USE OF CELLULAR MATRIX FOR FACIAL REJUVENATION

Dr. Madiha Ben Hassen, MD

Medical doctor, generalist.

INTRODUCTION

The restoration of facial volume loss, unrelated to age, is becoming a frequent request in aesthetic medicine.

Indeed, drastic and selective diets (high-protein diet, keto diet...) are exposing patients to a high risk of fat loss in the cheek, peri-orbital area and tear trough as well as resulting in a significant lack of hydration and dull skin appearance.

Commonly treated with hyaluronic acid (HA) injections or lipofilling, these young patients presenting with facial volume loss frequently refuse such procedures. A deep injection of Cellular Matrix was proposed as a therapeutic solution targeting both restoration of facial volume and improvement of skin laxity.

The presence of 40 mg of uncross-linked hyaluronic acid in the Cellular Matrix device makes it possible, in one easy step, to restore cheeks, peri-orbital area, tear trough and naso-labial folds when injected by cannula.

The combined effect of HA with Platelet-Rich Plasma (PRP) was sought to treat other symptoms, such as dehydration, lack of radiance and delayed healing. We present the results of a study with the aim of evaluating the safety of the use of PRP and hyaluronic acid (Regenkit-Cellular Matrix, Regenlab) to induce skin rejuvenation.

METHODS

In this study, 16 patients were treated by Cellular Matrix PRP-HA (CM-PRP-HA), mean age 35 (±9) years, presenting with facial volume loss, who received a deep injection of CM-PRP-HA in the subcutaneous fascia.

Each patient received 3 treatment sessions with an interval of 4 weeks between each session.

MATERIALS

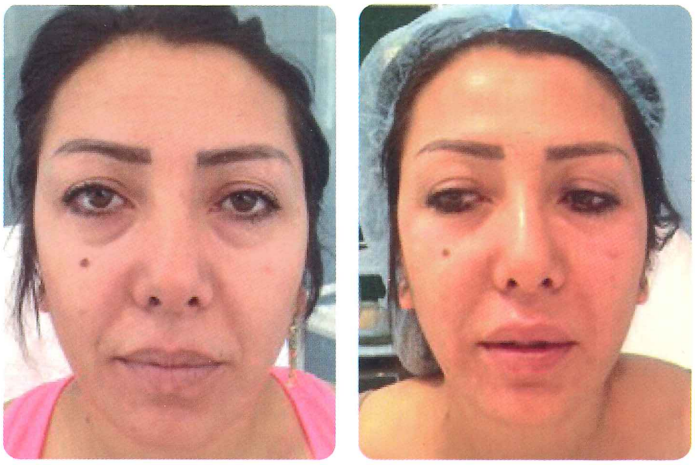
- Cellular Matrix kits (BCT-HA-3) with accessory devices (each tube allows the recovery of 3mL PRP+2mL HA from 10mL blood)
- Regenlab Centrifuge
- Canula softfil 25G/16/N- Ø0.5×16mm

RESULTS

PRP provides a tank of growth factors (EGF, VEGF, FGF, PDGF...) which will create a sustained effect on stem cell migration, differentiation and tissue regeneration. This effect is potentiated by the impact of hyaluronic acid on cell hydration and migration.

Following Cellular Matrix treatment, we observed reduced skin laxity and enhanced skin density especially in the cheeks (1 ml in each cheek), peri-orbital area (0.1 – 0.3 in each side) and nasolabial folds (0.3 - 0.6 in each side) area. Examples are shown in Figure 1.

Peri-orbital area



Skin Laxity

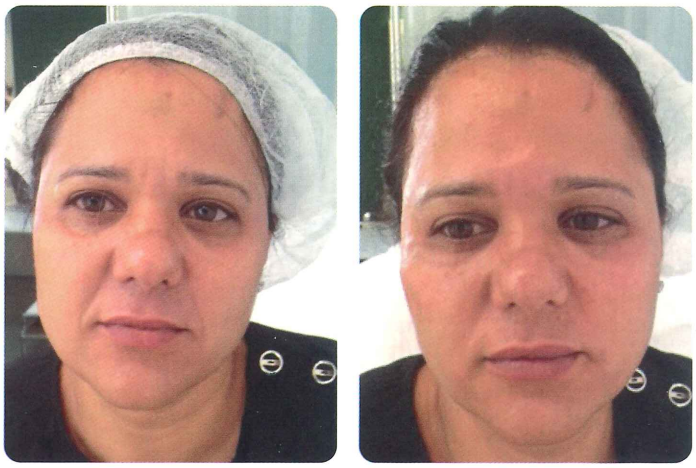


Fig. 1. Improvement in the skin of the periorbital area (A) and skin laxity (B) after PRP treatment. Images of the skin prior to treatment are on the left and after treatment on the right.

We observed that 80% of the patients involved in this study noticed a reduction of superficial wrinkles, such as peri-oral fine lines and crow's feet wrinkles, as well as an improvement of tissue tone and healthy appearance of the skin.

In conclusion, Cellular Matrix is a combination that restores the tissue by giving a bio remodeling and facelift effect.

PRP in Cosmetic Dermatology

Prof. Ashraf Badawi, MD, PhD

Professor of Dermatology, Laser Institute, Cairo University, Egypt
Visiting Professor of Dermatology and Laser applications, Szeged University, Hungary
Laser Consultant, Canada
Vice President of the European Society for Lasers and Energy-based Devices «ESLD»
President Elect of the European Society for Cosmetic and Aesthetic Dermatology «ESCAD»

The fountain of youth has been an age-old dream of humankind. People have long used extreme measures to prevent or reverse normal aging and senescence.

Accordingly, physicians implemented techniques to enhance or supplement rejuvenating material in their patients and claimed outstanding and often unbelievable results in order to outcompete their contemporaries.

Today, almost everyone is aware that there are modalities which can improve or delay the signs of aging affecting his/her face. In spite of the increasing demand for rejuvenation procedures, sometimes we as practitioners tend to forget about the basics and start to skip steps. Sometimes we do not pay attention to the pathophysiology of skin aging and start using a certain therapeutic tool with the hope of getting a satisfied patient, but this patient is not satisfied at the end. Why? Simply, because we did not consider "Rejuvenation", instead we performed procedures which lead to temporary improvement of the appearance of the skin but without any real structural and functional improvement.

When discussing any topic related to rejuvenation, it is essential to provide some basic knowledge about the process of skin aging and the changes happening in each of the skin layers. If we don't consider these changes, we lose our way and our patients won't be satisfied.

SKIN AGING

As with any other organ, the skin is subject to the gradual chronological senescence of aging. The biological consequences of aging are first apparent at the cellular level, where they act on a variety of targets. Even in people as young as 30, aging has an effect at the cellular level, but it is only in mid to old age that the process becomes visible, as the microscopic structural and functional changes accumulate, forming wrinkles and folds, for example. Aging of the skin can be attributed to intrinsic and extrinsic factors, both of which have an impact on surface and subsurface changes. Intrinsic aging is dictated by the genes of the person (Moschella and Hurley, 1992).

A very high percentage of age-associated cosmetic skin problems can be attributed to sun exposure. Changes to skin pigmentation are caused directly by sunlight. Sunlight is a spectrum of electromagnetic radiation encompassing visible light and wavelengths that are not visible to the human eye, high energy waves such as ultraviolet (UV) radiation. UV radiation is divided into two portions: A and B. UVA is nearest to visible light in the spectrum and extends from 320 to 400 nm in wavelength. It causes tanning and contributes to ageing of the skin. UVB extends from 280 to 320 nm in wavelength and is primarily responsible for sunburn, aging of the skin and the development of skin cancer. Photons of UV light strike the organic molecules of the skin and result in the formation of reactive oxygen species. These free radicals alter the structure and function of cellular membranes, polyunsaturated fats, proteins and DNA (Trautinger, 2001).

In terms of the skin, collagen and elastin are degraded and neo-collagenesis is inhibited. The DNA of living cells is also damaged (Fisher, 2005; Fisher et al., 1997).

The pigmentated lesions caused by sun exposure include solar lentigo, pigmented seborrheic keratoses, melasma and facial melanosis.

The intrinsic process of chronological aging results from thinning of the epidermis and dermis and loss of elasticity. This process affects all layers of the face, including subcutaneous tissue, the musculofascial system, the superficial musculoaponeurotic system, and the facial skeleton. The result is bony resorption, atrophy of subcutaneous fat, attenuation of the musculofibrous system, and alterations of the skin surface. The dermal-epidermal junction flattens, which results in loss of rete ridges and a thinner appearance to the epidermis. The dermis also becomes thin, with a decrease in elastic fibers, collagen production, vascularity, and ground substance. The biochemical alterations in collagen and elastin result in a dermis that is more lax yet less elastic and resilient. Collectively, these changes result in fine wrinkling of the skin and sagging of the tissues that overlay the facial skeleton (White et al., 1996).
Extrinsic photoaging causes degenerative changes in the skin that are superimposed on the normal chronologic aging process. Clinically, this process results in coarse and dry skin, deep wrinkles, sallowness, and dyschromia. Histologically, photoaged skin is characterized by epidermal hyperplasia and dysplasia, a thickened dermis with solar elastosis, actinic vasculopathy, decreased collagen fibers, and increased ground substance (Coopman et al., 1996).

SKIN REJUVENATION

Facial rejuvenation is becoming one of the most requested services in cosmetic dermatology and plastic surgery clinics. An important factor behind that is the media which is supported by the different manufacturers of skin care products, peels, fillers, neurotoxins and light-based systems. Today, almost everyone is aware that there are modalities which can improve or delay the signs of aging affecting his/her face.

During the past 15 years or so, practitioners and patients were eager to get a quick fix rather than rejuvenation.
Rejuvenation means the restoration of the structure and function of the skin.

Not every procedure we perform to enhance the appearance of the skin is considered rejuvenation. An example is the injection of neuromodulators. Neuromodulators can decrease expression lines, but they do not stimulate neo-collagenesis, they do not improve epidermal barrier function and they do not tighten the skin.

This is how we need to evaluate each and every technology we have in hand and decide if it leads to skin rejuvenation or if it is just a temporary fix where the end result is worse than the beginning due to failing to prevent the progress of skin aging.

PRP IN COSMETIC DERMATOLOGY

Platelet-rich plasma (PRP) is an autologous blood-derived product with an increased concentration of platelets in plasma, which are used to deliver supraphysiological levels of growth factors. Platelet-rich plasma has been used in many fields, including oral, maxillofacial, and plastic surgery. Its use in sports medicine has been increasing after recent evidence and media publicity suggested that it may augment the treatment of muscle strains, as well as tendon and ligament healing. With increasing media coverage on the use of PRP in cosmetic dermatology, it is paramount that practitioners understand the various methods of preparation and administration, potential clinical applications, and available clinical results to best counsel patients on its advantages and disadvantages.

In my experience, PRP has been always an excellent adjuvant to my rejuvenation techniques, whether it is a laser, microneedling or microneedling radiofrequency. In addition, I started to use PRP in the form of a PRP gel (Regenkit ACR Plus, Fig.1) as a more physiological replacement for fillers.

Figure 1: Autologous gel obtained with Regenkit ACR Plus.

The use of HA fillers carries a risk of vascular and non-vascular complications; that is not the case when injecting PRP.
With PRP, we observe a very gentle and favorable tissue response and we obtain a true rejuvenating effect.
There is a good synergy with other techniques like non ablative laser rejuvenation, microdermabrasion, skin microneedling and Radiofrequency.

In addition, I use it in combination with LED therapy for different cases of hair loss in males and females.
PRP is a procedure with which I never have a fear of allergic reactions or adverse events.

On the other hand, with a good quality product, we always get a good reproducible outcome.

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PRP in Cosmetic Dermatology

Dr. Amina Alamiri, MD

Dermatologist, aesthetic and laser specialist
ASDS, DASIL mentor - Dubai

Acne is a common dermatological condition resulting from multifactorial chronic inflammation of the pilosebaceous unit. It mainly occurs during adolescence causing great psychological stress impacting the quality of life if delayed or left untreated (Chawla, 2014; Chowdhary et al., 2019; Gollnick et al., 2003; Holland et al., 2004; Khunger, 2008).

Acne scarring occurs as a consequence of acne in the course of the disease. It is one of the most frequent causes of facial scarring and improvement of scars is a common request but it is the most challenging cosmetic procedure (Chawla, 2014; Khunger, 2008).

Classification of acne scars is necessary to assess the severity of cosmetic disfigurement and essential for choosing the therapeutic intervention (Chawla, 2014; Kubba et al., 2009).
Acne scars can be classified as atrophic or hypertrophic scars. Atrophic acne scars are more common than hypertrophic scars. Atrophic scars have been further sub-classified based on their width, depth and 3-dimensional architecture, namely ice-pick scars (V-shaped), boxcar scars (U-shaped) and rolling scars (W-shaped). Sometimes all three types of atrophic scars can be seen in the same patient and it may be difficult to differentiate between them (Chowdhary et al., 2019; Jacob et al., 2001; Kubba et al., 2009).

Goodman and Baron included all the morphological types of post acne scars and used a simple clinical examination as the tool to grade the scars on objective lines (Chawla, 2014; Goodman and Baron, 2006).

Grading of the atrophic scars as per the Goodman and Baron scale is as below:

Grade 1 — Macular erythematous, hypo or hyper pigmentation.
Grade 2 — Mild atrophy, not obvious at social distances of >50 cm or easily covered by facial make up or beard hair.
Grade 3 — Moderate atrophy, obvious at a social distance of >50 cm; not easily covered by make up or beard hair but can be flattened by manual stretching.
Grade 4 — Severe atrophy, not flattened by manual stretching of the skin (Chawla, 2014; Goodman and Baron, 2006).

Acne scar management demands a multimodal approach to deliver desirable outcomes. The therapeutic approach depends on several factors such as the type of scar, skin type, post procedure downtime and risk profile (Chowdhary et al., 2019).
Microneedling therapy is a recent addition to the treatment options for acne scars. It is a simple, inexpensive office procedure with no downtime. It is a procedure that uses a group of tiny needles that penetrate the skin and create micro-channels in the skin, whereas microneedling radio-frequency (MRF) is a combination of the two modalities of micro-needling and energy that allows penetration of needles and radio-frequency. The needles come with the option of being insulated or non-insulated. In the case of insulated needles, only the tip of the needle is exposed to deliver energy to the dermis at different levels depending on the depth of penetration. Heat produced by the RF energy leads to stimulation of collagen fibers and elastin, leading to significant improvement of skin quality and texture, helping in scar reduction and skin tightening (Gold and Biron, 2012; Hruza et al., 2009).

Subcision is a minor surgical procedure in which we use a nokor needle to cut the fibrotic tissue or strands in certain types of fibrotic scars. The needle's sharp edges are maneuvered under the scar to make subcuticular cuts. The new connective tissue formed during the course of normal wound healing after the incisions helps to achieve a flatter, more regular surface to the scars. The procedure must be repeated a number of times with an interval of 1–3 months between treatments (Chandrashekar and Nandini, 2010; Sánchez Viera, 2015).
Combining different modalities to remove acne scars is the treatment of choice for the past few years. Platelet-rich plasma (PRP) acts synergistically with growth factors in order to enhance the wound healing response and gives better outcomes and less side effects (Chawla, 2014; Chowdhary et al., 2019; Majid, 2009; Sharad, 2011).

METHOD AND MATERIALS

- The patients were evaluated, and grading of the acne scars was done using the Goodman and Baron scale.
- The different methods and the cost factor involved, benefits, duration, possible side effects and prognosis of the treatment were explained to the patients.
- For all patients, topical antioxidants like vitamin C, glycolic acid and retinoic acid were added post procedure according to the skin type.
- Informed consent was obtained.
- Digital photographs of the face were taken.
- The area of interest was anesthetized using a thick application of topical anesthetic cream for about 40-60 minutes prior to the procedure.
- Regular usage of sunscreens was advised.
- A minimum of 4-6 sessions with 4 weeks interval were done.
- First 4 sessions were performed with PRP, then repeated if necessary, every 3 to 6 months.
- Subcision was done once and repeated if needed.

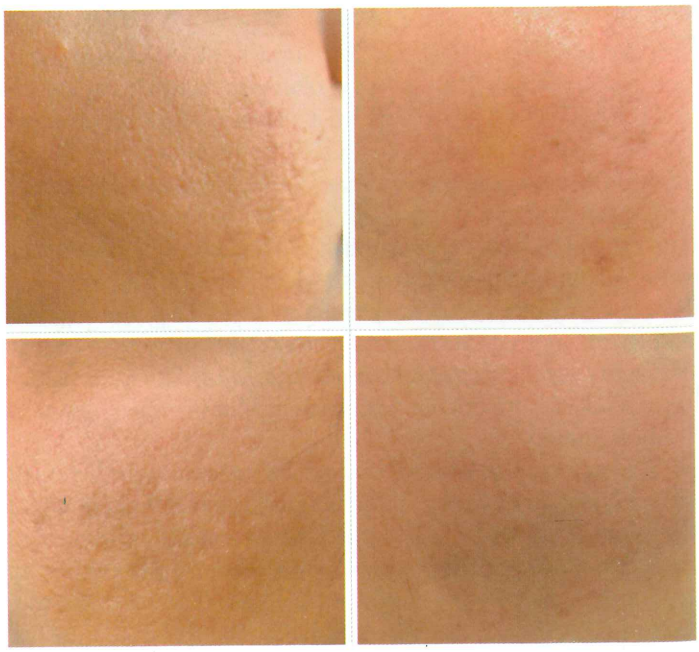
Micro-needling Protocol
- Microneedling with needles of 0.8-2.5mm length and needles on a roller drum were used.
- As a standard protocol, both sides of the face were subjected to microneedling with both topical and injected PRP.
- The microneedling was carried out in vertical, horizontal and both diagonal directions, about 4-5 times on the area that was affected.
- First pass was with needles of 2.5 mm length, then 1.5 mm length, then 0.8 mm length.
- We used 0.8 mm length needles on areas which were not involved.

MFR Protocol
- Microneedling fractional radio-frequency was done on the targeted area and whole face.
- The parameters used are listed below.
- Targeted area 2.5 mm.
- Over the forehead, bilateral temples and bony prominences, 0.8 - 1 mm needle depth was kept.
- Over the cheeks, multiple passes were given with needle depth 2.5mm for the first pass, 1.5 mm for the second pass, and 0.8 mm for the third pass, with minimal overlap.
- Energy was used according to needle depth.

The following images demonstrate the effectiveness of the treatment for the correction of acne scars.

Parameters	Values
Mode	Monopolar
Intensity/Energy	20 -50
Pulse width	50 - 200 ms
Depth	0.8 -2.5 mm
Passes	2-3
Interval between sessions	4 weeks

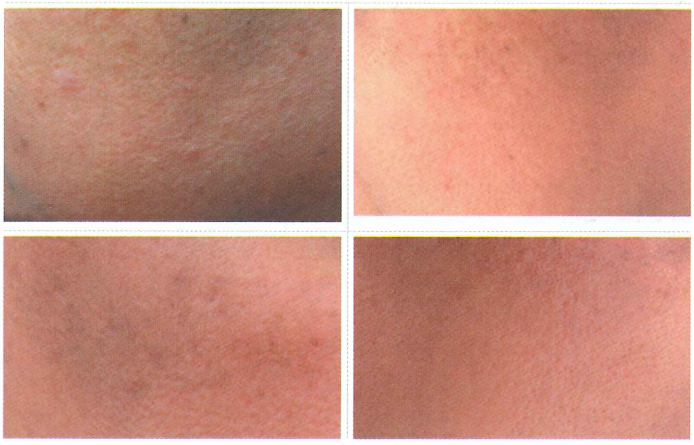
Case 1
43-year-old female with acne scars after 6 sessions of MRF with subcisions and PRP
Before After



Case 2

49-year-old female, photos of both sides of the cheeks after 4 sessions of derma pen and PRP

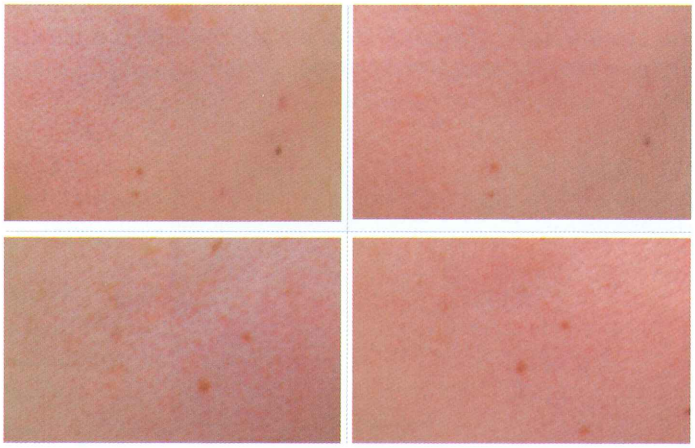
Before After



Case 3

35-year-old female with acne scars after 3 sessions of MRF and PRP

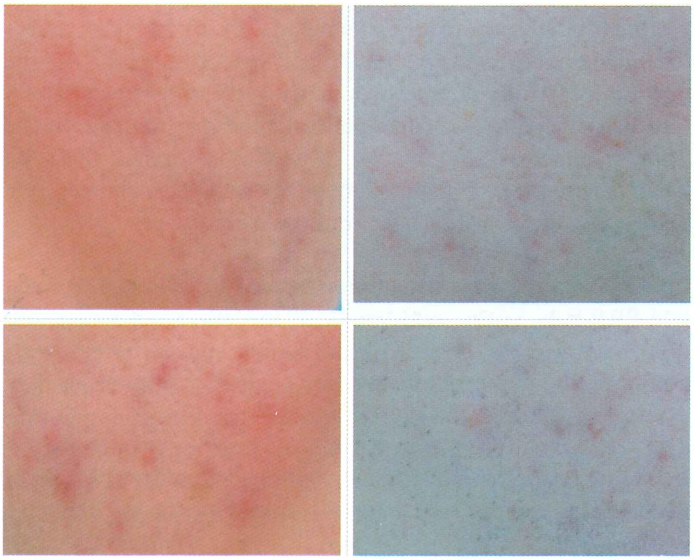
Before After



Case 4

24- year-old female, photos of both sides of the cheeks after 4 sessions of derma pen and PRP

Before After



CONCLUSION

The field of dermatology has seen various therapeutic innovations in the past decade, with PRP recently gaining significant interest as a treatment for acne scarring.

PRP efficacy in wound healing and regeneration as well as its ability to increase vascularity has motivated its use in the management of atrophic facial scars as a monotherapy as well as a combination therapy with other available treatment modalities (Gold and Biron, 2012; Goodman and Baron, 2006; Majid, 2009).

PRP is an autologous blood-derived product enriched in platelets, growth factors and chemo/cytokines delivered in a concentrated volume of plasma that has the potential to deliver a high concentration of growth factors to target tissues by virtue of the contents within the alpha and dense granules.

In my practice, combining different modalities always gives better results for many reasons. Treating acne scars with MRF and PRP gives the best result with 6 sessions combined with subcision if needed. Adding topical vitamin C results in improvement in firmness and smoothness of the skin as well as post inflammatory hyper-pigmentation.

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Tissue regeneration: angiogenesis is differentially modulated by platelet-derived products

Dr. Sarah Berndt, PhD

Department of Plastic, Reconstructive and Aesthetic Surgery, Geneva University Hospitals, Geneva, Switzerland.
Regen Lab SA, Le Mont-sur-Lausanne, Switzerland.

Vascularization of tissue structures is mandatory to ensure their survival. As the diffusion of oxygen within tissues is limited to 100-200 µm (Carmeliet and Jain, 2000), vascular ingrowth within a regenerating tissue will provide oxygen and nutrients in situ. In natural wound healing, angiogenesis is a tightly controlled vascularization process that allows the formation of new vessels from preexisting ones (Eelen et al., 2020).

In tissue regeneration, therapeutic angiogenesis aims at restoring a proper vascular system through the delivery of exogenous growth factors, cytokines and chemokines among others. Important angiogenic factors are VEGF, angiopoietin, FGF, HGF, PDGF, and TGF, although a myriad of other proteins are also known to be involved in blood vessel formation.

Current strategies in animal models of angiogenesis are the delivery of angiogenic genes, potent angiogenic proteins, or angiogenic progenitors or stem cells. However, the clinical translation of such approaches remains challenging (Chu and Wang, 2012). Another interesting option is the controlled delivery of angiogenic growth factors in situ, see Tayalia for review (Tayalia and Mooney, 2009). Most of the studies are based on the delivery of a single angiogenic factor (i.e., VEGF, FGF2, or others) combined with a synthetic or natural biomaterial as a delivery vector.

Using platelet concentrate is also an interesting option, as platelets are a reservoir of multiple growth factors that stimulate stem cell migration, differentiation and proliferation (Masoudi et al., 2016).

There is a growing number of publications showing the interest in using platelet derived bio-products in different fields of regenerative medicine (Acebes-Huerta et al., 2020). When a tissue is wounded, platelets are responsible for hemostasis and then play a key role in the healing process through their controlled release of growth factors. Platelets are recognized as blood vessel growth regulators from the early stages of vasculogenesis until the advanced stages of angiogenesis (Kisucka et al., 2006). Platelets carry a multitude of angiogenesis regulatory proteins in their α granules, and the normal set of angiogenesis regulators has been characterized in human platelets (Peterson et al., 2010).

In this work, we used a high throughput in vitro 3D angiogenesis assay (the fibrin bead assay or FBA) to model the angiogenic effect of platelet-derived preparations used in tissue engineering studies or in the clinic (Figure 1A). We tested a range of concentrations (5 to 40%) of standardized PRP prepared with the Regen Lab CuteCell PRP device (RegenPRP), CM-PRP-HA, a proprietary combination of RegenPRP and hyaluronic acid obtained with the Cellular Matrix BCT-HA tube, and a commercial preparation of platelet lysates (PLTmax, Millipore, SCM141, 100 mL, frozen preparation).

The FBA uses a culture of endothelial cells (EC) on the surface of ~200 µm-sized Cytodex-3 microspheres embedded in a 3D bovine fibrin matrix, with normal human dermal fibroblast (NHDF) used as feeder cells. These stromal cells provide various angiogenic growth factors: hepatocyte growth factor (HGF), transforming growth factor alpha (TGF-α), angiopoietin-1 (Ang-1), as well as matrix molecules, matrix-modifying proteins and matricellular proteins (e.g. procollagen C endopeptidase enhancer 1, secreted protein acidic and cysteine-rich (SPARC), transforming growth factor-β-induced protein Ig-H3 (βIgH3) and insulin-like growth factor binding protein 7 (IGFBP7)) (Nowak-Sliwinska et al., 2018).

In control conditions, sprouting of neovessels is apparent between days 2 and 3, and cultures are imaged on day 4. For the quantification of microvessel network sprouting, samples are automatically scanned with a high-throughput imager and analysis of the microsphere images is performed using a method we developed with the ImageJ software (Carpentier et al., 2020; Schneider et al., 2012). This assay represents a significant improvement over conventional, single-cell-type angiogenic assays, as the inclusion of multiple cell types more closely mimics the physiological environment (Nowak-Sliwinska et al., 2018).

The FBA is a model that mimics sprouting angiogenesis. The basic steps of sprouting angiogenesis include enzymatic degradation of the capillary basement membrane, endothelial cell (EC) proliferation, directed migration of ECs, tubulogenesis (EC tube formation), vessel fusion, vessel pruning, and pericyte stabilization.

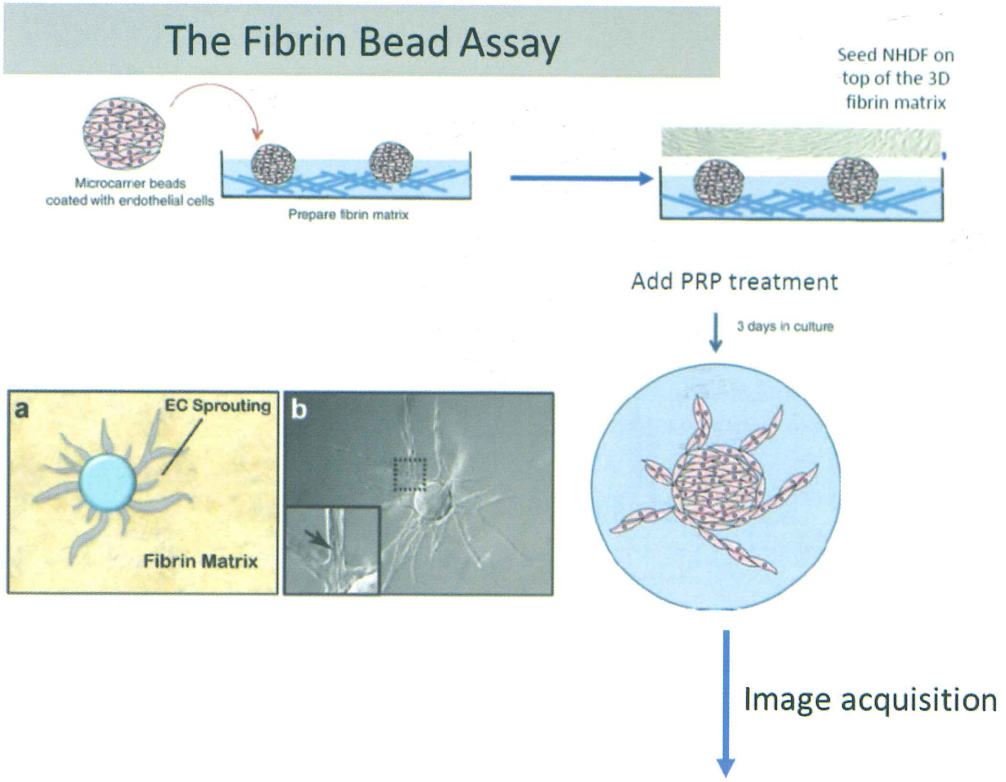
Platelet-derived preparations elicit different angiogenic responses when tested in the FBA where they act on different steps of the angiogenic process.

Thanks to the powerful automatic quantification method we have developed (Carpentier et al., 2020), we can assess the modulation of the morphometrical parameters of the neovessels formed in the fibrin matrix. Examples are the total length of the microvascular network, the anastomosis in the vessels (branches), the number of capillaries arising from the beads (anchorage), and the number of vessel tips showing the complexity of the network (extremities) (Figure 1B).

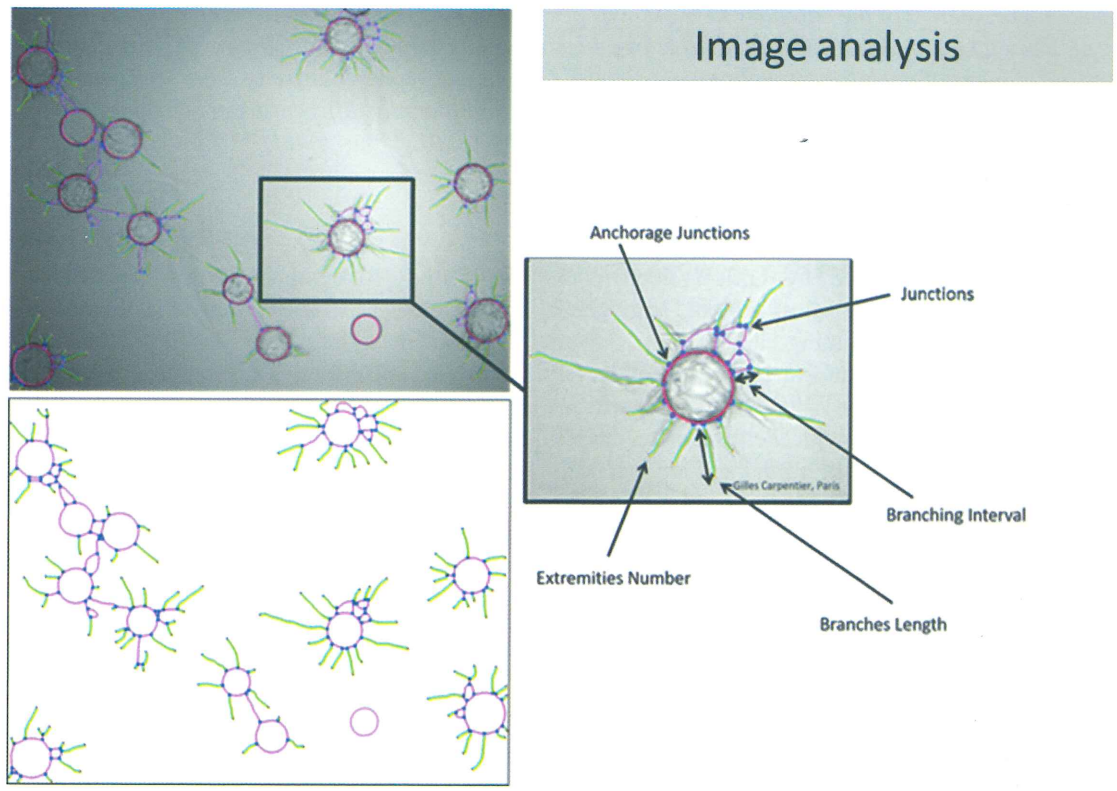
In our study, the most potent preparations were RegenPRP and CM-PRP-HA, as the total length of the neovascular network, total length of the branches, extremities and anchorage were highly stimulated compared to the control conditions (VEGF +/- heparin treatment) (Figure 2).

RegenPRP platelet preparations stimulated all steps of the angiogenic process, as massive sprouting of a branched

A.



B.



Adapted from [Jacobs, Cell Migration 2018] and [Berndt, Planta Medica 2018]

Figure 1. In vitro 3D fibrin bead assay (adapted from (Berndt et al., 2018; Jacobs and Gavard, 2018))
A. In this in vitro sprouting assay procedure, human umbilical vein endothelial cells (HUVEC) are coated on microcarrier beads and allowed to sprout into a bovine fibrin matrix in the presence of different platelet-derived preparations (PRP for example). B. Representative images of enhanced angiogenesis. Cytodex microcarrier beads coated with HUVECs exhibiting pseudocapillary growth after automatic analysis with a specific plugin developed for the Image J software (Schneider et al., 2012); some of the morphometrical parameters of interest are shown.

Figure 2.

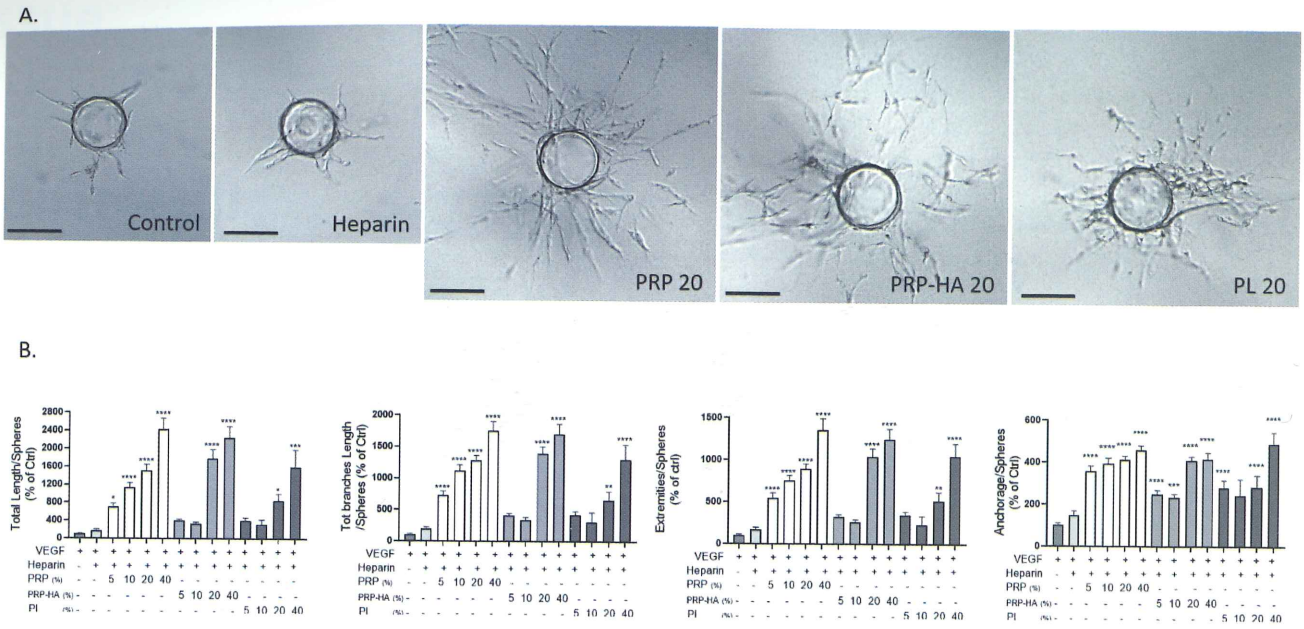


Figure 2. Platelet-derived products differentially modulate angiogenesis in the 3D fibrin bead assay.
*(A) 3D HUVEC cultures were treated with platelet-derived products (PRP, PRP-HA and PL) at different concentrations for 4 days. Representative pictures of massive enhancement of angiogenesis (PRP 20, PRP-HA 20) or slight endothelial proliferation from the EC coated beads (PL20) compared to control conditions (control and heparin) at day 4. Scale bar: 150 µm. (B) Quantification of morphometrical parameters of the capillary network was performed by a computerized method (thanks to Image J opensource software) on pictures taken on day 4. Representative parameters measured were total length, total length of branches, number of extremities and number of anchorage junctions per sphere. Graphs are representative of three independent experiments. One hundred spheres were quantified for each experimental condition. Significantly different from heparin as measured by one-way ANOVA followed by Dunett's multiple comparison test (*p<0.05, ** p< 0.01, ***p< 0.005, ****p<0.001).*

microvessel network was observed by optical microscopy (Figure 2A). RegenPRP was the most potent angiogenic preparation, significatively stimulating angiogenesis from 2 to 12-fold depending on the concentration of RegenPRP used and the parameter of interest (Figure 2B). Significant angiogenesis was already observed with the lowest RegenPRP concentration (5%). CM-PRP-HA also stimulated angiogenesis but to a lesser extent than RegenPRP. Platelet lysates had to be highly concentrated to elicit the same angiogenic response as RegenPRP and CM-PRP-HA.

RegenPRP and CM-PRP HA are freshly prepared autologous platelet preparations while platelet lysates are off-the-shelf, frozen platelet preparations. PLTMax is derived from multiple donor units collected at U.S. blood centers. RegenPRP and CM-PRP-HA contain living platelets that continuously deliver growth factors that stimulate the whole angiogenesis process directly on EC or indirectly (through a paracrine effect on NHDF). In addition, the plasma is a source of cell nutrients that sustain this process. The freezing and thawing process damages platelet integrity, thus platelet lysates are a mixture of growth factors and other platelet molecules in plasma. As growth factors have a short half-life, they are rapidly degraded in the culture medium after stimulation of the initial step of the angiogenesis process, i.e., endothelial cell proliferation. EC were stimulated and proliferated in the presence of platelet lysate, but proper vessel formation was not observed.

In this study, we demonstrate that RegenPRP and CM-PRP-HA are platelet preparations that lead to an efficient angiogenic response in a complex 3D physiological in vitro model. Controlled and effective angiogenesis is needed for proper tissue regeneration in situ. Platelet derived products are autologous biologics that drive angiogenesis in situ without the need for pre-vascularized exogenous materiel engraftment.

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The use of platelet-rich plasma in plastic and reconstructive surgery: the experience of the Henri Mondor Hospital

Dr. Barbara Hersant, MD

UNPUBLISHED CLINICAL STUDY

In a Phase II clinical trial, we compared the results of the group treated with Cellular Matrix with two groups of patients, one treated with PRP alone "RegenPRP" and another group of patients treated with hyaluronic acid "HA SkinVisc".

Ninety-three patients were included and evaluated in this randomized, controlled clinical study. Randomization was performed between the PRP and HA alone groups. The group that was treated with CM-PRP-HA showed a very significant improvement in the global facial appearance at 1, 3 and 6 months compared to the groups treated with PRP or HA alone ($p<0.0001$). In addition, the patients treated with CM-PRP-HA showed a relative increase of 20%, 24% and 17% in the FACE-Q score at 1 month, 3 months and 6 months after treatment, respectively.

Objective biophysical measures performed in the areas treated with CM-PRP-HA showed a significant improvement in skin elasticity for R5 at 3 months and 6 months compared to PRP or HA alone. Viscoelasticity (R6) was significantly improved at 3 months and 6 months and firmness (R7) was also significantly improved but only at 3 months when compared to the PRP or HA groups alone. No adverse events were reported.

The PRP and HA combination produced with the Cellular Matrix device and used in mesotherapy seems to be a promising treatment for facial rejuvenation with a very significant improvement in facial appearance, elasticity and firmness of the skin when compared to treatment with either PRP or HA alone. The combination of these treatments could have an additive and synergistic effect, thereby giving better results. Indeed, the beneficial effects of the PRP-HA combination may come from their similar biological and cellular mechanisms in cellular and tissue regeneration. In conclusion, the combination of PRP-HA in Cellular Matrix when used in mesotherapy seems to be a promising treatment for facial rejuvenation with an improvement in the elasticity and firmness of the skin with an additive and synergistic effect. Moreover, the hygroscopic properties of HA allow it to retain water (hydration agent), nutrients and growth factors, which helps to obtain better results in facial rejuvenation.

We strongly encourage the combined Cellular Matrix PRP-HA treatment due to its promising effects.

PRP improves fat grafting outcome

Dr. Ali Modarressi, MD

Privat Docent, Plastic, Reconstructive and Aesthetic Surgeon. Senior Consultant, University Hospitals of Geneva. Rue Gabriel-Perret-Gentil, 4, 1211 Geneva, Switzerland

The transplant of autologous fat tissue, so called lipomodelage, liposculpture or lipofilling has been known since the early 20th century. The principle of the technique is to transfer patient's own fat tissue from a donor site (e.g., abdomen, flanks, thighs) to a site where there is a volume deficit. Its first indications were for aesthetic surgery of the face. Fat grafting is also useful for tissue loss due to an accident, operation, a cancer resection, congenital disease or lipodystrophy.

In addition to a volumizing effect, the injected fat leads to a neoangiogenesis effect, improving the skin elasticity, and to an antiaging effect. This technique is thus also recommended for wound healing, scar reduction and radiodermatitis treatment. Moreover, during the last decade, fat grafting has been used more and more frequently for breast reconstruction and augmentation.

The main advantages of fat grafting are: (i) A long lasting result when compared to synthetic resorbable products, (ii) No risk of granuloma or allergic reactions which can be provoked by injection of permanent products (iii) A natural consistency and (iv) An improvement of cutaneous and subcutaneous trophicity.

On the other hand, the disadvantages of autologous fat grafting include (i) Its complexity of use, requiring a significant learning

curve compared to the prepared products, (ii) The morbidity and the necessity of a donor site, that sometimes may not provide sufficient material, and mainly (iii) The unpredictability of the remaining volume due to partial uncontrolled absorption of the fat transplant.

The survival rate and longstanding results depend partially on indications and patients but mostly on surgical technique. At present, the most used method for fat harvesting, purification and infiltration is the one described in detail by Coleman in 1986 (Coleman, 1991). This method considers the fragility of fat cells during the various steps of the treatment. However, even with the best surgical technique, the survival of the fat graft is unpredictable, with a variable resorption rate reported throughout the literature (10% to 90%).

To address this important disadvantage, we developed an innovative technique by adding autologous platelet-rich plasma (PRP) to fat grafts before injection. Based on recent literature, we hypothesized that adding PRP to the fat preparation may be a reliable way to bring appropriate nutrients to the graft in the early moments of transplantation to improve fat survival and render the result more predictable. PRP releases the native growth factors in their biologically determined ratios at the treatment site. The released growth factors stimulate angiogenesis, cell differentiation and proliferation leading to the reconstitution of the tridimensional matrix that allows the rearrangement of adipocytes into the correct 3D organization. This approach is completely autologous and immediately employed without any type of in vitro preconditioning or media supplement.

In a series of in vitro studies, we demonstrated that Regen PRP prepared by a standardized medical device with no activation (nPRP) significantly increases the proliferation of adipose-derived stem cells (Atashi et al., 2015). These cells are crucial for fat graft regeneration (Suga et al., 2010). In another study, we evaluated the effect of nPRP on the in vitro expansion of normal dermal fibroblasts (NHDF). NHDF cultured in medium supplemented with autologous nPRP showed dose-dependently significantly higher proliferation rates, are activated and show collective migration in a wound healing model (Berndt et al., 2019).

Animal models showed that the viability of adipocytes was significantly increased by the addition of 20% nPRP with an increase of tissue vascularity at day 90 (Atashi et al., 2019; Figure 1).

Several clinical cases also confirmed the improvement of fat graft survival by combination with PRP. Moreover, the liquefaction of the fat graft by addition of PRP renders the fat graft placement easier and more precise, particularly in delicate areas such as peri-orbital region or nose. Furthermore, patients present less bruising and inflammation reactions (Modarressi, 2013).

In conclusion, the combination of 20% non-activated PRP and 80% fat graft enhances lipofilling outcome cost-effectively and safely.

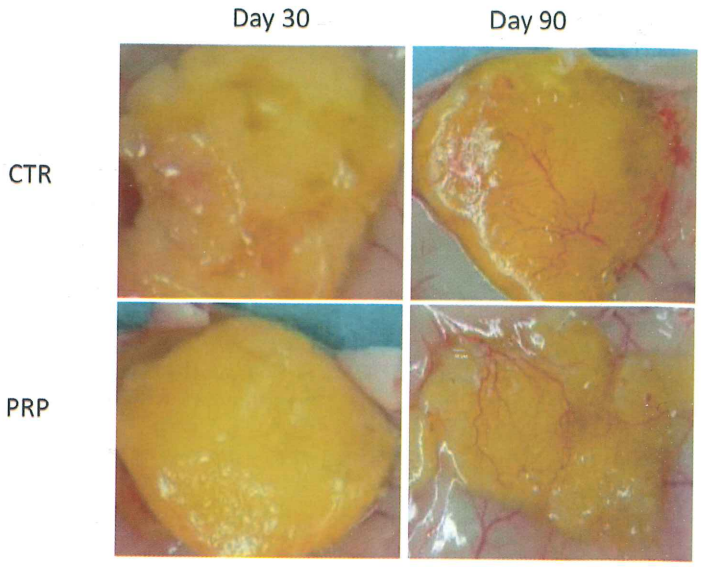

Figure 1 Macroscopic representative view of grafted fat on the scalp of mice on day 30 and 90 after grafting, showing more vascularization with 20% PRP. CTR: fat + 20% saline, PRP: fat + 20% non-activated-PRP.

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