Biotechnology for advanced skin therapy

Lonetá L. Lima[1]; Gislaine Ricci Leonardi[2]; Valéria de Souza Santos Holsback[2]; Ediléia Bagatin[3]; Natalia Cammarosano Mestnik[3]; Alice Teixeira Leite[3]; Rodrigo Alvarenga Rezende[1,4]; Jorge Vicente Lopes da Silva[1] lonetalima@gmail.com

[1]3D Technologies Research Group (NT3D), Renato Archer Center for Information Technology -CTI, Campinas, SP, Brazil

[2] Faculty of Pharmaceutical Sciences, University of Campinas – Unicamp, Campinas, SP, Brazil

[3]Department of Dermatology, Federal University of São Paulo-Unifesp, São Paulo, SP, Brazil

[4]Postgraduate Program in Biotechnology - Uniara, Araraquara, SP, Brazil

Abstract

Biotechnology uses cellular and biomolecular processes to develop technologies and skin products to improve patient life and health. Advanced skin therapy has benefited from the development of biotechnological ingredients, and biotechnological tools allow for the treatment of complex and recalcitrant skin disorders like genetic and inflammatory. Employing biotechnology in the development of advanced skin products can result in a more effective and less aggressive product, like biopharmaceuticals. This therapeutic strategy use delivery of cell, exosome, growth factor, gene, and nucleic acids, showing effects at multiple levels, among them, guiding cell migration and differentiation, modulating immune function, and stimulating skin repair and angiogenesis. Virtually, the biopharmaceutical administration is to guide the pathways for skin regeneration or to treat skin disorders. The topical application of biopharmaceuticals is patient compliance because it is a non-invasive route, and if the biopharmaceutical remains within the skin layers the effect may be non-systemic. However, the presence of the stratum corneum restricts the type and dose of biopharmaceuticals that can be administered. And it is noticed that biopharmaceutical application is restricted due to chemical and physical instabilities, enzymatic degradation, and short in vivo half-life. In this context, the use of microneedles conjugated with biopharmaceutics can promote biopharmaceutical stability, lead to appropriate pharmacokinetics, and target spatiotemporal release.

Keywords: Microneedle; Nanotechnology; Skin Bioengineering.

1. Introduction

This minireview presents the employment of biotechnology for advanced skin therapy. The methodology for the choice of the articles was the clinical relevance of the work. It begins with a short introduction to biotechnology and microneedle as enabling platforms for biopharmaceutical delivery. The second section summarizes the clinical application of skin regeneration and sequentially shows skin gene therapy.

Biotechnology and advanced skin therapy are closely linked through the production of high-quality active ingredients, which are more effective and safer.

In ancient, a primitive form of biotechnology was developed by farmers who established species of plants and animals of better quality by methods of cross-pollination or crossbreeding. The selective breeding of animals; the cultivation of crops; and the use of microorganisms to produce products such as cheese, yogurt, bread, beer, and wine are considered primitive forms of biotechnology(Gomes et al., 2020).

Nowadays, biotechnology is focused on the development of hybrid genes, followed by their transfer to different organisms. This field has important applications in health through the production of biopharmaceuticals(Gomes et al., 2020).

The classic definition of biopharmaceutical refers to medicinal products, among them therapeutics, prophylactics, and *in vivo* diagnostics, that are formulated with active ingredients inherently biological in nature and manufactured using biotechnology methods (Rader, 2008).

In this context, biopharmaceuticals used in advanced skin therapy are manufactured using living organisms or cells employing biotechnology fundamentals, involving genetic engineering, and bioprocessing.

Therefore, the biotechnology field connects many disciplines, such as pharmacy, medicine, biology, chemistry, and engineering, among others. The applications of biotechnology are extensive, and all industries can use their tools. Allowing the industries to produce new or better products faster and more efficiently(Gomes et al., 2020).

Biotechnological advances provided a better understanding of the relationship between genetics and biological function, elucidated the causes of certain skin disorders, and explored the association between genomic variation and drug response. Their advances ally with pharmacogenetics leading to personalized medicine, which seeks to find the right drug for the patient at the right dose(Foot et al., 2010; Gomes et al., 2020).

Modern biotechnology can supply the tools to treat rare and disabling skin disorders. For instance, an improved understanding of the genetics of Epidermolysis bullosa (EB) and advances in biotechnology have led to progress in the development of gene and cell-based regenerative therapies for EB(Subramaniam et al., 2022).

Especially in the treatment of severe, complex, and highly heterogenic diseases like cancer, autoimmune diseases, inflammatory, or infections, biopharmaceuticals are more effective than small molecule drugs due to their high specificity, potency, targeting ability, and reduced side effects(Witting et al., 2015; Zeb et al., 2020).

Biotechnology became an expanding field, there are many clinical trials employing products obtained by biotechnological processes, including therapeutic proteins, vaccines, and monoclonal antibodies for advanced skin therapy.

However, the delivery of biopharmaceuticals has been a challenge due to their specific physicochemical properties, short *in vivo* half-life, and instabilities.

Biopharmaceuticals, which consist of vaccines, whole microorganisms, cell, gene, cytokines, growth factors, monoclonal antibodies, and cultured skin has inherent diversity, randomness, and complexity due to their biological nature and biotechnological manufacture process(Rader, 2008; Sachdeva et al., 2016).

Since most biopharmaceuticals are hydrophilic macromolecules, they do not possess characteristics suitable for skin delivery(Zeb et al., 2020).

Biopharmaceuticals exhibit a complex structure with a high molecular weight (between 300 and 1,000,000 Da). Furthermore, biopharmaceuticals are labile compounds and susceptible to stability problems like aggregation and denaturation that can occur during manufacturing, storage, and administration which reduces their therapeutic activity. Repeated injections of high doses are inevitable to maintain therapeutic levels. That affects patient compliance and provokes adverse effects such as toxicity and immunogenicity(Witting et al., 2015).

Dissolvable and biodegradable microneedles (MNs) can adjust the properties and bioactivity of the encapsulated biopharmaceutical for improved pharmacokinetic and biodistribution profiles, reduced toxicity, controlled release, enhanced solubility and stability, and targeted delivery. Moreover, dissolvable and biodegradable MNs can be made to present a wide range of physicochemical and bioactivity attributes by altering their formulation, shape, size, and surface properties. 3D printing could be a robust, reliable, and scalable manufacturing alternative to produce both conventional and personalized medicine MNs. In this regard, there is a study showing the benefits of 3D printing for MN manufacturing for skin therapy(Lima et al.)

Employing MNs for the delivery of biopharmaceuticals results in practical benefits such as protecting from degradation in a hostile physiological environment, enhancing half-life and retention time, facilitating pass through the stratum corneum, providing site-specific delivery, and improving access to intracellular targets.

The concepts involved in the use of MN associated with biopharmaceuticals for advanced skin therapy are related to biostimulation for guide skin regeneration; microchannel creation for best perfuse topically the biopharmaceutical; microsurgery by target cell delivery; and microencapsulation for biopharmaceutical delivery.

Clinically, biopharmaceuticals have been studied for many skin treatments, among them skin regeneration, and skin genetic disorders. Therefore, follow will be emphasized these applications.

2. Skin regeneration

Biotechnology has impacted skin regeneration in several ways. Highlighting, the use of biotechnology methods to discover, develop, and produce ingredients for skin formulations and to evaluate the activity of these ingredients on the skin, for instance, allowed for improvement the skin regeneration treatments.

An important biotechnological ingredient in this way is the growth factor. Growth factors may act on specific cell surface receptors that subsequently transmit these cell signals to other intracellular components. The ability of growth factors to promote growth, differentiation, and cell division has attracted the attention of the pharmaceutical and cosmetic industry (Gomes et al., 2020).

The use of the growth factor for skin regeneration is an emerging and promising strategy. Advances in knowledge of the function of growth factors in wound healing and skin regeneration have aroused great interest in determining the growth factor's responsibility in the repair of skin structures.

It is observed that the endogenous functionalities of the growth factors decrease because of the reduction of skin cells during skin death, therefore, exogenous supplementation of growth factors can promote the repair of aging skin and regenerate it. A combination of growth factors together with antioxidants, matrix building agents, and skin conditioning agents can be effective in treating skin anti-aging(Gomes et al., 2020).

Growth factors can be applied topically or injected. Clinical trials have shown that topical application of animal growth factors, or the injection of autologous growth factors, can also increase collagen synthesis in the dermis. The purpose of administering topical or injectable growth factors is to increase the activity of the cells responsible for the remodeling of the dermis, and to delay or reverse the aging of the skin. For instance, the human epidermal growth factor (hEGF) can speed up the healing process and is also found to be effective in the treatments of wrinkles, age spots, and freckles(Gomes et al., 2020).

A study using the growth factors in conjunction with microneedling concluded that microneedling improved skin in many characteristics, and the conjugation between growth factors and microneedling helped improve skin texture and hydration to a higher degree. It was hypothesized that the growth factor decreases recovery time by downregulating inflammation and can continue stimulating collagen synthesis started by microneedling(Merati et al., 2020).

Growth factors acted as chemical messengers for triggering cell proliferation, reparative processes, and extracellular matrix formation, and microneedling created controlled microinjuries in the skin, inducing collagen formation, neovascularization, and wound healing. The use of growth factors conjugated with microneedling resulted in collagen remodeling and consequently skin regeneration(Merati et al., 2020).

Epidermal growth factor (EGF) is a polypeptide composed of 53 amino acids that triggers epithelial cell proliferation and division. It can enhance skin moisturization and the wound healing process. Despite the mechanism by which wrinkles arise and intensify being unclear, there have been many studies of EGF, and EGF receptors, and their associated with the cell responses. It is observed that when age increases the EGF receptor decline, suggesting that it is one of the mechanisms of the aging process. And the synthesis of fibronectin and collagen is reported to increase when EGF is administered to fibroblasts(Hae An et al., 2019).

In the Hae An et al. (2019) study, the treatment with an MN patch conjugated with EGF showed statistically significant improvements in wrinkle and skin moisturization compared with an MN patch alone ($p < 0.05$).

Similarly, to the growth factors, there is an interest in understanding the mechanisms of stem cells' self-renewal and differentiation, given their potential applications in regenerative medicine and aging studies. Remarkably, with aging, the functional capacities of stem cells decrease, resulting in reduced organ function and delayed tissue regeneration. Thus, the decline in stem cell function results in changes in the physiology of the tissue itself, which may affect the organism's health and normal functionality(Gomes et al., 2020).

A stem cell is an undifferentiated cell that can self-renew to replicate or can originate several specialized cell types. For instance, hematopoietic stem cells can differentiate into red blood cells, white blood cells, and platelets. Owing to the limited life cycle of most somatic cells, the

ability of stem cells to replace damaged somatic cells is crucial for the tissue homeostasis of the organisms(Gomes et al., 2020).

Stem cell extracellular vesicles or cell therapies, such as adipose-derived stem cells, mesenchymal stem cells, and bone marrow mesenchymal stem cells, have been used for skin advanced therapy due to the ability to repair and regenerate in cosmetic and reconstructive surgeries. The long-term safety and controllability of cell-based therapies remain controversial, making cell-derived extracellular vesicles such as exosomes or conditioned mediums preferable for most therapeutic applications(Liang et al., 2022).

There is a study to evaluate the efficacy of the conjugation of MN and human umbilical cord-derived mesenchymal stem cells conditioned media (hUC-MSCs-CM) in skin brightness and regeneration(Liang et al., 2022).

Noticed that the umbilical cord-derived mesenchymal stem cells conditioned media can form premature adipocytes, collagen type 1 and collagen type 2 which have anti-aging effects. Based on this, was explored the synergistic effect of MN combined with hUC-MSCs-CM against aging skin. The volunteers' satisfaction score and physician's global assessments score for facial regeneration were higher in MN plus hUC-MSCs-CM group, compared to MN alone group. (Liang et al., 2022).

3. Skin gene therapy

Gene therapy and genetic engineering are related technologies that involve altering the genetic composition of organisms. However, the distinction between them is based on purpose. Gene therapy pursues to alter genes to correct genetic defects and thus prevent or cure genetic diseases. While genetic engineering aims to modify the genes to enhance the capabilities of the organism beyond what is normal.

In essence, all types of skin diseases are candidates for gene therapy, ranging from inflammatory to genetic disorders. Inflammatory skin disorders represent one of the most persistent processes that are generally characterized by the activation of innate and adaptive immune responses through the production of proinflammatory cytokines. These disorders are generally considered to result from the inflammatory responses of the epithelial barrier of the skin against allergens and pathogens(Wan et al., 2021).

Gene therapy represents the only treatment option with the potential to cure severe skin diseases like (EB). Two promising forms of gene therapy are potentially feasible for EB, namely gene replacement and genome editing. While genome editing for genodermatoses remains at the preclinical stage, gene replacement approaches are clinically advanced and have been applied to a small number of patients with junctional and dystrophic forms of EB(Koller and Bauer, 2021).

The viral transduction of the wild-type transgene into skin stem cells, followed by autologous grafting of corrected epidermal sheets, led to the regeneration of stable skin. Recent developments regarding designer nuclease-based gene editing strategies enable the establishment of alternative options to restore the gene function in genodermatoses. This can be applicated in cases wherein genetic constellation hinders gene therapy-based gene replacement(Koller and Bauer, 2021).

Small interfering RNA (siRNA) has emerged as a promising therapeutic against a variety of pathological conditions including cancer, genetic disorders, and autoimmune diseases(Zeb et al., 2020).

siRNA can be delivered into skin after being coated on the surface of the solid MNs, embedded within the degradable polymeric MNs, injected through the hollow MNs, or smeared onto MN-treated skin with the aid of electroporation. However, transcending the stratum corneum is only the first step in the successful implementation of siRNA therapy with MNs. siRNAs need to be resistant to enzymatic degradation, enter target cells, and escape the endosome–lysosome degradation axis(Wang et al., 2020).

To forward this challenge, it was developed a nanoparticle-embedding MN system that contains a dissolvable hyaluronic acid matrix and mesoporous silica-coated up-conversion nanoparticles for achieving gene delivery, gene monitoring, and gene regulation. The mesoporous silica shell was used to load and protect siRNA while the up-conversion nanoparticle core allowed the tracking of MN skin penetration and nanoparticle diffusion through up-conversion luminescence or optical coherence tomography imaging. This approach allowed the monitoring of gene expression and efficient gene silencing of target mRNA in the 3D cell model of abnormal scar(Wang et al., 2020).

4. Conclusions

Biotechnological approaches which combine techniques of engineering and molecular biology have been employed for advanced skin therapy. The use of biopharmaceuticals for the treatment of skin disorders improves the outcomes due to their better performance.

The limitations of biopharmaceutical delivery, like chemical and physical instabilities, short half-life, enzymatic degradation, and inadequate skin permeation can be overcome by MN-based biopharmaceutical delivery. MNs can administer biopharmaceuticals with ease, painlessly, and safely without the need for specialized storage.

Therefore, biotechnology has excellent tools for the development of biopharmaceuticals for advanced skin therapy.

5. References

Foot, E., Kleyn, D., and Palmer Foster, E. (2010). Pharmacogenetics-pivotal to the future of the biopharmaceutical industry. *Drug Discov. Today* 15, 325–327. doi:10.1016/j.drudis.2010.03.004.

Gomes, C., Silva, A. C., Marques, A. C., Lobo, S., and Amaral, M. H. (2020). Aesthetic Medicines. 1–14.

Hae An, J., Jung Lee, H., Soo Yoon, M., and Hyun Kim, D. (2019). Anti-wrinkle efficacy of cross-linked hyaluronic acid-based microneedle patch with acetyl hexapeptide-8 and epidermal growth factor on Korean skin. *Ann. Dermatol.* 31, 263–271. doi:10.5021/ad.2019.31.3.263.

Koller, U., and Bauer, J. W. (2021). Gene Replacement Therapies for Genodermatoses: A Status Quo. *Front. Genet.* 12. doi:10.3389/fgene.2021.658295.

Liang, X., Li, J., Yan, Y., Xu, Y., Wang, X., Wu, H., et al. (2022). Efficacy of Microneedling Combined With Local Application of Human Umbilical Cord-Derived Mesenchymal Stem Cells Conditioned Media in Skin Brightness and Rejuvenation: A Randomized Controlled Split-Face Study. *Front. Med.* 9, 1–8. doi:10.3389/fmed.2022.837332.

Lima, L. L., Leonardi, G. R., Holsback, V. S. S., Bagatin, E., Mestnik, N. C., Leite, A. T., et al. Microneedle for skin bioengineering: concepts and approaches.

Merati, M., Woods, C., Reznik, N., and Parker, L. (2020). An Assessment of Microneedling with Topical Growth Factors for Facial Skin Rejuvenation: A Randomized Controlled Trial. *J. Clin. Aesthet. Dermatol.* 13, 22–27.

Rader, R. A. (2008). c o m m e n ta r y (Re) defining biopharmaceutical. 26, 743–751.

Sachdeva, S., Lobo, S., and Goswami, T. (2016). What is the future of noninvasive routes for protein-and peptide-based drugs? *Ther. Deliv.* 7, 355–357.

Subramaniam, K. S., Antoniou, M. N., McGrath, J. A., and Lwin, S. M. (2022). The potential of gene therapy for recessive dystrophic epidermolysis bullosa*. *Br. J. Dermatol.* 186, 609–619. doi:10.1111/bjd.20910.

Wan, T., Pan, Q., and Ping, Y. (2021). Microneedle-assisted genome editing: A transdermal strategy of targeting NLRP3 by CRISPR-Cas9 for synergistic therapy of inflammatory skin disorders. *Sci. Adv.* 7, eabe2888.

Wang, M., Han, Y., Yu, X., Liang, L., Chang, H., Yeo, D. C., et al. (2020). Upconversion Nanoparticle Powered Microneedle Patches for Transdermal Delivery of siRNA. *Adv. Healthc. Mater.* 9, 1–21.

doi:10.1002/adhm.201900635.

Witting, M., Obst, K., Friess, W., and Hedtrich, S. (2015). Recent advances in topical delivery of proteins and peptides mediated by soft matter nanocarriers. *Biotechnol. Adv.* 33, 1355–1369. doi:10.1016/j.biotechadv.2015.01.010.

Zeb, A., Rana, I., Choi, H. I., Lee, C. H., Baek, S. W., Lim, C. W., et al. (2020). *Potential and applications of nanocarriers for efficient delivery of biopharmaceuticals*. doi:10.3390/pharmaceutics12121184.