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Regenerative Medicine: A Review of Solutions in the Treatment of Skin Defects

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Abstract

The issue of skin defects is a major concern of almost every trauma surgeon after surgery. Despite numerous conventional methods and introduction of the reconstruction ladder, managing skin defects is still a challenge for the trauma surgeons. In recent years, parallel to the advances in the more conventional methods of skin repair, regenerative medicine has offered new and novel treatments. This article aims to explore these contemporary regenerative solutions as well as to review the conventional methods of treating skin defects.

Keywords

Regenerative Medicine, Skin, Wound, Defect

1. Introduction

As the largest organ in the body, skin plays a vital role in homeostasis [1] [2]. With more than 58 million lethal injuries worldwide each year, the issue of skin defects is a major concern that almost all trauma surgeons face [3] [4]. Skin tissue engineering is a modern domain in medicine which looks to provide an appropriate solution to this problem [5]. This article aims to explain practical knowledge of skin defects, explore contemporary regenerative treatments, and review the existing methods for the treatment of skin defects.

1.1. Skin's Cellular Structure

As each skin layer (including the epidermis, dermis and hypodermis) is recognized by its specific cellular structure [6], understanding this structure is key to

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applying regenerative medicine in treatment of skin defects.

The outermost layer, the epidermis, is rebuilt every 2 to 3 weeks [7]. Its source for cellular regeneration is in the basement membrane which provides an abundance of progenitor cells. Keratinocytes are the largest population of cells in the epidermis that originate from these progenitors [8]. Melanocytes produce skin pigments and are mostly located in the deep layer of epidermis [8]. Immune response in the epidermis is carried out by the Langerhans cells, which are considered part of the reticuloendothelial system [8].

The main layer, the dermis, is mostly composed of fibroblasts that produce collagen type I as the main structural component of the extracellular matrix (ECM). Dermis has a rich blood supply and innervation [9]. The skin's flexibility comes mostly from the elastic bundles in the dermis [7].

The deepest layer, the hypodermis, is mostly consisted of adipocytes. Aside from energy storage, they also facilitate thermoregulation. Adipocytes are a rich and easily accessible source of stem cells, growth factors and hormones [9].

1.2. Wound Types

Any disruption in the skin's integrity could be described as a wound. Wounds are classified based on their depth, complexity, age, origin, and etiology. Isolated loss of the epidermis and upper dermis, causes a superficial woundas seen in abrasions. More severe pathologiessuch as those resulting from a short fall, or a low-speed accidentmay involve the complete thickness of dermis (partial thickness wounds) or even extend to the subcutaneous tissue (full thickness wounds). The most severe and complicated wounds usually penetrate the underlying cavity, organ, or tissue (**Figure 1**).

A wound is considered acute if it has formed in the past 6 hours after which it would be referred to as an early wound. If more than 24 hours have passed since

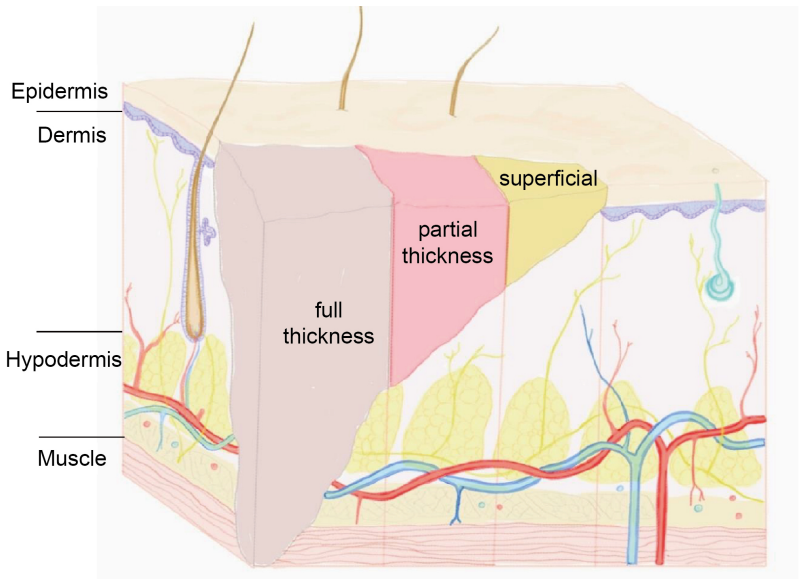

Figure 1. Structure of the skin and its relation to the thickness of the wound.

its formation, it will be classified as a late wound with rather different characteristics [10]. Furthermore, wounds can be classified into acute and chronic based on their healing potential. While acute wounds recover after a few weeks, chronic wounds fail to recover through the physiologic wound healing process [11].

1.3. Wound Healing

The wound heals through a complex collaboration between local and distant cells [12]. This process has been simplified into 4 phases: hemostasis, inflammation, proliferation, and remodeling (**Figure 2**) [13].

1) *Hemostasis*: The first phase involves local hemorrhage which is stopped through the mechanisms of hemostasis [14]. Platelets play a major role in this process. They are activated after contact with the exposed subendothelial matrix due to an interaction between the platelet's von Willebrand Factor and the subendothelial collagen fibers. In addition to the coagulation cascade due to platelet aggregation and their mediators causing the fibrin clot to form [15], an inflammatory cascade starts as a result of the local release of powerful chemo attractants such as thrombin, transforming growth factor (TGF)-β, platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) from the platelets [16]. Platelet secretions also contain various growth factors which stimulate fibroblasts and keratinocytes [17]. Meanwhile, injured blood vessels also begin to repair through muscular and endothelial proliferation [18].

2) *Inflammation*: Local release of chemokines and vasodilators like histamine along with increased vascular permeability begin cell recruitment which leads to a full blown inflammatory reaction [16]. Inflammatory cells mainly include neutrophils, circulating monocytes that differentiate into macrophages upon entering the wound tissue, and resident immune cells like mast cells, Langerhans cells, T cells and macrophages [19]. These cells are responsible for removal of the necrotic tissue, defense against pathogens either through phagocytosis [20] or by release of cytotoxic agents from neutrophils [21], stimulation of collagen production, angiogenesis, and re-epithelialization by macrophages. Indeed, macrophages play a pivotal role in transition to the proliferative phase [22].

3) *Proliferation*: principal events in this phase include fibroplasia, development of granulation tissue, wound contraction, matrix deposition, angiogenesis, and regeneration of hair follicles and nerve fibers [19] [23]. Major players in this

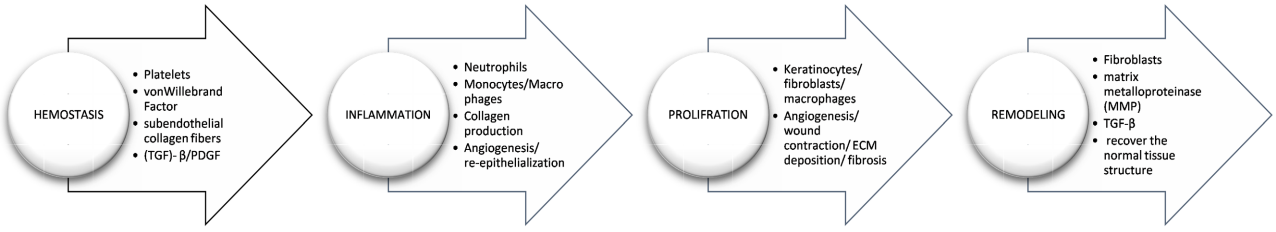

Figure 2. The 4 main phases of wound healing.

phase include keratinocytes, fibroblasts, macrophages, and endothelial cells. M2 macrophages are an important source of TGF-β which can promote different events of wound healing such as angiogenesis, wound contraction, ECM deposition and fibrosis through stimulating fibroblasts and mesenchymal cells, as well as chemotaxis. Fibroplasia is characterized by the formation of granulation tissue rich in type III collagen by fibroblasts [24] [25]. Angiogenesis occurs in the wound bed with the migration and proliferation of endothelial cells. Proliferation and migration of keratinocytes along wound edges is the hallmark of re-epithelialization [26]. Gradual wound contraction by myofibroblasts on the wound's border help wound closure [24].

4) *Remodeling*: This phase spans the whole process of wound healing [19]. Remodeling is meant to create a balance between ECM production, which has increased during the proliferation phase, and its breakdown. Fibroblasts are the principal cells responsible for ECM remodeling in the wound. The key enzymes in this phase are certain collagenases named matrix metalloproteinase (MMP) expressed by macrophages, fibroblasts and keratinocytes [27]. TGF-β is one of the stimulants for myofibroblasts differentiation. Gradually collagen type III is replaced by collagen type I, which is the main structural component of the dermis [27]. During the remodeling phase, an attempt to recover the normal tissue structure occurs, the cell number is significantly reduced and scar tissue forms.

In all the processes mentioned above, exogenous, and endogenous factors can influence the healing process. Systemic disorders, such as diabetes, immunosuppression, venous stasis, as well as the use of steroids and smoking can disrupt or delay the early closure of the wound. Furthermore there is the possibility of developing hypertrophic scars and keloids [28].

2. Conventional Treatments

Nowadays, a wide spectrum of techniques can be applied in reconstructive surgery. Herein, we briefly review the common approach to treating wounds, called the reconstructive ladder (Table 1). Logically, the simplest effective step of the ladder should be chosen.

2.1. Secondary Intention

This simple method is used in small wounds. As opposed to primary closure in

Table 1. Reconstructive ladder.

More Complex	Skin Flaps
	Tissue Expansion
	Skin Grafts
	Primary Closure
Simpler	Secondary Intention

which the wound edges are approximated with the help of sutures or other means, in secondary intention the wound is kept clean and may be covered by topical antibiotics or dressings while no sutures are used [29]. The wound closure in this method is achieved via layers of granulation tissue formed from the base of the wound upward [30]. Several different dressings exist for wounds managed via secondary intention while little concrete evidence exists for their superiority compared with others [30].

2.2. Primary Closure

This method is the second step of the reconstructive ladder. After proper sterilization and surgical debridement, the wound edges are closed with sutures. Tension on the wound edges should be avoided to reduce scaring, dehiscence, and infection. To gain better cosmetic results, the incision line is better to be placed parallel to the relaxed skin tension lines (RSTLs) [31]. These lines were first reported on by Borges and follow the natural orientation of the collagen fibers in the skin [32].

2.3. Skin Grafts

In large defects when primary closure cannot be used, the next step is skin graft. Grafts are further classified into split-thickness skin grafts (STSGs) and full-thickness skin grafts (FTSGs).

STSGs: It includes epidermis and part of the dermis that is detached from its own blood supply. The thickness of the dermis present can vary based on the conditions of the needed graft which further divides STSGs into thin, intermediate, and thick. STSGs are usually believed to have a higher chance of graft survival and "take" than the FTSGs. The donor site heals either by secondary intention or primary closure.

FTSGs: It includes epidermis, dermis, and part of the hypodermis. It is used in smaller wounds specially on the face or in places with hair or on the flexor surfaces. FTSGs are usually believed to cause less wound contracture and have better overall cosmetic results and hair growth than the STSGs. On the other hand, full thickness grafts do not survive as well as STSGs especially in the presence of infection and are more time consuming to prepare. Interestingly enough, the hair growth and hyperpigmentation usually observed in FTSGs can be a cause for patient dissatisfaction when occurring in inappropriate locations [33]. The donor site usually is an area with less cosmetic importance and must be closed with primary closure [34] [35] [36].

2.4. Tissue Expansion (TE)

In this technique the area surrounding the wound is expanded with a tissue expander (*i.e.*, a sack like structure capable of expanding via injections of fluid into it or some other mechanism), which provides the surgeon with extra amounts of skin capable of covering the defected area by being used as a flap and hence pro-

viding similar color and texture. In addition, there is no residual defect in the donor site [31] [34]. Since its introduction decades ago, a primary use for TE has been in breast reconstruction surgery following mastectomies. This remains true to this day while other indications of this technic have also gained popularity such as scalp defects and facial reconstructions [37]. While considered a popular option due to excellent cosmetic results and low cost, a lengthy treatment duration and a relatively high risk of complications such as infection exist as downsides [38] [39].

2.5. Skin Flaps

This is when a "flap" of tissue is transferred to an adjacent area along with its vascular attachments or the vasculature of the tissue may be anastomosed to the recipient vessels (free flaps). This method is used to cover low perfusion tissues such as bare cortical bone or tendon or cartilage, as well as in cases in which skin grafts have repeatedly failed [31]. Numerous factors may cause suboptimal results or failures of the flap, however, most of such causes can be summarized under the term "compromised perfusion". Prompt and effective measures to alleviate the cause of hypoxia can therefore make a big difference [40].

3. Skin Tissue Engineering (STE)

3.1. Cells

STE is based on 3 major components (STE triad): Cells, biomaterial and growth factors (as depicted in **Figure 3**) [3]. Cell-based therapy is useful as a single treatment method in STE [41]. First attempted in 1979, Rheinwald and Green, two pioneers of cellular therapy in tissue engineering (TE), used cultured autologous epithelial cells for a patient who suffered extensive burn wounds [42].

Cells can be divided into different categories according to different characteristics such as the source of cells and degree of differentiation. The cells can be either autologousor allogeneic, and both differentiated or stem cells can be used in cell-based therapy. [43].

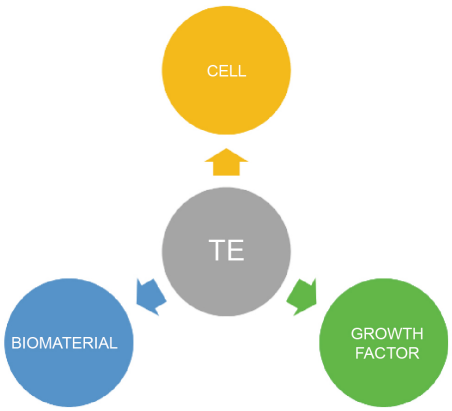

Figure 3. STE components: cells, biomaterials, and growth factors.

The main goals of cell-therapy in STE are to increase the speed and quality of wound healing and to decrease wound contracture and scar formation [44]. Among the differentiated cells, keratinocytes and fibroblasts are the most popular [45]. However, stem cells are generally considered a superior choice in STE due to their self-renewal capacity and multilineage differentiation potential. If properly managed, stem cell therapies can be expected to produce a complete tissue and organ similar to the normal skin [46] [47].

The most widely used stem cells in STE researchare bone-marrow derived stem cells, adipose-derived stem cells, umbilical cord cells, and mesenchymal stem cells [48]. Mesenchymal stem cells and adipose-derived stem cells are among the most widely used cells in research due to their characteristics and signaling pathways [46].

3.2. Biomaterials

A right choice of scaffolds and biomaterials has a very important role in treatment. Scaffolds are 3-dimensional (3D) structures made from synthetic or natural biomaterials [49], that is, bio-based substances compatible with living cells, that act as a natural extracellular matrix (ECM) for the tissue. In the proper scaffold, cells can proliferate and differentiate until reaching the goal of producing normal functional tissues. The scaffold should be compatible with the cells in different aspects such as mechanical, physical, chemical, etc., each of which is unique for each different type of tissue. For example, a scaffold suitable for skin tissue should be biocompatible and biodegradable and also have physical characteristics such as elasticity and mechanical strength close to natural skin. Finally, a scaffold should result in proper cell adhesion and placement [50] [51] [52] [53].

Generally, there are 4 main ways of producing scaffolds: 1) use of ECM secreting cell sheets 2) pre-made porous scaffolds made of synthetic or natural biomaterials 3) decellularized ECM scaffolds 4) cells entrapped in hydrogel [49].

Some of the most widely used natural biomaterials are chitosan, silk fibrin, collagen, fibrinogen, hyaluronic acid, gelatin, and elastin, and of the most widely used synthetic material in skin scaffolds we can name Poly Lactic Acid (PLA), Poly Lactic-co-Glycolic Acid (PLGA), Poly ε-Caprolactone, and Polyethylene Glycol (PEG). Some scaffolds can be composites of synthetic and natural components such as, Collagen-PCL, and Gelatin-PCL [54] [55].

3.3. Growth Factors

Growth factors are considered an important component of regenerative therapies and tissue repair. They stimulate cells and regulate their genetic makeup, leading them to proliferate and differentiate into the designated tissue cells. Such a signal can mimic neovascularization and cellular migration signals and assist the coordination and collaboration of cells. Based on the exact factors used, their utilization in TE can be beneficial for the wound healing process. However, they

may sometimes have negative effects on wound healing as cause fibrosis or unwanted wound contractions [44] [56]. To point out some of the most important factors inSTE, one can name Transforming growth factor beta (TGF-β), Vascular endothelial growth factor (VEGF), Platelet-derived growth factor (PDGF), Fibroblast growth factor (FGF), Hepatocyte growth factor (HGF), and Epidermal growth factor (EGF) [44]. A list of the most important STE components is available in **Table 2**.

3.4. STE Methods and Solutions

Skin substitutes are manufactured with the intention to help repair or cover the damaged tissues. However, none of such substitutes can perform all of the functions of normal skin [57]. These wound dressings usually comprise of both biosynthetic and unprocessed natural products such as allografts and xenografts [58].

They are classified into three main types:

Type I: Substitutes containing only epidermal components;

Type II: Substitutes that include dermal factors either obtained from processed skin or synthesized;

Type III: Substitutes made up of both the dermal and epidermal components (usually named composite skins) [59].

3.4.1. Type I: Epidermal Substitutes

1) Cultured Epidermal Autografts (CEA)

A process in which keratinocytes of the patient are proliferated *in vitro* and applied as a graft to cover the wound [60]. A small full-thickness biopsy is taken and the epithelial cells are isolated [61]; the cells are then cultured for 14 - 28

Table 2. The most important components in STE.

CELLS	BIOMATERIAL		GROWTH FACTORS
	Synthetic Material	Natural Material	
Keratinocytes	Poly (Lactic Acid) (PLA)	Chitosan	Transforming growth factor beta (TGF-β)
Fibroblasts	Poly (Lactic-co-Glycolic Acid) (PLGA)	Silk Fibrin	Vascular endothelial growth factor (VEGF)
Bone-marrow Derived Stem Cell	Poly (ε-Caprolactone)	Collagen	Platelet-derived growth factor (PDGF)
Adipose-derived Stem Cell	Poly (Ethylene Glycol)	Fibrinogen	Fibroblast growth factor (FGF)
Umbilical Cord Cell		Hyaluronic Acid	Hepatocyte growth factor (HGF)
Mesenchymal Stem Cell		Gelatin	Epidermal growth factor (EGF)
-		Elastin	-

days after which they are transferred to the wound bed [62] [63].

Application of CEAs is indicated in cases such as, full-thickness injuries (more than 50% of total body surface area (TBSA) burns), 30% - 50% TBSA burns when donor sites are limited, or burns in which the only accessible donor sites are in functional or aesthetic regions [63] [64]. Despite their indication in the aforementioned situations, CEAs have significant disadvantages which restrict their usage: it takes a long time and budget to culture the cells and they are also highly sensitive to bacterial colonization which leads to an ultimate poor graft success of only about 15% [62] [65] [66].

2) Spray Skin

A small split-thickness sample of the patient's skin is digested by enzymes producing a suspension including melanocytes, keratinocytes and other skin cells which is then sprayed on the wound [67]. This method can be beneficial in the treatment of various injuries, burns (partial or full thickness), and vitiligo [67] [68] [69] [70]. Spray skin dramatically diminishes postoperative pain, and needs less analgesics compared with traditional split-thickness grafting [71]. Under standard conditions, a round of treatment with spray skin is expected to be able to epithelialize a 10 to 15 cm^2 area of tissue in a period of one week [72].

3.4.2. Type II: Dermal Substitutes

Patients with full-thickness injuries such as extensive acute burns or chronic wounds are primary candidates for dermal and dermo-epidermal substitutes due to the lack of healthy donor sites for autografting. The first use of dermal substitute was in 1980s which was an acellular collagen-glycosaminoglycan-based substitute. Currently available as Integra Artificial Skin (Integra Life Sciences Corp., Plainsboro, New Jersey, United States) [73], it has shown premise in various clinical settings [74]; preventing wound contractions and offering better mechanical stability than cultured epithelial autografts [75].

Skin substitutes can be generally divided into cellular and acellular. Some of the most used cellular commercial products are Integra™, Biobrane®, Alloderm™, GraftJacket, and Matriderm®. As for the acellular substitutes, Dermagraft™ and TransCyte are among the most widely used. From a biomaterial point of view, dermal substitute scaffolds may have allogeneic, xenogeneic, or synthetic origin [76].

Despite their advantages, none of the acellular skin substitutes can provide regeneration of skin appendages, such as sebaceous, and sweat glands. Such a shortcoming, especially in large area damages, can cause great difficulties for patients. For a complete skin regeneration, a new generation of skin substitutes has been developed in which dermal follicular cells are used to construct structures mimicking natural skin appendages. Further advances in hair follicle reconstruction [77] and skin-on-chip products [78] can provide the solution for high-quality regeneration of skin structures and appendages. Considering the mechanisms in fibrosis generation, such approaches are likely to achieve minimal scarring and good cosmetic results [79].

As a newly developed dermal substitute, the bio-ink is expected to play a positive role in skin regeneration. This substitute is prepared by using an enzyme-digested decellularized skin extracellular matrix, in which basic structural and functional proteins of the ECM, bio reactive materials and growth factors, are preserved. This so called dermally derived "bio-ink" can create a 3D environment in which its residual skin-related proteins induce survival and proliferation of human skin-derived cells and skin development [80].

3.4.3. Type III: Dermo-Epidermal Substitutes

The most vital requirement of a skin substitute is the ability to reconstruct the epidermal barrier function (hence the primary use of epidermal substitutes discussed previously) of the skin. However, in cases of full-thickness skin injuries, replacement of both the epidermal and dermal layers are necessary for proper wound healing [81].

Currently the most similar and complex skin analogues or skin substitutes commercially available, are dermo-epidermal or composite skin substitutes [2] [55]. Bell *et al*. produced the first dermo-epidermal skin equivalent in 1981 [76] [82]. As their name implies, composite skin substitutes contain both epidermal and dermal layers [55] [83]. Use of both keratinocytes and fibroblasts in composite skin substitutes and their interactions leads to generation and excretion of different growth factors and cytokines which ultimately promotes wound healing process [55] [83].

Apligraf® is a composite allogenic skin substitute approved by FDA for the treatment of venous leg ulcers and neuropathic diabetic foot ulcers. It is composed of bovine type I collagen gel seeded with neonatal foreskin fibroblasts and a keratinocyte layer [1] [8] [84] [85] [86]. Although the use of such substitutes can cause significantly improved wound healing in comparison with other alternatives, there are downsides such as its high price, short lifespan of the substitute in the wound bed, and the possibility of disease transmission as it contains allogenic material [8].

OrCel® is another bilayer skin substitute composed of a collagen sponge seeded with keratinocytes and neonatal foreskin fibroblasts. It is used for treatment of partial-thickness skin wounds and provides sites of host cell migration in order to improve wound healing process [87]. In 2003, Still *et al*. examined the efficacy of OrCel® in donor site wound healing of severely burned patients. The results showed that in case of its use, the healing period was significantly shorter in comparison with Biobrane-L® [83]. The substitute is currently approved for the treatment of burns and blistering skin diseases [88].

Although dermo-epidermal skin substitutes are the most similar product to the natural skin available in the market, there exist a number of drawbacks which has hindered their broad adoption in the clinic. Among these, suboptimal vascularization of the grafted skin is an important issue plaguing the wide use of tissue engineered skin substitutes especially bilayer dermo-epidermal skin subs-

titutes [2].

4. Future Challenges

As a new multidisciplinary field, the realized potential of TE has increased by leaps and bounds over the past decade. Skin tissue engineering has been no exception and even though important limitations still remain, considerable developments and new technologies in recent years have led to significant marketing opportunities [9]. Of the remaining challenges is the long and complicated process of tailoring each product for each specific patient and skin damage [89]. Also, advancements in the field are further slowed by the long times needed for each product's governmental approvement. Limited shelf-life of the product is another problem that complicates distribution and supply [45].

All these challenges eventually lead to a very high consumer price which is not at all affordable for the masses and has hindered the method's wide use [9]. Also, massive costs associated with TE research projects often make them unfeasible to be supported solely by academic laboratories and government institutions, therefore necessitating the need for private investments. This eventually leads to a market controlled and monopolized by a few people and private investors which may negatively impact both the consumer and the science itself [90].

Finally, several functional limitations like decreased vascularization, poor mechanical strength, and formation of fibrosis or scar tissues as well as a probability of immune reaction or rejection due to host response leading to patient safety concerns casts doubt on the method's efficacy [8]. Skin appendages regeneration also remains elusive, warranting the need for a deeper understanding of skin tissue regeneration and self-organization [91].

5. Conclusions

Skin defects are one of the most challenging problems in the field of plastic surgery and regenerative medicine. The ability to create living human skin tissue provides clinicians with an invaluable tool to repair skin defects more effectively than conventional methods. This opportunity is provided by the skin tissue engineering (STE) knowledge and technology. STE is a multidisciplinary field of science that brings scientists and researchers from medical science, biomechanics, and bioengineering together with the aim of creating viable tissues *in vitro*.

While having the potential to significantly broaden the horizons of plastic and reconstructive surgery, STE is still at a young age and has a long way to go to completely fulfill this goal. There are still serious challenges to be solved and more research studies are needed to realize a future in which this newly emerged technology has replaced current conventional methods.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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