4. PRP FOR SKIN DISORDERS AND SKIN REJUVENATION

4.1 PATHOPHYSIOLOGY OF SKIN DISORDERS

Acne scars, skin aging, melasma, vitiligo and alopecia are skin disorders that can cause a considerable cosmetic problem and have an impact on self-esteem and/or quality of life (QoL) (Adil and Godwin, 2017; Kelly et al., 2016; Lai et al., 2017; Ngaage and Agius, 2018; Salzes et al., 2016). Thus, many individuals wish to treat them. Over 4.5 million nonsurgical procedures were performed in 2008 in the US for skin rejuvenation, and hair loss therapies are a growing billion-dollar industry, emphasizing the high demand from patients for such treatments (Schmidt, 2016; Sclafani, 2010). Current treatments for these skin disorders such as autologous fat or follicle transplants, peelings, laser therapy, UV therapy or pharmacological agents offer at best a temporary solution and can be invasive or of prolonged duration and can have significant side effects leading to poor patient compliance (Hesseler and Shyam, 2019a; Merchan et al., 2019). In contrast, standardized PRP prepared with dedicated medical devices is a safer treatment that is effective for skin regeneration. Moreover, few sessions are required and results last for a longer period due to PRP targeting the damaged structural and functional components of the skin and restoring their function.

4.1.1 Atrophic acne scars

Atrophic scars are characterized by depressions in the skin, reflecting an absence and disorganization of dermal collagen and elastin (Fig. 22). Atrophic scars represent at least 80% of acne scars and are classified according to the depth and size of the loss of collagen (Alser and Goutos, 2018). Histopathology of atrophic acne scars shows an extremely flattened-out thin epidermis, void of rete ridges and abnormal arrangement of collagen in the dermis (Plewig and Kligman, 2012). Clinical evaluation of atrophic acne scars is challenging due to the difficulty in differentiating between acne scars, as different types of scars may be present in the same patient. A global acne scarring grading system was developed by Goodman and Baron to allow healthcare professionals to compare their cases more accurately and to have a more objective discussion of the efficacy of operative interventions or therapies (Goodman and Baron, 2006). This scoring system was shown to be reproducible among observers, independent of their medical background, suggesting that patients can be assigned scores ether by physicians or nurses. However, not all investigators use this system for evaluating their results making comparisons between studies difficult.

Current treatments for acne scars aim to stimulate remodeling of the ECM and include laser resurfacing, chemical peeling, dermal fillers, dermabrasion, needling, subcision and punch excision (Hesseler and Shyam, 2019b; Merchan et al., 2019). However, the response to these treatments depends on the skin type and they can have side effects and risks, such as erythema, edema and hyperpigmentation and additional scarring.

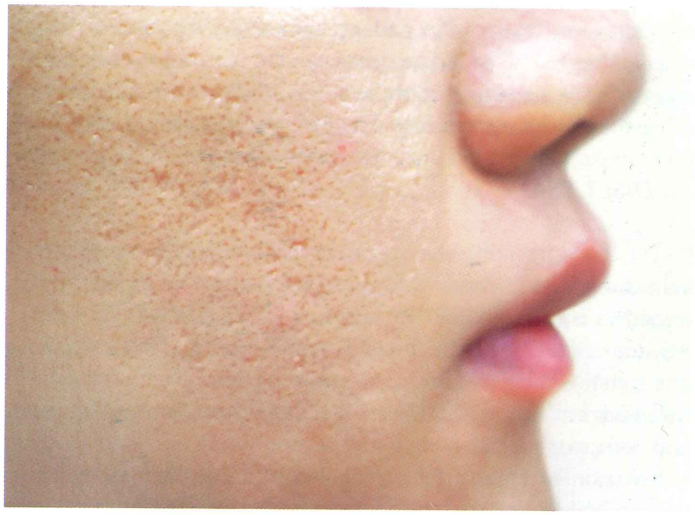

Figure 22: Examples of atrophic acne scars

4.1.2 Skin aging

Similar to atrophic acne scars, skin aging is associated with fat and collagen loss. Facial skin begins to sag as a natural consequence of aging, cellular fibroblast aging and defective mechanical stimulation in the aged tissue contribute to reduce collagen synthesis resulting in an irregular cascading appearance (O'Daniel, 2011; Varani et al., 2006). With aging, there is a decrease in the number of fibroblasts and a decrease in fibroblast production of collagen, hyaluronic acid (HA) and other components of the extracellular matrix, as well as an increase in the production of enzymes responsible for collagen fragmentation (Prikhnenko, 2015) and Fig. 23. As the concentration of HA decreases with aging, skin elasticity and the ability to retain water in the skin are decreased, leading to skin dryness and a reduction in the volume of the dermis (Lee et al., 2015). The process of skin aging eventually results in the formation of skin defects such as wrinkles, roughness, laxity, and pigmentation

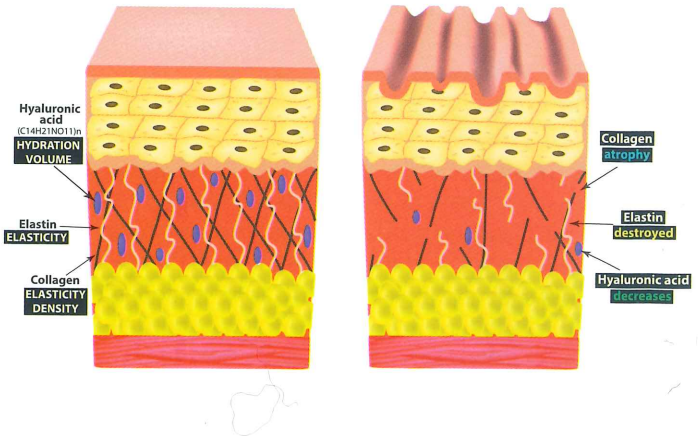

Figure 23: Skin aging processes

The aims of skin rejuvenation processes are to destroy specific layers of the epidermis and dermis and induce a wound healing response that stimulates fibroblasts to proliferate and generate new collagen and reorganize the skin architecture. Other approaches for skin rejuvenation include the use of HA to provide the skin with more elasticity, turgor and moisture (Papakonstantinou et al., 2012) and the use of autologous

fibroblasts or autologous fat transplantation to correct facial contour defects (Modarressi, 2013; Tang et al., 2016; Xiong et al., 2018; Zorin et al., 2016).

Treatment modalities such as radiofrequency, electro-optical synergy and laser treatments have been demonstrated to result in increased TGF-β production (El-Domyati et al., 2015). TGF-β signaling is important for ECM remodeling and is important for the wound healing process (Lichtenberger et al., 2016). Increased production of TGF-β1 was correlated with an increase in the levels of newly formed collagen (El-Domyati et al., 2015).

4.1.3 Melasma

Melasma is an acquired hyperpigmentary disorder with a genetic predisposition, affecting up to 30% of child-bearing women in some populations (Passeron and Picardo, 2018), Fig. 24. Histologically, melasma can be characterized as epidermal, dermal, or mixed according to the deposition of hyperactive melanocytes. The pathophysiology of melasma is not completely understood - its appearance during pregnancy (pregnancy mask) suggests involvement of female hormones and melasma frequently worsens in the summer season indicating that UV radiation, particularly the shorter wavelengths of visible light (blue-violet), is a triggering factor (Duteil et al., 2014; Passeron and Picardo, 2018; Regazzetti et al., 2018).

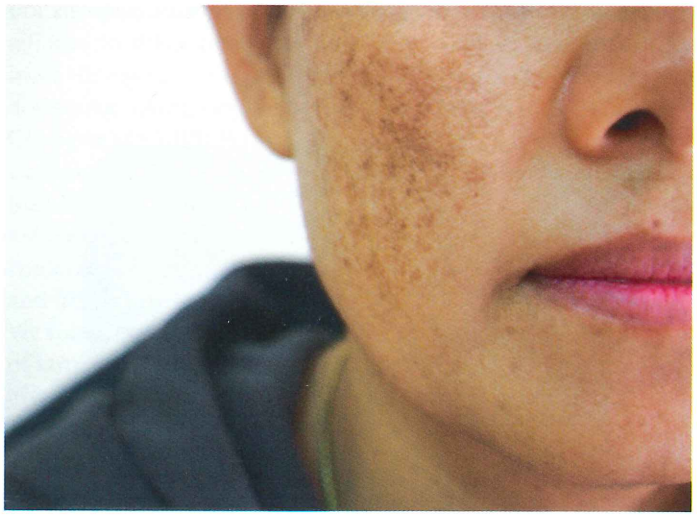

Figure 24: Melasma is a hyperpigmentary disorder

In the last decade, new insights into the pathophysiology of melasma have emerged. There is growing evidence that melasma is a photoaging disorder and not simply a pigmentation disorder (Passeron and Picardo, 2018). Melasma has several characteristics of a photoaging disorder, such as increased solar elastosis (Kang et al., 2002). Transcriptional profiling indicated that altered lipid metabolism is also involved in the pathogenesis of melasma and melasma patients were reported to have a decreased sebum secretion rate (Foolad et al., 2015; Kang et al., 2011). Melasma lesions were also reported to have impaired stratum corneum integrity and a delayed barrier recovery rate (Lee et al., 2012). At the molecular level, the Wnt signaling pathway has been identified as a key factor in melasma (Kang et al., 2011), which is expected due to its role in melanogenesis (Saito et al., 2003). TGF-β is also involved in the regulation of melanin synthesis (Kim et al., 2004; Nishimura et al., 2010) and melasma lesions have reduced expression of TGF-β compared to normal ones (Hofny et al., 2019b).

Current treatments for melasma are based on the assumption that melasma is a pigmentation disorder and include prevention by using anti-UVA and anti-UVB sunscreen and the use of topical bleaching agents. However, treatment proves challenging with inconsistent results and almost constant relapses (Passeron and Picardo, 2018). Due to emerging data indicating that melasma is a more complex disorder and that it shares similar modifications at the molecular level to some other skin disorders, the treatment approach for melasma needs to be reconsidered. Given the composition of PRP, it is possible that it contains the necessary growth factors to modulate the signaling pathways associated with melasma, and thus could be a viable treatment option for this disorder.

4.1.4 Vitiligo

Vitiligo is a pigmented disorder characterized by a loss of epidermal melanocytes and presents clinically as depigmented macules and patches (Taieb et al., 2007), Fig. 25. It may appear in either a segmented or non-segmented form. The mechanisms leading to vitiligo are still debated, but it is associated with autoimmune disorders and a genetic component is also recognized (Barbulescu et al., 2020; Speeckaert and van Geel, 2017). Both innate and acquired immunity have been implicated as has a decrease in the Wnt signaling pathway which is involved in melanocyte differentiation (Harris, 2015; Regazzetti et al., 2015; Speeckaert and van Geel, 2017). TGF-β1 has also been implicated due to its ability to decrease melanin synthesis (Kim et al., 2004).

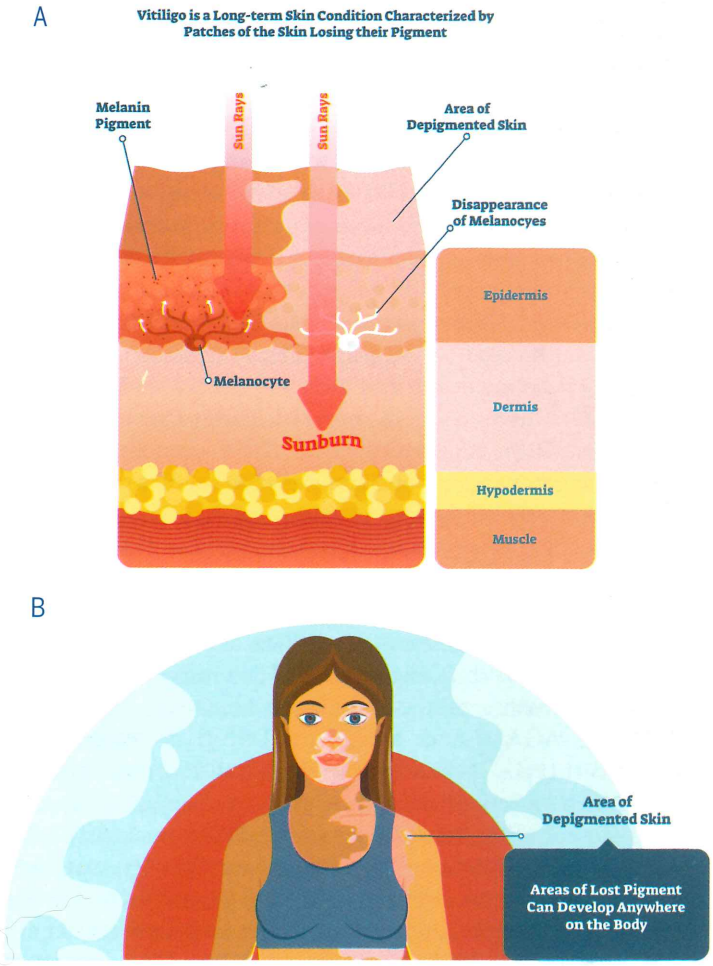

Figure 25: Vitiligo results from a loss of the melanin-producing melanocytes (A), resulting in the characteristic white patches of the disorder (B).

Current treatment approaches for vitiligo primarily target either the immune response (e.g., topical, or oral corticosteroids, topical immunomodulators) or stimulation of melanocytes (Afamelanotide, JAK inhibitors, Bimatoprost). Antioxidants have also been proposed as treatment options. However, while these treatments can halt progression of lesions, repigmentation is rarely observed, and if achieved, reoccurrence is likely once treatment stops (Speeckaert and van Geel, 2017). Surgery involving transplantation of pigment cells to the lesion is also used.

Narrow-band UVB (NB-UVB) is the current treatment of choice for vitiligo. It acts on melanocyte precursors in the bulge area of the hair follicle and induces their proliferation, differentiation and migration (Goldstein et al., 2015) and can also modulate the immune response (Barbulescu et al., 2020). However, it has a considerable cost for the patient, as 2-3 x weekly sessions for a maximum of 2 years are needed. Such demands on time and cost frequently lead to noncompliance with the treatment. The risk of cancer from such exposure has been discussed, but currently there is no consensus on what that risk may be. Because of PRP's actions at the cellular level, its demonstrated safety, and in general the need for fewer treatment sessions compared to other treatment modalities, it is being investigated as a treatment option for vitiligo, both alone and in combination with other treatments.

4.2 RATIONALE FOR THE USE OF PRP FOR SKIN DISORDERS

While the pathophysiology of the above-mentioned skin disorders is not fully understood, a common feature is the loss of the normal skin architecture, such as thinning of the epidermis or dermis or loss of rete ridges or loss of skin appendages. Inflammation to varying extents may also be present. At the molecular level there seems to be a loss or alterations in cell-cell communication, whether due to age-related reasons, or prolonged inflammation that can result in impaired wound healing, loss of skin structures or a dysregulated immune response. The Wnt signaling pathway has been implicated in the pathogenesis of acne scars, skin ageing, vitiligo, melasma and alopecia (Clayton et al., 2019; Dahl, 2012; Harris, 2015; Kang et al., 2011; Premanand and Rajkumari, 2018; Regazzetti et al., 2015; Zouboulis et al., 2008). Perturbation of Wnt signaling affects cell proliferation and differentiation (Lichtenberger et al., 2016). Another shared feature of these disorders are alterations in the production of transforming growth factor-beta (TGF-β). TGF-β has been implicated in the pathophysiology of skin aging (El-Domyati et al., 2015), atrophic acne scars (Moon et al., 2019) melasma (Hofny et al., 2019b; Kang et al., 2011), vitiligo (Tembhre et al., 2015; Tembhre et al., 2013; Tu et al., 2011; Zhou et al., 2015) and AGA (Lu et al., 2016). TGF-β is produced by several cell types including fibroblasts and macrophages and can act on a variety of cells. It is involved in cell proliferation and differentiation, remodeling of the ECM, melanogenesis and immunomodulation. Its function and activity can vary depending on its concentration and other growth factors in the surrounding milieu (Anitua et al., 2009; Lichtenberger et al., 2016; Moon et al., 2019; Narine et al., 2006). Thus, restoring cell-cell communication, improving the immune response, and inducing activation, proliferation and differentiation of dermal cells, such as fibroblasts, keratinocytes and stem cells may provide a new approach to treating these disorders. Hence there is great interest in using PRP to treat these disorders as it contains growth factors, such as TGF-β, needed for skin repair or rejuvenation or hair growth (Marx, 2004).

Given the key role that growth factors and cytokines play in many of these disorders, a standardized PRP preparation is crucial for evaluating the clinical benefit of PRP and reproducing the outcomes. TGF-β is one growth factor whose concentration in PRP has been shown to vary depending on the method of preparation (Anitua et al., 2009; Mazzocca et al., 2012; Mazzucco et al., 2009). Variations in the concentration of growth factors and other molecules in PRP could have a significant clinical impact.

A standardized preparation of PRP will consistently have the same increase of platelet concentration (within a given range), and the same level of contamination with red and white blood cells. Regen Lab kits (Fig. 26) are designed to produce RegenPRP, a PRP poor in leukocytes, with virtually no red blood cells and with a 1.5- to 2-fold increase in platelet concentration compared to baseline value in whole blood (see chapter 7). Some studies suggest the optimal range of platelet concentration factor is 2-6 -fold but this may be dependent on the technology used for PRP preparation, its resulting composition (e.g., whether leukocytes and red blood cells are present or not) and on the quality of the recovered platelets.

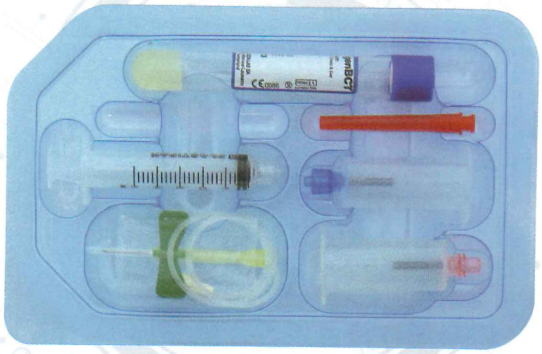

Figure 26: RegenKit-BCT-1

To obtain a PRP with a platelet concentration factor above 2-fold, a large volume of blood should be collected (30-60 ml) and platelet-poor plasma (PPP) should be discarded. This kind of protocol generally results in leukocyte-rich PRP with a variable level of red blood cell contamination. In some cases, an additional centrifugation step is needed. However, this might affect platelet quality and viability. By discarding a large volume of PPP, a highly supraphysiological platelet concentration is obtained.

The advantages of using highly concentrated PRP compared to PRP with a lower platelet concentration factor have not been demonstrated. PRP with platelets of high quality at a concentration close to physiological value, such as RegenPRP, is relevant for therapeutic use, since it does not affect tissue homeostasis, and the low level of cellular contamination reduces the risk of undesired inflammatory reactions.

Here we present the clinical studies that have used RegenKits to prepare RegenPRP and investigated the clinical benefit of RegenPRP for facial rejuvenation and treatment of acne scars. RegenPRP can be either used as a monotherapy or in conjunction with current treatment modalities to enhance treatment effects and reduce adverse events and downtime post-procedure. We also present results on other skin pathologies such as vitiligo and melasma for which PRP could be a new treatment option. Preliminary results using RegenPRP seem to confirm results obtained with other PRP preparations, which were mainly prepared using laboratory methods. Standardized protocols for dermatology indications remain to be developed.

4.3 REGENPRP FOR THE TREATMENT OF ATROPHIC SCARS

Abnormal changes in collagen and elastin fibers are found in the early development of acne lesions (Moon et al., 2019) and thus preventing the formation of scars may be better than a cure, as acne scars are difficult to treat. In this regard, as PRP is a safe and gentle product, it could be used in the early stages of acne, where its anti-inflammatory and anti-microbial properties may help prevent or limit the progression of the disease and the development of scars. Gómez et al., (2017) showed PRP had a positive effect on acne lesions.

For the treatment of acne scars, PRP is most often used as an adjunct to other treatment modalities such as fractional CO_2 laser therapy or microneedling to reduce the side effects and downtime (Alser and Goutos, 2018; Bhargava et al., 2019; Chang et al., 2019; Schoenberg et al., 2019). A meta-analysis of studies using PRP as an assisted therapy for acne scars found that PRP significantly improves outcomes and that the results are in agreement with histochemical findings of increased epidermal thickness, denser and more organized collagen fibers and finer elastic fibers (Hsieh et al., 2019).
We found two studies that have used RegenPRP for the treatment of atrophic acne scars. The treatment protocol for each study is given in section 4.9.

Two of the patients undergoing treatment for skin rejuvenation in the study by Redaelli et al., (2010) were also assessed for scar improvement following RegenPRP treatment by comparing images taken prior to the first session and at the 1-month follow-up and by a patient satisfaction questionnaire and the physician's impressions. The authors report that at the end of treatment the acne scars had almost disappeared. The patient questionnaire indicated that patients particularly appreciated the ease of use and safety of the technique. No serious and persistent adverse events were reported. The adverse events reported were transient and resolved quickly – bruising/ecchymosis (3% of patients), burning sensation for about 3 minutes after injection (70%). Mild erythema was reported by 80% of patients.

There is growing interest in the use of autologous fat grafting for the treatment of acne scars and it has been reported to be more effective than fractional CO_2 laser in their treatment (Azzam et al., 2013; Ghareeb et al., 2017). Fat can be harvested from various donor sites, such as the abdomen (Fig. 27). However, as discussed in section 3.8.4, the resorption rate of the transplanted tissue is unpredictable, thus PRP has been investigated as a means to improve autologous fat transplantation (Azzam et al., 2015; Modarressi, 2013; Xiong et al., 2018).

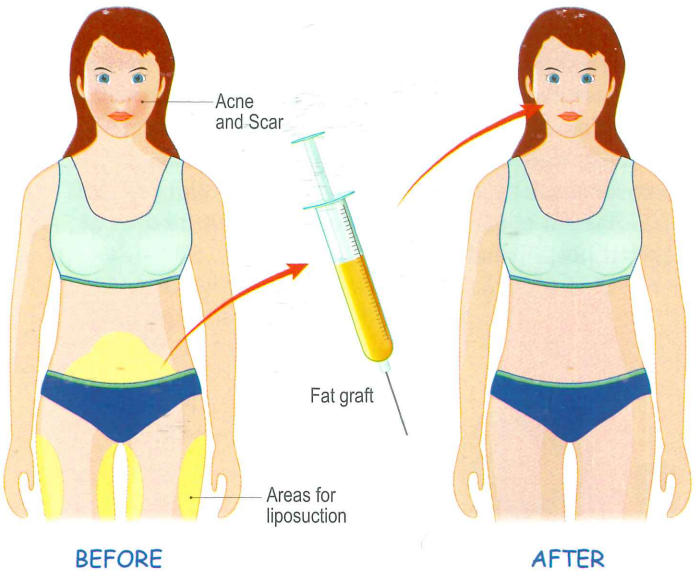

Figure 27: Autologous fat can be used alone or in combination with PRP for the treatment of acne scars

The concept of combining PRP with a fat transplant was developed by Regen Lab in the years 2004-2006 and patented in June 2006 (Turzi and Du Toit, 2008). Dr. Ali Modarressi at the University Hospital of Geneva (HUG) has conducted several in vitro studies for the determination of the best ratio of PRP to fat stem cells (Atashi et al., 2015a; Modarressi, 2013). See also the contributions by Dr Modarressi and Dr Bychkova and colleagues in section 4.10.

Tenna et al. (2017) conducted a study where 30 patients (Fitzpatrick type between II and IV) with atrophic acne scars on the cheeks were treated by infiltration of 10 cc nanofat (7cc) plus RegenPRP (3 cc). After treatment, patients were randomly divided into two groups. Group A underwent a fractional CO_2 laser resurfacing, and Group B served as a control (no additional treatment). The settings for the CO_2 laser were 15-watt, H-pulse, spacing 550 mcm. The patients underwent two treatment sessions, with a mean interval of 6 months. Patients were prescribed antibiotics for postoperative use and advised to use sun protection for 6 months after treatment. Patients attended a 1-month, 3-month and 6-month follow-up.

Treatment efficacy was assessed pre-and postoperatively by physical examination, photographs and an ultrasound scan with a 22-MHz probe to measure subcutaneous tissue thickness. The Italian version of the FACE-Q module was administered postoperatively to analyze satisfaction and aesthetic perception of the result.

The authors reported that on average postoperative thickness of subcutaneous tissue increased by 0.668 cm in group A and 0.63 cm in group B. There was no significant difference in patient satisfaction between the two groups. This study concluded that all patients benefited from the procedure of nanofat infiltration and PRP and were highly satisfied with the results. There was no additional benefit of adding fractional CO_2 resurfacing to improve skin texture.

4.4 REGENPRP FOR SKIN REJUVENATION

The interest in PRP for skin rejuvenation is due to the growth factors and other molecules it contains that can induce proliferation of fibroblasts, endothelial cells and adipose-derived stem cells which result in increased production of collagen, improved vascularization and improved fat volume (Atashi et al., 2015a; Atashi et al., 2015b; Berndt et al., 2019; Lai et al., 2018; Lei et al., 2019b) and Fig. 28.

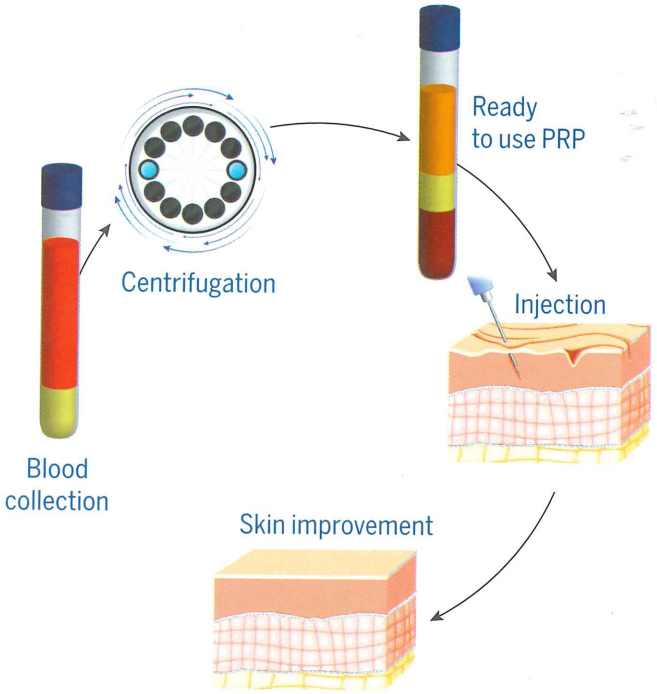

Figure 28: PRP for skin rejuvenation

PRP can also increase the production of HA – increased production of HA may improve the skin's volume and turgor and promote cell proliferation and extracellular matrix synthesis. It may also modulate the diameter of the collagen fibers (Hersant et al., 2017; Hsieh et al., 2019). In this regard, the platelet concentration of the PRP may need to be taken into consideration – according to (Hesseler and Shyam, 2019b), fibroblast proliferation is greatest when PRP contains 2-4-fold the peripheral platelet concentration, whereas HA production is greatest at a 2-fold concentration. RegenLab kits result in a PRP preparation with a platelet concentration about 2-fold more than the peripheral platelet concentration.

Regen Lab considers that the quality and viability of platelets in the PRP produced with all Regenkits is optimal, and does not recommend further platelet concentration for any RegenPRP treatment (Turzi and Du Toit, 2008).

PRP is also used to improve the outcomes with autologous fat transplantation due to its ability to induce neovascularization and improve the survival of the graft (Modarressi, 2013; Smith et al., 2019; Xiong et al., 2018). However, technical factors in graft preparation and administration vary significantly between in vivo studies and may explain observed differences in experimental and clinical outcomes (Luck et al., 2017). Thus, a standardized PRP preparation will facilitate the determination of the optimal PRP:fat ratio for grafting.

PRP can also be used in combination with other treatment modalities such as lasers and microneedling and hyaluronic acid for skin rejuvenation (section 3.8).

We found six published studies and one abstract that have used RegenPRP for skin rejuvenation purposes, including one study that demonstrated histological changes in the skin following treatment with PRP.

Abuaf et al. (2016) examined the effect of RegenPRP on skin rejuvenation by investigating its effect on dermal collagen. In this split-face study, 20 patients with Fitzpatrick skin type I to III received injections of 0.15 ml PRP into the papillary dermis (1.5 to 2.0 mm deep) of the right infra-auricular area. The injections were spaced about 1 cm apart. Saline was injected in the left infra-auricular area. Punch biopsies were taken before and after treatment for histological evaluation.

The authors found that collagen fiber bundles in the dermis as well as the number and thickness of elastic fibers increased on both sides after treatment, Histological analysis of biopsies revealed a significantly greater increase of elastic fibers and collagen in PRP-treated area compared with saline ($P<0.001$). The treatment was well tolerated, the only adverse events being mild erythema, mild burning, and ecchymosis that spontaneously resolved within 2 days. The severe erythema observed in 10% of the patients resolved within a week.

Redaelli et al. (2010) reported their results of a prospective case series of 23 patients treated with activated RegenPRP for skin rejuvenation, and scar improvement (two patients). Patients received 3 sessions of treatment with a 1-month interval between each session. During each session, PRP was injected intradermally in the upper third of the face, into the cheeks, in the nasolabial folds, and into the neck. Patients were assessed one month after the final treatment by comparing images taken prior to the first session and at the 1-month follow-up and by a patient satisfaction questionnaire and the physician's impressions. The authors report that in most of the patients there was a good improvement in skin texture and elasticity, and there was a volume increase at the injection site of nasolabial folds in 65% of patients. The patient questionnaire indicated that patients particularly appreciated the ease of use and safety of the technique. All patients reported a rejuvenation of their skin with improved texture, skin health and wrinkles and most were highly satisfied with the results. No serious and persistent adverse events were reported. The adverse events reported were transient and resolved quickly – bruising/ecchymosis (3% of patients), burning sensation for about 3 minutes after injection (70%). Mild erythema was reported by 80% of patients.

Tolba and Nasr (2015) conducted a study in 40 patients (10 were lost to follow up) to assess the use of autologous fat injection with RegenPRP as a method for facial augmentation using a ratio of RegenPRP:purified fat of 20%:80%. The volume of each injection ranged from 2 to 50 ml depending on the injection site. Half of the patients reported bruising in the treated area, and redness and swelling occurred in 10% of patients. These adverse events resolved within approximately 4 days of the procedure. Assessment of the final results performed by patients and plastic surgeons revealed that 70% of the patients were considered as excellent and 20% were satisfactory. 10% of the patients were

assessed as unsatisfactory. Ten patients required a top-up procedure to correct an inadequate volume augmentation from the initial procedure.

Alenichev et al. (2017) conducted a retrospective analysis to assess the efficacy of a combination of radiofrequency radiation administered via microneedling and RegenPRP for the treatment of age-related changes in the face and neck in 57 patients, aged 40-65 years. Patients received a total of three cycles at monthly intervals whereby a cycle consisted of a RF needle facelift followed by treatment with RegenPRP one week later. PRP was administered to the areas to be treated using a deep, regressive intradermal linear technique. Evaluation of the patients after treatment showed a significant decrease in the thickness of the epidermis and a significant increase in the thickness of the dermis as well as a significant improvement in the skin microrelief. Based on the visual assessment, there was a reduction in clinical indicators of chronoaging and photodamage of the skin, with changes being more significant in those under 49 years old. The patients reported a significant improvement in quality of life and 88-90% of patients reported good or very good efficacy, safety and comfort of the combined method.

Matyunin (2016) reported a case study of a patient who underwent a treatment for chronological and photo aging using a 2940 nm Er:YAG laser. RegenPRP was also included in the treatment to improve healing after the laser treatment. PRP was injected into the nasolabial folds and then to all areas of deep wrinkles. A cream consisting of a mixture of PRP and 5% Dexapenthenol was also provided to the patient with directions to apply it 6 times per day for 3-4 days. PRP therapy was repeated at the fourth, fifth and twenty-fourth month after the first procedure. At the end of treatment, the patient had better skin structure, and a reduction in rhytides, laxity and signs of photoaging.

4.5 CELLULAR MATRIX REGENPRP-HA COMBINATION FOR SKIN REJUVENATION

As discussed in section 3.8.1, hyaluronic acid is an important component of the skin, and has been used for a long time as a skin rejuvenation treatment (Patel, 2018). Recently, there is growing interest in the use of HA in combination with PRP for skin rejuvenation. We found two publications that have used a combination of RegenPRP and hyaluronic acid, specially prepared using the RegenLab Cellular Matrix device, for skin rejuvenation purposes.

Hersant et al. (2017) report their findings from a prospective clinical study of 31 patients using the combination of PRP and hyaluronic acid, specially prepared using the RegenLab Cellular Matrix BCT-HA tube (section 7.7.5) (Fig. 29). The resulting CM-PRP-HA solution was injected intradermally into the cheek. In addition, 1 ml was also spread on the cheek and followed by intradermal punctures using a 1-mm SkinRoller. Patients underwent 3 treatment sessions with assessments made after each sessions and 6 months after the last treatment. According to the FACE-Q scores used for assessment, there was a significant improvement at 6 months compared to baseline. No serious adverse events were reported.

Dzybova et al. (2017) examined the effectiveness of intradermal injections of RegenPRP compared to the Cellular Matrix CM-PRP-HA combination for the treatment of xerosis, wrinkles, disorders of pigmentation and secretory activity. One group of patients (n=12) were treated with RegenPRP and the other group (n=12) were treated with CM-PRP-HA. Hyperpigmentation decreased significantly in both groups, but to a greater extent in the group treated with CM-PRP-HA (52.3% vs 39.5%). The improvement in skin texture was also more pronounced in the CM-PRP-HA group (34.5% vs 24.6%). The skin elasticity index improved in both groups but was greater in the CM-PRP-HA (20.2% vs 14.9%). Therefore, although both RegenPRP and CM-PRP-HA are clinically effective and safe, CM-PRP-HA is more effective for the treatment of skin aging.

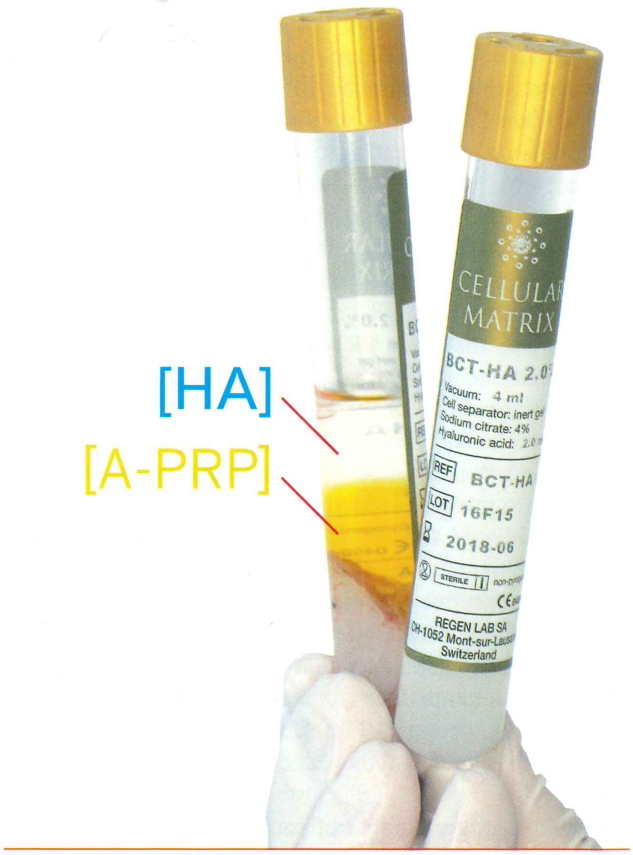

Figure 29 : Cellular Matrix BCT-HA tube

4.6 PRP FOR THE TREATMENT OF MELASMA

PRP has been evaluated as a potential treatment for melasma as it was shown to reduce hyperpigmentation in other skin conditions (Mehryan et al., 2014; Na et al., 2011). At the molecular level, PRP treatment was demonstrated to restore the levels of TGF-β in the epidermal and dermal layers of melasma lesions to almost the same levels as those seen in normal skin and to levels that were better than in perilesional skin (Hofny et al., 2019b). Clinical investigations into PRP as a treatment for melasma are still in the early stages. Here we present some published reports on PRP for the treatment of melasma, including one study using RegenPRP.

Cayırlı et al. (2014) reported significant improvement in epidermal hyperpigmentation in a patient presenting at their department for skin rejuvenation using RegenPRP. Approximately 1.5 mL of RegenPRP was injected into the papillary dermis of the face at each session (3 sessions with 15-day intervals). At the end of the third session of PRP treatment there was a >80% reduction in epidermal hyperpigmentation. In the follow-up period (6 months) there was no recurrence of melasma.

These findings are supported by another case study of a melasma patient who responded poorly to treatment for melasma and was subsequently treated with PRP for six sessions with intervals of between 3 and 10 weeks while continuing to use sunscreen and topical depigmenting cream. Six weeks after the last injection, the patient's Melasma Area Severity Index (MASI) score had almost halved and at the three-month follow-up there was no relapse of melasma (Garg et al., 2019b).

Yew et al. (2015) reported the findings from two patients who received two sessions of PRP treatment with a one-month interval in combination with a subthermolytic Q-switched Nd Yag laser with daily topical application of alpha arbutin. The method of PRP preparation is not described in this report. Patient 1 presented an immediate improvement after the first PRP treatment which continued to improve at the 6-month follow-up. Patient 2 showed a smaller improvement and at the 6-month follow-up the evaluators noted that the melasma had begun to reoccur.

Sirithanabadeekul et al. (2019) reported their findings from a split-face placebo controlled clinical trial, where 10 female patients were injected with PRP intradermally on one side of the face and saline on the other. PRP was prepared using a Korean device intended for the preparation of highly concentrated LR-PRP. Patients received injections every 2 weeks for a total of 4 treatment sessions. Clinical evaluations were performed at baseline, and at 2, 4, and 6 weeks, and 10 weeks. At week 6, the authors found a significant reduction in the mean melanin levels and wrinkle levels on the PRP-treated side. The mean MASI score in the PRP-treated side was significantly improved at week 10 compared to baseline and was significantly better than the control side. All side effects were mild and resolved within a few days. The authors concluded that PRP can partially improve melasma lesions although the effect was less than in other studies, possibly due to the type of PRP preparations (pro-inflammatory LR-PRP) or the age of the study participants.

In a study with a larger number of patients (n=23), Hofny et al. (2019a) demonstrated that three sessions of intradermal injection of PRP, prepared with a laboratory method, or microneedling followed by topical application of PRP at one-month intervals were both effective in improving melasma lesions at the follow-up session one month after the last injection. The authors found statistically significant better results in patients with epidermal melasma compared with the mixed type. Most patients experienced more pain on the side treated with microinjections but had less downtime compared to the side treated with microneedles. Almost 80% of patients were very satisfied or satisfied with treatment. A controlled study in which histological examination of biopsies from PRP-treated patients (n=20) and controls (n=9) were performed demonstrated that PRP improved the hyperpigmentation present in the epidermis of melasma patients likely by increasing the production of TGF-β (Hofny et al.; 2019b).

Taken together, these studies demonstrate that PRP may be an effective treatment for melasma. However, larger studies with standardized preparations of PRP as well as a longer follow-up are needed to determine the optimal number and frequency of PRP treatment sessions needed.

4.7 PRP FOR THE TREATMENT OF VITILIGO

Given PRP's ability to modulate the immune response and induce the proliferation, differentiation of fibroblasts and contribute to wound healing, as well as its role in influencing Wnt signaling in several dermatological indications, including vitiligo (Kang et al., 2011; Regazzetti et al., 2015; Xiao et al., 2019), there is interest in assessing the potential of PRP for the treatment of vitiligo, either alone or in combination with current and new treatment modalities.

Fractional CO_2 lasers are showing promise as a novel treatment modality for vitiligo (Kim et al., 2018; Yuan et al., 2016). As use of such lasers for treatment of acne scars and skin rejuvenation has been enhanced when used in conjunction with PRP, the efficacy of fractional CO_2 lasers when used in combination with PRP has also been assessed for treatment of vitiligo.

Kadry et al. (2018a) compared the effect of RegenPRP, Fr:CO_2, combined Fr:CO_2 with RegenPRP and exposure to sun (control) on intrapatient lesions (30 lesions for each treatment) in 30 patients with vitiligo. The most significant improvements were seen in the combination group, which resulted in a dramatic decrease in the number of lesions. The RegenPRP-treated lesions showed moderate improvements and mild improvements were seen in the Fr:CO_2 group. These findings were confirmed by histological evaluations of sections.

The findings from Kadry et al. (2018a) are similar to those of Abdelghani et al. (2018) who compared PRP alone, CO_2 alone, combined CO_2 and PRP and combined CO_2 and NB-UVB for the treatment of vitiligo. In the Abdelghani study, patients received 4 sessions of an intradermal PRP injection at three-week intervals. PRP was prepared using a double-spin laboratory method and activated prior to use. The best results were observed in the laser and PRP group, with 40% of patients achieving repigmentation of >75% and 60% of patients achieving repigmentation of >50%. In this study, PRP and Fr:CO_2 laser > Fr:CO_2 laser and NB-UVB > CO_2 laser alone > PRP alone. The finding that PRP alone was less effective than Fr:CO_2, which was the opposite to that seen by Kadry et al. (2018a), may be due to the different methods used for preparing the PRP.

Other treatment modalities explored in combination with PRP are NB-UVB, and excimer laser treatment which has been considered as an alternative to NB-UVB. Ibrahim et al. (2016) treated 60 patients with NB-UVB on one half of their body, and NB-UVB and PRP prepared using a two-spin laboratory protocol on the other. PRP was administered intradermally every 2 weeks for 4 months. The authors observed repigmentation in 25% of patients after the second session and in 43.3% of patients after the third session on the side receiving NB-UVB and PRP. In comparison, only 10% of the patients on the control side showed a response at the third session. After 3 months of follow-up, 55% of patients on the side treated with PRP showed an excellent response, whereas no patients on the control side showed an excellent or good response. The best response on the control side was a moderate response (6.7%) of patients, whereas 75% of patients in the PRP-treated side showed an excellent and good response. Moreover, at the 3-month follow-up, continued improvement was seen on the PRP-treated side, with no relapses observed. On the control side, depigmentation had started in half of the patients.

Khattab et al. (2019) compared excimer laser treatment with PRP prepared using a double-spin laboratory method to excimer laser treatment alone. They found a better response in the PRP and excimer laser treatment group compared to the excimer laser treatment alone. Histological analysis of biopsies revealed an absence of pigmentation prior to therapy, but post-therapy in the PRP treatment group there was expression of prominent melanin pigment whereas in the laser only group there was expression of weak melanin pigment.

PRP has also been used to suspend noncultured epidermal cell suspensions prior to transplantation and this has been demonstrated to significantly improve repigmentation, with repigmentation being seen as early as 2 weeks after transplantation (Garg et al., 2019a; Parambath et al., 2019).

Taken together, these findings show that PRP when used in combination with current or novel treatment modalities for vitiligo has a synergistic effect and has the potential to improve clinical outcomes and decrease the time needed for treatment. However, a standardized PRP preparation with knowledge of its composition is important to understand how to best use PRP for the treatment of vitiligo.

4.8 DISCUSSION

PRP offers a minimally invasive, low risk solution for many skin disorders. Clinical studies have demonstrated the potential of PRP, with patients being satisfied with the outcomes and clinically significant results with a few treatment sessions being sustained over many months or even a year and longer.

To date, most studies have assessed PRP in combination with other treatment modalities for the treatment of acne scars. Redaelli et al. (2010) and Nofal et al. (2014) are the only authors to our knowledge to publish outcomes of treatment with PRP alone on acne scars where it was shown to have a positive result, and to be as effective as a comparator treatment (Nofal et al., 2014). When used in conjunction with other treatment modalities, RegenPRP was also shown to be effective in improving outcomes. These findings support those from systematic reviews and meta-analyses that have concluded that PRP used in combination with other treatment modalities such as fractional CO_2 laser, microneedling, and autologous fat transplantation enhances treatment outcomes and reduces side effects of treatment such as erythema and edema, resulting in less downtime for the patient and in certain combinations can give even better outcomes than when PRP is used alone (Alser and Goutos, 2018; Hesseler and Shyam, 2019b).

These studies also demonstrated that RegenPRP, alone or in conjunction with other treatment modalities (Matyunin, 2016; Redaelli et al., 2010; Tolba and Nasr, 2015), can improve clinical outcomes when used for skin rejuvenation by inducing changes in the skin structure (Abuaf et al., 2016). In addition, we have the first published study of a PRP-HA preparation used effectively for skin rejuvenation (Hersant et al., 2017). These studies have demonstrated not only the efficacy of RegenPRP but also its safety, as no major adverse events have been reported.

These findings are in line with other published studies that have concluded that PRP has the potential to soften fine lines in cosmetically sensitive areas (e.g., crow's feet) and reverse degenerative soft tissue changes, particularly those resulting from extrinsic aging processes, such as ultraviolet exposure, with a high rate of patient satisfaction (Hausauer and Humphrey, 2019). When used alone or in conjunction with other treatment modalities PRP has been demonstrated to enhance the effects histologically as well as providing visual improvement (Shin et al., 2012). Although the effects may be modest, PRP treatment is well tolerated and appears to speed healing after fractional laser treatment (Maisel-Campbell et al., 2019; Shin et al., 2012). Adverse events, in order of frequency, are burning or warmth, erythema, pain, and bruising. All side effects are mild, transient, and self-remitting. No serious adverse events have been reported (Maisel-Campbell et al., 2019).

The two studies using RegenPRP with autologous fat transplantation showed clinical improvement and a large proportion of the treated patients were highly satisfied with the outcomes (Tenna et al., 2017; Tolba and Nasr, 2015). Adverse events like bruising, redness and swelling resolved quickly. Analysis of the scientific literature reveals that autologous fat transplants/grafting combined with PRP have given mixed results, with some studies reporting significant improvement while others failed to observe a difference (Park et al., 2012; Tolba and Nasr, 2015; Willemsen et al., 2018). These differences may be explained by different study methodologies, including fat graft preparation method and different ratio of PRP:fat, which may include volume of PRP at up to 50% of the mix. In addition, the heterogeneity of the PRP products used in these studies, with variable platelet concentration, quality of the recovered platelets and level of contamination with blood cells, should be taken into account (Luck et al., 2017). Some physicians vary the proportion of PRP combined with the fat transplant depending on the indication and area to be treated (Modarressi, 2013). Thus, a standardized preparation of PRP is important to enable the definition of other parameters needed for optimization of autologous fat transplantation combined with PRP and a consensus on the optimal PRP concentration and v/v ratio of PRP to fat to be used.

The first results from studies that have investigated the use of PRP for pigmentary disorders like melasma and vitiligo are promising and the use of PRP seems rational based on genomic studies that have demonstrated perturbations in the levels of growth factors as a possible contributing factor to these disorders. However, larger studies with standardized preparations of PRP as well as a longer follow-up are needed to determine the optimal number and frequency of PRP treatment sessions needed.

4.9 Regen Lab Medical Device Clinical Evaluation in Skin care

10 publications were identified including clinical data on 257 patients. Among them, RegenKits have been used for 4 different indications:

A. Acne scars (1 publication)
RegenKits have been used on 30 patients for the treatment of acne scars.

B. Bio-revitalization (7 publications)
RegenKits and Cellular-Matrix have been used on 196 patients to correct skin ageing.

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

D. Vitiligo (1 publication)
RegenKits have been used on 30 patients for vitiligo regression.

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 and resolved spontaneously. No serious AE was reported.

Performance: Generally, performance data showed that PRP treatment has a beneficial effect on 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:

Acne Scars

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
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, Panasiti V, Tirindelli M, Nobile C, Persichetti P. *Aesthetic Plast Surg* 2017; 41: 661-666	Comparative clinical study Follow-up: 6 months	Acne scars	30	RegenTHT

Treatment protocol	Key results on performance	Safety / adverse events (AE)
After preparation of the fat emulsion, 7cc of the fatty liquid was added to 3 cc of the patient's own PRP. This formulation was injected into soft tissue deformities using 19G cannulas. Patients were then divided into two groups. Group A underwent a fractional CO_2 laser resurfacing; Group B had no further treatment.	All patients benefited from the treatment. The post-operative thickness of subcutaneous tissue was 0.668 cm in group A and 0.63 cm in group B, and there was no significant difference between groups. Patients in both groups were very satisfied with the treatment.	Not formally assessed

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
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 aging	20	RegenBCT
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; 77: 584-586	Case series Follow-up: 6 months	Skin aging	31	Cellular Matrix BCT-HA

Treatment protocol	Key results on performance	Safety / adverse events (AE)
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). PRP was also injected in the right infra-auricular area. Saline was injected into the left infra-auricular area (control). On day 28 after injections, a biopsy was performed on the PRP-treated and control (saline injected area) site for histological analysis.	- Objective histopathological examination of biopsy 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 the PRP-treated side compared to the saline placebo-treated side was statistically highly significant ($p<0.001$). In fact, the improvement on the saline side was 46.01% vs. 89.05% on the PRP side.	No serious side effects reported. Mild and transient side effects such as bruising or ecchymosis, burning sensation, mild erythema, and severe erythema were observed.
PRP-HA was prepared as per instructions for use of Cellular-Matrix (2 ml of PRP and 2 ml of HA). Topical anesthesia was applied prior to deep intradermal injections of CM-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). The treatment procedure was repeated at 1-month intervals, for a total of 3 treatment sessions.	- 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).	No serious adverse event was reported.

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
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; 9: 466-72	Case series Follow-up: 4 months	Facial skin rejuvenation and acne scar	23	RegenBCT
Initial experience of face augmentation using fat graft-platelet rich plasma mix Tolba A, Nasr M. *Surg Sci* 2015; 6: 489-498.	Case series Follow-up: 24 months	Facial augmentation	40	RegenKit

Treatment protocol	Key results on performance	Safety / adverse events (AE)
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 at 1-month intervals. Injection points and quantities were standardized and given with different injection techniques including intra-dermal injections: -1 ml into the upper third of the face (0.5 ml forehead area and 0.5ml crow's feet area) -1 ml into the cheeks area (0.5ml per side) -1 ml into nasolabial and marionette areas (0.5 ml per side) -1 ml for the neck.	- Clinical photographs taken with the dermoscope showed an overall improvement of the skin of 30%. - 65.3% of 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%.	No serious infections or hematoma were detected. Mild erythema or burning sensation were reported in 70-80% of patients.
Purified fat was mixed with PRP (20% PRP: 80% purified fat). A blunt 18 or 17-gauge cannula was used for transfer of the fat-PRP mixture, with the PRP:fat mixture being injected into the pathway of the retreating cannula in tiny aliquots. Usually after 6 months, a second procedure was indicated.	The results were evaluated by the patient and surgeon comparing pre- and post-operative images for the degree of improvement. -70% of the patients were assessed as excellent, 20% were satisfactory and 10% were unsatisfactory	Bruising of the treated area occurred in 15 patients. Redness and swelling occurred in 3 patients. All issues resolved within an average of 4 days after the procedure.

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
Skin resurfacing with Er:YAG – the lost art of deep skin resurfacing Matyunin O. *Journal of the Laser and Health Academy* 2016; 1: 41-44	Case report Follow-up: 30 months	Skin aging	1	RegenBCT
Combined application of microneedle RF-therapy and injections by platelet-rich plasma activated by autological thrombin in aesthetic medicine Alenichev AY, Kruglova LS, Fedorov SM, Sharypova IV, Ast NA. *Russian Journal of Physiotherapy, Balneology and Rehabilitation* 2017; 16: 320-324	Retrospective Follow-up: 1- 2 years	Facial and neck skin aging and photodamage	57	RegenBCT/ RegenATS

Treatment protocol	Key results on performance	Safety / adverse events (AE)
This paper reports on a case of treatment of severely damaged skin utilizing a 2940 nm Er:YAG laser (Dualis SP, Fotona). PRP was applied on the treated areas and botulinum toxin was administered to the forehead and periorbital area. A cream was also prepared (a mixture of PRP and Bepanthen 5%) in a sterile container and handed to the patient with instructions to apply 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.	- The combination of planar (full-spot) and fractional Er:YAG revealed excellent aesthetic results. - In addition to better skin structure, there was a visible reduction of rhytides, laxity, and signs of photo aging.	Not formally assessed.
<u>Group 1 (n=26):</u> patients up to 49 years old <u>Group 2 (n=31)</u>: patients over 49 years old PRP and autologous thrombin were prepared using RegenKits. Autologous thrombin (AT) was used to activate the platelets in PRP. All patients received a radio-frequency (RF) needle facelift, and a combination PRP-AT injection. Patients underwent 3 treatment cycles at 1-month intervals. Each treatment cycle included the successive performance of the procedure with the following schedule i.e., an RF needle facelift, a one-week interval, and then a PRP-AT injection.	- There were significant improvements in the thickness of the epidermis, dermis and skin microrelief in both groups after treatment - The reduction of skin aging helped to improve the quality of life in both groups of patients. On average, the DSQL index was significantly improved by 77.1% in the group of patients up to 49 years old and by 69.4% in the patients over 49 years ($p<0.01$). - Approximately 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). - A clinically significant anti-aging effect in most patients lasts for quite a long time (1.5-2 years).	Not formally assessed.

Bio-revitalization

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
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 aging	24	Cellular Matrix BCT-HA

Melasma

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
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 hyper-pigmentation over the cheeks, perioral region, and forehead for 5 years	1	RegenTHT

Treatment protocol	Key results on performance	Safety / adverse events (AE)
Patients received intradermal injections of CellularMatrix 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): CellularMatrix Group 2 (n=12): PRP	- 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 CellularMatrix technology were confirmed by using dermoscopy and photographic data. Both PRP and CellularMatrix are efficient, however the CellularMatrix method is a safe and more effective for the treatment of skin aging and therefore can be recommended for broad clinical use.	No side effects were observed.

Treatment protocol	Key results on performance	Safety / adverse events (AE)
PRP was prepared as per instructions for use from 8 ml of whole blood. 32-G needle, mesotherapy technique, injections into 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, with an interval of 15 days between each session.	- At the end of the third session, >80% reduction in epidermal hyperpigmentation was observed. - No additional treatments or post treatment care (besides the use of a sunscreen) were prescribed. - No melasma recurrence during the 6-month follow-up.	Not formally assessed.

Vitiligo

Data Reference	Study design & follow-up	Medical indication/ pathology	N° of patients	RegenKit / RegenTube
Platelet-rich plasma versus combined fractional carbon dioxide laser with platelet-rich plasma in the treatment of vitiligo: a comparative study Kadry M, Tawfik A, Abdallah N, Badawi A, Shokeir H. *Clin Cosmet Investig Dermatol* 2018; 11: 551-559	Prospective, randomized, intrapatient, comparative controlled study Follow-up: 3 months	Vitiligo	30	RegenKit

Treatment protocol	Key results on performance	Safety / adverse events (AE)
PRP was prepared from 8 ml of whole blood. However, the centrifugation conditions for the preparation of RegenPRP were not correct. A 30G needle was used for superficial intradermal microinjections (0.1 ml per injection and spaced about 1 cm apart). Intrapatient lesions were randomly divided into four groups according to the treatment modality: PRP group, combined Fr:CO_2 with PRP group, Fr:CO_2 group, and control group. Patients received six treatment sessions at 2-week intervals for 3 months.	Both the combined Fr: CO_2 with PRP group and PRP group showed the highest significant improvement when compared to other modalities (P<0.001). A combination of Fr: CO_2 and PRP is more effective than either alone for the treatment of vitiligo.	Pain was the most common adverse event. Hyperpigmentation occurred in the Fr:CO_2 (26.66%) and combined Fr:CO_2 with PRP groups (6.66%). There were also reports of inflammation that developed in the Fr:CO_2 group and the combined Fr:CO_2 with PRP group. Ecchymosis in the PRP and combined Fr:CO_2 with PRP groups lasted for 3–5 days.