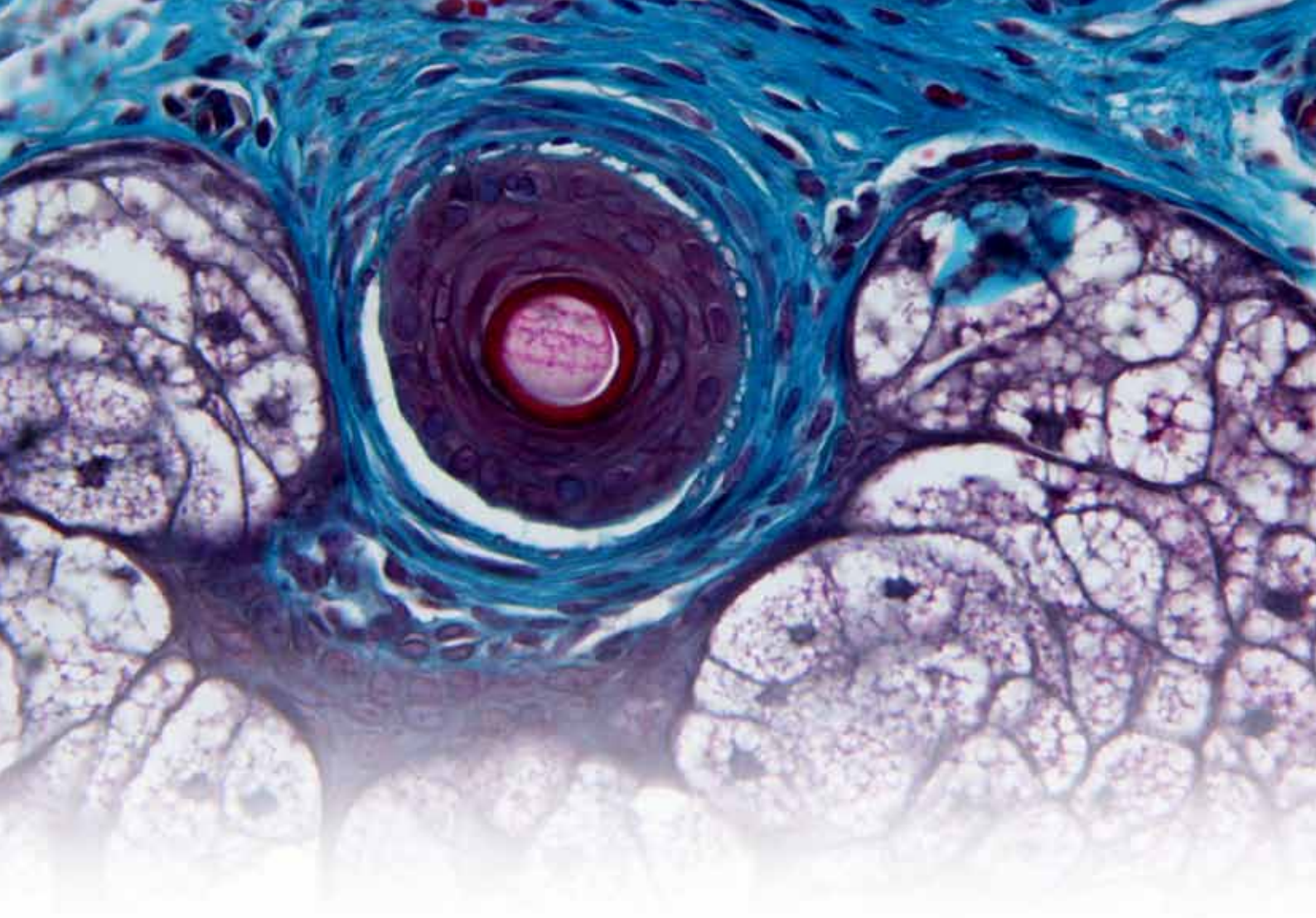
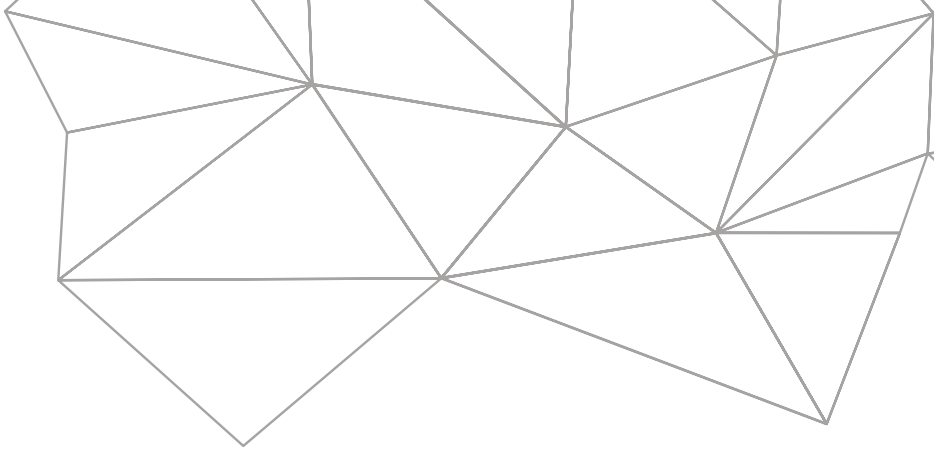


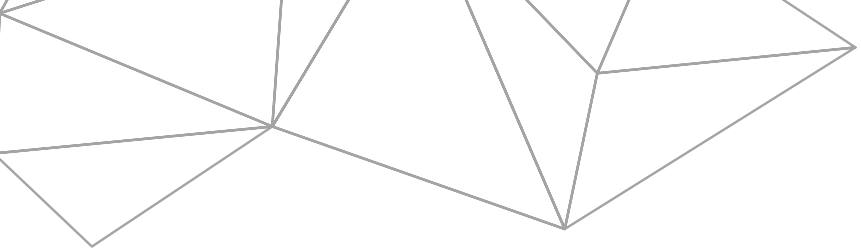
PRGF®-Endoret® Technology
Dermatology

bti®
Biotechnology Institute
Human Technology

Scientific Dossier

"Prince Felipe Award for Technological Innovation"





Edited By
BTI BIOTECHNOLOGY INSTITUTE

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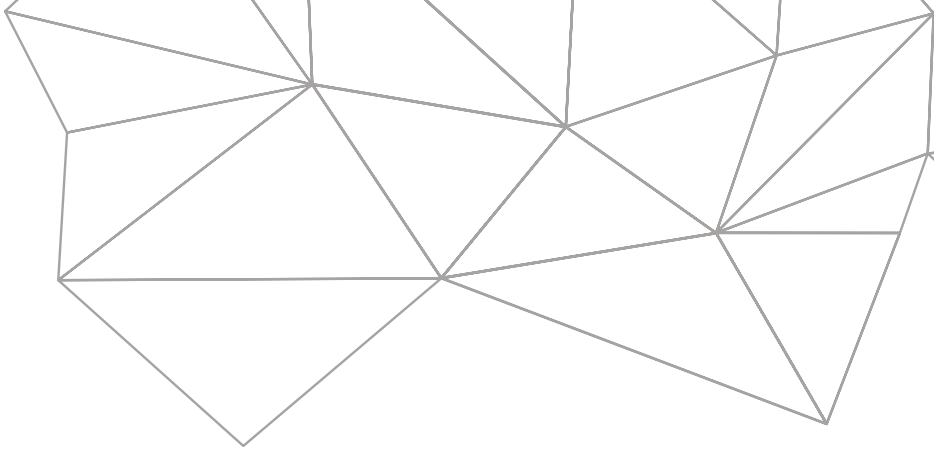
BTI Biotechnology Institute Design department

Research studies and material collection by BTI ImasD (Research) department



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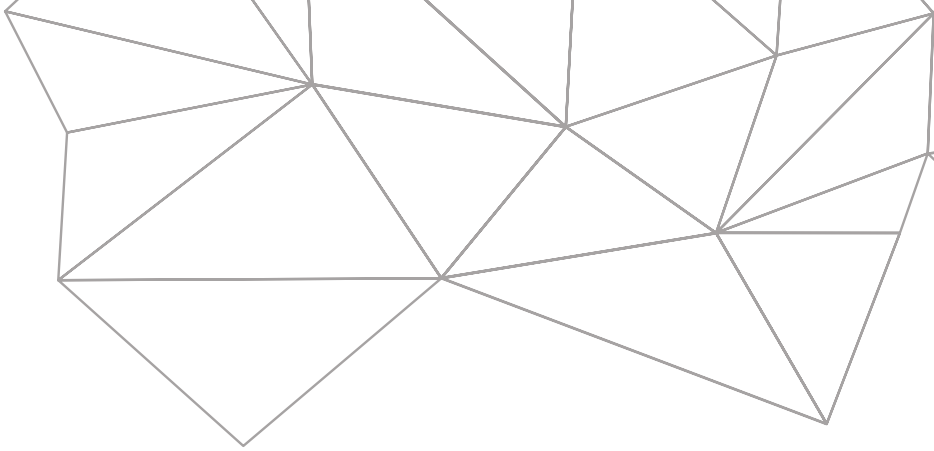


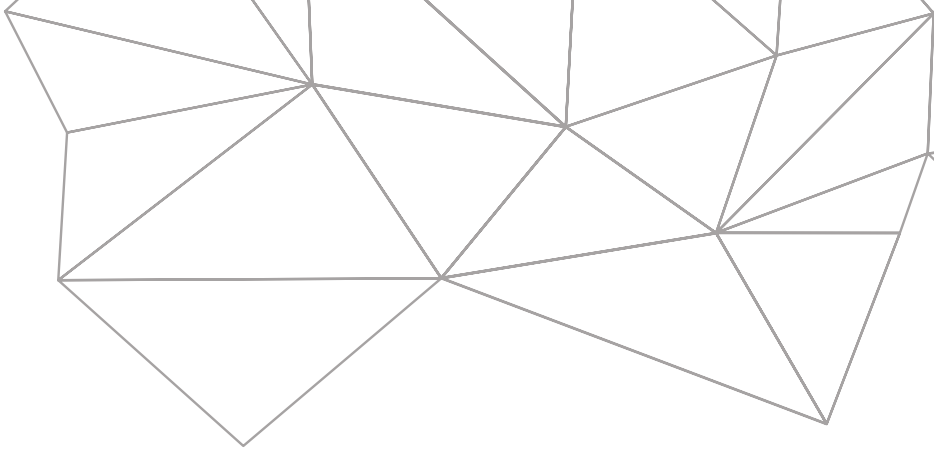
Introduction

This scientific dossier summarizes the set of indexed international articles published in recent years regarding the study and application of plasma rich in growth factors (PRGF®-Endoret®) in the area of wound closure and healing and in the field of regeneration and skin restoration. The results demonstrate the therapeutic potential and safety of this autologous technology in the treatment of vascular ulcers and skin biostimulation in regeneration and rejuvenation.

In addition, it highlights the vast amount of scientific evidences that supports the biosafety and effectiveness of PRGF®-Endoret® in other fields of medicine, highlighting the area of oral and maxillofacial surgery, oral implantology, orthopedic surgery and sports medicine.

This autologous technology has revolutionized the field of personalized regenerative medicine, as with the patient´s blood we can obtain different therapeutic formulations rich in growth factors which stimulate wound healing and tissue regeneration, reducing pain and inflammation.

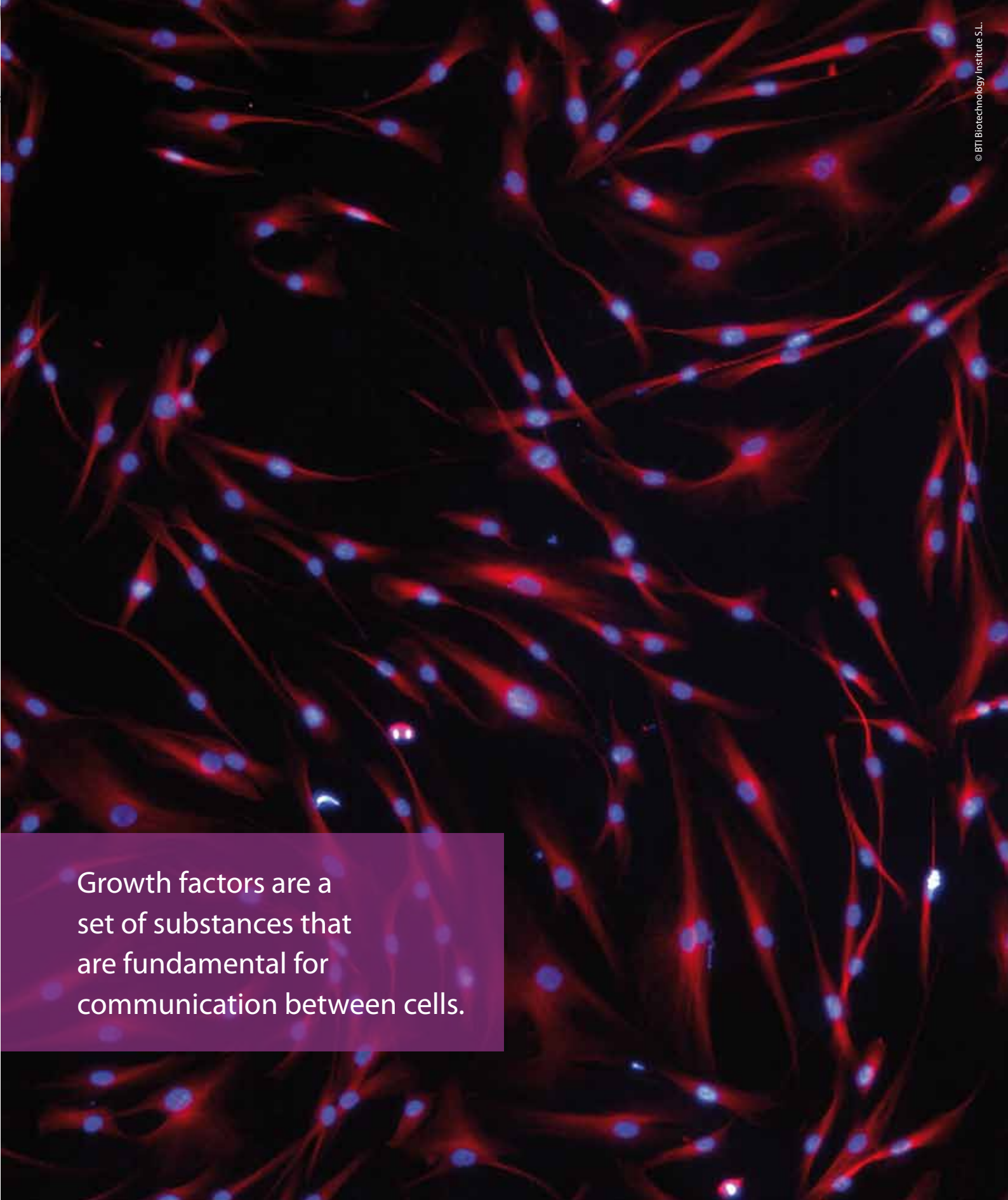
The scientific results, in both preclinical studies and clinical trials, show the potential of PRGF®-Endoret® in the treatment of skin lessions, in closing wounds and facial rejuvenation.



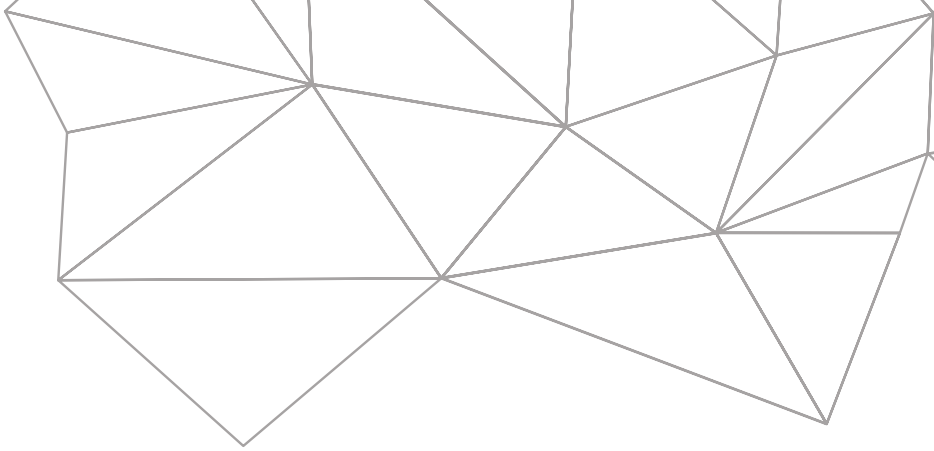
What are growth factors and how do they act?

Growth factors are a set of substances that carry out an important function in intercellular communication. They carry out a large number of biological functions among which cellular proliferation is important, though they also decisively affect cellular survival, migration, differentiation and even apoptosis.

Growth factors carry out their function at very low concentrations in body fluids and tissues, in the region of pico or nanograms. They act by binding to receptors located on the cell membrane that transmit the signal from the exterior to the interior of the cell, through the coupling of different protein kinases that are phosphorylated and which regulate a signalling cascade that ends up with the activation of one or more genes.



Growth factors are a set of substances that are fundamental for communication between cells.

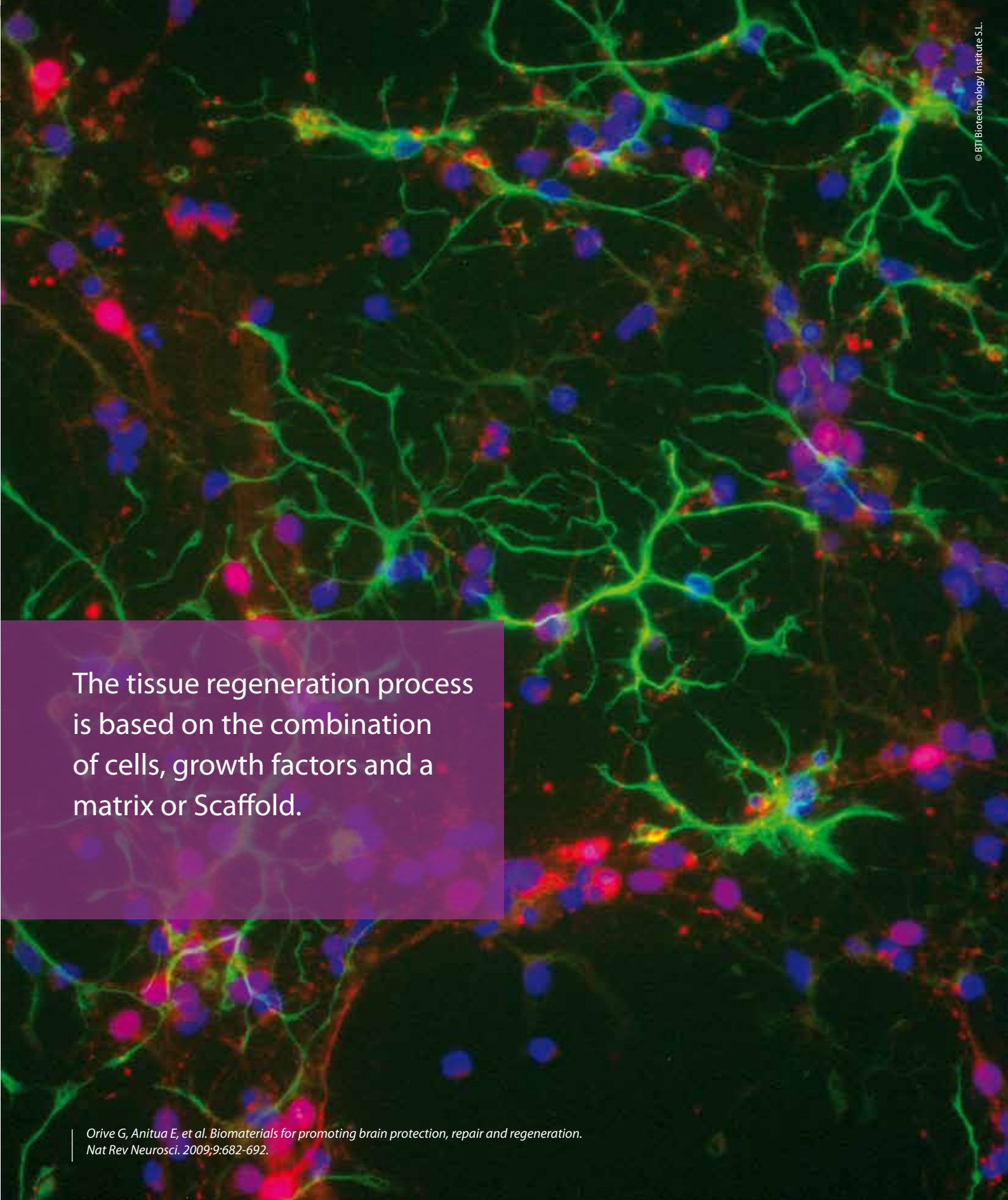


How can tissues be regenerated?

The process of tissue regeneration includes a complex set of biological events controlled by the action and synergy of a cocktail of growth factors. There are three agents involved in tissue regeneration: the cellular component, a combination of multiple biological mediators that include growth factors and cytokines among others and a matrix or "scaffold" that gives the new tissue under construction support.

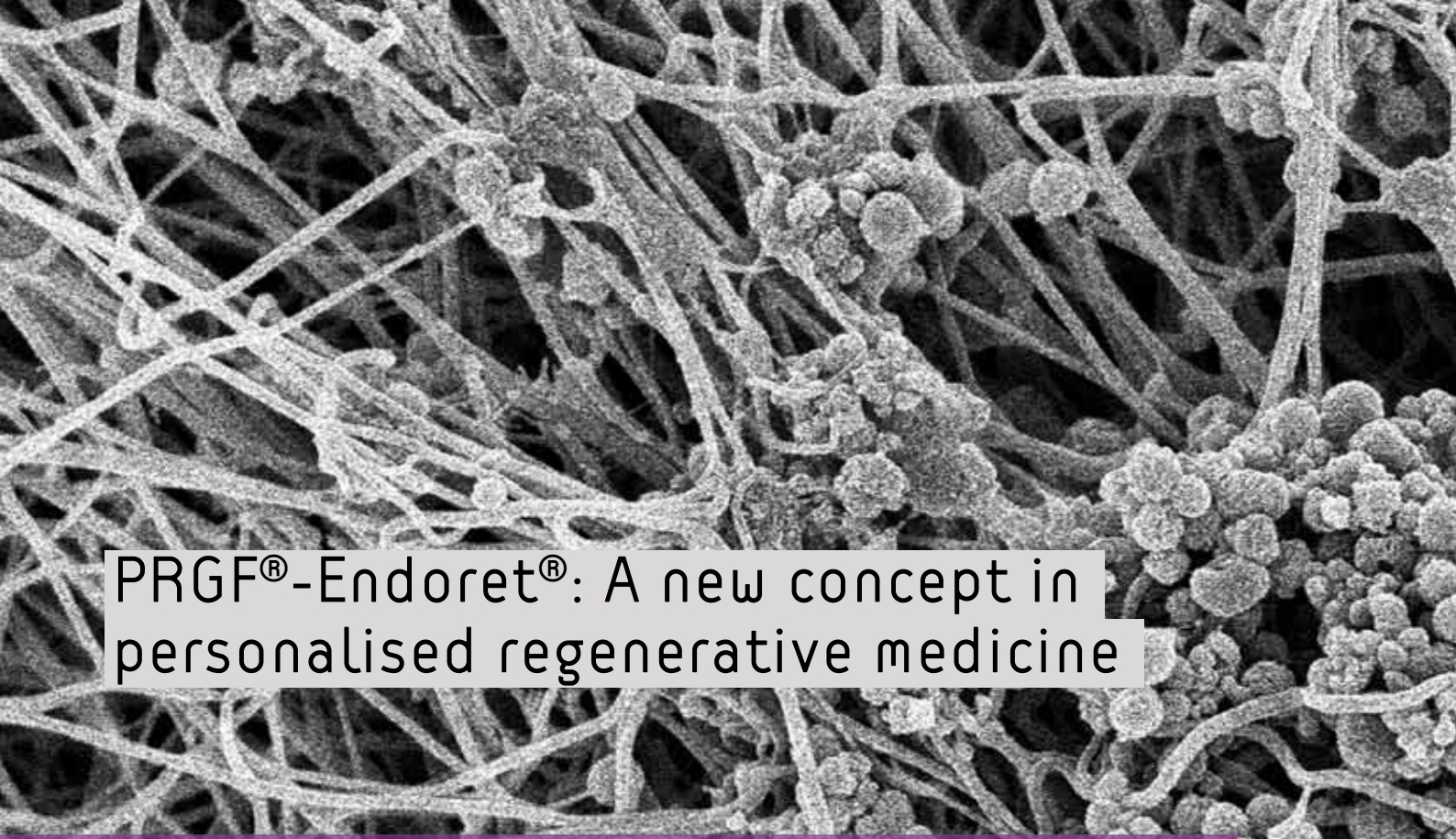
After an injury or tissue damage, they are activated and coordinate a large number of intercellular or intracellular paths with the aim of restoring the integrity of the tissue and its hemostasis. Growth factors are also necessary to promote angiogenesis or the formation of blood vessels that will supply oxygen and nutrients to the damaged tissue. Another fundamental aspect to be considered in the regeneration of a tissue is the development of a "scaffold" that acts as a provisional extracellular matrix and therefore houses the cells as well as locally presenting the biochemical, physical and structural signals that allow the anchorage of the cellular motility machinery.

Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003;83:835-870.



The tissue regeneration process is based on the combination of cells, growth factors and a matrix or Scaffold.

Orive G, Anitua E, et al. Biomaterials for promoting brain protection, repair and regeneration. Nat Rev Neurosci. 2009;9:682-692.

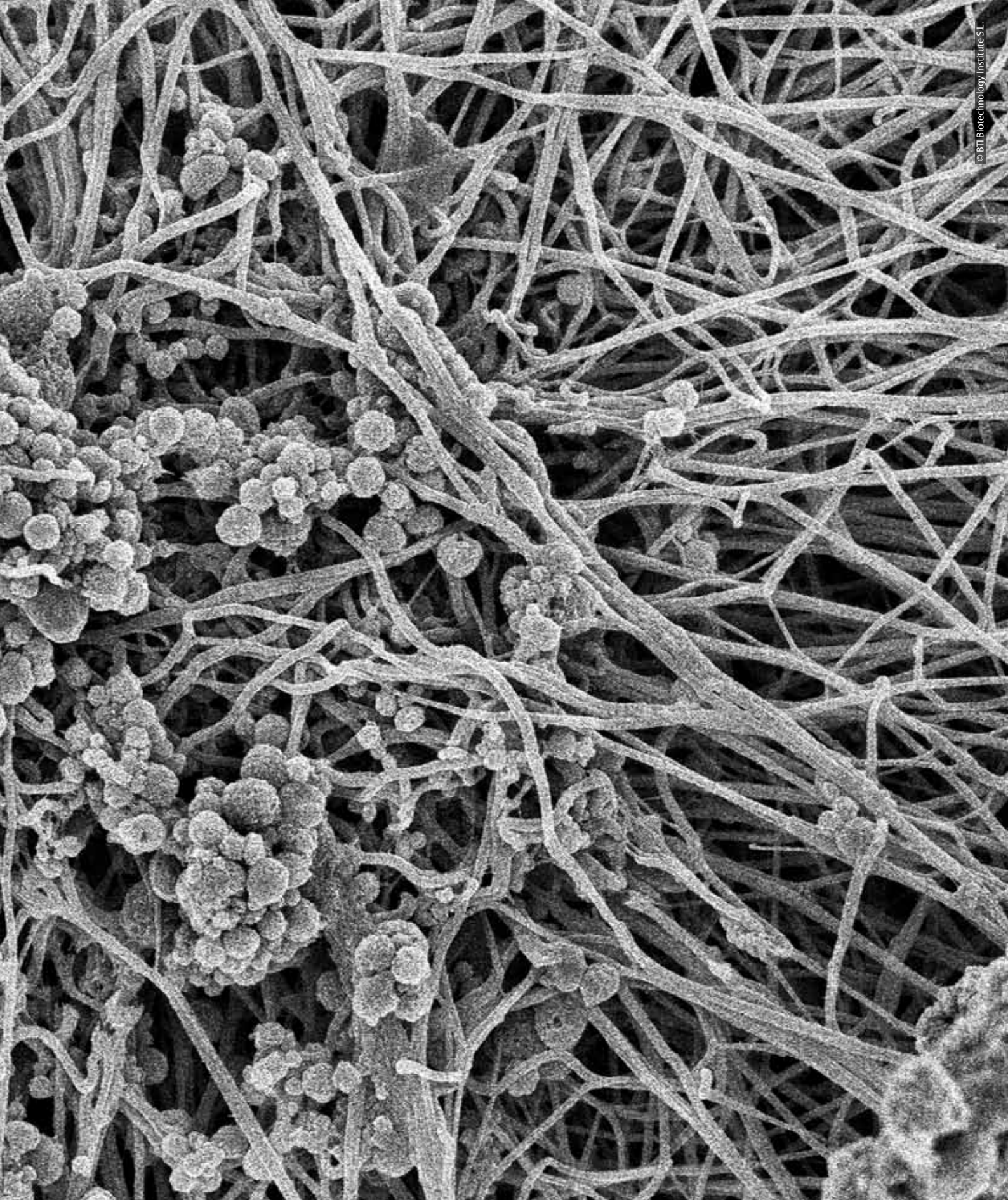


PRGF®-Endoret®: A new concept in personalised regenerative medicine

Over the last 15 years, the detailed study of platelets, of biological mediators contained in them and of the formulations aimed at allowing the administration and therapeutic use of growth factors and autologous biomaterials has allowed significant progress and has greatly increased the versatility and therapeutic possibilities of Plasma Rich in Growth Factors (PRGF®-Endoret®) technology. PRGF®-Endoret® technology is based on the preparation of platelet-rich 100% autologous plasma the application of which to damaged tissue areas speeds up the regeneration of a large number of tissues without any adverse effects.

Plasma rich in growth factors (PRGF®-Endoret®) is a personalized technology that has revolutionised the field of regenerative medicine. This article, published in one of the most prestigious journals in the field of biotechnology, summarizes the most important clinical results obtained with PRGF®-Endoret®. Its application over the last decade has extended to many fields of medicine, from oral and maxillofacial surgery to dermatology, cosmetics, orthopaedic surgery and sports medicine, and more recently to ophthalmology.

Anitua E, Sánchez M, Nurden AT, Nurden P, Orive G, Andía I. New insights into and novel applications for platelet-rich fibrin therapies. Trends Biotechnol. 2006;24:227-234.



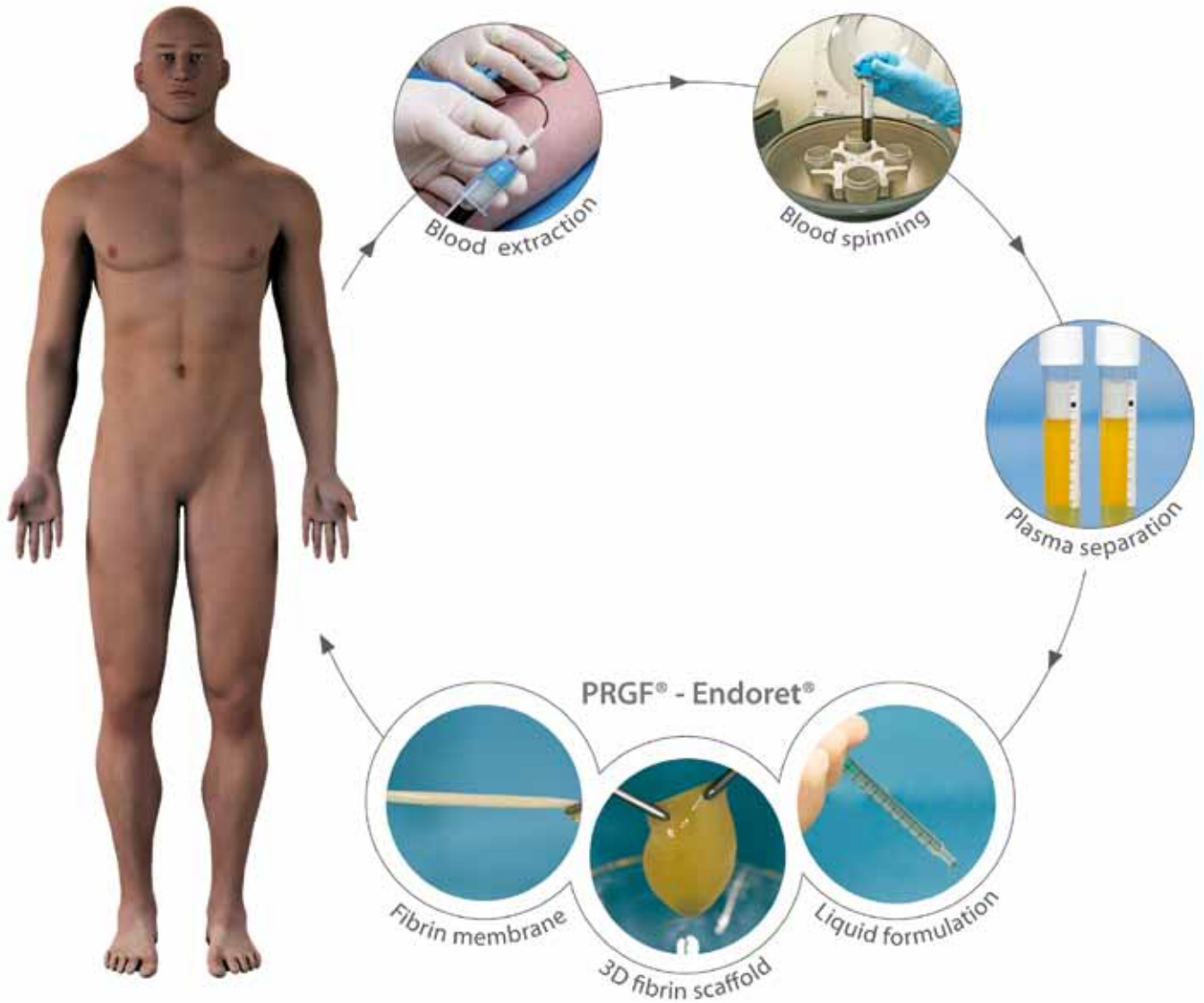
How is PRGF®-Endoret® obtained?

The set of therapeutic formulations of PRGF®-Endoret® are obtained by means of a simple protocol based on a tiny volume of the patient's blood. The blood is centrifuged in 9 ml citrated tubes allowing the separation of red and white blood cells from the platelet-rich plasma. The two fractions of PRGF®-Endoret® are separated from the rest of the blood components by means of the plasma transfer device (PTD). Later, and prior to its therapeutic application, the fractions of PRGF®-Endoret® are activated with calcium chloride, leading to a series of therapeutic formulations.

The process to obtain PRGF®-Endoret® is simple and easily reproducible.

DEMONSTRATION VIDEO
Scan this code with your mobile phone to watch the video.

Anitua E. Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants. Int J Oral Maxillofac Implants. 1999;14:529-535.



Versatility of PRGF®-Endoret®

This article, published in of the most important scientific journals in the field of biomaterials, focuses on the enormous versatility that PRGF®-Endoret® technology offers, as by using the patient's blood we can obtain up to 4 biocompatible formulations:

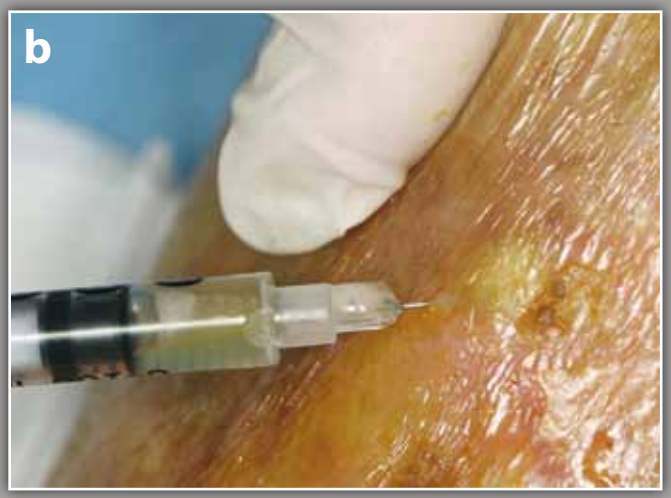
a. PRGF®-Endoret® supernatant: used to cultivate primary cells and stem cells in the laboratory, it is also the base of a new collyrium for treating a large number of pathologies of the occular surface.

b. Liquid PRGF®-Endoret®: Ideal for infiltrations in skin, musculoskeletal system tissues, TMJ, etc. It is the perfect tool to bioactivate dental implants and prostheses of all types with the aim of accelerating their osseointegration.

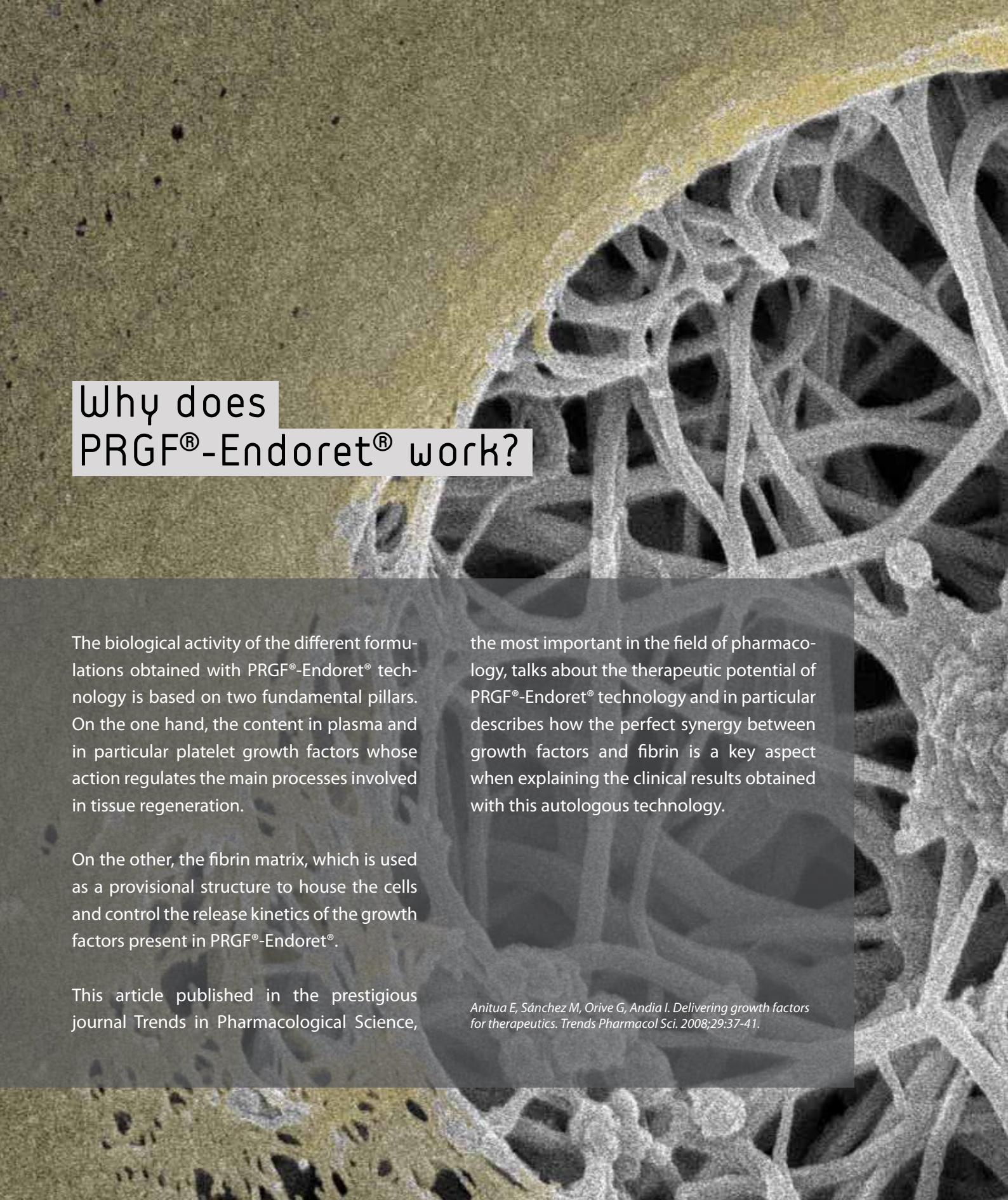
c. PRGF®-Endoret® clot or "scaffold" : Ideal to fill in defects and promote tissue regeneration: post-extraction sockets, treatment of ulcers, tissue engineering, etc.

d. Fibrin membrane: due to its hemostatic properties it is the best biomaterial to seal defects and stimulate epithelization.

Anitua E, Sánchez M, Orive G, Andía I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials. 2007;28:4551-4560.



The versatility of PRGF®-Endoret® technology allows you to obtain up to 4 autologous formulations.



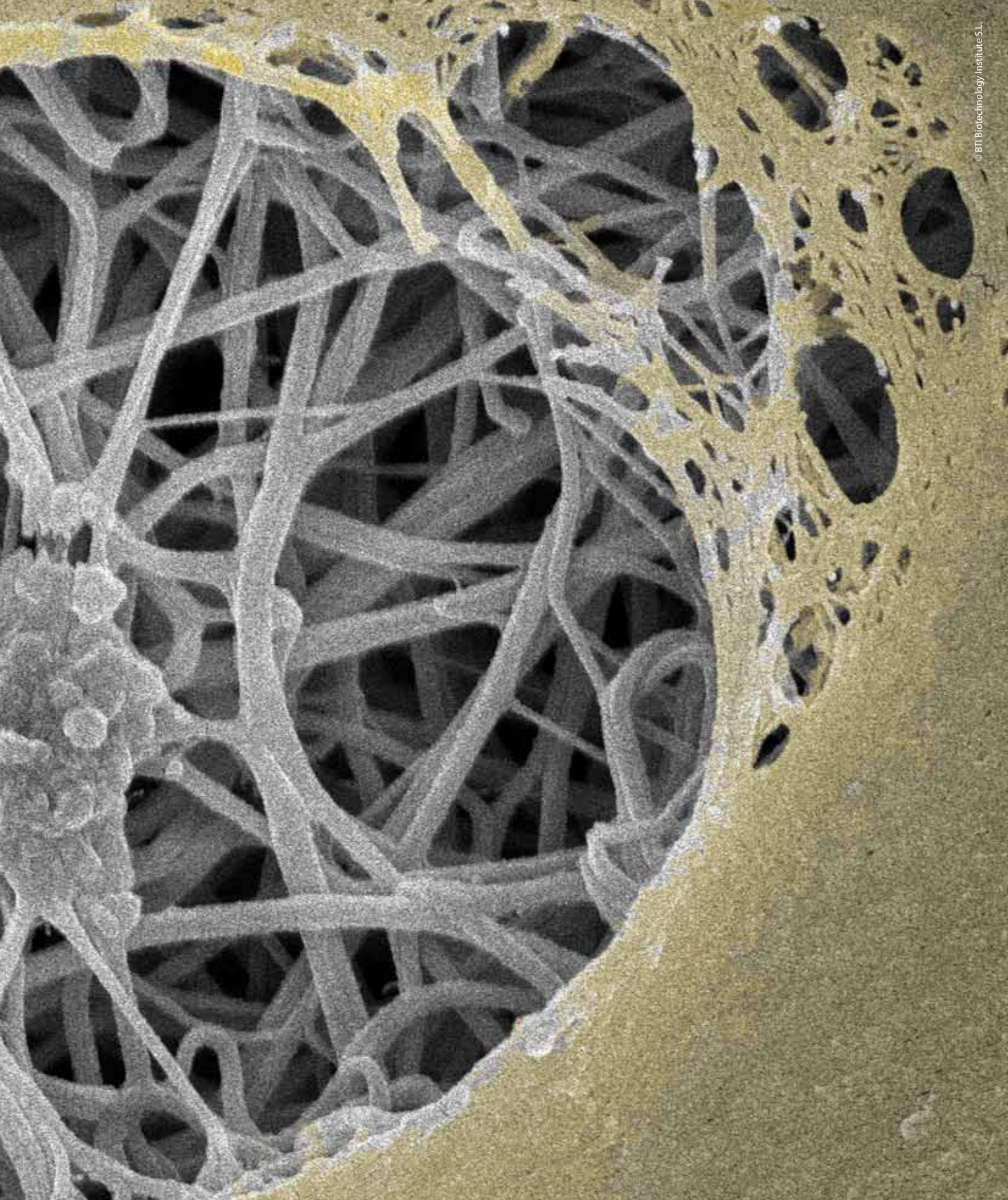
Why does PRGF®-Endoret® work?

The biological activity of the different formulations obtained with PRGF®-Endoret® technology is based on two fundamental pillars. On the one hand, the content in plasma and in particular platelet growth factors whose action regulates the main processes involved in tissue regeneration.

On the other, the fibrin matrix, which is used as a provisional structure to house the cells and control the release kinetics of the growth factors present in PRGF®-Endoret®.

This article published in the prestigious journal Trends in Pharmacological Science, the most important in the field of pharmacology, talks about the therapeutic potential of PRGF®-Endoret® technology and in particular describes how the perfect synergy between growth factors and fibrin is a key aspect when explaining the clinical results obtained with this autologous technology.

Anitua E, Sánchez M, Orive G, Andia I. Delivering growth factors for therapeutics. Trends Pharmacol Sci. 2008;29:37-41.



What makes PRGF®-Endoret® different to other platelet-rich plasmas?

PRGF®-Endoret® is the first 100% autologous platelet-rich plasma to be described in literature worldwide. It is, likewise, a pioneering technology in translational regenerative medicine. Over 15 years of research, added to its exclusive properties, make PRGF®-Endoret® a unique technique. PRGF®-Endoret® is prepared with small volumes of the patient's blood and does not require the use of thrombin or chemical agents for its activation. Unlike other products, it does not include white blood cells (leukocytes) in its composition, which gives it more effective anti-inflammatory properties. It is the most versatile technology, as its multiple formulations offer a large number of therapeutic applications.

In short, as shown by the series of letters to the editor published during recent years, we can define PRGF®-Endoret® as a platelet-rich autologous plasma whose effectiveness and safety have been widely proven. However, it is important to remember that not all platelet-rich plasmas are PRGF®-Endoret®.

DEMONSTRATION VIDEO
Scan this code with your mobile phone to watch the video.

Anitua E, Sánchez M, Orive G, Andía I. Shedding light in the controversial terminology for platelet rich products. J Biomed Mater Res A. 2009;90:1262-1263.

Sánchez M, Anitua E, Andia I. Poor standardization in platelet-rich therapies hampers advancement. Arthroscopy. 2010;26:725-726.

Anitua E, Sánchez M, Orive G. The importance of understanding what is platelet-rich growth factor (PRGF) and what is not. J Shoulder Elbow Surg. 2011;20:23-24.

Anitua E, Sánchez M, Prado R, Orive G. Plasma rich in growth factors: the pioneering autologous technology for tissue regeneration. J Biomed Mater Res A. 2011;97:536.

Anitua E, Sánchez M, Prado R, Orive G. The P makes the difference in plasma rich in growth factors (PRGF) technology. Platelets. 2011;22:473-474.

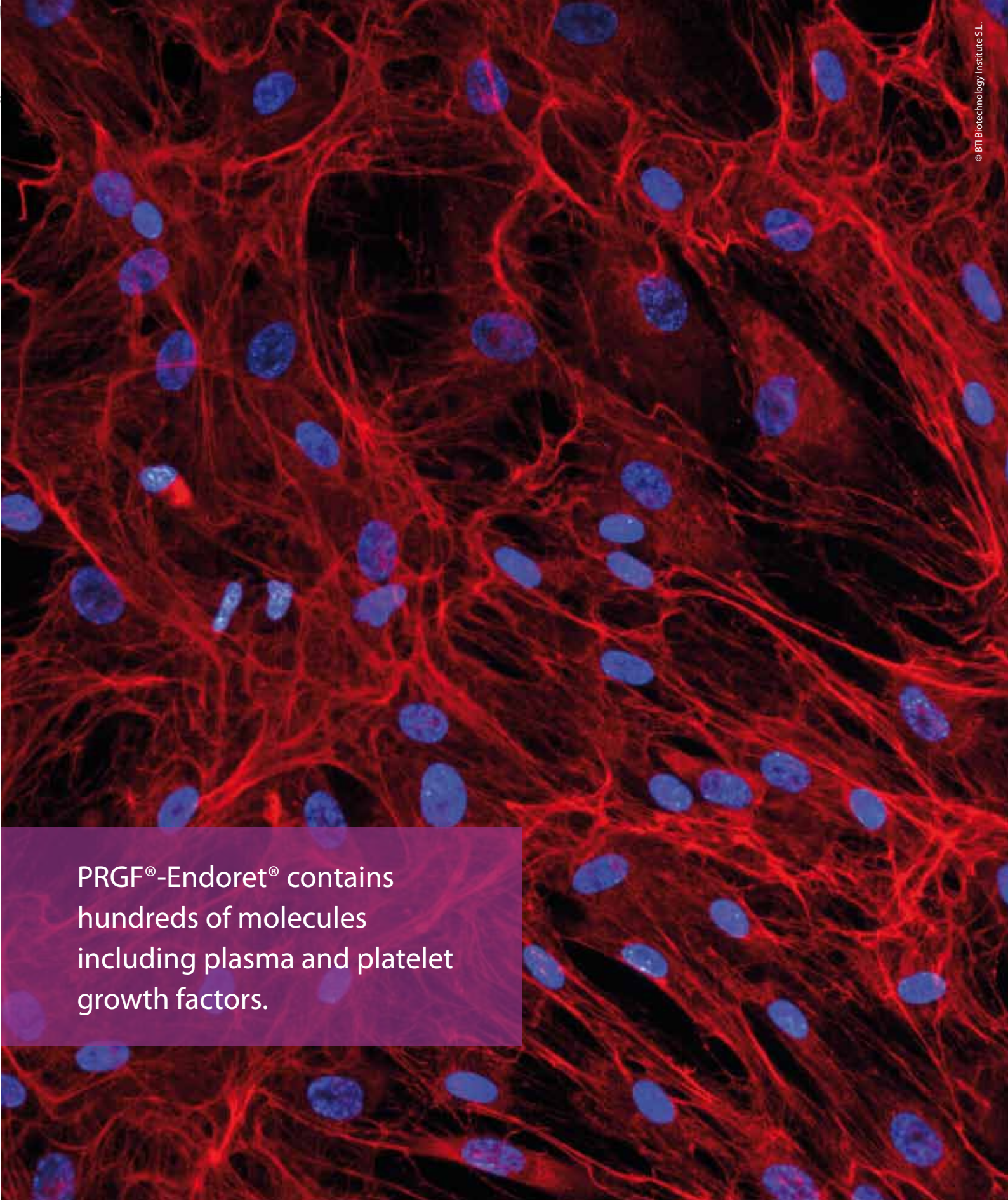
Anitua E, Sanchez M, Prado R, Orive G. The type of platelet-rich plasma may influence the safety of the approach. Knee Surg Sports Traumatol Arthrosc. 2012.

The pillars of PRGF®-Endoret®: growth factors

PRGF®-Endoret® contains a cocktail of autologous growth factors that proceed from both the plasma and the platelets. In fact, the platelets have a complex storage system in the form of intracellular granules that allow them to transport a large number of biologically active molecules. According to some authors, this list of proteins and peptides can come close to 500 molecules. Alpha (α) granules are the most abundant as there are around 40 to 80 alpha granules per platelet, but they are also the ones with the greatest retention capacity. In addition, they contain a series of antibacterial proteins that are generically called thrombocidines and which are lethal for a large variety of bacterial species.

However, it is important to remember that the plasma contains important growth factors and that the combination of the plasma and platelet factors is a key element in the biological action of PRGF®-Endoret®.

Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost. 2004;91:4-15.



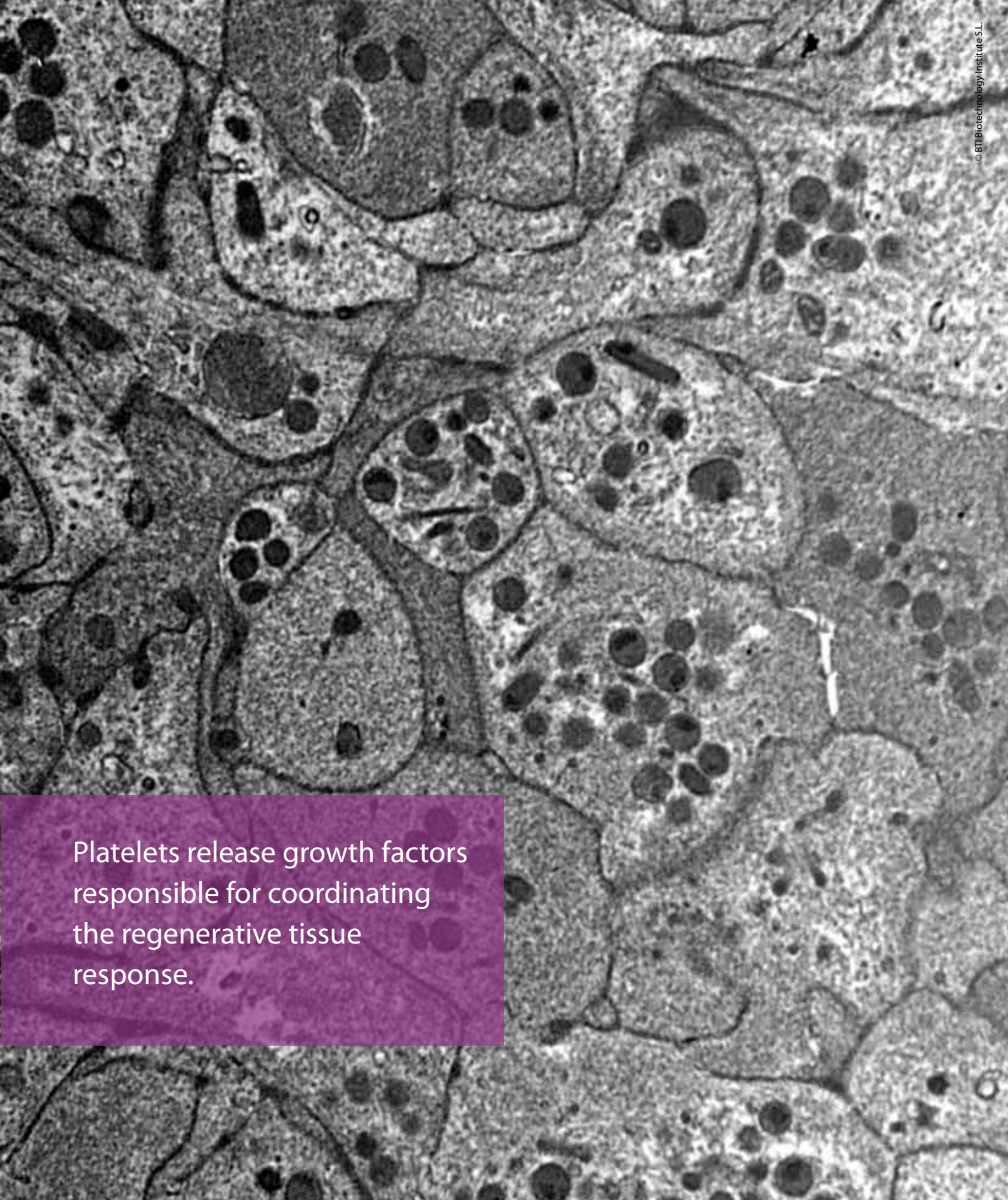
PRGF®-Endoret® contains hundreds of molecules including plasma and platelet growth factors.

Platelets and PRGF®-Endoret®

In a couple of review articles, our research team along with the Nurden Doctors from the Reference Centre for Platelet Disorders in France, have characterised the protein content of platelets in order to learn about the set of molecules present in PRGF®-Endoret® formulations.

Platelets release substances that promote tissue regeneration and which modulate both angiogenesis and inflammation. Important among other factors we have PDGF: platelet-derived growth factor, TGF-ß: transforming growth factor ß, bFGF: basic fibroblast growth factor, VEGF: vascular endothelial growth factor, EGF: epidermal growth factor or angiopoietin-1 among others. They release in parallel antibacterial molecules and specific growth factors that act on the mobilisation of progenitor cells from the bone marrow or from peripheral niches.

Nurden AT, Nurden P, Sanchez M, Andia I, Anitua E. Platelets and wound healing. Front Biosci. 2008;13:3532-3548.



Platelets release growth factors responsible for coordinating the regenerative tissue response.

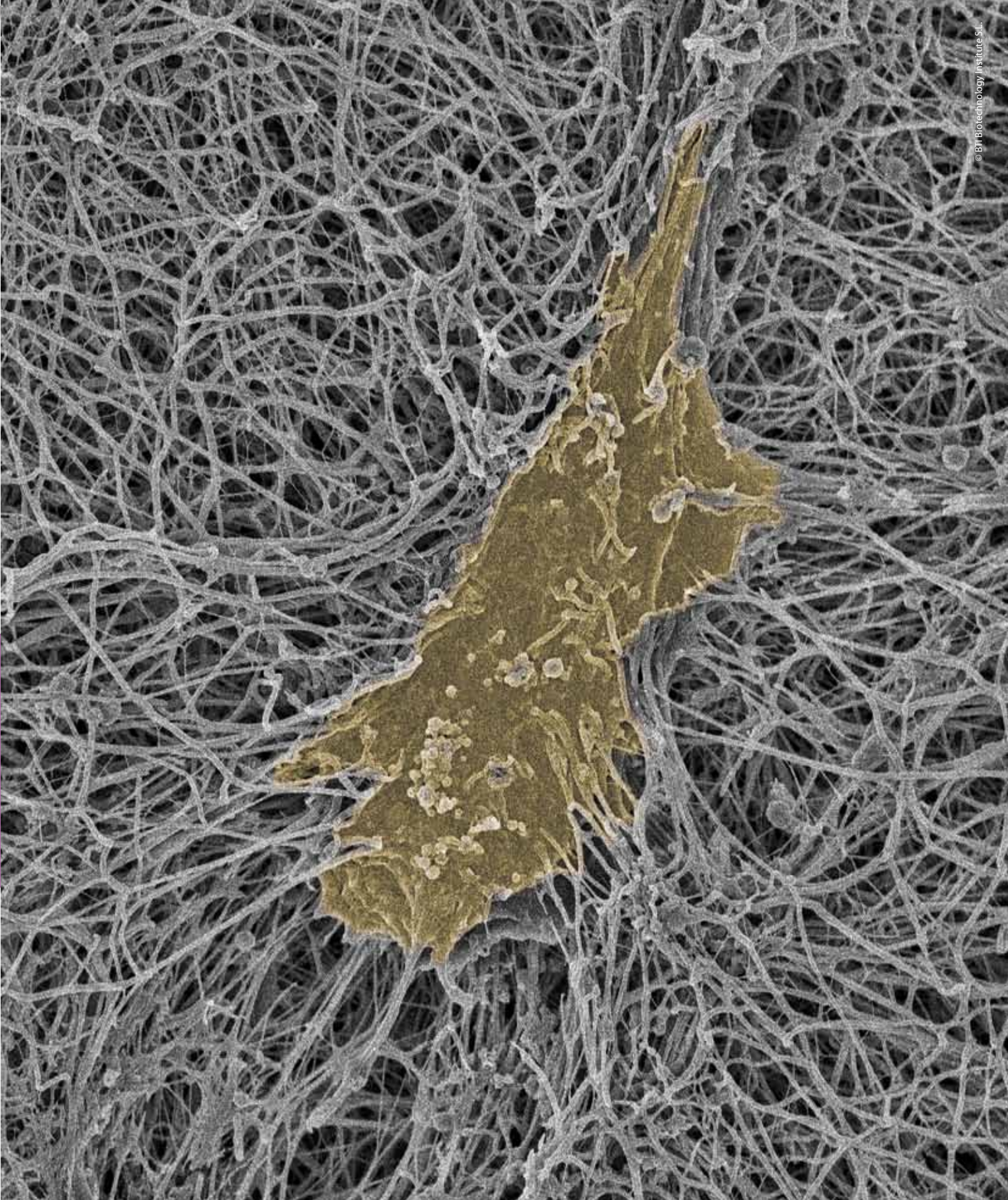
The pillars of PRGF®-Endoret®: fibrin as a biomaterial

Calcium acts as a cofactor in the activation process of PRGF®-Endoret®, which allows the conversion of the fibrinogen of the plasma into fibrin, generating a gel or clot with important biological functions. On the one hand, fibrin is an excellent matrix to maintain and house the cells, it acts as a provisional scaffold while the definitive tissue is regenerated and acts as a continuous growth factor release system. It is therefore a biocompatible and autologous sponge full of growth factors and cytokines that will permit a progressive release of them during several weeks.

The fibrin obtained with PRGF®-Endoret® technology is probably the best biomaterial for encouraging tissue regeneration.

DEMONSTRATION VIDEO
Scan this code with your mobile phone to watch the video.

Anitua E, Sanchez M, Nurden AT, Zalduendo M, de la Fuente M, Orive G, Azofra J, Andia I. Autologous fibrin matrices: a potential source of biological mediators that modulate tendon cell activities. J Biomed Mater Res A. 2006;77:285-293.



Biological and regenerative potential of PRGF®-Endoret®

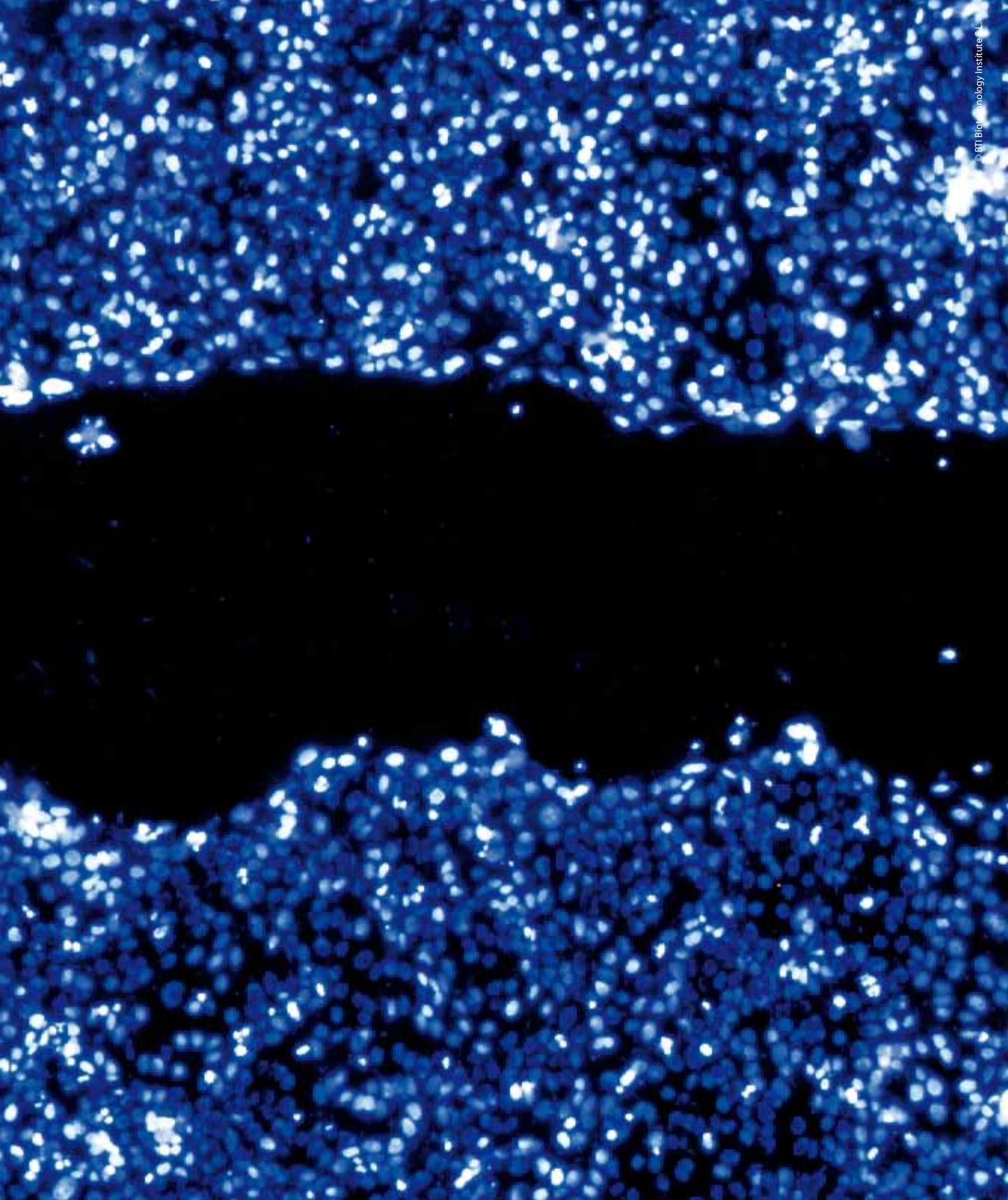
In over a decade of preclinical research, during which tens of cellular phenotypes were studied, we have managed to discover and understand the multiple biological functions that the set of therapeutic formulations of PRGF®-Endoret® carry out. The biological mediators of PRGF®-Endoret® stimulate and encourage such important processes for tissue regeneration as cellular proliferation and migration, chemotaxis (or the call from a distance for cells to go to the location of the injury), inflammation and the auto/paracrine synthesis of new molecules with biological activity.

DEMONSTRATION VIDEO
Scan this code with your mobile phone to watch the video.

Anitua E, Sanchez M, Merayo-Lloves J, De la Fuente M, Muruzabal F, Orive G. Plasma rich in growth factors (PRGF-Endoret) stimulates proliferation and migration of primary keratocytes and conjunctival fibroblasts and inhibits and reverts TGF-beta1-Induced myodifferentiation. Invest Ophthalmol Vis Sci. 2011;52: 6066-6073.

Anitua E, Prado R, Orive G. Bilateral sinus elevation evaluating plasma rich in growth factors technology: a report of five cases. Clin Implant Dent Relat Res. 2012;14:51-60.

Bendinelli P, Matteucci E, Dogliotti G, Corsi MM, Banfi G, Maroni P, Desiderio MA. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: mechanisms of NF-κB inhibition via HGF. J Cell Physiol. 2012;225:757-766.

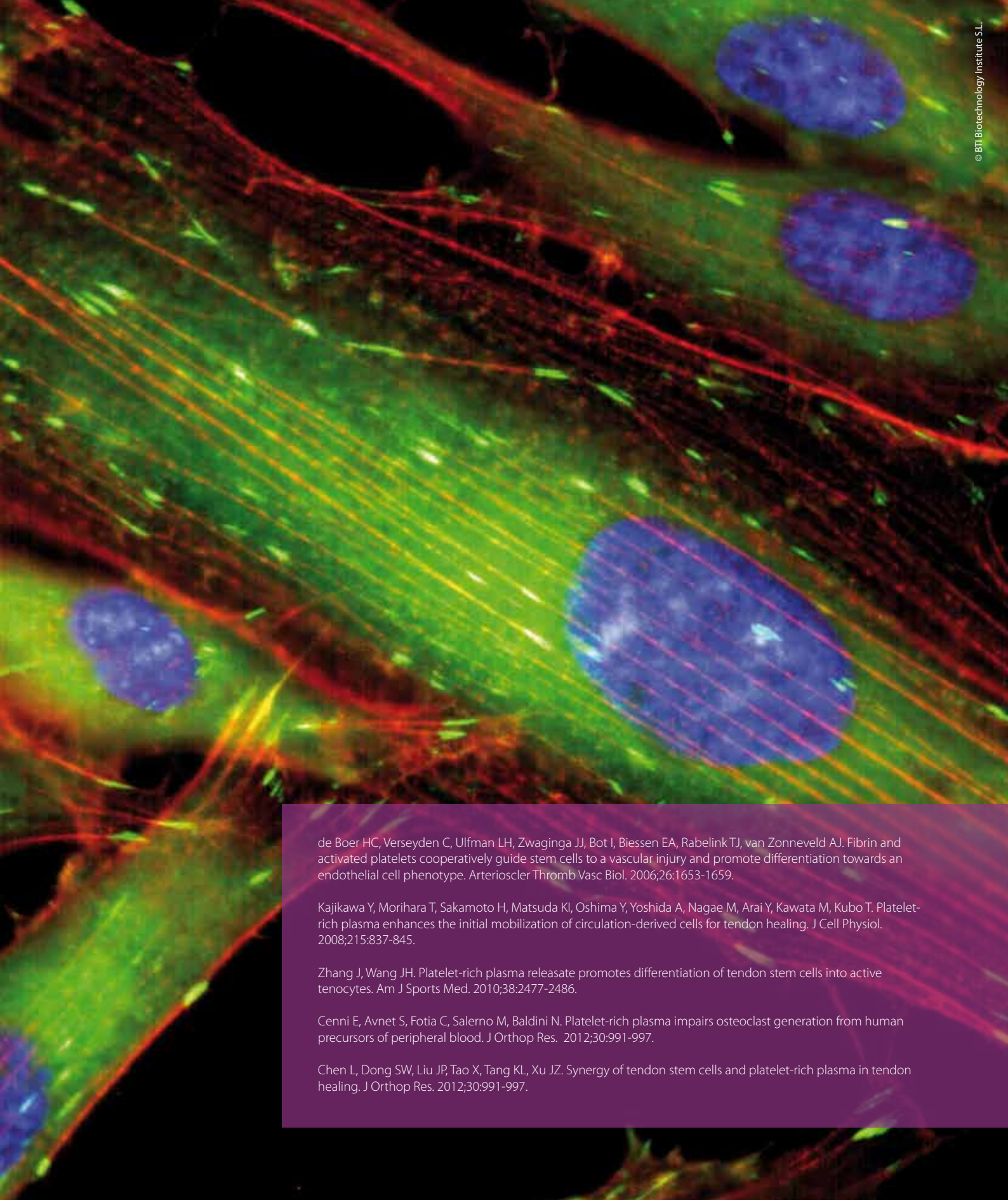


PRGF®-Endoret® in the field of stem cells

The growing interest in the range of biological options that PRGF®-Endoret® offers has even reached the field of stem cells. Stem or progenitor cells are characterised on the one hand by their unlimited capacity for proliferation, and on the other by the possibility of undergoing asymmetrical division (that is, self-renovation) maintaining their stemness while at the same time they can differentiate to diverse types of cells. There are different types of stem cells depending on their origin and their anatomical location.

There is evidence that the content of biologically active agents in PRGF®-Endoret® affects the mobilisation, adhesion, proliferation, survival, activation and differentiation of mesenchymal stem cells and other subtypes of precursor cells.

In addition, the cocktail of growth factors of PRGF®-Endoret® is an ideal resource for the cultivation and expansion of stem cells in the laboratory.



de Boer HC, Verseyden C, Ulfman LH, Zwaginga JJ, Bot I, Biessen EA, Rabelink TJ, van Zonneveld AJ. Fibrin and activated platelets cooperatively guide stem cells to a vascular injury and promote differentiation towards an endothelial cell phenotype. Arterioscler Thromb Vasc Biol. 2006;26:1653-1659.

Kajikawa Y, Morihara T, Sakamoto H, Matsuda KI, Oshima Y, Yoshida A, Nagae M, Arai Y, Kawata M, Kubo T. Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. J Cell Physiol. 2008;215:837-845.

Zhang J, Wang JH. Platelet-rich plasma releasate promotes differentiation of tendon stem cells into active tenocytes. Am J Sports Med. 2010;38:2477-2486.

Cenni E, Avnet S, Fotia C, Salerno M, Baldini N. Platelet-rich plasma impairs osteoclast generation from human precursors of peripheral blood. J Orthop Res. 2012;30:991-997.

Chen L, Dong SW, Liu JP, Tao X, Tang KL, Xu JZ. Synergy of tendon stem cells and platelet-rich plasma in tendon healing. J Orthop Res. 2012;30:991-997.

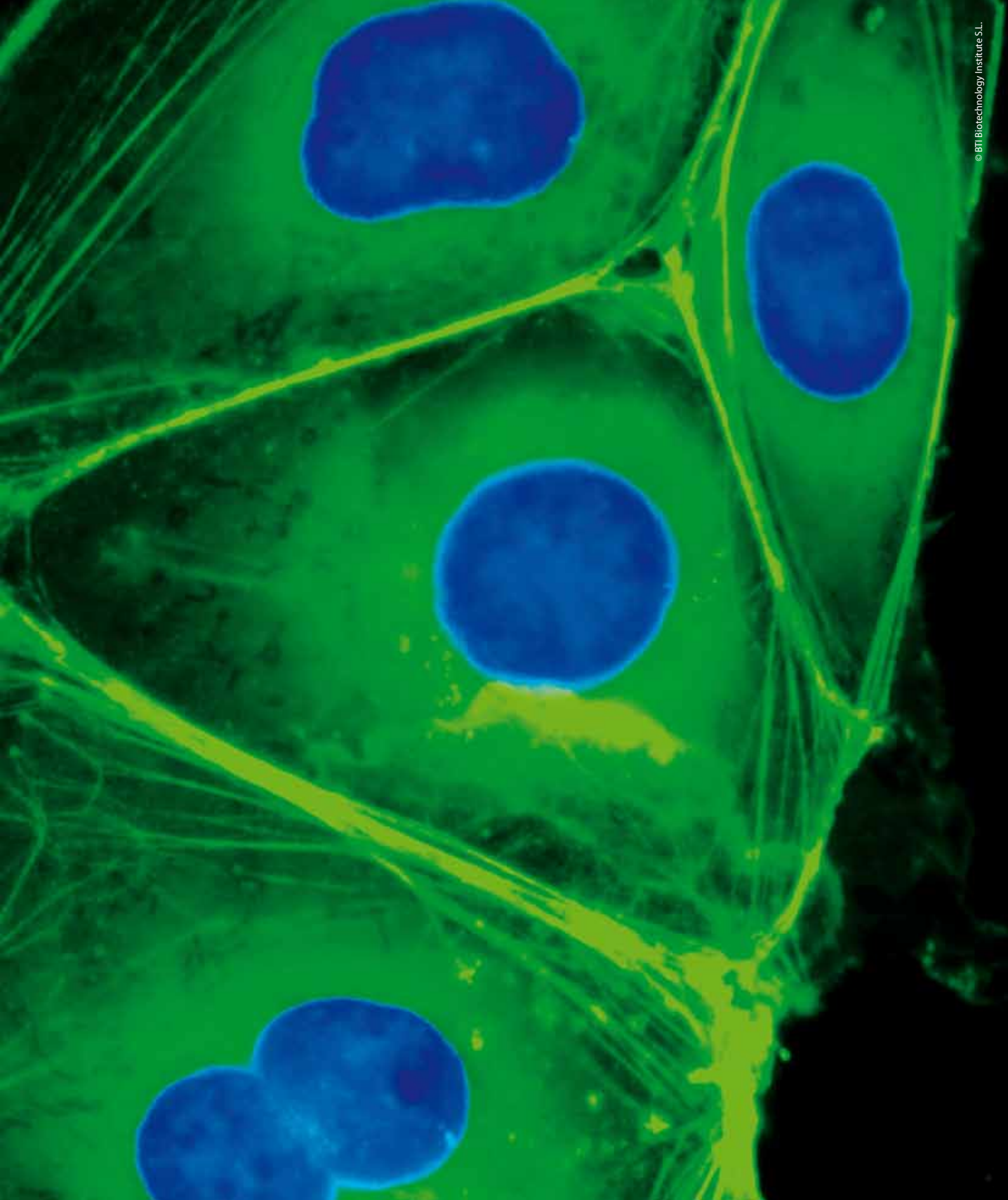
Mechanism of action of PRGF®-Endoret®

The use of growth factors and autologous fibrin for regenerative purposes represents a new approach to personalised medicine that a large number of patients could benefit from. In this paper, published in one of the most important journals of drug delivery, it is discussed the mechanisms of action through which PRGF®-Endoret® produces its multiple therapeutic effects.

The stimulation of cell proliferation and migration along with the call to circulating cells to come to the location of the injury are basic aspects of the action of PRGF®-Endoret®.

Likewise, also important is the angiogenic action of the growth factors which is crucial to start regeneration. Last of all, though no less important, its anti-inflammatory and antibacterial properties are a key element.

Anitua E, Orive G. Endogenous regenerative technology using plasma- and platelet-derived growth factors. J Control Release. 2012;157:317-320.



Bacteriostatic potential of PRGF®-Endoret®

Our research team has proven that PRGF®-Endoret® presents bacteriostatic activity with a large number of bacterial and fungal strains. This is because the platelets contain a series of antibacterial proteins called thrombocidines. These proteins are part of a wider family known as defensins, and they are of a cationic nature, which will allow them to bind to and alter bacterial membranes. In addition to thrombocidines, platelets transport and release other antimicrobial peptides among which we should mention platelet factor 4, RANTES, tissue activating peptide 3, the basic protein of platelets, thymosin β-4, and fibrinopeptides A and B.

In a recent paper we could see that the bacteriostatic potential of platelet-rich growth factors is due both to the antimicrobial peptides and to the fibrin, and not to the presence of leukocytes in their composition. In fact, the bacteriostatic effect of PRGF®-Endoret® is identical to that of a platelet and leukocyte-rich plasma. Another important conclusion of this study was to confirm how the inclusion of leukocytes notably alters the structure and uniformity of the fibrin matrix.

Anitua E, Alonso R, Girbau C, Aguirre JJ, Muruzabal F, Orive G. Antibacterial effect of plasma rich in growth factors (PRGF) against Staphylococcus aureus and epidermidis strains. Clin Exp Dermatol. 2012.

PRGF®-Endoret® presents bacteriostatic activity with a variety of bacterial strains.

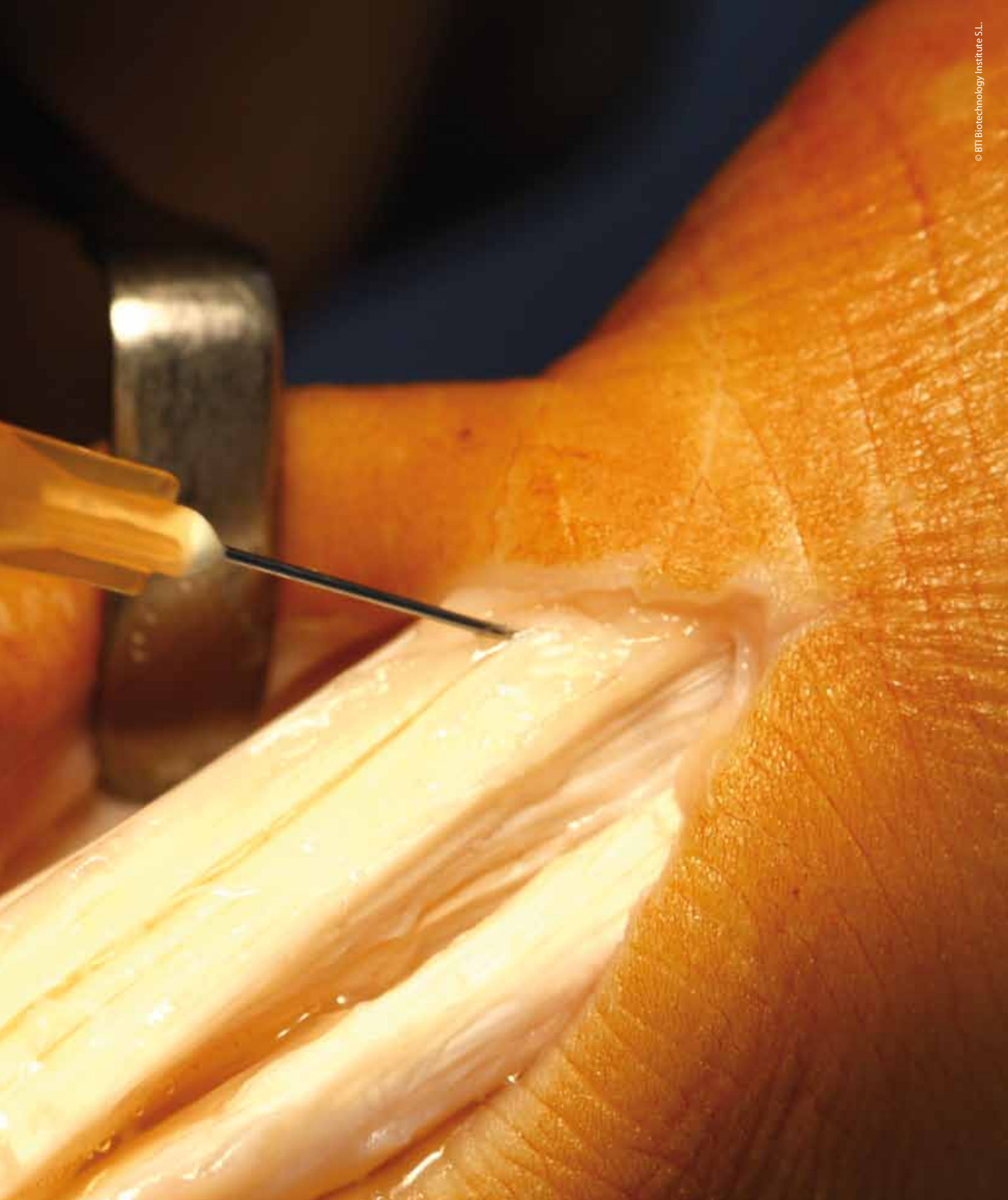
Therapeutic applications of PRGF®-Endoret®

This revision article is a reference in international bibliography as it is the journal with the greatest scientific impact. It is about the therapeutic potential of platelet-rich plasma, and in this specific case, PRGF®-Endoret®.

The use of growth factors and autologous fibrin for regenerative purposes represents a new approach to personalised medicine that a large number of patients could benefit from.

This dossier summarises the preclinical and clinical scientific articles that endorse the biosafety and efficacy of PRGF®-Endoret® in many fields of medicine.

Anitua E, Sánchez M, Orive G. Potential of endogenous regenerative technology for in situ regenerative medicine. Adv Drug Deliv Rev. 2010;62:741-752.



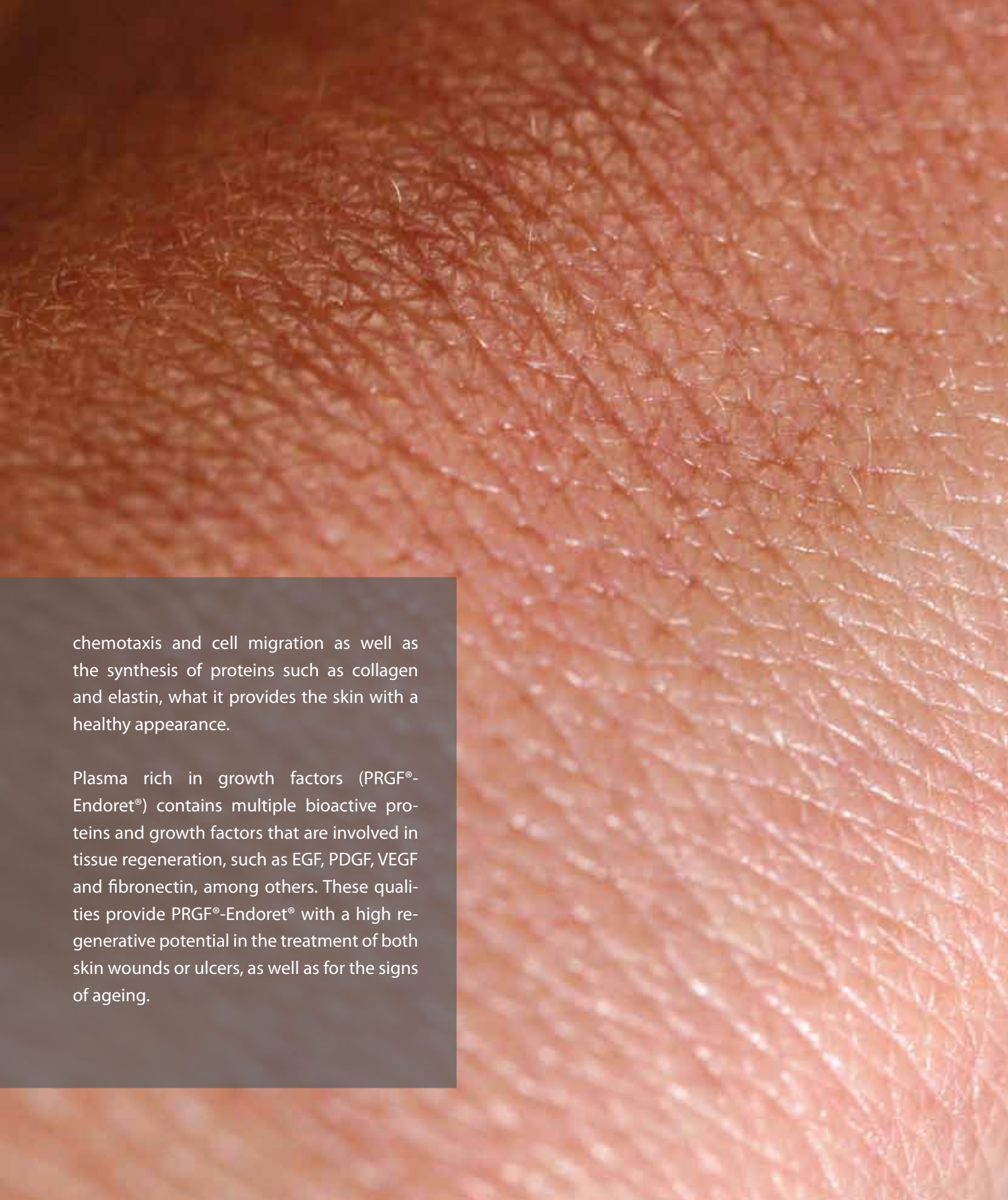
Dermatology and Facial Rejuvenation

The Skin.

The skin is the largest organ in the human body, occupying an area of approximately 2 m^2. The main function of the skin is to act as a protective barrier isolating the organism from the environment, protecting it from infections and dehydration. The skin is mainly composed of 3 layers: the epidermis, dermis and hypodermis.

The skin deteriorates with age, exposure to certain environmental factors (sun or low temperatures) and the development of certain diseases. As a result, skin losses hydration, elasticity and firmness; also its regenerative capacity, what it makes the skin wounds or ulcers to consistently delay its repair times.

To achieve an optimal state, the skin needs a balance between different factors (temperature, pH, hydration, elasticity, sebum production, scaling level, roughness, etc...). This condition ultimately depends on the right functioning of the cells that make up the different layers of the skin, including epidermal fibroblasts, corneocytes and adipocytes. To maintain the ideal microenvironment found in these cells, growth factors play a crucial role by promoting the proliferation,



chemotaxis and cell migration as well as the synthesis of proteins such as collagen and elastin, what it provides the skin with a healthy appearance.

Plasma rich in growth factors (PRGF®-Endoret®) contains multiple bioactive proteins and growth factors that are involved in tissue regeneration, such as EGF, PDGF, VEGF and fibronectin, among others. These qualities provide PRGF®-Endoret® with a high regenerative potential in the treatment of both skin wounds or ulcers, as well as for the signs of ageing.

Wound closure and healing

Skin ulcers are a common clinical problem that is increasing with the increase in average lifespan of the population. The etiology of ulcers is varied, since the ulcers may be a sign of cutaneous and systemic diseases. The most common ulcer is the one caused by venous peripheral vascular disease, though the ones caused by arterial and neuropathic alterations also have a high prevalence. Similarly, a group of systemic diseases are also closely linked with the appearance of ulcers, like diabetes.

The efficient and dynamic process of ulcer healing involves a complex series of active events, including hemostasis, inflammation, granulation, tissue formation, epithelialization, neovascularization, collagen synthesis and wound contraction. This process is altered with age and the development of certain types of diseases.

For over a decade, our research team has evaluated the safety and therapeutic potential of PRGF®-Endoret® technology in the treatment of skin ulcers. The set of preclinical evidence together with a variety of studies and clinical trials show that PRGF®-Endoret® is safe and effective when treating and healing vascular ulcers.

Wound closure and healing

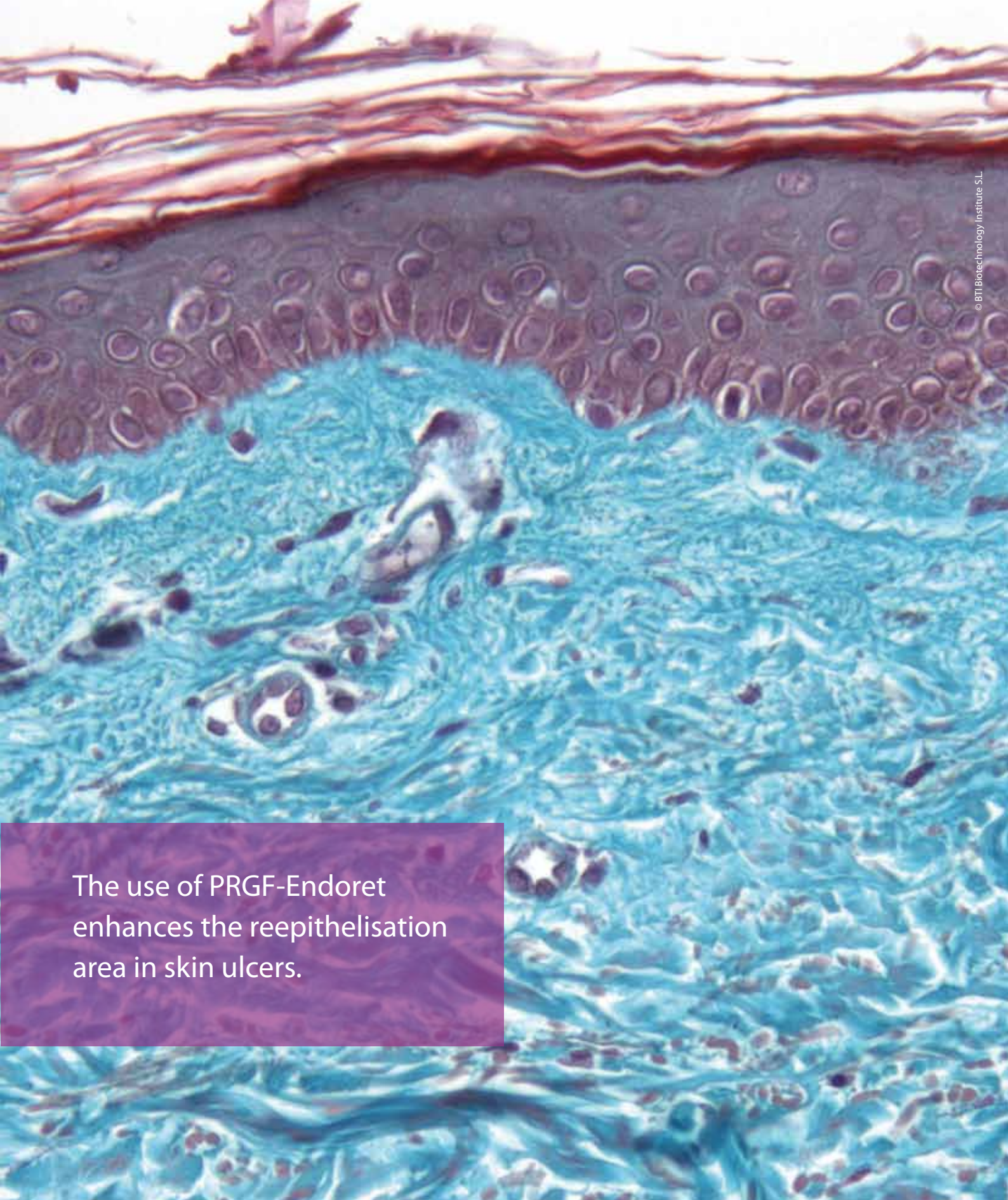
Preclinical Research

This study was conducted in 20 albino rabbits. Two similar wounds were made in the dorsal skin with a 6 mm diameter biopsy punch. One of the wounds was used as control (control wound) and no treatment was applied to it, while in the other one (study wound) the PRGF®-Endoret® fibrin obtained from the platelet richest fraction (F2) was applied.

After 7 days of treatment, it was observed that the reepithelialisation area was significantly greater in the wounds treated with PRGF®-Endoret®, and also, 40% of those wounds in which PRGF®-Endoret® was applied had resolved the inflammation process, while none of the control wounds presented full resolution.

This work is the first international preclinical study which demonstrates the effectiveness of plasma rich in growth factors (PRGF®-Endoret®) in the treatment of skin ulcers.

Molina-Miñano F, López-Jornet P, Camacho-Alonso F, Vicente-Ortega V. The use of plasma rich in growth factors on wound healing in the skin: experimental study in rabbits. Int Wound J. 2009;6:145-148.



The use of PRGF-Endoret enhances the reepithelisation area in skin ulcers.

Wound closure and healing

Clinical Research

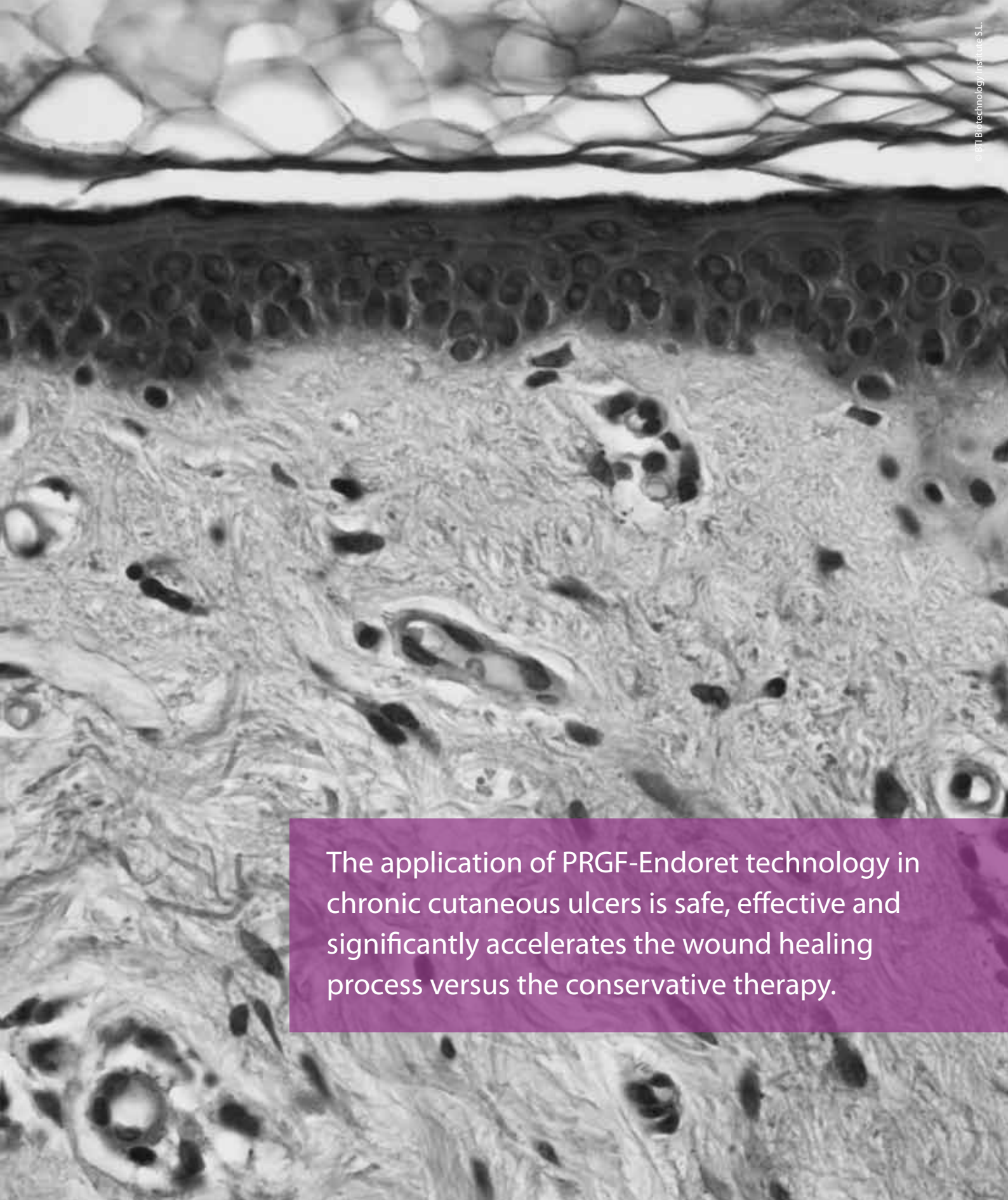
The goal of this preliminary clinical study was to test the efficacy and biosafety of plasma rich in growth factors (PRGF®-Endoret®) in the treatment of chronic ulcers. To do so, a full quantitative characterization of growth factors contained in PRGF®-Endoret® (PDGF, TGF-β, IGF, HGF, VEGF y EGF) was carried out. A randomized open-label controlled pilot clinical trial was designed to assess the effectiveness and safety of PRGF®-Endoret®.

Fifteen patients were randomly assigned to receive either topical treatment with PRGF®-Endoret® or conventional treatment (wound cleansing, debridement, moist saline gauze dressing). A total of 14 ulcers were treated distributed as follows: venous ulcers (64%), pressure ulcers (29%), and others (7%).

Results showed that at 8 weeks, the mean percentage of surface healed in the PRGF®-Endoret® group was 72.94% ± 22.25% whereas it was 21.48% ±33.56% in the control group ($p < 0.05$).

These results suggest that topical application of PRGF®-Endoret® is more effective than standard therapy in helping a chronic ulcer to heal.

Anitua E, Aguirre JJ, Algorta J, Ayerdi E, Cabezas AI, Orive G, Andia I. Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. J Biomed Mater Res B Appl Biomater. 2008;84:415-421



The application of PRGF-Endoret technology in chronic cutaneous ulcers is safe, effective and significantly accelerates the wound healing process versus the conservative therapy.

Wound closure and healing

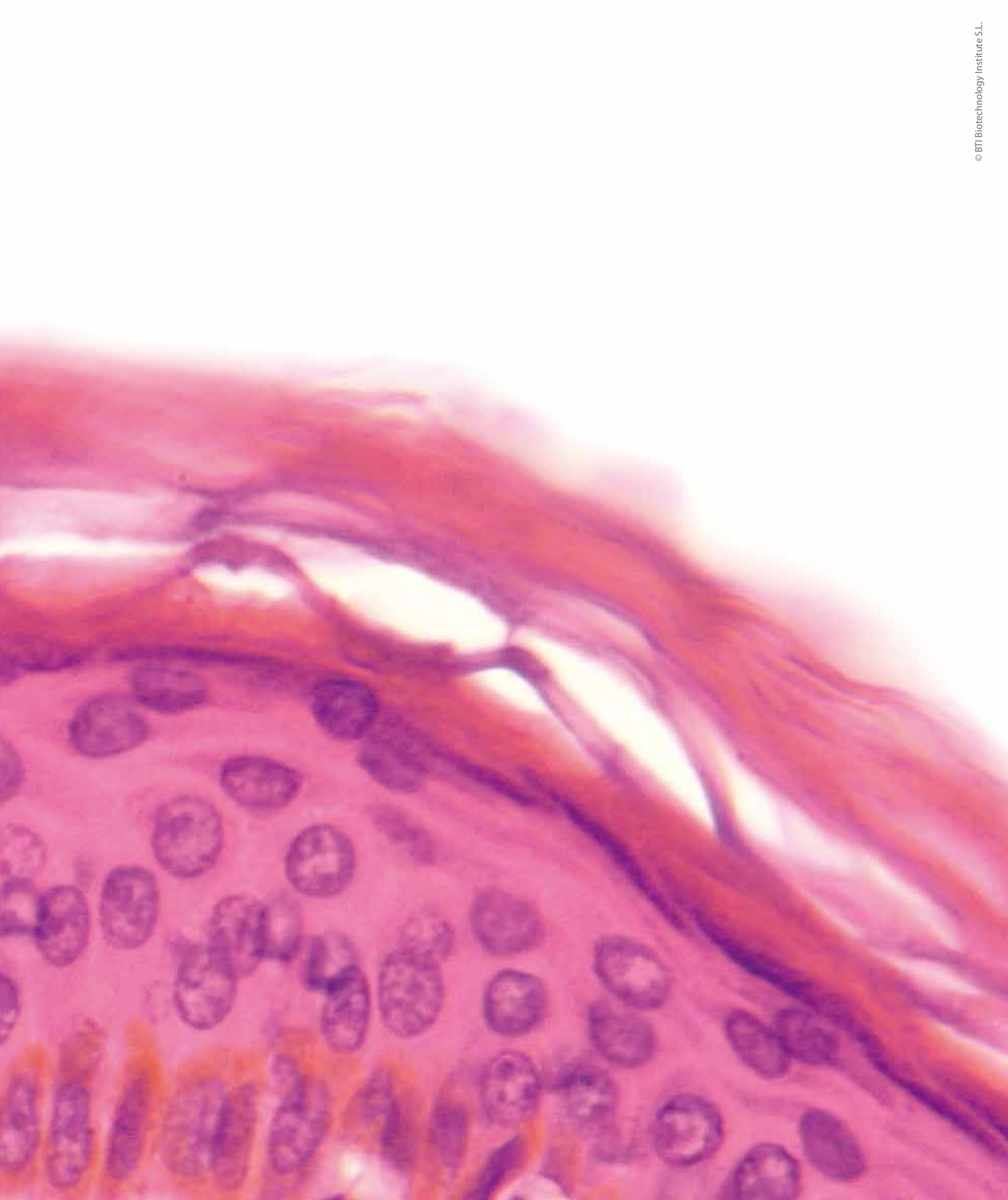
Clinical Research

Mal perforant ulcers are ulcers of torpid evolution that can lead to partial or total amputation of the affected limb. Usually, the incidence of ulcers is greater in individuals with an associated pathology, which reduces the regenerative capacity of the organism itself, as can be the case of diabetic patients.

This paper describes a clinical case of a patient with diabetic neuropathy and a severe mal perforant ulcer on her right foot. After 2 applications of PRGF®-Endoret® there was complete closure of the wound 10 weeks after the first application of this autologous preparation.

Although preliminary, this case report may shed light on the potential of PRGF®-Endoret® for accelerating the healing of severe mal perforant ulcers.

Orcajo B, Muruzabal F, Isasmendi MC, Gutierrez N, Sánchez M, Orive G, Anitua E. The use of plasma rich in growth factors (PRGF®-Endoret®) in the treatment of a severe mal perforant ulcer in the foot of a person with diabetes. Diabetes Res Clin Pract. 2011;93:e65-67.



Wound closure and healing

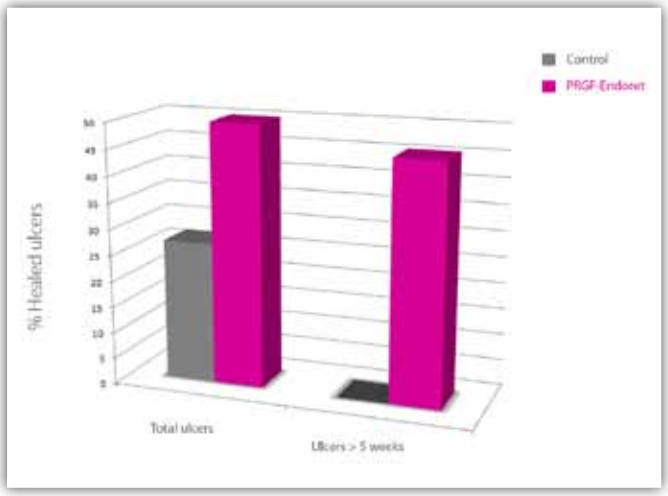
Clinical Research

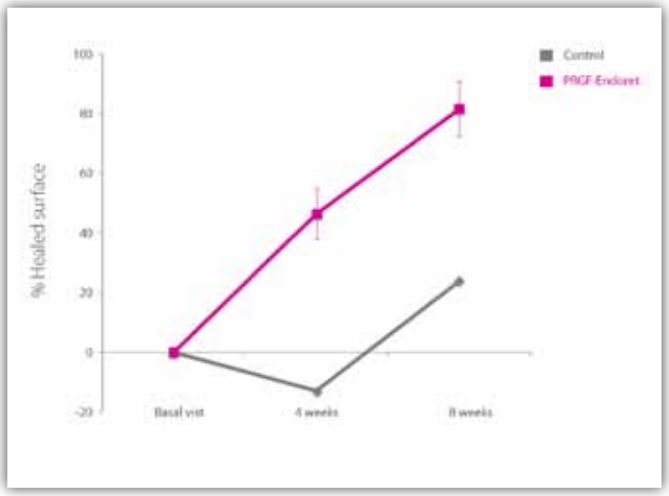
This clinical trial was conducted in order to evaluate the efficacy and safety of PRGF®-Endoret® in the treatment of skin ulcers of venous origin.

Twenty-two ulcers of 18 patients were included in a randomized pilot clinical trial, in parallel groups and controlled with conventional treatment (cleansing, debridement and saline wet cure).

Each patient attended once a week for 8 weeks follow-up to assess the injury condition and to apply whether the conventional treatment or PRGF®-Endoret® treatment according to randomization. The assessed variables were the number of ulcers healed at the end of the follow-up period, the area of injury, and the PUSH score.

At the end of the follow-up period, 50% of the ulcers treated with PRGF®-Endoret® were completely closed compared to 27% of the ones treated conventionally. By stratifying the analysis taking into account the wounds older than 5 weeks, the percentage of ulcers that reach complete healing was 45.5% in the experimental group versus 0% in the control group ($p = 0.035$). At 4 weeks follow-up, the percentage of recovered surface was 46.5%





in the group treated with PRGF®-Endoret® compared with 13% in the conventional group (p <0.001). After 8 weeks follow-up the percentage of recovered surface observed in the experimental group was 81.8% versus 23.9% in the control group (p <0.001).

These results demonstrate faster healing of skin ulcers treated with PRGF®-Endoret® with respect to conventional therapy.

Aguirre JJ et al. Eficacia del PRGF®-ENDORET® en el tratamiento de las úlceras cutáneas secundarias a insuficiencia venosa: Ensayo clínico aleatorizado controlado con tratamiento convencional. 2012.

The use of PRGF-Endoret significantly increases the percentage of total healing in ulcers older than 5 weeks. Likewise, the percentage of healed surface is greater in ulcers treated with PRGF-Endoret at 4 and 8 weeks.

Repair and Regeneration

Ageing is a multifactorial process that takes place during the last stage of the life cycle and is characterized by a gradual loss of functional ability in all tissues and organs of the body, and the consequent ability to adjust to environmental stimulus.

It is known that skin ageing is caused by degradation of the intercellular matrix, decreased vascularization, dysfunction of the annexed skin, fat atrophy, atrophy or muscle relaxation and repeated muscular contraction. Some fundamental factors are involved in these phenomena, like the inexorable passing of time and genetics. There are also a number of concurrent factors that are also involved in this process including sun exposure, diseases (general or skin related), hormonal situation, nutrition, weight changes, medications, toxic habits and cosmetics care. Nowadays, an increasing number of people worldwide face the challenge of maintaining a younger appearance. Facial rejuvenation has become socially acceptable and highly desirable for millions of people in the ageing population. This increased awareness of aesthetics is creating a heightened demand for novel cosmetic treatments aimed at hydrating and improving the appearance of the skin.

Repair and Regeneration

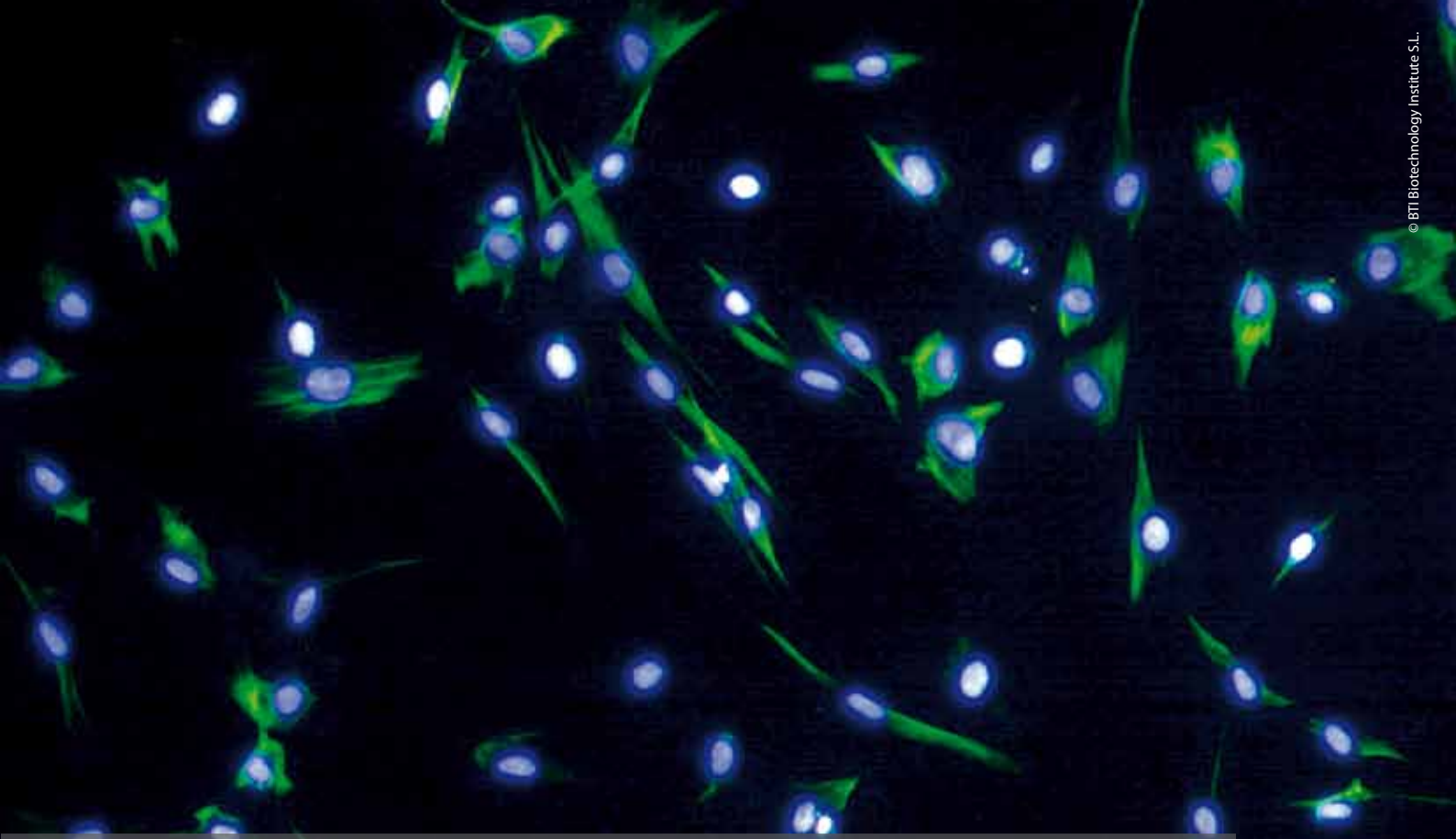
Preclinical Research

The use of plasma rich in growth factors (PRGF®-Endoret®) stimulates the proliferation and synthesis of various factors related to tissue regeneration in cultured epithelial fibroblasts.

The main objective of this study was to evaluate the effect of PRGF®-Endoret® in stimulating epithelial fibroblast proliferation related to epidermal regeneration. Various biological proteins secreted by cultured fibroblasts after stimulation with PRGF®-Endoret® were also studied. The different experiments were performed in primary epithelial fibroblasts cultures from different donors.

The results showed a significant increase in the process of epidermal fibroblasts proliferation after treatment with PRGF®-Endoret®. Analysis of the cultured cells after a treatment period of 72 hours showed an increase of anti-inflammatory factors (HGF), angiogenic (VEGF) and proteins related with the extracellular matrix remodeling (Procollagen-I, acid hyaluronic and TIMP-1).

Procollagen-I is a structural molecule in the dermis whose role is primarily to increase the skin resistance to forces such as tension and stretching. The hyaluronic acid in turn finds itself at high concentrations in the



interstitial fluid surrounding these collagen fibers acting as a lubricant and promoting its synthesis.

Furthermore, it is a molecule that is capable of retaining high levels of water, which in turn is an essential molecule in the epidermis hydration degree, providing the optimum levels of skin hydration, smoothness and firmness. Finally, TIMP-1 (metallopeptidase inhibitor 1) is an important metalloproteinases inhibitor, therefore plays a crucial role in the stability of the extracellular matrix that sorrounds the different cell types of the skin.

The results obtained in this study suggest that PRGF®-Endoret® significantly stimulates cell proliferation in skin fibroblasts and stimulates the autocrine expression of several factors involved in wound healing.

Repair and Regeneration

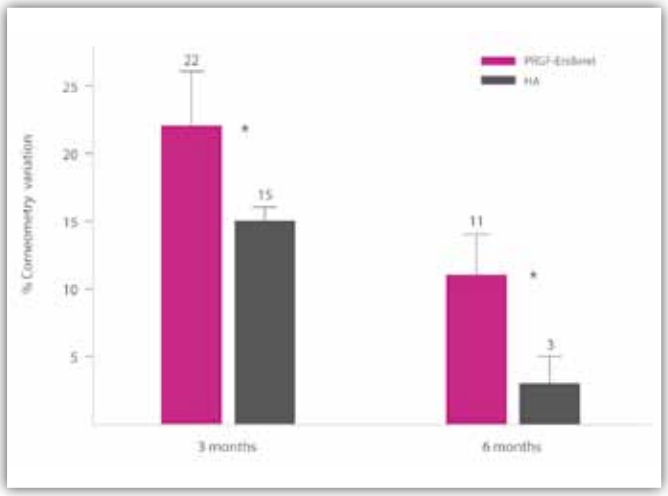
Clinical Research

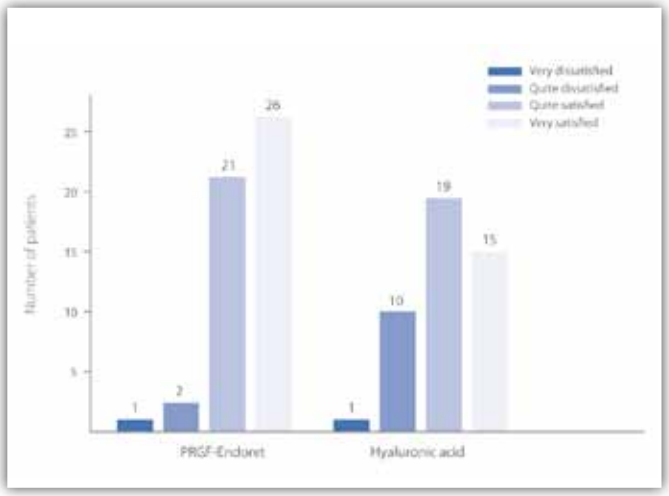
In this randomized clinical trial, we evaluated the efficacy and safety of PRGF®-Endoret® in skin regeneration compared with hyaluronic acid, widely accepted for the treatment of skin aging.

A total of 100 patients with external signs of skin aging were randomly assigned treatment with PRGF®-Endoret® or hyaluronic acid.

Two follow-up visits were carried out in which the following efficacy variables were measured: skin hydration degree, sebometry and skin pH; wrinkle severity and patient satisfaction were also assessed.

In both follow-up visits, skin hydration and pH values and patients´ satisfaction were significantly better in the group treated with PRGF®-Endoret® ($p < 0.05$) than in the control group. On the other hand, sebometry levels at 3 months significantly decreased in both treatment groups compared to baseline. Regarding the evolution of the depth of wrinkles, there was a significant improvement ($p < 0.001$) in both treatment groups at both follow-up visits. These results support the application of PRGF®-Endoret® in the treatment of age-related skin aging, obtaining excellent mid and long term results after its application.





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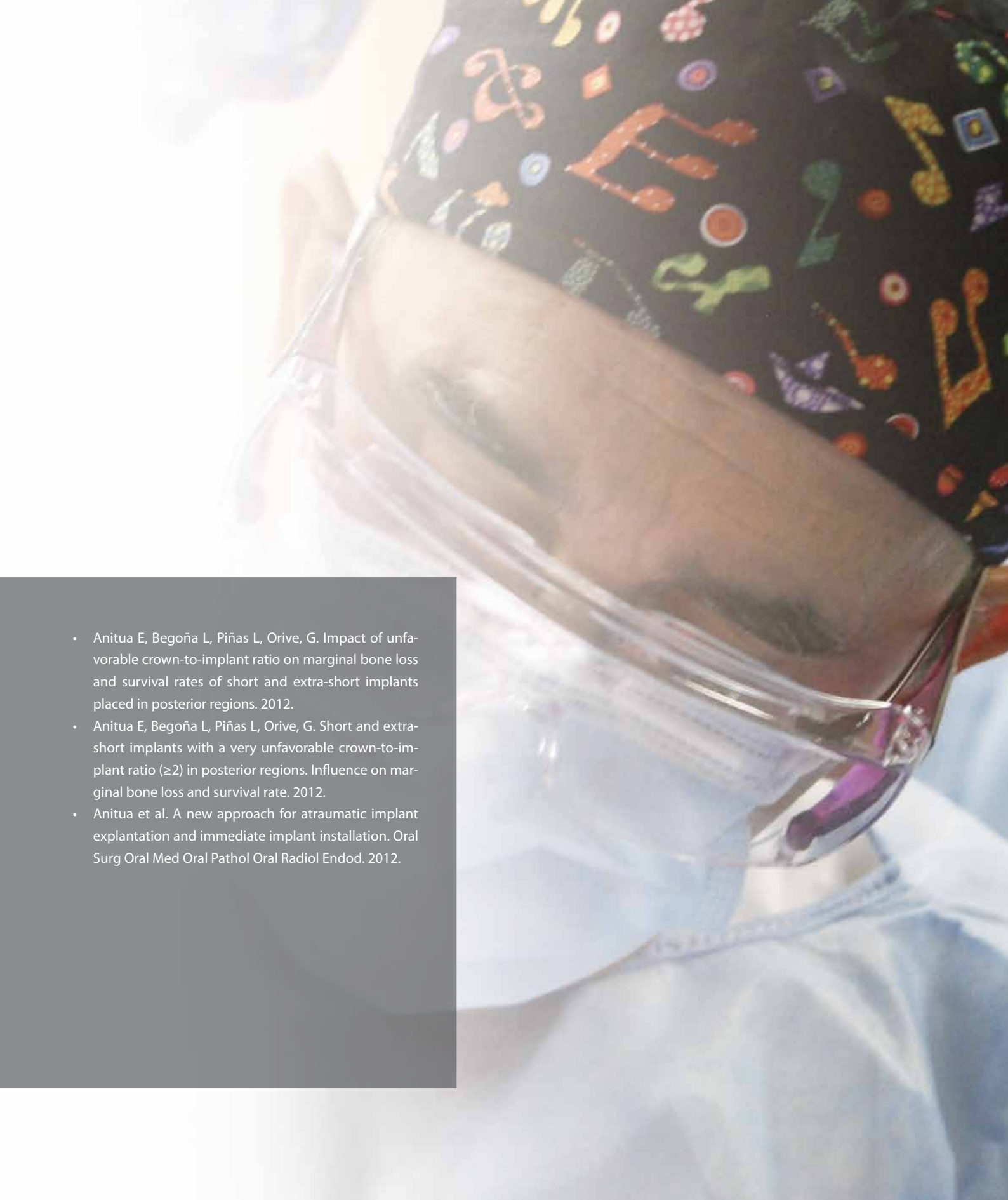

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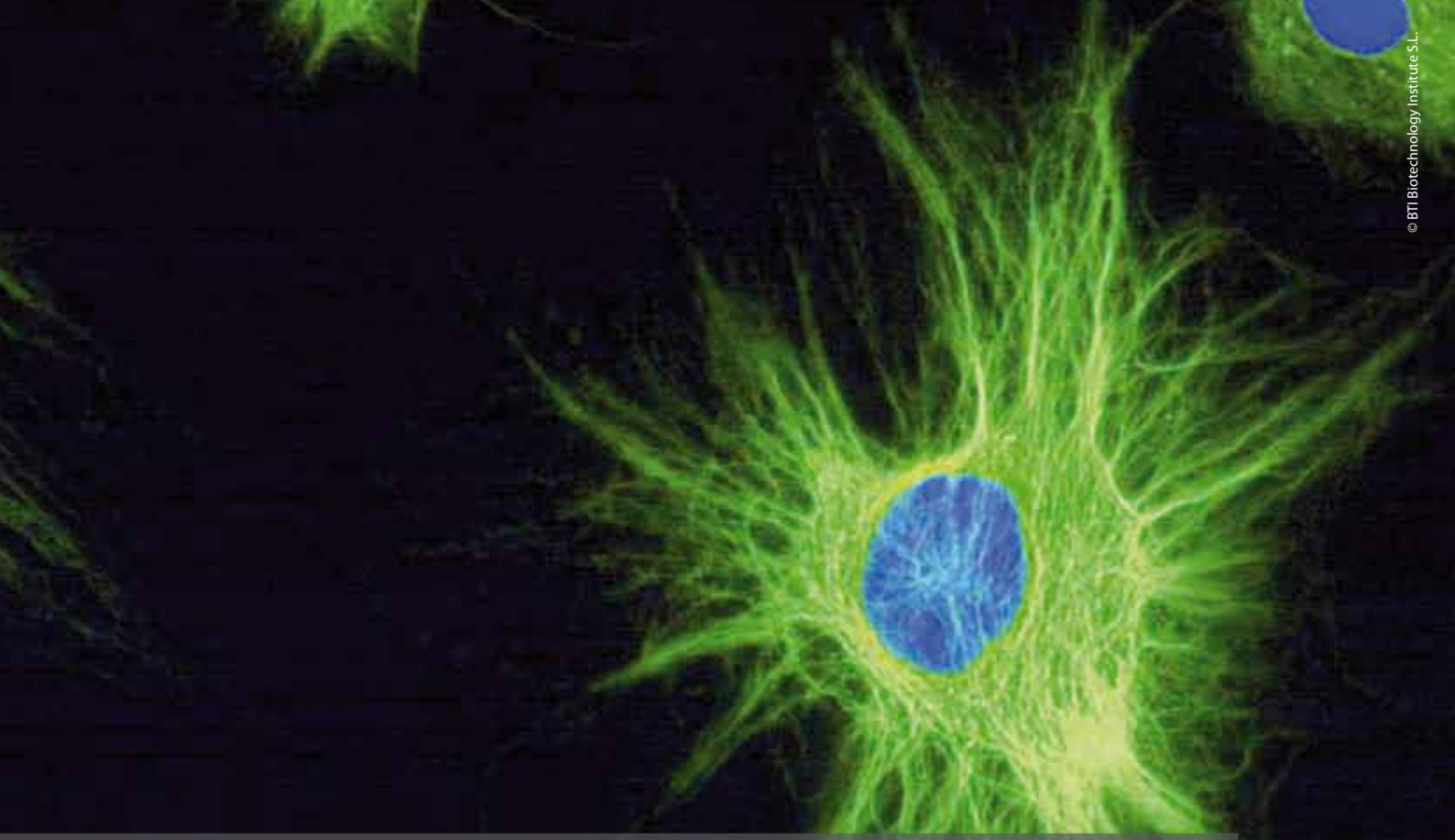

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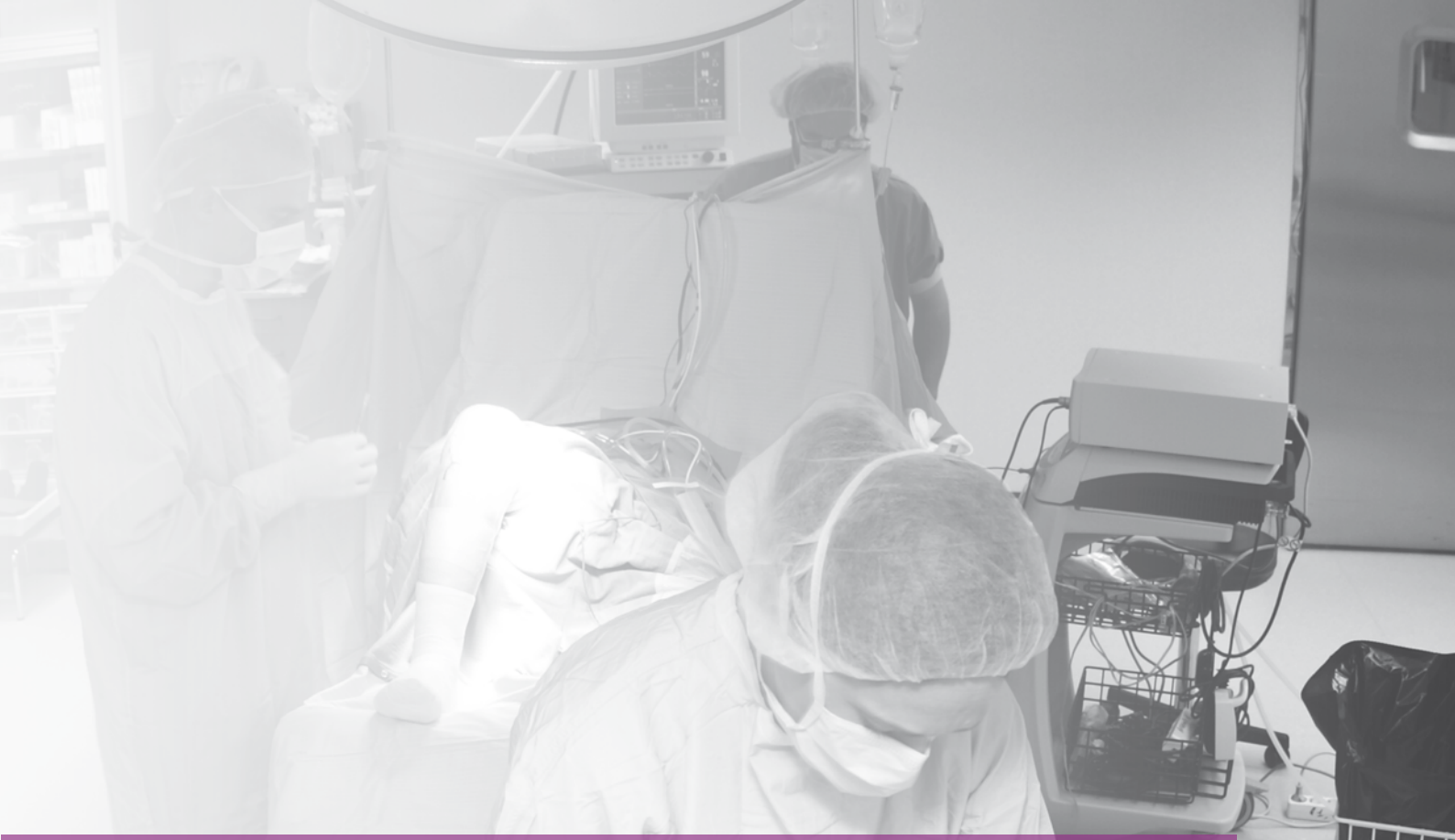

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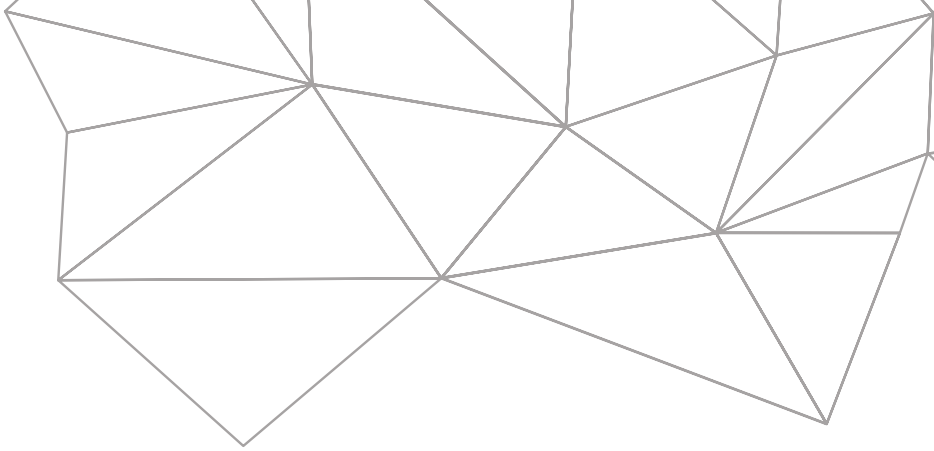

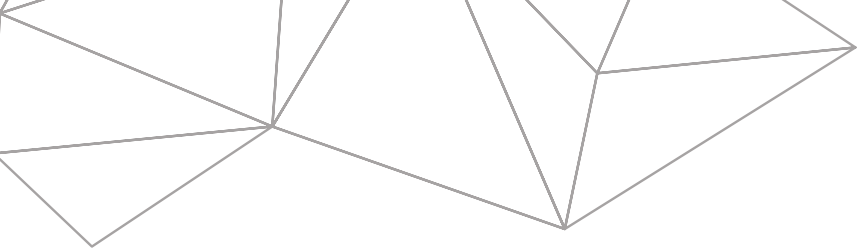

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