# 3. RATIONALE FOR PRP USE IN DERMATOLOGY

As our understanding of the pathophysiology of skin disorders increases, it is becoming clear that their etiology is multifactorial and that for effective treatment several issues will need to be addressed. PRP offers a holistic approach that may allow the simultaneous correction of several factors. In this section, we explore the scientific basis and rationale for the use of PRP in dermatology indications.

### *3.1 ACTIVATION, PROLIFERATION AND DIFFERENTIATION OF FIBROBLASTS*

Fibroblasts are responsible for producing the key components of the skin, such as collagen and extracellular matrix proteins. Fibroblasts also mediate the skin repair process, and they both respond to and produce growth factors needed for skin repair and direction of the skin repair process. They exhibit considerable plasticity but are sensitive to changes in the concentration of growth factors and will adapt their behavior accordingly (Thangapazham et al., 2014).

Loss of fibroblasts (e.g., skin aging) or alterations in the production of collagen or other key skin components is reflected as "damaged" skin. Since fibroblast dysfunction is associated with several skin disorders, Wong et al., (2007) suggested that providing the correct proportion of growth factors may be able to positively influence their activity. As platelets can release growth factors in a controlled manner and the plasma may contain molecules that modulate growth factor activity, PRP has thus been investigated as a means to induce fibroblast proliferation, production of growth factors and regeneration of the ECM.

Several studies have shown that growth of fibroblasts in PRP-supplemented medium enhances their proliferation (Anitua et al., 2009; Berndt et al., 2019; Cho et al., 2019; Cho et al., 2012; Kakudo et al., 2008; Kim et al., 2011; Noh et al., 2018). In most studies, highly concentrated PRP was prepared (4-8X) and PRP coagulation was activated by addition of autologous thrombin and calcium solution. The resulting clots were centrifuged, and the supernatant extracted from these clots was frozen and used to supplement the culture media. In these conditions, optimal proliferation of fibroblasts was achieved with 5% activated PRP supernatant. Proliferation decreased in a dose-dependent manner in the presence of 10% or 20% activated PRP supernatant (Cho et al., 2019; Kakudo et al., 2008; Kim et al., 2011). When using non-activated 1.6X RegenPRP directly in the culture media, supplementation with 20% PRP is optimal for fibroblast proliferation (Berndt et al., 2019) and Fig. 14.
RegenLab specifically designed and developed the CuteCell medical device for the standardized preparation of RegenPRP for in vitro applications (Berndt et al., 2019). Cell culture medium supplemented with CuteCell-PRP improved the proliferation of fibroblasts compared to the cells grown in fetal bovine serum (FBS)-supplemented medium (Berndt et al., 2019). PRP prepared using the CuteCell device was used in an autologous culture system to expand human dermal fibroblasts obtained from ten female patients undergoing abdominoplasty. The fibroblasts from the patients were grown in medium supplemented with CuteCell-PRP for 7 days, with no medium change required. The authors found that the proliferation of fibroblasts in PRP was dose-dependent, with the optimal dose being 20% PRP (non-activated). The proliferation rates of fibroblasts grown in 20% RegenPRP were almost 8 times better than those grown in 10% FBS (Fig. 14). This increased proliferation rate was reflected by changes in the cell growth cycle, but no chromosomal rearrangements or genomic instability were detected. The authors also observed that the morphology of the cells grown in PRP was spindle shaped and had a closer resemblance to cells grown in 3D matrix cultures or in vivo compared to cells grown in FBS, which had a regular flattened cell shape. This study demonstrated that expansion of autologous fibroblasts in PRP prepared from the patient is more efficient and with reduced risks compared to expansion in FBS-supplemented medium.

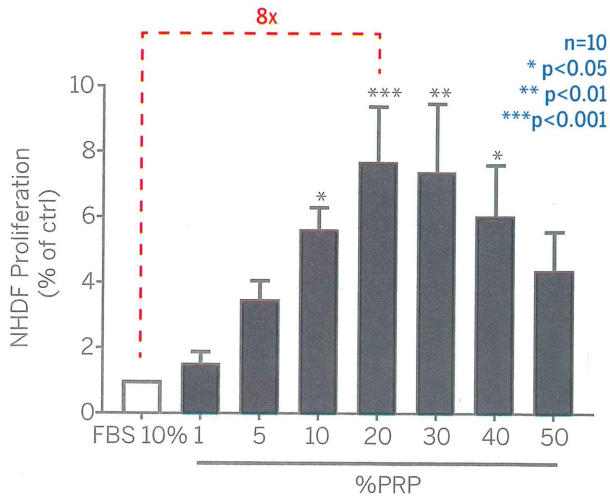



Figure 14: CuteCell PRP enhances proliferation of normal human dermal fibroblasts (NHDF)

One of the main goals of stimulating fibroblasts with PRP is to induce the synthesis of collagen and this has been demonstrated in several studies (Cho et al., 2019; Cho et al., 2012; Nicoletti et al., 2019). Induction of collagen synthesis is governed by the concentration of TGF-β1 and other molecules in the medium (Anitua et al., 2009; Cho et al., 2019; Cho et al., 2012; Kakudo et al., 2008; Kim et al., 2011; Moon et al., 2019; Narine et al., 2006). In this regard, consideration should be given to the method for preparing PRP, the concentrations of growth factors in it, the concentration of PRP being used and the evaluation time period when assessing the impact of PRP on fibroblast function as each growth factor displays a unique kinetic profile (Noh et al., 2018). Taken together, these studies show that treatment of dermal fibroblasts with PRP increases their proliferation and analysis of the culture medium shows an increase in several growth factors and cytokines needed for tissue repair and regeneration.

### *3.2 EXPANSION OF ADIPOSE-DERIVED STEM CELLS*

There is great interest in the use of adipose-derived stem cells (ADSCs) in regenerative medicine as fat is easier to harvest, widely available and contains higher concentrations of mesenchymal stem cells compared to bone marrow tissue (Atashi et al., 2015a). ADSCs can differentiate to fat, cartilage, muscle and bone tissue and can also be induced to form skin epithelium, neural cells, hepatocytes and pancreatic islets (James et al., 2016). ADSC, although numerous in fat tissue, may still need to be expanded to obtain sufficient numbers for transplantation. In addition, the clinical use of ADSCs needs to meet regulatory requirements.

Collagenase is needed to extract ADSC from fat tissue, This enzymatic digestion is considered by authorities as more than minimal, and thus the use of ADSC is considered as an advanced therapy medicinal product (ATMP). In addition, for their ex vivo expansion, it is highly desirable that they be cultured without xenogeneic supplements.

Atashi et al. (2015a) compared the proliferation of adipose tissue-derived mesenchymal stem cells (ADSCs) grown in basal medium supplemented with various concentrations (range: 1% to 60%) of either activated or non-activated RegenPRP with 10% FBS (Fig. 15). Cells grown in PRP had a better doubling time than cells grown in FBS (28 hours in 20% PRP, 56 hours in 10% FBS), retained their ability to differentiate to chondrocytes and other lineages and maintained their typical spindle fibroblast shape under all conditions. No abnormal karyotype was detected, indicating that growth in RegenPRP does not affect chromosomal stability of mesenchymal stem cells (MSCs) while maintaining their pluripotency and ability to differentiate. As discussed in section 3.1, Regen Lab has developed a new medical device, CuteCell, for the preparation of PRP to be used as an in vitro culture media supplement (Berndt et al., 2019). See also section 7.7.3. The findings from Atashi et al. (2015a) were explored further and they found that PRP appears to mainly induce proliferation of the ADSCs through the PDGFR/AKT and the TGFβR/Smad2 signaling pathways (Atashi et al., 2015b).

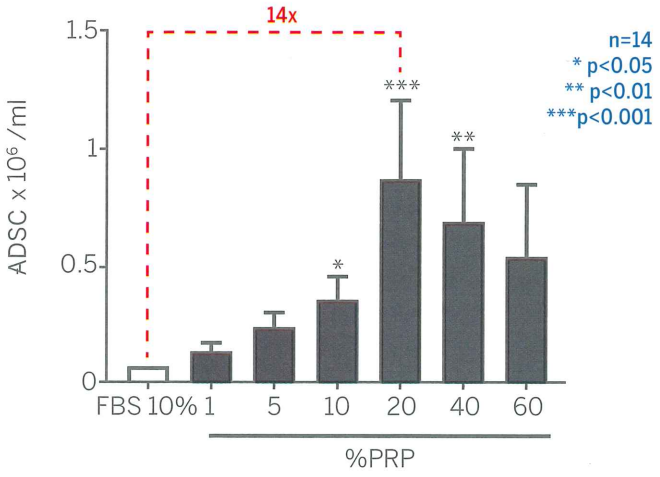



Figure 15: Effect of different concentration of RegenPRP on the proliferation of ADSC cultured over 10 days

Besides the interest in the use of ADSCs in aesthetic medicine, there is also interest in using ADSCs for the treatment of genitourinary disorders, alopecia and vitiligo, among others (Eshtiaghi and Sadownik, 2019; James et al., 2016; Owczarczyk-Saczonek et al., 2017; Stevens et al., 2018). PRP may be used as a growth supplement when needed, or alternatively may be co-administered with the ADSCs where promising results have also been achieved. Moreover, PRP supplementation could also be considered for the expansion of other stem cell populations, such as hair follicle stem cells (Gentile and Garcovich, 2019).

### *3.3 RECONSTRUCTION OR REMODELING OF THE EXTRACELLULAR MATRIX*

Current anti-aging strategies as well as those for improving the appearance of acne scars are aimed at increasing the synthesis of the ECM, particularly collagen, through the activation of fibroblasts (Jenkins, 2002), Fig. 16. The balance between matrix metalloproteinases (MMPs), which are enzymes involved in the selective degradation of collagen, and tissue inhibitors of MMPs (TIMPs) is essential to prevent excessive tissue degradation while still allowing removal of damaged tissue and regeneration. Restoring the balance of MMPs:TIMPs, which is altered in aged skin, acne and vulvar lichen sclerosus (de Oliveira et al., 2012; Saint-Jean et al., 2016; Shin et al., 2019; Yaykasli et al., 2013), may therefore be beneficial when considering treatment for these skin disorders. Autoimmune responses that target ECM proteins may also be a contributing factor in other disorders such as vulvar lichen sclerosus, which is associated with the development of scar tissue in the dermis (Chan et al., 2004; Erickson et al., 2016). Therefore, inducing remodeling of the ECM in such conditions may be a therapeutic approach worth exploring.

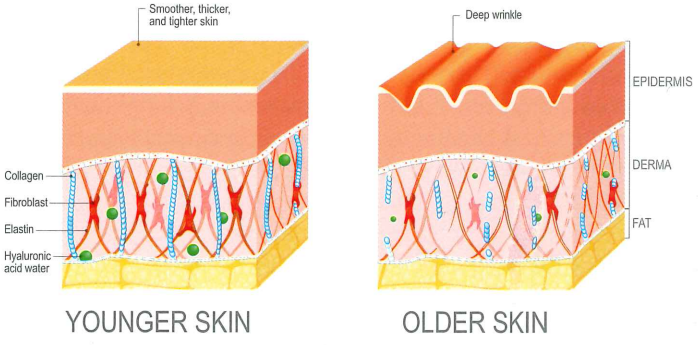



Figure 16: Over time, changes in the production and organization of collagen and elastic fibers contribute to the visible signs of skin aging. Inducing neocollagenesis and neoelastinogenesis in the dermis are among the anti-aging strategies in use. This approach is also being used for the treatment of acne scars.

There are a plethora of in vitro and in vivo studies showing that PRP increases the production of collagen (Cheng et al., 2012; Cho et al., 2011; Cho et al., 2012), stimulates fibroblast proliferation (Cho et al., 2019; Krasna et al., 2007), as well as angiogenesis (Giusti et al., 2009; Kakudo et al., 2014; Martinez et al., 2015). PRP has also been shown to upregulate MMPs (Cho et al., 2019; Cho et al., 2012; Kim et al., 2011). Platelets have been shown to produce tissue inhibitors of MMP-1 (TIMP-1) (Villeneuve et al., 2009). PRP has also been shown to modify the TGF-β/Smad signaling pathway which is critical for maintaining the structural and mechanical integrity of dermal connective tissue (Atashi et al., 2015b; Shin et al., 2019).

Histological examinations of skin from patients treated with PRP have shown modifications to the epidermis and dermis, such as increased thickness, better organization of collagen fibers and restoration of the skin architecture (Abuaf et al., 2016; Charles-de-Sa et al., 2018; Diaz-Ley et al., 2015; Draelos et al., 2019; Min et al., 2018; Na et al., 2011).
However, most in vitro studies investigating the ability of PRP to induce collagen synthesis use normal, healthy fibroblasts. Devereaux et al. (2018) highlighted the need to perform studies with PRP on impaired fibroblasts to demonstrate PRP's potential to repair damaged skin. The fibroblasts of aged skin display an altered morphology (Qin et al., 2017), which can be restored when grown in PRP-supplemented medium (Min et al., 2018). Wirohadidjojo et al. (2016) also showed that dermal fibroblasts exposed to chronic UV radiation demonstrate reduced

proliferation, collagen deposition and cellular migration, but that these processes could be partially restored (proliferation), completely restored (collagen deposition) or enhanced (migration) when the cells were grown in PRP-supplemented medium. A dose-dependent effect of PRP was observed for the latter two processes.

In conclusion, intradermal injection of PRP provides encouraging results for an overall improvement in skin texture, elasticity, and tone, and creates a volumizing effect that can lead to a significant volume correction of deep wrinkles such as the nasolabial folds and may enhance outcomes when used in combination with current treatment modalities aimed at remodeling of the ECM such as ablative fractional $CO_2$ laser therapy and microneedling (Chang et al., 2019; Hesseler and Shyam, 2019a; Hsieh et al., 2019; Schoenberg et al., 2019).

### *3.4 ANTI-INFLAMMATORY*

Inflammation and a dysregulated immune response are associated with several skin disorders and may be initiated due to alterations in skin homeostasis, such as hormonal changes that disrupt the skin microbiota or alter the production of sebum (e.g., acne, Fig. 17).

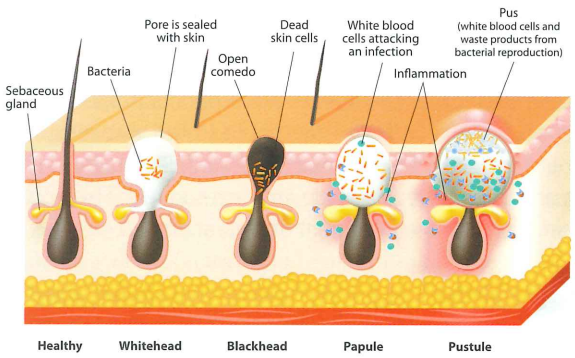



Figure 17: Acne is a well-known inflammatory skin disorder

The association between inflammation and the development of acne is well known (Kircik, 2014). Acne patients prone to scarring exhibit a different inflammatory profile in both clinically normal and inflamed skin to those not prone to scarring, with differences in expression of MMPs and TIMPs which affect remodeling of the ECM, as well as pro- and anti-inflammatory cytokines, particularly at later time-points when inflammation should be resolved (Carlavan et al., 2018; Holland et al., 2004; Moon et al., 2019; Saint-Jean et al., 2016). A prolonged inflammatory response is associated with decreased collagen deposition (Lee et al., 2013).

Inflammation and increased levels of Th17 cells have also been observed in cases of vitiligo and acne (Gokhale and Mehta, 1983; Kistowska et al., 2015; Zhen et al., 2016; Zhou et al., 2015). In addition, a micro RNA that is common to both vitiligo and vulvar lichen sclerosus has been linked to a pro-inflammatory response in vulvar lichen sclerosus (Limpers et al. 2014).

Chronic inflammation has also been proposed as an etiologic factor of androgenetic alopecia (AGA) (English, 2018; Katzer et al., 2019) with perifollicular inflammation being reported as an early step of the process that can lead to follicular miniaturization or loss of the follicle (El-Domyati et al., 2009; Jaworsky et al., 1992; Ramos et al., 2016; Sueki et al., 1999). Hair follicles from patients with AGA show signs of immune infiltrate (Mecklenburg et al., 2000). Therefore, transplanting hair follicles from the donor area with moderate to significant perifollicular inflammation could be responsible for follicular loss and poor hair growth post-transplantation. At least one third of AGA patients display such inflammation (Nirmal et al., 2013). Treatment of the scalp and the hair follicle units with PRP prior to or during the transplant procedure have given positive clinical outcomes (Garg and Garg, 2019; Garg, 2016; Navarro et al., 2018; Uebel et al., 2006), suggesting that PRP when used as a monotherapy or in conjunction with other treatments could be used as an anti-inflammatory agent to improve clinical outcomes in skin and scalp disorders.

In genitourinary disorders, chronic inflammation has been implicated in lichen sclerosus, with lichen sclerosus patients tending to have increased levels of proinflammatory cytokines (Fergus et al., 2020). Antibodies against extracellular matrix proteins, among others, have been detected. However, the etiology of the disease is not understood, and it is unknown whether these autoimmune antibodies contribute to the disease or are a consequence of it (Fergus et al., 2020). Chronic inflammation may also be associated with vaginal mesh exposure, whether due to the presence of a subclinical infection or issues associated with placement of the mesh (Nolfi et al., 2016).

How PRP may modulate inflammation and improve outcomes in skin disorders is not yet known. PRP is proposed to induce a mild inflammation at the injection site that stimulates ECM regeneration (Abuaf et al., 2016; Merchan et al., 2019; Papait et al., 2018) and has been shown to be anti-inflammatory in in vivo skin flap models (Chai et al., 2019; Wang et al., 2016) and in a rabbit model where precoating a polypropylene mesh with PRP prior to implantation was shown to reduce the inflammatory response (Parizzi et al., 2017). Chen demonstrated that the presence of neutrophils was enhanced in osteoarthritic groups of an animal model but significantly reduced in groups treated with PRP or HA or both (Chen et al., 2014). Anecdotal evidence from clinical studies suggests PRP may have some anti-inflammatory properties, e.g., improvement of acne pustules (Gómez et al., 2017), seborrheic dermatitis (Borhan et al., 2015) and scalp inflammation (Qu et al., 2019). When used in conjunction with current treatment modalities such as ablative $CO_2$ lasers or microneedling, PRP has been shown to reduce inflammation-associated side effects and downtime in most studies (Badran and Nabili, 2018).

In some cases, pain may also be associated with inflammation or neutrophil migration (Fiset et al., 2003). Guerid et al., (2013) observed that besides improving the time for healing, patients treated with RegenPRP in the wound bed had a significant decrease in pain resulting from a skin graft, and that this decrease was enhanced when a combination of RegenPRP and keratinocytes was used. The authors suggested that the analgesic effect may be due to platelets releasing or stimulating the release of substances with an analgesic effect. Indeed, PRP has been demonstrated to have anti-nociceptive activity, linked, at least in part, to their endocannabinoids and related compound content (Descalzi et al., 2013).

### *3.5 ANTI-MICROBIAL AND MODULATION OF THE SKIN MICROBIOTA*

PRP is rich in platelets and depending on the method used for preparation may be leukocyte-rich or leukocyte poor. Platelets have been shown to release antimicrobial peptides from their granules. Whether leukocytes also contribute to the antimicrobial activity of PRP is still debated (D'Asta et al., 2018). PRP has demonstrated anti-microbial activity against both Gram-positive and Gram-negative bacteria, however, its effectiveness against different bacteria varies and it may be at best bacteriostatic (Del Fabbro et al., 2016; Intravia et al., 2014; Jafarzadeh et al., 2016; Varshney et al., 2019). Additional studies to delineate the role of the various components of PRP to its antimicrobial activity against the skin microflora (Fig. 18) are warranted, particularly their antimicrobial activity against the phylotype of *Cutibacterium acnes* associated with acne. Prysak et al. (2019) reported an association between granulocyte number in the PRP and its potency against *C. acnes*. A pilot study of a patient with bore moderate papulopustular acne demonstrated that PRP reduced the size and number of papules, pustules and comedones on the skin and the papules and pustules improved from moderate to mild after treatment. PRP also had positive effects on the skin lesions (Gómez et al., 2017). Thus, the anti-microbial properties of PRP may alleviate the symptoms of acne and reduce the risk of scarring and help restore a healthy balance of microbiota on the skin.

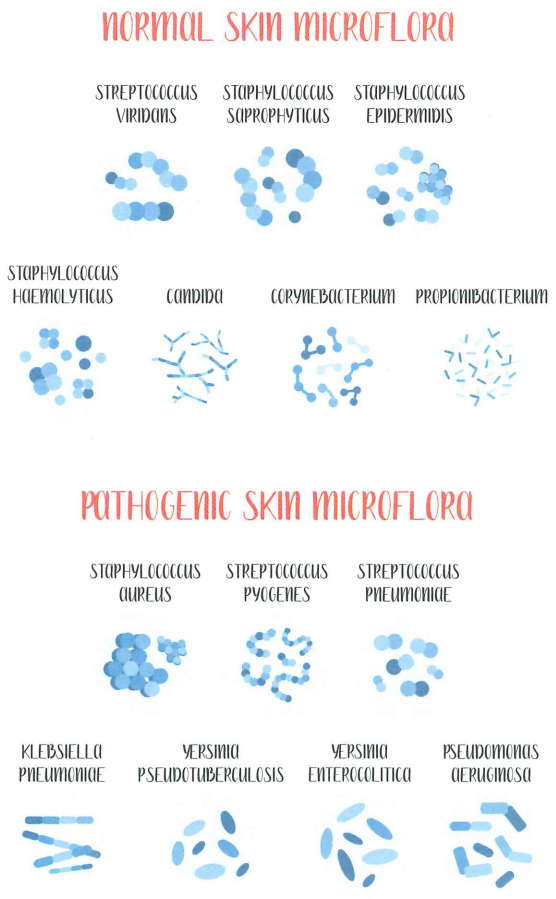



Figure 18: Normal and pathogenic skin microflora

A study of the microbiome in the hair follicle of AGA patients also showed increased abundance of *Propionibacterium acnes* in the middle and lower compartments of miniaturized vertex hair, while the microbiome of non-miniaturized follicles was similar to healthy controls (Ho et al., 2019). The increased presence of *P. acnes* in the hair follicle may be a cause of an elevated immune response and may be a contributing factor to the pathogenesis of AGA (Ho et al., 2019). Modifications of the hair follicle microbiome may also be associated with other forms of alopecia (Lousada et al., 2020). It remains to be seen what effect PRP has on the microbiome of the hair follicle.

Lactobacilli are the predominant species in the vagina of premenopausal women where they contribute to maintaining a low vaginal pH and the production of anti-microbial and anti-inflammatory products provide a first line of defense against invading pathogens (Amabebe and Anumba, 2018). The levels of free glycogen in the vagina are correlated with the abundance of Lactobacilli, therefore as the levels of free glycogen decrease with age, the composition of the vagina microbiota changes and the pH rises, leaving the vagina more susceptible to colonization by other species, including pathogenic ones (Mirmonsef et al., 2015; Photiou et al., 2020). In this case, PRP may be able to restore the Lactobacilli population indirectly through its regenerative effects on the skin, whereby restoration of normal skin function will increase the levels of free glycogen thereby increasing the resident Lactobacilli population.

The anti-microbial properties of PRP may also be of interest for implant devices, whereby pre-coating the device with PRP prior to placement may reduce the risk of infection. In an animal model, pre-treatment of the implantation site with PRP reduced the number of bacterial colonies and improved bone healing (Li et al., 2013). Such procedures might be of interest where surgical meshes are used, such as for pelvic organ prolapse repair, to prevent the development of low-grade infections which may lead to chronic inflammation and vaginal mesh exposure.

We can currently only speculate that the anti-microbial or microbiome-modulating properties of PRP may be manifested directly and/or indirectly, through its direct effect on microbial growth or indirectly by the changes PRP induces in other skin components. For example, improved skin function in the vagina or modification of sebum production can lead to changes in the composition of the skin flora and favors the presence of commensal rather than pathogenic bacteria.

### *3.6 HAIR GROWTH*

Hair growth and the hair cycle is controlled through several signaling pathways and involves communication between several components of the hair follicle and external factors (Chen and Chuong, 2012). Any disruption of these signaling pathways, whether due to alterations in hormones, disease, or medication, can lead to hair loss. The role of androgens in hair loss is well documented and increases in testosterone result in modification of the production of signaling molecules involved in hair growth, such as IGF and PDGF (Ashton et al., 1995; Motamer et al., 2019). Loss of vascularization is an early step in AGA and alopecia areata (AA) and AGA hair follicles show reduced expression of VEGF, an important growth factor for angiogenesis (Goldman et al., 1995; Simonetti et al., 2004). Angiogenesis is also important for normal progression through the anagen phase of the hair cycle (Mecklenburg et al., 2000; Yano et al., 2001) and to prevent atrophy of important skin components such as hair follicles (Goldman et al., 1995).

PRP contains many growth factors that are known to influence the hair growth cycle (Table 2) and it is thought to function by stimulating the initiation/extension of the anagen phase and

promoting vascularization (Girijala et al., 2018; Gupta and Carviel, 2016). For example, IGF-1 is found in PRP and may have a growth-promoting function in the anagen phase and its anti-apoptotic effects may delay entry into catagen phase; thereby increasing the number of hairs in the anagen phase (Girijala et al., 2018; Rodrigues et al., 2019; Vasserot et al., 2019; Weger and Schlake, 2005). PRP also contains several growth factors involved in angiogenesis such as VEGF, PDGF, HGF. Several in vitro and in vivo studies have demonstrated that PRP can stimulate angiogenesis (Martinez et al., 2015). Clinical studies have demonstrated increases in the number of follicular bulge cells and follicles, epidermal thickening, improved vascularization, and a higher number of Ki67+ keratinocytes in scalps treated with PRP (Gentile et al., 2015).

Table 2: Selected growth factors involved in key cell processes relating to hair growth

| Growth Factor | Function | Reference* |
|---|---|---|
| **Epidermal growth factor (EGF)** | Cell proliferation and differentiation<br>Activates the proliferation and causes transdifferentiation of hair stem cells and produces new follicular units | Katsuoka et al., 1987<br>Langer and Mahajan, 2014*<br>Girijala et al., 2018* |
| **Fibroblast growth factor (FGF)** | Increased expression of FGF-7 prolongs anagen phase of hair growth cycle<br>bFGF promotes proliferation of papilla cells, which is important for elongation of the hair shaft<br>Cell proliferation, migration, differentiation, angiogenesis | Li et al., 2012b<br>Katsuoka et al., 1987<br>Girijala et al., 2018*<br>Girijala et al., 2018; Yun et al., 2010* |
| **Hepatocyte growth factor (HGF)** | Control of hair growth by elongating the hair follicle and preventing induction of catagen phase | Girijala et al., 2018; Lindner et al., 2000* |
| **Insulin-like growth factor 1 (IGF-1)** | Induction and prolongation of anagen phase of hair cycle | Langer and Mahajan, 2014* |
| **Platelet-derived growth factor (PDGF)** | Cell proliferation and differentiation<br>Coordinates wound healing process<br>Adult hair follicle dermal stem cell maintenance and self-renewal<br>Angiogenesis | Langer and Mahajan, 2014; Li et al., 2012b*<br>Cross and Mustoe, 2003<br>Gonzalez et al., 2017<br>Girijala et al., 2018* |
| **Transforming growth factor β1 (TGF-β1)** | Modulates inflammation<br>Stimulates human dermal fibroblasts<br>Induces collagen synthesis<br>Hair folliculogenesis and maturation | Cross and Mustoe, 2003<br>Langer and Mahajan 2014*<br>Girijala et al., 2018*<br>Girijala et al., 2018* |
| **Vascular endothelial growth factor (VEGF)** | Hair cycle<br>Angiogenesis<br>Stimulates human dermal fibroblasts<br>Stimulates dermal papilla cells | Mecklenburg et al., 2000<br>Girijala et al., 2018*<br>Langer and Mahajan, 2014*<br>Li et al., 2012a |

*see references within these articles for original publications

Li et al. (2012a) reported that human dermal papilla cells cultured in PRP-supplemented medium had higher levels of expression of the regulators pERK (cell proliferation) and pAkt (apoptosis) as well as higher levels of Bcl-2 (anti-apoptosis), β-catenin (hair follicle development and hair growth cycle) and FGF-7 (prolongation of anagen phase and delayed entry to the catagen phase). A genomic analysis examining the effect of PRP on human dermal papilla cells revealed over 500 genes and Long non-coding RNAs (lncRNAs) that were differentially expressed in dermal papilla cells exposed to PRP compared to those that were not. These genes were enriched in proliferation-associated pathways, such as those involved in the cell cycle and DNA replication, suggesting that PRP can contribute to the proliferation of dermal papilla cells (Shen et al., 2017). PDGF-signaling was also shown to be critical for dermal papilla stem cell function (Gonzalez et al., 2017).

PRP may have a different role to play in alopecia depending on the disorder in question, the stage of the disease or the particular aims of treatment, e.g., hair thickening or transition to anagen phase (Hausauer and Jones, 2018) since the signaling pathways affected differ depending on the stage of the disease (Martinez-Jacobo et al., 2018a; Martinez-Jacobo et al., 2018b). Vasserot et al. (2019) discuss three approaches to addressing hair loss (i) prolongation of the anagen phase or delaying entry to the catagen phase – this approach is best suited to early stages of hair loss (ii) reactivation of the miniaturized hair follicle for mid-stage AGA (iii) hair neogenesis for late stage AGA or alopecia areata. Clinical studies suggest that PRP is most effective in the earlier stages of AGA (Qu et al., 2019). Nonetheless, it may still have a role to play in later stages of AGA where it can be used as an adjunct to other therapies such as follicular unit transplantation to reduce inflammation and facilitate establishment of the new follicles (Garg, 2016; Garg and Manchanda, 2017; Navarro et al., 2018; Uebel et al., 2006). Although PRP is also being considered for other forms of alopecia, there is not yet sufficient data available to determine the most effective way of using PRP for these conditions.

### *3.7 MODIFICATION OF SEBUM*

Alterations in the sebaceous gland and sebum production have been associated with several skin indications. For example, AGA patients tend to have oily scalps possibly due to an enlarged sebaceous gland (Kure et al., 2015; Qu et al., 2019), alterations in sebum production during puberty are associated with the development of acne (Clayton et al., 2019) and loss of sebaceous glands may be a contributing factor to skin aging (Kim et al., 2014; Sakuma and Maibach, 2012). Lipid metabolism was identified as the major biological process affected in melasma (Kang et al., 2011).

To date, no clinical trials have specifically examined the effect of PRP on sebum production. However, some observations have been made. In one study, PRP was shown to significantly reduce the levels of oil secretion in the scalp after six consecutive injections of PRP (Qu et al., 2019). Geldenhuys and Hudson (2016) reported that patients injected with PRP and autologous cultured fibroblasts in the nasolabial fold showed a change in sebum quality (number of sebaceous glands, density of secretions and average weight of secretions) of the skin.

### *3.8 PRP COMBINED WITH OTHER TREATMENTS FOR SKIN DISORDERS*

#### *3.8.1 Hyaluronic acid*

Hyaluronic acid (HA) is an important component of the skin and is a major contributor to the formation of a resilient gel-like ground substance that resists compressive forces (Fig. 19). It contributes to tissue hydrodynamics by creating space for the movement of cells. It is believed to regulate the diffusion of micronutrients, metabolites and hormones between cells, and stimulate fibroblast migration, proliferation and collagen production (Edwards and Fantasia, 2007).

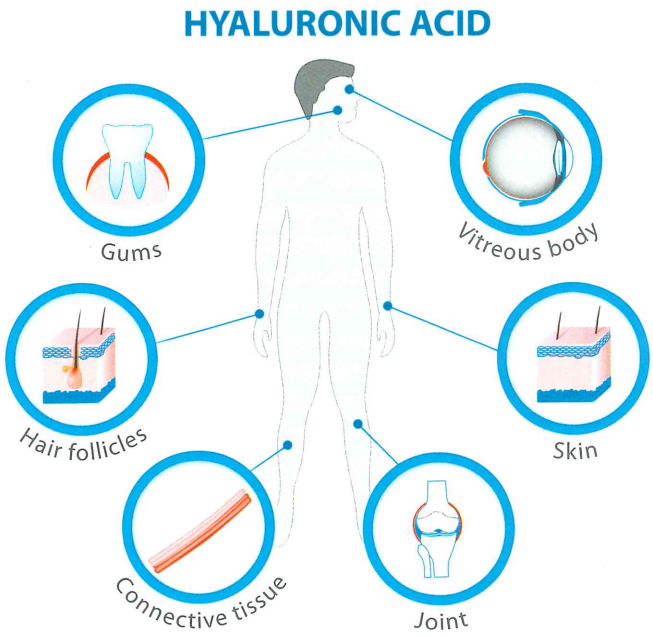



Figure 19: Hyaluronic acid is an important component of many parts of the body, including the skin and articular cartilage.

For several years, injections with non-crosslinked HA have been performed in dermatology to provide the skin with more elasticity, turgor, and moisture. According to the results of an expert meeting held in May 2007 during the 16th Congress of the European Academy of Dermatology and Venerology, in Vienna, Austria (Wiest and Kerscher, 2008), treatment with native HA can improve dry, aged skin as well as extrinsically damaged skin, with positive effects on skin elasticity, turgor, firmness and water content of the stratum corneum. All experts were of the opinion that selection criteria for such a treatment should be fine, superficial wrinkles and/or early stages of elasticity loss. Cheeks, neck and décolleté are favorable sites to be treated with native, non-crosslinked HA, but the treatment is also suited for the peri- and infra-orbital area, perioral skin region, as well as for the back of the hands. This proposition was confirmed by a number of more recent studies evaluating the potential of non-crosslinked HA as a hydrating agent for the treatment of crow's feet (Choi et al., 2017), cheek, neckline and perioral skin (Taieb et al., 2012), as well as the periorbital region (Succi et al., 2012).

There are several reasons for considering using a combination of PRP and hyaluronic acid for the treatment of skin disorders.

- PRP can induce production of hyaluronic acid in fibroblasts (Anitua et al., 2007) as well as having many other positive effects on fibroblasts and other skin cells with demonstrated clinical benefits for skin rejuvenation.
- Studies have shown that a PRP-HA combination is more potent than either alone in osteoarthritic models (Chen et al., 2014) and wound healing (Cervelli et al., 2010). In vitro studies have shown that when PRP is incubated with HA, the release of growth factors is increased after 5 days (Iio et al., 2016).
- When PRP combined with HA coagulates, the structure of the resulting fibrin network is different and has a larger porosity than a fibrin clot without HA. This creates a better environment for cells, allowing easier cell migration and proliferation (Smith et al., 2007).

Therefore, as PRP and HA target different pathways and have different functions, when used together they may have a synergistic effect when used as a therapeutic approach for healing, inflammation or analgesic purposes, as already demonstrated by Cervelli et al. (2010) and Chen et al. (2014).

One study has also reported treatment with both PRP and HA to correct signs of skin aging on face and neck regions (Ulusal, 2017). However, the treatment protocol was not standardized between patients, as the number of injection sessions varied between 1 and 8. Subjects who received a higher number of treatment sessions were more satisfied with the treatment and showed greater improvement. Given the variety in the protocol, it was not easy to assess the effectiveness of the PRP and HA combination globally; however, digital clinical photographs, wrinkle severity scale score and subjects' satisfaction showed that the PRP association with HA provided satisfactory results for rejuvenation of treated regions from the first treatment. PRP/HA association was safe, with no significant side effects and no inflammation or allergic reactions were reported. Temporary mild facial edemas were observed, and some patients reported ecchymosis, which lasted about 10 days.

However, it is important to note that the use of empirical combinations of HA with PRP on patients is not allowed, as the safety and efficacy of such combinations should be first demonstrated. To respond to this demand, Regen Lab has developed a new medical device platform: Cellular Matrix.
Cellular Matrix kits are EU certified class III medical devices specifically designed for the preparation of RegenPRP in combination with HA in a closed-circuit system (section 7.7.5). They allow the concomitant use of the two therapies in a single injection, in conformity with good practice and regulatory requirements. The safety and clinical efficacy of this specific combination has been demonstrated (Vischer et al., 2018).

To date, there are few reports on the use of Cellular Matrix in the literature for dermatological indications (Hersant et al., 2018; Hersant et al., 2017), section 4.5) and genitourinary disorders (Aguilar et al., 2016; Hersant et al., 2018; section 6.3). However, additional reports on its use are provided by several of the physicians that have contributed to this book.

### *3.8.2 Energy-based devices*

The aim of energy-based devices, such as fractional radiofrequency or fractional ablative $CO_2$ lasers, is to disrupt the collagen and other structures in the dermis and stimulate fibroblasts to induce a regenerative response (Mehta-Ambalal, 2016), Fig. 20. The rationale for combining PRP with these therapies is that PRP may augment the regenerative response and help reduce inflammation-associated side-effects associated with energy-based devices. This may lead to a reduction in patient downtime after treatment.

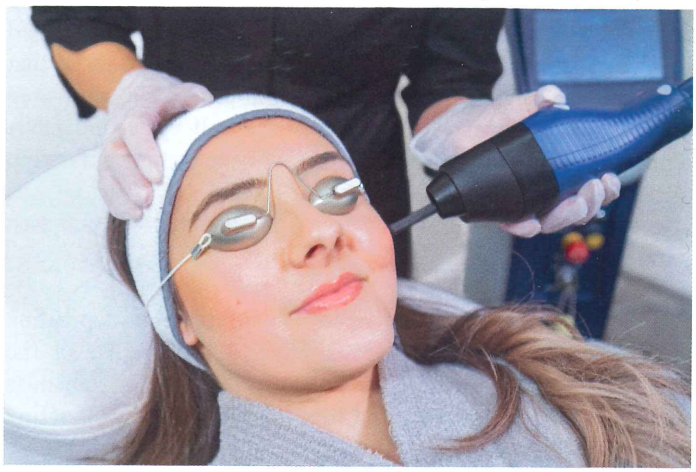


Figure 20: Energy-based devices, such as fractional ablative $CO_2$ lasers, aim to disrupt the extracellular matrix and induce a regenerative response.

Shin et al. (2012) found that a combination treatment with PRP and fractional laser resulted in objective improvement of skin elasticity, a lower erythema index, and an increase in collagen density compared to fractional laser treatment alone. A review of the literature also concluded that when PRP is used as an adjuvant to laser therapy it gives a positive clinical result (Badran and Nabili, 2018).

A systematic review concluded that adjuvant PRP accelerated healing after fractional laser resurfacing. Although the degree of improvement was typically less than 50%, patients generally reported high satisfaction (Maisel-Campbell et al., 2019).

A systematic review and meta-analysis concluded that the results indicated a higher response and patient satisfaction rates for combined $CO_2$ laser and PRP treatment for acne scarring as well as shorter durations of posttreatment erythema, edema, and crust formation after the combined treatment than after laser-only treatment (Chang et al., 2019; Hesseler and Shyam, 2019b). However, another study found that PRP did not improve the scar quality compared to laser treatment alone, although the downtime and inflammation associated with laser treatment was significantly reduced on the PRP-treated side (Kar and Raj, 2017).

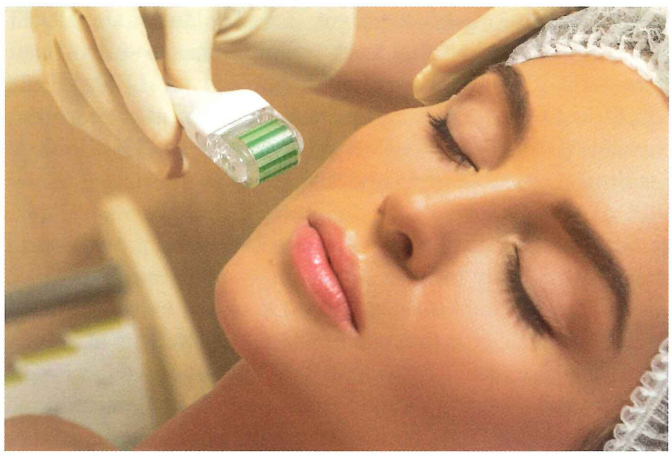
Combinations of $CO_2$ or lasers or narrow band-UVB with PRP have also been effective for the treatment of vitiligo (Hesseler and Shyam, 2019a; Kadry et al., 2018a; Khattab et al., 2019).

Laser therapy has also been investigated for vulvovaginal atrophy (VVA)/genitourinary syndrome of menopause (GSM), including in breast cancer survivors (Knight et al., 2019). At least one study has demonstrated promising results when laser therapy is used in combination with PRP for the treatment of vaginal atrophy (Gaspar et al., 2011). However, there is a lack of data relating to the safety and efficacy of laser therapies for vaginal rejuvenation

procedures and currently there is no formal guidance for their use (Digesu et al., 2019).

### *3.8.3 Microneedling*

Microneedling has been investigated for its effects on atrophic acne scars, skin rejuvenation, hypertrophic scars, keloids, striae distensae, androgenetic alopecia, melasma and acne vulgaris (Ramaut et al., 2018). Microneedling is a safe, cost-effective, and efficacious treatment option for a variety of dermatologic conditions in all skin types (Bonati et al., 2017). Microneedling involves rolling microneedles of varying length across the skin. The needles penetrate the skin to various depths and induce mechanical injury to the dermis thereby inducing a wound healing or regenerative response (Fig. 21). Microneedling is thought to induce a similar regenerative response to PRP and combining these two approaches may improve outcomes, although it is not yet clear whether they have a synergistic effect. Application of PRP prior to or after microneedling may allow PRP to penetrate the skin to different extents depending on the length of the needle used (Sasaki, 2017).



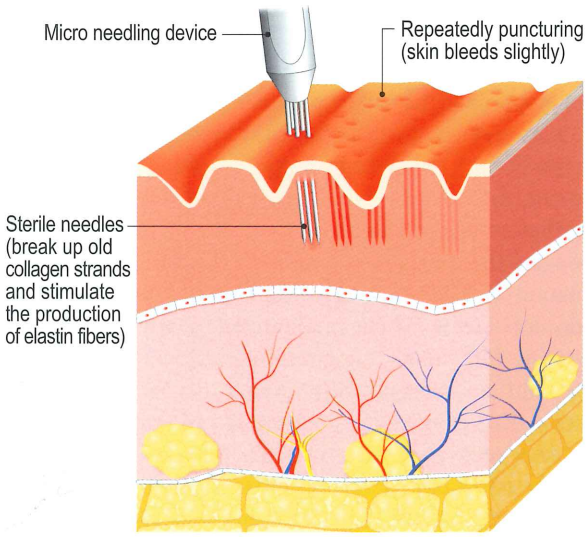



Figure 21: Microneedling involves rolling microneedles of varying length across the skin (A). These punctures to the skin induce a wound healing or tissue regenerative response (B).

Microneedling alone or in combination with PRP has demonstrated efficacy for skin rejuvenation, scar management and alopecia (El-Domyati et al., 2018a, b; Sasaki, 2017). A review of the literature suggests that PRP in combination with microneedling can improve the cosmetic outcomes of microneedling without increasing the risk of adverse events (Hashim et al., 2017). Microneedling can also be combined with PRP and other treatment modalities like radiofrequency for skin rejuvenation and management of acne scars (Alenichev et al., 2017); see also the contributions by Dr. Alenichev and Dr. Alamiri in this book). A combination of microneedling and PRP was shown to be an effective treatment for melasma, and was less painful that PRP microinjections using mesoneedles (Hofny et al., 2019a).

Recent studies have also investigated the combination of microneedling with low-level laser therapy combined with PRP for the treatment of alopecia, which gave promising results (Gentile et al., 2020).

PRP in combination with microneedling was also reported to be more effective than PRP alone or minoxidil monotherapy in patients with AGA (Jha et al., 2019; Shah et al., 2017).

### *3.8.4 Autologous fat transplantation*

Autologous fat grafting is used extensively in reconstructive and aesthetic plastic surgery for the correction of soft-tissue volume defects (Luck et al., 2017). However, a major limitation of this approach is the variability in the survival of the graft, with resorption rates ranging from 10-90% (Modarressi, 2013). This is thought to be in part due to inadequate vascularization of the transplanted fat tissue resulting in necrosis of the transplanted adipocytes (Smith et al., 2019). Modarressi 2013) hypothesized that since PRP is rich in growth factors and other factors involved in neovascularization adding it to fat preparations would provide an appropriate source of nutrients at the early stage of transplantation and contribute to improved survival of the graft. In addition, PRP may release anti-inflammatory cytokines that prevent degradation of the graft (Seyhan et al., 2015) and PRP factors may enhance the differentiation of preadipocytes to the mature form (Cervelli et al., 2012; Kakudo et al., 2008). In vivo, adding RegenPRP to fat grafts prior to injection significantly increased adipocyte viability and tissue vascularity in a mouse model (Atashi et al., 2019). These studies show that PRP can improve the outcome of autologous fat transplantation. However, further work is needed to understand the role of PRP in improving autologous fat transplantation as many factors can affect the outcome of this process (Luck et al., 2017).

### *3.8.5 Ex vivo expansion of autologous fibroblasts for the treatment of atrophic scars and skin rejuvenation*

Atrophic scars and the effects of skin aging are associated with the loss of collagen. These issues can be temporarily addressed using dermal fillers. However, once the filler is resorbed any benefit is lost and the dermal defect reverts to its original appearance. Weiss et al. (2007) proposed novel collagen production and remodeling of the preexisting extracellular matrix in scarred tissue as a means to improve skin appearance. Fibroblasts are the major producer of collagen in the dermis, therefore there is great interest in using autologous fibroblasts to treat skin defects, such as atrophic acne scars and skin aging.

Although collagen production and the number of fibroblasts are significantly reduced in the elderly, the proliferative potential of fibroblasts remains throughout life, meaning it is possible to obtain autologous fibroblasts for treatment purposes regardless of age (Bayreuther et al., 1992; Fisher et al., 2008). Autologous fibroblasts have been authorized for use in Russia since July 2010, and in the USA since July 2011 for the correction of changes associated with aging skin (Schmidt, 2011; Zorin et al., 2017). In Europe, autologous fibroblast therapy is considered an Advanced Therapy Medicinal Product with a Hospital Exemption (ATMP-HE) product, and thus does not require a centralized marketing authorization from the European Medicines Agency Berndt et al., (2019). However, their use in Europe is regulated under Regulation (EC) No 1394/2007 of the European Parliament and Council of 13 November, 2007 (Yano and Tsuyuki, 2015) and their production should follow the European Commission. EudraLex - Volume 4 - Good Manufacturing Practice (GMP) guidelines. (https://ec.europa.eu/health/documents/eudralex/vol-4_en, last updated 6 July 2020).

Clinical studies have consistently shown a progressive improvement in skin defects when patients are treated with autologous fibroblasts. Statistically significant improvement tends to appear around 6 months after treatment (although improvement may be apparent earlier) and progressive improvement can be seen for as long as 24 months after treatment and in some subjects may last as long as 5 years (Bajouri et al., 2020; Boss et al., 2000; Weiss et al., 2007; Zorin et al., 2016). Areas treated with fibroblasts have a thicker, denser layer of collagen in the dermal region, and show a progressive increase in dermal thickness. These changes manifest themselves as a decrease in the number and depth of wrinkles, improved elasticity, reductions in large rhytid and depressed facial scars, or improved response rate for acne scars and nasolabial folds (Bajouri et al., 2020; Boss et al., 2000; Geldenhuys and Hudson, 2016; Machalinski et al., 2016; Munavalli et al., 2013; Watson et al., 1999; Weiss et al., 2007; Zorin et al., 2017; Zorin et al., 2016). Transplanted fibroblasts have not displayed any mitogenic potential and there is no evidence of any inflammatory reaction, indicating that they are safe to use (Watson et al., 1999; Zeng et al., 2014; Zorin et al., 2016). Patient satisfaction with these treatments is also high, with 92% of patients reporting being pleased with the results, and 70% satisfied with the long-term results (Boss et al., 2000).

Despite the promising results from autologous fibroblast transplantation, some obstacles remain for this treatment approach. There is a time and cost factor involved due to the length of time required to prepare and grow the cells (up to 22 weeks). In addition, a supply chain needs to be established if the clinician doesn't have the facilities to culture the fibroblasts. Because a decrease in the culture time and a cheaper alternative to FBS would be both more economical and reduce risk of infections and inflammation, PRP has been considered as an alternative cell-culture medium supplement to boost the proliferation of fibroblasts and other cells in culture. We and others have demonstrated that PRP is significantly better than FBS at inducing proliferation of fibroblasts (Berndt et al., 2019; Kim et al., 2011). Regen Lab has developed a new medical device, CuteCell (section 7.7.3), to prepare a standardized PRP for in vitro applications. CuteCell-PRP enhances the proliferation of fibroblasts and reduces the time in culture as well as the number of medium changes. Berndt et al., (2019) demonstrated that the proliferation rates of fibroblasts grown in 20% PRP were almost 10 times better than those grown in FBS. This increased proliferation rate was reflected by changes in the cell growth cycle, but no chromosomal rearrangements or genomic instability were detected. This study demonstrated that expansion of autologous fibroblasts in PRP prepared from the patient is more efficient and with reduced risks compared to expansion in FBS-supplemented medium (Berndt et al., 2019).

### *3.8.6 Finasteride and Minoxidil for alopecia*

While many pharmacological treatments have been described for male AGA (MAGA), only two commercially available therapies are currently Food and Drug Administration (FDA)-approved: the 5α-reductase inhibitor finasteride, administered orally, and the potassium channel opener minoxidil, administered topically. Finasteride is an inhibitor of 5α-Reductase, an enzyme that converts testosterone into dihydrotestosterone (DHT), which is responsible for the hair follicle miniaturization in MAGA. The efficacy of finasteride has been well established and is supported by several systematic reviews and meta-analyses (Adil and Godwin, 2017; Kelly et al., 2016; Lolli et al., 2017). It was shown to decrease serum, prostate, and scalp DHT by 60-70%, leading to a noticeable improvement in about 30% of the patients (Kelly et al., 2016; Lolli et al., 2017). Consistently, interruption of the treatment was followed with progression of baldness due to DHT levels rising back to normal levels, emphasizing the importance of long-term, if not life-long, observance (Kelly et al., 2016; Lolli et al., 2017). Owing to the controversial involvement of androgens in female AGA (FAGA), finasteride is only used off-label due to poor clinical evidence and important safety issues.

The exact mechanism of action of minoxidil on hair growth is still unclear but probably involves potassium channel opening, which leads to an increased cutaneous blood flow and enhanced levels of VEGF and hair growth promoters in DPCs (Kelly et al., 2016). The efficacy of topical minoxidil to treat MAGA as well as FAGA has been established by several double-blind, randomized, and placebo-controlled trials. Moreover, recent meta-analysis studies have confirmed the high quality of evidence for use of minoxidil to treat AGA in both sexes (Kelly et al., 2016). In MAGA, the superiority of the 5% minoxidil solution over the 2% solution was established in randomized controlled clinical trials, while both dosages had similar efficacy against FAGA (Kelly et al., 2016).

The rationale for combining these treatments with PRP for the treatment of alopecia is that many patients may already be using these treatments and may be reluctant to stop their use due to the risk of progression of the disorder upon cessation. In addition, these treatments differ in their mode of action from that of PRP, thus a combination may be more effective than either treatment alone. However, there is limited published data available on the combination of PRP with minoxidil or finasteride for the treatment of alopecia. A recent randomized, controlled clinical study comparing PRP alone, topical minoxidil alone, PRP with topical minoxidil, and normal saline found that PRP alone was more effective than minoxidil alone for the treatment of male pattern baldness, and that a combination of minoxidil and PRP may prolong the effect of PRP on hair growth (Singh et al., 2020). These findings support other observations that PRP could be used in combination with other treatment modalities to improve hair density (Ho et al., 2020; Juhasz et al., 2020).

As PRP alone has been demonstrated to be effective and safe and does not interfere with pharmacological treatments, but may not have the capacity to address all the issues associated with AGA, Ferrando et al., (2017) strongly recommended the use of PRP as

a coadjuvant treatment in patients with AGA who are no longer responding to the standard pharmacological treatments. Other authors have also supported the use of PRP in combination with other therapies for the treatment of alopecia (Anitua et al., 2019).

### *3.9 DISCUSSION*

PRP is an interesting therapeutic approach for the treatment of skin disorders. The rationale for its use for the treatment of skin disorders is manifold:

- Stimulation of cells involved in maintenance of skin structure and function such as fibroblasts and stem cells
- Regeneration of the ECM through induction of neocollagenesis and neovascularization
- Provision of growth factors and other biomolecules that may be needed to restore or circumvent perturbed cell signaling pathways
- PRP contains molecules that have immunomodulatory and analgesic properties

As an autologous product, PRP is safe to use and it also been demonstrated to be safe to use in conjunction with other treatment modalities for skin disorders where it may even enhance their benefit. The combination of PRP and hyaluronic acid, which can be easily and safely prepared using the Cellular Matrix device, may be of particular interest for skin rejuvenation, the treatment of acne scars and some of the symptoms associated with genitourinary disorders. PRP may also be considered when patients are no longer responding to or are unable to follow established treatment regimens.