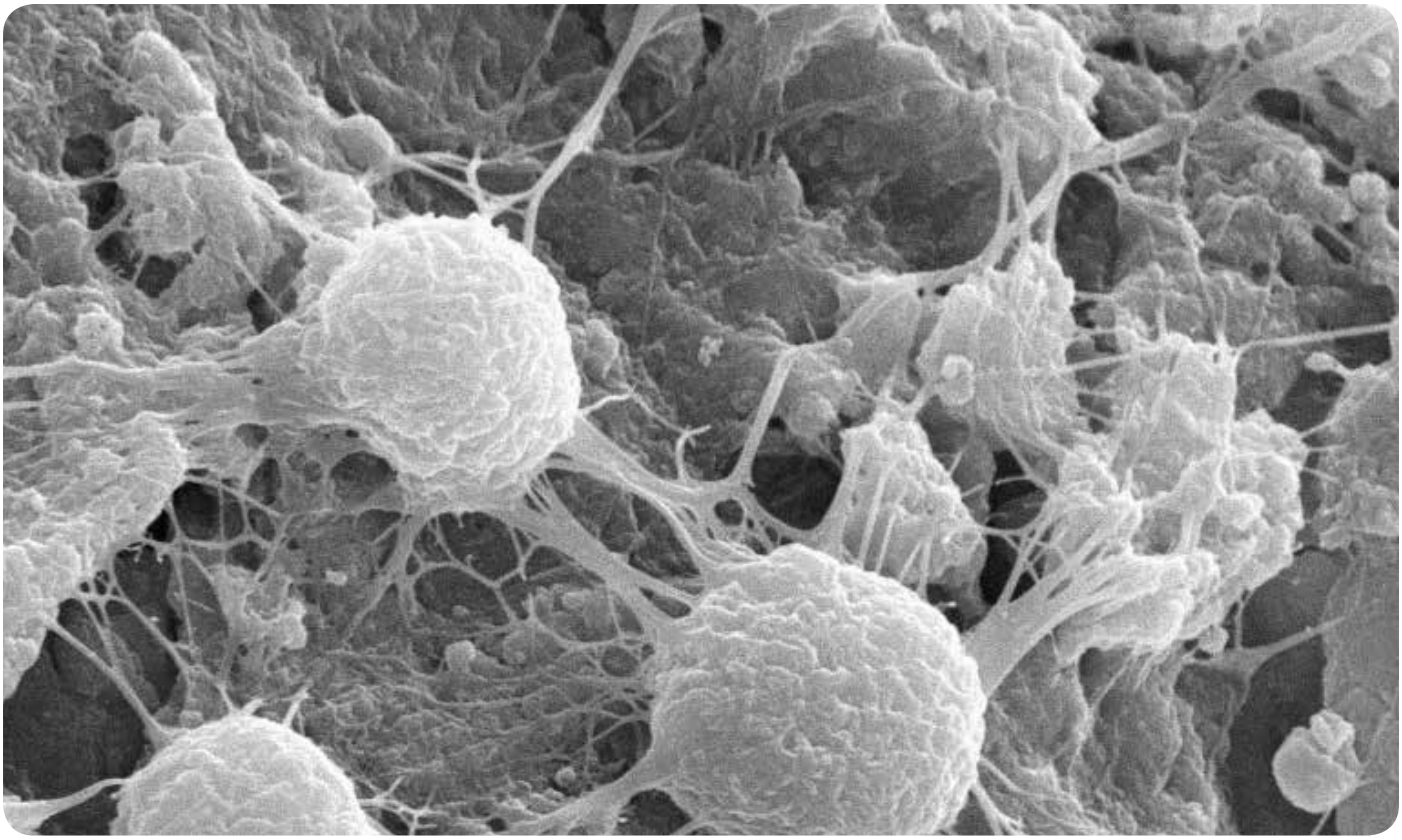


Figure 18: Scanning electron microscopy of platelet fibrin matrix gel (PRP-ATS) that is clinically used as biological glue. Platelet gel containing MNC was obtained using a mixture of PRP, autologous thrombin and a bone marrow cell preparation processed using Regen BCT, RegenATS, and RegenTHT medical devices, respectively. Courtesy Dr Susan Lim, Singapore.

bone substitute. The overall clinical success rate of this procedure was 86.5%, with three conversions to total hip arthroplasty.

Dr. Alberto Gobbi showed that repair of chondral injury using a HA-based scaffold with activated bone marrow cell concentrate provides good clinical outcomes and durable cartilage repair at medium-term follow-up that is superior to that achieved by microfractures (Gobbi et al., 2016).

1.6.2 Risks related to the use of bone marrow cell concentrates

General risks associated with the preparation of bone marrow cell concentrates have been defined as those associated with the bone marrow aspiration procedure. Complications related to bone marrow aspiration and trephine biopsy occurred in only 0.08% of total reported procedures; they included the following (Bain et al., 2005):

- Haemorrhage
- Infection
- Persistent pain
- Serous leak persisting for 6 up to days

Other risks related to the procedure of bone marrow aspiration include:

- Allergic reaction to anaesthesia
- Trocar tip break in the patient's bone

Up to now, 10'000 RegenExtracell BMC kits have been used, without any reported major side effect.

1.7 COMBINATION CELLULAR THERAPIES

PRP can be used to improve cellular therapies and sustain graft bio-integration.

1.7.1 PRP combined to fat

Auto-transplantation of adipose tissue is commonly used for the treatment of tissue defects in plastic and reconstructive surgeries. The reduced survival of a large percentage of the transplanted adipose tissue remains an unsolved issue. This is at least in part due to accelerated apoptosis of the implanted pre-adipocytes (D'Esposito et al., 2015). Recently, there has been increased interest in the co-application of PRP and fat grafts. It has been hypothesized that adding PRP to fat preparation may be a reliable way to bring appropriate nutrients at the early stage of transplantation to improve fat survival during soft tissue reconstruction and render the result more predictable. Platelet-released growth factors stimulate angiogenesis, cell differentiation and proliferation leading to reconstitution of the 3-D matrix that allows the rearrangement of adipocytes into the correct 3-D organization (Modarressi et al., 2013). In vitro studies have demonstrated that PRP can significantly induce the proliferation of adipose tissue-derived stem cells (Atashi et al., 2014). Dr. Valerio Cervelli and his team have successfully used autologous fat mixed with PRP prepared with RegenKits to improve healing of wounds of various origins, as well as for the treatment of traumatic scars (Cervelli et al., 2010, 2012).

1.7.2 PRP combined to bone marrow cell concentrate

Bone marrow cell concentrate is an attractive therapeutic option for cartilage and bone defect healing because it contains multipotent stems cells with the potential for diverse differentiation (see section 1.6). PRP contains a multitude of growth factors and can stimulate bone marrow MSCs to proliferate and differentiate. PRP, therefore, represents the ideal media to promote bone marrow stem cell survival, differentiation and proliferation for orthopedic reconstructive surgery (Figure 18).

To exploit the potential offered using bone marrow concentrates enriched with PRP, Regen Lab produces RegenKit Extracell Glue kits designed for bone marrow cell concentration using the separating gel technology for optimal stem cell isolation. The bone marrow cell concentrate is combined with PRP ATS and bone substitute to obtain a bone putty enriched in stem cells.

1.8 COMBINATION OF PRP AND HYALURONIC ACID: CellularMatrix

CellularMatrix is Regen Lab's latest innovation. It is the sole certified medical device that allows the combination of HA and PRP in respect to medical device and health regulations. Indeed, HA is an implantable medical device and PRP a biological drug. Consequently, they should not be combined by the operator, as modifications of medical devices or biological drugs are not authorized.

CellularMatrix allows the rapid and safe preparation of PRP in the presence of a high quality, non-cross-linked HA produced by bacterial fermentation. HA creates a cell friendly matrix in which platelets are suspended. This biologically enriched network facilitates cell migration and proliferation to the treated site.

As schematized in Figure 19, the hypothesis that the association of PRP and HA would provide synergistic clinical benefit for the treatment of OA arises from the fact that the effects of PRP and HA are based on complementary mechanisms of action. This favors the transition from a downward spiral ("vicious cycle") in which inflammation and degeneration induce pain and joint stiffness to a situation where PRP can reverse the catabolic environment, leading to regulation of inflammation and symptom relief. PRP/HA combination prepared with CellularMatrix (CM-PRP-HA) is successfully used for intra-articular injections for the symptomatic treatment of articular pain and the improvement of mobility (Abate et al., 2015, Seleem et al., 2017, see Chapter 4 for details; Barac et al., publication in progress, Renevier et al., to be published in 2018;).

The CM-PRP-HA combination is also used in the field of skin care for intra-dermal injections for the hydration of dehydrated and wrinkled skin, where it has been shown to prolong the effects of PRP, thereby improving skin tone and quality (Hersant et al., 2017; see Chapter 4).

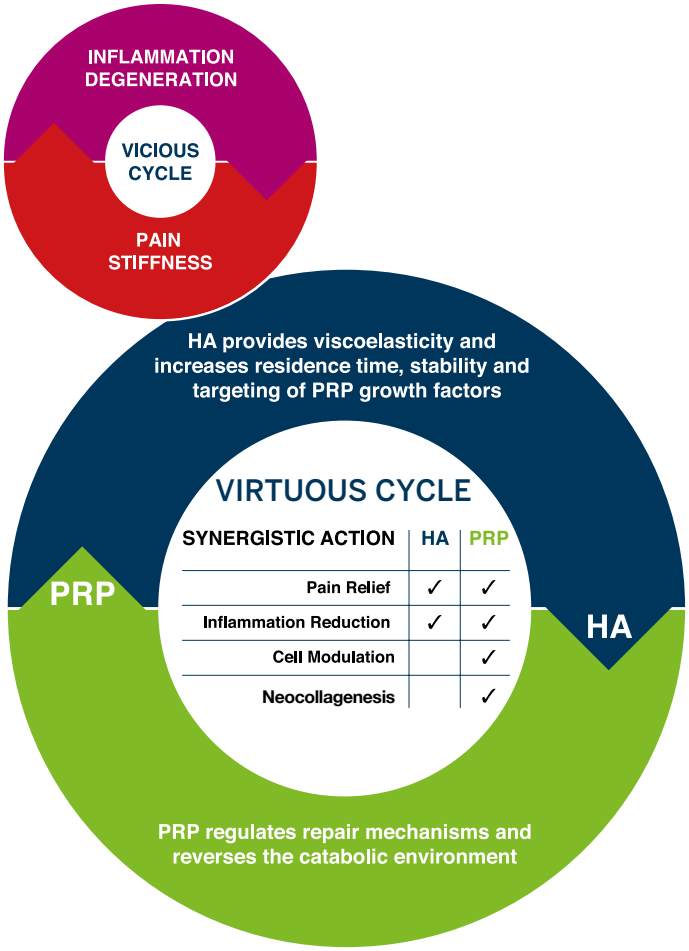


Figure 19: Complementary mechanisms of action of HA and PRP provide synergistic benefit for the treatment of osteoarthritis and other degenerative joint diseases.

1.8.1 Articular pain and loss of mobility

Articular pains are one of the most common health concerns among people 65 and older. They can be defined as pains related to a disorder in one of the structures of the joint: the cartilage, the subchondral bone, the joint capsule, the synovial membrane, menisci, tendons, ligaments and periarticular muscles. Joint disorders associated with pain can have either an inflammatory, a traumatic, or a degenerative origin. There are more than 150 diseases and progressive syndromes that affect joints (Fusco et al., 2016) and are characterized by joint pain and stiffness, sometimes associated with redness, swelling and a decreased range of motion. Osteoarthritis represents by far the most common cause of articular pain (Warner et al., 2016).

OA is considered a degenerative disease (Figure 20) that affects all articular joints and is associated with degeneration of the joint, subchondral sclerosis and inflammation of the synovial membrane (Fusco et al., 2016). The evolution of this condition is characterized by the following symptoms: pain, joint cracking/ popping, stiffness, deformity, loss of mobility and especially in the case of knee osteoarthritis (KOA), swelling (synovial effusion). The disease is related to aging and generally

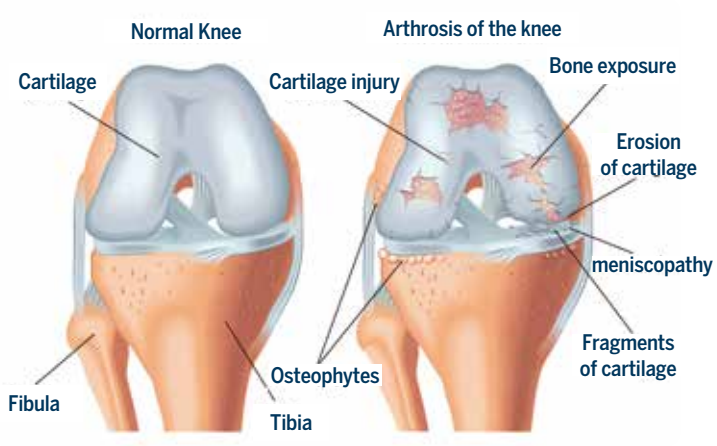


Figure 20: Osteoarthritis is a degenerative disease in which articular cartilage is worn causing pain and immobility.

affects the joints that are subject to stress, such as the knee, hips, small joints of the hand, and the cervical and lumbar spine (Fusco et al., 2016). OA can lead to functional impairment and deformities, thereby causing degradation of quality of life and may result in disabilities. Due to the fact that the knees and hips are weight bearing, osteoarthritis of these joints is often more disabling for persons who are affected by this condition. OA is the most common joint disorder in the United States. Among adults 60 years of age or older, the prevalence of symptomatic KOA is approximately 10% in men and 13% in women. The number of people affected with symptomatic OA is likely to increase due to the aging of the population and the obesity epidemic (Zhang et al., 2010).

A healthy joint is composed of 2 bone ends covered by cartilage (hyaline cartilage). This allows for shock absorption and for the bones to slide over one another with ease, thus ensuring the joint mobility. Synovial fluid (synovium) that surrounds the cartilage acts as lubrication and nutrition for the articular cartilage: it is mainly composed of HA, a glycosaminoglycan which binds to water molecules which in turn will result in a very viscous solution thereby giving the synovial fluid its shock-absorbing properties. It has been shown that the rheological properties of synovial fluid decrease with age and in patients suffering from OA, which may cause symptoms of pain and physical loss of function. OA has multiple causative agents, many of which are not yet fully understood. However, we know of a number of risk factors including; age, gender, genetics, obesity, joint trauma, and certain sports or professional activities. Conventionally, there is a distinction between so-called primary OA, for which there is no obvious predisposition, and secondary OA, related to previous trauma or joint diseases. The secondary type represents the most common form of OA. There are many and varied possibilities for treating articular pains presented in Figure 21.

Non-pharmacological treatments:

The National Institute for Health and Care Excellence (NICE) and Osteoarthritis Society International (OARSI) guidelines recommend that the first-line therapy for OA focuses on conservative therapies with an emphasis

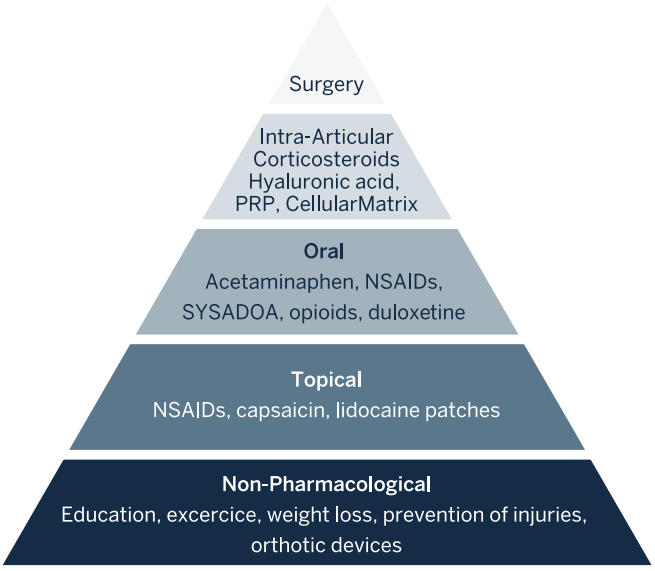


Figure 21: Multimodal management of osteoarthritis.
Modified from: www.medicographia.com/2013/10/osteoarthritis-treatments-where-do-we-stand-at-the-moment/

towards lifestyle modifications, including weight loss, water intake, land-based exercise, strength training and instilling self-management skills for a chronic disease process (Yu et al., 2016). Other conservative treatment modalities include biomechanical interventions aiming at supporting the limbs such as orthoses.

Oral analgesics:

Existing pharmacologic treatments mainly target pain with minimal, if any, effects on disease progression. Topical or oral non-steroidal anti-inflammatory drugs (NSAIDs) or Coxibs have taken over from paracetamol as the first-line analgesic medications (Yu et al., 2016; da Costa et al., 2016). However, they only have a limited effect over time.

Oral analgesic risks:
• NSAIDs are associated with important adverse effects.

A comparative effectiveness review (Chou et al., 2011) indicated that NSAIDs, when compared to placebo, are associated with an increased risk of:
• Serious gastrointestinal injury
• Cardiovascular injury
• Renal injury

A risk analysis based on a retrospective case-control survey of emergency admissions for upper gastrointestinal disease in two UK general hospital provided estimates of the frequency of serious gastrointestinal complications from NSAIDs (Table 2). Celecoxib, in the same review, appeared to have a lower risk of ulcer complications compared to non-selective NSAIDs but had a moderately higher risk of cardiovascular complications.

Slow-acting anti-rheumatic drugs:

Slow-acting anti-rheumatic drugs such as glucosamine and chondroitin sulphate have a delayed action on the pain and joint function. The precise mechanisms

Age range (years)	Chance of gastrointestinal bleed due to NSAID	Chance of dying from gastrointestinal bleed due to NSAID
	Risk in any one year is 1 in:	
16-45	2'100	12'353
45-64	646	3'800
65-74	570	3'353
≥ 75	110	647

Table 2: One-year risk of gastrointestinal bleeding due to NSAID (data from Blower et al., 1997, recalculated in Moore et al.,1999, and in Bandolier et al., 2011; found in Chou et al., 2011)

of action are unknown but may involve promotion of maintenance and repair of cartilage. Glucosamine has shown to increase proteoglycan synthesis. In Europe, it is available as a prescription drug, while in USA it is considered a dietary supplement and is not regulated as a pharmaceutical (Chou et al., 2011). Although still widely used in clinical practice for their chondroprotective effect, glucosamine and chondroitin sulphate have moderate efficacy and primarily allow for the reduction of analgesic and anti-inflammatory drug doses. A double-blind randomized placebo-controlled trial showed that the combination of glucosamine and chondroitin resulted in a statistically significant reduction in joint space narrowing at 2 years of use. The reduction in knee pain over the study period, however, was not statistically significant compared to placebo (Fransen et al., 2015).

Slow-acting anti-rheumatic drug risks:
A review of the available literature demonstrated excellent safety profile that is equal to placebo in most studies (Fibel et al., 2015).

Intra-articular injections of corticosteroids:

Intra-articular corticosteroid injections represent an appropriate additional treatment option against OA, especially when analgesics and anti-inflammatory drugs fail to relieve an inflammatory flare. They appear to be an effective way to decrease pain in the short-term and should be used when signs of inflammation arise (Fibel et al., 2015).

According to a meta-analysis performed by Dr. Nicholas Bellamy (Bellamy et al., 2006), the comparison of intra-articular corticosteroid and intra-articular placebo provides evidence for pain relief and global assessment at one week post-injection. There was evidence at 2 or 3 weeks post-injection that corticosteroid was more effective than placebo in pain reduction, but a lack of evidence for benefit in functional improvement. From 4 to 24 weeks post-injection, there was no compelling evidence of benefit, although some mid and late stage benefits in favour of corticosteroids were noted. Therefore, it appears that the beneficial effects of intra-articular corticosteroids are rapid in onset but may be relatively short lived (approximately 1 to 3 weeks).

Intra-articular injection of corticosteroids risks:
From a meta-analysis of 27 trials including 1767 patients in total, it was found that 13% of patients who used intra-articular corticosteroids experienced side effects (Jüni et al., 2015).

Risks related to the intra-articular injection itself include the following:
• Pain
• Intra-articular infection, sometimes leading to severe sepsis. This risk is however very low, provided aseptic technique is strictly maintained. The reported frequency of joint infection after intra-articular corticosteroid injection according to a survey performed among rheumatologists in 1999 is 4.6/100'000 (Pal et al., 1999). Lack of aseptic technique at the time of injection is thought to be the main cause. In general, infections are considered to be no more than the random expression of a small risk inherent to the procedure, if it is found to have occurred despite proper aseptic precautions. In such cases, infection is thought to be due to pathogenic organisms that were present in deeper-lying parts of the skin not accessible to aseptic disinfection (Holland et al., 2012). No epidemiology study has been conducted to determine the extent to which the improvement of aseptic techniques would influenced the above-mentioned frequency.

• Inaccuracy of the injection, which might contribute to the incidence of local tissue damage (atrophy of soft tissue and fat). A study revealed that accuracy at the knee and shoulder, which are among the most commonly injected joints, was poor (Jones et al., 1993).

Complications of intra-articular corticosteroids, although uncommon, include the following:
• Post-injection flare (reddening of the skin)
• Crystal-induced synovitis (inflammation of joint membrane)
• Tissue atrophy (wasting of tissue)
• Fat necrosis
• Calcification (deposition of calcium salts in tissue)
• Steroid arthropathy (acceleration of cartilage damage)
• Vascular necrosis (death of blood vessels)
• Hematoma (swelling caused by accumulation of blood)

In rare cases, absorption of intra-articular corticosteroids from the joint through the body may result in:
• Fluid retention
• Hyperglycemia
• Hypertension

Hyaluronic acid:

HA is a naturally occurring linear polysaccharide, widely distributed in human tissues, where it constitutes the major part of the extraCellularMatrix. It is mostly found in epithelial, connective, and neural tissues, and plays important biological roles. HA is a major component of synovial fluid and it is known that its concentration and its molecular weight is lower in synovial fluids from patients suffering from OA. Intra-articular injections of exogenous hyaluronic acid solution (visco-supplementation treatment) are currently the treatment of choice for OA, especially of the knee, due to HA's ability to relieve symptoms for several months at a time. HA injections are

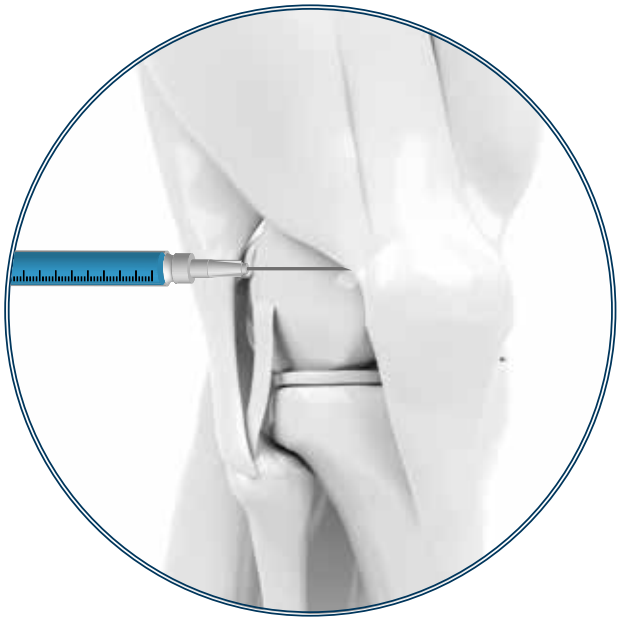


Figure 22: Viscosupplementation. This treatment involves injecting hyaluronic acid lubricant into the joint space of the knee.

designed to exert a mechanical action on the cartilaginous structures of the joints, leading to a reduction of the pain and an improvement of the joint function (Figure 22).

Because of its unique medical and physical properties, HA is used in various medical fields, ranging from aesthetic medicine to orthopedics. Particularly, its ability to retain water at up to 1000x its volume makes it the ideal substance for ensuring hydration and for adding volume to the skin, while its high viscoelastic properties make it an interesting tool for restoring the rheological properties of the synovial fluid in the osteoarthritic joints. As the major component of the synovial fluid, HA assumes an important role in the viscoelastic properties of the articular cartilage, improving shock absorption, as well as lubrication of the joints. OA is both characterized by a decrease in the concentration of HA and a reduction of its molecular weight, which may lead to an increase of pain and a loss of joint functions.

Therefore, intra-articular injections of HA, referred to as viscosupplementation, represent one of the most sought after therapeutic approaches to treat osteoarthritic symptoms. The concentration of HA and its molecular weight within the joints contribute to pain relief and improvement of the joint mobility. It has been demonstrated that locally injected HA is rapidly degraded, so it is thought that the long-term clinical benefits may be due to its ability to promote the de novo synthesis of a high molecular weight HA, rather than just replacing the depolymerized endogenous HA.

In vitro studies and in vivo clinical studies demonstrate that exogenous HA may also mediate therapeutic effects in OA by a number of other biochemical actions within the joint, including induction of proteoglycan aggregation and proteoglycan synthesis, inhibition of inflammatory mediators, and analgesic activity (Moreland, 2003).

For almost two decades, chemically modified HA derivatives have been developed to increase their molecular weight and thus their residence time within the joint. The resulting so-called hylans, made of chemically cross-linked HA molecules, exhibit a higher viscosity and consequently higher half-life in the joint, suggesting a potentially better effectiveness. Whether hylans do really show a greater efficacy is still controversial, as available data is not sufficient to be able to draw reliable conclusions (Reichenbach et al., 2007; Jüni et al., 2007).

A systematic review in 2011 showed evidence of a modest but significant efficacy of intra-articular HA for KOA compared to a placebo by week four post-injection with a moderate clinical significance at week eight and continued residual benefit until 24 weeks (Bannuru et al., 2011).

This finding is confirmed by a systematic literature search performed by Regen Lab on PubMed database, aiming at evaluating performance and safety of HA versus a saline placebo. It demonstrated that a majority of the selected studies (14 out of 22) conclude that HA is at least partially- superior to a saline placebo, in terms of symptoms relief, and that the therapeutic effects may last for a relatively long period, ranging from several weeks to several months (Altman, 2009; Day 2004; Huang , 2011; Huskisson, 1999; Navarro-Sarabia, 2011; Petrella, 2008; Altman, 2011; Karlsson, 2002; Petrella, 2002; Pham, 2004; Dixon, 1988; Lohmander, 1996; Kotevoglu, 2006).

Nevertheless, some meta-analyses (Lo et al., 2003; Modawal et al., 2005) highlight the fact that the extent of these positive effects appears to be only modest form the clinical point of view. Indeed, the authors showed that even though the outcome parameters exhibited statistically significant differences in favor of HA over the saline placebo, pain relief and functional improvement do not seem to represent an important clinical benefit. One reason may be that there was a generally high placebo effect. Possible explanations for it include synovium aspiration (arthrocentesis), as well as the injections per se, which are known to provide beneficial effects by themselves, making it difficult to distinguish them from the beneficial effects of the HA.

In addition, HA has been shown to have a high rate of non-responding patients (about 50% of the patients, depending on the studies and the HA used (Berenbaum et al., 2012; Maheu et al., 2011; Pavelka et al., 2011), making PRP a first intention treatment.

In conclusion both the above-mentioned review and literature search indicate that intra-articular injections of HA represent an efficient and safe approach for the treatment of OA, particularly if it considered within the framework of a global treatment, which also includes medication-based and non-medication-based therapeutic interventions. Furthermore, because HA injections have no known medication interactions, it is a good option for patients on multiple medications (Fibel, 2015).

A recently conducted literature search on a HA with similar characteristics to the one contained in CellularMatrix (Ostenil®, TRB Chemedica, Switzerland) identified 12

studies (Pons, 2007; Tikiz, 2005; Oliveras-Moreno, 2008; Fuchs, 2006; Yiasemidou, 2016; Skwara, 2009; Man'shikova, 2007, Pourbagher, 2005; Dreiser, 2012; Tsvetkova, 2010; Roos, 2010; Idrissi, 2012). In total, 694 patients suffering from various degenerative joints conditions, including knee OA (62%), hip OA (17.5%), hallux rigidus (5.5%), temporomandibular joint disorders (7%) and rhizarthrosis (8%) were treated with Ostenil® or Ostenil®Mini according to different treatment courses (one to five intra-articular injections). All studies, except two case reports on acute pseudoseptic arthritis, showed that pain relief could be obtained after the first intra-articular injection, and was continuously improved with an increased number of injections. Additionally, functional improvement could also be obtained. Generally, HA effects could last for up to 6 months. In all studies, the product proved to be safe and well-tolerated, as no serious adverse reactions were observed. The only exceptions to this concerned two cases of pseudoseptic arthritis (Roos et al., 2004; Idriss et al., 2012) that occurred several days after a second intra-articular injection of Ostenil® in the knee.

Hyaluronic Acid Risks:

- Risks associated with intra-articular injection itself include:
- Pain
- Infection
- Inaccuracy of injection

Risks of infection and inaccuracy of injection are however very low, provided strict aseptic technique and ultrasound guidance are used (see Corticosteroids section).

- Although articular HA injections are associated with few, tolerable complications, Puttick et al (Puttick, 1995) reported that local acute reaction following HA injections can be observed in up to 11% of injected knee (27% of patients), characterized by:
- Pain
- Swelling
- Warmth

Frequency of adverse reactions, however, greatly varies according to the HA used. Indeed, it was found, that: (see Table 3)

In most patients, however, these reactions could be treated with NSAIDs, ice compression and sometimes cortisone (Aydin et al., 2017).

One very rare, but severe complication following intra-articular injection of HA is acute pseudoseptic arthritis, which is characterized by:

- Intra-articular effusion with severe pain most often within 24h and 72h of administration
- Requirement for two or three injections prior to the development of immune sensitization
- Absence of infectious agents and calcium pyrophosphate crystals in the synovial fluid aspirate
- Possible elevated mononuclear cell level in the synovial fluid
- Disease prognosis that is not self-limiting and that requires treatment in nearly all cases (arthrocentesis, intra-articular steroid injection, and NSAIDs)

Among various case series evaluated in the literature, the hylan G-F 20 product (Synvisc®) appears to be the injectable HA form that most frequently results in pseudosepsis. Only three cases wherein pseudosepsis was observed with Ostenil®, only one with Curavisc® and only one with Genvisc® (Aydin, et al., 2017; Roos et al. 2004; Idrissi et al., 2012).

Platelet-rich plasma:

More recently, injections of autologous PRP were found to be an attractive treatment option, due to the mechanism of action and the autologous nature of the product. Indeed, the action of PRP is not based on the sustainable viscoelasticity of the product, but on the biological stimulation of mesenchymal stem cells (MSCs) and cartilaginous cells.

TESTED PRODUCT	TREATMENT GROUP	NUMBER KNEE OA SUBJECTS TREATED	NUMBER SUBJECTS WITH DEVICE RELATED AE'S	% SUBJECTS WITH DEVICE RELATED AE'S
Gel One (Supartz®, Artz®, Artzal®) Seikagaku Corp. (Strand, 2012)	Gel One	249	67	26.9
	Placebo Control	128	33	25.8
Orthovisc Anika Therapeutics (Anika Therapeutics internal data)	Orthovisc	562	42	7.5
	Placebo Control	296	23	7.7
Monovisc Anika Therapeutics (Anika Therapeutics internal data)	Monovisc	184	13	7.1
	Placebo Control	185	10	5.4
Ostenil® TRB Chemedica	Ostenil	430	7	1.6
	Placebo control	(*)	(*)	(*)
ArthroVisc40 Regen Lab SA (RegenLab SA ongoing clinical studies)	ArthroVisc40	204	Disclosure at publication	Disclosure at publication
	Placebo control	(**)	(**)	(**)

Table 3: Safety profile of several CE-Marked injectable HA for the treatment of knee osteoarthritis. (*) Studies with Ostenil (TRB Chemedica) did not include placebo control.(**) Ongoing clinical studies with ArthroVisc40 (Regen Lab SA) do not include placebo control.

A number of in vitro studies have already shown the impact of isolated growth factors on the chondrogenic stimulation and differentiation of MSCs. For example, it was shown that MSCs produce significantly more proteoglycans and type II collagen when cultured in presence of TGF-α (Barry et al., 2001), while bFGF induces chondrogenic proliferation and differentiation of MSCs (Stevens et al., 2004). However, due to their method of production, the use of recombinant isolated growth factors is today impossible as it represents a potential threat for to human health. Therefore, PRP represents a safe alternative to recombinant growth factors, releasing an autologous and appropriate cocktail of growth factors within a natural and physiological range at the site of injection. Drengk showed that PRP has a proliferative effect on autologous chondrocytes and mesenchymal stem cells in an in vitro study (Drengk et al., 2009).

Clinical studies evaluating the efficacy of PRP for the treatment of articular pain demonstrated significant effects on pain relief and functional improvement, especially for those patients with lower degrees of OA, with safety of use and no SAE (SAE) attributable to the treatment. See Chapter 4 for results with PRP prepared with Regen Lab devices on osteoarthritis.

Platelet-rich plasma risks:
Risks associated with the phlebotomy include the following (according to WHO guidelines):
• Hematoma formation (the most common complication of venipuncture)
• Infection
• Nerve damage
• Vasovagal reaction, syncope and fainting
• Extensive bleeding
• Pain at blood sampling site
Risks associated with the intra-articular injection itself include:
• Pain
• Infection
• Inaccuracy of injection

Risks of infection and inaccuracy of injection are however very low, provided strict aseptic technique and ultrasound guidance are used (see Corticosteroids section and hyaluronic acid section).
Nonspecific adverse effects include:
• Local pain
• Stiffness
• Syncope
• Dizziness
• Headache
• Nausea
• Sweating
• Tachycardia
According to Patel et al (Patel et al., 2013, competitor device), nausea and dizziness occurred in 22.2% and 44% patients in single and double injection groups, respectively.

Other reported adverse effects include:
• Mild post-injection pain (16% of patients according to Vaquerizo [Vaquerizo et al., 2013], competitor device)
• Slight swelling
• Temporary mild worsening of knee pain after PRP injections (10% patients, according to Spakova [Spakova et al., 2012], competitor device).

All these adverse effects self-resolved within 48 hours. No severe complication was reported in all publications considered.

In accordance with what has been found in the literature, no serious adverse reaction has been reported so far with RegenKits following intra-articular injections. Side effects only consist of mild and transient localized reactions that resolved spontaneously.

Stem cells:

MSCs are multipotent cells that can be isolated from several human tissue types. The immunomodulatory, reparative, and anti-inflammatory properties of MSCs have been tested in a variety of animal models and appear to have potential clinical applications which include tissue repair (Fibel et al., 2015). For example, a study by Dr A. Singh from the Banaras Hindu University used scaffold-free MSCs obtained from bone marrow in an experimental model of osteoarthritis in rabbits by direct intra-articular injection. Twenty weeks after the injection, rabbits treated with the MSCs showed a lower degree of cartilage degeneration, osteophytes formation and subchondral sclerosis than a control group treated with medium without MSCs (Singh et al., 2014).

In a pilot study by Dr. Lluis Orozco form the Institute for Regenerative Medicine in Barcelona, Spain, 12 patients suffering from KOA were treated by intra-articular injections with expanded bone marrow MSCs and with a one-year follow-up. Patients exhibited rapid and progressive functional improvement that ranged between 65% to 78% at one year. Furthermore, there was an improvement in cartilage quality together with a highly significant decrease of poor cartilage areas, as measured by imaging techniques (Orozco et al., 2013). These encouraging results were further confirmed in a meta-analysis gathering both preclinical and clinical studies (Filardo et al., 2013). However, the poor methodology and small number of patients involved in the trials included in this meta-analysis highlight the need for future randomized controlled studies.

Stem Cell risks:
A meta-analysis performed to evaluate the safety of MSCs-based treatments only identified a significant association between MSCs administration and transient fever (Lalu et al., 2012). More investigations are required to assess safety of stem cell therapy.

Promising new treatment:
CellularMatrix combination of PRP and HA

There is currently a growing interest for the combination of PRP and HA injections as a treatment option for articular pain.

The combination of PRP and HA injections for the treatment of articular pain and loss of mobility was

evaluated through a systematic literature search conducted on PubMed database by Regen Lab SA. A summary of the most important clinical findings are described below.

A study conducted by Dr. José. F.S.D. Lana (Lana et al., 2016) prospectively compared the experimental group, treated with a combination of PRP and HA injections (33 knees), to control groups, treated with PRP or HA only. In this study, the group treated with the PRP/HA combination received an injection of HA first, followed by an injection of PRP. Patients were suffering from early to moderate KOA (I-III OA grade, according to Kellgren and Lawrence grading scale). Treatment was administered under local anesthesia and ultrasound-guidance; each patient received 3 intra-articular treatment using the lateral mid-patellar approach, at a 2-week interval. Results suggested that the PRP/HA combination provided an added benefit compared to each product administered individually, as the improvement in pain and physical function scores were significantly higher. Regarding inflammation, PRP/HA or PRP alone were the groups whose C-reactive protein (CRP) level was both lower and more stable, when compared to HA during the entire course of the study.

A study conducted by Dr. Chandirasegaran Saturveithan (Saturveithan et al., 2016) retrospectively compared the experimental group, treated with a combination of PRP and HA intra-articular injections (56 knees) to a control group treated with HA only. Treated patients were suffering from moderate to severe knee OA (III-IV OA grade, according to Kellgren and Lawrence grading scale). Results suggested that the combination of PRP and HA injections was more effective than HA injections, in terms of pain and physical function. No adverse event was reported during the study period.

Dr. Szu-Hsuan Chen (Chen et al., 2016) reports three cases on the use of consecutive PRP and HA injections in elderly patients suffering from bilateral advanced knee OA and who were not responsive to intra-articular HA therapy. Treatment administration protocol was customized to each patient's need and based on their improvement. Generally, all patients benefited from the treatment and experienced mainly pain relief and functional improvement. Each patient showed an increase in the joint space width in both left and right side of the medial tibiofemoral compartment, as demonstrated through objective X-ray measurements. The results suggest that the PRP/HA combination may induce local cartilage regeneration and that this therapy may be an effective ultimate chance for patients whose last option might be a total arthroplasty.

Taken together, these data are all supportive of the use of the PRP and HA combination as an infiltrative treatment for articular pain and loss of mobility. However, it is not known how PRP interact with HA when the two products are injected consecutively. In addition, there is no safety assessments for this type of procedure.

CellularMatrix

CellularMatrix was designed by Regen Lab to offer to medical practitioners a regulatory compliant medical device for the safe combination of PRP with HA and the injection of this combination in a single procedure.

A pilot multicenter study Renevier et al., 2014 and Renevier et al., to be published in 2018) was conducted in France (to evaluate the safety and efficacy of CM-PRP-HA combination obtained with CellularMatrix A-CP-HA Kit in a total of 77 patients with II-III OA grade, according to Kellgren and Lawrence grading scale and who had failed to respond adequately to previous treatment with HA alone. The treatment consisted of a series of 3 intra-articular injections scheduled at D0, D60 and D180 into the affected knee of CM-PRP-HA. The primary efficacy criterion was the variation of pain at walking, as assessed with the Western Ontario and McMaster Universities Arthritis Index (WOMAC) A1 score between baseline and D270. The treatment with CM-PRP-HA significantly reduced pain at walking between baseline and D270. The percentage of responders according to the criteria of the Outcome Measures in Rheumatology Clinical Trial and Osteoarthritis Research Society International was 94.4%. CM-PRP-HA provided long-lasting benefits for half of the patients and allowed avoiding surgery for almost 80% of them at four years.

The study conducted by Abate (Abate et al., 2015) retrospectively compared patients treated with CM-PRP-HA combination obtained with CellularMatrix A-CP-HA Kit (40 patients) to a control group treated with PRP only in patients with mild to moderate knee OA (II-III OA grade, according to Kellgren and Lawrence grading scale). The treatment was based on 3 intra-articular injections administered at weekly intervals. Clinical results based on validated questionnaires for the evaluation of OA symptoms showed a statistically significant improvement compared to baseline over a six-month follow-up period for both groups. In this study, the side effects observed were similar for both groups These adverse reactions appeared at the injection site and were characterized by temporary pain, heat and redness, which were solved in less than 24-48 hours, and did not require the administration of any medications. This study was not able to show a significant difference between the two treatments probably because the injection interval of one-week for both PRP and the CellularMatrix PRP/HA combination (CM-PRP-HA) was not optimized and too short. Indeed, it has been shown that optimal injection protocol for Regen Lab PRP is with one-month interval and for CM-PRP-HA the recommended protocol is with a two-month interval.

The study conducted by Seleem (Seleem et al., 2017) compared patients treated with CM-PRP-HA combination obtained with CellularMatrix A-CP-HA Kit (50 patients) to a control group treated with HA only in patients with mild to moderate knee OA (0-III OA grade, according to Kellgren and Lawrence grading scale). The treatment was based on 3 intra-articular injections administered at a three-week interval. Patients were prospectively, clinically evaluated before the treatment and at 2, 6 and 12 months follow-up visits by WOMAC score. At each follow up visit, both groups showed a highly significant improvement when compared with the basal assessment. The infra-group comparison showed, at each follow up evaluation, a significantly higher

improvement for the group treated with CM-PRP-HA in respect to HA alone. Similar results were obtained by Dr. Branco Barac (Barac et al., publication in progress).

1.8.2 Skin dehydration

Human skin ageing is a complex biological process, resulting from 2 biologically independent processes: the first one is intrinsic ageing, which affects the skin in the same pattern as it affects all internal organs; the second one is extrinsic ageing, which results of exposure to external factors, mainly ultraviolet (UV) irradiation. Intrinsic skin ageing is influenced by hormonal changes that occur with age, such as the gradual decreased production of sex hormones from the mid-twenties and the diminution of estrogens and progesterone associated with menopause. It is well established that the deficiency in estrogens and androgens results in collagen degradation, dryness, loss of elasticity, epidermal atrophy and wrinkling of the skin.

Fibroblasts represent the major cell type in the dermis, where they have a key role in producing and maintaining the extracellular connective tissue that is crucial to maintain the youthful appearance of skin. Fibroblast dysfunction and a reduction of fibroblast biosynthetic activity are major factors involved in the skin ageing processes. With ageing, there is a decrease in the number of fibroblasts and a decrease in fibroblast production of HA, collagen, and other components of the extracellular matrix, as well as an increase in the production of enzymes responsible for collagen fragmentation (Prikhnenko et al., 2015).

Skin ageing is associated with loss of skin moisture. The key molecule involved in skin moisture is HA (Papakonstantinou et al., 2012), due to its hydrophilic nature. Because concentration of HA decreases with ageing, elasticity and ability to hold water in skin are decreased, leading to decreased volume of dermis, and

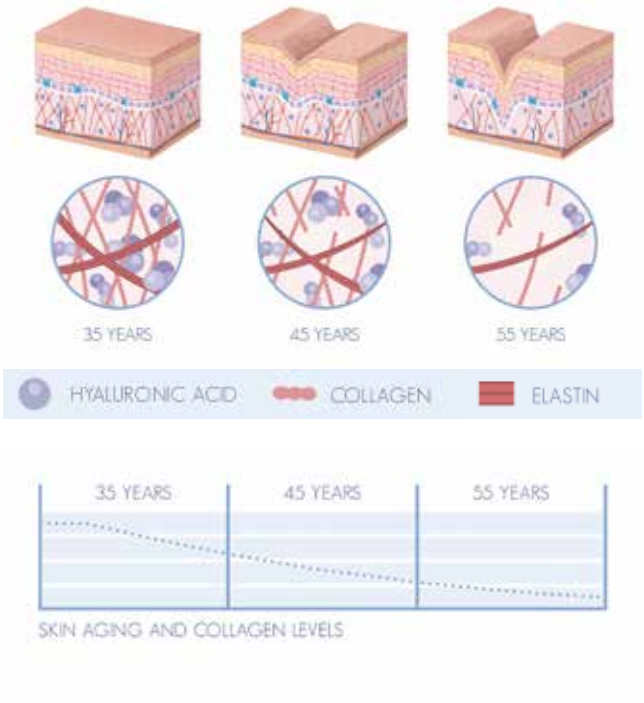


Figure 23: The gradual loss of key structural components such as collagen, elastin and hyaluronic acid is a sign of ageing.

increased tendency of wrinkles and skin laxity formation (Lee et al., 2015) (Figure 23).

Besides both systemic and topical estrogen therapies that have been shown to prevent skin collagen decline, restoring skin elasticity, increasing skin hydration and alleviating delayed wound healing (Shu, 2011), several other options are currently available. Among them, the intradermal microinjection of biological substances (mesotherapy) is one of the most popular techniques, as it allows active and essential ingredients to come directly into contact with the dermal fibroblast cells (Prikhnenko et al., 2015). Goals of such treatments are to increase the biosynthetic capacity of fibroblasts, inducing the reconstruction of an optimal physiologic environment, the enhancement of cell activity, the synthesis of collagen, elastin and HA. The desired final effect is a firm, bright, and moisturized skin (Iorizzo et al., 2008; Sparavigna et al., 2015). The range of available mesotherapy solutions is wide, including vitamins and vitamin-like substances, amino acids and related compounds, minerals, nucleosides, coenzymes, antioxidants, HA and PRP. The most frequently used substances include natural non-crosslinked HA, as well as PRP.

Hyaluronic acid:

Hyaluronic acid, as an important component of the skin, is a major contributor to the formation of a resilient gel-like ground substance that resists compressive forces. It contributes to tissue hydrodynamics by creating space for the movement of cells. It is believed to regulate the diffusion of micronutrients, metabolites and hormones between cells, and stimulate fibroblast migration, proliferation and collagen production (Edwards et al., 2007).

For several years, injections with, non-crosslinked HA, have been performed in dermatology to provide the skin with more elasticity, turgor and moisture. According to the results of an expert meeting held in May 2007 in the course of the 16th Congress of the European Academy of Dermatology and Venerology, in Vienna, Austria (Wiest et al., 2008), treatment with native HA can improve dry aged skin, as well as extrinsically damaged skin, with positive effects on skin elasticity, turgor, firmness and water content of the stratum corneum. All experts were of the opinion that selection criteria for such a treatment should be fine, superficial wrinkles and/or early stages of elasticity loss. Cheeks, neck and décolleté are favorable sites to be treated with native, non-crosslinked HA, but the treatment is also suited for the peri- and infra-orbital area, perioral skin region, as well as for the backs of the hands.

This was confirmed by a number of more recent studies evaluating the potential of non-crosslinked HA as a hydrating agent for the treatment of crow's feet (Pak, 2014), cheek, neckline and perioral skin (Taieb et al., 2012), as well as the periorbital region (Succi et al., 2012).

A literature search performed by Regen Lab SA on PubMed database on HA with similar characteristics to Regen Lab SkinVisc and to the HA present in the CellularMatrix device was conducted. Reports of clinical data on safety and performance of non-crosslinked HA were obtained from 126 patients who received intra-dermal HA injections for

the improvement of wrinkles and the hydration of skin.
A summary of the most important clinical findings is described below:

A study conducted by Dr. Chang S. Pak (Pak et al., 2014) took into consideration the comparison between two substantially different products: a crosslinked HA and a non-crosslinked HA with a large group of patients (72 subjects) in a randomized, double-blind clinical trial. Product performance was assessed up to three months by clinical photographs, investigator judgment, changes in wrinkle severity and during resting state according to lateral canthal line severity scale, and by visual analogue scale. The non-crosslinked HA (Yvoire-Hydro®) was used to improve hydration of dehydrated and wrinkled skin with intra-dermal injections. 95% of patients in both groups showed an improvement in Crow's feet wrinkles.

Safety data: 2 severe adverse events (SAE) were reported, pneumonia and ligament disorder occurred in two subjects and needed in-hospital care, with no causal relationship between the SAE and study devices, according to the investigator's clinical evaluation. Finally, they were no unexpected adverse device event (UADE) nor other notable adverse event (AE).

This study provides a positive contribution concerning the use of non-crosslinked HA to treat dehydrated crow's feet wrinkles.

A study conducted by Dr. Maryna Taieb (Taieb et al., 2012) took into consideration the use of a non-crosslinked HA for both skin hydration and elasticity in a prospective, multicenter clinical study on 34 patients. Subjects were divided in two groups based up on the method of injection. Skin measurements, such as physical and visual parameters and skin images, were assessed with instrumental probes. It was found that subjects treated with depot technique improved overall skin hydration with statistical significance to pre-treatment condition, and all the subjects were satisfied with their allocated treatment. Contrarily, subjects treated with nappage technique were almost all unsatisfied (86%), with no significant difference in skin areas with respect to screening, and adverse effects were more frequent.

Safety data: AEs were reported in 14 subjects, which included mainly ecchymosis, papules, and hematoma. All the AEs were related to injection technique rather than the product. All AEs were transient, with a mean duration of four days, and there were no sequelae.

This study provides a positive contribution concerning the use of non-crosslinked HA for the overall hydration of dehydrated skin, suggesting also that product performance is strictly related to the injection technique.

A study conducted by Dr Isabella B. Succi (Succi et al., 2012) took into consideration the use of a non-crosslinked HA as a treatment for skin hydration and elasticity in the periorbital area in a prospective, open-label, pilot study on 20 patients. Clinical evaluation was assessed by a blinded, independent evaluator who recognized a 25-50% improvement in skin brightness, texture, and turgor of the treated areas.

Safety data: approximately 76% of patients had a hematoma in the treated area. This was the longest-lasting adverse event which lasted at maximum 7 days. Erythema was the adverse effect with the shortest duration and lowest frequency during the entire treatment period. All adverse events occurred during the study were reversible and non-serious.

This study provides a positive contribution concerning the use of non-crosslinked HA for the overall hydration of dehydrated skin in the periorbital region.

Hyaluronic Acid risks:
Injection-related risks include the following:
- Pain
- Ecchymosis
- Erythema
- Bruising
- Bleeding
- Hematoma
- Infection

Other risks include:
- Allergic reactions/hypersensitivity
- Inflammatory reactions
- Papules

According to Succi who treated the periorbital region with non-crosslinked HA of non-animal origin, these adverse effects occurred with the following frequencies:
- Mild but tolerable pain during injection (80% of the patients)
- Medium-sized papules that lasted five days (most of the patients)
- Edema of a mean duration of six days (80% of the patients)
- Hematoma that lasted on average seven days (89% of the patients after the first session, 61% after the final session)
- Erythema that lasted on average one day: 10%-30%, according to the session.

Similar frequencies were found by Dr. Martine Baspeyras (Baspeyras et al., 2013), who observed reactions such as hematoma, edema, papules, erythema or transient inflammatory reactions in 87.7% of the patients treated with a non-crosslinked HA-based mesotherapy product.

Platelet-rich plasma :

There are plethoric *in vitro* studies or investigations conducted in animal models showing that PRP increases the production of collagen (Cheng, 2012; Cho, 2010; Cho, 2012), stimulates fibroblast proliferation (Krasna et al., 2007), as well as angiogenesis (Kakudo et al., 2014; Giusti et al., 2009).

The intradermal injection of PRP provides effective encouraging results for an overall improvement in skin texture, elasticity, and tone, and creates a volumizing effect that can lead to a significant volume correction of deep wrinkles (such as the nasolabial folds).

Platelet-rich plasma risks:
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The intradermal injection of PRP provides effective encouraging results for an overall improvement in skin texture, elasticity, and tone, and creates a volumizing effect that can lead to a significant volume correction of deep wrinkles (such as the nasolabial folds). See Chapter 4 for results with PRP prepared with Regen Lab devices used for skin care. To our best knowledge the Abuaf study (Abuaf et al., 2016) is the sole study that has demonstrated with histological evidence the increase in collagen in the dermis of patients treated with PRP.

Platelet Rich Plasma risks:
Risks associated to the phlebotomy include the following (according to WHO guidelines):
• Hematoma formation (the most common complication of venipuncture)
• Infection
• Nerve damage
• Vasovagal reaction, syncope and fainting
• Extensive bleeding
• Pain at blood sampling site
• Allergies (latex, iodine, alcohol)

Injection-related risks include the following:
• Pain
• Ecchymosis
• Erythema
• Bruising
• Bleeding
• Hematoma
• Infection
Other risks include:
• Burning sensation

According to Dr. Eman Nofal (Nofal et al., 2014, competitor device), adverse effects related to PRP treatment occurred with the following frequencies:
• Mild bruises: 6.7%
• Pain: mild: 40%, moderate: 20%; severe: 40%
According to Redaelli (Redaelli et al., 2010, Regen Lab device), adverse effects related to PRP treatment occurred with the following frequencies:
• Bruising: 3%
• Burning sensation:70% Mild
• Erythema: 80%
According to Dr. Pedram Mehryan (Mehryan et al., 2014, competitor device) adverse effects related to PRP treatment occurred with the following frequencies:
• Transient burning / ecchymosis (60%)
According to Dr. Boo Kyoung Kang (Kang et al., 2014, competitor device), adverse effects related to PRP treatment occurred with the following frequencies:
• Mild pain: 20%
• Immediate erythema: 50%
• Purpura of the injection site: 30%
• Mild oedema: 25%

In all publications reviewed, no case of serious complications (infection or hematomas) were reported. CellularMatrix combination of PRP and HA Promising new treatment for skin care

Even though to a lesser extent than for intra-articular injections, the intuition that the association of both PRP and HA could represent a treatment option with added benefit than each product separately has emerged for skin care treatments, too.

Indeed, Dr. Betul G. Ulusal (Ulusal et al., 2016) investigated the combination therapy of PRP and HA on a series of 94 females to correct signs of skin aging on face and neck regions. The PRP/HA combination was injected in small quantity into the deep dermis and hypodermis (about 5-6 mm of depth). The treatment protocol was not standardized between patients, as the number of injection sessions varied between 1 and 8. Subjects who received a higher number of treatment sessions were more satisfied with the treatment and showed greater improvement. Given the variety in the protocol, it was not easy to assess the effectiveness of the PRP and HA combination globally; however, digital clinical photographs, wrinkle severity scale score and subjects' satisfaction showed that the PRP association with HA provided satisfactory results for rejuvenation of treated regions from the first treatment. PRP/HA association was safe, with no significant side effects and no inflammation or allergic reactions were reported. Temporary mild facial edemas were observed and some patients reported ecchymosis, which lasted about 10 days.

Dr. Barbara Hersant and Prof. Jean-Paul Méningaud (Hersant et al., 2017) investigated the effects on skin firmness and elasticity of the PRP/HA combination obtained with CellularMatrix (CM-PRP-HA). Thirty-one patients were treated with three injections one month apart using the mesotherapy injection technique. Patients' skin was assessed at baseline and over a 6-month follow-up period using both objective parameters with a Cutometer and subjective criteria using the FACE-Q scale. Significant improvement between baseline and 6 months was observed for both the FACE-Q scores and the biophysical measurements obtained with the Cutometer, with no serious adverse reaction.

1.8.3 Urological and gynecological indications

The combination of PRP/HA ob-tained with CellularMatrix is current-ly clinically assessed for various uro-logical and gynecological indications:
- Genitourinary syndrome of menopause (GSM)
- Lichen sclerosus
- Stress urinary incontinence

Preliminary results from a case se-ries conducted by Dr. Hersant (Her-sant et al., 2018) on 20 patients suf-fering from vulvovaginal atrophy in-dicate that only one intra-mucosal injection session of the CM-PRP-HA combination could improve vaginal trophicity and hydration. This sug-gests that CM-PRP-HA could repre-sent a new therapeutic option for GSM, especially for those patients who cannot benefit from hormonal therapy.

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2. REGEN LAB MEDICAL DEVICE DESCRIPTIONS

2.1 APPLICABLE STANDARD AND GUIDANCE DOCUMENTS

The RegenKit® Product Families (Table 4) and CellularMatrix (Table 6) have been designed, manufactured, and packaged with consideration of the relevant harmonized European and other international standards to ensure that they are in accordance with the current international guidelines and to meet the Essential Requirements of the European Medical Device Directive 93/42/EEC (MDD) and new Medical Device Regulation 2017/745 (MDR).

2.2 REGENKIT® PRODUCT FAMILIES

2.2.1 Device description

RegenKits are defined as "hematology tubes and accessories packaged for a bedside and extemporaneous and medical single use".

This section covers the description of:
1. RegenKit devices:
- RegenKit®-BCT Family
- RegenKit®-THT Family
- A-CP® Kit Family

2. RegenKit Accessories:
- RegenET
- RegenCT
- Spray Applicator
- Accessory Set

Family	Name	Use	Punction site
A-CP Kits Family	A-CP-Kit-1/2/3	PRP	Peripheral blood
	A-CP-Kit-2 Plus	Platelet gel /Autologous glue	
	A-CP-Kit-3 ATS	Autologous Thrombin Serum	
RegenKit BCT Family	RegenACR-C Plus One	Platelet gel / Autologous glue	Peripheral blood
	RegenACR-C Plus		
	RegenACR-C Cellular Mask	PRP	
	RegenACR-C Classic		
	RegenACR-C Extra		
	RegenKit-BCT-1/2/3/4		
	RegenKit-BCT-1/2 Plus	Platelet gel /Autologous glue	
	RegenKit Ophthalmology	PRP	
	RegenKit Surgery	Autologous glue	
	RegenKit-ATS-1/2/3	Autologous Thrombin Serum	
	RegenKit Extracell Adipocyte	PRP + cellular extract	
RegenKit THT Family	RegenKit-THT-1/273/4	PRP	Peripheral blood
	RegenKit-THT-2 Plus	Platelet gel	
	RegenKit Surgery	Autologous glue	
	Regen Fibrin Polymer 1/2	Fibrin clot	
	RegenKit Extracell Glue	Autologous glue enriched in bone marrow cells	Bone marrow blood & Peripheral blood
	RegenKit Extracell BMC/BMC2	Bone marrow concentrate	Bone marrow blood
	RegenKit Extracell Membrane	Suturable Fibrin membrane	Peripheral blood
Accessories Family	RegenET	Evacuated tube	Peripheral blood
	RegenCT	Citrate-containing tube	
	Regen Spray Applicator	N/A	
	Accessory set	N/A	

Table 4: List of all RegenKits

The RegenKit devices allow for the preparation of autologous PRP and of other plasma-derived products, such as autologous serum, in a closed-circuit system with minimal steps by a trained physician.

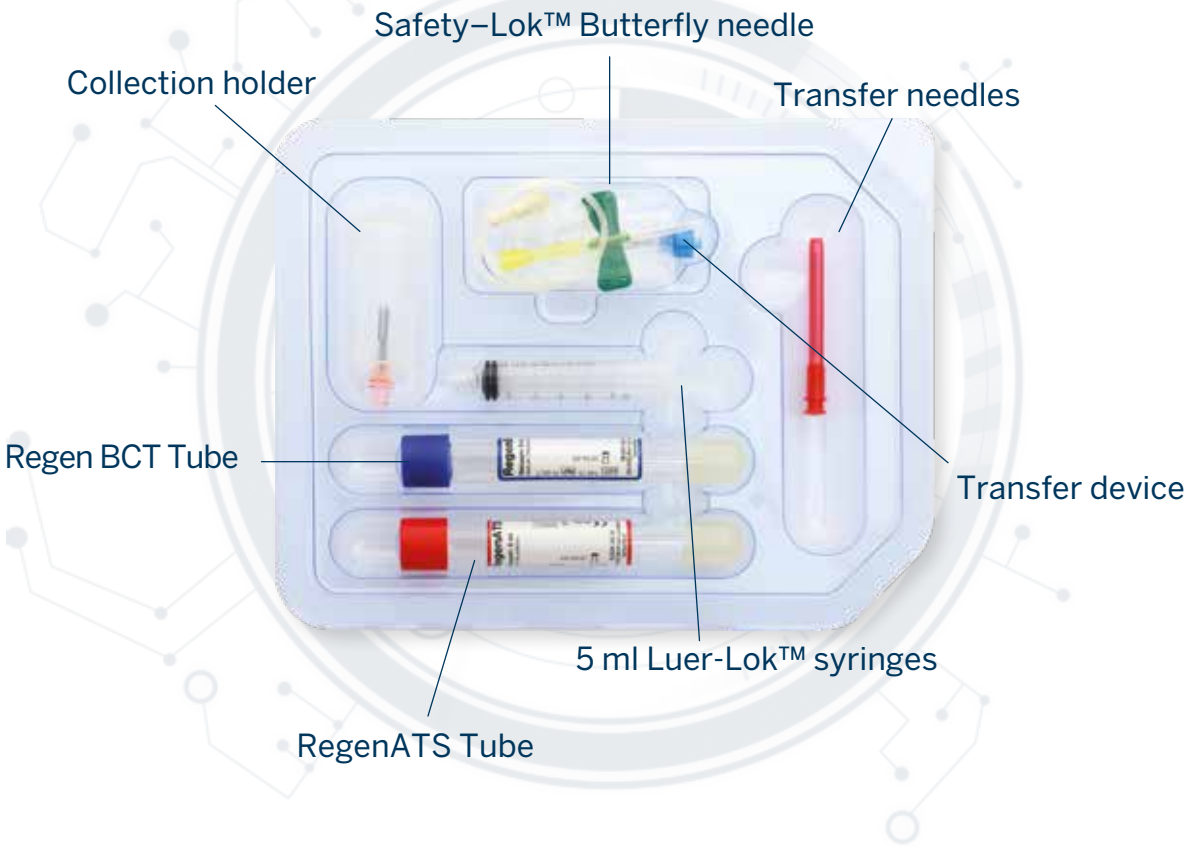

Figure 24: Regen Lab medical device. The example presented is a RegenKit-BCT Plus kit containing sterile disposable tubes, butterfly needle, transfer devices and syringes, packaged in a single-use double blister for RegenKit -1,-2 & -4 while RegenKit -3 that contains only 3 tubes is packaged in single blisters.

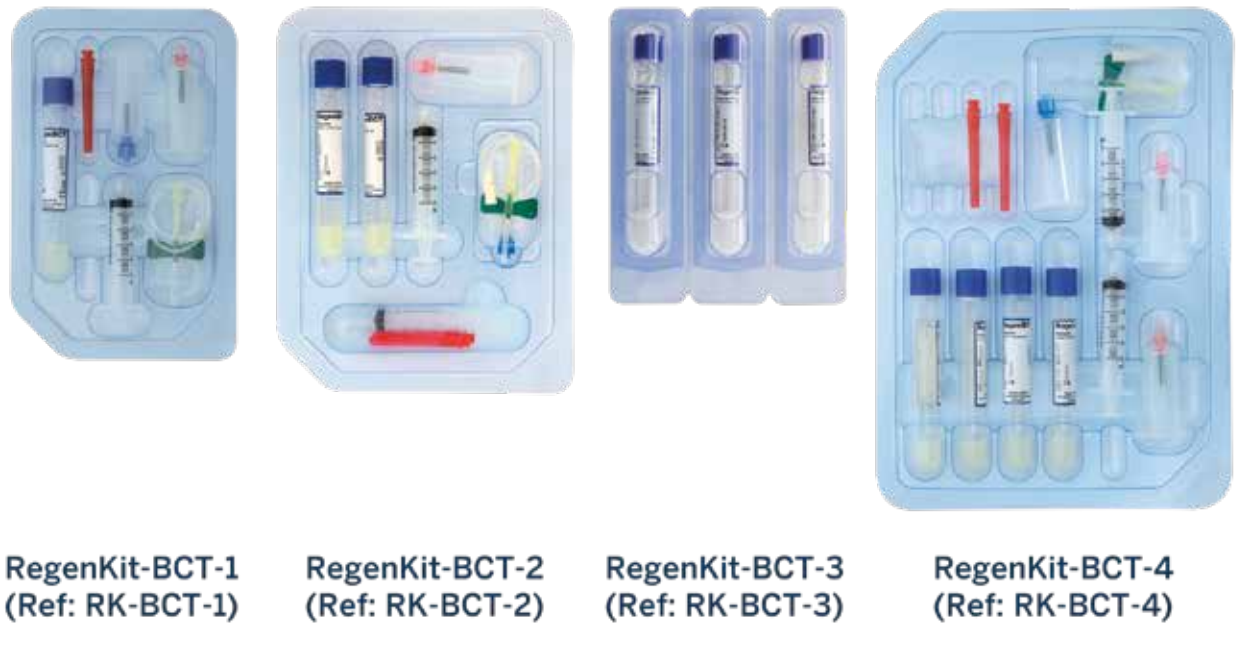

Figure 25: RegenKit-BCT Product Family.

Regen Lab offers different devices for the preparation of platelet-rich plasma and other plasma-derived products (see Figure 26, 27 and 28) These devices differ from each other by the composition of the separator gel, the volume of blood withdrawn, the presence or not of sodium citrate solution and the color of the cap.

All Regen™ tubes consist of pharmaceutical-grade class I borosilicate and use vacuum for automated blood collection. The hallmark of the Regen™ technology is a thixotropic separating gel composed of a mixture of polymers for gravimetric plasma separation (elimination of RBC and WBC). RegenTHT, Regen BCT and A-CP are devices designed for the preparation of PRP and contain a sodium citrate anticoagulant solution above the separating gel. The RegenATS device (figure 27) does not contain anticoagulant. In this device, the plasma coagulates and a serum rich in activated thrombin is extracted from the plasma clot.

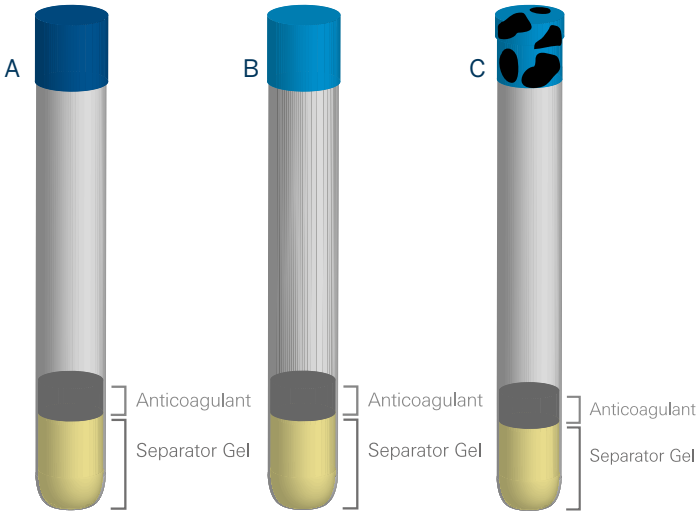

Figure 26:
Both Regen BCT (A) and A-CP (B) tubes are devices that collect 10 ml of blood for the preparation of around 5.5 ml of leukocyte poor PRP.
The RegenTHT device (C) collects 8 ml of blood and produces around 4.5 ml of a PRP enriched in MNC (lymphocytes and monocytes) but poor in granulocytes.

Besides the tubes, RegenKits contain off the shelf material intended for phlebotomy or bone marrow aspiration and PRP transfer, necessary to maintain the close-circuit system.

Figure 27: RegenATS tube collects 10 ml of blood for the preparation of around 4 ml of serum rich in activated thrombin serum (plasma-derived product)

Accessories kits: Some accessories are supplied as kits: RegenCT tubes (Ref: R-CT-3), RegenET tubes (Ref: R-ET-3) (figure 28), RegenSpray Applicator (Ref : R-A/NAC1) and Accessory Set for blood collection (ref: BCA-Set3).

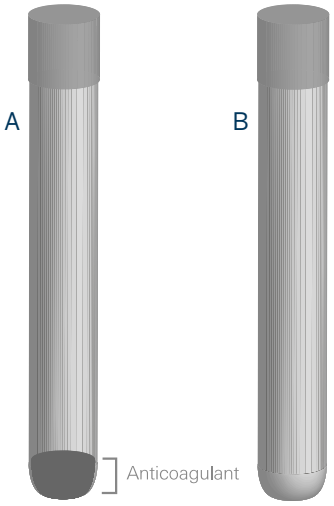

Figure 28:
A. RegenCT (Accessory tube for aspiration and transfer of bio-logical tissues; vacuum for col-lection of 10 ml / Anticoagu-lant: Sodium Citrate)
B. RegenET (Accessory tube for aspiration and transfer of bio-logical tissues; vacuum for col-lection of 10 ml / Anticoagu-lant: None.)

Recently, Regen Lab launched CuteCell, a new set of products to prepare PRP (CuteCell-PRP), serum (CuteCell-serum) and MNC (CuteCell-MNC) to be used exclusively in vitro in a research laboratory setting.

These new products offer an autologous alternative to Fetal Bovine Serum (FBS) in culture media for cell therapies. Results and rationale for the use of these products are further explained in Chapter 3.

Each Regen tube is evacuated in order to allow automatic blood withdrawal. Tubes are closed with a bromobutyl rubber (BBR) stopper covered by a safety polypropylene cap.

RegenKits (except the Spray Applicator and Accessory Set) are used in combination with a centrifuge, pre-set with the specific centrifugation parameters for PRP preparation mentioned in the Instructions for Use (IFU). The combination of the device with the centrifuge is considered to be a standard combination.

RegenKits (except the Accessory Set) are packaged in single use, double or single blistered kits (Tyvek®/PETG Polyethylene terephthalate glycol), terminally sterilized by gamma ray irradiation. The Accessory Set components remain in the original primary packaging from the supplier and are already provided sterile. Only the final packaging and IFU are performed by Regen Lab, in compliance with MDD 93/42/EEC, Article 12, and MDR 2017/745.

2.2.2 Principles of Operation of RegenKits

RegenKits allow for the extemporaneous preparation of autologous PRP and of other plasma-derived products from the patient's own blood.

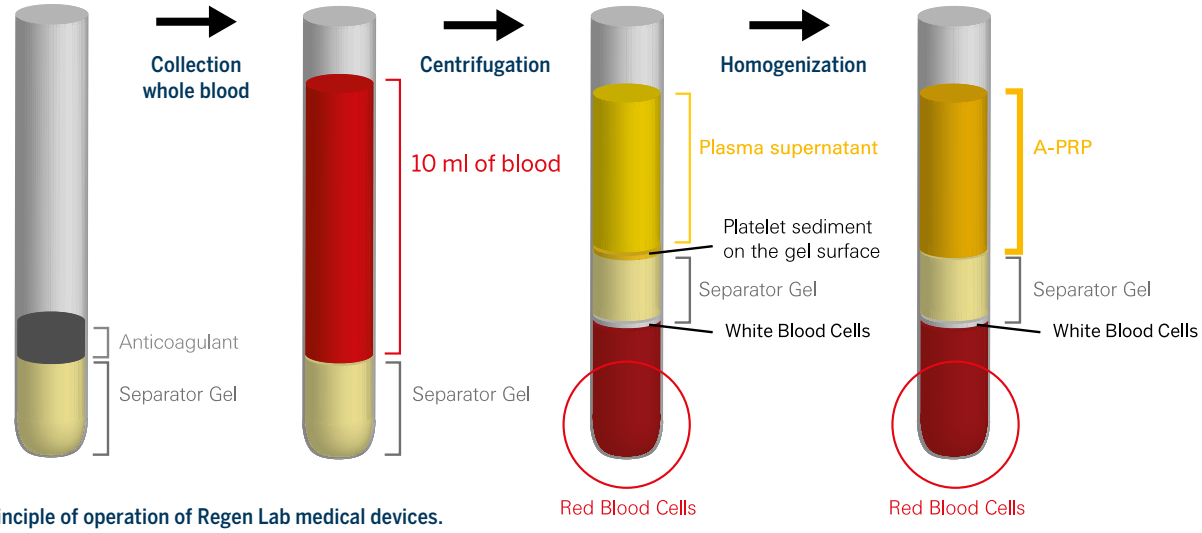

Figure 29: Principle of operation of Regen Lab medical devices.

Blood consists of cellular elements (red and white blood cells and platelets) and a liquid part called plasma. The plasma represents around 55% of the blood volume. The remaining volume consists of red blood cells (around 44%), white bloods cells (<1%) and platelets (0.2%). White blood cells can be divided into two main populations, granulocytes and mononuclear cells, monocytes and lymphocytes, (MNC), which represent around 70% and 30% of the white blood cells, respectively. See section 1.2.1 for more detailed information.

Blood components have specific densities and thus can be separated using centrifugal force. In increasing order, the specific gravity of blood components in g/cm is 1.026 for plasma, 1.040 to 1.060 for platelets, 1.065 to 1.070 for mononuclear cells, 1.075 to 1.096 for granulocytes and 1.090 to 1.110 for red blood cells (Immunology, Luttmann, W., et al., Immunology 2006; Academic Press). This blood property is used to isolate platelets and plasma from the patient's own blood. The resulting product is called platelet rich plasma, or PRP.

Regen Lab devices intended for PRP preparation consist of evacuated glass tubes containing a thixotropic gel with a specific density designed for platelet or platelet and MNC isolation, and anticoagulant solution. The gel acts as a mechanical separator of blood components, and sodium citrate solution as blood anticoagulant. Thixotropy is a physical property of some gels which are thick under static condition, become more fluid when submitted to physical stress (in this case, the centrifugal force) and regain their original consistency when the physical stress stops.

Thanks to the vacuum, a pre-set volume of blood is directly and gently collected into the tube. Blood is mixed with the anticoagulant by gentle inversion of the tube. The blood-filled device is then centrifuged at a relative centrifugal force of 1500 g for 5 (A-CP and Regen BCT) or 9 minutes (RegenTHT), depending on the family of devices. During centrifugation, the separator gel becomes more fluid, thanks to its thixotropy, and thus can migrate upwards in the device while the red and white blood cells migrate downwards between the separating gel and the glass wall. The separating gel remains in one block and does not mix itself with the blood. The different blood components separate themselves according to their specific densities and the separator gel intercalates itself between them at the level of its own specific density. At the end of the centrifugation, the separator gel regains its solid consistency and forms a solid barrier that mechanically separates the blood components. It isolates the platelets and the plasma in the upper part of the device, while RBC and most of WBC (more specifically the granulocytes) are trapped below the separator gel in the lower part of the device. The platelets form a thin sediment on the upper surface of the gel. In order to obtain re-suspension of platelets in the plasma supernatant, the tube must be gently and repetitively inverted (see steps of preparation in Figure 29). The resulting PRP is then collected using a blood transfer device connected to a syringe. At this point, the preparation is ready to be used by the physician. As it is the gel that separates blood components, PRP isolation in Regen Lab devices is specific and operator independent, thus reliable and reproducible. The process is automated and produce a standardized PRP with constant high platelet recovery and low level of red and white blood cell contamination.

Regen Lab supplies three types of PRP devices: (1) Regen BCT, (2) A-CP and (3) RegenTHT, . These devices produce a PRP with a high platelet recovery (> 80% for Regen BCT and A-CP devices and > 95% for RegenTHT devices) and an optimal depletion of undesired cellular contaminants (RBCs and granulocytes). The recovered WBCs are mostly mononuclear cells. The main difference between these devices lies in the specific density of the two kinds of separator gels that are used within each device and the level of mononuclear cells that are recovered. In A-CP and Regen BCT tubes, the separator gel intercalates itself below the platelets and above the WBC producing a leukocyte poor PRP. In RegenTHT tubes, the separator gel has a slightly higher density, that allows not only the high recovery of platelets but also of the mononuclear cell fraction (that contain the mesenchymal stem cells found in bone marrow) while granulocytes are trapped under the separating gel with the red blood cells. The resulting mononuclear cell recovery in PRP is 20% to 30% with A-CP and Regen BCT devices and 70% to 80% with RegenTHT devices (Table 5).

	Cellular element recovery (%) and concentration factor (x) compared to baseline values in peripheral blood						
	Platelets	Red blood cells	White blood cells	Mononuclear cells	Lymphocyte	Monocyte	Granulocyte
RegenTHT	~95% 1.7-1.8x	<1% 0.011x	30-40% 0.6X	70-80% 1.3x	70-75% 1.4x	50-55% 0.9x	10-15% 0.3x
RegenBCT & A-CP	~ 80% 1.6x	<0.3% 0.007x	10-13% 0.2X	20-30% 0.5x	25-35% 0.6x	~ 10% 0.2x	3.5% 0.06x

Table 5: Summary of cellular composition in PRP prepared with RegenTHT tubes, Regen BCT tubes and A-CP tubes. RBC: Red Blood Cells; WBC: White Blood Cells; MNC: mononuclear cells (lymphocytes and monocytes).

As mononuclear cells represent a minor population in whole blood, total white blood cell concentration in PRP prepared with Regen Lab devices remains below the physiological value in venous blood. Consequently, PRP prepared with Regen Lab devices are all considered as leukocyte-poor PRP, even RegenTHT PRP that should be qualified as MNC-rich PRP, but not as leukocyte-rich PRP as the total WBC level in RegenTHT PRP remains below the baseline concentration in blood. Accordingly, PRP prepared with Regen Lab devices are classified P2-Bα PRP according to PAW PRP classification system (DeLong et al., 2012), which is based on 3 components: (1) the absolute number of Platelets, (2) the manner in which platelets Activation occurs, and (3) the presence or absence of White cells. In that system, platelet concentration is measured in platelets per microliter and categorized as follows: P1, less than or equal to baseline level; P2, greater than baseline levels to 750'000 platelets/microliter; P3, greater than 750'000 platelets/µl to 1'250'000 platelets/µl; and P4 greater than 1'250'000 platelets/µl. Total WBC content is identified as either above (A) or below/equal to (B) baseline level. Because inclusion or exclusion of neutrophils is an important variable, a subcategory for neutrophil count has been created: if neutrophils are included, then α (above) is added; if neutrophils are excluded, then α (below) is added. Finally, if an exogenous external activator is used, it is documented with an "x". Endogenous activation is not given a designation (Figure 30).

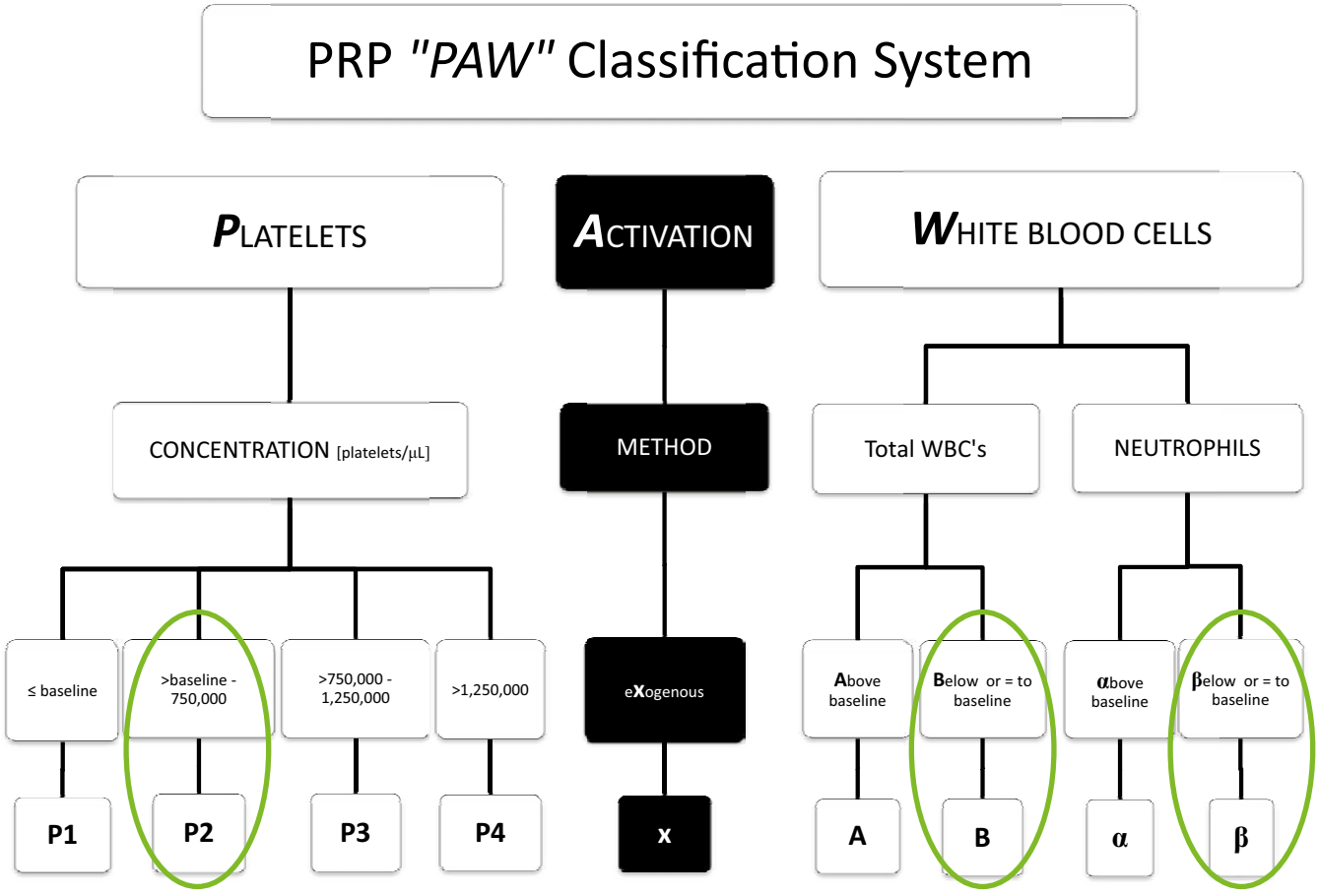

Figure 30: PAW classification system. RegenTHT A-CP and Regen BCT PRP are natively P2-Bβ PRP. Concentrations can be increased by platelet poor plasma (PPP) removal to obtain P3-Bβ PRP with Regen BCT and A-CP tubes, and P3-Aβ PRP with RegenTHT tubes. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. Arthroscopy 2012; 28(7): 998-1009.

The RegenATS device (figure 27) differs from the Regen Lab PRP devices as it does not contain anticoagulant over the separating gel. Consequently, RegenATS tubes should be centrifuged at 1500 g with no delay, directly after blood collection. As with the other Regen Lab devices, blood components are separated according to their specific density in the RegenATS tubes and the thixotropic gel intercalates itself below the plasma and over the red and white blood cells. As there is no anticoagulant, the coagulation cascade occurs in the isolated plasma and a fibrin clot is formed over the separating gel at the end of the centrifugation. Serum is extracted from this clot. This serum contains the enzymes of the coagulation cascade in their active form, and among them, activated thrombin. Thrombin is the enzyme that converts plasma fibrinogen into fibrin monomers and which polymerize and form the clot. The serum produced from the patient's own blood using the RegenATS device is thus a source of autologous activated thrombin, that is used to initiate the coagulation process by counterbalancing the effect of citrate in PRP prepared in parallel with Regen Lab PRP devices. The RegenKit- Surgery and RegenKit Plus allow the simultaneous preparation of PRP and ATS.

To obtain either a biological glue or platelet gel, PRP and autologous thrombin serum are combined together in a sterile vessel or with the RegenSpray Applicator (Figure 31). The glue or gel can then be applied topically or injected directly into the wound bed.

Figure 31: PRP+ATS preparation with RegenSpray Applicator.

Because of the ability of Regen Lab separating gels to recover mononuclear cells with platelets and plasma, Regen Lab devices can also be used to process bone marrow aspirate. The recommended device for this application is the RegenTHT tube as it has a higher performance regarding MNC recovery. Like blood, bone marrow aspirate consists of a suspension of blood cells but also of stem cells, precursors and immature cells of the different cell lineages produced by the bone marrow, in plasma. When bone marrow is centrifuged in Regen Lab devices, mature red and white blood cells as well as most immature blood cells migrate below the separating gel, while platelets and the mononuclear cell fraction remain above the separating gel and can be recovered within the plasma. The mononuclear cell fraction of bone marrow contains not only mature blood mononuclear cells but also hematopoietic progenitor cells and mesenchymal stem cells. RegenTHT tubes has been shown to recover mesenchymal stem cells with high efficiency (> 87%) which is the highest yield when compared to other system for bone marrow processing..

2.2.3 Mode of action of PRP

PRP is an autologous preparation from the patient's own blood playing the role of growth factor reservoir during treatment. Indeed, platelet activation induces alpha-granule degranulation, releasing synthesized pre-packaged growth factors (Marx et al., 2004; Nurden et al., 2011; Eppley et al., 2006). The active and orchestrated secretion of these growth factors is initiated by contact of platelets with the extracellular matrix proteins. Once released, these growth factors induce different cell signaling cascades that activate angiogenesis, cell proliferation and differentiation, and new matrix synthesis for tissue regeneration (Roubelakis et al., 2014). Importantly, PRP, in addition to its effect on tissue repair, also reduces inflammation and pain (Monteleone et al., 2000; Descalzi et al., 2013).

Therefore, PRP is an active ingredient that promotes and accelerates healing of soft and hard tissues and exerts analgesic effects.

2.2.4 Mode of action of bone marrow aspirate concentrates

Due to their ability to differentiate into various lineages, including osteoblasts and chondrocytes, bone marrow MSCs have been proposed as a new option for treatment of bone and articular cartilage defects. Autologous bone marrow contains not only stem cells and precursors cells as a source of regeneration tissue, but also accessory cells that support angiogenesis and vasculogenesis by producing several growth factors (Giannini et al., 2009). Therefore, treatment with bone marrow aspirate concentrates is used to stimulate bone healing in skeletal defects through both osteogenic precursors, which will repopulate the damaged tissue, and cytokines and growth factors secreted by the transplanted cells (Hernigou et al., 2009; Nandi, 2009).

2.2.5 Intended use of the device

• RegenKit-THT Family, RegenKit-BCT Family and A-CP Kit Family have the same intended use:
Preparation of autologous PRP and of other plasma-derived products
• Accessories have the following intended use:
- RegenET kits and RegenCT kits: Aspiration and transfer of biological tissues
- Spray Applicator kit: sterile, single use device for applica-tion of two non-homogenous fluids or liquids to a treatment site as deemed necessary by the clinical use requirements

2.2.6 Intended user

The preparation of the PRP must be performed by a physician or under the supervision of a physician familiar with the equipment and procedure.

2.2.7 Claims

- The devices are sterile and non pyrogenic
- All kit components in contact with blood are biocompatible accord-ing to ISO 10993.

Performance:
- The devices allow a high recovery of platelet of at least 80% (high above the US-FDA requirements of 50%), with low contamination of red blood cells
- Ease of handling in a time-effective way
- Reproducible PRP preparation, not-operator dependent

2.2.8 Warning and Precautions

Use appropriate safety precautions to protect yourself from needles and beveled cannulas. Do not re-cap the needle after use and discard directly in the biological hazard container. Follow the manufacturer's instructions when using the centrifuge. Do not use the sterile components of the kits if the packaging is open or damaged. All kits contain single-use devices only: do not reuse. The physician must be familiar with the equipment and with the procedure before using the kit. The patient must be informed about the general risks associated with the treatment and of possible adverse effects. The PRP must be prepared from fresh blood and must be used within four hours (extemporaneous use only).

NOTE: FOR SINGLE-USE ONLY. Throw away the entire kit after use, using the method of elimination for potentially contaminated blood products.

2.2.9 Contraindications

Absolute contraindications:
Patients with hereditary or acquired hematologic/coagulation disorders such as:

- Platelet dysfunction syndrome
- Critical thrombocytopenia
- Impaired coagulation
- Drepanocytosis
- Porphyria

Patients suffering from:
- Severe metabolic or systemic dis-orders
- Septicemia or acute infection

Relative contraindications:
- Malignancy, particularly with he-matologic or bony involvement, metastatic disease
- Auto-immune diseases (Hashimo-to, rheumatoid arthritis, lupus, etc.)
- Patients who have taken, within 3 days, aspirin or other medications that alter platelet function (Plavix, Omega 3, Vitamin C)
- Recent fever or illness
- Anemia (HGB <10g/dl)

2.3 CELLULARMATRIX: COMBINATION OF PRP AND HA

2.3.1 Device description

CellularMatrix family contains two devices:
- A-CP HA Kit, composed of three A-CP HA tubes
- BCT-HA Kit, composed of three BCT-HA tubes

A-CP-HA tubes and BCT-HA tubes are an incremental improvement of devices manufactured by Regen Lab designed to prepare PRP only (A-CP tube and Regen BCT tubes, respectively).

Family name	Cellular Matrix	
Brand names	BCT-HA Kit	A-CP-HA Kit
References	BCT-HA-3	A-CP-HA-3
Classification Justification	Class III	
Components	3x BCT-HA tube	3x A-CP-HA tube
Classification	Hematological concentrate system kit, platelet concentration) Categories: Single-use devices	
Description	A collection of sterile devices designed to prepare platelet-rich plasma (PRP) or platelet-rich fibrin matrix (PRFM) from an autologous blood specimen for application to soft-tissue wounds and injured bone to accelerate healing and/or achieve hemostasis. It typically includes a blood withdrawal set, tubes, and syringes. Blood is collected by venous puncture and processed in a centrifuge, increasing the concentration of platelets within the plasma or fibrin matrix; this may be done at the point-of-care. The glue-like product is applied to the surface of the injured tissue or bone where its adherent properties form a hemostatic barrier and enhance regeneration. This is a single-use device.	

Table 6: Nomenclature and classification of the CellularMatrix kits

CellularMatrix kits are only composed of three A-CP-HA or BCT-HA tubes as detailed in Table 6 and Figure 32B and 32C, respectively.

The difference between the A-CP-HA Kit and the BCT-HA Kit are following tubes specifications:
- Blood withdrawal volume (4 or 6 ml)
- Tube length (110 or 125 mm)

Packaging is the same for both kits, except the color of the cap and tube labelling. Intended use is the same for both kits. The devices are packed in double blisters.

CellularMatrix tubes are designed to be used for the safe and rapid preparation of autologous PRP combined with non-crosslinked HA gel 2%. CellularMatrix tubes are manufactured by Regen Lab using the same separating gel technology as for the A-CP and Regen BCT tubes. HA is added to the tubes composing the CellularMatrix kit to form a cellular friendly 3D matrix when combined with PRP.

PRP/HA combination is currently used for intradermal and intra-articular treatments. All associations of autologous PRP and HA treatments reported in the literature are however either obtained after sequential administration of PRP and HA, or by manual ex vivo mixing of both components without using a dedicated device. CellularMatrix is the only CE-Marked medical device placed on the market that allows a treatment with autologous PRP combined with HA in an extemporaneous, one-step and closed-circuit procedure.

Each glass tube composed of pharmaceutical-grade class I borosilicate uses a vacuum for blood collection into the tube containing 2% (w/v) non-crosslinked HA. Also included is the thixotropic gel composed of polymers for plasma separation (elimination of red and white blood cells), located above the hyaluronic acid. On top of the thixotropic gel is the anticoagulant sodium citrate 4% (w/v) solution (see Figure 32A).

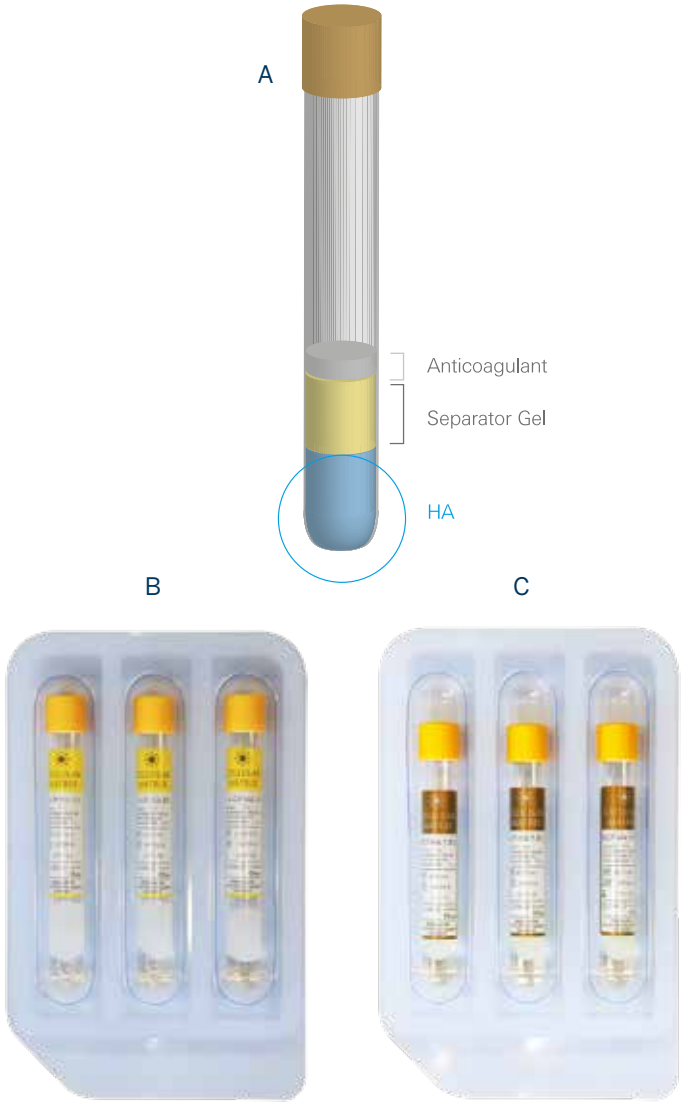

Figure 32: A. Individual CellularMatrix tube. B. CellularMatrix A-CP-HA Kit for knee osteoarthritis and skin care. C. CellularMatrix BCT-HA Kit for knee osteoarthritis and skin care.

Each tube uses a pre-measured vacuum to draw the patient's blood. Vacuum volume depends on the tube type. Tubes are sealed with a BBR stopper covered by a safety polypropylene cap. BCT-HA and A-CP-HA tubes are sterilized by moist heat at 121°C for 15 minutes (ISO 17665). CellularMatrix tubes are sterile, non-pyrogenic and single-use. Every three months, bioburden tests are performed on representative kits by a Swiss certified laboratory. Furthermore, endotoxin level (Limulus amebocyte lysate [LAL] test) is verified on each batch of tubes. All tubes are first packaged in a sterile, individual single-use blister (PETG/Tyvek) and then packaged together in a second single-use blister (PETG/Tyvek) (double packaging) (see Figure 32B and C).

The device is used in combination with a standard blood collection set for blood withdrawal and a transfer device connected to a standard Luer Lock syringe for PRP recuperation.

Regen Lab SA provides an Accessory Set (REF: BCA-SET-3), composed of 3x Blood Collection Set and 3x Blood Transfer devices, regrouped in a carton box, compliant with Medical device Directive 93/42/EEC, Article 12.

2.3.2 Principle of operation

CellularMatrix allows for the extemporaneous preparation of autologous PRP in presence of HA.

Following blood withdrawal, the tube is placed into a centrifuge and centrifuged for 5 minutes at 1500 g force at room temperature. During this centrifugation, the gel migrates to form a barrier that mechanically separates the plasma and platelets from the other blood components (RBCs and WBCs). The mechanism allowing the separation of the PRP fraction from the RBCs and WBCs exploits the specific property of the thixotropic gel to change its shape when a force is applied to this material (in this case, centrifugal force). When the gel's shape is modified, it allows the blood cells (with higher density) to migrate to the bottom of the tube, while platelets (with lower density) stay above the gel. This supernatant containing plasma and concentrated platelets is commonly called PRP. In addition, the HA gel also migrates to the top of the tube and sits above the PRP fraction. This location allows for an easy mixing of the HA and PRP after centrifugation, by gentle and repetitive inversions and rotations of the tube. The final PRP/HA mixture is collected using the blood transfer device (supplied in accessory set BCA-Set-3) and a syringe. At this point, the preparation is ready to be used by the physician.

CellularMatrix allows for at least 60% platelet recovery (see corresponding PRP devices performances in Table 5), with low contamination of RBCs and WBCs, appropriately mixed with 2 ml of a 2% non-crosslinked hyaluronic acid solution. Platelets recovered with CellularMatrix are fully viable/ functional for 4 hours after preparation.

2.3.3 Mode of action of PRP/HA combination prepared with CellularMatrix

As described in chapter 1 and in section 2.2.3, PRP is involved in tissue regeneration by secreting biologically determined ratios of growth factors in an orchestrated manner.

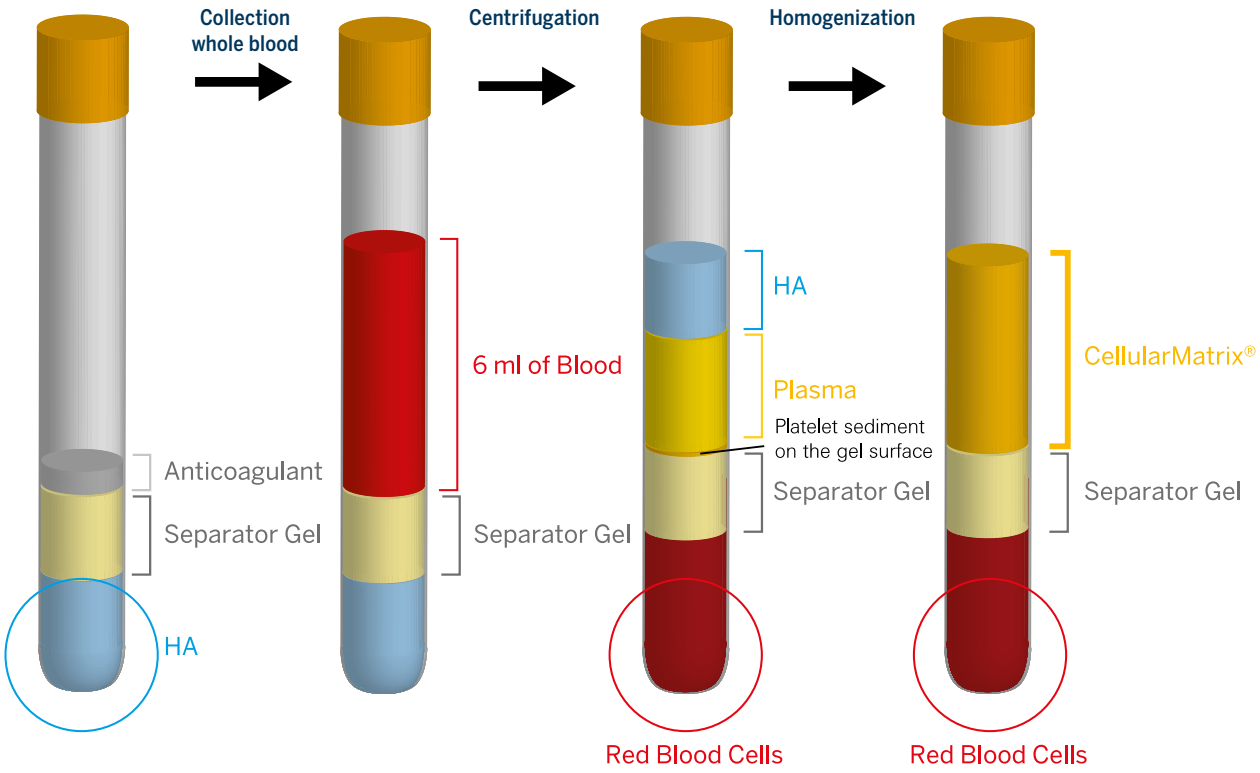

Figure 33: Different steps of preparation of the PRP-HA mix with CellularMatrix tubes.

HAs similar to the one present in CellularMatrix are a well-known therapy used for intra-articular injections and intradermal indications.

Furthermore, HA has been utilized in recent years in various medical fields due to its natural constitution in human connective tissues. It is an anionic, non-sulfated glycosaminoglycan considered an optimal biomaterial for tissue engineering (Fujioka-Kobayashi et al., 2016). Therefore, while growth factors have well recognized roles in the wound healing cascade, the viscoelasticity, hygroscopicity, bio-compatibility and immuno-compatibility of HA confer it with a facilitating role that may potentiate or maximize tissue response to growth factors (Xie et al., 2011). When HA is added to PRP, this has demonstrated to exert stronger anti-inflammatory effects by inflammatory suppression and enhanced regeneration compared with HA or PRP alone (Chen et al., 2014 & 2016).

In 2015, Dr Michele Abate (Abate et al., 2015) conducted a retrospective comparative study between a patient group treated with PRP prepared from autologous patient blood and a patient group treated with a combination of PRP/HA obtained with CellularMatrix. The authors conclude that the presence of HA improves PRP properties, by creating a pericellular bioactive scaffold around the cells that increases the platelet residence time and improve the delivery of their growth factor on the target cells.

No negative interaction between PRP and HA have been described in the scientific literature so far.

In March 2016, three years after CE MARK and commercial launch of A-CP-HA Kit, and two years after CE MARK and commercial launch of BCT-HA Kit, a new version of the CellularMatrix kit was developed at physician's request after the study conducted by Dr. Abate in 2015. The new version allows for the collection of a larger volume blood and thus, a higher amount of PRP in the final PRP/HA preparation. Therefore, there are two different devices within the CellularMatrix kit family, with slightly different PRP/HA ratio. The PRP/HA ratio of the homogenous preparation obtained with the A-CP-HA tube is approximately 60% PRP 40% HA, and the BCT-HA tube prepares a PRP/HA combination with a final ratio of 50% PRP 50% HA.

It is the physician's responsibility to decide whether they want to inject a combination of PRP/HA with a ratio of 60% PRP – 40% HA obtained with the A-CP-HA tube or a combination of PRP/HA with a ratio of 50% PRP – 50% HA obtained with the BCT-HA tube.

In 2016, Dr. Abate conducted a study to confirm the hypothesis that better results could be achieved by increasing the volume of PRP in the PRP/HA ratio. Performance and safety of the preparation containing 3 ml PRP + 2 ml HA was assessed in patients with knee OA. Data obtained was compared with those previously obtained with 2 ml PRP + 2 ml HA and supports the idea that a higher proportion of PRP mixed with HA can further improve the clinical performance of the PRP/HA combination. In terms of pain relief and joint function, statistically better outcomes were reported for the PRP/HA combination with a final ratio of 64% PRP – 36% HA (A-CP-HA Kit) compared to the PRP/HA combination with a final ratio of 50% PRP – 50% HA (Abate et al., 2016, unpublished preliminary data).

2.3.4 Intended use of the device

Both devices, the BCT-HA Kit and the A-CP-HA Kit have the same indication of use:
• Intra-articular injections into the knee for symptomatic treatment of articular pain and mobility im-provement.
• Intra-dermal injections for hydra-tion of dehydrated and wrinkled skin tissues

2.3.5 Intended user

The preparation and injection of the PRP/HA combination must be performed by a physician or under the supervision of a physician familiar with the equipment and procedure.

2.3.6 Warnings and Precautions

Intradermal injections
Injections of the PRP/HA preparation must be performed on clean skin (exempt of make-up) using the same precautions as any other intradermal injection. Sensitive skin may be pretreated using a local anesthetic cream. This product is intended to be injected in small doses with nappage and filler technique; papula injection technique is not recommended. The patient must avoid UV rays and long exposure to the sun or to the heat (such as saunas) during 1 week after the treatment.

Intra-articular injections
Injections of the PRP/HA preparation into the joint cavity must be performed with the same precautions as any other intra-articular injection, and preferably using imaging control. Joint effusion, if present, must be removed before injecting the PRP/HA preparation. The patient must respect a delay of 1 hour without physical activity after the injection and avoid strenuous or weight bearing activities for 48 hours following the intra-articular injection.

General precautions:
The patient must be informed about the general risks associated with the treatment and the possible adverse effects. Injections must be performed by the physician and under his responsibility. The physician must be familiar with the equipment and with the procedure before using the kit. Follow the manufacturer's instructions when using the centrifuge. Strict aseptic technique must be followed during phlebotomy and injection. The PRP/HA preparation must be prepared from fresh blood and must be used within 4 hours (extemporaneous use only). Use appropriate safety precautions to protect yourself from needles. Do not re-cap the needle after use and discard directly in the biological hazard container. Throw away each tube after use using the method of elimination for potentially contaminated blood products.
Do not inject intravenously or for any other application than described in the intended use. Do not use if the packaging and /or the tube are open or damaged. Do not use the sodium citrate solution or other tube component alone. Do not use after the expiration date. This product is for single use only: do not re-use.

2.3.7 Contraindications

• Ascertained hypersensitivity to one of the components
• Serious disease such as cancer or infection in joint or in the treated area
• The administration to patients suf-fering from inflammatory joint diseases and autoimmune dis-eases such as rheumatoid arthri-tis or Bechterew disease is not recommended
• The administration to children, pregnant or lactating women is not recommended

2.4 DEVICE HISTORY AND REGU-LATORY STATUS

2.4.1 RegenKits

The RegenKits are CE-marked since 2003 and patented final versions were introduced in the market in 2006 after three years of clinical evaluation, in Europe and outside Europe. At that time, RegenKits were exclusively composed of RegenTHT tubes and accessories.

In 2009, the Regen BCT tubes and RegenATS tubes were CE-Marked and commercialized. In 2011, the A-CP tubes were CE-Marked and commercialized.

2.4.2 CellularMatrix

A-CP-HA Kit and BCT-HA Kit are CE-marked since July 22, 2013 and February 12, 2014, respectively, and have been sold in Europe since those dates.
Since the devices first commercialization in Europe and in over 38 countries worldwide, no reportable incident has occurred.

2.5 DEGREE OF NOVELTY

2.5.1 RegenKits

We defined the degree of novelty of RegenKits as 4 (Innovation, see Table 7). Indeed, since the first idea in the 80's of using PRP in medicine, different ways of preparing PRP have emerged, from conventional blood centrifugation to commercial systems, and the methodology continues to broaden.

In this context, RegenKits represent an efficient concept (i.e. the fractioning of whole blood into its composite parts to allow isolation of plasma with platelet concentration above the baseline) and significant impact for the patient, allowing to provide an extemporaneous and sterile preparation of PRP in a rapid, safe, reliable and standardized manner, that can be used immediately for clinical purposes, according to the physician's choice. To comply with this objective, RegenKit devices contain one, two, or three Regen tube(s) for plasma separation, together with the standard materials for phlebotomy or bone marrow aspiration, and PRP transfer, necessary to maintain the close-circuit.

2.5.2 CellularMatrix

We defined the degree of novelty of CellularMatrix as 5 (major innovation, see Table 7) for the following reasons. First, from a technological point of view, CellularMatrix is the first CE-marked tubes designed to prepare PRP in presence of a non-crosslinked hyaluronic acid in Europe. Second, from a clinical point of view, the CellularMatrix technology presents a strong clinical impact. It significantly improves the clinical practice by providing a safe and easily prepared mixture of PRP and hyaluronic acid in a one-step procedure and improves the patient's condition by providing a new therapeutic option with great potential.

Degree of novelty	Type of novelty	Innovation where the dominant is:		
		Technological		Clinical
5	Major innovation	Breaking technology	and	Strong clinical impact
4	Innovation	Breaking technology	or	Strong clinical impact
3	Substantial novelty	Incremental technology	and	Moderate clinical impact
2	Moderate novelty	Incremental technology	or	Moderate clinical impact
1	Lacking or minor novelty	Known technology	and	Unchanged clinical impact

Table 7: Degree of novelty for a medical device (from http://ansm.sante.fr/; see Agence nationale de sécurité du médicament et des produits de santé (ANSM) website for definitions).

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3. REGENLAB MEDICAL DEVICES VALIDATION

3.1 SAFETY

3.1.1 Biocompatibility

In order to comply with the European Directive (93/42/EEC) and its amendments for demonstrating safety and performance of medical devices, Regen tubes (RegenTHT tubes, Regen BCT tubes, RegenATS tubes, A-CP tubes), BCT-HA tubes and A-CP-HA tubes were submitted for biological evaluation testing.

According to the International Organization for Standardization (ISO) 10993-1, these tubes have to be tested on the basis of the following features:

The device is an external communicating device, indirectly in contact with blood path.

The contact duration is inferior to 24h since it corresponds to the phlebotomy step and PRP preparation.

Consequently, Regen tubes, BCT-HA tubes and A-CP-HA tubes were tested according to ISO 10993 biocompatibility standards. These tests were performed by ISO 17025-accredited laboratories and are listed in Table 8.

Tests performed on RegenTubes, BCT-HA tubes and A-CP HA tubes				
Standard	Test	Results for Regen tubes	Results for BCT-HA tubes	Results for A-CP HA tubes
ISO 10993-5	Cytotoxicity	Passed	Passed	Passed
ISO 10993-4	Hemolysis	Passed	Passed	Passed
ISO 10993-10	Intracutaneous irritation	Passed	Passed	Passed
ISO 10993-10	Maximization sensitization	Passed	Passed	Passed
ISO 10993-3	Reverse mutation	Passed	Passed	Passed
ISO 10993-11	Acute systemic toxicity	Passed	Passed	Passed
ISO 10993-11 and ISO 10993-12	Pyrogen test	Passed	Passed	Passed

Table 8: Biocompatibility tests performed on Regen tubes, BCT-HA tubes and A-CP-HA tubes. All tests were performed by Biomatech Namsa (Chasse-sur-Rhône, France).

Borosilicate glass Type 1 tubes are certified as pharmaceutical grade (USP, Ph.EUR).

The safety of the sodium citrate solution was also investigated by an external consultant (Biomatech Namsa, France). This safety assessment shows that the quantity of sodium citrate anti-coagulant present in Regen tubes, BCT-HA tubes and A-CP-HA tubes cannot provide any ancillary or toxic effect in the recommended conditions of use.

Additionally, according to the international standard ISO 10993-1, the implantable part of the BCT-HA and A-CP-HA tubes (hyaluronic acid) has to be tested on the basis of the following features:

- This part of the device is implant-able, injected in tissues.
- The contact duration is superior to 24h but inferior to 30 days since most of the final preparation is resorbed after 4 weeks (see implantation test below).

The results of these biocompatibility studies are summarized in Table 9.

Tests performed on the hyaluronic acid gel present in BCT-HA tubes and A-CP HA tubes			
Standard	Test	Product tested	Results
ISO 10993-3	Genotoxicity (MicroNucleus)	HA gel (2% w/v), Ref. ARV-HA40	Passed
ISO 10993-3	Genotoxicity (MLA)	HA gel (2% w/v), Ref. ARV-HA40	Passed
ISO 10993-3	Genotoxicity (Reverse mutation)	HA gel (2% w/v), Ref. ARV-HA40	Passed
ISO 10993-5	Cytotoxicity	HA gel (2% w/v), Ref. SKV-HA40	Passed
ISO 10993-10	Intracutaneous Irritation test	HA gel (2% w/v), Ref. SKV-HA40	Intermediate
ISO 10993-10	Maximization Sensitization Test	HA gel (2% w/v), Ref. SKV-HA40	Passed
ISO10993-11	Acute Systemic Toxicity test	HA gel (2% w/v), Ref. SKV-HA40	Passed
ISO10993-11	Subacute systemic toxicity	HA gel (2% w/v), Ref. SKV-HA40	Passed
ISO 10993-4, ASTM F756	Hemolysis test	HA gel (2% w/v), Ref. SKV-HA40	Slightly hemolytic

Table 9. List of biocompatibility tests performed on the hyaluronic acid gel manufactured by Regen Lab and present in BCT-HA tubes and A-CP-HA tubes. All tests were performed by Biomatech Namsa (Chasse-sur-Rhône, France).

3.1.2 Animal testing on the final product (BCT-HA tubes only)

Local tissue effects of BCT-HA and A-CP-HA tubes were assessed through 2 implantation studies as follows:

• In the first study, local effects of the PRP/HA combination pre-pared with the BCT-HA tube fol-lowing subcutaneous, intradermal and intra-articular injections in the rabbit were evaluated. The test product (PRP/HA combination prepared with the BCT-HA tube) was compared with two control products (PRP prepared with a Regen tube and HA alone) at 4 and 12 weeks post-injection. In this study, the HA alone used as one of the control products was exact-ly the same as the one contained in the PRP/HA combination pre-pared with the BCT-HA tube. The rabbits were sacrificed and local effects after injection were evalu-ated macroscopically and histo-pathologically.

No local tissue effect was macroscopically observed after injection of the test product. No product was visible after 4 or 12 weeks.

Histopathologically, the test product did not induce any evidence of local irritation after four and 12 weeks. Following intradermal and intra-articular injections, the test product was fully degraded from 4 weeks post-injection. Following subcutaneous injections, the test product was fully degraded at most sites at four and 12 weeks.

• In the second study, the local ef-fects of the PRP/HA combination prepared with the A-CP-HA tube were compared to an HA already placed on the market (Sinovial, IBSA, Switzerland) following intra-articular injection. Additionally, the local effects of the PRP/HA combination prepared with the A-CP tube were compared to the PRP/HA combination prepared with the BCT-HA tube following subcutaneous injection. Macro-scopic and histopathological as-sessments were performed at one and four weeks post-injection for both sites.

No macroscopic nor microscopic local tissue adverse effects were observed at 1 and 4 weeks after intra-articular and subcutaneous injections. For both groups, no remarkable inflammatory reactions were observed in the subcutaneous sites, nor in articular tissues (cartilage, subchondral bone tissues, ligaments, menisci, synovial membranes and fluids). Slight signs of mineralization were observed in the cruciate ligaments which were higher in the Sinovial control group than in the A-CP-HA group and that may be due to the experimental procedure.

In both sites, degradation of the test treatment was complete after four weeks.

The results of these studies are summarized in Table 10.

Animal testing on the final product				
Standard	**Test**	**Implantation duration**	**Product tested**	**Results**
ISO 10993-6	Implantation	12 weeks	BCT-HA Tube (Ref. BCT-HA) RegenBCT Tube (Ref.R-BCT) Regenvisc syringe (Ref. Regen-HA)	Passed
ISO 10993-6	Implantation	4 weeks	A-CP-HA Tube (Ref. A-CP-HA) BCT-HA Tube (Ref. BCT-HA)	Passed

Table 10. Implantation tests performed on A-CP-HA or BCT-HA devices according to requirements of ISO 10993-6 standard. All tests were performed by an independent ISO-17025-accredited laboratory (Chasse-sur-Rhône, France).

Taken together, both studies demonstrate the safety of both BCT-HA and A-CP-HA tubes following skin and intra-articular injections. They show that the implanted combination samples were mainly absorbed within 30 days post-injection and that no severe adverse effects were observed at the site of injection.

3.1.3 Other safety aspects

All components of Regen tubes are verified to be exempt of substance or material from animal origin.

Moreover, Regen tubes are also exempt of drug or any substance able to provide an ancillary action according to the definition of the Directive 2001/83/EC. The sodium citrate anticoagulant has been assessed for its absence of ancillary effect *in vivo* and has led to a classification of RegenKits in Class IIb according to the Rule 3 (Annex III of 93/42/EEC Directive).

No other applicable aspect (such as radiations, mechanical aspects, electrical safety or electromagnetic compatibility) were identified in RegenKits Family.

RegenKits composition and manufacturing process are also free of latex and phthalates. No other safety aspect was identified.

3.2 EFFICACY

In order to assess the performance of our medical devices, we performed extensive efficacy validation of our devices with our Quality Assurance (QA) department. Results are presented in the 3.2.1 Bench testing section.

From a biological point of view, Regen Lab carried out thorough characterization of PRP prepared with RegenKits and the combination of PRP and HA prepared with CellularMatrix kits in terms of performance, as demonstrated by *in vitro* studies (section 3.2.2 Performance studies).

3.2.1 Bench testing

Bench testing includes verification and validation of the system. It includes internally performed tests as well as tests performed by an accredited laboratory (Table 11). Test performed in order to demonstrate safety and performance are listed below.

- Performance test in-vitro (see section 3.2.2)
- Viscosity and functionality tests
- Dosage test
- Sterility and LAL tests
- Glass tube Mechanical testing
- Closed-circuit tests (PRP sterility)
- Transport validation

Design Matrix Table: CellularMatrix A-CP-HA Kit

User Requirements		Description	Acceptance criteria
§ 1	Easy and rapid preparation	The device should be easy-to-use and allow rapid preparation of the autologous platelet-rich plasma (PRP) and Hylauronic acid (HA) mix.	- Short centrifugation time: < 10 minutes - Rapid obtention of the final PRP-HA mix (whole process < 30min) - Fewest manipulations possible
§ 2	Preparation from a small sample	The PRP should be prepared from a small sample of blood.	- < 20 ml of patient blood - Draw volume has to be stable along shelf life
§ 3	HA as vehicle of PRP	The HA has to possess the adapted properties in order to be a vehicle for the PRP. The HA has to increase PRP's viscosity.	- Composition of HA solution : 1.8 to 2.2 % (w/v) - Increase of PRP viscosity by HA: > 0 mPa.s
§ 4	Safe device	The device must be safe for the health care provider and the patient. The device has to be non-pyrogenic and biocompatible. The interior of the tube has to be sterile.	- Bacterial endotoxins: < 2.75 EU/tube - Biocompatibility: compliance with the ISO 10993 standards - Device must be sterile according to ISO 11737-2
§ 5	Avoid cross contamination	The device has to preserve the PRP-HA mix from cross contamination.	- Fewest manipulations possible - PRP has to be sterile before patient injection

Table 11.Example of a design matrix file for A-CP-HA kit that summarizes all the verification and validation tests done in the bench testing procedure.

Verification	Verification data
As stated in the Instructions For Use (IFU), the centrifugation takes 5 minutes with a RCF of 1500g, as demonstrated and validated in performance test. All the manipulations are processed in closed circuit, which allow the rapid obtention of the final PRP:HA mix. Manipulation of the device in only 4 steps: 1) Preparing the patient care (5 min) 2) Collecting the patient blood by pre-determined vacuum (5 min) 3) Centrifugation (5 min) 4) Resuspending the PRP fraction and mixing with HA (2 min) 5) Collecting the PRP-HA mix fraction (2 min) The whole preparation process remains inferior to 30 minutes and with the fewest manipulations possible, including automatic blood filling by pre-determined vacuum. Use analysis confirmed and validated the easy-useness of device and the rapid obtention of PRP-HA mix	- **A-CP HA TUBE PERFORMANCE TESTING**_Rev03_04.03.2016 (Technical File Cellular Matrix - Annex V) '- **Cellular Matrix Use Analysis Report 2016_01** UseRep-01-2016, rev01, 13.04.2017 (Technical File Cellular Matrix - Annex K)
Use analysis report confirms that the draw volume that is aspirated is lower than 20ml. Stability report (Time 0) confirm that the draw volume of blood that can be aspirated in the tube is well in the range of 4 ml +/- 20% (mean 4.63 ml). This value is well inferior to 20 ml. This criteria is also confirmed by Performance testing, in which blood was collected from 23 volunteers. In any case, by the design of the A-CP-HA tube, the maximal volume of the glass tube (before HA, gel and citrate filling) is 12 ml, so a volume of blood superior to 20 ml is not possible (see Technical drawings of the tube in the Datasheet of the A-CP-HA tube).	- **Cellular Matrix Use Analysis Report 2016_01** UseRep-01-2016, rev01, 13.04.2017 (Technical File Cellular Matrix - Annex K) '-**Cellular Matrix Kits stability Closure report** nr 2016-09_ACPHA-BCTHA, rev04, 19.12.2017 (Technical File Cellular Matrix - Annex W) '- **A-CP HA TUBE PERFORMANCE TESTING**_Rev03_04.03.2016 (Technical File Cellular Matrix - Annex V) '- **68. DataSheet - A-CP-HA Tube** (Technical File Cellular Matrix - Annex C)
In vitro performance testing results show that with a composition of HA of 2% and a ratio PRP-HA 50-50, the addition of HA will increase the viscosity of the PRP solution (with an initial viscosity of 0 mPa.s). Results of the sodium hyaluronate concentration tested in 3 batches of BCT-HA tubes (equivalent to A-CP-HA tubes, because with the same manufacturing process) performed by Caref Laboratories, shows that the HA solution well-ranges between 1.8 to 2.2 %, with a mean of 1.94% of sodium hyaluronate. Clinical evaluation demonstrates that HA has the adapted properties in order to be a vehicle for the PRP in order to prolong PRP effect.	- **Conclusion of tests performed on the viscosity of HA gel (BCT-HA and A-CP HA projects)**, Ver.03, 2012-08-01 (Technical File Cellular Matrix - Annex V) - **Dosage testing on sterile HA syringes report** rev00_22.02.2017 including Analysis report No 161059, 161060 and 161061 from accredited Laboratory CAREF (Technical File Cellular Matrix - Annex V) - **Clinical Evaluation Report Cellular Matrix**, v2.2, 10.01.2018 (Technical File Cellular Matrix - Annex Y)
Endotoxin detection test by LAL method, method validated according to USP 32 NF 27 by an accredited laboratory (NAMSA, Biomatech) and controlled on each batch (on the Certificate of Analysis). Biocompatibility testing according to ISO 10993 biocompatibility standards by ISO 17025-accredited laboratories: - Biocompatibility tests were performed on the RegenBCT tubes, BBR stopper and the native hyaluronic acid and showed compliance to ISO 10993-1 requirements. - All components of the A-CP-HA tube are non toxic and non mutagenic under the conditions tested. Hyaluronic acid is not listed as mutagenic. - Equally, the final HA-PRP preparation obtained with this tube was tested for implantation (according to the international standard ISO 10993-6) and showed no induction of local adverse effects. - Component interactions were assessed and no leachable or degradation product requires application of ISO 10993-17 standard. Sterilization: the sterilization process by moist heat has been validated to comply with ISO 17665 standard requirements (>121°C during at least 15 min). These parameters are controlled and recorded during each sterilization cycle according to Steam sterilization SOP 7C/PROD023 to comply with the validation. An other verification of the sterilization parameters is done at the moment of the final inspection before batch release. Use safety of the device on user and patient is validated through the Use Analysis recorded in Use analysis report. Safety of the device is also demonstrated and validated through the Clinical Evaluation, including PMS data.	- **LAL test (protocol and report) on HA tubes and syringes** Protocol nr 20160628, rev.00, 2016-06-08 (Technical File Cellular Matrix - Annex V) - **Validation of the endotoxin detection test by LAL method**, rev1_29.03.2017 including NAMSA Test Report 219367, validation of the LAL test A-CP-HA tube, 02.08.2016 (Technical File Cellular Matrix - Annex V) - **Biocompatibility closure report Cellular Matrix BCT-HA Kit and A-CP-HA Kit**, Rev04, 2017-03-28 (Technical File Cellular Matrix - Annex X) **Sterilization validation:** - Moist heat Sterilization Validation Plan (Report N°VP-MOISTHEAT-B2Sup-2016-02 V1),v.1,dated of 20.01.2016. -Cellular Matrix Tubes Sterilization Validation Final Report (Report N°Final-Moist heat-Cell Mat tubes-2016-06), v.1,dated of 01.07.2016. (Technical File Cellular Matrix - Annex T) -SOP 7C/PROD023 **Steam Sterilization procedure** (Technical File Cellular Matrix - Annex T) - **Cellular Matrix Use Analysis Report 2016_01** UseRep-01-2016, rev01, 13.04.2017 (Technical File Cellular Matrix - Annex K) -**Clinical Evaluation Report Cellular Matrix** v.2.2, 10.01.2018 (Technical File Cellular Matrix - Annex Y)
The design of the device leads to 4 main steps of preparation in closed circuit: 1) Collecting the patient blood 2) Centrifugation 3) Resuspending the PRP fraction and mixing with HA 4) Collecting the PRP-HA mix fraction From blood harvest up to PRP-HA injection, the PRP-HA preparation remains in closed circuit. No more manipulation is required for this preparation. Use Analysis report also validates the fewest manipulation to obtain PRP without any contaminationof the PRP. PRP sterility has been validated and documented in a study of sterility and stability of PRP in RegenCollyrium and a rational demonstrates the applicability to PRP-HA mix. Safety of the device is also demonstrated and validated through Use Analysis. Safety of the device is also demonstrated and validated through the Clinical Evaluation, including PMS data.	- **A-CP HA TUBE PERFORMANCE TESTING**_Rev03_04.03.2016 (Technical File Cellular Matrix - Annex V) - **Rational to demonstrate sterility of PRP-HA mix prepared with BCT-HA or A-CP HA tube** - 2017-03-03 including annex :Study report: Test of sterility and stability of PRP in RegenCollyrium , Study 2012-07_RegenCollyrium-GP rev02 (Technical File Cellular Matrix - Annex V) - **Cellular Matrix Use Analysis report 2016_01** UseRep-01-2016, rev01, 13.04.2017 (Technical File Cellular Matrix - K) - **Clinical Evaluation Report Cellular Matrix** , v.2.2, 10.01.2018 (Technical File Cellular Matrix - Annex Y)

Design Matrix Table: CellularMatrix A-CP-HA Kit

Safety Requirements		Description	Acceptance criteria
§1	Single use	The device should be designed and labeled for single use.	- Design: single use - Label: single use
§2	Sterile and non-pyrogenic	The device (tube) should be sterile and non-pyrogenic.	- Inside of the tube must be sterile according to ISO 11737-2 - Endotoxin level < 2.75 EU/tube and <20 EU per kit
§3	Packaging specifications	Device packaging should be adapted to protect the device from transport-induced damage.	-Transport test: bubble -tensile-visual tests and draw volume (>3.20 ml) must be conform after transport test according to 11607-1
§4	Manufacturing conditions	Device should be manufactured in accordance with current good manufacturing practices for medical devices, thus under clean manufacturing conditions to maintain bioburden under acceptable limits.	- Bioburden: regularly tested, action limit set at < 200 CFU/device - Microbiological test acceptance criteria according to ISO 14698 - Cleanrooms qualification acceptance criteria according to 14644
§5	Safe components	Purchased components for the device should be verified before release for manufacturing.	-Supplier qualification - Components : entrance acceptance controls - All acceptance criteria of each component should be documented
§6	Final products controls	Final products controls should be performed on the manufactured device to ensure the safety.	- Mandatory final products controls that match with device specifications should be documented in release SOP

Table 11.Example of a design matrix file for A-CP-HA kit that summarizes all the verification and validation tests done in the bench testing procedure.

Verification	Verification data
The design of the device does not allow several uses. Indeed the A-CP-HA tube, composed by the cell separator gel and the HA gel, can only be used once: - As soon as blood is harvested, no vacuum in the tube is able to allow a new blood harvest - The quantity of Sodium citrate is limiting, and cannot anticoagulate 4 ml of blood again after it's mixed the first time with blood. - As soon as the tube is used a first time, the implantable HA gel is definitively mixed in the final preparation and harvested, so this HA gel cannot be mixed again with a new PRP. Moreover, Instructions for Use (IFU) provided with the device have the mention "for single use only" and are marked with the corresponding symbol. This symbol stating "single use" is also printed on the outer carton box and on the different labels.	- **Labels of Cellular Matrix A-CP HA** according to labeling instructions INS 7C/PROD015 (Technical File Cellular Matrix- Annex E and Annex F) - **IFU_A-CP-HA-3**- According to IFU Management procedure SOP 7C/QA003 (Technical File Cellular Matrix - Annex E and Annex F)
According to the sterilization validation, sterilization parameters allow obtaining a sterile device (only inside of the tube is sterile) with a SAL 10^{-6}. Moreover, sterilization process is verified for each lot of tubes and each autoclave run (according to SOP 7C/PROD023). According to internal procedures, each batch of tubes is tested for presence of bacterial endotoxins (pyrogenicity) using the LAL methog.	- **Cellular Matrix Tubes Moist Heat Sterilization Validation** (Technical File Cellular Matrix - Annex T), including : -Validation Plan N° VP- MOISTHEAT-82Sup -2016-02 V1 -Final Report N° Final-Moist heat-Cell Mat tubes-2016-06 - **Steam sterilization procedure for Medical Device** (SOP 7C-PROD023) - 2016-03-03 (Technical File Cellular Matrix - Annex T) - **LAL test (protocol and report) on HA tubes and syringes Protocol nr 20160628** rev00 2016-06-28 (Technical File Cellular Matrix - Annex V) - **Validation of the endotoxin detection test by LAL method**, rev1_29.03.2017 including NAMSA test Report 219367, validation of the LAL test A-CP-HA tube, 02.08.2016 (Technical File Cellular Matrix - Annex S)
Packaging validation showed that the packaging materials and processes were able to guaranty sterile microbiological maintenance according to ISO 11607-1. Packaging integrity testing (after transportation) showed that package was able to protect the device from transport-induced damages under routine transport conditions according to ISO 11607-1.	**Packaging Validation** (Technical File Cellular Matrix - Annex S) **Validation Blistering Machine M285**, including: -Validation Plan n°M285 VP 2017-05 -Operational qualification M285 _ OQ_2017-05 -Performance qualification M285 _ PQ_2017-05 -FINAL REPORT CLOSURE,# M285_Closure _2017-05 **Transport validation** (Technical File Cellular Matrix - Annex S) - Validation final report closure Kit transportation test, Report N°RKT-CR_2016, v1_17.10.2016
Bioburden is tested quarterly on the products by an ISO 17025-accredited laboratory (according to SOP 7C/PROD023). When action limit is trespassed, the cause of bioburden increase is investigated and the product is not conform only if SAL of 10^{-6} is not reached. Good manufacturing practices are implemented in manufacturing procedures (see SOP 7C/PROD022, SOP 7C/PROD019, SOP 7C/PROD008, SOP 7C/PROD014) as part of the quality system. Additionnally, all steps of manufacturing are processed in ISO 7 clean rooms and critical steps are performed under ISO 5 laminar flows. Manufacturing facility is cleaned according to an approved schedule and by using validated cleaning agents and methods, according to cleaning SOP 6D/QA019. Microbiological tests are performed regularly on manufacturing equipments and surfaces according to Environmental Control SOP 6D/QA007.	- **Steam sterilization procedure for Medical Device** (SOP 7C/PROD023) (Technical File Cellular Matrix - Annex T) - **Manufacturing of Hyaluronic Acid(HA) gel, HA tubes and HA syringes** (SOP 7C/PROD022) (Technical File Cellular Matrix - Annex M) - **Production of kits with steam sterilised components on Blister Pack Machine M285** (SOP 7C/PROD019) (Technical File Cellular Matrix - Annex M) - **Management of people and product flows** (SOP 7C/PROD008) (Technical File Cellular Matrix - Annex M) - **DESIGN OF AREA** (SOP 7C/PROD014) (Technical File Cellular Matrix - Annex M) - **Cleaning and Hygiene** (SOP 6D/QA019) (Technical File Cellular Matrix - Annex U) - **Environmental Control** (SOP 6D/QA007) (Technical File Cellular Matrix - Annex U)
All components are systematically stored in quarantine up to release by QC staff in material stock. Incomong tests are performed on critical components according to SOP 7C/QA012. Release of the components is processed in accordance with SOP 7C/QA001. Allcritical components are tested for their safety before release for manufacturing. Supplier qualification according to SOP 7D/AUD002.	- **Batch release procedure** (SOP 7C/QA001) (Technical File Cellular Matrix - Annex M) - **Acceptance test for incoming materials** (SOP 7C/QA012) (Technical File Cellular Matrix - Annex M) - **Supplier qualification** (SOP 7D/AUD002) (Technical File Cellular Matrix - Annex R) - **Supplier list** managed according to SOP 7D/AUD002
According to SOP 7C/PROD022, the A-CP-HA tube included in Cellular Matrix A-CP-HA Kit is controlled for several parameters before release of the batch. These final controls are also indicated on the Certificate of Analysis of each batch manufactured. Moreover, various in-process controls are planned during manufacturing of the product, according to manufacturing procedure (SOP 7C/PROD022). Final release of the finished products is performed according to Batch release procedure SOP 7C/QA001.	- **Manufacturing of Hyaluronic Acid(HA) gel, HA tubes and HA syringes** (SOP 7C/PROD022) (Technical File Cellular Matrix - Annex M) - **Batch release procedure** (SOP 7C/QA001) (Technical File Cellular Matrix - Annex M)

3.2.2 Performance studies

3.2.2.1 Purpose and objective of testing

The performance testing was conducted according to the US FDA requirements for PRP producing medical devices to determine the cellular specificities and the stability of the PRP obtained with the tested device, and the viability and functionality of the platelets after blood processing with the device. PRP samples were prepared with the blood from 60 volunteers (30 women and 30 men).

3.2.2.2 Parameters tested

For the RegenTHT, Regen BCT and A-CP:

In the RegenTHT, Regen BCT and RegenA-CP tubes, blood components are separated by centrifugation according to their relative density. A thixotropic gel allows the sequestration of the red blood cells in the lower part of the tube while the plasma and the platelets are above the gel. PRP was prepared with blood from 60 volunteers. For each PRP preparation the following values were determined:

- Volume obtained
- pH
- Blood cell and platelet concentration
- Platelet recovery and concentration factor

Platelet functionality and viability in PRP were also tested according to the US FDA requirements on 12 donors by evaluating:

- P-selecting expression on platelet membranes in response to specific activation
- Hypotonic stress response of platelets
- Platelet aggregation in presence of collagen

All measures were done at time of PRP preparation (T=0H) and four hours later (T=4H) in order to control the stability of the platelet preparations.

For the RegenATS:

This tube is similar to the Regen BCT tube (device for PRP preparation), except that it does not contain anticoagulant. This device has been designed for the preparation of autologous thrombin to induce coagulation of PRP preparation. With or without the addition of calcium gluconate, the combination of the 2 preparations allow the coagulation process to occur, forming a fibrin clot and platelet gel. The RegenATS uses the same technology to separate the RBCs. However, as it does not contain anticoagulant, the normal coagulation cascade occurs in the tube during the centrifugation. This results in the formation of a fibrin clot above the separating gel.

When the centrifugation time is prolonged for 9 or 10 minutes, the fibrin clot collapses on the surface of the gel and the serum containing the activated thrombin is extracted.

In order to precisely quantify the thrombin in our samples, we used a modification of the *in vitro* assay for the quantitative determination of prothrombin in human plasma from Hyphen BioMed, France.

We then assessed *in vitro* fibrin platelet gel formation with the autologous thrombin mixed with PRP and calcium gluconate.

3.2.2.3 Performance testing results for THT and Regen BCT / A-CP medical devices

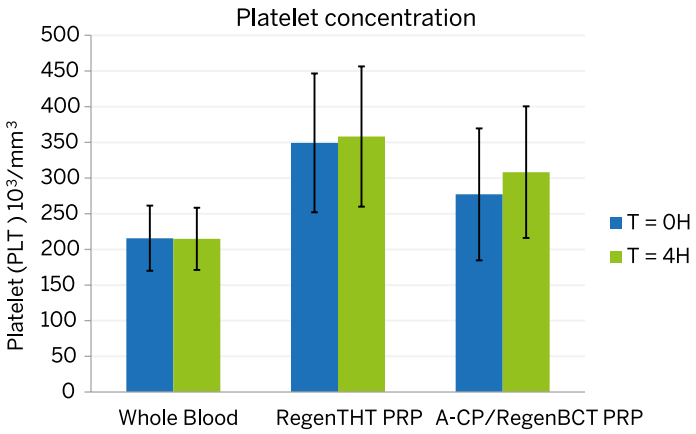


Figure 34: Platelet concentration following blood processing with the RegenTHT device and the Regen BCT / A-CP HA devices is 1.8 and 1.6 times over the baseline values in blood, respectively.

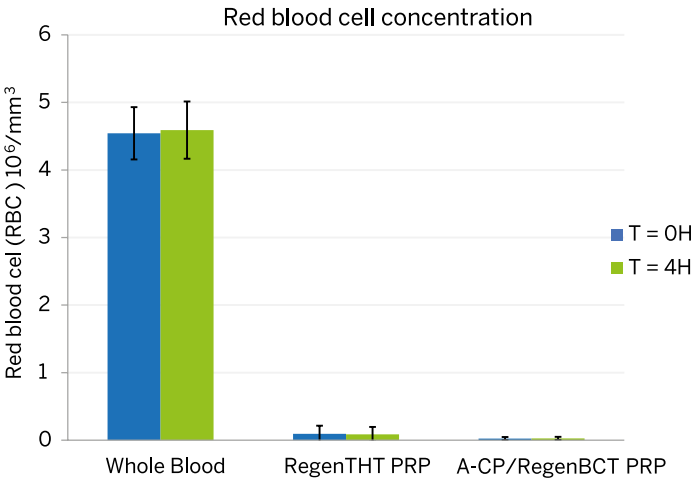


Figure 35:Red blood cells are depleted by 99% and 99.7% following blood processing with the RegenTHT device and the Regen BCT / A-CP devices, respectively.

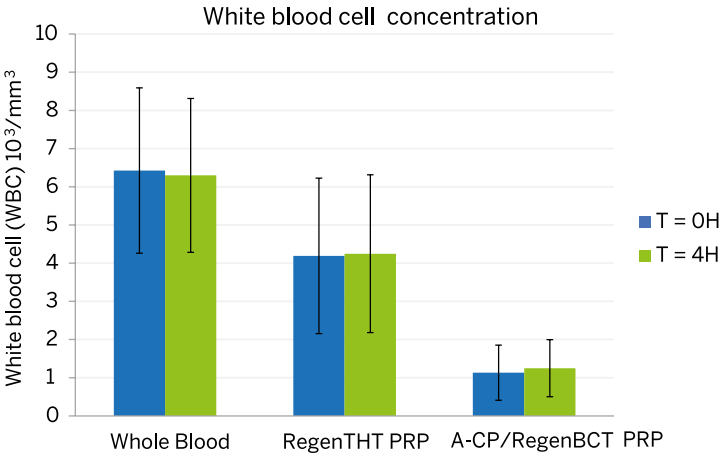


Figure 36: White blood cells are depleted by 60 to 70% following blood processing with RegenTHT device, while they are depleted by 87 to 90% with Regen BCT / A-CP devices.

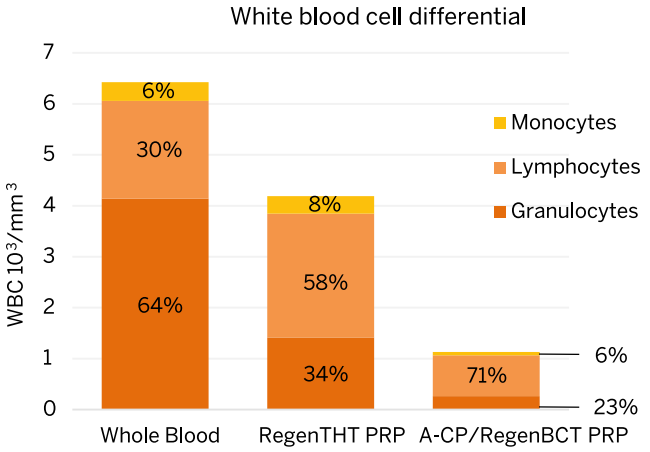


Figure 37: The remaining white blood cells in PRP preparations obtained with all Regen Lab's devices are mostly lymphocytes and monocytes. In these preparations, there is a preferential depletion of the proinflammatory granulocytes:85 to 90% of the granulocytes are depleted following blood processing with Regen THT device, while 95% of the granulocytes are depleted with the Regen BCT / A-CP devices.

In regards to the functionality testing, the platelets retrieved from Regen Lab kits are able to:

- Resist hypotonic stress
- Respond to specific ADP activa-tion by an increased expression of P-selectin
- Aggregate in presence of collagen

These biological properties are maintained during the four hours of the experiment, attesting the integrity and the functionality of the platelets.

As an example of functionality testing, we present below the results obtained for the RegenTHT device (See Figure 38).

All the recovery rates of the Regen Lab devices has been compiled in Table 12.

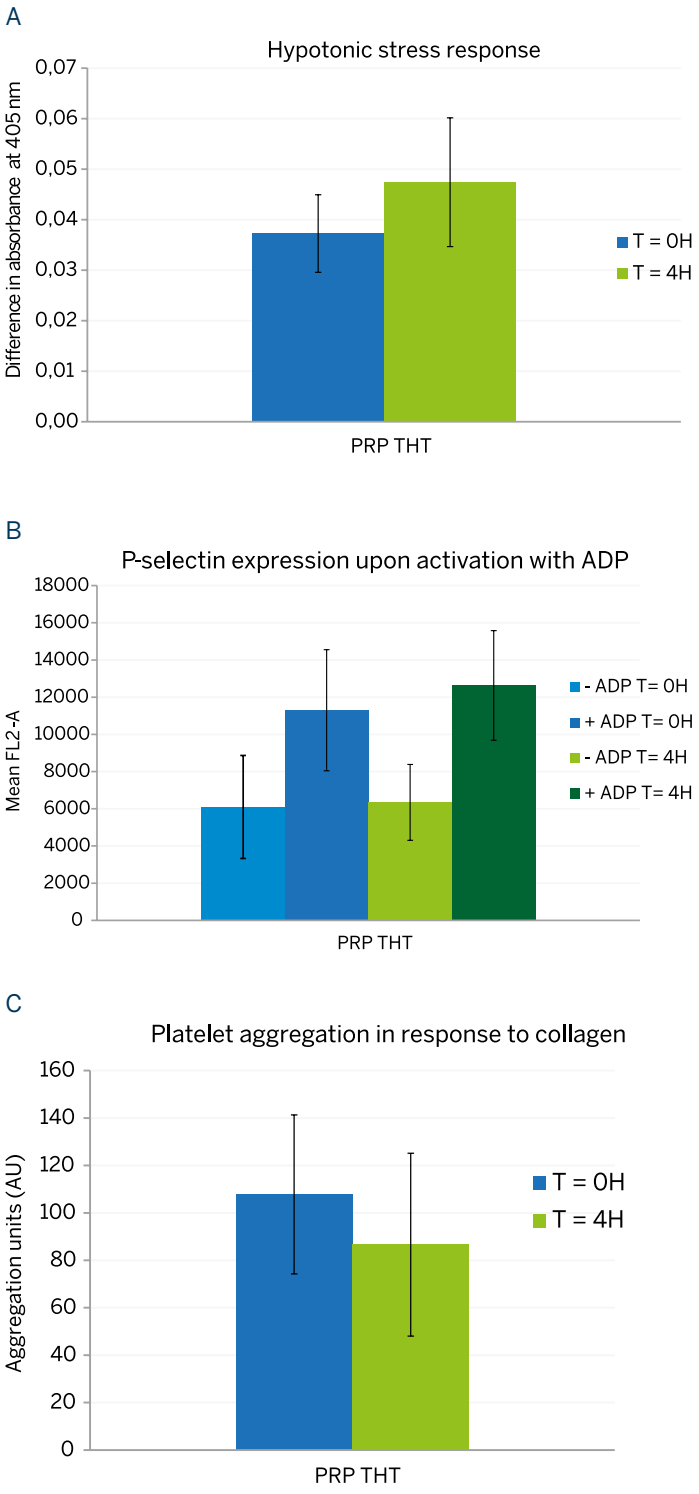
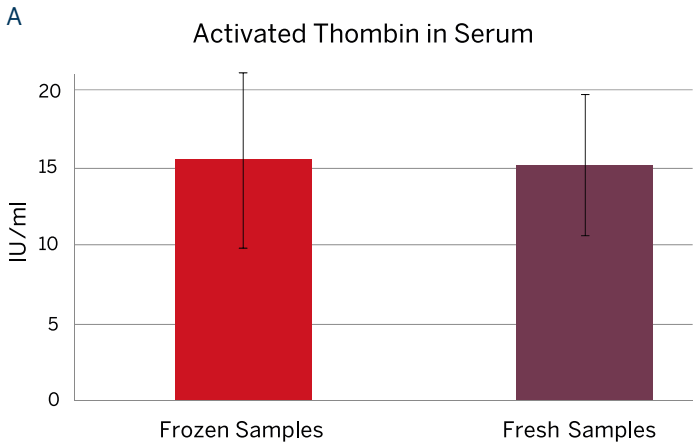


Figure 38: A. Platelet response to hypotonic stress at T=0H and after T=4H. B. P-selectin (CD62P) expression upon activation with ADP at T=0 and after T=4H. C. Platelet aggregation in presence of collagen at T=0 and after T=4H.

	Cellular element recovery (%) and concentration factor (x) compared to baseline values in peripheral blood						
	Platelets	Red blood cells	White blood cells	Mononuclear cells	Lymphocyte	Monocyte	Granulocyte
RegenTHT	~95% 1.7-1.8x	<1% 0.011x	30-40% 0.6X	70-80% 1.3x	70-75% 1.4x	50-55% 0.9x	10-15% 0.3x
RegenBCT & A-CP	~ 80% 1.6x	<0.3% 0.007x	10-13% 0.2X	20-30% 0.5x	25-35% 0.6x	~ 10% 0.2x	3.5% 0.06x

Table 12. Summary of cellular composition in PRP prepared with RegenTHT tubes, Regen BCT tubes and A-CP tubes. RBC: Red Blood Cells; WBC: White Blood Cells; MNC: mononuclear cells (lymphocytes and monocytes).

The autologous serum prepared with RegenATS tubes contained activated thrombin with a concentration around 15 IU/ml (Figure 39A). These sera can be used as a natural coagulation activator of PRP allowing for the formation of a platelet-rich fibrin gel (Figure 39B) when combined with PRP and calcium gluconate.



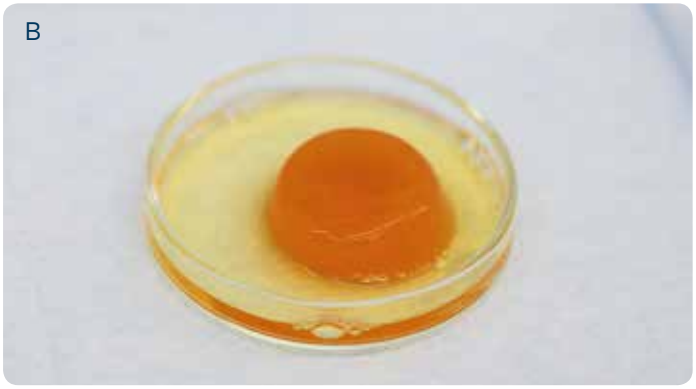


Figure 39: A. Concentration of activated thrombin obtained with ATS devices. B. Platelet gel obtained following a combination of autologous thrombin serum and calcium gluconate.

3.2.2.4 New line of product for *in vitro* cell therapy research: CuteCell

On the basis of former *in vitro* studies initiated by Regen Lab (Figure 40), a new line of products has been recently developed.

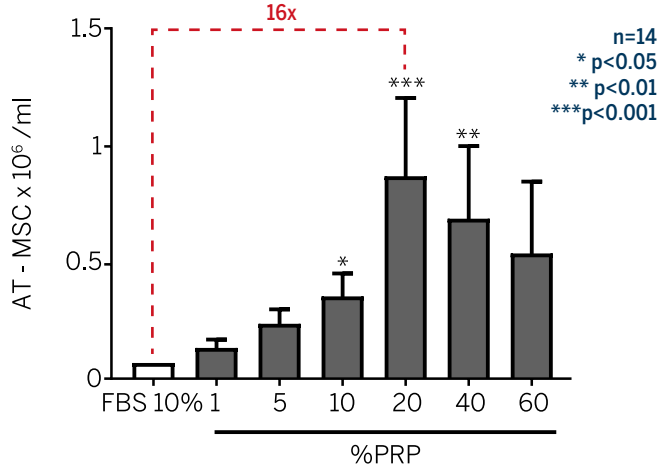


Figure 40: AT-MSC culture supplements prepared from the patient's own blood drastically enhances *in vitro* proliferation in comparison to the classical culture medium prepared with 10% FBS. Data from Atashi et al. Tissue Engineering. Part C. 2014

Results of these studies highlighted the proliferative effect of RegenPRP obtained from the patient's own blood on adipose tissue-derived mesenchymal stem cells (AT-MSC) and on Normal Human Dermal Fibroblasts (NHDF) from the same patient. Indeed, as depicted in Figure 40 AT-MSC supplemented with PRP prepared from the patient's own blood enhances by 16 times *in vitro* proliferation in comparison to the classical culture medium prepared with 10% FBS over 10 days, The same was observed when NHDF were cultured for 7 days with PRP prepared from the same patient (Figures 38, 39 and 40).

Therefore, a line exclusively dedicated to *in vitro* human cell culture for tissue engineering and cell therapy purposes has been designed and named Cutecell.

The main advantage of this research line product is that it offers an autologous alternative to the use of FBS in cell cultures, ensuring safe and animal product-free conditions for *ex vivo* expansion of autologous cells for therapeutic use.

These devices were developed with the same Regen Lab technology platform, but for *in vitro* research purposes. Three devices are available: CuteCell-PRP, CuteCell-MNC and CuteCell-serum (Figure 41).

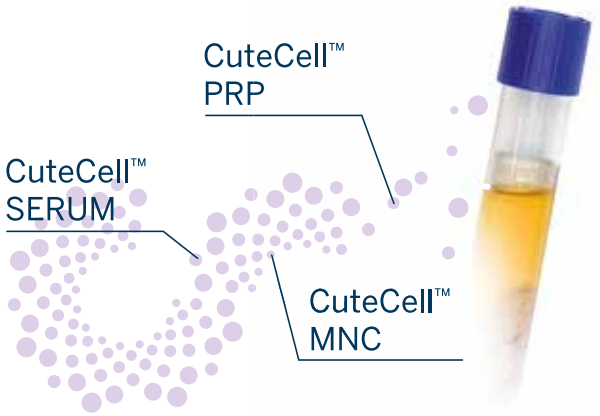


Figure 41: New product line developed by RegenLab for *in vitro* cell therapy laboratory research

CuteCell products are currently in validation through 3 collaborations with University Hospital laboratories in 3 different countries:

One based in Geneva (CuteCell SKIN at HUG, Plastic Surgery Dr Modarressi), the second in Montpellier (IRMB, Prof. Jorgensen) and the last one in the USA (The Feinstein Institute for Medical Research, Northwell University).

• The CuteCell SKIN project is an experimental research project where patient's own skin cells are cultivated in the presence of its own PRP to provide nutrients and growth factors to speed up skin cell growth *in vitro*. The main ad-vantage of this system is the safe-ty: it is a 100 % autologous cul-ture system. The patient's own cells are expanded *in vitro* with a clinical endpoint to return back to *in vivo*, for example as a skin graft, without any exogenous an-imal component interference (Figure 42).

CuteCell SKIN Flowchart

100% autologous system
From *in vivo* to *in vitro*

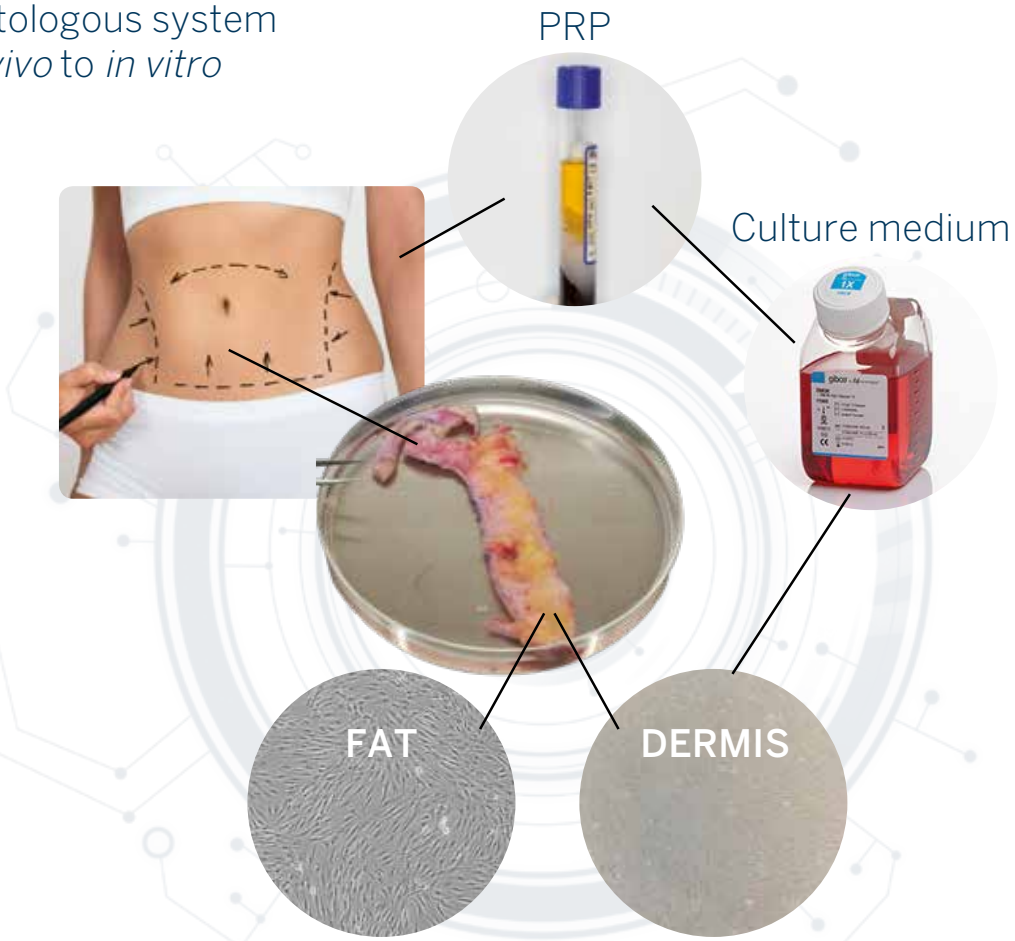
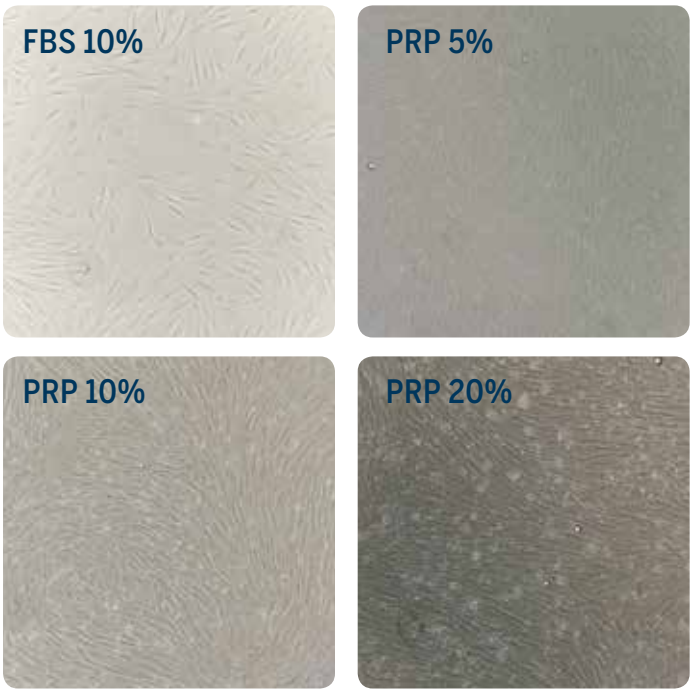


Figure 42: CuteCell SKIN flowchart. During surgical abdominoplasty, a piece of skin is retrieved from the patient as well as blood from venous puncture. In the laboratory, cells from skin (fat or dermis parts) are isolated for *in vitro* cultures. The patient's blood is processed with the CuteCell PRP device. The prepared PRP can be added to the culture medium in replacement to FBS to expand patient's cells *in vitro*.

A PRP speeds up the growth of skin cells



B

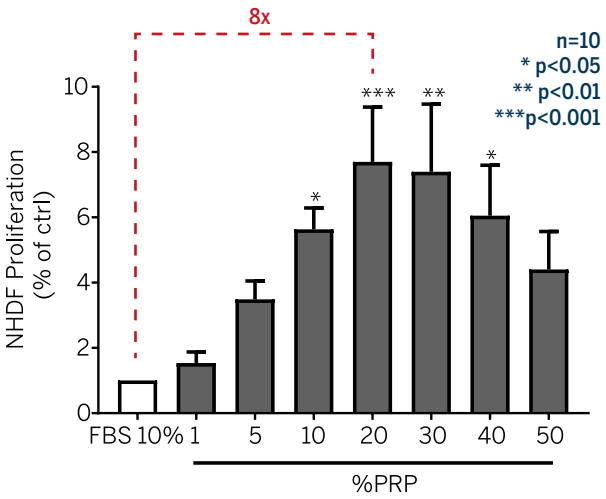


Figure 43: A. Bright field optical photography of Normal Human Dermal Fibroblast (NHDF) in the presence of FBS 10% or PRP (5-20%) after 6 days of culture. Magnification 10x. Pictures are representative of one donor. B. Proliferative effect of increasing PRP concentrations in comparison (1-50%) to FBS 10% (n=10 different patients) on NHDF cultures for 7 days without medium change in a complete autologous system (cells and PRP from the same patient).

• The second advantage of the CuteCell PRP device is the effica-cy as we demonstrated that au-tologous PRP treatment is more potent than FBS for inducing fi-broblast cell proliferation.

A small number of cells retrieved from a piece of skin sample can be exponentially expanded *in vitro* in a shorter period of time in comparison to classical culture conditions with FBS (Figure 43).

• Autologous PRP treatment of dermal fibroblast also promotes other cellular responses like cell-matrix interactions and migration. Right after PRP treatment, fibro-blasts are activated, they de-creased their adhesion to matrix proteins in order to proliferate and migrate (Figures 38 and 39).

Conclusion of the CuteCell SKIN research project:
Autologous PRP treatment promotes major cellular responses involved in tissue repair including fibroblast proliferation, matrix adhesion and migration.

This culture setting will be interesting for cell therapy researches where classical methods for maintenance and *in vitro* expansion of cells rely on the use of xenogeneic media. These methods provide limited expansion and cells tend to lose clonal and differentiation capacity upon long-term passaging. Thus, human cells expanded in their autologous CuteCell PRP optimized medium in the absence of xenogeneic serum may have improved therapeutic potential *in vivo*.

Summary of CuteCell products research applications:

• CELL THERAPY: The use of stem cells, pluripotent cells or repro-gramming adult cells in cell ther-apy for treatment of diseases re-quires cells to be grown in *ex vivo* conditions. CuteCell-PRP and CuteCell-Serum are efficient products to replace FBS in autol-ogous cell therapy.

• TISSUE ENGINEERING: The re-generation of damaged tissue re-quires the creation of a scaffold in which the patient's own cells can proliferate and maintain the bio-logical function of each tissue type. The combination of CuteCell PRP and autologous thrombin re-trieved from the CuteCell serum allows the formation of platelets embedded in a tridimensional fi-brin scaffold. This matrix rich in growth factors can support cell proliferation and tissue regenera-tion.

• IMMUNOTHERAPY: The use of lymphocytes to fight against can-cer requires that they are taken from the patient's own body. The lymphocytes are then activated *ex vivo*, before being reinjected into the patient. The CuteCell system MNC allows for the quick and easy isolation of lymphocytes to be used in immunotherapy.

Right after PRP treatment, fibroblasts are activated and decreased their adhesion to matrix proteins in order to proliferate and migrate

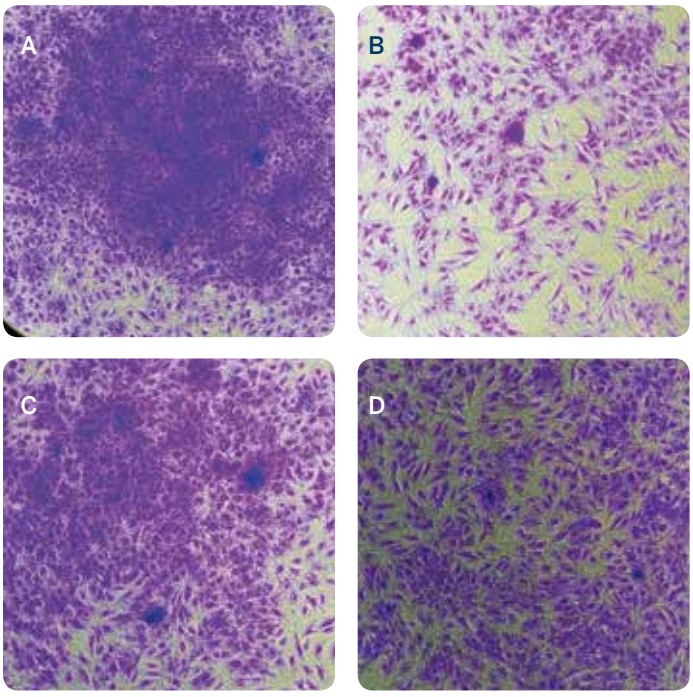


Figure 44 : Short-term PRP effect of NHDF adhesion to laminin (A and B) and collagen I (C and D). NHDFs were stimulated with FBS 10% (A and C) or PRP 10% (B and D) for 4h. Unattached cells were removed, and adherent cells were evidenced by crystal violet staining.

PRP promotes a rapid collective fibroblast migration, one of the hallmarks of wound healing.

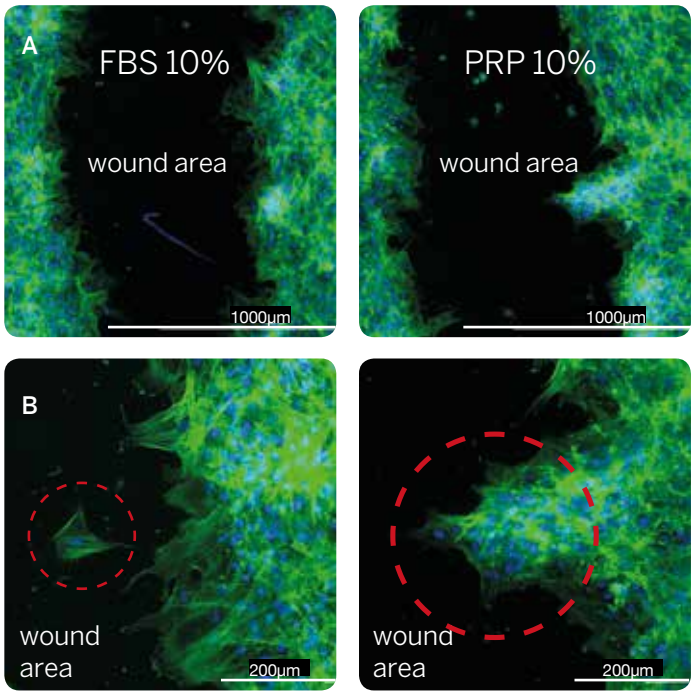


Figure 45: Comparative cellular effects of PRP 10% treatment on cell migration in NHDFs cultures. A. Migrating fibroblasts narrowed the width of the scratch zone after 8h as depicted with an immunofluorescence staining with Phalloidin. Cell Migration front is equally distributed along the scratch border in FBS 10 % while it is less homogeneous in PRP 10% treated cells. B. Zoom in pictures showing isolated cell migration in FBS 10 % cultures and collective cell migration in PRP 10% cultures.

3.3 SHELF LIFE OF REGENPRP AND CELLULARMATRIX TUBES

The stability parameters (tube stability, packaging, sterility) that insure the safety and performance of Regen tubes, A-CP HA tubes and BCT-HA tubes along their shelf life were assessed. Tested parameters were investigated on products stored in normal conditions at room temperature or, if not available, over an accelerated aging period at 50°C, equivalent to storage for 30 months at room temperature (5°C to 30°C).

Based on available stability data, all the parameters mentioned above are in the limit values at 24 months or older. The shelf life is validated for both devices at 24 months from manufacturing, and the maximal storage temperature is validated at 30°C. The minimal storage temperature is validated at 5°C as technical (non-freezing) limit.

The following stability aspects of the A-CP-HA Kit and BCT-HA Kits were investigated:
• Performance and functional criteria:
- Pre-determined tube vacuum
- Performance of the cell separator gel
- Stability of the HA
• Safety criteria:
- Predetermined tube vacuum
- Sterility

Test	Test item / Measured parameter	Type of test	Storage period	Conclusion of tests
Vacuum stability test	RegenBCT tube (containing the same raw materials than RegenATS and A-CP tubes)/ Vacuum of the tube	Real time ageing of RegenKits	36 Months	Vacuum of the Regen BCT tube has been verified to be stable for 1097 days (36 months) at 30°C. A non-significant variation (+ 4.5%) in vacuum measures has been detected.
Vacuum stability test	RegenTHT tube/ Vacuum of the tube	Real time ageing of RegenKits	44 months	The vacuum of RegenTHT tubes remained within acceptance criteria for several years and did not vary more than 4.3% between different lots. The variability in the same lot did not exceed 11%, but might be attributed to imperfect storage conditions. These data indicate that the RegenTHT tube vacuum remains highly stable for at least 44 months at room temperature.
Performance stability test	A-CP tube BCT tube ATS tube	Real time ageing of RegenKits	36 Months	Tests demonstrated a platelet recovery superior to 50% (US-FDA requirements) for the tubes. This shows that this product is stable over time, as its performance for PRP preparation and platelet recovery is maintained even after 36 months of storage at room temperature. Therefore, it was demonstrated that RegenBCT tubes are stable for a longer time than the shelf life defined by the Manufacturer, REGEN LAB SA.
Performance stability test	RegenTHT tube	Real time ageing of RegenKits	42 months	Tests demonstrated a platelet recovery superior to 50% (US-FDA requirements) for the tubes. This shows that the product is stable over time, as its performance for PRP preparation and platelet recovery is maintained even after 42 months of storage at room temperature. Therefore, it was demonstrated that RegenTHT tubes are stable for a longer time than the shelf life defined by the Manufacturer, REGEN LAB SA.
Stability of sterility	RegenACR-C Extra kit (same association Tyvek-blister than all RegenKits) / Sterility of the packaging of the RegenKits	Real time ageing of RegenKits	41 Months	In sum, it was demonstrated that: RegenKits packaging integrity is maintained at room temperature for a period of 41 months from its manufacturing date. The data indicates that RegenKits products packaged in double sealed trays are stable for 41 months at room temperature, maintaining their safety characteristics. As a consequence, the Company intends to label the RegenKits with a maximal expiration date of 24 months from its manufacturing date, taking into account that the RegenKits expiration date may not exceed the Regen tubes expiration date.

Table 13. Table of the shelf life testing for A-CP HA and BCT-HA tubes.

ANNEXE - PROTOCOLS FOR CLINICAL EVALUATION

MUSCULOSKELETAL / Cartilage	**MUSCULOSKELETAL** **Cartilage** Ostheoarthritis Per session: 1 PRP device Ostheoarthritis 1 Cellular Matrix tube per session	**Preparation protocol** Inject into the articulation, under ultrasound guidance , if necessary When present, articular effusion should be withdrawn prior to injecting PRP Recommend the patient to respect a delay of 1h without physical activity after the injection, and to avoid strenuous or weight bearing activities for 48 hours following the intra-articular injection Inject into the articulation, under ultrasound control, if necessary When present, articular effusion should be withdrawn prior to injecting PRP-HA Recommend the patient to respect a delay of 1h without physical activity after the injection, and to avoid strenuous or weight bearing activities for 48 hours following the intra-articular injection	**Application protocol** According to Dr Gobbi's study (2012): Three injections one month apart Optional: repeat treatment at 1 year (D0, D30 and D60), if necessary According to Dr Renevier's study: Two injections 2 months apart Optionally: a 3rd injection 6 months after the onset of the treatment
MUSCULOSKELETAL **Tendons/Muscles/ Ligaments**	**SPORT MEDICINE** - Sport lesions (e.g. tendons, muscle, ligaments and plantar fascitiis) Per session: 1 PRP device	**Injection Protocol** Inject directly into the lesion, under ultrasound control using the retrograde fashion Optional: supplement the treatment with perilesional, in the tendinous sheath and peritendinous injections. Only inject small volumes not to cause a dissection of the lesion	**Frequency/Follow-up** According to Dr Abate's study: One session For recalcitrant tendonitis: a second or even a third injection might be necessary, with a minimum delay of one month between two injections
ORTHOPAEDICS SURGERY **Bone reconstruction**	**ORTHOPAEDICS SURGERY** **Bone reconstruction** Possible Indications Non-union (pseudarthrosis) Delayed union Bone cysts RegenKit Extracell BMC 1-4 Bone Marrow/ MSC devices (RegenTHT)	**Preparation protocol** Rinse syringes and trocar with a heparinized saline solution (100 U/ml) Collect medullary blood with the trocar and syringes (20 to 40 ml) Transfer medullary blood into RegenTHT devices Centrifuge 8' at 1600g Gently invert the devices several times in order to resuspend the cellular elements in plasma Collect the medullary plasma (= bone marrow concentrate) into a syringe connected to a transfer device.	**Application protocol** See Dr De Biase, Prof Capanna and Dr Civinini's studies.

ORTHOPAEDICS SURGERY / Bone reconstruction	**ORTHOPAEDICS SURGERY** **Bone reconstruction** Possible Indications Non-union (pseudarthrosis) Delayed unions Prosthetic revisions (Hip/Knee) Osteonecrosis (AVN, avascular head, necrosis) Bone cysts Vertebral fusion (e.g. spondylolisthesis) Bone loss (e.g. bone tumor) Osteotomy (bone lengthening) Cartilage defects RegenKit Extracell Glue 1-4 Bone Marrow/ MSC devices (RegenTHT) 1-2 PRP devices 1 device to get autologous activated thrombin (RegenATS)	**Preparation protocol** Medullary blood: Rinse syringes and trocar with a heparinized saline solution (100 U/ml) Collect medullary blood with the trocar and syringes (20 to 40 ml) Transfer medullary blood into RegenTHT devices Centrifuge 8' at 1600g Gently invert the devices several times in order to resuspend the cellular elements in plasma Collect the medullary plasma (= bone marrow concentrate) into a syringe connected to a transfer device. Venous blood: Prepare PRP and the autologous activated thrombin as described in the Instruction for Use Mix them in the following ratio: 70% PRP – 30% autologous activated thrombin. Combine this mix with the bone marrow concentrate and with a scaffold, according to the surgeon's needs.	**Application protocol** See Dr De Biase, Prof Capanna and Dr Civinini's studies.
ORTHOPAEDICS SURGERY **Ligaments, tendons and cartilage reconstruction**	**ORTHOPAEDICS SURGERY** **Ligaments, tendons and cartilage reconstruction** Suture consolidation (e.g. tendons) RegenKit Extracell Membrane 2 PRP devices Calcium Gluconate 10% 2 centrifugations	**Preparation protocol** Collect the PRP prepared with 2 PRP devices and transfer it into the glass snap-cap flask Add 5 ml of calcium gluconate 10% Alternative to obtain a more resistant coagulum: only use 2 ml of calcium gluconate 10% Centrifuge for 10' at 1500g Take the coagulum out of the flasks using sterile pliers Alternative to obtain a flat fibrin membrane: centrifuge for 20 to 30' Remember that the coagulum or the fibrin membrane is polarized, with platelets settled at the bottom of the flask	**Application protocol** See Dr Giannotti's study.

TRICHOLOGY	**TRICHOLOGY** - Hair Loss 1 or 2 PRP tubes - Follicular Unit Extraction (FUE) 1 or 2 PRP tubes	**Injection Protocol** Apply anesthetic cream on the area to be treated 1 hour prior to injection Inject non-concentrated, non-activated PRP prepared with 1 or 2 PRP device(s) with 27-32G needle, point by point or nappage technique (U225 mesogun recommended, 1.5-4mm depth Recommend the patient not to wash the treated area in the next 4 hours 3-4 weeks prior to grafting: pre-treat graft donor and receiver sites with PRP mesotherapy injections Hair graft day: collect FUE and place in PRP for follicular nourishment until reimplantation Directly post-grafting: apply PRP topically on donor and receiver sites 3-4 weeks post-grafting: treat graft donor and receiver sites with PRP mesotherapy injections	**Frequency/Follow-up** According to Dr Shapiro and Dr Ho's study : One initial treatment consisting of PRP injection for 2 to 6 months. Maintenane treatment every 3 to 6 months
SKIN CARE / SCARS	**SKIN CARE / SCARS** - Keloid scars 1 - 2 PRP tubes	**Injection Protocol** According to Dr Hersant's study: Following total surgical excision of the keloid: Intradermal injection of PRP prepared with 1 or 2 PRP device(s) into both edges of the wound (healthy, extralesional tissue)	**Frequency/Follow-up** According to Dr Hersant's study: One session / every 4 weeks until inflammation is reduced and induration disappears
PLASTIC & RECONSTRUCTIVE SURGERY	**PLASTIC & RECONSTRUCTIVE SURGERY** Weight loss sequelae (e.g. abdominoplasty, thigh lift, brachioplasty) Breast reduction Split-thickness skin graft Autologous Glue 2 PRP devices 1 device to get autologous activated thrombin (RegenATS) Calcium Gluconate 10% (not supplied) 1 Regen Spray Applicator	**Preparation and application protocol** According to Dr Hersant's protocol: Collect PRP from 2 PRP devices in the 10-ml syringe. Collect 2 ml of autologous activated thrombin prepared from 1 RegenATS device and 1 ml calcium gluconate in the 3 ml-syringe. Assemble the spray applicator. Apply drop by drop onto the area to be treated: Weight loss sequelae: onto the undermined subcutaneous space before suturing the flap Breast reduction: onto the entire surface of the subcutaneous tissue before suturing the flap Thigh lift/brachioplasty: under the suture in 2 layers Split-thickness skin graft: onto the split-thickness skin graft For large areas, spread the mix with a sterile glove	**Frequency/Follow-up** This a unique, per-operative application

WOUND CARE	**WOUND CARE** Superficial Wounds e.g. Leg ulcers Autologous Glue 2 PRP devices 1 device to get autologous activated thrombin (RegenATS) Calcium Gluconate 10% (not supplied) 1 Regen Spray Applicator	**Preparation and application protocol** Collect PRP from 2 devices in the 10-ml syringe. Collect 2ml of autologous activated thrombin prepared from 1 RegenATS device and 1 ml calcium gluconate in the 3 ml-syringe. Assemble the spray applicator. Apply drop by drop onto the clean and debrided wound.	**Frequency/Follow-up** According to Prof Humbert's case: Repeat the treatment each week until full closure of the wound. The treatment interval can be extended to 2-3 weeks if the wound responds well to the treatment.

WOUND CARE	**WOUND CARE** -Deep Wounds e.g. Diabetic Foot ulcers Biological dressing: 2 PRP devices 1 device to get autologous activated thrombin (RegenATS) Calcium Gluconate 10% (not supplied) or 2 Cellular Matrix devices 1 device to get autologous activated thrombin (RegenATS) Calcium Gluconate 10% (not supplied)	**Preparation and application protocol** According to Dr Clavel's study: In a small sterile vessel, gently mix: PRP from 2 PRP devices 0.5 to 1 ml calcium gluconate 10% 1 to 2 ml autologous activated thrombin prepared with 1 RegenATS device Let stand for 10' without manipulation for clot formation Carefully transfer the clot from the vessel to the clean and debrided wound Alternative for upward facing deep wound: directly inject the mixture and allow to jellify in situ Optional: PRP biological dressing can be combined with point by point liquid PRP injections in the bed and edges of the wound to stimulate healthy surrounding tissues Cover the clot with layers of Vaseline gauze to keep moisture. Fix this primary dressing with Steri-Strips. Cover the primary dressing with sterile gauze to absorb possible exsudates. This secondary dressing can be changed as often as deemed necessary. The paraffin gauze in direct contact with the biological dressing and the wound should not be removed until the next treatment except in case of infection. In case of infection, the wound should be cleaned and treated for the infection before resuming PRP treatment.	**Frequency/Follow-up** According to Dr Clavel's study: Repeat the treatment after one week, then the interval can be increased to 2 or 3 weeks or even more Repeat until full closure of the wound has occurred

WOUND CARE / Burns	**WOUND CARE** Deep 2nd degree / partially 3rd degree burns Autologous Glue 2 PRP devices 1 device to get autologous activated thrombin (RegenATS) Calcium Gluconate 10% (not supplied) 1 Regen Spray Applicator	**Preparation and application protocol** Collect PRP from 2 devices in the 10-ml syringe. Collect 2ml of autologous activated thrombin prepared from 1 RegenATS device and 1 ml calcium gluconate in the 3 ml-syringe. Assemble the spray applicator. Apply drop by drop onto the clean and debrided wound.	**Frequency/Follow-up** According to Dr Beustes Stefanelli's case, repeat the treatment each week until full closure of the wound. The treatment interval can be extended to 2-3 weeks if the wound responds well to the treatment. .
UROLOGY / Male dysfunctions	**UROGENITAL** Lapeyronie's disease Per session: 1 Cellular Matrix device + 1 PRP device	**Preparation protocol** According to Dr Virag's protocol: Perform a penile local anaesthesia Prior to injection, apply mechanical action to the fibrotic and/or calcified plaques with a 22G and/or 18G needles Inject a mix of PRP and PRP-HA prepared from a PRP device and a Cellular Matrix device, respectively, into the tunica albuginea's diseased sites under ultrasound control Apply a slightly compressive dressing Recommend the patient to avoid intercourses for the 48 hours	**Application protocol** 5 injection sessions 2 weeks apart According to clinical outcomes, two additional monthly sessions, scheduled 3 months after the onset of the treatment can be performed and repeated every 3 months, if necessary.
SEXUAL MEDICINE / Gynecology	**SEXUAL MEDICINE** **Gynecology** Sexual Rejuvenation Per session: 1 PRP device OR 1 Cellular Matrix device	**Preparation protocol** According to Dr Selih-Martinec's protocol: Inject 1ml directly into the clitoris with 27G needle Inject 2-3ml injected suburetheraly with 23-27G needle	**Application protocol** Two injection sessions three months apart Repeat treatment every 12-18 months

PELVIC FLOOR DISORDERS / Gynecology	**SEXUAL MEDICINE** **Gynecology** Stress Urinary Incontinence Per session: 1 PRP device OR 1 Cellular Matrix device Lichen sclerosus Per session: 1-2 Cellular Matrix device(s) OR 1-2 PRP device(s) Vulvovaginal atrophy Per session: 1 PRP device OR 1 Cellular Matrix device	**Preparation protocol** According to Dr Behnia-Willison 's protocol: Inject the preparation into the anterior lower one third of the vagina and the periurethral area with a 27G needle According to Dr Behnia-Willison's study: Inject the preparation with a 27G needle in a fanning motion to break the scar and fibrotic tissue and retrograde injection of PRP or PRP-HA into any affected area of the external genitalia, including labia majora, labia minora, clitoris and clitoral hood; volume depends upon size of affected area According to Dr Behnia-Willison's protocol: Inject 1 cm below the hymen at the level of the fourchette and bevel deep into the labia minora with a short 30G needle. Inject into the anterior, posterior and lateral walls of the vagina with a 27G spinal needle	**Application protocol** Three injection sessions 4 to 6 weeks apart Treatment: every 12-18 months Three injection sessions 4-6 weeks apart One additional injection session at 12 months Three treatment sessions 3 weeks apart Treatment: every 12-18 months
OPHTHALMOLOGY	**OPHTALMOLOGY** - Dry Eye Disease 1 or 2 PRP device(s) - Persistent ocular epithelial defects 1 or 2 PRP device(s)	**Preparation Protocol** Under a laminar flow air hood: • transfer PRP prepared from one or two tube(s) to the RegenCollyrium container, previously placed in the lower part of a RegenClip (supplied separately) • Put the RegenCollyrium dispenser onto the RegenCollyrium container and seal thewhole thing using the upper part of the RegenClip	**Application protocol** According to Dr Conca's study: Topical instillation onto the ocular surface: 1 drop into each eye / 2 hours for 1 month According to Dr Adorno's study: Topical instillation onto the ocular surface: 1 drop into each eye, 3 times/day

4. REGENLAB MEDICAL DEVICES CLINICAL EVALUATION

Therapeutic area		Medical indications	RegenKit references	Number of patients treated with RegenKits	Pages
Musculo-skeletal (MSK) and sports medicine	**Cartilage**	Knee osteoarthritis (OA)	RegenBCT / RegenTHT Cellular Matrix A-CP-HA	696	
		Hip osteoarthritis (OA)	RegenTHT	58	
		Hemophilic arthropathy	RegenTHT	12	
		Osteochondral or cartilage degenerative lesions	RegenTHT	102	
	Tendons	Tendinopathy	RegenBCT / RegenTHT	287	
	Muscles	Exercice-induced muscle damage	RegenBCT	12	
	Total			**1167**	
Orthopaedic surgery	**Bone reconstruction**	Bone nonunion	Regen Extracell BMC / Regen THT	230	
		Osteonecrosis	Regen Extracell BMC / Regen THT	54	
		Femoral fracture	Regen Extracell BMC / Regen THT	1	
	Ligaments, tendons and cartilage reconstruction	Tendons	Regen Extracell Membrane / Regen THT	49	
		Osteochondral lesions	RegenTHT	110	
	Total			**444**	
Spine and Nerve surgery		Carpal tunnel syndrome	RegenTHT / RegenATS	31	
		Various Neuropathy/Neuralgia	RegenBCT	3	
		Spine fusion and lumbar spine surgery	RegenTHT / RegenATS	46	
	Total			**80**	
Trichology		Androgenetic alopecia	RegenBCT	141	
	Total			**141**	
Skin Care	**Scars**	Skin scars	RegenBCT	23	
		Keloid scars	RegenBCT	17	
	Bio-revitalization	Skin ageing	RegenBCT / RegenATS Cellular-Matrix BCT-HA	96	
		Facial rejuvenation and acne scars	RegenBCT	23	
	Melasma	Melasma	RegenTHT	1	
	Total			**160**	
Plastic and reconstructive surgery		Facial rejuvenation/augmentation	RegenBCT Cellular-Matrix A-CP-HA	80	
		Atrophic scars	RegenTHT	70	
		Skin graft	RegenBCT / RegenATS RegenTHT	44	
		Wound lesions	RegenTHT / RegenATS	45	
		Various surgeries	RegenBCT / RegenATS	51	
	Total			**290**	

4.1. PUBLISHED CLINICAL STUDIES WITH REGENKITS AND CELLULARMATRIX

Key results from published clinical studies with RegenKits and CellularMatrix devices for each medical indication are described and summarized in the following tables.

A total of 108 publications have been retrieved in the past 10-year period. This analysis allowed to identify specific therapeutic areas in which RegenKits and CellularMatrix were used on a total number of 3351 patients.

Therapeutic area		Medical indications	RegenKit references	Number of patients treated with RegenKits	Pages
Wound care	**Chronic wound**	Diabetic foot ulcers	RegenTHT RegenATS RegenBCT	79	
		Chronic skin diseases	RegenATS	1	
		Bleeding ulcer	RegenTHT	1	
		Tendons exposure/injuries	RegenTHT / RegenATS RegenBCT	40	
	Surgical wound	Sternal or LVAD wound infections	RegenTHT	425	
	Burns	Burnt tissue	RegenTHT / RegenATS RegenBCT / RegenATS	33	
	Total			**579**	
Sexual medicine	**Gynecology**	Lichen sclerosus	RegenBCT Cellular Matrix BCT-HA	69	
		Vaginal mesh exposure	RegenBCT / RegenATS	3	
		Dyspareunia (female sexual dysfunction)	RegenBCT	11	
		Vulvo vaginal atrophy	Cellular Matrix BCT-HA	21	
	Fertility	Ovarian regeneration/ folliculogenesis activation	RegenBCT	12	
	Male dysfunction	Peyronie's disease	RegenBCT Cellular Matrix BCT-HA	103	
	Total			**219**	
Dentistry, Maxillofacial and otorhinolaryngology		Tooth avulsion	RegenBCT	10	
		Maxillary or mandibular lesions/defects	RegenExtracell BMC /RegenTHT RegenTHT RegenTHT / RegenATS	185	
		Tympanic perforation/cochlear implants	RegenTHT RegenBCT	15	
		Nose lesions	RegenExtracell BMC /RegenBCT RegenBCT	26	
	Total			**236**	
Ophtalmology		Macular degeration/ocular defect	RegenBCT	35	
	Total			**35**	
Total number of patients treated with RegenKits				**3351**	

4.1.1 MUSCULOSKELETAL AND SPORTS MEDICINE

29 publications were identified including clinical data on 1425 patients.
Among them, RegenKits have been used for 3 different subtypes of indications:

1.A. Cartilage (18 publications)
RegenKits and CellularMatrix have been used on 868 patients for the treatment of cartilage diseases, including knee and hip osteoarthritis (OA), arthropathies, hemophilic arthropathy and osteochondral lesions.

1.B. Tendons (9 publications)
RegenKits have been used on 287 patients to treat both insertional and non-insertional tendinopathies (Achilles, patellar, and elbow) and other tendon injuries such as rotator cuff foot print tear and glenoid labral tear.

1.C. Muscles (2 publications)
RegenKits have been used on 12 patients to evaluate the effect of intra-muscular PRP injections following exercise-induced muscle damage.

CARTILAGE					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Knee osteoarthritis treatment using PRP mix with HA. 6 months follow-up. Blums K *et al.* *International student conference health science.* 2017 (Abstract)	Prospective Randomized Controlled clinical trial Follow-up: 6 months	Knee OA, Grade I-III Kellgren-Lawrence	43	Cellular Matrix A-CP-HA	PRP+HA group (16 pts): Cellular matrix, 3 injections 0,2 and 6 months Control group (27 pts): only HA, 3 injections 0, 1 and 2 weeks
Intra-articular hyaluronic acid vs platelet-rich plasma in the treatment of hip osteoarthritis Di Sante L, Villani C, Santilli V, *et al.* *Medical ultrasonography.* 2016; 18:463-8	Prospective Randomized Controlled trial Follow-up: 16 weeks	Treatment of hip OA, II-III radiographic grade Kellgren and Lawrence	43	RegenTHT	PRP group (21 pts): 8 ml of patient blood, centrifugation 9 min at 3100 rpm to obtained 4ml of PRP were used for i.a injection Note: PRP was not prepared as per instructions for use, RegenTHT tube was centrifuged twice. Control group (22 pts): Na-HA 3 i.a injections with weekly intervals were performed under US-guidance with 20-gauge (9 cm) spinal needle into the anterior synovial recess at the junction of the femoral head and neck.

Safety: Generally, no adverse events (AE) were reported apart temporary local pain and discomfort during or after the injection procedure. No serious complication was observed during the injection procedure and the post-treatment period.
Performance:
Cartilage: Performance data showed that intra-articular PRP +/-HA injections was effective for the treatment of cartilage disease with great improvement of pre-treatment symptoms, which included mainly articular pain, joint stiffness and limited joint function. In addition, i.a PRP +/- HA may be valid alternative to drug therapy with analgesics and anti-inflammatory drugs or corticosteroid injections.
Tendons: PRP treatment was effective, all treated patients reported a significant reduction in pain and improvement in function even if patients had failed to response to previous conservative treatments, and active people can return to physical activity.
Muscles: Performance data showed that intra-muscular PRP injections improved muscle recovery, by increasing iron (Fe) levels, from the induced injury.

These data contribute to the safety and performance profile of PRP prepared with RegenKits and PRP-HA prepared with CellularMatrix and support their use for the treatment of cartilage lesions, osteoarthritis, tendinopathies and muscles injuries, even for patients' refractory to standard treatments.

The key results described in publications are summarized in the following table:

CARTILAGE	
Key results on performance	**Safety / adverse events (AE)**
- Both treatments were efficient KOOS score (symptoms, pain, activity and quality of life) between baseline and 6 months. ➔ 1 injection of PRP+HA provides less improvement than 3 injections of HA; but PRP+HA provides clinically and statistically improvement of knee OA at 6 months: improvement of 10 points of KOOS score (symptoms, pain, activity and quality of life).	Not formally assessed.
Clinical evaluation performed with VAS pain score and WOMAC questionnaire: - VAS pain score was significantly improved in short-term follow-up (4-week) in PRP group. - WOMAC clinical scores were improved, but no statistical difference was found between follow-up and pre-treatment scores in PRP group. - Improvement of VAS and WOMAC scores in HA group. ➔ Intra-articular PRP had an immediate effect on pain that was not maintained at longer term follow-up.	No complications related to the i.a injections were registered during the treatment and follow-up period.

CARTILAGE					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
The effects of repeated intra-articular PRP injections on clinical outcomes of early osteoarthritis of the knee Gobbi A, Lad D, Karnatzikos G. *Knee Surg Sports Traumatol Arthrosc.* 2015; 23:2170-7	Prospective Randomized clinical trial Level II Follow-up: 24 months	To evaluate long-term performance of i.a PRP to early knee OA, grade I-II Kellgren-Lawrence	93	RegenACR-C Extra / RegenBCT	PRP (93 pts): 8ml of patient blood drawn into evacuated tube containing 1 ml sodium citrate 4% and a thixotropic gel selector for separation of blood components. Single-step centrifugation. About 4ml of PRP obtained. All patients received a cycle of 3 i.a PRP injection into the knee joint, administered with 1-month interval. 12 months after, 38 patients randomly chosen among the 93, received a second i.a treatment cycle. Treatment was arthroscopically administered through supra-patellar approach.
Intra-articular injection of platelet-rich plasma versus hyaluronic acid viscosupplementation in treating haemophilic arthropathy of the knee joints. Li TY *et al.* *Haemophilia*. 2016 (Poster and abstract)	Prospective Randomized Controlled clinical trial Follow-up: 6 months	Haemophi-lic arthro-pathy of knee joints	22	RegenTHT	PRP group (11 pts): single intra-articular injection of 2 ml of PRP Control group (11 pts): 5 weekly intraarticular injections of HA (ARTZDispo)
Intra-Articular injections for degenerative cartilage lesions of the knee: platelet rich plasma vs hyaluronic acid Papalia R, Franceschi F, Carni S, *et al.* *Muscles, ligaments and tendons journal* 2012; 2 (3 – Suppl):67	Prospective Randomized Comparative study Follow-up: 6 months	Knee degenerati ve cartilage lesions	150	RegenTHT	Group 1 (50 patients): Regen Lab PRP Group 2 (50 patients): 'Home Made' PRP Group 3 (50 patients): Hyaluronic acid Patients received 3 intra-articular injections of 5.5ml of PRP or 4 intra-articular injections of HA (interval between injections in not available from the abstract)

CARTILAGE	
Key results on performance	**Safety / adverse events (AE)**
-Significant improvement on all clinical score KOOS, VAS, MARX and TEGNER clinical scores, PRP treatment gave encouraging results when compared to baseline data (p<0.001). -Significantly further improvement in the symptomatic response (MARX and VAS score) was observed in a group of 38 patients who received a second cycle of injections (p<0.001). - At 2 years, the scores declined in both groups but remained above the pre-treatment value with no significant difference between the groups. ➔ Intra-articular PRP injections into the knee for symptomatic early stages of OA are a valid treatment option. There is a significant reduction in pain and improvement in function after 12 months, which can be further improved at 18 months by annual repetition of the treatment.	Not formally assessed.
- In the PRP group, a significant reduction in pain from haemophilic arthropathy of knee was observed for 6 months (F = 17.47, p < 0.01). - WOMAC score was significantly improved for 6 months after injection (F = 4.78, P < 0.01). - Significant improvement in synovial hyperemia was also achieved (F = 4.16, p < 0.01). - The comparative analysis of the two treatments showed no significant intergroup difference. ➔ PRP injections showed longer efficacy than HA injections in reducing pain and improving knee function.	No severe adverse events were reported.
According to the evaluation of IKDC, KOOS and VAS pain score (numerical data are not available): - Both PRP groups reported higher score improvement than HA group at 1 and 6 months, but clinical outcomes were higher when the PRP was prepared with a dedicated medical device (RegenKit®) that PRP prepared with other methods. ➔ PRP is markedly more effective than HA. In particular, RegenLab PRP injections provide significantly better outcomes than home-made PRP.	Not formally assessed.

CARTILAGE					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Intra-articular injections of platelet-rich plasma combined with hyaluronic acid versus hyaluronic acid alone in treatment of knee osteoarthritis Seleem N. A., Elsheree E., Elhosary, A. A. and Salama N. M. *European journal of pharmaceutical and medical research.* 2017,4(4), 608-615.	Prospective Randomized Controlled clinical trial Follow-up: 12 months	Early to moderate knee OA, grade 0-III Kellgren-Lawrence	100	Cellular Matrix A-CP-HA	PRP+HA group (50pts): A-CP HA Kit 4ml of PRP+HA preparation obtained as per instructions for use. Comparative group (50 pts): HA (Sinovial forte 1.6%, 32mg/2ml) Product was injected in the intra-articular knee cavity through lateral approach with a 22-gauge needle. Each patient underwent 3 injection sessions administered with 3 weeks interval.
Effects of platelet-rich plasma on pain and muscle strength in patients with knee osteoarthritis Wu Yung-Tsan, Hsu Kao-Chih *et al.* *Am J Phys Med Rehabil.* 2018 Apr;97(4):248-254.	Prospective Randomized Controlled clinical trial Follow-up: 6 months	Bilateral knee OA, stage I-II Ahlback classification	20 (40 knees)	RegenTHT	PRP group / 20 knees: 10 ml of patient blood drawn into RegenTHT tubes. Single-step centrifugation. 4 ml of PRP were obtained. Control group / 20 knees: Saline injection (the contralateral knee of the same patient) Single intra-articular of 4 ml PRP or saline injection into the intra-articular joint space of the knee 1 cm above the tibial plateau and 1 cm lateral to the patellar tendon towards the femoral notch.
Efficacy and safety profile of a compound composed of platelet-rich plasma and hyaluronic acid in the treatment for knee osteoarthritis (preliminary results) Abate M, Verna S, Schiavone C, Di Gregorio P, Salini V. *European journal of orthopaedic surgery & traumatology.* 2015; 25:1321-6.	Retrospective Comparative clinical experience Follow-up: 6 months	Efficacity and safety of PRP or CM in mild-to-moderate knee OA, grade II–III Kellgren-Lawrence	80	Cellular Matrix A-CP-HA or RegenBCT	PRP+HA group (40 pts): 4 ml of PRP+HA preparation obtained as per instructions for use of A-CP-HA. Retrospective comparative group (40 pts): 4 ml of PRP only, prepared as per instructions for use of BCT. Each patient underwent 3 weekly intra-articular injections by lateral approach, in sterile conditions.

CARTILAGE	
Key results on performance	**Safety / adverse events (AE)**
- At each follow-up visit (2, 6 and 12 months) statistical significant improvement was observed in both groups (PRP+HA and HA only) when compared to basal assessment (both group: $p < 0.0001$). - Infra-group comparison showed that the clinical results obtained in the PRP+HA group were statistically significant superior when compared to the HA control group, at each follow-up visit (2, 6 and 12 months; $p < 0.0001$). ➔ IA injections of PRP-HA showed more efficacy than HA injections in reducing pain and symptoms of mild to moderate knee degenerative osteoarthritis.	Not formally assessed.
- The PRP group showed a significant reduction in the WOMAC-pain and -total scores compared to normal saline group ($p < 0.05$). - Although a significantly greater percentage of knee strength (extensor > flexor) was found in the PRP group during a longer follow-up period, PRP treatment resulted in insignificant differences in muscle strength compared to normal saline. ➔ PRP treatment significantly improves pain, stiffness, and disability in patients with knee OA compared to normal saline treatment.	Neither obvious complications, nor adverse effects related to the injections were observed during the treatment and follow-up period in both groups.
- Both PRP+HA and PRP treated patients showed, at 1, 3, 6 months vs baseline, a statistically significant reduction of VAS pain level a rest and at movement, improvement of function –KOOS, reduction in NSAID consumption. - No significant differences between groups in clinical and functional parameters were observed. - At the end of the study (6 months), 82% of the patients treated with PRP+ HA and 77% of those with PRP expressed their judgment as extremely/very satisfied from the treatment received. ➔ The efficacy of PRP+HA (2 ml of PRP + 2 ml HA) was the same of single PRP but administered in higher volume (4-5ml). We may infer that hyaluronic acid works synergistically and improves the activity of several molecules contained in PRP.	Mild adverse events (pain, heat, redness) were observed after injection in 2/40 PRP+HA and 3/40 PRP group respectively. All events lasted 24–48 hours and did not require the use of any medication. In the majority of the patients these have been considered correlated to the synovial inflammation due to OA at baseline.

CARTILAGE					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Short-term clinical results of intra-articular PRP injections for early osteoarthritis of the knee Huang Po-Hua, Wang Ching-Jen, Chou Wen-Yi, *et al.* *International Journal of Surgery*. 2017, vol. 42, p. 117-122	Retrospective Comparative study Follow-up: 12 months	To assess repeated intra-articular PRP injections for early knee OA, grade I-III Ahlback radiographic classification	127	RegenACR-C / RegenTHT	All patients were treated with PRP. - 64 patients (98 knees) received 1x i.a PRP injection - 18 patients (27 knees) received 2x i.a PRP injections administered with monthly interval - 45 patients (66 knees) received 3x i.a PRP injections administered at monthly interval The skin over the injection site was prepped and draped, and the PRP was injected in sterile condition using a 23G needle through the anterolateral portal approach.
The influence of platelet rich plasma on synovial fluid volumes, protein concentrations, and severity of pain in patients with knee osteoarthritis Chen, C. P., Cheng, C. H., Hsu, C. C., Lin, H. C., Tsai, Y. R., & Chen, J. L. *Experimental Gerontology*. 2017 Jul;93:68-72	Case series Follow-up: 6 months	Mild-moderate knee OA, grade II-III Kellgren-Lawrence with supra-patellar bursitis (caused by accumulation of synovial fluid)	24	RegenACR-C Classic/ RegenTHT	5 ml of PRP for injection obtained after centrifuge 15min at 2900 rpm. Note: RegenTHT tubes were centrifuged for 15min and not as per instruction for use. All procedure was performed under ultrasound-guidance. First, synovial fluid (SF) was aspirated from the supra-patellar bursa, then PRP was administered in the intra-articular knee cavity using the lateral approach. All patients were treated with 3 i.a injections of PRP administered with monthly interval.
Platelet Rich Plasma (PRP) in osteoarthritis Gobbi A, Karnatzikos G *et al.* *Platelet-Rich Plasma Regenerative Medicine: Sports Medicine, Orthopedic, and Recovery of Musculoskeletal Injuries. pages* 231-236. 2014 (Book)	Case series Follow-up: 12 months	Mild to moderate knee OA grade I-III Kellgren-Lawrence. (40 patients underwent previous intervention for cartilage lesion)	80	RegenACR-C Extra / RegenTHT	PRP was prepared as per instruction for use. Topical anesthetic skin refrigerant was applied locally before the injections. All patients received 2 intra-articular infiltrations of PRP within monthly interval by supra-patellar approach.

CARTILAGE	
Key results on performance	**Safety / adverse events (AE)**
Treatment performance was based on pain score, range of motion (ROM), WOMAC questionnaire: - At 12 months post treatment, all three groups had significant improvement when compared to the pre-treatment values ($p < 0.05$). - Amongst the 3 injections group, pain scores were significantly less ($p < 0.001$) and more functional score ($p < 0.004$) and WOMAC scores ($p < 0.001$) when compared to the other two groups at 12 months follow-up. - No improvement was observed according to the ROM score. ➔Three injections at monthly interval appear to show better results in short-term clinical follow-up.	Not formally assessed.
- Knee pain was evaluated with Lequesne Functional Index, being 12.21 ± 2.44 and decreased to 4.33 ± 0.78 at final follow-up. - Significant decreases in volume and concentration of SF protein was observed after the completion of the 2nd PRP injection ($p<0.05$). - Proteins associated to inflammation decreased at least 2-fold in concentrations. ➔ At least two monthly PRP injections may be beneficial for treating patients with minor to moderate knee OA combined with supra-patellar bursitis.	Not formally assessed.
- All patients showed significant improvement in all scores at 12 months and returned to previous activities including sports (KOOS, Tegner, VAS, ADL, QOL, IKDC and MARX scores). - No significant difference between operated and non-operated patients. ➔ PRP could act as a new hope as a preventive agent in patients with chronic and degenerative disease of knee by diminishing pain and improving symptoms and quality of life.	No adverse reactions as infection, swelling, acute pain or any major complications were noted.

CARTILAGE					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Platelet-rich plasma treatment in symptomatic patients with knee osteoarthritis: preliminary results in a group of active patients Gobbi, Alberto *et al.* *Sports Health* 4.2. 2012: 162-172.	Case series Follow-up: 12 months	Mild to moderate knee OA, grade I-III Kellgren-Lawrence	50	Regen ACR-C / RegenTHT	8ml of patient blood drawn into evacuated tube containing 1 ml sodium citrate 4% and a thixotropic gel. Single-step centrifugation. About 4ml of PRP obtained. All patients receive 2 i.a PRP injections into the knee joint, administered with 1-month interval, by suprapatellar approach. Group 1 (25 pts): no previous intervention Group 2 (25 pts): previous operative intervention for cartilage lesion.
Infiltrative treatment with Platelet Rich Plasma (PRP) in gonarthrosis Mangone Giuseppe *et al.* *Clinical Cases in Mineral & Bone Metabolism*. 2014; 11.1	Case series Follow-up: 12 months	Mild to moderate knee OA, grade II-III Kellgren-Lawrence	72	RegenTHT	14ml of patient blood was collected into 2 evacuated tubes containing 1ml sodium citrate and a thixotropic gel selector for separation of blood components. About 3ml of PRP obtained, activated with 0.5-1% calcium gluconate Note: RegenTHT tubes were centrifuge for 12 minutes at 3200rpm. All patients received 3 i.a PRP injection, administered with 3-week interval by antero-medial or antero-lateral approach.
Autologous platelet gel for tissue regeneration in degenerative disorders of the knee Napolitano Marcello, *et al.* *Blood Transfus* 2012; 10.1(72-77).	Pilot study Follow-up: 6 months	-Early to moderate knee OA grade I-II-III (13 pts) -Cartilage degenerative lesions (14 pts)	27	Regen Fibrin Polymer 2/ RegenTHT	8ml of patient blood was collected into an evacuated tube containing 1ml sodium citrate and a thixotropic gel selector for separation of blood components. 5ml of PRP obtained, activated with calcium gluconate 10%. All patients received 3 i.a PRP injections (5 ml) administered with 1-week interval Group 1 (13 pts): knee OA Group 2 (14 pts): cartilage lesions

CARTILAGE	
Key results on performance	**Safety / adverse events (AE)**
- All patients showed significant improvement, respect to baseline, in all clinical scores (VAS pain score, IKDC, KOOS, Marx, Tegner) at 6-month; maintained during the entire follow-up (12-month). - Results did not show any significant difference between patients that had surgery before i.a PRP and patients that were treated with i.a. PRP only. ➔ The PRP treatment showed positive effects in patients with knee OA. Operated and nonoperated patients showed significant improvement by means of diminishing pain and improved symptoms and quality of life.	No adverse reactions (swelling or acute pain) or any major complications (eg, infection) were noted.
-Clinical evaluation based on VAS sore (pain at rest as well as during walking) and WOMAC scale, PRP provided statistical significant improvement in the first month after treatment and clinical improvements were maintained during the entire 12-month follow-up period. - WOMAC Pain and stiffness improved till the 3rd month ($p < 0.05$) - WOMAC Stiffness decrease in a very statistically significant way during the first month ($p < 0.005$). ➔ PRP injections can be considered a valid method in the control of pain, stiffness and joint function.	Not formally assessed.
- All patients (both groups) obtained a clear improvement of the NRS score for the evaluation of subjective pain after PRP injections and until 6 months. - Improvement in OA symptoms at 6-month respect to baseline scores (WOMAC pain, stiffness and function) in both groups. - No comparative evaluation between groups. ➔ This pilot study indicates that PRP treatment was safe and effective, clinical scores showed that all patients statistically significant improved their symptoms.	None of the patients had any adverse effects or allergic reactions.

CARTILAGE					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
PRP Injection for the Painful Hips: The Taiwan Series Pan JL *et al.* *Healthy Aging Research.* 2015 (Poster and abstract)	Case series Follow-up: 6 months	Painful advanced hip arthropathies	37	RegenTHT	Each patient got at 3 intraarticular injections of 4-5 ml of PRP for a full course, two weeks apart. In addition of PRP, several collateral treatments (based on subjective complains and clinical findings) could help optimizing the outcomes: (1) ultrasound-guided hydrodissection of the ipsilateral lateral femoral cutaneous and/or superior cluneal nerves; (2) ultrasound-guided hip intraarticular hyalonate (ARTZ dispo, Japan) injections; (3) intra-fascial injections over ipsilateral iliotibial band and/or gluteal fasciae; (4) ipsilateral hip periarticular entheseal PRP injections.
Etude pilote d'un dispositif médical intra-articulaire innovant dans la prise en charge de la gonarthrose symptomatique fémoro-tibiale grade II-III radiologique après échec d'un AH Renevier JL and Marc JF *Communication Poster / Revue du Rhumatisme.* 2014; 81S, Al 29-A387 (Abstract)	Case series Follow-up: 9 months	Mild to moderate knee OA after HA failure, grade II-III Kellgren-Lawrence	71	Cellular-Matrix	PRP+HA: 4 ml of PRP+HA preparation obtained as per instructions for use (centrifuge 5 min at 3400 rpm). All patients received 3 i.a PRP injection, administered at D0, 2 months and 6 months.
The use of autologous PRP for the treatment of degenerative and inflammatory arthropathy and tendinopathy Stigliano MA, Visin F, Sargentini V, Pizzi T. *61° National Congres A.I.Pa.C.Me.M., Caserta Italy.* 2011 (Abstract)	Case series Follow-up: 6 months	Degenerative arthropathies and acute or chronic tendon disease (resistant to conventional treatment)	38	RegenTHT	PRP was prepared as per instructions for use. All patients were treated with a cycle of 2-5 intra-articular PRP injections (4-5ml) administered with 2-3 weeks of interval.

CARTILAGE	
Key results on performance	**Safety / adverse events (AE)**
➔ The successful rate of PRP (painful improvement >50%) is about 70%.	No severe adverse events were reported. Pain syndrome post injections were reported in less than half of patients.
- 87,3% of patients presented clinical improvement at the end of the study. - Significant decrease in WOMAC score at 9 months (5.93 at D0 to 2.03 at 9 months). - The decrease in WOMAC score was significant between D0 and 2 months and between 2 months and 6 months. ➔ PRP is an efficient non-surgical treatment for the refractory HA knee OA.	No adverse events reported.
- 91% of patients were satisfied with the treatment and the 90% improved the articular function. Results were stable up to the 6th month of follow-up. - X-ray result showed complete disappearance of inflammation, however no improvement was found in the degenerative joint. - Tendon segments were responsive to the PRP infiltrative treatment, including functional recovery. - After the 2nd injection ultrasounds showed healing from the inflammatory process. ➔ The use of the PRP constitutes a valid therapeutic option for the treatment of pain during degenerative arthropathies and inflammatory tendinopathies.	No complication reported, no AE reported.

CARTILAGE					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Use of PRG in joints affected by hemophilic arthropathy: our experience Dominijanni A, Monterosso G, Santoro RC, Brescia A. *Transfusion and Apheresis Science.* 2014; 50-S9–S30. (Abstract)	Case report Follow-up: Until end of treatment	Hemophi-lic arthro-pathy, Chondrom alacia (grade II/III)	1	RegenTHT	PRP was prepared as per instructions for use. The patient was treated with a cycle of three US-guided intra-articular injections of PRP at weekly intervals. The patient was in supine position and the ankle to be treated flexed in neutral position; about 4 ml of PRP was injected at each treatment session.

TENDONS					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Synergistic activity of platelet rich plasma and high-volume image guided injection for patellar tendinopathy. Abate M, Di Carlo L, Verna S, *et al.* *Knee Surg Sports Traumatol Arthrosc.* 2018, Mar 31.	Prospective Randomized Controlled trial Follow-up: 6 months	Patellar tendinopat hy (PT)	54	RegenBCT	The therapeutic procedures were performed in sterile conditions and under US-guidance. <u>Group 1: HVIGI (n=18):</u> high volume image guided injections of saline (30 ml). A 21G needle was inserted immediately at the interface between the tendon/paratenon and the fat pad. <u>Group 2: PRP (n=18):</u> 4 ml of PRP. A 21G needle was inserted into the degenerated tendon and small autologous pure PRP depots were left at the site of most damaged areas, and then proximal and distal. PRP is prepared as per instructions for use. <u>Group 3: HVIGI + PRP (n=18):</u> Both administrations of saline and PRP were performed with the same modalities in an unique session. The procedure in all groups was repeated after 2 weeks.

CARTILAGE	
Key results on performance	**Safety / adverse events (AE)**
- Clinical assessment at the end of treatment showed a clear decrease in pain and an improvement in joint function (Pettersson Score improved from 7 to 3). ➔ This result provides preliminary data on the efficacy of PRP for the treatment of hemophilic arthropathy.	No AE reported. No side effect or allergic reaction were reported.

TENDONS	
Key results on performance	**Safety / adverse events (AE)**
- In the short term both treatments showed comparable efficacy, whereas in the medium term the positive effects of high-volume image-guided injections gradually diminished and platelet rich plasma showed greater efficacy. - Better results (reduced pain, improved function and increased number of subjects who exhibited optimal recovery [> 20 points in VISA-P score]) were observed when both procedures were associated. ➔ The contemporaneous administration of platelet rich plasma and high-volume image guided injections of saline treatments, which influence tendon repair by means of different mechanisms, grants a greater improvement for patellar tendinopathy.	All the treatments were well tolerated, and no significant adverse reactions were registered, with the exception of slight discomfort after injection (pain, swelling) which lasted few hours.

TENDONS					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Conservative treatment for Insertional Achilles Tendinopathy: platelet-rich plasma and focused shock waves. A retrospective study. Erroi D, Sigona M, Suarez T, *et al.* *Muscles Ligaments Tendons J.* 2017, May 10;7(1):98-106.	Retrospective Controlled study Follow-up: 6 months	Chronic insertional tendinopathy (IAT)	45	RegenBCT	Group 1 (n=24) control group: Patients received three sessions of extracorporeal shock-wave therapy (ESWT) at weekly interval. Each session consisted of 2400 impulses administered with an energy flux density ranged from 0.17 to 0.25 mJ/mm2 using a focused electromagnetic shock wave device (ModulithR SLK) Group 2 (n=21) PRP group: patients received two autologous pure PRP injections (2 ml of PRP after removed PPP) over two weeks (1 injection per week for two weeks) under ultrasound guidance. PRP was prepared as per instructions for use.
Are Platelet Rich Plasma Injections More Effective in Tendinopathy or Enthesopathy? Papalia R, Zampogna B, Vadala G, *et al.* *J Pain Relief*. 2017, 6:3	Retrospective study Follow-up: 3 years	Tendinopathy (insertional or not)	139	RegenTHT	PRP was prepared as per instructions for use from 8 ml of whole blood. Group A (n=31): insertional tendinopathy Group B (n=108): non-insertional tendinopathy. Specifically, once the site of pain had been identified at ultrasound, the skin was scrubbed and a needle was introduced perpendicularly to the axis of the probe, parallel to the tendon, along its side, within the paratenon. Taking care not to enter the body of the tendon, PRP was injected. Injections to rotator cuff tendons were performed through the sub acromial space with the forearm in internal rotation, and the needle perpendicular to rotator cuff tendons.
Platelet Rich Plasma Therapy in Non-insertional Achilles Tendinopathy: The Efficacy is Reduced in 60-years Old People Compared to Young and Middle-Age Individuals Salini V, Vanni D, Pantalone A, Abate M. *Frontiers in aging neuroscience* 2015; 7:228.	Retrospective study Follow-up: 12 months	Recalcitrant non-insertional Achilles tendinopathy	44 (29 young age 39.5 ± 6.9 and 15 elderly age 61.5 ± 5.3)	RegenBCT	PRP was prepared as per instructions for use from 8ml of whole blood. All patients were treated with injections of 4 ml of PRP at several sites into the degenerate tendon areas, once a week for 3 weeks. Injections were performed in sterile conditions, under ultrasound guidance and with a 21G-needle. No use of local anaesthesia. *Note:* All treated patients had failed to respond to conservative treatments.

TENDONS	
Key results on performance	**Safety / adverse events (AE)**
- Intra-group analysis showed a significant improvement of VISA-A and VAS scores in both groups at all time-points. No differences between groups were observed for VAS and VISA-A scores at all time-points, excepted for VISA-A at 4-months in favor of ESWT group (P=0.049). - Patient satisfaction increased progressively (>70% at 6 months), without differences between two groups. ➔ Both ESWT and PRP injections are effective, safe and comparable in the treatment of insertional Achilles tendinopathy in physically active people.	No clinically relevant side effects were detected in both groups. 5 patients in the PRP group complained local pain and discomfort soon after injection and gradually disappeared. In the ESWT group, transient reddening of the skin occurred after treatment, but no bruising was seen.
- Significantly lower values between pre-treatment and follow-up pain scores associated to a significant improvement in recorded segmental scores at all-time points were found in the patients affected by entesopathy compared to tendinopathy ($p<0.001$). - Conversely, the treatment was not effective in patients with pure tendinopathy at the shoulder and ankle level. ➔ This is a pilot study comparing outcomes after injection of PRP for management of insertional tendinopathy and tendinopathies of the main body of the tendon, with better results in patients with insertional tendinopathy.	No serious AE were reported.
- Throughout the whole length of the study, a significant increase of VISA-A[1] score was seen in both groups (from 50.3 to 76.1 in the young group, and from 48.7 to 61.1 in the elderly group). - Infra-groups comparison showed better results in young patients, compared to the aged counterpart. - At 12 months, 18/25 (72%) young and 6/12 (50%) elderly patients were very/extremely satisfied from the treatment. ➔ PRP treatment provides satisfactory results in subjects with Achilles recalcitrant non-insertional tendinopathy reducing pain and improving function. PRP is less effective in aged people. 1 VISA-A : Victorian Institute of Sports Assessment- Achilles questionnaire	No complications related to the injections or severe adverse events were observed during the treatment and follow-up period.

TENDONS					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Infiltration of Autologous Growth Factors in Chronic Tendinopathies Crescibene A, Napolitano M, Sbano R, Costabile E, Almolla H. *Journal of blood transfusion* 2015; 2015:924380	Case series Follow-up: 2 years	Achilles' tendon (14 pts.) Patellar tendinopathy (7 pts.)	21	Regen Fibrin Polymer 2 / RegenTHT	PRP was prepared as per instructions for use from 8ml of whole blood. PRP was activated with 10% CaGlu. All patient received local anesthesia with 2ml of 2 % lidocaine and were treated with 3 US-guided tendinous infiltrations of PRP, weekly given, with a 22G needle. Half of the PRP was injected into the tendon and the rest into the peritendinous area. *Note:* All treated patients had previously undergone other treatments such as intake of NSAIDs, rehabilitation program, laser therapy, corticosteroids injections, with unsatisfactory outcome.
Twenty-two elbows tendinites treated with platelets rich plasma after failure with the usual treatment Le Coz J. *Journal de Traumatologie du Sport* 2011; 28:83-9	Case series Follow-up: Between 9-22 months	Epicondylitis (19 pt.) and chronic tricipital tendinitis (3 pt.)	22	RegenTHT	PRP was prepared as per instructions for use from 8 ml of whole blood. 2 ml of upper plasma were removed before resuspending platelets. PRP treatment was applied in the tendon, without local anaesthesia, using point-by-point and nappage technique with a 6mm x 27G needle: 10 pt. received 1 injection of PRP 9 pt. received 2 injections of PRP 3 pt. received 3 injections of PRP
Case Report: Neotendon regeneration and repair of gluteus tendon tear at 1- year follow-up after ultrasound guided platelet rich plasma tenotomy Doss AX. *F1000 Research* 2014; 3.	Case report Follow-up: 12 months	Gluteal Tendinopathy	1	Regen BCT	Percutaneous tenotomy of minimus tendon was performed into the foot print and the adjacent gluteal cuff under real time imaging guidance and 4–5 ml of autologous PRP was infiltrated through a 22 G. Repeated percutaneous tenotomy with PRP of the medius tendon was performed in a similar manner 12 days later.

TENDONS	
Key results on performance	**Safety / adverse events (AE)**
- PRP treatment was effective in numerous tendon pathologies: all patients gained complete functional recovery. - Ultrasound scans showed visible reduction in tissue irregularity in 86% of infiltrated tendons. The result is confirmed by complete functional recovery of the patients, with painful symptomatology disappearing. - The patients showed a clear pain reduction, along with an enhanced VISA[2] score after the 24-month follow-up, equal to 84.2 points on a scale of 0 to 100. ➔ The present clinical experience provides evidence to suggest that PRP infiltration is a valid option to patients with chronic tendinopathy who did not benefit from other treatments. 2 VISA : Victorian Institute of Sports Assessment questionnaire	A good tolerance to the use of PRP was observed, with the absence of any adverse effects related to such therapy.
- All patients included in this study were not responding to corticoids treatment and had previously undergone other treatments with unsatisfactory outcome. - All patients benefited from the treatment and the 86% were satisfied or highly satisfied with the PRP therapy. - There was no evidence of relapse but continuous improvements during follow-up period. ➔ The use of PRP for tendinitis can be considered very encouraging because of the simplicicty of treatment and the long-term benefits.	No adverse events were reported apart temporary local pain during or after PRP injection.
- Symptoms improved within 4 weeks. - At 6 months post treatment, the patient could walk without limitation. - At 12 months daily pain had resolved, with moderate pain only on ascending stairs. There was minimal greater trochanteric tenderness and no limitation of hip abduction. - Ultrasound of the gluteal tendons revealed that neotendon tissue had replaced the degenerative tendinotic tissue with obliteration of the previously known tear defect. ➔ In this patient, percutaneous tendon repair under imaging guidance with autologous PRP tenotomy resulted in neotissue tendon regeneration of a gluteal tendon tear.	Not formally assessed.

TENDONS					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Neotendon infilling of a full thickness rotator cuff foot print tear following ultrasound guided liquid platelet rich plasma injection and percutaneous tenotomy: favourable outcome up to one year Doss AX. *F1000Research* 2013, Jan 24;2:23	Case report Follow-up: 12 months	Bilateral shoulder pain due to tendon tear, patient not responding to corticosteroid injection	1	RegenBCT	PRP was prepared as per instructions for use from 8ml of whole blood. Injection was performed under direct ultrasound (US) guidance. 5ml 1% lignocaine was injected into the superficial soft tissues, subacromial bursa and the supraspinatus tear as local anesthetic. Then, about 4-5 ml of PRP was injected with a 22G-mneedle into the tear and its margins with simultaneous percutaneous tenotomy directed into the footprint of the anterior facet of the greater tuberosity.
Glenoid Labral Tear: follow up case series on ultrasound guided autologous platelet rich plasma in conjunction with a progressive rehabilitation program Vander Kraats R, Doss AX. *F1000Research* 2012; 1.	Case reports Follow-up: -Case 1: 18 months -Case 2: 13 months	Glenoid labral tear, deep ache of the shoulder joint	2	RegenBCT	-Case 1: Under US-guidance, 5 ml of 0.5% bupivacaine was injected into the paraglenoid soft tissues, followed by 8ml of PRP into the glenohumeral synovial cavity near the posterior inferior quadrant of the torn glenoid labrum. Also, 2ml of PRP were injected into the acromioclavicular joint. 2 weeks after, the patient started physiotherapy program. -Case 2: 5ml of 1% lignocaine and 6ml of PRP (products were mixed) were injected under direct US-guidance into the glenohumeral synovial cavity through the posterior glenohumeral recess. Two weeks after the patient started exercise program.

TENDONS	
Key results on performance	**Safety / adverse events (AE)**
- Marked reduction in pain with improvement in shoulder movement at 8 weeks. - At the 7 and 10 months follow up, US imaging showed neotendon infilling in place of the tear defect. The lateral tear margin retracted with mild interval attrition of the supraspinatus tendon. - At 12 months, the patient returned to a completely pain free state, along with full range of movement with no dysfunction. ➔ This result support first evidence that PRP may be an alternative treatment option in some cases of full thickness rotator cuff tears.	Not formally assessed.
-Case 1: 2-week post PRP treatment, VAS pain score showed 85% improvement (from 7/10 to 0/10) along with functional recovery and pain free sports activities. 4-week post PRP treatment considerable improvement in pain and function of the shoulder. Final follow-up at 18 months, the patient reported complete resolution of all symptoms and returned to sports engagements. -Case 2: immediately resolution of shoulder pain symptoms post PRP injection. 2-week after the PRP injection VAS pain score was improved of 65% (from 6/10 to 0/10), and the patient started overhead activities and rehabilitation program. 6-week post PRP overhead activities could be performed with minimal pain. Final follow-up at 13-month, all symptoms had resolved with adequate functional level to compete in high grade sports activities. ➔ This report offers evidence that PRP with appropriate rehabilitation is a viable, low risk alternative to corticosteroid injections or surgical procedures in glenoid labral tears.	No AE was reported, and injections were well tolerated by both patients.

MUSCLES					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Single dose of intra-muscular platelet rich plasma reverses the increase in plasma iron levels in exercise-induced muscle damage: A pilot study Punduk Z, Oral O, Ozkayin N, Rahman K, Varol R. *Journal of Sport and Health Science* 2016; 5:109-14.	Prospective Randomized Controlled pilot study Follow-up: 4 days post exercise	Exercise-induced muscle damage	12, male healthy volunteers	RegenACR-C / RegenBCT	Prior treatment administration (24-h), all patients underwent delayed onset muscle soreness exercise. Patients were randomized in 2 groups: Group 1 / PRP group (n=6): 4 ml of PRP was prepared from 8 ml of whole blood after a centrifugation 9 min at 3500 rpm. Note: the centrifugation was not performed as per instructions for use. Group 2 /control group (n=6): 4 mL of saline solution. All injections were performed using a 20G needle into the painful region of the non-dominant arm under sterile aseptic conditions.
Exercise induced muscle damage may be improved by a single dose of intra-muscular platelet rich plasma: a pilot study Punduk Z, Oral O, Aydinoglu R, Ozkayin N. *Central European Journal of Sport Sciences and Medicine* 2014; 8:19-26	Pilot study Follow-up: 5 days post exercise	Exercise-induced muscle damage	6, male healthy volunteers	RegenACR-C / RegenBCT	4 ml of PRP was prepared from 8 ml of whole blood after a centrifugation 9 min at 3500 rpm. Note: the centrifugation was not performed as per instructions for use. Participants non-dominant arms were treated post-24 hours delayed onset muscle soreness exercise. 4ml of PRP was injected using a 20G needle into the region of the biceps brachii of the non-dominant arm under sterile aseptic conditions. The contralateral arm did not receive any treatment (control arm).

MUSCLES	
Key results on performance	**Safety / adverse events (AE)**
- PRP administration resulted in improved muscle recovery from injury by reversing the observed increase in *Fe* levels. - *Fe* levels decreased during the 1-4 days postexercise muscle damage (p<0.01). - Intramuscular PRP injection had no effect on creatinine kinase levels, which means that there is no myotoxic effect. ➔ PRP administration improves inflammation by reversing the increase in the iron levels post-exercise without displaying any myotoxicity and may have a role to play in the recovery of exercise-induced muscle damage.	Not formally assessed. However, results showed that intramuscular PRP injection is not myotoxic.

- The control arm (no PRP injection) displayed significantly higher VAS pain scores and greater muscle soreness compared to the PRP arm between days 2–5, difference was found statistically significant (p<0.05). - Flexor peak torque values were higher in PRP administered arm compared to the control arm. ➔ Intramuscular administration of PRP may improve muscle strength recovery and also, it may have a role to play in accelerating exercise induced muscle damage recovery.	Not formally assessed.

4.1.2 ORTHOPAEDIC SURGERY

12 publications were identified including clinical data on 600 patients.
Among them, RegenKits have been used in addition to surgery for 2 different subtypes of indications:

2.A. Bone reconstruction (8 publications)
RegenKits have been used on 285 patients during orthopaedic surgery for the treatment of bone nonunion, osteonecrosis of the femur, bone lesions, osteochondral lesions, large cavity bone defects of pseudo-tumoral origin and also during arthroscopic repair.

2.B. Ligaments, tendons and cartilage reconstruction (4 publications)
RegenKits have been used on 159 patients during surgery for the treatment of tendons injury, cartilage lesions, and postero-superior rotator cuff tear ligament lesions.

BONE RECONSTRUCTION					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Polytherapy with bone marrow concentrate, "scaffold" and autologous growth factors to promote healing in bone lesions pseudotumoral. De Biase P, Pecchioli O, Campanacci DA, *et al.* *Arch Ortop Reumatol* 2013; 124:27-9.	Clinical experience 12-year experience Follow-up: 1 year - 2 years	Large cavity bone defects of pseudo-tumoral origin	103 (131 surgeries)	RegenKit Extracell BMC / RegenTHT	All patients were treated with a bone substitute, combined with autologous bone marrow concentrate (BMC). To support bone regeneration, platelet-rich plasma (PRP), in addition to demineralized bone matrix (DBM), autograft, allograft or synthetic scaffold, were added.
Ten-Year Experience Of Non Unions Treated With Autologous Bone Marrow Concentrate And Osteogenic Protein-1 De Biase P, Biancalani E, Capanna R, Pecchioli O. *EFORT congress.* Geneva; 2016: #1341 – Free Papers. (Abstract)	Case series 12-year experience, no information about each patient follow–up	Bone nonunions: -26 tibia -25 femur -9 humerus -4 ulna -3 radius -1 clavicle -1 ankle -1 astragalus	70 (79 cases)	Regen Extracell BMC / RegenTHT	Patients history: all treated patients had failure of a previous autologous graft treatment for nonunion (range 2-6 treatment). All patients were treated with - allograft or enriched with autologous bone marrow concentrate (BMAC), - Osteogenic Protein 1 (OP-1) - or a combination of both (more recalcitrant nonunion).

Safety: Generally, no intraoperative or postoperative complications occurred. Among all treated patients, it has been reported only one case who developed infection of the site and required further intervention.
Performance:
Bone: Performance data showed that Bone marrow concentrate (BMC) treatment was shown to have 80% of clinical success and healing.
Ligaments, tendons and cartilage: Performance data showed that PRP was effective, treated patients presented better clinical outcomes and functional improvement.

Safety and performance clinical data of bone marrow concentrate (BMC) or PRP prepared with RegenKits, suggest that autologous BMC preparation or PRP represent valid autologous therapies to be used during orthopaedic surgery for bone reconstruction and ligament, tendon and cartilage reconstruction.

The key results described in publications are summarized in the following table:

BONE RECONSTRUCTION	
Key results on performance	**Safety / adverse events (AE)**
- Healing was achieved without recurrence of the lesion in 78.76% of patients. - DBM appeared to be ineffective since the healing rates did not differ in its absence or presence in the graft (72% vs 67%, respectively). On the contrary, PRP showed a positive effect and 87% of the cases in which it was used healed successfully. ➔ These positive results showed that autologous PRP and BMC are effective and represent a valid alternative to traditional tissue engineering techniques for the treatment of bone defects.	Not formally assessed.
- In the cases where OP-1 was used in the absence of BMAC a healing rate of 91% was observed, whereas if OP-1 was used in combination with BMAC, the success rate decreased to 62.5%. The results are related to the severity of the lesions, for more recalcitrant non-unions with ≥ 3 previous surgery where treated with a combination of all growth factors. - 20% of patients were treated by percutaneous infiltration of BMAC without other factors and, in these cases, 83% of successful healing was observed. - Patients treated with open surgery healed in 79% of cases. ➔ Based on more than 10 years' experience, in 79 cases, of using a graft enriched with BMAC and/or OP-1, the surgical technique has proven to be safe for the patient, non-invasive and effective.	Not formally assessed.

BONE RECONSTRUCTION					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Scaffolds combined with stem cells and growth factors in healing of pseudotumoral lesions of bone De Biase P, Campanacci DA, Beltrami G, *et al.* *Int J Immunopathol Pharmacol* 2011; 24:11-5	Clinical experience Follow-up: 2 years (up to 5 years for benign tumor cases)	Large cavity bone defects of pseudo-tumoral origin	63 (76 surgeries)	RegenKit Extracell BMC / RegenTHT	Group 1 = 17 patients: treated with percutaneous infiltration of autologous bone marrow concentrate (BMC). Starting from 2005, demineralized bone matrix was used in association to autologous BMC. 1 or 2 percutaneous infiltration of autologous BMC were administered to achieve complete healing. Group 2 = 46 patients: underwent open surgery.
The use of autologous bone marrow concentrate for the treatment of pseudoarthrosis: literature review and case series. Maccari L. *Thesis Università Di Pisa;* 2013. Original article in Italian	Retrospective observational study Follow-up: 10 months (average)	Pseudo-arthrosis or bone nonunion: -12 femur -10 tibia -2 fibula (calf bone) -4 humerus -3 ulna	31	RegenKit Extracell BMC / RegenTHT	21 patients were treated with RegenKit Extracell BMC. Bone marrow cell concentrate was mix with 10ml of bone matrix and inserted in the lesions. This product was use during the surgical procedure. External fixator or plaque was also used for the surgical procedure.
The use of an injectable calcium sulphate/calcium phosphate bioceramic in the treatment of osteonecrosis of the femoral head Civinini R, De Biase P, Carulli C, *et al.* *Int Orthop* 2012; 36:1583-8.	Case series Follow-up: 20 months (average)	Osteonecrosis of the femoral head (early stage)	31 (37 hips)	RegenKit Extracell BMC / RegenTHT	Standard debridement procedure, removal of large volume of necrotic lesions using a Percutaneous Expandable Reamer. 40 ml of bone marrow was obtained from the anterior iliac crest with a 10-mL syringe. After separation with 2 centrifugations, 15-20 ml of bone marrow concentrate (BMC) were obtained and injected into the head. After, the necrotic area was filled with $CaSO_4/CaPO_4$ injectable composite and let it harden *in vivo* (approx. 20-30 min).

BONE RECONSTRUCTION	
Key results on performance	**Safety / adverse events (AE)**
- Group 1: 8/17 patients were considered clinically and radiographically healed with obliteration of the cyst (5 with a single percutaneous infiltration of BMC and 3 needed a second infiltration for complete healing). 9/17 did not heal and received a conventional open surgical technique and healed subsequently. - Group 2: 41/46 patients healed after the treatment while 5 patients did not heal and required further surgery. - Medium time of healing = 6 months in group 1 and 3 to 6 months in group 2. - DBM appeared to be ineffective since the healing rates. ➔ These positive results showed that autologous BMC is effective and represents a valid alternative to traditional tissue engineering techniques for the treatment of bone defects.	Not formally assessed.
- Radiographic healing was achieved in 66.7% of cases, healing time post-surgery was in between 3-10 months. ➔ The use of bone marrow concentrate represents a valid support for surgical treatment.	No complications have been reported regarding the use of Regenkit Extracell BMC.
- All patients statistically significant improved Harry Hip score (HHS) (mean of 68 to 86 at the end of follow-up; $p<0.05$) compared to pre-treatment. - 29/37 hips improved initial stage of the pathology without further collapse (86% clinical success). - After clinical failure, 3 patients underwent further total hip replacement. ➔ This treatment seems to relieve hip pain and prevent the progression of osteonecrosis of the femoral head in the majority of the cases.	No complications related to the procedure were reported during or after the surgical procedure.

BONE RECONSTRUCTION					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Regenerative Medicine In The Treatment Of Osteonecrosis Of The Hip: A Comparative Clinical Study On The Use Of Autologous Cell Concentrates In The Presence Or Absence Of BMP De Biase P, Biancalani E, Capanna R, Campanacci D, Pecchioli O. *EFORT Congress.* Geneva; 2016: #1343 - Free Papers. (Abstract)	Case series 5-year experience and follow-up	Osteonecrosis of the proximal femur (stage II, ARCO[3] classification)	23 (28 hips)	Regen Extracell BMC / RegenTHT	Core decompression was performed with X-Reamer (tunnel of 9mm in diameter) in the femoral neck up to the necrotic area, which was removed using the incorporated expandable milling cutter. The void was then filled with a combination of BMAC and an injectable allograft, with or without the addition of Osteogenic-Protein 1 (OP-1). The femoral neck area of the bone tunnel was then sealed with a calcium phosphate bone substitute. -11 hips: treated with BMAC + allograft + OP-1 -16 hips: treated with BMAC + allograft -Bilateral patients: only one vial of OP-1 on the contralateral side.
Long bone nonunions treated with autologous concentrated bone marrow-derived cells combined with dried bone allograft. Scaglione M, Fabbri L, Dell'Omo D, Gambini F, Guido G. *Musculoskeletal surgery* 2014; 98:101-6.	Case series Preliminary study Follow-up: 7 months (average)	Bone nonunion	19	RegenKit Extracell BMC / RegenTHT	Autologous bone marrow-derived cells were harvested from the iliac crest and prepared as per instructions for use. All patients underwent surgery, at the final step bone matrix preparation with bone marrow concentrate + 10 cc of demineralized bone was inserted to promote osteosynthesis.

3 ARCO = Association Research Clinical Osseous

Surgical treatment of neglected hip fracture in children with cerebral palsy: case report and review of the literature. Toro G, Moretti A, *et al.* *Clinical Cases in Mineral and Bone Metabolism* 2017; 14(3):317-323	Case report Follow-up: 12 months	Femoral neck fracture in hemiplegic cerebral palsy child	1 (child)	RegenKit Extracell BMC / RegenTHT	A capsulotomy with postero-lateral flap preservation was performed to obtain a freshening fracture surfaces with a direct reduction. 3 cannulated screws were inserted with a transverse one in the calcar. Autologous bone marrow stem cell concentrate, obtained by an iliac crest aspiration needle, was prepared using RegenKit as per instructions for use. The stem cells concentrate was then applied in hip fracture gap as a gelled membrane. Finally, a cancellous bone auto-graft from the greater trochanter was used to fill the fracture gap.

BONE RECONSTRUCTION	
Key results on performance	**Safety / adverse events (AE)**
- At 5 years, resolution of necrotic areas was observed in 79% of cases overall. - Functional parameters of the treated hips (range of motion and strength) did not change significantly due to early stages of the necrosis. - A healing rate of 82% was observed in cases treated with BMAC, allograft and OP-1, which was not statistically significantly different from the results observed when using BMAC and allograft alone (79% healing). - Negative outcomes (bilateral patients, regards the contralateral side treated with OP-1 only) were related to the lack of a mineralized scaffold; suggesting that the collagen scaffold of OP-1 alone is not sufficient. ➔ We strongly recommend BMAC as a safe and effective preparation when combined with a mineralized scaffold, when a curettage of the necrotic lesion is performed, thereby leaving a void which is larger than just the reaming hole.	Not formally assessed.
- Radiographic clinical evaluation showed that 15/19 patients achieved complete healing within 6.2 m in average, with complete remission of clinical symptoms. The last 4 patients did not show radiographic improvement but only resolution of symptoms. - Overall, 18/19 patients benefited from the treatment. ➔ The use of growth factors and autologous mesenchymal stem cells through the enforcement of system for tissue regeneration is a valid and innovative biotechnology technique for the treatment long bone non-unions.	A single case developed infection of the site and required further intervention.
- 6 months after surgery: a complete fracture healing and no signs of osteonecrosis. At 12 months after surgery, the patient was able to walk without pain and aids. Hip flexion contracture was completely resolved, whereas a slight equinus (<10°) and a lower limb discrepancy of 0.5 cm were observed. - The X-ray and MRI evaluations confirmed the bone healing and the absence of osteonecrosis. ➔ The use of cannulated screws with cancellous bone graft and MSCs is a viable treatment option in cerebral palsy patients.	Not formally assessed.

TENDON, LIGAMENT, CARTILAGE RECONSTRUCTION					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Use of Platelet-Leukocyte Membrane in Arthroscopic Repair of Large Rotator Cuff Tears A Prospective Randomized Study Gumina S, Campagna V, Ferrazza G, *et al.* *J Bone Joint Surg Am* 2012; 94:1345-52	Prospective Randomized Controlled Study Level I Follow-up: 13 months	Large full-thickness postero-superior rotator cuff tear	80	RegenTHT	All tears were repaired using an arthroscopic single-row technique. Subacromial decompression was performed in all patients. <u>PRP membrane group (40 pts):</u> PRP was mixed with CaGlu and batroxobin. This preparation was centrifuged 20-30 min at 1500 g to obtain a round membrane: Ø = 13 mm, 3-4 mm thick. The membrane was positioned between the abraded footprint area and the lateral edge of the rotator cuff so that it could not be displaced or moved during the successive phases of the rotator cuff suturing. <u>Note:</u> RegenTHT tube was centrifuged at 120g for 10 min and then at 1500g for 30 min to prepare the autologous membrane. <u>Control group (40 pts):</u> only debridement.
A comparison of clinical results between microfracture technique with the use of PRF intra-operative injection, PRP post-operative injection and microfracture only for osteochondral lesion of the knee Papalia R, Vadala G, Franceschi F*, et al.* *Bone & Joint Journal Orthopaedic Proceedings Supplement.* 2014; 96-B:124-	Randomized Controlled Clinical experience Follow-up: 12 months	Osteochondral lesion of the medial or lateral compartment of the knee	90	RegenTHT	All patients received arthroscopic debridement and Microfractures and were randomised into 3 groups: <u>Group A: PRF (30 pts):</u> Received microfractures and intraoperative PRF injection (Vivostat) <u>Group B: PRP (30 pts):</u> received microfracture and 3 i.a injections of 5.5 mL PRP (Regen Lab) <u>Group C: control (30 pts):</u> received microfracture only.

TENDON, LIGAMENT, CARTILAGE RECONSTRUCTION	
Key results on performance	**Safety / adverse events (AE)**
- The use of the platelet membrane improved repair of rotator cuff integrity compared to the control group (p=0.04). - However, functional improvements were similar in both groups. - Rotator cuff retears were observed only in the group of patients in whom the membrane had not been used. ➔ In conclusion, the use of platelet-leukocyte membrane led to a slight improvement, as assessed by MRI, in the repair integrity of large tears involving the supraspinatus tendon, although this improvement was not associated with a better objective functional outcome.	Not formally assessed.

Clinical assessment was based on IKDC, KOOS and VAS score: - Patients who underwent post-operative PRP i.a injections (Group B) reported significant improvement in clinical scores, than those undergoing arthroscopic microfracture alone (P<0.005) of Group C; but lesser than intraoperative PRF group at 6 months and 1 year follow up. ➔ This result suggests that PRP injections as adjunct to microfracture may offer better clinical outcomes over microfracture alone.	Not formally assessed.

TENDON, LIGAMENT, CARTILAGE RECONSTRUCTION					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Treatment of Tendon Injuries of the Lower Limb with Growth Factors Associated with Autologous Fibrin Scaffold or Collagenous Scaffold Giannotti S, Dell'Osso G, Bottai V, *et al.* *Surg Technol Int* 2015; 26:324-8	Case series Follow-up: 13 months	Tendon injury	9	RegenKit Extracell Membrane /RegenTHT	The surgical technique has provided, as appropriate, the terminoterminal tenorraphy, techniques of plastics of rotation flap, reinsertion with suture anchors, and in one case tendon augmentation with cadaver tissue. Procedure customized according to each patient need as: infiltration of PRP or insertion of a fibrin matrix preloaded with PRP or insertion of a collagen patch soaked with PRP. (single treatment)
Our clinical experience with ACT and AMIC. [Unsere klinische Erfahrung mit ACT und AMIC] Bachelin P. *Orthopädie & Rheumatologie* 2011; 1:16-8.	Case series Follow-up: Up to 5 years (30 patients)	Osteochondral lesions	80	RegenTHT	PRP was used in addition to autologous chondrocytes transplantation (ACT, 48 patients.) and autologous matrix induced chondrogenesis (AMIC, PRP soaked in the matrix for 5-10 min and then implanted, 32 patients.)

TENDON, LIGAMENT, CARTILAGE RECONSTRUCTION	
Key results on performance	**Safety / adverse events (AE)**
- Clinical and functional recovery was observed in all cases. All patients were able to perform activities of daily living. - No cases of re-rupture, no cases of intolerance to the implanted materials. - Ultrasound and MRI scans showed also good tendon structure. ➔ Biological support has become crucial in cases of repair of tendon injuries that are degenerative lesions.	No intraoperative or postoperative complications occurred during the study period.
5 years follow-up results on 30 patients showed that: - 25 patients: good / very good results and were satisfied with the treatment received. - 4 patients: satisfied but sport activities were reduced. - 1 patient was satisfied but did not practice a sport activity. ➔ These new regenerative treatments provide a real alternative to the prosthetic joint set, especially for cartilage damage to younger patients seeking joint-preserving defect reconstruction, and also to reducing costs.	Not formally assessed.

4.1.3 SPINE AND NERVE SURGERY

8 publications were identified including clinical data on 110 patients.

Among them, RegenKits have been used in addition to surgery on 80 patients for the treatment of vertebral fusion, carpal tunnel syndrome, trigeminal neuralgia or generally used during neural surgery.

SPINE AND NERVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Six-month efficacy of platelet-rich plasma for carpal tunnel syndrome: A prospective randomized, single-blind controlled trial Wu, Y. T., Ho, T. Y., Chou, Y. C., *et al.* *Scientific Report.* 2017 Dec;7(1):94.	Prospective, Randomized, Controlled study Follow-up: 6 months	Carpal tunnel syndrome	60	RegenTHT	PRP was prepared from 10ml of whole blood and centrifugation into Regen device (3400 rpm, 15 min). -Group PRP (30 pts.): 3ml of PRP were injected under US-guidance using the in-plane ulnar approach. The ulnar artery was identified using Doppler imaging, and a 25-gauge needle was passed from the ulnar side of the wrist. 2 mL of PRP was injected to peel the nerve off the flexor retinaculum via hydrodissection; 1 mL of PRP was delivered to the inferior part of the median nerve (MN) and the MN was peeled from the underlying subsynovial connective tissue. -Group control (30 pts): received a night splint.
The use of platelet gel in postero-lateral fusion: preliminary results in a series of 14 cases Landi A, Tarantino R, Marotta N, *et al.* *Eur Spine J* 2011; 20 Suppl 1:S61-7.	Case series Comparative study Follow-up: 6 months	Vertebral fixation or postero-lateral fusion	14	RegenTHT	PRP was obtained from 16 ml of whole blood and was activated with calcium gluconate and ethanol 95% (PRP gel). Right side of the lesion (PRP gel treatment): the platelet gel is combined with fragments of autologous bone fashioned and to synthetic bone during surgical operation. Left side: control

4 BCTQ = Boston Carpal Tunnel Syndrome Questionnaire (s = severity and f = function);
FP = Finger pinch; SNCV = Sensory nerve conduction velocity; DML = Distal motor latency; CSA = Crosssectional area; Pre = Pretreatment.

Safety: No signs of peripheral nerve damage or nerve trauma was observed during the procedure and post-treatment period. No serious AE was reported.
Performance: Performance data showed that PRP treatment was effective, treated patients could benefit mainly of pain symptoms relief and improvement in mobility.

These results suggest that PRP may be a safe and effective treatment for medical indications such as vertebral fusion, carpal tunnel syndrome, trigeminal neuralgia; or generally used during neural surgery.

The key results described in publications are summarized in the following table:

SPINE AND NERVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
- With respect to pre-treatment, a significant improvement in all clinical scores (VAS pain score, BCTQs, FP, SNCV, DML, CVA)[4] measured was observed in the PRP and control groups at all follow-up assessments ($p < 0.05$). - There was significantly greater improvement in the PRP group at all follow-up time points in the VAS scores, BCTQ scores, and CSA of the median nerve (not for FP and SNCV); this trend became more pronounced along with follow-up duration. ➔ The study demonstrates that PRP is a safe modality that effectively relieves pain and improves disability in the patients with CTS. The efficacy of PRP for CTS seems to be a potentially worthwhile area for further study in peripheral neuropathy.	No AE reported. No side-effects or nerve trauma were observed.
- Complete bone fusion was achieved in all patients within 6 months. PRP treated side showed fasted bone deposition, especially during the first 3 months after treatment: <u>Bone density:</u> 3months: 345 HU vs 558 HU (PRP) 6months: 634 HU vs 760 HU (PRP) ➔ The autologous platelet preparation used seems to accelerate bony deposition and to promote tissue healing, increasing bone density at the level of posterior-lateral arthrodesis. Moreover, this preparation has low production costs and is easy to apply.	Not formally assessed.

SPINE AND NERVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Posterolateral arthrodesis in lumbar spine surgery using autologous platelet-rich plasma and cancellous bone substitute: an osteoinductive and osteoconductive effect Tarantino R, Donnarumma P, *et al.* *Global spine journal* 2014; 4:137-42	Prospective Comparative study Follow-up: 12 months	Surgery for: lumbar stenosis (15 pts) or lumbar body fractures (5 pts) / degenerative or traumatic origins	20	RegenTHT	PRP was prepared as per instructions for use. PRP was activated with 2ml of calcium gluconate. Right hemifield (PRP treatment): Implantation of cancellous bone substitute (25x50x35 mm) soaked with PRP obtained directly in the operating theater. Left hemifield (control): Implantation of cancellous bone substitute soaked with saline solution.
Autologous bone marrow concentrate combined with platelet-rich plasma enhance bone allograft potential to induce spinal fusion. Vadalà G, Di Martino A, Russo F, *et al.* *J biol regul homeost agents.* 2016 oct-dec; 30(4 suppl 1):165-172.	Prospective Comparative study Follow-up: 12 months	Isthmic or degenerative spondylolisthesis, or stenosis involving the lumbar spine	12	RegenTHT/ Regen ATS	→ Autologous BMC preparation: from 60 ml of BM aspirate from the posterior iliac crest with multiple punctures and centrifugation. → PRP and thrombin: from 16 ml of whole blood and centrifugation 20 min at 1500g. ATS from 8 ml oh whole blood. → PRF: 6 ml from120 ml by Vivostat system. During surgery in aseptic conditions, autologous PRP (7mL) was mixed with autologous BMC (7 mL), 4 mL of autologous Thrombin and 4 mL of calcium gluconate to obtain a gel of autologous cells. The gel was placed inside a scaffold of corticocancellous bone. Autologous PRF (6 mL) was then sprayed locally all over the graft to promote tissues adhesion. Posterolateral Spine fusion: At surgery, a standard midline approach was used, and decompression performed. On the left posterolateral side of the spine, allograft plus autologous BMC and PRP was used (treatment group), while on the right-side allograft alone was used (control).

SPINE AND NERVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
- Bone density after 6 months was significantly improved respect to pretreatment and to the left control side ($p<0.05$). - The difference of bone density between sides was no significative at 12 months post treatment. ➔ PRP used in combination with cancellous bone substitute increases the speed of bone production in posterolateral arthrodesis joining osteoinductive and osteoconductive effect.	Not formally assessed.
- Cumputed tomography scans at 12 months, showed good fusion on right masses (allograft alone) in 4 patients and poor in 6 patients; good left masses (allograft + BMC + PRP) in 9 patients and poor in 1 patient ➔ The differences detected between right-side and left-side masses show an advantage in adding BMC and PRP to the bone allograft to increase spinal fusion rate.	No significant intraoperative or postoperative complications or adverse effects due to the bone marrow aspiration.

SPINE AND NERVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Case Series Report: Ultrasound Guided Autologous Liquid Platelet Rich Plasma - A new treatment option for Complex Regional Pain Syndrome and Reflex Sympathetic Dystrophy? Doss AX. *WebmedCentral ORTHOPAEDICS* 2014; 5:WMC004621	Case reports Follow-up: Case 1: 13 months Case 2: 3 months	Complex regional pain syndrome and reflex sympathetic dystrophy	2	RegenBCT	Case 1: A series of US-guided injections of 4-8ml of PRP was performed into the talonavicular joint, anterior talofibular ligament (ATFL) and anterior tibiofibular syndesmosis. Injection were performed 2-4 weeks apart. At 8-month visit the patient complained persisting pain in the untreated posterolateral ankle in the region of the calcaneofibular ligament (CFL). Patient CFL was treated similarly with PRP. Case 2: series of US-guided infiltrations of 8ml PRP photo-activated (Adilight 2, Adistem), was performed at 2-week intervals into the right anterolateral gutter with percutaneous tenotomy of the ATFL, followed by infiltration into the postero-lateral gutter and percutaneous tenotomy of the CFL subsequently. Two weeks later, 5cc of PRP prepared from clotted platelet rich plasma and 7cc of PRP was placed into the right anterior ankle joint recess under US-guidance twice, 2-week apart.
Trigeminal Neuralgia Treatment: A Case Report on Short-Term Follow up After Ultrasound Guided Autologous Platelet Rich Plasma Injections Doss AX *WebmedCentral NEUROLOGY.* 2012; 3(5):WMC003381	Case report Follow-up: 6 months	Trigeminal neuralgia	1	RegenBCT	5 ml of PRP was obtained from 8 ml of whole blood. 1.5 ml of the upper plasma was removed before platelet resuspension. PRP was then mixed with 0.5ml of 1% lignocaine. With aseptic technique, using a 25G needle this mixture was injected around the distal branches of the maxillary nerve under US-guidance. -First 2 injections were done 5 days apart, -Second set of 2 injections were performed after 11 days, 9 days apart -Last injection was administered 7 days after.

SPINE AND NERVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
Case 1: Symptoms of peroneal neuropathy gradually improved over the first couple of months with progressive improvement in her mobility. At 8 months, reported improvement in the treated areas. At 13 months: pain symptoms improved for the first time in 5 years, improved range of motion of the ankle joint, improved flexion of toes. FADI clinical score showed 39.5% of improvement. Intake of pharmaceutical drugs for pain relief was reduce along with the clinical improvement. Case 2: After the first four weeks the patient reported significant improvement in nocturnal skin discolouration and swelling. At 3 months, she was able to stand for longer and perform day to day activities with a noticeable change in the gait.	Not formally assessed.
- PRP injections were effective, for 6 months post-PRP, the patient has had a normal social and work life with resolution of symptoms. ➔ This is the first report of ultrasound guided PRP of the distal branches of the trigeminal nerve in Trigeminal Neuralgia. The Doss Trigeminal Neuralgia Treatment is a very low risk, outpatient clinic procedure that can be repeated and should be further evaluated in a large patient group with long term follow-up.	PRP treatment was safe, no signs of peripheral nerve damage.

SPINE AND NERVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Ultrasound-guided perineural injection with platelet-rich plasma improved the neurophysiological parameters of carpal tunnel syndrome: A case report Kuo YC, Lee CC, Hsieh LF *J Clin Neurosci*. 2017 Oct;44:234-236.	Case report Follow-up: 6 months	Carpal tunnel syndrome	1	RegenTHT/ RegenATS	PRP was prepared by taking 10 ml of venous blood, which was then mixed with 2 ml of thrombin. Centrifugation 15min at 3400 rpm. 5 ml of PRP was administered by ultrasound-guided perineural injection into the carpal tunnel was performed. second PRP injection to her left carpal tunnel 2 weeks later. The injection procedure was an in-plane approach with technique of hydrodissection, from distal to proximal at the wrist.
Platelet-rich plasma: the role in neural repair Giorgetti M, Siciliano G. *Neural regeneration research* 2015;10:1920-1	Clinical experience Follow-up: Unknown	Severe carpal tunnel syndrome	Un-known	RegenKit Extracell Membrane /RegenTHT	Platelet-rich plasma (PRP) membrane was obtained from centrifugation of autologous blood, as described in the instructions for use. PRP membrane was positioned during a surgical release for severe median nerve compression at the wrist.

SPINE AND NERVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
- Pain, paresthesia, and hypesthesia of both hands decreased gradually over 2 weeks. - Follow-up nerve conduction study of both median nerves 3 and 6 months after the injection revealed significant improvements in the distal motor and sensory latencies as well as sensory nerve action potential and coumpound muscle action potential amplitudes. ➔ If patients with CTS are refractory to conservative treatments, ultrasound-guided PRP injection may be an option.	No adverse reactions were observed.
No clinical results presented.	No safety data were reported.

4.1.4 TRICHOLOGY

5 publications were identified including clinical data on 141 patients.

Among them, RegenKits have been used on all patients for the treatment of androgenetic alopecia (AGA).

TRICHOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Platelet-rich plasma injection is effective and safe for the treatment of alopecia Betsi E-E, Esnault G, Kalbermatten D, Tremp M, Emmenegger V. *Eur J Plast Surg* 2013; 36: 407-12	Case series Follow-up: 3 months	Androgenetic alopecia (AGA)	42	RegenACR-C Extra / RegenBCT	PRP was prepared as per instructions for use from 16 ml of whole blood. 1 ml of upper plasma was removed before platelet resuspension. Anaesthetics cream was used, and a cold roller was applied during the injections. PRP was injected using 32 G or 30.5 G needle. Each patient underwent 6 sessions of treatment every 2 weeks.
Autologous platelet rich plasma as a treatment of male androgenetic alopecia: study of 14 cases Borhan R, Gasnier C, Reygagne P. *J Clin Exp Dermatol Res* 2015; 6: 292	Case series Follow-up: 4 months	Androgenetic alopecia (AGA)	14	RegenBCT	PRP was prepared as per instructions for use from 8 ml of whole blood. Each patient received 4 series of PRP injections, at W0, W3, W6 and W12. Follow-up at W16. PRP was injected on superficial dermis over the vertex. Every injection consisted of 0.05 to 0.1 ml of PRP (4/5 ml in total) using a 32G needle.
Study of Platelet-Rich Plasma Injections in the Treatment of Androgenetic Alopecia Through an One-Year Period Gkini MA, Kouskoukis AE, Tripsianis G, Rigopoulos D, Kouskoukis K. *J Cutan Aesthet Surg* 2014; 7:213-9	Case series Follow-up: 12 months	Androgenetic alopecia (AGA)	20	RegenBCT	PRP was prepared as per instructions for use from 16 ml of whole blood. After removal of 2 ml of upper supernatant plasma from each tube, 3 ml of PRP was resuspended by gentle inversions. Total amount of yielded PRP was about 6 ml. PRP was activated with calcium gluconate in a 1:9 ratio. After local anaesthesia and scalp cleaned, PRP (0.05-0.1 ml/cm^2, 27 G needle) was injected into the androgen-related areas of the scalp (men) and into the problematic areas (women); nappage was made in a depth of 1.5-2.5 mm. 3 Sessions of treatment with intervals of 3 weeks + a booster session at the 6th month.

Safety: Safety data included mainly erythema, headache and drowsiness, and mild pain due to scalp sensitivity to the injection procedure; all effects were transient and solved spontaneously. No serious AE was reported.
Performance: Performance data showed that PRP treatment has a beneficial effect for areas of the scalp affected by androgenetic alopecia, improving quality, density and strengthens of hair, and reducing simultaneously the hair loss.

These data contribute to the safety and performance profile of PRP prepared with RegenKits for androgenetic alopecia indication of use.

The key results described in publications are summarized in the following table:

TRICHOLOGY	
Key results on performance	**Safety / adverse events (AE)**
- Treatment was effective, negative hair pull test after the 3rd session (90.5% pts. were positive prior treatment). - Patients were overall satisfied and significantly improved quality and volume of their hair. Poor results were reported in 1 patient who was previously classified type VI-VII of Norwood scale. - After 3 months, the hair volume remained stable, and there was a high overall patient satisfaction (7 on a scale of 1–10). ➔ PRP injections are simple and efficient, have minimal morbidity with a low cost-to-benefit ratio and can be regarded a valuable alternative for the treatment of alopecia.	Treatment was safe, no remarkable adverse effects were reported apart scalp sensibility and drowsiness during the 1st session. After, patients got more comfortable that the anaesthetic cream was no longer needed.
- TrichoScan assessment showed an increase in hair density in 11 patients. We observed a decrease of hair density in 3 patients. Globally the number of new hair follicles was 33 and the number of vellus hairs was 16. - Self-assessment questionnaires showed: texture improvement 14/14, volume improvement 5/14, decreased shedding 10/14, growth back 7/14. DLQI questionnaire showed improvement of patient life quality (8/14). - Photographic results were not representative. ➔ The study confirms the potential interest of using PRP in AGA.	Few side effects such as erythema or headaches have been reported. Two patients with seborrheic dermatitis noticed an improvement with PRP (probably due to its anti-inflammatory effect). Darkening of hair was observed in two patients with blond hair.
- Hair loss remarkably decreased reaching normal values at 3 months after treatment. Also, hair density increased (baseline: 143.10±31.07 and 12-months: 153.70±39.92; p<0.001) reaching the greatest value at the 3rd month (170.70±37.81) after treatment and remained stable up to 1 year of follow-up. - Patients were satisfied with a mean result rating of 7.1 on a scale of 1-10. - 85% of patients reported an improvement in hair quality and thickness flash, while 65% reported an increase in hair density. ➔ PRP injections may have a positive therapeutic effect on male and female pattern hair loss without remarkable major side effects. Further studies are needed to confirm its efficacy.	All treated patients felt mild pain, despite application of local anaesthesia. After PRP treatment, 25% of them felt a mild pain feeling, lasting 4 hours, while the 60% had scalp sensitivity during first hair wash after treatment injections. None reported any worsened hair shedding, infection or ecchymosis.

TRICHOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Trichologic response of platelet-rich plasma in androgenetic alopecia is maintained during combination therapy. Ho A, Sukhdeo K, Lo Sicco K, Shapiro J. *J Am Acad Dermatol.* 2018; Mar 23. pii: S0190-9622(18)30473-0.	Retrospective study Follow-up: 2 months	Androgenetic alopecia (AGA)	24	RegenBCT	PRP was prepared as per instructions for use from 8 ml of whole blood. Initial scalp treatment consisting of PRP on two consecutive months. Scalp injections, directed to areas of alopecia, deposited 0.1mL PRP approximately 1cm deep and spaced 1cm apart. Patients were then reassessed; if hair density increased greater than 10 hairs/cm2 over baseline, monthly treatments were continued for four additional months followed by maintenance injections every 3-6 months. All PRP patients used topical 5% minoxidil and 83.3% used oral anti-androgen medications.
Correlation between individual inflammation genetic profile and platelet rich plasma efficacy in hair follicle regeneration: a pilot study reveals prognostic value of IL-1a polymorphism. Rossano F, Di Martino S, Iodice L, Di Paolo M, Misso S, *et al.* *Eur Rev Med Pharmacol Sci.* 2017 Nov;21(22):5247-5257.	Retrospective study Follow-up: 12 months	Androgenetic alopecia (AGA)	41	RegenBCT	6 ml of PRP was prepared as per instructions for use from 16 ml of whole blood. PRP was activated with CaGlu in a 1:10 ratio. Note: PRP was frozen at -20°C until application session. Using a 27-G needle 0.1 ml/cm2 of PRP was injected into the hair loss-related areas (frontal, occipital, parietal) of the scalp in men and into the problematic areas in women, using BD-Luer LokTM 1 ml syringes. Nappage technique was executed in a depth of 1.5-2.5 mm. Our protocol proposed 3 treatment sessions with an interval of 3-5 weeks. After 6 months from the beginning, a booster session was also performed.

TRICHOLOGY	
Key results on performance	**Safety / adverse events (AE)**
- Positive response to PRP was seen in 70.8% of patients two months after initial injections. - Hair density after PRP showed a statistically significant increase from baseline on the anterior crown (+24.5 hairs/cm2, p=0.022). Hair density with PRP increased greater than 10% over baseline in 62.5% of patients and greater than 20% over baseline in 33.3% of patients. One patient had an increase in hair density of more than 50%. - Changes in hair shaft diameter did not reach statistical significance. ➔ Androgenetic alopecia patients opting for PRP are typically on multiple modalities and any newly developed therapy will likely be incorporated as an additional treatment. PRP may also serve to benefit patients' unresponsive to medical therapy.	Not formally assessed.
- PRP treatment was clinically satisfactory. Significant increase rate in hair density was noticed after the third month of treatment in 32/41 (78%) of the subjects. - Statistically significant differences were found in the distribution of IL-1a, IL-1b between responder and non-responder patients, whilst for IL-6 and IL-10 any statistically significant differences were found. - For IL-1α, the frequency of C/C genotype was higher in responder (66%) than in non-responder patients (22%). On the contrary, in females, this research showed a negative correlation. ➔ Our pilot study demonstrated a correlation between the individual genetic inflammatory profile and the efficacy of the PRP treatment in males. On the contrary, in females, it showed a negative correlation. IL-1a could be used as a prognostic value for PRP efficacy.	Not formally assessed.

4.1.5 DERMAL

10 publications were identified including clinical data on 160 patients.
Among them, RegenKits have been used for 3 different subtypes of indications:

5.A. Scars (keloids, stretch marks) (2 publications)
RegenKits have been used on 40 patients for the treatment of scars such as keloids or traumatic scars.

5.B. Bio-revitalization (7 publications)
RegenKits and Cellular-Matrix have been used on 119 patients to correct skin ageing or malar area atrophy.

5.C. Melasma (1 publication)
RegenKits have been used on 1 patient for melasma regression.

SCARS (KELOIDS AND STRETCH MARKS)					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
The contribution of PRP injection in the sequelae of cutaneous scars of the face [L'apport de l'injection du PRP dans les séquelles de cicatrices cutanées de la face] Berrada El-Azizi Dounia *Thèse N°043 – Marrakech.* 2017 (Thesis)	Case series Follow-up: 5 months	Skin scars of the face, traumatic origin (assault, accident)	23	RegenBCT	PRP was prepared from 8 ml of whole blood and centrifugation 10 min at 1200 RPM. Note: The PRP was not centrifuged as per instructions for use. After local anesthesia, 3 (78.26% of patients) to 5 (21.74% of patients) PRP injection was performed on the scar area perpendicularly slightly inclined to the sides with 1-month interval according to the scar.
Efficacy of Autologous Platelet Concentrates as Adjuvant Therapy to Surgical Excision in the Treatment of Keloid Scars Refractory to Conventional Treatments: A Pilot Prospective Study. Hersant B, SidAhmed-Mezi M, Picard F, *et al.* *Ann Plast Surg.* 2018 May 14.	Pilot study Follow-up: 24 months	Keloid scars, refractory to conventional treatments	17	RegenBCT	PRP was prepared as per instructions for use from 8 ml of whole blood. After complete resection of the keloid scar, the first injection was administered before closure. Platelet-rich plasma was injected with a 27-gauge needle in the dermis of the margins and in the lesion resection bed and edges while avoiding injection into the healthy edges of the epidermis. Three additional injections were administered with a 1-month interval. An anesthetic topic was applied 1 hour before the injection.

Safety: Generally, safety data include mainly burning sensation, ecchymosis, bruising, erythema, redness, tenderness and swelling. The effects observed were transient and of mild to moderate severity, localized to the treated area; which solved spontaneously. No serious AE was reported.
Performance: Generally, performance data showed that PRP treatment has a beneficial effect for healing, improving skin health/ structure and facial age-related lines and for melasma regression, with a high patient satisfaction of the received treatment.

These data contribute to the safety and performance profile of PRP prepared with RegenKits and PRP-HA prepared with Cellular-Matrix and support their use for skin care medical indications of use.

The key results described in publications are summarized in the following table:

SCARS (KELOIDS AND STRETCH MARKS)	
Key results on performance	**Safety / adverse events (AE)**
- The mean Vancouver score was initially at 8.33 with 87% of inflammatory scars, 52.2% red and 34.8% purple, 39.1% hard scar, 43.5% solid and little stretch scars. - At the last PRP session, the mean Vancouver score decreased to 2.59 with 91.3% non-inflammatory scars, 69.6% with normal skin colour and 21.7% pink colour. 65.2% of scars became supple, with only 21.7% indurated and 13.1% firm. - The average length and width remained almost unchanged, but scar thickness was decreased from 3mm to 1mm. ➔ The injection of PRP is a new and promising modality for the treatment of sequelae of scars of the face without risk of side effects.	Not formally assessed.

- 9 lesions (53%) were completely healed and therefore did not relapse whereas 5 lesions (29%) completely relapsed after treatment and 3 lesions (18%) partially relapsed. - The VSS score significantly decreased from 8.18 before treatment to 3.82 at 2 years ($P < 0.0001$). The VSS score improved in 16 patients (88%). - Pruritus, a symptom associated with the keloid scar, disappeared in 60% of patients 2 years after treatment. - The patient satisfaction questionnaire showed that 9 patients (53%) were very satisfied with the result, 2 patients (12%) were moderately satisfied, and 6 patients (35%) were not satisfied. ➔ Injecting PRP after keloid scar resection is safe and efficient treatment, although the recurrence rate was high. This treatment allows a new alternative in these patients with difficult-to-treat keloid scars.	No adverse event was reported during the injection sessions and 2 years after treatment. Regarding pain assessed using VAS during injections of platelet concentrates, the mean score was 4.35.

BIO-REVITALIZATION					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Histologic evidence of new collagen formulation using platelet rich plasma in skin rejuvenation: a prospective controlled clinical study Abuaf OK, Yildiz H, Baloglu H, Bilgili ME, Simsek HA, Dogan B. *Ann Dermatol* 2016; 28:718-24	Prospective Controlled clinical study Follow-up: 1 month	Skin ageing	20	RegenBCT	PRP was injected to the upper site of this right infra-auricular area and all face. Saline was injected into the left infra-auricular area (control). On the day 28 after injections, biopsy was performed on the PRP-treated and control (saline injected area) site. Anaesthetic cream was applied. Approximately 2 ml of PRP was injected into the dermis of the face (about 0.15 ml per injection) using a 30G-needle for superficial micro- injections by the mesotherapy technique 'point by point'. Injections were spaced about 1 cm apart. The injections were administered into the papillary dermis (1.5∼2.0 mm deep).
Combined use of micro-needling RF treatment with injections of Platelet-Rich Plasma activated by autologous thrombin in aesthetic medicine Dzybova E., Kruglova L., Alenichev A. *P0120 – 14th EADV Spring Symposium, Brussels, Belgium*, 2017 (Abstract)	Case series Follow-up: 1- 2 years	Facial and neck skin ageing	20	RegenBCT/ RegenATS	Group 1 (n=10): patient up to 49 years old Group 2 (n=10): patients over 50 years old All patients underwent the combined PRP-activated platelets by autologous thrombin, and the micro-needle RF (Radio-frequency) lifting treatment.
Research of clinical effectiveness of the new cellular technology CellularMatrix Dzybova E., Kruglova L., Alenichev A. *P0119 – 14th EADV Spring Symposium, Brussels, Belgium,* 2017 (Abstract)	Case series Follow-up: 6 months	Skin ageing	24	Cellular Matrix BCT-HA	Patients received intradermal injections of Cellular-Matrix or PRP to treat xerosis, wrinkles, disorders of pigmentation and secretory activity. All patients were divided into two groups comparable by basic morphofunctional parameters. Group 1 (n=12): Cellular-Matrix Group 2 (n=12): PRP

BIO-REVITALIZATION	
Key results on performance	**Safety / adverse events (AE)**
- Objective histopathological examination of biopsies samples showed an increase in the number and thickness of elastic fibers and collagen content with both saline and PRP ($p<0.001$). - The improvement in PRP treated side respect to saline placebo treated side was statistically highly significant ($p<0.001$). In fact, in the saline site, 46.01% improvement was found vs. 89.05% in PRP side. ➔ PRP application could be considered as an effective (even a single application) and safety procedure for facial skin rejuvenation.	No serious side effects reported. Mild and transient side effect such as bruising or ecchymosis, burning sensation, mild erythema, and severe erythema were observed.

- The reduction of skin ageing helped to improve the quality of life in both groups of patients. On average, the DLQI index improved ($p<0.01$) by 76.6% in the group of patients up to 49 years old and by 70.4% in the patients over 50 years. - More than 90% of patients regardless of age noted a good/very good efficiency and comfort of use of the combined method (same results obtained from physician). - Clinically significant anti-ageing effect in most patients lasts for quite a long time (1.5-2 years). ➔ The correction of facial and neck skin ageing, based on the combination of the PRP-treatment, which activates platelets by autologous thrombin, and the micro-needle RF lifting treatment, is a highly effective method thus can be recommended for a widespread use in clinical practice.	Not formally assessed.
- Hyperpigmentation decreased significantly in both groups but to a greater extent in group 1: by 52.3% in group 1 and by 39.5% in group 2. - The improvement of skin texture was also more pronounced in group 1: 34.4% in group 1 and 24.6% in group 2. - The skin elasticity index improved in both groups by 20.2% and 14.9% respectively. - The clinical effectiveness and advantages of the Cellular-Matrix technology were confirmed by using dermoscopy and photographing data. ➔ Both PRP and Cellular-Matrix are efficient, however the Cellular-Matrix method is more effective and safe in the treatment of skin ageing and therefore can be recommended for wide clinical use.	Any side effects were observed.

BIO-REVITALIZATION					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Efficacy of autologous platelet-rich plasma combined with hyaluronic acid on skin facial rejuvenation: A prospective study. Hersant B, SidAhmed-Mezi M, Niddam J, *et al.* *J Am Acad Dermatol.* 2017 Sep;77(3):584-586.	Case series Follow-up: 6 months	Skin ageing	31	Cellular Matrix BCT-HA	PRP-HA was prepared as per instructions for use of Cellular-Matrix (2 mL of PRP and 2 mL of HA). Mesotherapy was performed using topical anesthesia (EMLA) at 0, 1, and 2 months by injecting 4mL of the mixture per cheek. The first step consisted of deep intradermal injections of PRP-HA using a 1-mL syringe with a 32G needle every 5 mm per cheek. The second step consisted of spreading 1 mL of the mixture per cheek followed by intradermal punctures made by using a grid with punctures every 1 mm and a 1-mm SkinRoller (Aesthetic Group).
Face and neck revitalization with platelet-rich plasma (PRP): Clinical outcome in a series of 23 consecutively treated patients Redaelli A, Romano D, Marcianó A. *J Drugs Dermatol.* 2010 May;9(5):466-72	Case series Follow-up: 4 months	Facial skin rejuvenation and acne scar	23	RegenBCT	PRP was prepared as per instructions for use from 16 ml of whole blood. 2 ml of PRP were activated with $CaCl_2$ 10%. All patients were treated with 3 sessions of PRP injections administered with 1-month interval. Injection points and quantities were standardized and given with different injection techniques including intra-dermal injections: -1ml into the upper third of the face (0.5ml forehead area and 0.5ml crow's feet area) -1ml into the cheeks area (0.5ml per side) -1ml into nasolabial and marionette areas (0.5ml per side) -1ml for the neck.

BIO-REVITALIZATION	
Key results on performance	**Safety / adverse events (AE)**
- Comparison of satisfaction with appearance using FACE-Q scores showed significant improvement at 6 months compared with baseline (44.3 versus 52, p=0 .03). - Similarly, biophysical measurements showed significant improvement for skin elasticity (p=0.036) from baseline (by R5 software). ➔ The combination of PRP-HA obtained by Cellular-Matrix for facial revitalization have clinical benefits on skin firmness and elasticity.	No serious adverse event was reported.
- Clinical photographs taken with the dermoscope showed an overall improvement of the skin of 30%. - 65.3% or patients were satisfied or very satisfied with the treatment. - All patients reported overall rejuvenation of their skin including better skin texture, improved skin health and wrinkles. - Clinical photographs taken with the dermoscope showed an overall improvement of the skin of 30%. ➔ Face and neck revitalization with PRP is a promising easy-to-perform technique in face and neck rejuvenation and scar attenuation. Further work needs to be carried out to investigate its exact mechanism of action.	No serious infections or hematoma were detected; mainly mild erythema or burning sensation were reported in 70-80% of patients.

BIO-REVITALIZATION					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Skin Resurfacing with Er:YAG – the Lost Art of Deep Skin Resurfacing Matyunin Oleg *Journal of the Laser and Health Academy* 2016, No.1, pp.41-44.	Case report Follow-up: 30 months	Skin ageing	1	RegenBCT	This paper reports on a case of treatment of a severely damaged skin utilizing a 2940 nm Er:YAG laser (Dualis SP, Fotona). Additionally, PRP was applied on the treated areas and botulinum toxin administered to the forehead and periorbital area. A cream was prepared (a mixture of PRP and Bepanthen 5%) in a sterile container and handed to the patient. The patient then applied the cream 6 times per day for 3-4 days. PRP therapy was repeated as described above in the 4th, 5th and 24th month after the first procedure.
Malar Area Goisis M, Stella E, Di Petrillo A. *Injections in Aesthetic Medicine: Springer;* 2014: 73-88	Clinical experience Follow-up: Not reported	Correction of age-related malar atrophy	Not report-ed	RegenACR-C Extra / RegenBCT	All subjects (unknown number) were treated with the same protocol. Injected product contained: 1.5 ml PRP + 0.05 ml CaGlu + 0.5 ml anaesthesia + 1.5 ml calcium hydroxylapatite. Product was injected deep in the malar area (1 treatment session only). Note: RegenBCT tube was spun at 1500g for 15 min and not as per instructions for use.

MELASMA					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Regression of melasma with platelet-rich plasma treatment Cayirli M, Caliskan E, Acikgoz G, Erbil AH, Erturk G. *Ann Dermatol* 2014; 26:401-2	Case report Follow-up: 6 months	Melasma, epidermal hyperpigm entation over the cheeks, perioral region, and forehead for 5 years	1	RegenTHT	PRP was prepared as per instructions for use from 8 ml of whole blood. 32-G needle, mesotherapy technique, injections in to the papillary dermis (1.5~2.0 mm deep). Patient underwent 3 injection sessions. Approximately 1.5 ml of PRP was injected at each session, spaced of 15 days.

BIO-REVITALIZATION	
Key results on performance	**Safety / adverse events (AE)**
- The combination of planar (full-spot) and fractional Er:YAG revealed excellent aesthetic results. - In addition to better skin structure, there is a visible reduction of rhytides, laxity, and signs of photo aging. ➔ PRP could improve the deep skin resurfacing using laser resurfacing.	Not formally assessed.
➔ PRP treatment was effective to improve facial age-related lines, supported by clinical photograph which showed good aesthetic results.	No adverse effects were reported during the course of this clinical experience, beside expected mild tenderness and sporadic swelling on the malar area.

MELASMA	
Key results on performance	**Safety / adverse events (AE)**
- At the end of the 3rd session, >80% reduction in epidermal hyperpigmentation was observed. - No additional treatments or post treatment care (beside the use of a sun-screen) was prescribed. - No melasma recurrence during 6-month follow-up. ➔ The regression of melasma after PRP treatment is an interesting finding. Controlled clinical trials are needed to confirm this preliminary observation.	Not formally assessed.

4.1.6 PLASTIC AND RECONSTRUCTIVE SURGERY

9 publications were identified including clinical data on 353 patients.

Among them, RegenKits and Cellular-Matrix have been used in addition to surgery on 290 patients for the treatment of atrophic acne or traumatic scars, for improvement of periocular area and facial augmentation, for skin graft, for breast reduction and abdominoplasty, and for regeneration of skin defect or severe wound with bone exposure.

PLASTIC AND RECONSTRUCTIVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Treatment of traumatic scars using fat grafts mixed with platelet-rich plasma, and resurfacing of skin with the 1540 nm nonablative Cervelli V, Nicoli F, Spallone D, *et al.* *Clin Exp Dermatol* 2012; 37:55-61	Prospective Randomized Comparative study Follow-up: 6 months	Scars from traumatic origin	60	RegenTHT	Group 1: Fat + PRP (20 pts.): patients were treated with fat combined with PRP (20-30%) during months 1 and 3. Group 2: laser (20 pts.): patients received 4 sessions (one per month) of laser treatment with the 1540 nm nonablative laser Group 3: laser + fat + PRP (20 pts.): patients were treated with nonablative laser therapy during months 1 and 3 delivered 7 days after the fat graft / PRP treatment. Globally, PRP was prepared from 18 ml of whole blood. PRP was activated with autologous thrombin (ATS). Note: RegenTHT was centrifuged at 1100g for 10min and not at 1500g as per instructions for use. 50-100ml of fat was harvested from the abdominal region using Coleman's technique. Between 5 and 50 mL of the fat / PRP solution were used, depending on the defect to be corrected.

Safety: The tolerance and acceptance of the treatments were excellent, and no undesirable effects were noted except transient and mild bruising, erythema, redness and swelling. Also, hyperpigmentation was seen in 4 patients, and disappeared within 3 months.
Performance: Performance data showed that PRP +/-HA treatment has a beneficial effect for treating scars and achieve complete healing and for improvement of thickness of tissue, with a high patient satisfaction of the received treatment.

These data contribute to the safety and performance profile of PRP prepared with RegenKits and PRP-HA prepared with Cellular-Matrix and support their use in plastic and reconstruction surgery of skin or wound.

The key results described in publications are summarized in the following table:

PLASTIC AND RECONSTRUCTIVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
- All treatments were effective, no new scarring occurred as a result of the procedures. - The greatest clinical results were found in patients of group 3 whose wound healing was significantly superior (22% compared to group 1 and 11% compared to group 2), with clear improvement in texture, color and scar contours. - At 6 months follow-up, 50 patients (84%) evaluated scars improvement as 'good-excellent' and 10 patients. as 'poor-fair'. ➔This combined treatment appears to be safe and effective for treating scars, but further immunohistochemical studies and histological analysis are needed to explore its potential.	Side-effects were mild and were limited to transient erythema (duration of 2–3 days) and oedema (1– 2 days). Temporary hyperpigmentation was seen in 4 patients, but this disappeared within 3 months.

PLASTIC AND RECONSTRUCTIVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Autologous keratinocyte suspension in platelet concentrate accelerates and enhances wound healing – a prospective randomized clinical trial on skin graft donor sites: platelet concentrate and keratinocytes on donor sites Guerid S, Darwiche SE, Berger MM, *et al.* *Fibrogenesis Tissue Repair.* 2013 Apr 9;6(1):8	Prospective Randomized Controlled clinical trial Level I Follow-up: Until complete wound healing and closure	Skin graft	45	RegenTHT	PRP was prepared as per instructions for use. PRP was transferred into a sterile tube and activated with 10% $CaCl_2$. Group 1 - PRP (15 pts.): application of PRP 13.5 x 10^6 plt/cm^2 of wound + 3 layers of paraffin gauze and elastic bandage Group 2 – PRP + keratinocytes (15 pts.): application of PRP+ keratinocytes 13.5 x 10^6 plt/cm^2 + 80000 ker/cm^2 of wound + 3 layers of paraffin gauze and elastic bandage Group 3 – control (15 pts.): application of three layers of paraffin gauze and bandages.
Autologous Platelet-Rich Plasma/Thrombin Gel Combined with Split-Thickness Skin Graft (STSG) to Manage Postinfectious Skin Defects: A Randomized Controlled Study. Hersant B, SidAhmed-Mezi M, Bosc R, Meningaud JP. *Adv Skin Wound Care.* 2017 Nov;30(11):502-508.	Prospective Randomized Controlled clinical trial Follow-up: until complete healing	Skin defects	27	RegenBCT/ RegenATS	Each patient had 24 to 48mL of blood drawn, depending on the degree of skin loss. Autologous PRP was collected after centrifugation at 1500g for 5 min. A second centrifugation at 1500g for 5 min was performed for isolating autologous thrombin (Regen ATS tube). PRP was then mixed and activated with ATS using a Regen spray applicator with a 10:3 ratio (PRP:thrombin) forming the A-PRP/thrombin gel. In all patients, time between surgical debridement and the skin graft ranged from 15 days to 1 month. In each patient, a 0.2-mm meshed STSG (Split-Thickness Skin Graft) was harvested with a dermatome on the inner surface of the thigh. The meshed STSG was performed with a skin graft mesher, and the ratio for graft expansion was 1:1.5, which increased the surface area by 50%. Experimental group (n=14): During STSG surgery, the wound bed was sprayed with A-PRP/thrombin gel according to Regen procedure. Control group (n=13): STSG only

PLASTIC AND RECONSTRUCTIVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
- Wound healing was significantly shortened in PRP and PRP + K groups compared to control (P < 0.01). - Patients from the PRP group also experienced a significant reduction of the pain resulting from the skin graft donor sites compared to the control group, an effect further enhanced in the PC + K group (P < 0.01). - Data showed a statistically detectable advantage of using PC + K over PC alone (P < 0.01). G1: 7.2 d + pain reduction G2: 5.7 d + pain reduction G3: 13.9 d ➔ The results demonstrate the positive contribution of autologous platelets combined with keratinocytes in stimulating wound healing and reducing pain.	No infection was noted. The tolerance and acceptance of the treatments were excellent, and no undesirable effects were noted.
- In the experimental group, patients achieved a rate of skin graft success of 89.6%, whereas in the control group, the graft success was 76.8% (P = 0.01). - The complete healing time was significantly shorter in the experimental group compared with the control group: 37.9 days and 73.7 days, respectively (P=0 .01) ➔ A-PRP/thrombin gel combined with STSG could improve the healing time of complex and difficult wounds and thereby provide a basis for healing skin loss without adverse reactions and with minimal recovery time.	During the follow-up, no recurrence or complication occurred in either group. In the experimental group, no adverse reaction, infection, allergy, or additional complication was observed.

PLASTIC AND RECONSTRUCTIVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Comparative study using autologous fat grafts plus platelet-rich plasma with or without fractional CO_2 laser resurfacing in treatment of acne scars: analysis of outcomes and satisfaction with FACE-Q Tenna S, Cogliandro A, Barone M, *et al.* *Aesthetic Plast Surg.* 2017 Jun;41(3):661-666.	Prospective Randomized Comparative clinical study Follow-up: 12 months	Chronic atrophic acne scars	30	RegenTHT	PRP was prepared as per instruction for use. Group A (15pt.): PRP + nanofat infiltrations combined with fractional CO_2 laser resurfacing Group B (15pt.): PRP + nanofat infiltrations A 3-mm Coleman aspiration cannula was then used to harvest adipose tissue with manually generated negative pressure. Emulsification of the fat was achieved by shifting the fat between two 10-cc syringes connected to each other by a female-to-female Luer-Lock connector. After 30 passes, the fat changed into an emulsion. After this emulsification process, the fatty liquid (7 cc) was added to 3 cc of the patient's own PRP. The fat grafts were then transferred into 1-ml syringes for transfer into soft tissue deformities by means of 19-gauge cannulas. The patients underwent two treatments with a mean interval of 6 months.
Use of platelet-rich plasma and hyaluronic acid in the loss of substance with bone exposure Cervelli V, Lucarini L, Spallone D, *et al.* *Adv Skin Wound Care* 2011; 24:176-81	Prospective Controlled study Follow-up: 12 months	Lower-extremity wound with bone exposure	30	RegenTHT	PRP was prepared from 16 ml of whole blood and then, activated with CaGlu and second centrifugation to obtain PRP gel. Group 1: PRP-HA (15 pts.): after debridement, PRP gel was applied on the wound which was subsequently closed with HA dressing and silicone film. A secondary dressing was used to improve the venous return. Group 2: HA (15 pts.): patients were treated with HA dressing only.

PLASTIC AND RECONSTRUCTIVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
- The improvement of thickness of subcutaneous tissue was 0.668 cm in group A and 0.63 cm in group B. All patients in both groups had a treatment benefit, but without a significant difference between the two groups. - The FACE-Q module confirmed the impact of treatment of facial acne scars in social life and relationships. The results obtained with the FACE-Q postoperative module in both group A and group B and found no significant difference ➔ Subcutaneous infiltration with nanofat + PRP seems to be effective to improve atrophic scars, either alone or combined with fractional CO_2 laser resurfacing.	Not formally assessed.
- This study shows the superior efficacy of the combination of PRP + HA *vs* HA alone to manage wounds following bone exposure ($p<0.001$). - Mean re-epithelialization time was 8.1 weeks for 73.3% of patients treated with PRP + HA, *versus* 30% patients treated with HA dressing only. ➔ These data confirm the evidence of using PRP technology in the healing of both soft- and hard-tissue wounds. Moreover, the satisfaction of the patient confirms the quality of this study's results.	Not formally assessed.

PLASTIC AND RECONSTRUCTIVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Nanofat grafting compared to hyaluronic acid and PRP treatment of the lower lid and tear trough: clinical outcome. Goisis M, Stella E, Di Petrillo A, Rosset L. *JSM Ophthalmology* 2015; 3(1):1027	Comparative study Follow-up: 3 months	Rejuvenation of periocular area	40	Cellular-Matrix A-CP-HA / and RegenACR-C Extra / RegenBCT	<u>Group A (20 pts):</u> treated with a combination of PRP+HA (A-CP-HA Kit). <u>Group B (20 pts):</u> treated with PRP+fat. <u>Note:</u> RegenBCT was centrifuged at 1500g for 15 min and not as per instruction for use. Fat was harvested with a multi- port 1, 5-mm cannula with sharp side holes of 1 mm in diameter (Tulip Medical Products) and then, emulsified by Tonnard's technique (30 passes). PRP was mixed to autologous purified fat tissue (20% PRP - 80% fat). All injections were performed intra-dermally with a 30-gauge needle or with a 27-gauge 4cm long blunt cannula in the lower lid and/or tear trough. One treatment session only.
Initial experience of face augmentation using fat graft-platelet rich plasma mix Tolba A, Nasr M. *Surgical Science* 2015; 6:489-98.	Case series Follow-up: 24 months	Facial augmentation	40	RegenBCT	PRP was prepared as per instructions for use. Fat tissue was harvested and purified, by withdrawing the plunger (2 - 3 mm), negative pressure was provided, back and forth hand movement gathered fat into the syringe through an opening located on the lateral side of a cannula (2 - 3 mm). Fat-PRP mixture (20% of PRP to 80% of purified fat) was injected with a 1 ml syringe by using a blunt 18- or 17G cannula with one distal aperture just proximal to the tip. A second procedure after 6 months was indicated when the outline of facial contour was not satisfactorily improved. All patients received an autologous fat injection between 1 and 3 times into 1 to 3 areas of their faces. The volume of each injection was ranging from 2 to 50 ml.

5 POSAS score: Patient and Observer Scar Assessment Scale (POSAS) score, which was the sum of the scores obtained using 2 scales: the Patient Scar Assessment Scale and the Observer Scar Assessment Scale.Both scales contained 6 items that were scored numerically on a 10-point scale.

PLASTIC AND RECONSTRUCTIVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
- Both treatments gave a positive effect in the treated areas with similar clinical benefit. 85% of patients were satisfied or highly satisfied with the allocated treatment, for each group. - The PRP+HA preparation and procedure is minimally invasive (requires 4 ml of blood withdrawn only) and faster respect to the association of PRP+fat (8ml of blood withdrawn to obtain PRP, fat harvesting and purification). ➔ Fat transposition techniques, autologous fat grafting, nanograft and hyaluronic acid fillers are reliable choices for tear trough correction of the lower eyelid. Furthermore, we can affirm that it's still easier and faster the treatment with HA and PRP compared to the Nanofat Grafting added to PRP.	No fat cysts, no infections, no foreign body reactions, no permanent discolorations, or other side effects were observed.
Clinical results are available for 30 patients, as 10 were lost to follow-up: - 70% of patients were evaluated as excellent, 20% of patients were satisfied with the treatment while the remaining 10% was not. ➔ Fascial fat graft is biocompatible, inexpensive, readily available, non-migratory with long term results. However, even with the best technique, the survival rate is still quite variable and unpredictable. The addition of PRP to fat grafts represents many advantages with a simple, cost-effective and safe method.	No indurations, no cysts, no infections or visible scar in this series. Bruising of the treated area occurred in 50% of patients, and redness with some swelling occurred in 10% of patients, all of which resolved within an average of 4 days after the procedure. No surface irregularities, such as lumpiness or unevenness.

PLASTIC AND RECONSTRUCTIVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Efficacy of autologous platelet-rich plasma glue in weight loss sequelae surgery and breast reduction: a prospective study Hersant B, SidAhmed-Mezi M, Lapadula S, Niddam J, Bouhassira J, Meningaud JP. *Plastic and reconstructive surgery Global open* 2016; 4: e871	Pilot study with Retrospective control Follow-up: 12 months	Breast reduction surgery, abdominoplasty, or limb lifting	51	RegenKit Surgery / RegenBCT RegenATS	Experimental group (n=51): application of A-PRP glue on the entire surface of the subcutaneous tissue at the time of suture. Control group (n=24): retrospective patients, without PRP Between 24 and 48 mL of blood was drawn from each donor depending on the indication. Autologous PRP was collected after centrifugation at 1500g for 5 min. A second centrifugation at 1500g for 5 min was performed for isolating autologous thrombin (Regen ATS tube). PRP was then mixed and activated with ATS using a Regen spray applicator with a 9:3 ratio (PRP:thrombin) forming the A-PRP/thrombin gel. Abdominoplasty with umbilical transposition: PRP glue was applied in the undermined subcutaneous space, followed by placement of blade drains, closure in 2 layers, and application of a compressive dressing. Brachioplasty and thigh lift: liposuction was performed in the resection cutaneous zone to preserve lymphatic vessels, rigorous haemostasis, cutaneous resection, and application of PRP glue under the suture in 2 layers. No drainage. Breast reduction: glandular cutaneous resection was performed according to the Wise pattern breast reduction technique. The flap with nipple was an internal posterosuperior pedicle. PRP glue was sprayed at the subcutaneous tissue and applied under the suture. The suture was made in 2 plans. No drainage. A semimodeling dressing was used.

PLASTIC AND RECONSTRUCTIVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
Primary criteria was the incidence of postoperative seroma or hematoma: - After <u>abdominoplasty</u>, 37.5% of patients (3/8) in the control group experienced seroma and hematoma complications *versus* 12.5% of patients (2/16) in the PRP glue group (*P* = 0.55 and *P* = 0.25, respectively). - After <u>limb lifting</u>, 50% of patients experienced postoperative complications in the control group *versus* no patient in the PRP glue group (*P* = 0.03). - After <u>breast reduction</u>, no patient experienced complication in the PRP glue group *versus* 25% of patients in the control group experienced hematoma (*P* = 0.04). - The scar quality assessed with the POSAS score[5] 12 months after surgery showed no statistical differences between the groups. ➔ By improving hemostasis and tissue adherence, the PRP glue seems to prevent seroma formation after limb lifting and hematoma after breast reduction. The quality of wound healing did not seem to be improved.	No PRP-related side effects reported.

PLASTIC AND RECONSTRUCTIVE SURGERY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Tissue regeneration in loss of substance on the lower limbs through use of platelet-rich plasma, stem cells from adipose tissue, and hyaluronic acid Cervelli V, De Angelis B, Lucarini L, *et al.* *Adv Skin Wound Care* 2010; 23:262-72	Retrospective study Follow-up: 12 months	Lower limb lesions	30	RegenTHT/ RegenATS	PRP was prepared as per instructions for use from 16 ml of whole blood. Fat was harvested through abdominal cavities (3 mm) using Coleman microcannula technique. Centrifuged fat was mixed with 0.5 ml of activated PRP to form an adipose platelet gel and injected with a 1ml microcannula into the lesion (intralesionally, intratendonally or perilesionally). Then, PRP was activated with autologous thrombin (ATS) in a 9:1 ratio. Also, CaGlu was used for topical use of PRP gel. PRP gel was sutured to the tendons and wounds were covered with a 3D polymerized HA-medicated biological dressing.

PLASTIC AND RECONSTRUCTIVE SURGERY	
Key results on performance	**Safety / adverse events (AE)**
- All patients significantly improved their wounds during the first 3 weeks. - 47% of patients achieved complete wound healing within 6 weeks 57% of patients within 3 months. - Also, immunohistochemistry results showed an increase in cells proliferation. ➔ Fat+PRP guarantees a temporary barrier with multiple functions: reduction of pain, infection, and wound exudate and maintenance of a moist wound environment.	No AE reported. Intraoperative and postoperative complications were observed in 2 multidrug-resistant patients with diabetes; those patients reported a partial response to the treatment with long postoperative time.

4.1.7 WOUND CARE

12 publications were identified including clinical data on 1269 patients.
Among them, RegenKits have been used for 3 different subtypes of indications:

7.A. Chronic wound (7 publications)
RegenKits have been used on 121 patients for the treatment of diabetic foot ulcers and wound with tendon exposure.

7.B. Surgical wound (3 publications)
RegenKits have been used on 425 patients to prevent sternal wound infections and infectious wounds due to the ventricular assist device.

7.C. Burns (2 publications)
RegenKits have been used on 33 patients to treat burnt tissues.

CHRONIC WOUND					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Use of Platelet Rich Plasma and Hyaluronic Acid in the Treatment of Complications of Achilles Tendon Reconstruction Gentile P, De Angelis B, Agovino A, Orlandi F, Migner A, Di Pasquali C, Cervelli V. *World J Plast Surg* 2016; 5(2); 124-132	Comparative study Follow-up: 1 month	Achilles tendon injuries resulting from post-surgical complications subsequent to tenorrhaphy	20	RegenBCT	PRP was used in combination to polymerize HA. Patients were divided in 2 groups, respect to the treatment received: Group 1 (PRP, 10 pt.): After infiltration of a local anesthetic and/or sedation, curettage of the wound was performed. The PRP was activated by CaCl, CaGl or autologous thrombin. PRP is injected intra-tendons, intra-lesional and peri-lesional after activation. PRP gel was applied to the lesion and cover with 3D-polymerized HA (as biological dressing). HA is left in place for 15-20 days. In order to reduce the interference with veno-lymphatic regeneration process, an elastic compressive bandage was applied on the limb. Note: RegenBCT were centrifuged ad 1500g for 10 min and not for 5 min as per instructions for use. Group 2 (control group, 10 pt.): treated with curettage and HA applications in the bed of the ulcers. Medications based on HA were removed after 15 days.

Safety: Generally, no serious complication was observed during the procedure and the post-treatment period.
Performance: Generally, performance data showed that PRP treatment has a beneficial effect for wound care medical indications, which contributes to accelerate wounds healing time and wounds closure, even after failure of standard therapy and to prevent infections.

These data contribute to the safety and performance profile of PRP prepared with RegenKits and support its use for wound care.

The key results described in publications are summarized in the following table:

CHRONIC WOUND	
Key results on performance	**Safety / adverse events (AE)**
- PRP + HA treatment was effective in healing and regeneration of soft and hard tissues: complete wound healing of 4 patients after 21 days, 8 patients after 30 days, with a progressive significant reduction of the score from 15 to 21 days (p<0.001) and from 21 to 30 days (p<0.001). - Healing time was shortened, treated area preserved a satisfying strength in plantar flexion and extension of the ankle: improvement in texture, more rapid healing, good cutaneous elasticity, with a significant reduction of the costs of hospitalization and pain. - Functional rehabilitation in terms of deambulation and joint mobility was complete. ➔ The use of PRP and HA together best significantly the formation of granulation and tissue regeneration as it helps to prevent infection.	Not formally assessed.

CHRONIC WOUND					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
CO_2 laser for the treatment of diabetic foot ulcers with exposed bone. A consecutive series of type 2 diabetic patients Monami M, Mirabella C, Scatena A, Nreu B, Zannoni S, Aleffi S, Giannoni L, Mannucci E *J Endocrinol Invest* 2017 Aug;40(8):819-822.	Prospective pilot study Follow-up: 3 months	Diabetic foot ulcers with exposed bone	14	RegenTHT	Patients were divided in 2 group, according to the treatment: CO_2 laser alone (9 pts.): after washing the ulcers, CO_2- laser was used for producing discontinuities on periosteum of exposed bone. The laser beam (wavelength 10,600 nm) was used in Ultrapulsed mode, with repeat mode of emission (0.05s on/0.70 s off), with 80 W power, providing trains of single pulses with energy of 4 J each. After bleeding, dressing was placed on the bone surface for 5–7 days. Procedure was repeated up to 6 times, 1-week interval, and patients were evaluated weekly. PRP + CO_2 laser (5 pts.): treatment described above was associated with PRP. After bleeding, PRP was applied on an adequate-sized Hyalofil® dressing placed on the bone surface. The procedure was repeated up to 4 times, 1-week interval.
Use of platelet rich plasma and hyaluronic acid on exposed tendons of the foot and ankle Cervelli V, Lucarini L, Spallone D, Brinci L, de Angelis B. *J Wound Care* 2010; 19:186, 188-90	Retrospective study Follow-up: 1 month	Wound with tendon exposure	30	RegenTHT /RegenATS	PRP was prepared as per instructions for use from 16 ml of whole blood. PRP was injected intralesionally, intratendonally or perilesionally. PRP was activated with autologous thrombin (ATS) in a 9:1 ratio. Also, CaGlu was used for topical use of PRP gel. PRP gel was sutured to the tendons and wounds were covered with a 3D polymerized HA-medicated biological dressing.

CHRONIC WOUND	
Key results on performance	**Safety / adverse events (AE)**
- CO_2 laser alone: 4 pts. healed, and 1 patient developed granulation tissue covering entirely bone surface. Out of the 4 patients who did not heal, 1 underwent minor amputation. - PRP + CO_2 laser: 2 pts. healed within 3 months, and 2pts. developed granulation tissue covering entirely bone surface; the 5th patient did not show any improvement and underwent amputation. ➔ The results of the present series of uncontrolled observations are encouraging, with a 43% healing rate within 3 months. It should be recognized that all patients enrolled would have probably undergone amputation, if this new technique had not been applied.	Not formally assessed.
- Wounds closure was successfully achieved during 30 days of follow-up (or earlier) for 28 patients and they were all satisfied with the treatment. - 2 Patients experienced wound opening at the end of the assessment period with skin graft scar contracture in one case. - No infection occurred; contrary, PRP most probably reduces the infection rate in soft tissue defects. ➔ The results add to growing evidence that PRP and HA are useful adjunctive treatments for open wounds by the rapid formation of granulation tissue was facilitated and shorter healing times.	No case of infection was recorded during the treatment period. The one complication reported was related to the skin graft scar contracture and not to PRP treatment.

CHRONIC WOUND					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Effectiveness of Platelet-Rich Plasma to Enhance Healing of Diabetic Foot Ulcers in Patients With Concomitant Peripheral Arterial Disease and Critical Limb Ischemia Kontopodis N, Tavlas E, Papadopoulos G, *et al*. *Int J Low Extrem Wounds.* 2016 Mar;15(1):45-51.	Retrospective study Follow-up: Until wound closure	Diabetic foot ulcers, patients suffering from arterial disease	72	RegenATS	All ulcers were firstly cleansed with saline solution, and when required, surgical debridements were removed from the necrotic tissue. PRP gel was applied on the wound bed and some remaining liquid PRP was also placed on the wound bed. Subsequently, the treated area was covered with moist saline gauze dressing. Treatment was applied twice a week until complete wound healing, otherwise for 16 weeks. Note: RegenATS tube were centrifuge 8 min at 3000 and not 10 min as per instructions of use. Patients were divided into 2 groups based on the severity of PAD according to ankle and/or to pressure measurements: Group A (42 pts): Fontaine classification stages I, IIa, and IIb Group B (30 pts): Fontaine classification stages III and IV
Autologous platelet rich plasma (PRP) and and derived diabetis skin disease Bioulac B. *J Méd Esth et Chir Derm* 2012; 39:223-5	Case reports Follow-up: Until wound closure	Diabetic foot ulcers	2	RegenBCT	1 single injection of 5 ml of PRP into the ulcer and into the surrounding tissue (peri- and intra-lesional injection) with a 30G needle. After treatment, Flammazine cream was applied all over the wound and subsequently covered with an occlusive dressing that was changed every 3 days. Pt.1: 8 months chronic heel wound Pt.2: Necrotic ankle ulcer
Treatment of pilonidal sinus disease with autologous platelet-rich plasma Filomia D, Ventura C, Crescibene A, Almolla J, Napolitano M. *G Ital Dermatol Venereol* 2013; 148:704-6	Case report Follow-up: 6 months	Painful bleeding ulcers 4 years post-fistulectomy	1	Regen Fibrin Polymer 2 / RegenTHT	PRP was prepared as per instructions for use from 8 ml of whole blood. PRP was activated with 10% CaGlu and centrifuged 15 min to obtain platelet gel. Skin area was cleansed with normal saline solution and local anesthesia was injected. Necrotic tissue was removed, platelet gel was directly applied on the bleeding wounds and covered with conventional gauze dressing. This protocol was repeated 10 times on weekly basis.

CHRONIC WOUND	
Key results on performance	**Safety / adverse events (AE)**
-52 patients significantly improved ulcer with > 90% reduction in ulcer area. In 6 patients the ulcer area reduction was between 50% and 90%. In 14 patients there was no benefit from therapy and subsequently they underwent 6 minor and 8 major amputations (20% amputation rate). Finally, limb salvage rated 89% ($p<0.001$). - With regard to clinical improvement (ulcer area reduction >50%), there were 36/42 (86%) improved ulcers in group A and 22/30 (73%) in group B, and this difference was not statistical significant ($P = 0.23$). - Limb salvage was significantly greater among patients in group A ($P < .001$). ➔ PRP could serve as a useful adjunct during management of diabetic foot ulcers even in diabetic patients with unreconstructable arterial disease.	There were no complications from application of GFs observed in this series.
- Treatment was effective on both patients with healing at 21 days and 3 months respectively. ➔ PRP injections promoted wound healing with an initial good cicatrization and subsequent wound closure.	Not formally assessed.
- Treatment was effective compared to previously failure of multiple medical therapy. - Ulcer stopped secretion and reached healing. - Pain significantly decreased from 8 (baseline) to 2 (end of treatment) and to 0 at final follow-up visit. - SF-12[6] health questionnaire = 28 (baseline), 34 (end of treatment) and 180 (final follow-up), the patient resumed his normal social activity. ➔ The platelet gel is a minimally invasive procedure which can be used to accelerate the healing process, it has an analgesic effect too.	At no time there were adverse effects.

CHRONIC WOUND					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Severe hidradenitis suppurativa treatment using platelet-rich plasma gel and Hyalomatrix Nicoli F, Balzani A, Lazzeri D, *et al.* *International Wound Journal*. 2015 Jun;12(3):338-43	Case report Follow-up: 12 months	Chronic skin disease (severe cystic acne lesions on face and back)	1	RegenATS	Wide surgical excision of the nuchal area (15×10 cm) under general anesthesia and resection of lesions were performed. From 40 ml of whole blood, RegenATS tubes were centrifuged and PRP gel was applied over the wound area and the remaining liquid was injected into the perilesional area. Wound was then covered with a non-woven mesh dressing composed of 80% esterified HA. Subsequent dressing changes were done on weekly intervals for 2 months.

SURGICAL WOUND					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Platelet-rich plasma inside the sternotomy wound reduces the incidence of sternal wound infections Serraino GF, Dominijanni A, Jiritano F, *et al.* *Int Wound J.* 2015 Jun;12(3):260-4.	Prospective Controlled study Follow-up: 1 year	Superficial and deep sternal wound infection post-sternotomy	1093	RegenTHT	All patients received full median sternotomy and a cardiac surgery procedure with extracorporeal circulation. <u>Group 1 PRP (422 pts.):</u> After PRP preparation as per instructions for use, 2 ml of upper plasma were discarded, PRP was activated with 0.5 ml of $CaCl_2$ or CaGlu. PRP was applied on the sternum before closure of subcutaneous tissue. <u>Group 2 control (671 pts.):</u> median sternotomy without application of PRP.
Ventricular assist device abdominal driveline infection: Treatment with platelet-rich plasma Jiritano F, Serraino GF, Cristodoro L, Renzulli A. *J Thorac Cardiovasc Surg* 2013; 145:e69-70	Case reports Follow-up: Until complete wound closure	Abdominal driveline infection due to long-term use of LVAD[7]	2	RegenTHT	Pt.1) *Escherichia coli* grew within erythematous skin, the patient did not respond to antibiotics. Protocol: the exit site wound was dressed topically with autologous PRP every 3 days for 5 times. Pt.2) *Acinetobacter Baumannii* and *Staphylococcus aureus* grew in the abdominal exit site, the infection worsen after 2 weeks of antibiotics and VAC therapy. Protocol: topical application of PRP and wound dressing every 3 days for 2 weeks.

CHRONIC WOUND	
Key results on performance	**Safety / adverse events (AE)**
- The combination of HA + PRP enhanced tissue proliferation and promote wound healing. - The patient was discharged from the hospital after 24 hours. - Complete wound healing was achieved in 2 months. - The patient was followed-up to 1 year and no recurrence, infection or wound dehiscence was observed. ➔ Application of PRP gel with HA is a new concept in the management of hidradenitis suppurativa. Its application is easy, compatible and provides excellent results without additional morbidity.	No AE reported. No recurrence, infection or wound dehiscence was observed 1 year following the surgical procedure. No scar contractures, itching, pain, irritation, hypertrophic scars or restriction of neck movements were observed or reported in this patient.

SURGICAL WOUND	
Key results on performance	**Safety / adverse events (AE)**
The two groups were homogeneous for preoperative and intraoperative risk factors. PRP Treatment was significantly more effective: - Only one patient in PRP group developed deep sternal wound infection compared with 10 patients in control group (*P* =0.043). - Occurrence of superficial sternal wound infections was significantly lower in PRP group than in control group (*P* =0.006). - The cost of a preventive treatment with PRP is definitely lower than 1-day hospital stay. ➔ PRP provided the surgeon a potential tool to help preventing a dreadful complication such as deep or superficial sternal wound infections.	No adverse reaction was recorded in patients who received PRP treatment.
Pt.1 → At the 28th post-operative day the patient achieved good tissue healing with complete wound closure. Pt. 2 → At the end of the treatment the infection disappeared, and the tissue healed completely ➔ In our 2 cases, the topical application of autologous PRP helped in the treatment of abdominal driveline infection refractory to antibiotic treatment alone.	No signs of skin infection were found around the driveline.

SURGICAL WOUND					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Ventricular assist device driveline infection: treatment with platelet-rich plasma Jiritano F, Serraino GF, Rossi M, Dominijanni A, Brescia A, Renzulli A. *Ann Thorac Surg* 2013; 96:e37-8	Case report Follow-up: 2 years	Driveline infection: retroauricular dehiscence of pedestal wound 3 weeks after implantation of LVAD[8]	1	RegenTHT	*Staphylococcus epidermidis* was grown in the wound. The wound was surgically debrided and then dressed topically with autologous PRP every 3 days for 2 weeks of treatment.

BURNS					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Autogenous platelet-rich plasma gel with acellular xenogeneic dermal matrix for treatment of deep II-degree burns. Hao T, Zhu J, Hu W, Zhang H, *et al.* *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi.* 2010 Jun;24(6):647-9. (Abstract)	Case series Follow-up: 3-4 weeks	Deep II degree burns	30	RegenTHT /RegenATS	All patients were treated with tangential excision surgery, one side of the wounds were covered with autogenous PRP gel and acellular xenogeneic dermal matrix (PRP group), the other side of the wounds were covered with acellular xenogeneic dermal matrix only (control group).
Therapeutic protocol using growth factors in electrocution wounds--case reports and review of the literature Teodoreanu RN, Popescu SA, Lascar I, Vulturescu V, Grigore A. *Rom J Morphol Embryol* 2014; 55:473-82	Case reports Follow-up: Until wound closure	Burnt tissue post-high voltage electrocution injuries	3	RegenBCT/ RegenATS	PRP was activated with calcium Gluconate (CaGlu) and autologous thrombin. A volume of 0.9 mL of PRP was mixed with 0.1 mL Calcium Gluconate or Thrombin, in order to obtain 1 mL of activated PRP. The treatments were performed in a seven days interval. Affected areas were treated with insulin (topical application), PRP (topical application, mesotherapy or papule injections) or untreated area (control), with or without skin grafting.

SURGICAL WOUND	
Key results on performance	**Safety / adverse events (AE)**
- After 2 weeks of treatment, granulation tissue was observed, and direct wound closure was performed under local anaesthesia. ➔ This study supports the possibility that the topical application of autologous PRP could be a potential solution to prevent or treat driveline infections.	The treatment was safe, no recurrence of infection has been observed after 2 years of follow-up.

BURNS	
Key results on performance	**Safety / adverse events (AE)**
- At 7 days after operation, the infection rate in PRP group (2/30) was significantly lower than that in control group (5/30, $P < 0.05$). - The healing times were significantly lower in PRP group than that in control group (18 vs 22 days respectively, $P < 0.05$). - The healing rates at 14 days and 21 days were 75% and 88% in PRP group and were 62% and 73% in control group, showing significant difference ($P < 0.05$). - PRP group was superior to control group in elasticity, color, appearance, softness, scar formation, and healing quality. ➔ Autogenous PRP gel with acellular xenogeneic dermal matrix can accelerate the wound healing of deep II degree burns as well as alleviate the scar proliferation.	Not formally assessed.
- Local increase of granulation tissue in the 48 hours post PRP treatment with visible accelerated compared to the untreated area or the insulin treated area. - The PRP-treated area presented an intense proliferation of inflammatory elements, an improved vascularization and an agglomeration of cellular. - Shortening of hospitalization after PRP by about seven days. - No lesion reoccurrence. ➔ PRP limits the development of ischemic areas, and shortens surgical and pharmacological treatment, as well as the duration of hospitalization.	Not formally assessed.

4.1.8 GYNECOLOGY AND UROLOGY

11 publications were identified including clinical data on 219 patients.
Among them, RegenKits have been used for 3 different subtypes of indications:

8.A. Gynecology (7 publications)
RegenKits and Cellular-Matrix have been used on 104 women for the treatment of vulvo-vaginal atrophy, vaginal mesh exposure, lichen sclerosus, and dyspareunia (female sexual dysfunction)

8.B. Fertility (2 publications)
RegenKits have been used on 12 women to stimulate ovarian regeneration and folliculogenesis activation in peri-menopausal women.

8.C. Male dysfunctions (2 publications)
RegenKits and Cellular-Matrix have been used on 103 men for the treatment of Peyronie's disease.

GYNAECOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Use of Platelet-rich Plasma for vulvovaginal autoimmune conditions like Lichen sclerosus Behnia-Willison F, Pour NR, Mohamadi B *et al*. *Plastic and reconstructive surgery Global open* 2016; 4: e1124	Case series Follow-up: 12 months	Lichen sclerosus unresponsive to steroid treatment	28	RegenBCT	PRP was prepared as per instructions for use. PRP was injected under local anaesthesia (lignocaine, 23%; tetracaine, 7%) to any affected areas of the external genitalia, including the labia majora, labia minora, clitoris, and clitoral hood. The injection was carried out using a 27-gauge needle in a fanning motion to break the scar and fibrotic tissue and retrograde injection of PRP in the tissue. Patients received 3 PRP treatments, 4 to 6 weeks apart and again at 12 months.
An innovative approach to treating vaginal mesh exposure after abdominal sacral colpopexy: endoscopic resection of mesh and platelet-rich plasma; initial experience in three women Castellani D, Valloni A, Piccirilli A, Paradiso Galatioto G, Vicentini C. *Int Urogynecol* 2017; 28: 325	Case series Follow-up: 12 months	Vaginal vault mesh exposure after laparoscopic ASC with concomitant hysterectomy	3	RegenBCT/ RegenATS	Transvaginal endoscopic resection of the extruding prosthetic fragment was conducted by using Iglesias resectoscope and Gyrus device. PRP and autologous thrombin serum (ATS) were prepared according to instructions for use: 4 ml PRP + 1 ml calcium gluconate + 1 ml activated thrombin. PRP was infiltrated along the margins of the breach created by the resection with a 25G spinal needle. PRP and ATS gel was infiltrated along the margins of the breach created by the resection.

Safety: Generally, minimal-moderate pain was reported in few patients and this was limited to the first 24 hours post-treatment. No serious AE was reported.
Performance: Generally, performance data showed that PRP treatment has a beneficial effect for several gynaecology medical indications; also, PRP was shown to be effective in patients who are not responsive to other therapies such as steroids treatment. In addition, the combination PRP+HA was shown to be effective for the treatment of vulvo-vaginal atrophy in women and Peyronie's disease in men.

These data contribute to the safety and performance profile of PRP prepared with RegenKits and PRP-HA prepared with Cellular-Matrix for uro-gynaecological indications.

The key results described in publications are summarized in the following table:

GYNAECOLOGY	
Key results on performance	**Safety / adverse events (AE)**
Based on the Australian Pelvic Floor Questionnaire 24 and colposcopy results: - Almost all patients showed clinical improvement in the size of their lesions, and in 8 cases, lesions totally disappeared after treatment. - Symptoms disappeared in 15 of the 28 patients after treatment, with no need for further steroid therapy in 23 patients. 13 women experienced partial symptom relief. ➔PRP presents a potential alternative to topical steroids for treatment of vulvovaginal autoimmune conditions such as lichen sclerosus.	Patients reported minimal to moderate pain. During the 24 hours after the procedure, 26 patients (92.9%) reported pain scores of 2 to 3; the remaining patients reported scores of 5 and 7, respectively. No cases of infection, bleeding, hematoma, or other adverse outcomes.
- 30 days post-PRP injections, physical urogynecological and vaginoscopy examinations showed tissue response to the treatment. Full recovery was obtained already 6-month post injections. - All women recovered sexual function, and nobody experienced relapsed pelvic organ prolapse at 1- year follow-up. ➔ Bipolar plasmakinetic resection (BPR) combined with PRP is an effective technique for treating vaginal mesh exposure with conservation of anatomical results and sexual function. PRP represents an alternative method to suture stitches, being decisive in solving problems of responsiveness in hysterectomy surgeries.	No complications were observed during surgery. 2 patients complained of mild vaginal itching lasting for 1 month.

GYNAECOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Experience in management of patients with lichen sclerosus and HPV infection of the vulva Chernova NI, Bagaeva MI, Stovbun SV *et al.* 2016, *Article in Russian (translated)*	Case series Observational study Follow-up: 1 month	Lichen sclerosus accompagnied by co-infections (HPV)	40	RegenACR-C Extra / RegenBCT	PRP was prepared according to instructions for use. 4-8 ml (two vacuum tubes) of platelet-rich plasma (PRP) was injected in the affected area with a 30G needle using a mesotherapy technique. PRP + infrared laser radiation + panavir gel. Treatment course included 10 sessions (on a daily basis). A total of 2-3 courses have been carried out with 4-week long intervals.
Efficacy of injecting platelet concentrate combined with hyaluronic acid for the treatment of vulvovaginal atrophy in postmenopausal women with history of breast cancer: a phase 2 pilot study. Hersant B, SidAhmed-Mezi M, Belkacemi Y, Darmon F, *et al.* *Menopause: The Journal of The North American Menopause Society* 2018, Vol. 25, No. 10	Pilot study Follow-up: 6 months	Vulvo-vaginal atrophy (VVA)	20	Cellular Matrix BCT-HA kit	The PRP+HA combination obtained with the Cellular Matrix BCT-HA Kit as per instructions for use. After an anesthetic ointment, 4mL of PRP-HA was injected into the vestibule (posterior wall of the introitus) and the first 3 cm of the posterior wall of the vagina using a point-by-point technique. The injections were made every 5mm using a 27G caliber needle and a 1-mL syringe, bevel upwards and tangential injection 60° into the mucosa and lamina propria using a speculum or by laterally opening the vaginal walls with the fingers if the participant could not tolerate a speculum.
A pilot study of the effect of localized injections of autologous platelet rich plasma (PRP) for the treatment of female sexual dysfunction Runels C, Melnick H, Debourbon E, Roy L. *J Women's Health Care* 2014; 3-4	Case series Follow-up: 12 weeks	Female sexual dysfunction (dyspareunia)	11	RegenBCT	5 ml of PRP was obtained from 10 ml of whole blood. PRP was activated with 0.5 ml of 10% $CaCl_2$ to form a platelet-rich fibrin matrix (PRFM). 2 PRFM injections administered with 27-G needle: one into the interior vaginal wall, between vagina and urethra, and the other one into the clitoris.

GYNAECOLOGY	
Key results on performance	**Safety / adverse events (AE)**
- All patients reported a decrease in the intensity of itching and burning on the day following the injection. - On the 3rd-7th day they reported reduced edema, moisturized skin and mucosa of the vulva. - On the 10th day of treatment, dysuria symptoms disappeared (8 women) or significantly decreased (16 women). All patients reported an improvement in the quality of sexual activity. - The results continued to improve during the first month and after 6 months. ➔ Combination therapy using PRP, infrared laser radiation and Panavir gel for external and topical application in patients with early signs of lichen sclerosus was effective in 90% of cases (36/40).	No complications were seen after the treatment.
- The vaginal health index score increased significantly from baseline and 1-month post treatment and carry on increasing at 3 and 6 months. - Functional results (volume of vaginal secretions, elasticity and epithelial integrity, moisture rate and vaginal pH) improved significantly after treatment. - The quality of life of patients improved significantly after treatment to reach 17% of improvement at 6 months (FSD score). - 19/20 patients were satisfied by the treatment (VAS score). ➔ The injection of PRP-HA appeared to be a promising method to improve the trophicity and hydration of vaginal mucosa for the treatment of VVA in postmenopausal breast cancer survivors with contraindications to hormone therapy.	No adverse events were observed or reported by the participants.
- Clinical findings from FSDS-R and FSFI patient's questionnaires suggested a trend of improvement after injection. 71% of patients improved from "distressed" to "not distressed", with positive changes in sexual-related dysfunctions (desire, arousal, lubrification, orgasm). - Improvement in satisfaction and pain were noted but were not statistically significant. ➔ The preliminary results of this pilot study suggest that specifically placed intravaginal and intraclitoral PRP injections could be an effective method to treat certain types of female sexual dysfunction, especially in the areas of desire, arousal, lubrication and orgasm.	No serious AE reported. Side effects were observed in 2 patients and including sexual arousal with urination and continuous sexual arousal, ejaculatory orgasm, and spontaneous orgasm. Except for ejaculation, these responses only lasted 1 to 2 weeks. All side effects solved spontaneously.

GYNAECOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Novel technique of vulvo-vaginal Rejuvenation by lipofilling and injection of combined platelet-rich-plasma and hyaluronic acid: a case-report Aguilar P, Hersant B, SidAhmed-Mezi M, *et al.* *SpringerPlus* 2016; 5:1184	Case report Follow-up: 3 months	Vulvo-vaginal rejuvenation	1	Cellular Matrix BCT-HA kit	Vaginal lipofilling and perineal rejuvenation with injections of the PRP+HA combination obtained with the Cellular Matrix BCT-HA Kit. Fat tissue was harvested by Coleman's technique in the lower abdomen area and was then centrifuged at 1500g for 1 min. 12 ml of whole blood was harvested for PRP+HA preparation. 4 ml pf PRP+HA was obtained after a centrifugation at 1500g for 5 min. 16 ml of purified fat cells were injected into two linear antero-posterior tractus in the posterior vaginal wall and 10 ml of the PRP+HA solution in the perineum focusing in the episiotomy scar.
Platelet-rich plasma (PRP) for the treatment of vulvar lichen sclerosus in a premenopausal woman: A case report. Franic D, Iternička Z, Franić-Ivanišević M. *Case Rep Womens Health*. 2018 Apr 16;18:e00062.	Case report Follow-up: 4 months	Lichen sclerosus unresponsive to steroid treatment	1	Cellular Matrix BCT-HA	PRP-HA was prepared as per instructions for use. Anesthetic gel was applied to the vulva and left in place for 10 min. Then 4 ml of PRP was injected subdermally with a 23 G needle in the affected region. The therapy was repeated two months later. This time, two tubes of Cellular-Matrix RegenKit were used, to obtain 8 ml of PRP.

GYNAECOLOGY	
Key results on performance	**Safety / adverse events (AE)**
- Pre-operative Stabbatsberg score was 15/40. - 3 months after treatment symptoms improved significantly. Flatus incontinence disappeared completely. Vaginal appearance was tighter with a diminished vaginal caliber. Clinical improvement was also observed in vaginal mucosa trophicity and in episiotomy Scar, which was softer and more hydrated. - At 3 months Stabbatsberg scale score was 30/40 (100% improvement). ➔ Vulvo-vaginal rejuvenation lipofilling and an injection of combined platelet-rich-plasma and hyaluronic acid is a minimally invasive technique that is safe and easy to perform. Further studies are necessary to assess more thoroughly the effectiveness and safety of this procedure.	No atrophy or hardening was found, nor any signs of infection.
Before treatment, the patients showed a validated International Consultation, on Incontinence Questionnaire – Vaginal Symptoms (ICIQ-VS) score of 42, a sexual symptom score at 42, and a quality of life score at 8. The satisfaction Female Sexual Function Index (FSFI) score was 3.6. - After treatment, the epidermis was nearly normal and upper dermal cellularity had been restored. The patient felt comfortable and was symptom-free. - Her sex drive had returned, and her quality of life had increased significantly. This was confirmed by her ICIQ-VS total score at 7, sexual matters score at 0, and quality-of-life score also at 0. Her full-scale FSFI score was now 32.6. ➔ In conclusion, our case highlights that PRP seems a promising new treatment for female genital LS, as it promotes regeneration and leads to cessation of symptoms, which is generally not achieved with the standard current therapy options.	Not formally assessed.

In addition, 6 articles pending for scientific publications or conferences were identified including clinical data on 245 patients. Among them, RegenKits have been used for the treatment of stress, urge and coital incontinence and mesh exposure. Generally, no major side effects were reported, some urinary tract infections (UTI) occurred. Performance data showed that PRP treatment in case of failure of laser or in combination with laser has a beneficial effect with clinical improvement.

GYNAECOLOGY					
Data to be submitted for publications/conferences					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Fractional Co2 laser for treatment of stress urinary incontinence Behnia-Willison & Nguyen Submitted and accepted for publication in *European journal of Obstetrics and Gynecology* 2018	Prospective cohort study	Stress urinary incontinen-ce	58	RegenBCT after failure of Co2 laser	Laser treatment of the entire vaginal canal was used. In failed cases, periurethral injection of PRP (in Ten women) was performed. Three PRP treatments was realized one month apart.
Fractional Co2 laser and PRP for treatment of stress urinary incontinence in severe atrophic vagina Behnia-Willison & Nguyen pending publication *European journal of Obstetrics and Gynecology* 2018	Prospective cohort study	Stress urinary incontinen-ce	57	RegenBCT	Laser treatment of the entire vaginal canal was used and periurethral injection of PRP was performed. Three PRP treatments was realized one month apart.
Fractional Co2 laser and PRP for treatment of urge urinary incontinence in severe atrophic vagina Behnia-Willison & Nguyen pending publication *European journal of Obstetrics and Gynecology* 2018	Prospective cohort study	Urge incontinen ce	62	RegenBCT	Laser treatment of the entire vaginal canal was used and anterior vaginal wall injection of PRP was performed. Three PRP treatments was realized one month apart.

GYNAECOLOGY	
Data to be submitted for publications/conferences	
Key results on performance	**Safety / adverse events (AE)**
Objective assessment: Positive cough test and urodynamic study Subjective assessment: Australian Pelvic Floor Questionnaire at baseline, 3 months and 12 months after treatment ➔ SI improvement: 75% improvement.	No major side effect reported, x2 UTIs only, 1 genital herpes flare.

Objective assessment: Positive cough test and urodynamic study Subjective assessment: Australian Pelvic Floor Questionnaire at baseline, 3 months and 12 months after treatment ➔ SI improvement: 65% improvement.	No major side effect reported; x2 UTIs only
Objective assessment: Positive cough test and urodynamic study Subjective assessment: Australian Pelvic Floor Questionnaire at baseline, 3 months and 12 months after treatment ➔ 74% improvement of urge incontinence.	No major side effect reported; x3 UTIs only

GYNAECOLOGY					
Data to be submitted for publications/conferences					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Fractional Co2 laser and PRP for treatment of coital urinary incontinence Behnia-Willison & Nguyen pending publication *European journal of Obstetrics and Gynecology* 2018	Prospective cohort study	Coital incontinence	34	RegenBCT	Laser treatment of the entire vaginal canal was used and anterior vaginal wall injection of PRP was performed. Three PRP treatments was realized one month apart.
Pelvic Floor Medicine with Co2 laser and PRP: Chance, Change, Challenges Behnia-Willison & Nguyen Submitted to *AGES XIX Pelvic Floor Symposium Conference, Brisbane , Australia* 2018	Prospective cohort study	Stress and urge incontinence	SI: 62 UI: 66 CI: 34	RegenBCT	Periurethral injection of PRP and laser treatment of the entire vaginal canal were performed. Three PRP treatments was realized one month apart.
Treatment of vaginal mesh exposure with platelet rich plasma and Regen ExtraCell membrane Behnia-Willison Submitted to *AGES XIX Pelvic Floor symposium conference, Brisbane, Australia* 2018	Prospective cohort study	Symptomatic mesh exposure	34	RegenKit Surgery / Regen Extracell membrane	Patients were given topical Oestrogen cream for 3 months, if the mesh exposure was still a problem they were treated with PRP, if the problem persisted they underwent surgical excision of the mesh.

GYNAECOLOGY	
Data to be submitted for publications/conferences	
Key results on performance	**Safety / adverse events (AE)**
Objective assessment: Positive cough test and urodynamic study Subjective assessment: Australian Pelvic Floor Questionnaire at baseline, 3 months and 12 months after treatment. ➔ 54% improvement of coital incontinence.	No major side effect reported; x2 UTIs only
Objective assessment: Positive cough test and urodynamic study Subjective assessment: Australian Pelvic Floor Questionnaire at baseline, 3 months and 12 months after treatment. ➔ SI: Improvement of 62% at 12 months UI: improvement of 60% at 12 months CI: Improvement of 50% at 12 months	SI: No major side effect reported, x2UTIs only, 1x genital herpes flare. UI: No major side effect reported, x3 UTIs only.
6 women improved with Oestrogen alone. 4 patients had PRP after Oestrogen has failed, with complete resolution. 14 women had surgical excision and injection of the primary closure site with PRP, 3 patients needed repeat excision of the mesh. 10 patients opted to wait and see and do nothing.	No major side effect.

FERTILITY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
First data on in vitro fertilization and blastocyst formation after intra-ovarian injection of calcium gluconate-activated autologous platelet rich plasma Sills E, Rickers N, Li Xand Palermo G *Gynecological Endocrinology*, 2018 Feb 28:1-5.	Case series Follow-up: 2 weeks	Infertility treatment (blastocyst formation)	4	RegenBCT	From 8 ml of whole blood (centrifuged 5 min at 1500g), less than 3 ml of supernatant (PPP) was aspirated before recapping the vial for gentle tube inversion. PRP activation was achieved with calcium gluconate. Direct injection into ovarian stroma with a 17G needle. The active PRP was slowly introduced. 1 ml of sample was deposited under the ovarian capsule.
Ovarian rejuvenation and folliculogenesis reactivation in perimenopausal women after autologous platelet rich plasma treatment Pantos K, Nitsos N, Kokkali G, *et al.* *ESHRE (European Society of Human Reproduction and Embryology). Annual Meeting. Helsinki, Finland;* 2016 (Abstract and poster)	Case series Follow-up: 3 months	*In vitro* ovarian rejuvenation and folliculogenesis reactivation in peri-menopausal women	8	RegenACR-C Classic / RegenBCT	PRP was prepared as per instructions for use. PRP was infused into the ovaries using a transvaginal ultrasound-guided Injection technique. When a follicle of >16 mm was observed, ovulation triggering was achieved with 5000 IU of hCG and follicle aspiration was performed 32 hours later by the transvaginal route.

FERTILITY	
Key results on performance	**Safety / adverse events (AE)**
- Increase in AMH hormone after treatment (no significant) and decrease in FSH ($p<0.01$), sufficient to permit retrieval MII oocytes. - IVF occurred, and all patient had at least one blastocyst suitable for conservation. ➔ Evidence of improved ovarian function was noted in all who received intraovarian PRP, possibly as early as two months after treatment. Additional research is needed to clarify (and enhance) which PRP components are responsible for altered ovarian function, and to identify predictive characteristics for patients most likely to benefit from this intervention.	No serious AE was reported, only mild pain.

- The successful ovarian rejuvenation was confirmed by the menstrual cycle restoration 1 to 3 months after the ovarian PRP treatment. - Oocyte retrieval was successful in all cases, resulting in 2.50±0.71 follicles of 15.20±2.05 mm diameter, 1.50±0.71 oocytes and 1.50±0.71 MII oocytes. - All mature oocytes were inseminated by ICSI and the 1.50±0.71 resultant embryos were cryopreserved at 2pn stage until transfer. ➔ PRP therapy may extend the fertility potential of perimenopausal women, rendering oocyte donation IVF cycles as an ultimate option.	Not formally assessed.

MALE DYSFUNCTIONS					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Evaluation of the benefit of using a combination of autologous platelet rich-plasma and hyaluronic acid for the treatment of Peyronie's disease Virag R., Sussman H., Lambion S. and de Fourmestraux V. *Sex Health Issues,* 2017 Volume 1(1): 1-6	Case series Observational study Follow-up: Up to 10 months	Penile deformations and/or curvatures due to plaques palpated in the tunica albuginea (TA)	90	RegenBCT and Cellular Matrix / BCT-HA	All patients underwent 3-steps penile local anaesthesia. 4 ml of PRP and 4 ml of a combination of PRP/HA were prepared with a RegenBCT tube and a BCT-HA tube, as per instructions for use. Both preparations were collected in a syringe and mixed together and injected into the TA's diseased sites under Duplex Ultrasound control. All patients underwent 4 treatments administered with 15-day interval. If needed, treatment was repeated each 3 months.
A new treatment of lapeyronie's disease by local injections of plasma rich platelets (PRP) and hyaluronic acid. Preliminary results Virag R, Sussman H, Lobel B *e-mémoires de l'Académie Nationale de Chirurgie*, 2014, 13 (3) : 096-100	Case series Observational study Follow-up: 9 months	Lapeyronie's Disease (8 patients with curvatures angles >50° and 5 patients with deformities such as strictures and/or deviation <30°)	13	Cellular Matrix BCT-HA	8 ml of the PRP+HA combination (prepared with 2 Cellular Matrix BCT-HA tubes, as per instructions for use) were injected with 18-gauge or 25-gauge needle. All intra-lesional injections were performed under ultrasound-guidance; local anesthesia was applied before treatment administration All patients received 4 treatment sessions administered within 2 months with 2 weeks interval.

MALE DYSFUNCTIONS	
Key results on performance	**Safety / adverse events (AE)**
- Deformation and thickening of the TA one month after 4 treatment sessions (n = 84) and at the last follow-up visit (n = 54) showed a significant decrease: mean gain percentage of degree =39.65 ± 24.83% and mean gain percentage of mm = 26.22 ± 10.29%. - Presence of calcifications (n=28) did not change significantly the results but a decrease was detected in 6 patients. - Peyronie's disease questionnaire was significantly improved in all domains: 62.2% of the patients felt a decrease in their deformation, 43.3% judged that their erections were better, 46.7% thought that sexual activity was easier and 67.8% considered that the treatment improved their initial condition. ➔ Repeated intralesional injections of PRP-HA improve significantly penile deformation and thickening of the TA. This fully natural treatment may stand as a first line treatment of PD.	No serious adverse events were recorded. Ecchymosis was present in 16.7% of the patients and marked hematoma in 10%. Two patients suffered from transient hypotension after local anesthesia. One patient stopped the treatment after 4 sessions because of worsening symptoms.

- The combination of PRP and HA was effective for the majority of patients presenting Peyronie's disease. 10 Patients (77%) got improvements with a 30° of decrease in penis curvature. - Also, density and size of plaques was decreased in 7 patients (53%). - US-images showed five reductions in echogenicity and plaques volume and reduction in density of albuginea images. ➔ Positive results of this short preliminary series of patients treated by intralesional injections of PRP+HLA in LaPeyronie's disease are encouraging for the methodology (simplicity) and the practice (innocuity and efficacy).	Treatment was safe, one patient developed a hematoma, probably related to dorsal vein punction of which was solved with local ointment.

4.1.9 DENTISTRY, MAXILLOFACIAL AND OTORHINOLARYNGOLOGY

10 publications were identified including clinical data on 299 patients.

Among them, RegenKits have been used on 236 patients for the treatment of tooth avulsion, fracture of atrophic mandibles or other maxillary and mandibular lesions/defects/atrophy, chronic osteomyelitis of the nasal root, tympanic membrane perforation, empty nose syndrome, severely hearing-impaired and atrophic alveolar bone of the maxilla (or mandible).

DENTISTRY, MAXILLOFACIAL AND OTORHINOLARYNGOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
New technologies for the prevention and treatment of empty nose syndrome: minimally invasive and regenerative surgery with PRL Di Rienzo Businco L, Di Mario A, Tombolini M, Laurino S, Crescenzi D, Radici M. *Aesthetic Medicine* 2016; 2:25-32.	Prospective Randomized controlled study Follow-up: 12 months	Empty nose syndrome	46	RegenBCT	Group A (23 pts. Medical treatment, control): intranasal spray with a solution of salt-bromineiodine thermal water (3 spurts per nostril 3 times daily) together with the nightly application of a nasal unguent Group B (23 pts. PRP experimental treatment): endoscopic reconstruction of inferior turbinates with PRP (activated with $CaCl_2$ 10%) mixed with purified autologous fat (PRL: platelet rich lipotransfert), followed by medical treatment as described for Group A. The purified fat was obtained after the transumbilical extraction with Coleman's technique and inserted aseptically into a syringe of 1ml mixed with PRP.
The role of bone marrow aspirate cells in the management of atrophic mandibular fractures by mini-invasive surgical approach: Single-institution experience Mannelli, G., Arcuri, F., Conti, M., Agostini, T., Raffaini, M., & Spinelli, G. *Journal of Cranio-Maxillofacial Surgery* 2017, May;45(5):694-703.	Prospective Randomized Comparative study Follow-up: 12 months	Fracture of atrophic mandibles caused by: falls / vehicle accidents / assaults	35 (26 of them with bilateral fractures)	RegenKit Extracell BMC / RegenTHT	All fractures were treated by open reduction, the type of osteosynthesis varied among the two groups: Group A (17 patients): Bone graft + standard osteosynthesis technique and autologous stem cells. Mini-invasive skin incision approach. BMC+PRP+ activation with thrombin and $CaCl_2$; this preparation was inserted in the periosteum once the osteosynthesis plates were positioned and fixed. BMC was prepared as per instructions for use from 32 ml of BM. PRP was obtained after centrifugation of 200 ml collection bag containing citrate anticoagulant. Group B (18 patients), control: Bone graft + large reconstructive osteosynthesis plates.

Safety: Safety data included mainly mild to severe pain during the post-operative day, local wound complications and diffused mandibular and cervical hematoma (in 5 patients). Regarding patients treated for the empty nose syndrome, few sporadic cases of nasal burns and minimal discharge mixed with blood after nose-blowing, requiring oral paracetamol, have been reported.
Performance: Performance data showed that Bone marrow concentrate (BMC) and PRP preparation promote faster recovery post-surgical intervention with limited post-operative complications compared to standard osteosynthesis technique. In patients treated for severe maxillary atrophy, BMC and PRP preparations promoted osseointegration of the graft and sufficient bone formation for further implant placement.

Safety and performance clinical data of BMC and PRP, prepared with RegenKits, suggest that these autologous and fresh cells preparations represent valid option for the dental, maxillofacial and otorhinolaryngology treatments.

The key results described in publications are summarized in the following table:

DENTISTRY, MAXILLOFACIAL AND OTORHINOLARYNGOLOGY	
Key results on performance	**Safety / adverse events (AE)**
- Only patients treated with PRL showed a statistically significant improvement ($p<0.05$) in the subjective nasal symptoms and the endoscopic nasal objectivity after surgery. - Almost complete reepithelialization of the mucosal surface of the turbinate along with reduction of the inflammatory part of the submucosa after 12 months have been observed in the areas subjected to a reconstruction with PRL. ➔ Turbinate reconstruction with PRL is a safe, simple and effective procedure characterized by a very low invasiveness with easy availability to autologous biological tissue and no collateral effects.	No cases of epistaxis, nor any general or local complications in the nasal sites treated with PRL (synechiae, crusting formation) were found. Few sporadic cases of nasal burns and minimal discharge mixed with blood after nose-blowing, requiring oral paracetamol.
- Lower incidence of postoperative complications in group A (not significant): Group A) 10 patients reported mild to moderate pain during their first postoperative day; 1 patient showed extended cervical hematoma and 1 patient showed wound dehiscence. Group B) 2 patients reported local severe pain during the postoperative stay, together with local wound complications (dehiscence and infection). 5 patients presented diffused mandibular and cervical hematoma. 2 patients showed non-union of the fracture. - Faster recovery with an overall hospital stay 1 day shorter in group A compared to standard technique (not significant). ➔ Mini-invasive open approach with autologous bone graft (BMC) is a safe and useful procedure for atrophic mandibular fractures ensures a fast recovery and lower complication rate when compared to standard technique.	No major intraoperative complications were seen in any patients.

DENTISTRY, MAXILLOFACIAL AND OTORHINOLARYNGOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
The Effect of PRP-enriched Gelfoam on Chronic Tympanic Membrane Perforation: A Double-blind Randomized Clinical Trial. Saeedi M, Ajalloueian M, Zare E, Taheri A, Yousefi J, *et al.* *Int Tinnitus J.* 2017 Dec 1;21(2):108-111.	Prospective Randomized Controlled clinical trial Follow-up: 12 months	Tympanic Membrane (TM) perforation	24	RegenBCT	Intervention group (n=12): tympanoplasty +PRP-enriched gel foams Control group (n=12): tympanoplasty + normal saline-enriched gel foams PRP was prepared as per instructions for use from 8 ml of whole blood. Small (1 and 2 mm) and two large (1 to 2 mm more than size of perforation) gel foams were prepared and enriched with PRP (or saline) in galipot. Following anesthesia, sharp rosen was used for trimming of TM perforation margins and gel foams were put in the middle ear. Then one of the large gel foams was located in middle ear, under TM, and another one was fixed in ear canal on the TM. Ear canal was filled with small gel foams and Tetracycline mesh.
Mesenchymal stem cells in post-surgical cavities of large maxillary bone lesions. Bertolai R, Catelani C, Signorini M, Rossi A, Giannini D. *Clin Cases Miner Bone Metab.* 2016; Sep-Dec;13(3):214-220.	Controlled study Follow-up: 24 months	Large maxillary bone lesions	20	RegenKit ExtraCell Glue / RegenTHT, Regen ATS	Test group PRP+MSC (10 pts): The bone marrow was withdrawn of the iliac crest to prepare stem cells. MSC and PRP were prepared as per instructions for use. Finally, autologous thrombin serum was added to induce platelet activation. All this was placed inside the residual bone cavity after enucleation and surgical debridement. After that the flap was sutured. Control group (10 pts): enucleation and surgical debridement
Bone graft and mesenchymal stem cells: clinical observations and histological analysis Bertolai, R., Catelani, C., Aversa, A., Rossi, A., Giannini, D., & Bani, D. *Clinical Cases in Mineral and Bone Metabolism,* 2015; 12:183-7.	Controlled study Follow-up: 3 months	Severe maxillary atrophy, all patients required bilateral maxillary sinus augmentation	20	RegenKit ExtraCell Glue RegenTHT, Regen ATS	Each patient was randomly assigned a "test" side and a "control" side: "Test" side: composed by corticocancellous freeze-dried bone allograft (FDBA) chips engineered in a bone marrow mesenchymal stem cells (MSCs) concentrate. In the sterile surgical field, a container was filled with lyophilized bone tissue. The bone was then rehydrated by soaking in freshly prepared PRP and then absorbed with concentrated MSCs. Finally, autologous thrombin serum was added to induce platelet activation. MSC and PRP were prepared as per instructions for use. "Control" side: graft composed by corticocancellous freeze-dried bone chips.

Key results on performance	Safety / adverse events (AE)
- Complete TM healing was seen in 8 (66.67%) patients in intervention group and 3 (25%) patients in control group three months after intervention (p = 0.031). - At the endpoint of the study, intervention group had a mean hospitalization duration of 5 ± 1.28 hours; while it was 4.25 ± 0.62 hours in control group (p = 0.087). - Treatment and hospitalization costs were the same for both intervention and control group. ➔ Addition of PRP to conventional gelfoams used in TM perforation repair increases the complete healing rate of TM perforation with less morbidity and complications.	No complications were seen in intervention group; while three otorrhea cases were recorded for control group in the first follow up after intervention which were resolved by medication.
- Patients with mandibular lesions showed a rapid and very good healing with an 85-90% ossification of the major defect at 12 months and patients with maxillary bone lesions showed good tissue healing but a large loss of vestibular bone. - At 8 months after surgery histological examination of bone showed a complete new bone formation. - Radiographic controls of test group showed a faster bone healing than the control group (12 months vs 24). ➔ Through the use of stem cells, a greater ossification of the residual cavity (85-90%) was observed at 12 months after surgical enucleation in contenitive defects.	No complications were observed.
- Signs of bone remodelling were observed with light microscopy in all samples, no sign of inflammatory cells. - Histology samples at 3 months showed successful osseointegration of the grafted FDBA conditioned with MSCs and PRP characterized by a higher presence of the cellular component compared to the “control” sides. The grafted bone trabeculae, when detectable, were completely imprisoned inside new formed bone, in direct contact with it and without interposition of connective tissue. - The newly-formed bone was sufficiently stable to allow safe placement of endosseous implants. ➔ In conclusion, this study provides further support to the notion that FDBA bio-engineered with MSCs and PRP can significantly improve bone formation as compared with FDBA alone and can represent the treatment of choice for sinus floor elevation.	No complications occurred during the surgical procedures. None of the enrolled patients complained about pain or other dysfunctional symptoms.

DENTISTRY, MAXILLOFACIAL AND OTORHINOLARYNGOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Platelet gel: applications in dental regenerative surgery Forni F, Marzagalli M, Tesei P, Grassi A. *Blood Transfus* 2013; 11: 102-7	Observational study Follow-up: Up to 6 years	Atrophic alveolar bone of the maxilla or mandible	133 (304 implants)	RegenTHT	PRP was prepared as per instructions for use. 2 ml of upper plasma were removed before resuspending platelets. PRP was transferred into an IVD tube and 0.5 ml of $CaCl_2$ or CaGlu were added to for a clot in about 10 min (manipulation under laminar flow). Note: the PRPgel was frozen until required! All patients were treated equally with the insertion of a bone graft. The graft was composed as follows: 5-10% platelet gel (frozen, cut into pieces) 15-20% autologous bone from the oral cavity 70-80% BIO-OSS (0.25-1mm) + fibrin clot supernatant. The graft was implanted to fill the defect / cavity and then covered with an absorbable membrane (BIOGIDE). 3-4 Months later the implant was inserted.
The effects of the combined use of platelet-rich plasma and xenograft on alveolar socket healing. Traini T, Isaia B, Isaia F and Isaia L *Biomed J Sci & Tech Res* 2018; Volume 3- Issue 1	Case series Follow-up: 4 months	Tooth avulsion	10	RegenBCT	4-5 ml of PRP was prepared as per instructions for use from 10 ml of whole blood. PRP was transformed on gel, clot or membrane according to instructions for use. After surgical procedures for tooth extractions, the alveolar sockets were filled with a mixture of plasma A-PRP®gel and particles of ABB in 20% /80%ratio. Furthermore, A-PRP®clot/ membrane was used to cover the grafted area.
Jaw bones regeneration using mesenchymal stem cells. A single-center experience. Colangeli W, Riccelli U, Giudice A, *et al.* *Ann Ital Chir.* 2017; 89:20-23.	Case series Follow-up: 6 months	Maxillary and mandibular lesions/defects due to benign dentigerous cyst, followed by bone regeneration	5	RegenKit Extracell BMC / RegenTHT	Autologous stem cells (ASC) was obtained with RegenKit EC BMC, from bone marrow (BM) extracted from the iliac crest (the superior posterior iliac spine) and centrifugation 8 mon at 3400 rpm. 5 ml of calcium gluconate was added to 15 ml of ASC concentrate. The mixture was put in the maxillary lesion and closed the defect with absorbable sutures.

DENTISTRY, MAXILLOFACIAL AND OTORHINOLARYNGOLOGY	
Key results on performance	**Safety / adverse events (AE)**
- Tempopary bone graft provided viable bone, enough to ensure primary stability for implant placement in 97% of patients, with minimum discomfort and pain. - X-ray evaluation showed a zone of mature bone. Histological evaluation at the 4th month showed immature osteoid tissue with thin trabeculae of vital bone including osteocytes nuclei and associate with fibro-connective tissue. ➔ The use of PRP in oral surgery seems to decrease the need for autologous bone grafts collected from extra-oral sites, avoiding cutaneous incisions and reducing the extent of mucosal incisions. The technique for producing PRP that we used minimized the discomfort of the patient.	No complications or side effects were reported.
- From a practical point of view, the use of A-PRP®gel to mix the particles of ABB greatly facilitates and improves the local application of the mixture compared to saline or blood used conventionally. - The pain, the bleeding and the postoperative swelling were reduced so much that the patients did not take any pain medications. - According to a comparison of results of Vance et al., the bone regenerative index and the density structural index were significantly better in the present study (BRI: 2.12 (study) vs 1.89 (Vance); DSI: 2.7 (study) vs 2.3 (Vance); P<0.001). ➔ The ABB-PRP was useful in alveolar socket preservation, demonstrating a significant increase of bone regenerative index and structural bone density. The alveolar socket preservation appears to be a more efficient procedure when ABB is mixed to PRP gel and membrane clot is used.	No AE was reported.
- At 6 months, progressive improvement of the mucosa layer. - According to computed tomography, all patients showed an excellent bone regeneration. - According to Hounsfield scale, all patients had high density new trabecular bone (mean density of 1137 HU) and a volume of newly formed bone at 2.44 cm^3. ➔ Bone regeneration using MSC from autologous BM is a valid treatment option for maxillary bone defects.	There were no intraoperative or postoperative complications. No dehiscence of the surgical wound or infection was observed. Pain was well controlled.

DENTISTRY, MAXILLOFACIAL AND OTORHINOLARYNGOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Successful Treatment of Intractable Facial Osteomyelitis with Autologous PRP and Gentamicin Roemer A, Warnecke A, Lenarz T, R. O. Stolle S Clin of Otorhinolaryngology, 2017 ;1:2 011	Case series Follow-up: 6 months	Chronic osteomyelitis of the nasal root	3	RegenKit Extracell BMC / RegenBCT	PRP was prepared as per instructions for use with Extracell BMC kit after a centrifugation 5 min at 5000 rpm. PRP was harvested in a double syringe and the other syringe was filled with calcium gluconate and gentamicin. After surgical debridement of the necrotic bone and tissue, the gelatinous matrix containing gentamicin and the factors stored in the platelets is created directly after instillation within the infection area. The following treatments included repeated transdermal application of PRP, calcium gluconate and gentamycin application once weekly without debridement. Cas 1: 7 treatment applications Cas 2 / 3: 5 treatment applications
Biohybrid cochlear implants in human neurosensory restoration. Roemer A, Köhl U, Majdani O, Klöß S, Falk C, *et al.* *Stem Cell Research & Therapy,* 2016; 7:148	Case series Follow-up: 6 months	Severely hearing-impaired treated with biohybrid cochlear implants	3	RegenTHT	After in vitro studies and tests, cochlear implants were coated with autologous mononuclear cells derived from the bone marrow (BM-MNC). After the standard surgical procedure (retroauricular approach, mastoidectomy, posterior tympanotomy, and cochleostomy) the sternal puncture was performed and the BM-MNC were isolated directly using RegenTHT tubes as per instructions for use from 10 ml of BM aspirate. An aliquot of 0.5 ml of the progenitor cell suspension was mixed with 0.5 ml of the fibrinogen component of the two-component fibrin adhesive (Tisseal, Illinois, USA). The cochlear implant electrode was pulled through this solution and thereafter through the thrombin component of the two-component fibrin adhesive in order to cover the electrode with a thin layer of cells trapped within. This biohybrid electrode was inserted immediately in a standard procedure.

DENTISTRY, MAXILLOFACIAL AND OTORHINOLARYNGOLOGY	
Key results on performance	**Safety / adverse events (AE)**
- None of the treated patients showed a progression of osteomyelitis due to radiological aspects. All patients showed regular trabecular structure of the bone with reorganization of cortical bone. - After 1 month, 2 patients noticed a reduction of skin sensitivity, an overall increase in well-being and reduced fatigue. - Patient 3 furthermore showed a soft tissue closure of an over years existing fistula of the orbit to the sinus. ➔ Platelet rich plasma is widely used and showed great sufficiency and safety. Activated PRP in combination with gentamicin is a promising novel approach for the control of difficult to treat facial osteomyelitis.	No sign of subdermal scarring or osteoblastic proliferation. The redness of the skin was reduced with each treatment. Directly after treatment, no pain or other AE. After one week, 2 patients noted slight discomfort.
- All three patients had developed satisfactory speech perception and showed similar impedances on both sides. - In one of the patients, the speech perception with the biohybrid implant exceeded the performance on the other ear (patient 2), in one it was similar (patient 1), and in one the standard implant outperformed the biohybrid implant (patient 3). ➔ This safety study demonstrated the feasibility of autologous progenitor cell transplantation in humans as an adjuvant to cochlear implantation for neurosensory restoration. Further studies are necessary to prove the effect of BM-MNC.	None of the subjects demonstrated any adverse effects 5 months after implantation.

4.1.10 OPHTHALMOLOGY

2 publications were identified including clinical data on 35 patients.

Among them, RegenKits have been used on all patients for the treatment of ocular epithelial defects and dry age-related macular degeneration.

OPHTHALMOLOGY					
Data Reference	**Study design & follow-up**	**Medical indication/ pathology**	**N° of patients**	**RegenKit / RegenTube**	**Treatment protocol**
Cell surgery and growth factors in dry age-related macular degeneration: visual prognosis and morphological study Limoli PG, Limoli C, Vingolo EM, Scalinci SZ, Nebbioso M. *Oncotarget.* 2016; 7(30): 46913-46923	Prospective Comparative study Follow-up: 6 months	Dry age-related macular degeneration (AMD)	25 (35 eyes)	RegenBCT	Patients were divided in 2 groups according to retinal thickness average (RTA). Group A (10 pts/14 eyes): RTA < 250 µm Group B (15 pts/22 eyes): RTA ≥ 250 µm PRP was prepared according to manufacturer instructions for use. Fat tissue was collected by Coleman technique, and ADSC are prepared from fat. The distance between the grafted autologous cells and choroid was reduced by deep sclerectomy to favour the paracrine secretion of the autologous cells into the choroidal flow. The suprachoroidal pocket was built to accommodate the graft and saturated with a mixture of ADSCs and SVF. The adipose pedicle was infiltrated with PRP gel obtained by centrifugation of the blood material, separation of the component, and its platelet degranulation.
Platelet-rich plasma as treatment for persistent ocular epithelial defects Ronci C, Ferraro AS, Lanti A, *et al.* *Transfus Apher Sci* 2015; 52:300-4	Case series Follow-up: 2 years	Ocular epithelial defect (severe corneal pathology)	10 (13 eyes)	RegenBCT	PRP was prepared as per instructions for use from 16 ml of whole blood. Note: 1 ml aliquots of PRP were stored at –20° C until use. (PRP manipulations were performed under laminar flow hood). 9/10 patients were treated with autologous PRP and the other was treated with homologous PRP. All patients were treated with topical instillation of autologous PRP: 1 drop eye/3 times per day for at least 2-4 weeks.

4.2. Unpublished preliminary results from ongoing clinical studies

As part of post-market surveillance (PMS) activities, RegenKits and Cellular-Matrix are currently evaluated in post-market clinical follow-up studies (PMCF).

The objective of PMCF studies is to proactively collect performance and safety clinical data on the CE-marked RegenKits, to confirm both safety and performance of the devices according to the intended use and to contribute to the risk management process of the devices to address issues linked to potential residual risks or to detect emerging risks.

Safety: No AE has been reported in both clinical experiences.
Performance: PRP treatment was effective both during surgical procedure and as local application for the healing of ocular epithelial defects.

These data contribute to the safety and performance profile of PRP prepared with RegenKits and support preliminary data for ocular epithelial defects and dry age-related macular degeneration indications of use.

The key results described in publications are summarized in the following table:

OPHTHALMOLOGY	
Key results on performance	**Safety / adverse events (AE)**
- The best corrected visual acuity for far distance, close-up visus and average retinal sensitivity increased in both groups. - Patient compliance analysis showed that 6 months after surgery, 52.78% of patients recorded better vision, 38.89% no change and 8.33% worsening. - Significant improved was observed in the group with higher RTA (group B). ➔ Cell therapy by autologous cell graft in the suprachoroidal space seems to afford a functional improvement in retinal residual cells, proportional to their condition, with positive consequences on patient visual performance. Including in severe cases the contribution of GFs to the residual cells of the retina allows the optimization of rehabilitation and recovery of lost capacity with appropriate magnifying systems.	No adverse effects were reported in any case and mean values of the intraocular pressure recorded before and after surgery did not change significantly.
- All patients improved their clinical condition during follow-up, all epithelial defects healed or improved starting from the 2nd week of treatment, as demonstrated by fluorescein staining, photograph and disappearance of symptoms. - In patients with severe dry eye (SDE), best corrected visual acuity and fluorescein staining improved only during the PRP eye drop treatment. ➔ Topical instillation of autologous PRP was effective in healing ocular epithelial defects. High level of platelet counts seems not required to treat corneal lesions.	No adverse events related to PRP treatment were reported during the follow-up visits.

Below, are shown unpublished preliminary clinical results currently available on the use of Regen PRP and Cellular-Matrix.
e identified including clinical data on 35 patients.
Among them, RegenKits have been used on all patients for the treatment of ocular epithelial defects and dry age-related macular degeneration.

4.2. UNPUBLISHED PRELIMINARY RESULTS FROM ONGOING CLINICAL STUDIES

As part of post-market surveillance (PMS) activities, RegenKits and Cellular-Matrix are currently evaluated in post-market clinical follow-up studies (PMCF).

The objective of PMCF studies is to proactively collect performance and safety clinical data on the CE-marked RegenKits, to confirm both safety and performance of the devices according to the intended use and to contribute to the risk management process of the devices to address issues linked to potential residual risks or to detect emerging risks.

Below, are shown unpublished preliminary clinical results currently available on the use of Regen PRP and Cellular-Matrix.

Study Investigator(s) Center(s)	Study design	Patients Nr.	Diagnosis	Treatment schedule	Follow-up duration	Assessment methods	Results (performance & safety)
Renevier JL et al, to be published in 2018	Post-marketing Clinical Follow up data collection – Cellular matrix used per IFU in patients suffering from mild to moderate osteoarthritis of the knee Device: Cellular-Matrix A-CP-HA Kit Sponsor-initiated	77 patients (83 knees ; 7 patients bilateral) 57,8% M 42,2% F, 40-85yr	Patients suffering from knee osteoarthritis grade II-III according to Kellgren and Lawrence classification (43.4% grade II, 56.6% grade III), and not responding to previous HA treatment / visco-supplementation.	All patients received a treatment based on 3 i.a injections of the Cellular matrix (A-CP-HA), given at Day 0, Month-2 and Month-6, respectively. If joint effusion was present, the synovial liquid was aspirated before injecting the HA+PRP treatment intrarticularly.	9 months following first treatment injection Evaluation at D0, M2, M6 and M9 + Long-term evaluation (3-4 years)	WOMAC Index (patient questionnaire to assess articular pain, joint stiffness and joint function)	Performance: Pain at walking (WOMAC A1) was statistically significantly improved being 5.87 at baseline and decrease down to 1.89 at 9-months. Treatment was also well tolerated, transient pain at the injection site was reported in 7 knees and immediately solved with application of ice-packing Decomposed WOMAC index between baseline and the end of follow-up period: WOMAC A (pain): Day 0 = 23.24 9-month = 7.9 WOMAC B (stiffness): Day 0 = 10.34 9-month = 6.18 WOMAC (physical function): Day 0 = 68.36 9-month = 28.5 % responders according to OMERACT-OARSI criteria (assessed on 60 knees): YES (94.4%) and NO (5.6%), whose 51 knees (83.6%) were strictly responders. Long-term evaluation on 62 patients: 50% still perceive benefits 4 years after the treatment and 79% could avoid knee surgery. Safety: No serious adverse events were reported. 13.25% of patients experienced minor adverse reactions such as pain on injection, pain post-injection and oedema solved within 1-3 days.

Study Investigator(s) Center(s)	Study design	Patients Nr.	Diagnosis	Treatment schedule	Follow-up duration	Assessment methods	Results (performance & safety)
Clavel et al, ongoing (interim data)	Post-marketing Clinical Follow up study – Prospective, controlled, randomized RegenKits used per IFU in patients suffering from diabetic foot ulcer grade 2A or 3A Device: RegenKit-BCT Plus Sponsor-initiated	47 patients: 24 treated with autologous gel (PRP+ATS) + 23 patients treated with conventional treatment (standard dressing) 91.5% M 8.5% F	Diabetic foot ulcer	24 patients were treated 2 or 3 applications of autologous gel over a 6 week-period (gel renewal every 2 or 3 weeks); 23 patients were treated with 2 or 3 applications of standard dressing	12 weeks	Primary endpoint: % of total wound closure after a 6-week treatment period Secondary endpoints: Mean time-to-healing of the wound, qualitative assessment of the wound (re-epithelialization), patients's satisfaction	Performance: At the end of the 6-week treatment period, 75% of the patients treated with autologous gel had a complete wound closure, whereas only 26,1% of the patients treated with standard dressing has a complete wound closure. Mean time-to-healing is 37.88. days for the PRP-treated patients, and 43.83 days for the control group Safety: No side-effects were reported

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CONCLUSION

In this book, we fully described how our innovative devices empower the doctors with the perfect tool to standardize the patient's fresh cell preparations.

We have proven that thanks to our technology, patients are now allowed to expect very good outcomes for the treatment of their chronic and surgical wounds, dry eye syndrome and other ophthalmic conditions, sport-related wounds and even alopecia, as demonstrated through the clinical studies Regen Lab has strived to set-up over the past few years.

Cell biology research widens now our knowledge and comprehension of many diseases and drives the development of new therapies. Among them, cell therapies carry hopes for repairing or replacing the damaged and diseased tissues in the body without getting the risk of rejections.

As a precision medicine for cancer, immunotherapy opens recently a brand-new era with the goal of manipulating T-Cell activity to overcome malignancy.
We are now jumping into this exciting field of research by focusing our research projects on the development of new standardized cell therapy protocols.

For these research projects, we just developed a new line of product that we elegantly named CuteCell.

Those devices were designed to specifically isolate curative lymphoid cells and to keep them alive and kicking together with the more advanced cultures methods where the platelets will play a pivotal role.

Research projects are ongoing with renown cell therapy centers at the HUG in Geneva or E-Cell in Montpellier.

Regenerative medicine encompasses a wide range of techniques aiming at repairing or even replacing damaged or aged tissues. Among them, autologous platelet-rich plasma (PRP) is the simplest one and the more efficient. This technique is based on the intrinsic abilities of the human body to repair a tissue lesion.

Currently, there is a growing interest in using PRP, due both to the fact that it represents a safe and natural alternative compare to more invasive forms of treatments, and the promising results it has demonstrated so far, for a large amount of indications.

PRP is becoming a key player in the medical world with millions of patients treated every year. Although limitations related to a lack of standardized procedure for its preparation make it difficult to compare available clinical data. Consequently, Regen Lab SA developed the only one technology capable of providing a solution to this challenge of standardizing fresh cell preparations.

Based on more than 10-year experience, this book was written to help professionals involved in regenerative medicine to better understand PRP and cell therapies use. In addition, this book is intended for physicians who practice and conduct research in disciplines where PRP represents a therapeutic option, but it is also intended for anyone seeking reliable and up-to-date information on this technology.

Antoine Turzi & REGEN LAB team
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