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STEM CELLS IN
DERMATOLOGY
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STEM CELLS IN DERMATOLOGY AND SKIN ANTI-AGING

Amy Forman Taub and Kim Pham highlight the use of defensins as a new therapy for skin rejuvenation



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ABSTRACT

Multipotent stem cells have paved the way for new applications and deeper understanding in the field of regenerative medicine and the pathophysiology of aging. During skin aging, cumulative photodamage, exhaustion of endogenous stem cell populations, mechanical stress, and increased fibrosis leads to skin with decreased epidermal thickness and compromised dermal integrity. Various studies characterizing dermatological stem cell populations

and their coordination with one another during wound healing have uncovered how these components can act as a skin aging therapy. Located in the hair follicle isthmus, Lgr6+ stem cells act *in utero* to create all aspects of the epidermis. During wounding, Lgr6+ cells create new keratinocytes and new epidermal basal stem cells that provide long-lasting contributions to the skin post-wounding. Activation of these cells during wounding are mediated by defensin peptides, which normally function in the

skin epithelium's innate immunity. Unlike prior skin care regimens and treatments that activate and stimulate aged cells of the skin, defensins mobilize normally quiescent and relatively undamaged stem cells. Thus, the targeted specificity of defensins to Lgr6+ stem cells may be exploited to provide a more effective approach to skin aging therapy. Studies pertaining to defensin's efficacy on skin aging have been conducted, highlighting its potential as a new therapy for skin rejuvenation.

SINCE THE DISCOVERY OF MULTIPOTENT stem cells by Till and McCulloch in 1961^{1,2}, further elucidation of stem cells' functions have been identified as both facilitating development of new cells and maintaining homeostasis of current normal cells. The activity of stem cells is stimulated by the start of tissue dysfunction. During aging, DNA accumulates damage, impairing protein homeostasis, cell function, and communication as well as normal organ physiology³. Another key hallmark to aging is the exhaustion of the endogenous stem cell population, which aids in maintaining tissue homeostasis and repair of injured tissues. Since aging is so intimately tied to stem cell integrity, one of the major goals of stem cell biology and regenerative medicine is how one can use these cells to reverse aging and the associated dysfunctions that comes with it.

Stem cells are undifferentiated or partially differentiated cells that are capable of dividing and generating differentiated and proliferative cells (Figure 1). Stem cells range from pluripotent cells that are found in the inner cell mass of pre-implantation blastocysts or isolated from other sources to unipotent progenitors such as fetal tissues, birth-associated tissues, or adult tissues. Several advances have been made to apply the unique traits of this variety of stem cells types. These include establishment of embryonic stem cell lines (ESC) via *in vitro* fertilization, the reprogramming of differentiated adult cells to induced pluripotent stem cells (iPSC), and the generation of cloning stem cells (somatic nuclear transfer stem cells, SNTSC). Other strategies include the creation of parthenogenetic stem cells (hpSC), isolation of stem cells from fetal tissues (including neural stem cells or retinal progenitor cells), and separation of birth-associated stem cell populations

KEYWORDS

Stem cells, anti-aging skin care, pore reduction, epidermal thickness, melanin, wrinkles, defensin, growth factors, peptides, plant stem cells, conditioned medium

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including cord blood stem cells or placental stem cells. Although these different modes of pluripotent and fetal stem cells provide great potential for treating aging and age-related diseases, there are several associated disadvantages. Pluripotent and fetal stem cells may be tumorigenic⁴, possess genetic instability, and are often tied to ethical and regulatory debate⁵. Even though iPSCs bypass the ethical issues of embryonic stem cells, they still possess the same mutations and damage that the donor cells had, which can decrease its ability to proliferate and respond to its respective niche⁶. Stem cells isolated from birth-associated tissues have limited ability to proliferate, with directions of differentiation and therapeutic potential resulting in limited areas of applications. An alternative method that is being explored is the use of pharmaceuticals to modulate endogenous stem cell populations to leverage their respective mechanism of cell-signaling and communication.

Dermatologic epidermal stem cells

There has been great interest in understanding the regulation and coordination of the stem cells found within the skin in order to repair aged skin (Figure 2). Through wound healing and genetic knock-out experiments, several stem cell populations have been elucidated in the skin that have applications to regenerative medicine²⁸. Within the epidermis lay basal epidermal stem cells that proliferate and maintain epidermal turnover and homeostasis^{29,30}. Other stem cells that are involved in transient repair of skin wounds (although they do not contribute skin's homeostasis on a daily basis) are hair follicle stem cells. These follicular based stem cells include Lrig1+ stem cells, (residing in the junctional zone of the hair follicle and contributing to the infundibulum), Gli1+ stem cells, (maintaining sebaceous glands), and Lgr6+ stem cells (acting as skin's master stem cells)³¹. The Lgr6+ cells are termed 'master' cells as they >



Figure 1 Types of human stem cells

PLURIPOTENT stem cells

- Embryonic stems cells (ESC)
- Induced pluripotent stem cells (iPS)
- Cloning stem cells (CSS)
- Other

Applications

- Injectable drugs

Product development

- Regulatory issues (All)
- Ethical issues (ESC, CSS)
- Genetic instability issues (ESC, iPS, CSS)
- Infection risk (All)
- Tumorigenic issues (ESC, iPS, CSS)
- 5-10 years from lab research to clinic

FETAL & BIRTH-associated stem cells

- Neural stem cells
- Placental stem cells
- Umbilical cord stem cells
- Other

ADULT stem cells

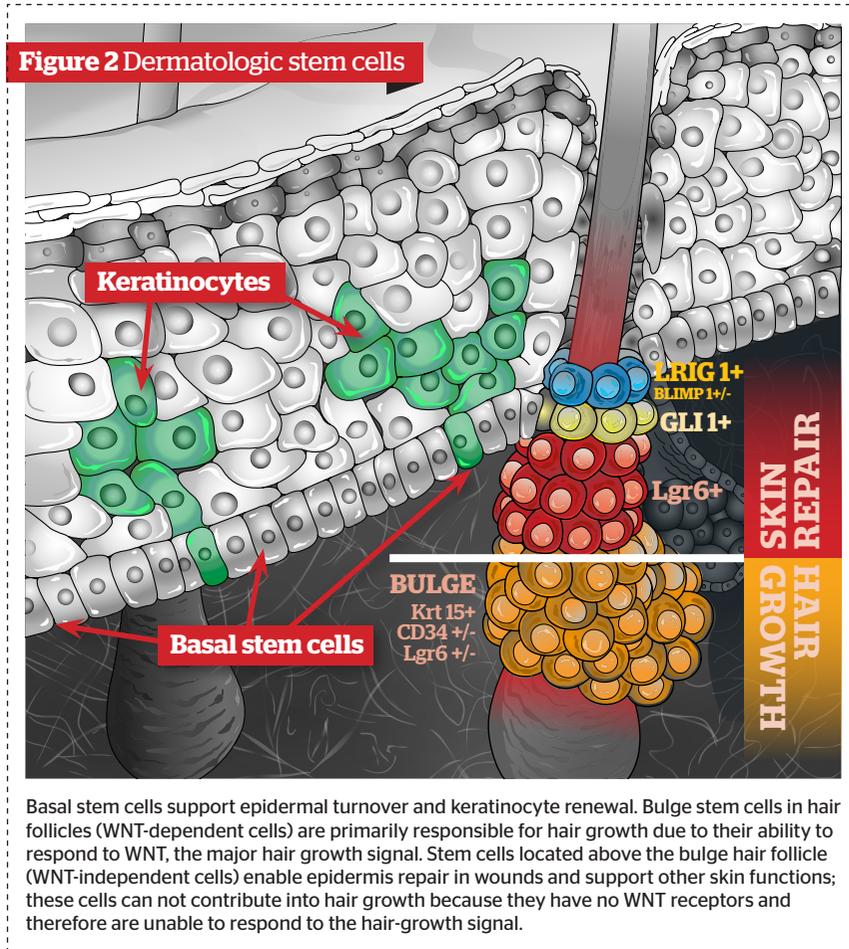
- Basal stem cells
- Hair-follicle stem cells
- Mesenchymal stem cells (MSC)
- Other

Applications

- Injectable drugs
- Targets for stimulation

Product development

- Minimal or no risks
- Quick development

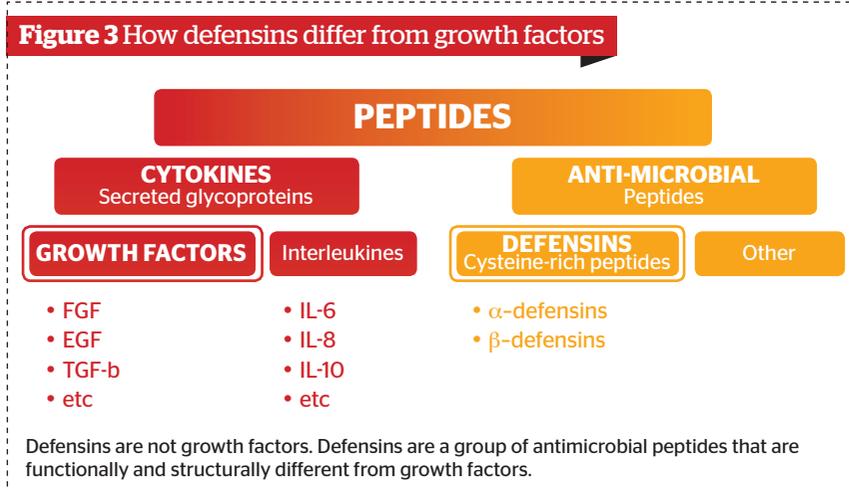


Basal stem cells support epidermal turnover and keratinocyte renewal. Bulge stem cells in hair follicles (WNT-dependent cells) are primarily responsible for hair growth due to their ability to respond to WNT, the major hair growth signal. Stem cells located above the bulge hair follicle (WNT-independent cells) enable epidermis repair in wounds and support other skin functions; these cells can not contribute into hair growth because they have no WNT receptors and therefore are unable to respond to the hair-growth signal.

▷ are the ones that create the entire epidermis and appendages early *in utero*¹⁸. Within the stratum basale are basal cells that act as the stem cells for epidermal homeostasis.

Lgr6+ stem cells

As mentioned earlier, Lgr6+ cells are multipotent cells found within the hair follicle above the bulge that actively cycle to contribute to the epidermis and sebaceous



gland¹⁹. When investigating the development of these cells, Snippert et al. observed that Lgr6+ is first expressed embryonically in the early placode (embryonic structures that give rise to structures such as hair follicles and teeth) and remains expressed during hair development¹⁹. Thus, Lgr6+ cells are considered primitive epidermal stem cells by establishing the epithelial placode, confirming their multipotency in adult skin.

Lgr6+ cells involvement in wound healing, as well as its multipotency, are two key interests in the study of skin aging and regeneration. It is known that Lgr6+ cells can migrate into the wound center to aid in reepithelialization¹⁹.

“ Though not comprehensive, some of the major sources that lead to skin aging include UV damage, environmental insults, inflammation, and an increase in reactive oxidative species in comparison to antioxidant load. ”

Activation, migration, and eventual proliferation of these cells are triggered by cytokines that are secreted by neutrophils for pathogen defense¹⁹.

Defensins

Defensins are a group of antimicrobial peptides that are functionally and structurally different from growth factors (Figure 3). Defensins are peptides secreted by the skin epithelium and are of importance to Lgr6+ mediated skin healing. This peptide comes from a family shared with α-defensins that serves

multiple functions: β-defensins provide innate immunity by deterring microbial colonization on the skin surface¹⁹, and induce wound healing by recruiting Lgr6+ stem cells to create new basal stem cells in the wound and thus stimulate the creation of new keratinocytes in the wound bed¹⁹ (Figure 4).

One application proposed and studied for this peptide is the use of intestinal α-defensins on the skin to stimulate Lgr6+ stem cells. Lough et al., found that healing was enhanced in murine skin wounds upon induction of α-defensin 5 as observed by rapid wound closure and hair follicle neogenesis within the wound bed. This enhancement of healing was mediated by the recruitment of Lgr6+ cells. Due to these results, use of α-defensins would be particularly useful in large-scale wounds or burns where the local stem cell niche is removed, and β-defensins are no longer present on the skin surface to induce wound healing¹⁹. Additionally, since Lgr6+ is involved in new keratinocyte production, α-defensins could also have applications in reversing skin aging¹⁹.

Aging and applications

Aging is considered the decline or deterioration of physiological functions often attributed to accumulated alterations in the genome, decreased telomere length, protein and cellular damage, increased inflammation

and cell senescence, exhaustion of endogenous stem cell populations, and issues with intercellular communication³.

Though not comprehensive, some of the major sources that lead to skin aging include UV damage, environmental insults, inflammation, and an increase in reactive oxidative species in comparison to antioxidant load^{15,16}. Overall, the damage created by these different sources leads to the deterioration and damage of epidermal tissue and loss of thickness as well as the loss of collagen and elastin in the dermis¹⁷. Though they may appear as distinct events, aging and wound healing have commonalities due to similar genetic and cellular pathways which compensate and replenish. During the initial phase of wound healing, inflammation arises via reactive oxygen species¹⁷. In the same manner, skin aging is often associated with the increase in the presence of reactive oxidative species¹⁷.

The insights from wounding studies demonstrate the gaps observed in adult skin healing and provide mechanisms to recapitulate the same processes seen in fetal skin regeneration, which appears to be due to a different pathway and results in scarless healing. Elucidations of these different mechanisms have potential applications in the reversal and delay in skin aging. One treatment that has been proposed is the use of mesenchymal stem cells in the placenta or umbilical cord¹⁸. Advantages of the use of these extra-embryonic cells include their similarity to embryonic stem cells, multipotency, and higher efficacy in regeneration when compared to adult-derived mesenchymal stem cells. Despite these benefits, there are issues with controlling the differentiation plan of these cells, and little information is known about how mesenchymal cells participate in fetal wound healing¹⁹.

Another growing field in terms of skin therapies is the use of growth factors to induce keratinocyte and collagen proliferation. Growth factors are regulatory peptides that participate in cell to cell signaling as well as intracellular signaling, such as chemotaxis, division, and differentiation²⁰. These proteins can be produced by fibroblasts, platelets, keratinocytes, and immuno-modulatory cells. In comparison to other peptides that aid in intercellular signaling, these proteins are defined by possessing a targeted

response. This is beneficial during post-skin wounding where these growth factors can diffuse into the wound bed and aid in repair by inducing collagen proliferation, promoting angiogenesis, stimulating cell migration and division, and reducing local inflammation²¹.

The understanding of growth factors in aging skin was elucidated through the studies of skin wound healing²². Here, growth factors were found to act in repair by mediating in the inflammatory, granulation, and remodeling stages seen after wounding. In this case, multiple growth factors like VEGF, TGF- β , and IL-8 coordinate to resolve the wound²². One of the main goals seen during this event is for growth factors to re-establish the extracellular matrix, and ensure collagen and elastin production is made²³. With that in mind, the function and mechanism of growth factors in wounds can be translated in its therapeutic use to skin aging where growth factor count is diminished, and the aged skin possesses a reduced collagen network. Specifically, growth factors can decelerate aging by stimulating keratinocytes to produce more growth factors that can promote collagen synthesis as well as keratinocyte division¹⁷.

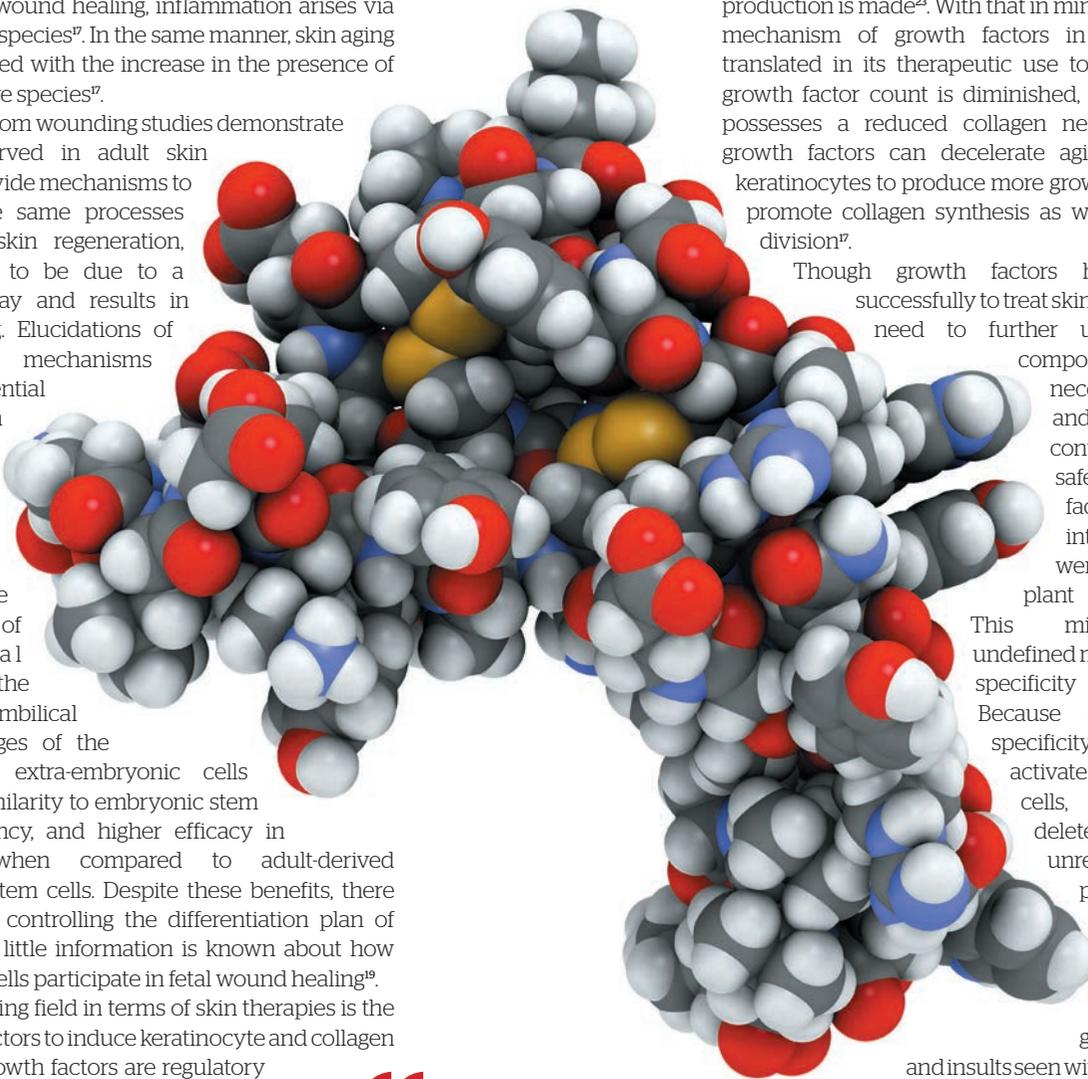
Though growth factors have been used successfully to treat skin aging, there is still a need to further understand which

components are necessary for efficacy and to clarify some controversies over safety. Initial growth factors introduced into cosmeceuticals were derived from plant stem cell sources.

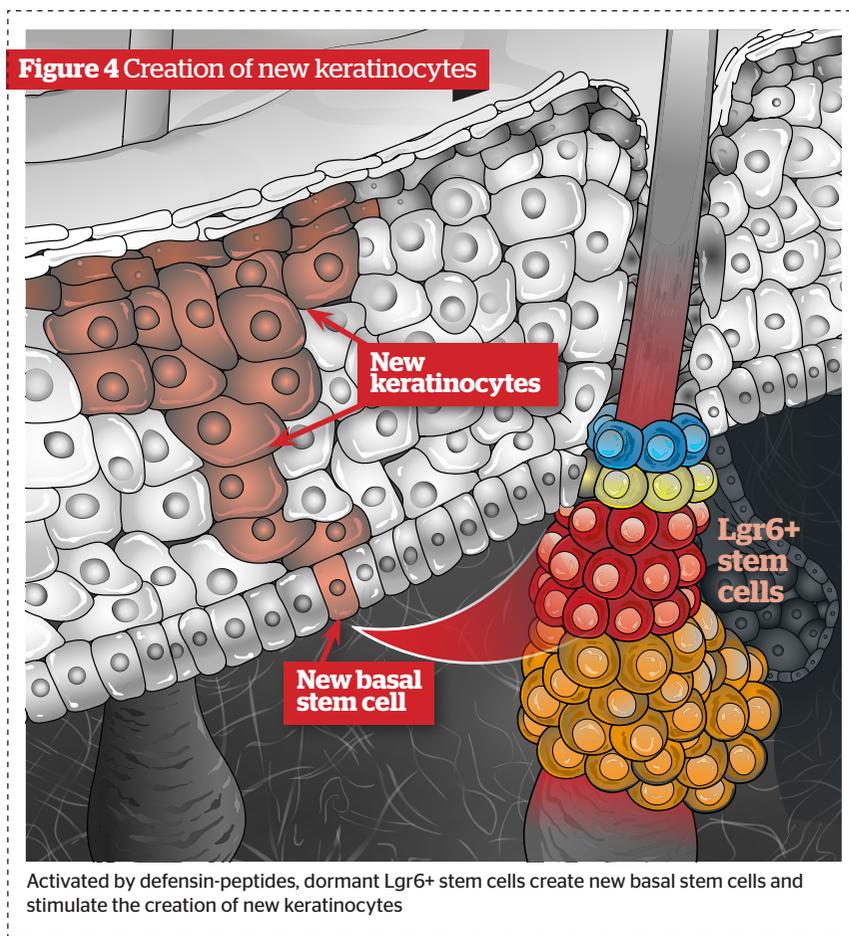
This mixture contains undefined molecules with non-specificity and low efficacy. Because of the lack of specificity, this treatment can activate a wide array of cells, which could be deleterious if unregulated. Moreover, plant stem cells act on the host's old basal stem cells that may still possess the genetic alterations

and insults seen with the accumulative effects of internal aging and photoaging. Since

then, other applications of growth factor therapy have been created, such as the use of conditioned medium growth factors. Here, there is more efficacy on age reversal or deceleration compared to plant stem cells, but like its predecessor, this strategy contains undefined growth factors that are non-specific and only target aged cells of the skin²⁴. Because of these drawbacks, more well-defined growth factors were the next step in skin aging therapy. >



“Growth factors are regulatory peptides that participate in cell to cell signaling as well as intracellular signaling, such as chemotaxis, division, and differentiation.”



▷ In comparison to the preceding two treatments, there is a defined growth factor that is given for treatment leading to greater control of application and results. However, there is still non-specificity involved with using this approach, and again, these growth factors only activate on aged skin cells (Table 1).

Though these three treatments have been considered for their role in decelerating skin aging, there are still disadvantages involved with their use. The most popular products are derived from the supernatant of cell cultures or the cytoplasmic contents of fetal epithelial cells. These products contain a great many biologically active substances, and it is not known which contribute to the desired effect or even an undesired effect, such as tumorigenesis (Figure 5A). Another drawback is the lack of standardization seen in what growth factors and proteins are being made and applied to⁷.

Applications of Lgr6+ stem cells and defensins

A new approach to aid in skin aging could be the use of defensins to activate Lgr6+ stem cells (Table 1). Unlike past treatments, defensins would only target Lgr6+ cells, as opposed to many potential targets that may be helpful but also may be deleterious or even tumorigenic in skin tissue (Figure 5B); the authors were not able to find any

“A new approach to aid in skin aging could be the use of defensins to activate Lgr6+ stem cells.”

publications with respect of involvement of defensins in cancer-related pathways. Moreover, some tissues respond to tumor growth by enhanced expression of defensins as a natural protective immune response²⁸. Studies also show the ability of defensins to suppress tumor growth both *in vitro* and *in vivo*^{26,29}. In addition, Lgr6+ cells

are quiescent compared to basal stem cells and reside in the isthmus, which is not as directly exposed to UV radiation. Therefore, Lgr6+ cells would have accumulated fewer mutations and damage than basal stem cells. Thus, by activating these cells, there would be differentiation and proliferation of less damaged keratinocytes.

In a six-week pilot study, it was observed that there was a global improvement in wrinkle reduction and decreased skin oil production in the 22 subjects that used synthetic α -defensin 5 and β -defensin 3 based skin care regimen³⁰. To affirm these findings, a placebo-controlled, double-blind study across multiple medical centers was carried out with 45 subjects for 12 weeks. The results of this study followed those from the pilot, suggesting some potential for the use of defensins as a skin therapy³¹. Though further investigation must be undertaken to fully understand the mechanisms behind defensins and skin repair, this therapy provides a new avenue for a more targeted treatment in skin aging.

Conclusion

Currently, different skin therapies are emerging to treat

WHAT IS THIS?	PLANT STEM CELLS	GROWTH FACTORS FROM CONDITIONED MEDIUM	GROWTH FACTORS (DEFINED)	TARGET-SPECIFIC STEM CELL ACTIVATORS
FORMULATION	UNDEFINED: mixture of active molecules.	UNDEFINED: mixture of growth factors - everything that cells release during <i>in vitro</i> culture.	DEFINED: pre-defined Composition of growth factors.	DEFINED: pre-defined composition of natural peptides.
TYPE OF ACTIVATION	NON-SPECIFIC: 'switch-on of everything'-mechanism. Low efficacy.	NON-SPECIFIC: 'switch-on of everything'-mechanism. Contains TGF-beta, potent cancer trigger.	NON-SPECIFIC: 'switch-on of everything'-mechanism.	HIGHLY-SPECIFIC: peptide (defensins) activate specific stem cells (Lgr6+ stem cells).
WHAT IT DOES	FORCES 'OLD' and 'exhausted' skin cells work even harder than before.	FORCES 'OLD'™ and 'exhausted' skin cells work even harder than before.	FORCES 'OLD' and 'exhausted' skin cells work even harder than before.	CREATES 'NEW' and 'fresh' skin cells using resource of our own body.

and reverse the signs of aging. One approach is the utilization of growth factors to activate cell populations in the skin⁷. Initially starting with plant stem cells, to conditioned medium growth factors, and finally to defined growth factors, there is increasing specificity in the growth factors being applied, but there are several disadvantages to these three treatments. First is the lack of specificity to target cells, such that these stem cells and growth factors can activate cells that are not usually involved in skin rejuvenation and be deleterious or tumorigenic. Additionally, there are concerns about the efficacy and safety of these treatments as the composition of growth factors are not fully defined, and there is a scarcity of clinical research to affirm how effective these treatments are. Another aspect to their disadvantage is that all three activate aged basal stem cells that have accumulated photo-damage, genetic mutations, and epigenetic alterations. By activating these cells, the differentiated keratinocytes will still possess these damages, thus not decelerating aging at an optimal rate.

Nevertheless, new findings demonstrate that particular stem cell populations in the hair follicle can facilitate wound healing by creating long-term keratinocyte progenitors as well as appendages like the hair follicle and sebaceous gland. One population of note is Lgr6+ stem cell located in the hair follicle isthmus. This multipotent stem cells act as skin's master stem cells and in cases where there is wounding or other insults, these cells can proliferate and reprogram to epidermal fates and create new basal stem cells and, eventually, new keratinocytes⁸. In order for Lgr6+ cells to migrate into the wound bed, defensins must be present to target and activate these cells. β -defensin peptides are produced by the skin in cases where innate immunity is needed. Not only does it have immunomodulatory qualities but it can specifically act on Lgr6+ cells for migration and proliferation onto the wound bed⁹.

Using this mechanism further applications can be put to use in terms of skin aging therapy. Synthetic β -defensin 3 or α -defensin 5 have some advantages over previous growth factors treatments⁹. Each application will have a known composition as only defensins, and a vehicle is necessary. Since defensins specifically target Lgr6+ cells, there will not be issues of inappropriate

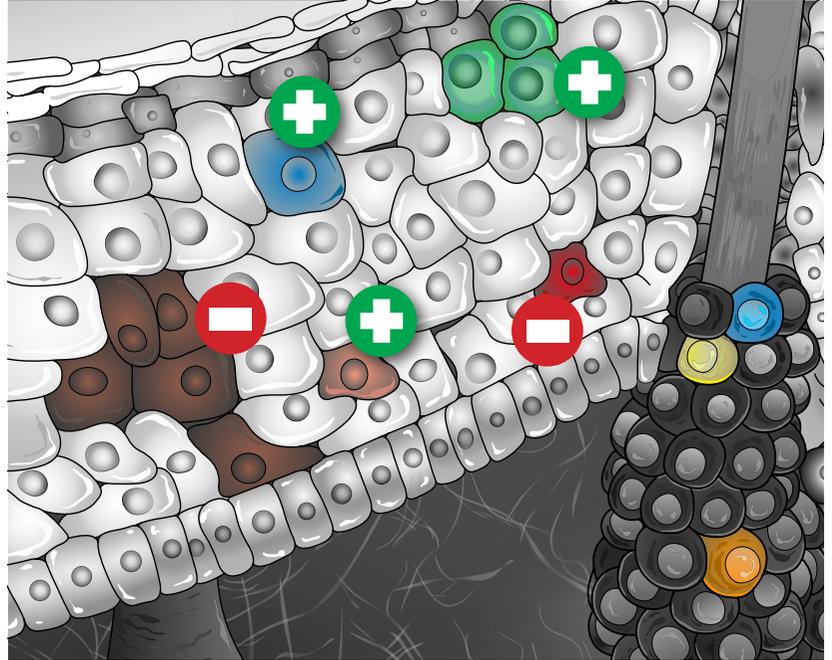
“The utilization of growth factors to activate cell populations raises concerns about the efficacy and safety of skin therapy treatments as the composition of growth factors are not fully defined.”

activation of other cell types. This approach would also activate a stem cell population that can produce basal stem cells and keratinocytes with less genetic damage and more signaling responsiveness in comparison to the keratinocytes that were derived from aged basal cells. Pilot studies have demonstrated that a composition of >

Figure 5 Non-specific vs specific targeting

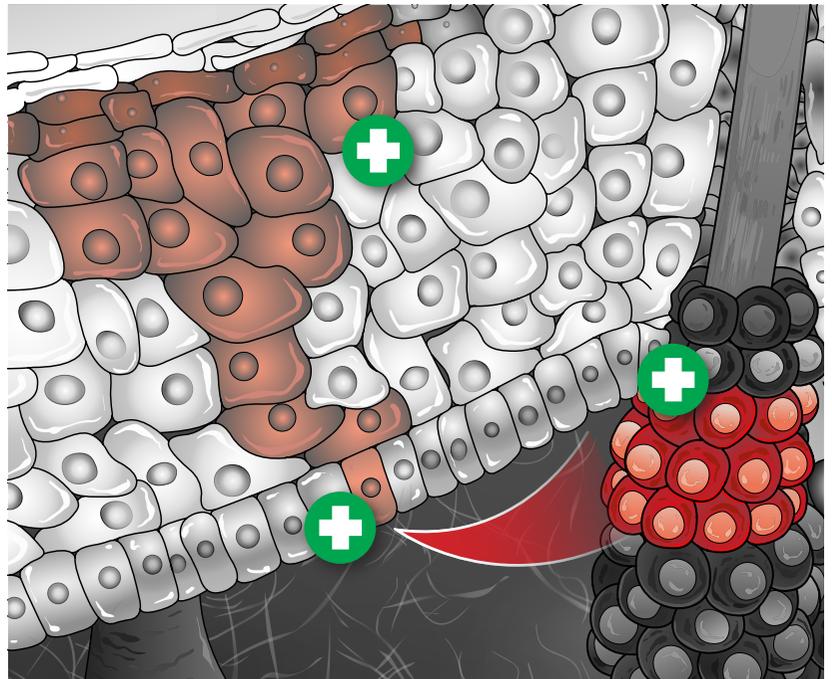
A) GROWTH FACTORS

Non-specific activation, 'switch on of everything' -mechanism

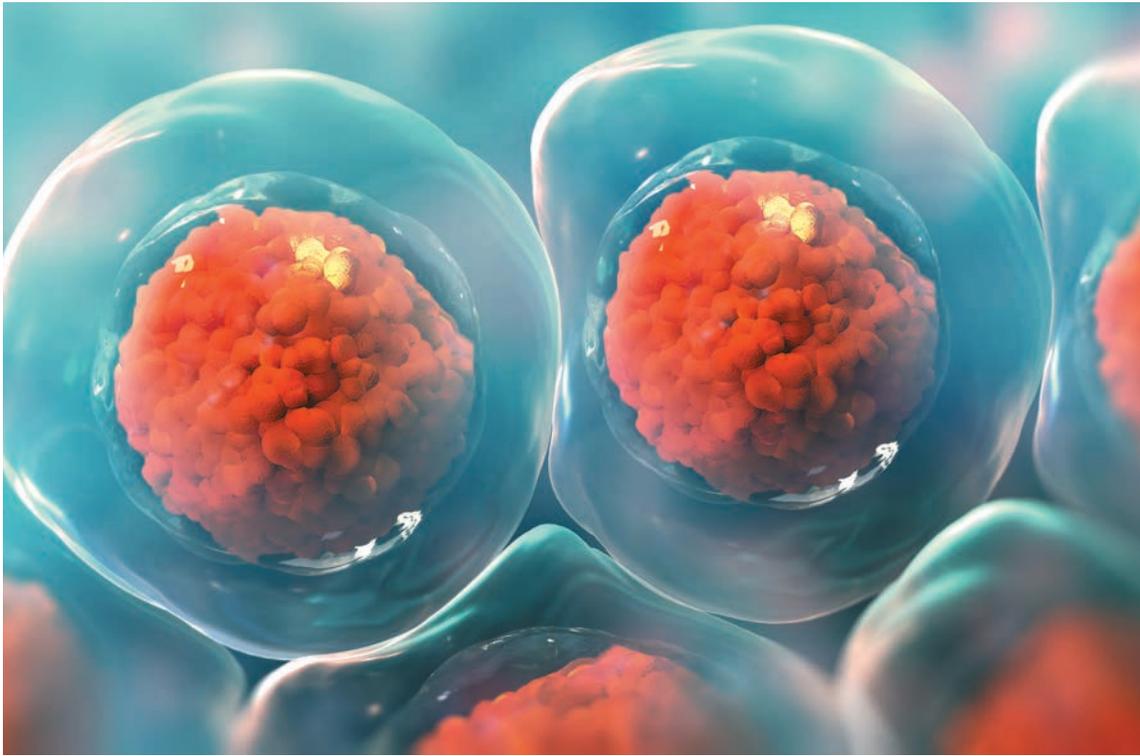


B) DEFENSINS

Activation of specific cell type to do a specific job



(A) Due to the variety of functions, growth factors have an ability to activate and stimulate the different cells in skin including potentially 'unstable' tumorigenic cells, therefore the activation by growth factors is non-specific and is based on an 'switch-on of everything'-mechanism. (B) Defensins activate only one specific cell type in skin, Lgr6+ stem cells, thus representing a target-specific activation



Key points

- 1 Stem cells are undifferentiated or partially differentiated cells that are capable of dividing and generating differentiated and proliferative cells
- 2 Within the epidermis there are stem cells that proliferate and maintain epidermal turnover and homeostasis
- 3 Within hair follicles, there are specific stem cells known as Lgr6+ cells, that lay dormant unless there is trauma or other insult to the skin
- 4 Lgr6+ stem cells are stimulated to proliferate when turned on by a messenger peptide known as a defensin, usually released by neutrophils
- 5 Defensin based skin care has shown in double blind placebo controlled studies to be very effective for thin skin, hyperpigmentation, enlarged pores and wrinkles

▷ defensins, topically applied on intact skin, dramatically improve the overall quality of epidermis and comprehensively address the visible signs of aging skin. The observing effect may be caused by defensin-activated repopulation of epidermis with new and 'healthy' basal cells following the increase of epidermal mass. Normalized/refreshed epidermis may enhance the performance of dermis renewal and function.

speaking. She also has a small equity in the company. She was not paid to produce this paper. Kim Pham was paid an honorarium to assist in this publication.

► **Figures 1-5** Data © Dr Taub; **2, 4 & 5** illustrations © David 'Spike' McCormack

► **Table 1** Data © Dr Taub

► **Declaration of interest** Dr. Taub has been paid by Medicell Technologies for research conducted as well as honoraria for

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