

Innovations in Epidermal Tissue Engineering

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Abstract. This paper reviews recent advances and challenges in epidermal medical engineering and scaffold material research. The basic elements of tissue engineering include seed cells, scaffold materials and bioactive factors. Current skin tissue engineering techniques offer innovative therapeutic avenues for the repair of large wounds, mainly through suitable tissue scaffolds. The paper also discusses the principles of skin regeneration, including skin structure and function, the process of skin regeneration, and explores the potential of stem cells and exosomes in scar-free healing. Future research directions will focus on developing high-performance biomaterials and scaffolds, optimising stem cell delivery methods, finding optimal stem cell sources and maintaining stem cell activity. In particular, the combined use of natural polymers such as filipin proteins and sodium alginate is expected to create composites that more closely resemble extracellular matrices, thus better promoting cell proliferation and differentiation. In addition, the study of stem cells and exosomes offers new ways to achieve scar-free healing, potentially improving healing efficiency and reducing scar formation when combined with bioscaffolds.

Keywords: Epidermal medical engineering; bioscaffold; stem cell; skin regeneration.

1. Introduction

The skin serves as a crucial interface between organisms and the external environment, enveloping the body in a protective layer. Characterized by a stratified structure, the epidermis plays vital roles in thermoregulation, sensory perception, hydration retention, and defense against environmental threats. While the epidermis possesses inherent self-repair and renewal capabilities, severe skin injuries, such as burns, can compromise its regenerative capacity, posing significant health risks [1]. Thus, epidermal tissue engineering emerges as a pivotal branch of regenerative medicine, dedicated to developing artificial skin to replace or repair skin defects, with profound implications for burn treatment, wound healing enhancement, and the restoration of skin structure and function.

Tissue engineering encompasses essential elements: seed cells, scaffolding materials, and bioactive factors. Seed cells are fundamental to this field, as obtaining a sufficient quantity and quality of these cells is critical for successful applications. The seed cells must possess the ability to form new tissue structures and can be categorized into three types: autologous, allogeneic, and xenogeneic, each presenting distinct advantages and challenges. Adult stem cells, including hematopoietic, bone marrow, neural, adipose-derived, and skin stem cells, are favored for their ethical viability and relative ease of development and differentiation [2]. Scaffolds serve as temporary three-dimensional structures that facilitate cell attachment, growth, and migration. Ideally, these scaffolds should exhibit excellent biocompatibility, appropriate porosity, adjustable degradation rates, and suitable mechanical properties. Bioactive factors, including growth factors and cytokines, regulate cell behavior and can enhance healing outcomes by stabilizing the degenerative environment or promoting tissue regeneration [3].

Current skin tissue engineering techniques provide innovative therapeutic avenues for repairing large wounds, primarily through suitable tissue scaffolds. Scaffold materials fall into natural or synthetic categories, with composites of multiple materials predominantly used in clinical practice today. Over the past four decades, a diverse array of tissue-engineered skin products has been developed for treating both acute and chronic wounds, categorized based on their tissue structure into epidermal products like EpiCel®, dermal scaffolds such as AlloDerm® and Integra®, and composite dermal products like PermaDerm®. These products can also be classified by clinical use,

distinguishing temporary skin substitutes (dressings) like DermaGraft® from permanent grafts such as EpiCel®, AlloDerm®, and PermaDerm® [4].

Currently, the only permanent skin substitutes that effectively close wounds are autologous tissue-engineered skins using autologous skin cells [4]. Research into stem cells and their derived exosomes offers promising avenues for scarless wound healing, potentially enhancing healing efficiency while reducing scar formation when combined with bioscaffolds. Future research directions in skin tissue engineering should focus on developing high-performance biomaterials and scaffolds, improving stem cell delivery methods, identifying optimal stem cell sources, and maintaining stem cell viability [5].

2. Skin Regeneration Principles

2.1. Skin Structure and Function

The skin is the largest organ in the human body, comprising approximately 16% of total body weight. It plays a crucial role in maintaining internal homeostasis and acts as a barrier against external stimuli [6]. The integrity of the skin is vital for overall health, as injuries from chronic conditions, burns, trauma, or surgical procedures can lead to significant impairment and emotional distress [7,8]. The skin's structure can be divided into two primary layers: the epidermis and the dermis. The epidermis, which includes hair follicles, sebaceous glands, and sweat glands, provides structural integrity, immunity, and nutritional support to the integumentary system. The dermis is characterized by a higher concentration of extracellular matrix (ECM), as well as vascular and mechanoreceptive elements, while the subcutaneous adipose tissue serves as an energy reservoir and a consistent source of growth factors [9].

2.2. Processes of Skin Regeneration

Wound healing in the skin is a complex process involving multiple cellular, humoral, and molecular pathways [10]. Under normal conditions, the skin can repair damaged areas and promote the formation of a new epidermis through the proliferation of cells in the basal layer.

The first stage of trauma response is the hemostatic phase. This phase aims to minimize blood loss through vasoconstriction and thrombus formation. The hemostatic process is initiated when blood components interact with the endothelial lining of blood vessels, leading to platelet adhesion, aggregation, and the formation of an initial hemostatic thrombus [11]. Thrombus formation is essential for hemostasis and also promotes the release of complement proteins and growth factors that facilitate wound healing by providing a supportive scaffold [12].

Following this, the inflammatory phase occurs. Inflammation during wound healing involves a complex interplay of cellular and molecular interactions orchestrated by the immune system, which includes macrophages, neutrophils, lymphocytes, and various signaling molecules. This phase begins after hemostasis when blood vessels dilate due to coagulation and complement cascade reactions, with bradykinin and other inflammatory mediators playing crucial roles [13]. Leukocytes and macrophages migrate to the wound site to eliminate bacteria and foreign materials, releasing growth factors and cytokines that promote regeneration. During this time, inflammatory symptoms such as redness, swelling, heat, and pain become evident around the wound.

Subsequently, stem cells located above the wound begin to proliferate and form new skin tissue, entering the regeneration phase. Basal cells divide to produce new keratinocytes, melanocytes, and sweat gland cells, among others. These cells gradually migrate toward the center of the wound, ultimately replacing the existing damaged tissue [14].

3. Research on Seed Cells

Cells utilized in tissue engineering encompass both stem cells and various other cell types, with stem cells being the most crucial seed cells. Stem cells can be classified into embryonic stem cells

and adult stem cells based on their developmental stage. Adult stem cells are considered the most clinically valuable seed cells for tissue engineering due to their ethical advantages and relatively straightforward differentiation conditions compared to embryonic stem cells [2].

In addition to stem cells, other cell types can serve as seed cells in tissue engineering, including endothelial cells, epithelial cells, fibroblasts, osteoblasts, osteoclasts, keratinocytes, preadipocytes, adipocytes, and tendon cells. Although these differentiated cells generally exhibit lower proliferative capacities, they still play significant roles in the field of tissue engineering.

3.1. Source of Seed Cells

Tissue engineering employs a diverse range of seed cell sources, including autologous, allogeneic, xenogeneic, and stem cells. Currently, autologous cells derived from the patient are regarded as the optimal source. These cells can be expanded in vitro to sufficient quantities for constructing engineered tissues. The use of autologous cells minimizes the risk of immune rejection following in vivo transplantation, making them highly favored by researchers and the most commonly used source in tissue engineering studies. Conversely, xenogeneic cells, which are derived from animals, represent another potential source. Given that many human tissues lack a sufficient supply of their own cells, researchers are exploring the use of xenogeneic cells as viable seed cell sources [15].

3.2. Isolation and Cultivation of Seed Cells

Cell culture techniques in tissue engineering are primarily categorized into three methods. The first method is the independent culture method, in which endothelial cells and mesenchymal stem cells are extracted from fresh tissues, expanded, and cultured separately before being sequentially layered, starting with mesenchymal cells followed by endothelial cells. The second method is the mixed culture method, where endothelial cells and mesenchymal cells are collected together during extraction, then separated and expanded individually in vitro, and finally layered for implantation. The third method is the co-culture method, which involves the direct co-culturing of endothelial cells and mesenchymal cells without separation post-extraction, followed by the implantation of the mixed cell population onto a scaffold [2].

4. Clinical Application and Research of Scaffold Materials

4.1. Requirements for Scaffold Materials

Biomaterials must meet several essential criteria: they should support cell adhesion on their surfaces and possess a structure that allows cells to grow within them, enabling normal proliferation and retention of differentiation functions. Additionally, they should exhibit controlled degradation, ensuring that both the material and its degradation products are non-cytotoxic and do not provoke a host immune response. Furthermore, the materials should be widely available, cost-effective, and exhibit good biocompatibility, with no risk of disease transmission or ethical concerns.

Regarding scaffold pore structure, materials should feature high porosity to facilitate cell adhesion, extracellular matrix regeneration, cell spreading, and the transport of nutrients and waste products. Sufficient space within the scaffold allows for cell growth and migration, promoting the formation of homogeneous tissues. Finally, the three-dimensional scaffold structure should possess adequate mechanical strength to provide structural support and templates for the cells.

4.2. Classification and Properties of Scaffold Materials

Scaffold materials can be classified primarily into synthetic scaffolds and bio-based scaffolds. Synthetic scaffold materials, often bioactive polyester-based, demonstrate good plasticity and biocompatibility. However, a notable limitation is their lack of cellular recognition signaling, which adversely affects cell adhesion and the specific activation of certain genes.

In contrast, bio-based scaffold materials can replicate the environment of the natural extracellular matrix, promoting cell adhesion and growth. While these materials are characterized by excellent biocompatibility, they often exhibit poor mechanical properties, which can restrict their applications. Therefore, it is crucial to weigh both the benefits and limitations of scaffold materials when making selections.

4.2.1 Synthetic Skin-like Tissue Engineering Scaffold Materials

The main class of synthetic scaffold materials consists of polyester-based materials such as polycaprolactone, polylactides, polyhydroxyalkanoates, and polycarbonates. Among these, poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(hydroxyglycolic acid) (PGA), and copolymers of lactic and glycolic acid (PLGA) are the focal points of research and have received approval from the US FDA for use in artificial skin [16]. For instance, Nam et al. utilized ammonium bicarbonate as a porogenic additive to create PLLA porous spongy scaffolds with high porosity, achieving an average diameter of connected pores around 200 μm . They also successfully fabricated PLLA, PLGA, PDLLA, and other scaffolds with varying pore sizes by controlling multiple parameters in a thermally induced phase separation process using a dioxane-water solvent system [17].

Among synthetic polymers, polycaprolactone is widely employed in tissue engineering, with its biocompatibility enhanced through protein coatings [18]. By selectively creating pores in the scaffold material, the attachment points for cells can be increased, improving overall biocompatibility. Sadeghi et al. prepared optimized electrospun fibrous membranes by coaxial electrospinning of poly(caprolactone) and poly(vinylpyrrolidone), subsequently removing the poly(vinylpyrrolidone) to leverage the differences in hydrophilicity. Characterization of the physicochemical properties and in vitro testing revealed that cells efficiently penetrated the samples, thereby enhancing cytocompatibility [19].

4.2.2 Bio-based Skin Tissue Engineering Scaffold Materials

A significant drawback of collagen gels and sponges, commonly used as artificial skin scaffold materials, is their inadequate mechanical strength. Integra, an artificial skin product developed by Integra Life Sciences, features a 2-mm-thick porous membrane composed of bovine collagen and glycosaminoglycans in its lower layer, covered by a 0.22-mm-thick silicone membrane in the upper layer. This product has undergone extensive clinical testing for treating third-degree burns and has demonstrated a significant reduction in wound healing time [20]. In China, researchers like Yang Jun combined collagen extracted from fresh pig skin with chondroitin sulfate to manufacture collagen sponge membranes using vacuum drying technology. This membrane served as a scaffold for in vitro culture of human epidermal cells, with experimental results indicating that the cells were able to grow and proliferate effectively, ultimately forming a complete cell layer [21].

Acellular dermal matrices (ADM) have emerged as a prominent focus in tissue engineering, as they reduce immune rejection by removing cellular components from skin tissue, leaving only the extracellular matrix. Utilizing this technology, Integra Life Sciences has developed Alloderm, a porous membrane primarily composed of bovine collagen and glycosaminoglycans, covered by a thin silicone rubber membrane, which has been widely used in the treatment of severe burns [22]. However, the limited availability and risk of disease transmission associated with human donor skin for ADM preparation have prompted researchers to explore alternative sources, such as pig skin. In China, researchers like Xiao Shichu et al. are actively investigating the preparation methods, cell culture, and clinical applications of cell-free dermal matrices, finding that xenogeneic cell-free matrices can support the growth of epidermal cells [21]. Currently, enhancing the permeability of ADMs and promoting vascularization are key research priorities in this field [23].

4.3. Multifunctional Composites: SF/SA Composite Porous Materials

Natural polymers, derived from living organisms or the environment, are sustainable and environmentally friendly. In the field of skin tissue engineering, two primary categories of natural

polymeric materials are commonly utilized: proteins and polysaccharides. Protein-based materials include collagen, elastin, sericin, and fibronectin, while polysaccharide-based materials encompass chitosan, sodium alginate, chondroitin sulfate, and cellulose [24,25]. Numerous studies have demonstrated that combining two or more natural polymers can yield composites that closely mimic the extracellular matrix (ECM), effectively inducing and regulating cell proliferation and differentiation, thereby enhancing cell adhesion and growth. High-purity natural polymers typically exhibit controlled degradation properties, good biocompatibility, and low immunogenicity, and their abundant availability can significantly reduce manufacturing costs, making them widely applicable as biomedical materials in skin tissue engineering [26].

Silk proteins are particularly notable due to their rich sources, low cost, non-irritating nature, good oxygen permeability, and controlled biodegradability. In vitro, silk proteins support a variety of cell adhesion, growth, and differentiation, while in vivo, they demonstrate excellent histocompatibility. Although regenerated silk materials can be unstable and water-soluble, their stability can be significantly enhanced under specific processing conditions, broadening their applications. Processed silk materials also possess outstanding mechanical properties, making them a promising class of medical biopolymers.

Sodium alginate is a natural polysaccharide that can be formulated into films, porous materials, microspheres, nano-nonwoven nets, and gels for biomedical applications. Materials derived from sodium alginate exhibit good cytocompatibility, low immunogenicity, and bioinertness.

While both silk proteins and sodium alginate hold significant research potential, they have inherent limitations when used independently, which restricts their effectiveness as biomaterials in tissue engineering. Over the past few decades, researchers have sought to mimic the structure of the ECM by combining silk proteins and sodium alginate for tissue engineering applications. This approach has led to numerous studies aimed at developing biomaterials with reparative functions. For instance, Li Q. et al. prepared a filipin (SF) and sodium alginate (SA) hydrogel suitable for controlled drug release. They initially created porcine cardiomyocyte extract (PPM) to modify the SF/SA hydrogel. Comparative investigations of three components—SF, SF/SA, and SF/SA/PPM—revealed that the SF/SA/PPM ternary composite hydrogels exhibited shorter gelation times, more stable gel morphologies, and a more porous internal structure, which are advantageous for drug loading and release [27]. Cytocompatibility experiments indicated that this material could support the adhesion and growth of bone marrow mesenchymal stem cells (BMSCs), providing a solid foundation for further research on controlled drug release.

5. Conclusion

This paper reviews current advancements and challenges in epidermal medical engineering and scaffold material research. As a vital branch of regenerative medicine, epidermal tissue engineering focuses on the development of artificial skin for the replacement or repair of skin defects, which is crucial for treating burn wounds and injuries, promoting wound healing, and reconstructing skin structure and function.

Future research will prioritize the development of biomaterials and scaffolds with enhanced properties, the optimization of stem cell delivery methods, the identification of ideal stem cell sources, and the preservation of stem cell activity. Notably, the combined use of natural polymeric materials such as silk proteins and sodium alginate holds promise for creating composites that closely resemble extracellular matrices. These composites are expected to better induce and regulate cell proliferation and differentiation, thereby enhancing cell adhesion and growth.

Furthermore, stem cells and their derived exosomes represent an important avenue in the pursuit of scarless wound healing, with the potential to improve healing efficiency and reduce scar formation when integrated with bioscaffolds. Exploring the development of biomaterials with reparative functions, refining stem cell delivery techniques, identifying optimal stem cell sources, and maintaining stem cell viability will be key research directions in the field of skin tissue engineering.

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