Chapter 15

Tissue Engineering for Skin Replacement Methods

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Additional information is available at the end of the chapter

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Abstract

The skin is the biggest structure of the body, and it plays a significant role in maintaining the unity of the body environment. The skin is important for the endurance of the organism as an outer coat for the thermal regulation and hydration preservation. With the intention of helping these significant utilities, the skin continually experiences regeneration and holds the capability to overhaul wound by repair and regeneration of several kinds of skin stem cells. Noteworthy, development has been accomplished throughout the recent times in the generation of engineered skin alternates which imitate human skin cells in vitro for replacement or modeling. Conversely, existing new skin alternatives do not reinstate completely the healthy skin anatomy and suffer from deficiency of natural supplements in skin covering, sebaceous glands, hair follicles, and sweat glands. Improvements in stem cell biology and skin morphogenesis show significant potentials to evidently advance the engineering of skin replacements which would preferably be vague from normal skin. This chapter reviews these developments in the in vivo and in vitro techniques of engineered and manufactured skin scaffold biomaterials.

Keywords: skin tissue engineering, scaffolds, skin, skin alternates, in vitro skin models, in vivo skin models, biomaterials, skin regeneration, skin renewal

1. Introduction

Tissue engineering is growing as a novel area in biomedical engineering which purposes to redevelop newfangled material for substituting problematic or injured tissues. In order to accomplish this, not only is a basis of cells necessary but also a simulated extracellular matrix (ECM) that the cells which may be reinforced should exist. Human skin signifies about one-tenth of the body form, and injuries like physical distress, infection, burn, disease, or operation to a portion of this main organ carry intense penalties. Tissue engineering

of skin substitutes signifies a potential foundation of improved treatment in fighting acute and chronic skin wounds. Currently, there are no significant prototypes of engineered skin which entirely duplicate the composition, structure, organic constancy, or visual environment of healthy skin. Skin alternates should carry some important physiognomies that comprise being simple to use and implement to the wound location; deliver vital blockade utility with suitable aquatic fluidity; be willingly adherent; have fitting corporeal and mechanical possessions; experience regulated deprivation; be disinfected, nontoxic, and nonantigenic; and induce negligible inflammatory effect. Moreover, they should join to the congregation with nominal damaging and agony and ease angiogenesis, whereas yet being cost operative. The eventual aim of tissue engineering is to gratify most if not all of these standards when creating original, clever skin replacement [1].

2. Structural and progressive provisions

The skin covers epidermal and dermal sheets pervaded via a multifaceted vascular and nervous system. The hypodermis is located underneath, made by moveable linking tissue and fat. Epidermal basal cells and stem cells existing in the basal layer and hair follicles are in control of an unremitting progression of epidermal regeneration. Additional cell varieties that exist in the epidermis comprise melanocytes, Langerhans cells, and Merkel cells [2].

The dermis carries two stratums: a superior papillary layer carrying a reedy organization of collagen fibers and a dense inferior reticular layer with profuse collagen fibers similar to the superficial of the skin. The dermal extracellular matrix is made mostly of collagen, elastin, and reticular fibers. The key constituent of the dermis is the fibroblasts that deliver continuous excretion of the collagen and proteoglycan matrix [2].

Fetal wound repair shows a lack of scarring and fibrosis. This progression is categorized via negligible irritation and renewal of healthy collagen deposition and skin adnexa. The development dynamic outline in fetal renewing skin is significantly diverse from the adult one, being described via advanced intensities of transforming growth factor (TGF)-β3 and minor stages of platelet-derived growth factor (PDGF), TGF-β1, and TGF-β2 [2].

3. Aims of skin tissue engineering

Tissue-contrived skin is a noteworthy improvement in the arena of wound healing. It has primarily been advanced related to the limits linked with the utilization of autografts and allografts where the contributor site agonizes from aching, contamination, and blemishing. Lately, engineered skin substitutes have been covering extensive submission, particularly in the circumstance of injuries, where the main preventive issue is the obtainability of autologous skin. The expansion of an imitated skin enables the action of patients with burns and several skin-associated disorders [2]. The existing review contributes an inclusive outline of the improvements and upcoming forecasts of skin alternates for tissue overhaul and renewal.

4. Current skin substitutes

Autologous keratinocytes may be obtained and cultivated into interconnected layers of the epithelium which may be displaced onto big skin deficiencies on the suffering individual. Clonogenic keratinocytes, defined as holoclones, may be obtained from the skin and consecutively proliferated in culture for more than 140 replications and have revealed to be bona fide multipotent stem cells founded on their aptitude to renovate manifold lines in the skin [3, 4].

These embedded stem cells inside of these epithelial expanses provide repair and regeneration of the epidermis. Developing the epidermal stem cells over fibrin environments or allogeneic dermis has established to be beneficial. The funding materials have significantly enhanced the receiving amounts of the implants, advanced the affluence of managing and operation of the implants, and reduced the wound refutation and scarring. Cultivation of autologous epidermal stem cells simplifies to obtain large epithelial areas for transfer from a minor skin biopsy; therefore, this method needs more than a few weeks [5].

Mounting the stem cells on a substance drops the period necessary to brand outsized epithelial layers from a minor skin biopsy for the epithelia on the material which does not necessitate to attain full confluence of the previous replacement. Furthermore, epidermal stem cells on fibrin environments or allogeneic dermis converse the capability to renew the usual rolled dermal/epidermal connection and the artificial ration of the dermis, named the papillary dermis. Nevertheless, these epidermal stem cell implants lack renovation of a complete practical skin. Epidermal adjuncts, containing hair follicles, sebaceous glands, or sweat glands, are not redeveloped after transferring these implants of epidermal stem cells, signifying that multifaceted epithelial and mesenchymal connections are essential to generate additions. Additionally, the implantations do not reinstate the automated possessions or visual form of the novel skin. Improvements in stem cell biology and skin morphogenesis have the prospective to expand the manufacturing of the skin that may interchange the typical utility and esthetics of healthy skin [5].

5. Skin stem cells

Up to now, scientists have recognized numerous diverse sorts of skin stem cell covering epidermal stem cells, hair follicle stem cells, melanocyte stem cells, mesenchymal stem cells, and recently identified human newborn foreskin stem cells. Epidermal stem cells are in charge for routine regeneration of the dissimilar stratums of the epidermis. These stem cells exist in the basal layer of the epidermis [6]. Hair follicle stem cells safeguard continuous renewal of the hair follicles. They can also restore the epidermis and sebaceous glands in case of injury. Hair follicle stem cells originated through the hair follicles [7].

Melanocyte stem cells are in control of melanocyte revival which is a kind of pigment cell. Melanocytes generate the pigment melanin and so carry a significant part in skin and hair follicle pigmentation. It is not yet clear where these stem cells are located [8]. Studies also indicate

another type of stem cell, known as mesenchymal stem cells, which can be established in the dermis and hypodermis. Mesenchymal stem cells conquer lymphocyte production in vitro and extend skin graft endurance in vivo [9].

Another stem cell that resides in the skin is recently established and named as human newborn foreskin stem cells. They carry pluripotency, and they are capable to turn into different cell types. They show fibroblastic shape; however, they express both mesenchymal stem cell markers and some of the hematopoietic stem cell markers [10].

6. In vivo applications

Autologous skin transfer is now the scientific main protocol for full-breadth skin injuries covering burn damages. Before grafting, primary editing is a significant portion of the handling of burn wounds, as skin temperature-denatured proteins have to be detached to avoid numerous difficulties like contamination, manifold organ impairment condition, hypertrophic mutilation development, unrestrained inflammatory reaction, or infection with pathogenic microorganisms. Microbes could utilize the eschar as a basis of nutriments and are particularly damaging to seriously burnt individual, as this damage also triggers a provisional destruction of cell-related and humoral immunity [11].

Autologous split skin grafts (SSGs) are reaped with a dermatome which separates the epidermis and an artificial portion of the dermis. Residual epidermal cells in the enduring dermis of the SSG giver site will recreate an epidermis. Subsequent to the submission of an SSG to a full-width wound, its vessels unite with the capillary system in the removed wound. This "graft take" is vital for a correct source of nourishment and brings implant endurance. The divided skin contributor place patches up in 1 week and may be utilized for SSG collecting up to four times; though, continual reaping is linked with blemishing at the contributor sites in addition to long hospital visits. Furthermore, in the situation of a wider damage, contributor sites are tremendously restricted and might leave the individual with very small unharmed skin to produce sufficient autologous SSGs. An initial and enduring wound healing is wanted, as it outcomes in negligible or no scarring difficulties, poorer impermanence, and improved practical extended duration outcomes [12]. Oppositely, wound healing postponement is straightly relative to vigorous hypertrophic scarring. In order to indicate the difficulty of restricted SSG reaping sites, an interconnecting method is applied that expanses the implant and so may cope a superior wounded region at the expenditure of cosmetic and practical result [13].

Additional option is the usage of allografts, for a provisional deterrence of liquid loss or infection of the wound. Allografts integrate into serious injuries and deliver ache relief. Therefore, moral as well as protection matters endure, as the severe broadcast for virus-related illnesses and consistent disinfection methods cannot entirely eradicate the probability of infectious mediator conduction. When compared to autologous SSGs, a leading trouble of allografts is that they consent the patients for a long time with wounds likely to problems. Ultimately, allografts experience immunogenic refusal, and the location of wound requires to be enclosed with an autologous SSG. Deferred refusal may happen in people with wide injuries because

of their clinically repressed immune reaction and, nonetheless, finally may be activated via the extremely immunogenic epithelial cells of the allograft throughout its vascularization. Consequently, there is a countless requirement for a substitute that may deliver a more enduring clarification [13].

7. Tissue-engineered skin alternates

Manufactured cell free along with allogeneic cell comprising skin alternates delivers a conceivable resolution to the difficulty of donor implant scarcity. The engineered skin alternatives propose defense from liquid loss and infection while transporting dermal matrix constituents, cytokines, and evolution elements to the wound bed, increasing usual host wound therapeutic answers. Bioengineered skin alternatives may be utilized as impermanent covers once wound damaged tissue up to there is an autograft accessible. Subsequent to assimilation, these assemblies persevere in the wound throughout healing or even afterward. Cell-free biomaterial-related skin alternates may be utilized in integration with autografts as a defensive cover over interconnected autografts to fund their income in addition to arouse the wound bed in the spaces or to expand implant engraftment in parts of pressure. Nevertheless, in contradiction of autografts, tissue-engineered allogeneic skin implants may tolerate the danger of conveying like hepatitis B virus (HBV) or human immunodeficiency virus (HIV). One benefit over autologous in vitro engineered skin alternates is that they have decreased industrial prices [14].

In order to manufacture epidermal replacements, a skin biopsy of 2–5 cm^2 must be picked up from the individual. This may be joined with the first debridement of the injured person. Consequently, the epidermis is detached from the dermis, and solitary keratinocytes are chemically discharged and cultivated on mitotically incapacitated mouse fibroblasts (**Figure 1**). The utilized development media cover fetal calf serum and other essential additions; conversely, it is also likely to enlarge these cells in xenogeneic-free situations. There are numerous revisions analyzing epithelial allografts such as Celaderm; conversely, the efficiency and protection of these harvests have to be established in organized scientific trainings. Along with these custom-built concepts, there have been several laboratories manufacturing cultivated epithelial allografts. Allogeneic crops carry the benefit of abridged industrial charges equated to autologous crops. Yet, an inadequacy of both harvests is that they demonstrate deprived attachment levels that may bring the creation of wounds [15].

On behalf of the management of full-width burns, mutually the epidermal and the dermal layers of the skin require to be substituted, as the action with expanded epidermal (keratinocyte) layers would end in a mediocre conclusion. In contradiction of cultured epidermal layers, engineered dermal concepts can inhibit wound shrinkage, and they deliver a better constancy. The dermal and epidermal counterparts should be submitted successively, as decent dermal vascularization via the debrided wound bed requires to be attained previously to submission of the epidermal stratum. There is an extensive diversity of advertised dermal concepts, both natural and artificial. Some of these alternates are chemical-treated allografts

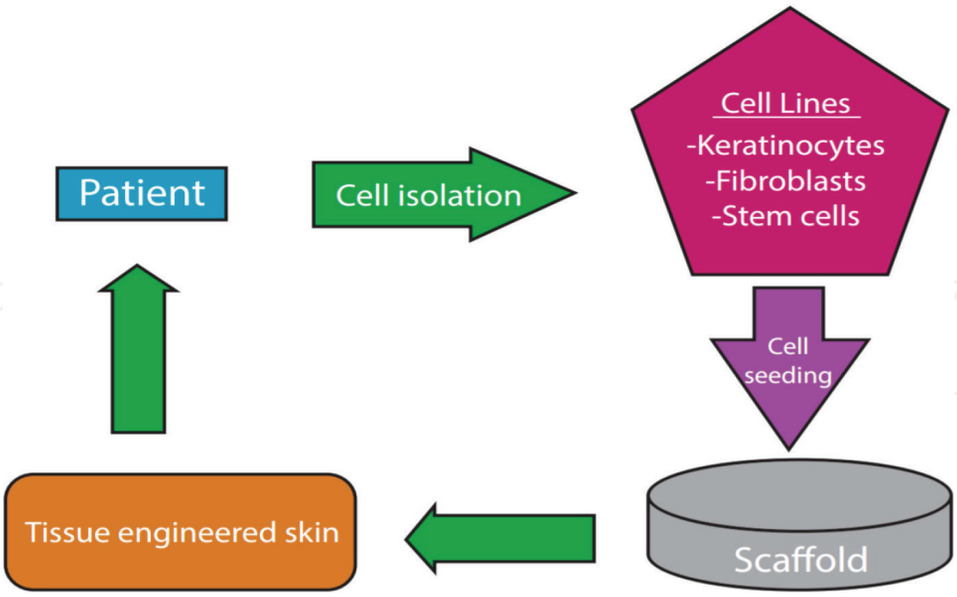

Figure 1. Representation of perspectives of skin tissue engineering. Primary keratinocytes, fibroblasts, and stem cells are obtained from human contributor tissues that are afterward in vitro expanded previously to seeding onto appropriate scaffold materials.

such as Alloderm, deficient for cellular rudiments that are important for the immunogenic refusal [16]. Besides, Dermagraft contains human foreskin fibroblasts, expanded in a fissionable polyglactin network. In these alternates, cells secrete extracellular matrix (ECM) proteins, a variation of growth factors and cytokines into the wound till they experience usual programmed cell death 1 or 2 weeks post-embedding [17].

The most progressive and refined concepts that are accessible for scientific utilization are alternates that imitate epidermal along with dermal sheets of the skin. Even though imitating the histo-architecture of healthy skin, the epidermal/dermal skin alternates would be thought as provisional structurally effective wound layers. Skin replacements deliver growth factors, cytokines, and ECM for host cells; control wound remedial; and may consequence in active pain relief. Main drawbacks are the elevated industrial charges and their insufficiency to heal the wound enduringly regarding to tissue refusal. The immunogenic acceptance of a host in the direction of allogeneic fibroblasts is controversially deliberated. There are different revisions sustaining the theories that allogeneic fibroblasts are individual autologous keratinocytes and are passable for the creation of a perpetual epidermal-dermal skin alternate [18].

8. In vitro applications

Tissue-engineered human skin has been technologically advanced to replicate the main fundamental and practical features of normal skin. In this background, they allow not only the examination of essential procedures in the skin but also the risk valuation of several chemicals which are locally presented to the skin deprived of the necessity to utilize animal models. Outcomes obtained from experimentations showed in animal models are mostly restricted regarding the alterations in the metabolism and the functional architecture. In vitro tests in two-flat monolayer

cultivation of human cells are also of nominal significance because of the absence of multifaceted cell-cell and cell-ECM connections [18]. Conversely, manufactured skin alternates may eliminate these difficulties via utilizing human cells which are organized in a 3D physical background, letting the interface of the dissimilar cell sorts with one additional and nearby matrix [12].

Presently, skin alternatives are utilized in pharmaceutical investigation and in rudimentary examination. In these experimentations, skin alternatives attend as consistent archetypal schemes to classify irrigative, toxic, or destructive possessions of chemicals that arise into communication with the human skin. In basic investigation, skin replacements may assist to explain essential progressions in the skin like the inducements that bring the creation of the epidermis, the cross talk among dissimilar cell sorts, the conservation of the stem cells, the development of wound healing, and the contamination with diverse types of pathogens. One excessive benefit of skin replacements is that the cellular arrangement is entirely manageable via the scientist. Therefore, a specific cell sort may be precisely combined or misplaced to regulate the significance of the cell kind in the organic progression under investigation [12]. Skin tissue engineering may be examined with several in vitro models shown in **Table 1**.

Up to the present time, several sorts of skin alternatives have been established via dissimilar scientific groups. These skin alternatives may be categorized in two kinds. The primary one contains keratinocytes applied on an artificial or collagen transporter faking only the human epidermis. The subsequent one contains a dermal sheet of human fibroblasts entrenched in numerous types of scaffolds [12].

In vitro skin models	Cell foundation	References
Melanoma model	Melanoma mesenchymal cells, fibroblasts, keratinocytes	[19]
UV radiation and phototype	Keratinocytes, melanocytes, and fibroblasts	[20]
Wound healing model	Fibroblasts and keratinocytes	[21]
Psoriasis model	Keratinocytes and fibroblasts	[22]
Full-thickness model	Epithelial sheath, fibroblasts, and keratinocytes	[23]
Ex vivo model	No requirement of cells	[24]

Table 1. In vitro skin tissue engineering models.

9. Full-thickness in vitro models

Although the excessive mainstream of the skin alternates utilized in pharmaceutical investigation is made of an epidermal sheet, these skin replacements could then be developed via the totaling of a dermal stratum covering fibroblasts. In this perspective, fibroblasts have only lately started to have more consideration. It was revealed that skin fibroblasts are distant from being uniform, and it was guessed that a few of the chronic wounds are related to an

alteration in the arrangement of the fibroblasts. It was displayed that fibroblasts clearly effect keratinocyte development in vitro, most probably because of the circumstance that these cells discharge solvable growth factors. In normal skin, the communication among fibroblasts and keratinocytes covers a key role in progressions like wound healing and the creation of the base membrane [1].

Utilizing skin alternatives, it was established that fibroblasts carry a vital part in the normal epidermal histology (**Figure 2**). In the absence of fibroblasts, the keratinocyte differentiation harshly changes and outcomes only in some sheets of extremely distinguished epithelial cells. Fascinatingly, keratinocytes carry a significant influence on the expansion of fibroblasts. This interface of epidermal and dermal cells is theorized because of a double paracrine tool that controls the development of keratinocytes and fibroblasts. Due to the theory, keratinocytes discharge IL-1 which rouses the skin fibroblasts to secrete keratinocyte growth factor (KGF) and granulocyte-monocyte colony-stimulating factor (GM-CSF) that sequentially effect the production of the keratinocytes. Moreover, dermal fibroblasts carry an important part in the renovation of the skin and in the tightening of acute wounds, and they can upturn the struggles of keratinocytes to toxic chemicals. Regarding the outcomes, one could determine that for obtaining significant data from toxicological in vitro experiments. Oppositely, epidermal replacements might be more appropriate for the determination of the diffusion constant through the skin. In monotonous in vitro diffusion studies, a specific part of skin alternates

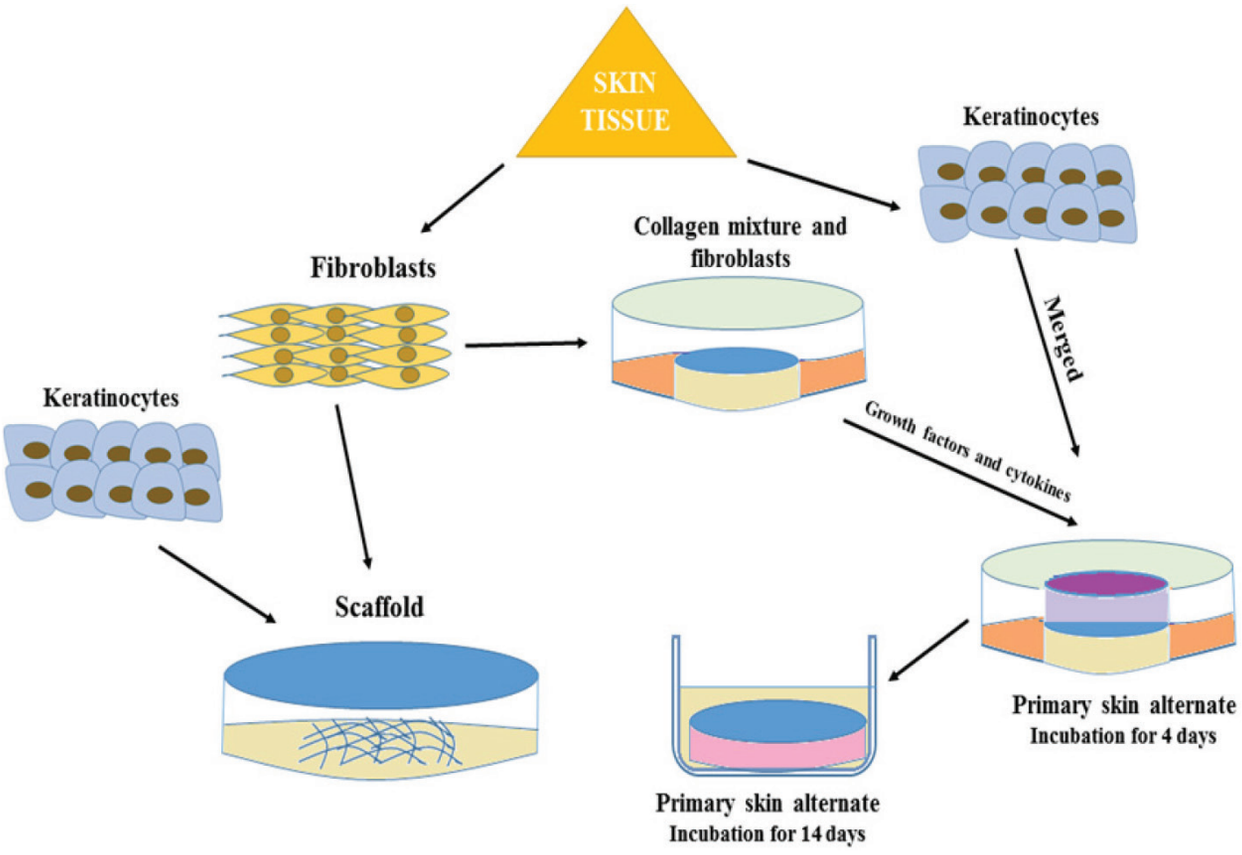

Figure 2. Utilization of skin fibroblast and keratinocyte for skin tissue engineering.

divorces a contributor from an acceptor compartment. Collagen-related full-breadth skin alternates are not ultimate for that kind of tests since they do not cover the entire external part regarding to a low mechanic pliability, consequently finishing in open superiorities, over that the constituent under study may flexibly diffuse [12].

10. Skin gene therapy

The aptitude to hereditarily alter cells utilized to organize skin alternates allows ex vivo gene treatment methods to treat cutaneous illnesses and damages. Nonetheless, gene therapy stays as an unsatisfied potential of cell treatments with skin alternatives. Still, designated analysis of gene therapy in skin wounds has been accomplished recently. Initial models utilizing overexpression of angiogenic growth factors with duplication-incompetent retroviral vectors established viability for constructive distribution of physically dynamic composites such as VEGF and PDGF, with a capacity for prompt wound healing in diminished wounds. Therefore, usage of retroviral gene transmission for the action of hematopoietic syndromes was linked with expansion of leukemia regarding the addition of mutagenesis, an outcome which has fundamentally banned the method from potential deliberation [25].

Parallel threats have been recognized in lentiviruses which have also decreased their forthcoming custom in therapeutics. Replacements to viral tools have been industrialized that comprise plasmid transfection for expression of endogenous antimicrobial peptides like cathelicidin. Additional lately advanced methods such as gathered frequently interspaced short palindromic repeat (CRISPR) arrangement enable site-specific genome editing, greatly reducing the risk of insertional mutagenesis. In addition if gene therapies are managed in allogenic cells that are eradicated immunologically after a restricted period of time, then threats can be minimalized [25]. Nonviral skin gene transfer techniques are listed in **Table 2** individually.

Transfer techniques	Therapeutic mediator	Submission reasons	References
Direct injection	Cytosine-phosphate-guanine class C/ immunestimulatory sequence oligodeoxynucleotides	Tumor treatment	[26]
Electroporation	Antisense oligodeoxynucleotides	Wound healing	[27]
Electroporation	Chimeric RNA/DNA oligodeoxynucleotides	Hair follicle manipulation	[28]
Topical	Liposome-coated DNA	Expressions of growth factors, cytokines, and hormones	[26]
Biolistic	Naked DNA	Immunization	[29]

Table 2. Nonviral skin gene therapy methods.

Induced pluripotent stem (iPS) cells may progress the competence of genome excision. The application of iPS cells includes deterioration of donor cells to a pluripotent state in vitro, growth of cell quantities, and adjustment of cell populace in the direction of a distinguished phenotype of concern. The arrangement of genetic adjustment methods, permitting accumulation of healthy genes or alteration of mutated genes, with iPS knowledge delivers the aptitude to fix hereditary illnesses [25].

Skin alternates consequent to skin stem cells also carry a potential for practicable gene therapy for inactivating inherited illnesses of the skin, like epidermolysis bullosa. The epidermis is systematized into epidermal multiplying components which are self-renovated via at least one epidermal stem cell an propose that transport of epidermal stem cells from the basal stratum of the epidermis for gene treatment might consequence in enduring expression of the transgene [26]. Ex vivo transduced keratinocytes of holoclones have been revealed to have transgene expression which continues for more than 150 cell productions in culture and, more significantly, have been publicized to express the transgene protein once implanted in epidermal stratums in vivo. Autologous epidermal stem cells obtained in culture via development of holoclones were retrovirally interacted with laminin 5 and were effectively relocated in people with junctional epidermolysis bullosa. The implants renewed a healthy epidermis at day 8, and the usual epidermis was preserved during 1 year of continuation [30].

11. Scaffold biomaterials in tissue engineering of the skin

In order to manufacture a body-compatible scaffold, it is important that the scaffold does not cause any acute or chronic response in the body. The scaffold must have a surface that is suitable for cell attachment so that it can replace the damaged tissue and help creating new tissue. If the biomaterial used in the making of the scaffold is biodegradable, newly regenerated tissue can replace the scaffold. Therefore, it is crucial that the scaffold is compatible with the skin tissue. For a scaffold to hold, it must have certain physical and mechanical properties and have a certain chemical structure in the surface. Researchers may use different biomaterials such as collagen, chitosan, hyaluronic acid, and poly(lactic acid) (PLA) in tissue engineering to build scaffolds [31].

11.1. Chitosan

Chitosan is one of the materials that is used in tissue engineering, which is used in wound healing. It is biodegradable, biocompatible, and nontoxic. In addition, it has hemostatic activity. It is also advantageous that chitosan is antibacterial. Chitosan can be used in stimulating collagen synthesis, and its electrostatic function can speed up the healing process. Sponges and gels that are made from chitosan are utilized in the healing process of full-width burn wounds. Chitosan loses its effect in acidic environments, and since wound healing is an acidic incident, cross-link agents may be used to stabilize chitosan structures [32].

11.2. Hyaluronic acid

Hyaluronic acid is a lineal polysaccharide made of repeating disaccharide elements of N-acetyl-glucosamine and n-glucuronic acid. Hyaluronic acid can be found in human skin and is known to be speeding up the healing process. Apart from these, it is observed that hyaluronic acid amount is increased in fetal skin and wounds in case of scar-free healing. Hyaluronic acid is a material with so much perks in scaffolding such as expanding the possibilities of cross-linking, delaying the biodegrading of materials, and more control over mechanical aspects of the process. Also, hyaluronic acid offers more incorporation of cell adhesion ligands and growth factors in the making of scaffolds. Aquatic uptake ability, flexibility, and biocompatibility of the scaffold are some of the properties that are made possible and enhanced by HA [32].

11.3. Collagen

Collagen is a naturally found protein that can enhance the structural integrity. Collagen can be found in human skin tissue and mostly created by fibroblasts and myofibroblasts. In the body parts that are under stress and used often, for example, the skin, tendons and bones, collagen can be found in fibrils. One of the most common types of collagen which is also seen widely in scar tissues as well as the dermis, fasciae, and tendons is type I collagen. There are 20 variations of collagens, and only types II, III, V, and XI can make up fibrils. Collagen is one of the most used materials that have been utilized in skin tissue engineering, and only recently it has been possible to create a model that can promote human capillary-like network [33].

It is an excellent material for scaffolding because of its ability to boost cell attachment, migration, proliferation, and differentiation. It is preferred in medical applications as a primary material since it is excellent in biocompatibility, biodegradability, and weak in antigenicity. Scientific researches provided recombinant human collagen, and it proves to be a more dependable foundation for collagen which is not animal based. Human-based collagens are used in scaffolding, and they show promising results in efficiency for manufacturing skin, cartilage, and periodontal ligaments. Permeable collagen matrices with specific structural, biochemical, and biotic characteristics are interesting materials for tissue engineering, and introducing glycosaminoglycans may add to these characteristics because they are constituents of ECM proteins [34].

11.4. Silk

Silk is a biopolymer that is found in nature and has been used in medical applications for centuries. It contains filament core protein, named fibroin, and a glue-similar coating with sericin proteins. Silk can be composed into many forms such as films, fibers, meshes, and sponges, and these forms have been used in many incidents, show great promise in supporting stem cell union, multiplying, and distinction in vitro, and are known to be boosting tissue repair in vivo. Skeletal structures such as bone, ligament, cartilage, and connective tissues such as the skin have been engineered using 3D silk fibroin scaffolds in stem cell-related tissue manufacturing [35].

11.5. Fibrin glue

For some time now, fibrin glue has been used for medical applications such as plastic surgery and reconstructions as an adhesive compound. It is antibacterial, as well as it boosts hemostasis. Apart from these, fibrin helps grow keratinocyte and fibroblast in vitro and in vivo and therefore promotes cellular movement in the wound. It has been observed that endogenous fibrin clots to create a temporary matrix in a purpose of promoting angiogenesis in the primary stage of wound healing. It is recognized that some growth factors are increased during the wound healing process to promote angiogenesis. Vascular endothelial growth factor (VEGF) is one of these. Furthermore, if fibrin is preferred as a dermal substrate for an alternative of cultivated skin, it upsurges the discharge of VEGF, thus indorsing angiogenesis [34].

11.6. Artificial fragmental polymers

A few of the artificial fragmentable polymers utilized as permeable scaffolding constituents cover polyethylene glycol (PEG), poly(lactic acid) (PLA), polyglycolide (PGA), poly(lactic-co-glycolic acid) (PLGA) [34], polycaprolactone (PCL), poly(D,L-lactic acid or D,L-lactide) (PDLLA), polyester elastomer (PEE) founded on polyethylene oxide (PEO), and polybutylene terephthalate (PBT). There are some synthetic polymers with biodegradable properties that are highly preferred as permeable scaffolding constituents such as polyethylene glycol (PEG), poly(lactic acid) (PLA), polyglycolide (PGA), poly(lactic-co-glycolic acid) (PLGA) [35], polycaprolactone (PCL) [17], poly(D,L-lactic acid or D,L-lactide) (PDLLA), polyester elastomer (PEE) based on polyethylene oxide (PEO), and polybutylene terephthalate (PBT) [37].

A superlative absorbent scaffold in skin tissue engineering should be the one which imitates the normal surroundings for skin development over suitable cell penetration, propagation, and differentiation. It should be biodegradable and penetrable to oxygen, aqua, and nutrition interchange and must be defensive contrary to contamination and injury [38]. Up to the present time, there have been numerous kinds of absorbent scaffolds defined for skin tissue renewal, and most of them may be branded as fibrous permeable scaffolds. Nevertheless, there are some spongy or foamy scaffold sorts with advanced absorbency that may be utilized as operative concepts for skin renewal. Supreme of these scaffolds has collagen as a foundation, and then keratinocytes or fibroblasts are planted into the scaffolds [39].

When choosing a porous scaffold to be used in skin tissue engineering, one must look for some properties and characteristics to create optimum conditions that resemble the usual background for skin development of the most over suitable cell permeation, creation, and distinction. The ideal scaffold also should safeguard against contamination and injury. There have been plenty of porous scaffolds with various forms to this day that are described for the regeneration of skin tissue, and most of these may be seen as fibrous absorbent scaffolds. Other than these scaffolds, there are also various types of spongy or foamy scaffolds which have higher porosity and can be used in skin regeneration. Collagen is the main ingredient in most of these porous scaffolds with keratinocytes or fibroblasts that are seeded into the scaffolds [36].

Scaffolds are designated in severe burns and skin deficiencies persuaded because of the elimination of tumors or skin implanting in patients experiencing necrotizing fasciitis owing to

bacterial contaminations [40]. Meanwhile, some original sponge scaffolds in arrangement with biomaterials like human keratin and polyvinyl alcohol/chitosan have also been described for their utilization as operative skin alternatives. Scaffolds have been used in medical applications such as acute burns and skin defects. Apart from these, some novel sponge scaffolds when used with biomaterials like human keratin and polyvinyl alcohol/chitosan have also been used since they are so effective as skin substitutes [1].

Nanofibrous scaffolds are extensively utilized for firming along with lenient tissue engineering submissions, and they also perform as tools for the regulated distribution of drugs and numerous biological particles in the arrangement of proteins and DNA [41]. Numerous usual and synthetic polymers have been applied for nanofiber constructions to generate fibrous scaffolds for biomedical applications [42]. These nanofibers are occasionally precisely functionalized via a basic interference or coating method or with superficial implanting polymerization by adding ligands and adhesive proteins on the nanofiber shallow. Combination of drugs, development factors, and genes straightly into the polymer elucidation throughout electrospinning is also a training for precise discharge possessions. Current methods for integrating therapeutic mediators or bioactive particles comprise coaxial electrospinning, suspension electrospinning, and alterations by external absorption or chemical conjugation [43]. In lieu of soft and hard tissue engineering and its submissions, nanofibrous scaffolds are widely used as well as deliver drugs in a controlled manner. Synthetic and natural polymers have been used for nanofiber manufactures to harvest rubbery scaffolds for biomedical presentations [44].

12. Discussion

The largest part of the human body is the skin. One of its key responsibilities is to protect the body from the environmental influences. When this shield is lost both acutely and chronically, it must repair itself to avoid termination of the life of its carrier. When the skin is wounded, re-epithelialization of the wounded surface occurs [1, 2, 15–17]. Another important subject is that extensive wounds require an additional protection layer. This layer helps preventing desiccation and infections. It also guides cells to repair the wound and improve healing rates. This requirement led to the evolution of biologic and synthetic dressings and skin substitutes [14]. Wounds that occur due to massive burning made inventing different kinds of temporary and permanent skin alternatives necessary. This was needed because the patient could not repair its skin by itself. To be successful in long-term recovery, certain properties from both dermal and epidermal skin should be considered [9]. There is no example where an artificial skin is better than the original. Because of this, tissue engineers must focus their efforts on producing a universal skin that would be the best alternative in the shortest amount of time [13, 19].

A scaffold is used to create the three-dimensional structure for cell interactions and ECM production. It carries proper cytokines and growth factors to the target area [18]. It also supports the structure and functions of the newly formed tissue. There are certain criteria for a scaffold to provide all of the above [34, 36, 39, 41]: it should have a proper internal structure and surface that can support cellular behaviors such as adhesion, differentiation, and proliferation [30]; it should have mechanical properties alike to the ones in the repair site [30]; and

it should be biocompatible. Its properties rely on the modifications that were applied to it and the nature of the biomaterial. Sponginess and pore scope of polymeric scaffolds display a critical role in tissue regeneration [38]. These considerations have been comprehensively deliberated and covered in different publications. Porous organization of scaffolds is important with regard to their allowance of migration, adhesion, and multiplication and also diffusion of wastes, oxygen, and nutrients [31]. It is certain that larger pores are used for supplying nutrients and removal of wastes. Small pores on the other hand provide surface area for cell adhesion. In order to generate dissimilar pore sizes and porosities, used techniques vary from salt discharge, modified lyophilization, phase separation, application of numerous freezing temperatures, application of various acoustic heaviness amplitudes, and application of different electrospinning rates [36].

Reports show several types of scaffolds used in skin tissue regeneration. Even though there are some setbacks, they have been notably successful for repairing tissue and wound healing. They are also successful for delivering regular supply of nutrients and oxygen to cells, due to their variable porosities [36]. Mechanical strength and biocompatibility may be a subject of concern for fabrication of scaffolds, but composite and ceramic types have a promising future. Various biomaterials, both natural and synthetic, are used separately or in combination to create scaffolds. Combinations of these materials overcome the issues with biocompatibility, biodegradability, and mechanical strength [33]. Collagen, cellulose, and chitosan are some of the examples for natural biomaterials [34]. They are found as either polysaccharide or proteins. These are highly biocompatible and easily degraded due to their similarities to the natural ECM. This makes them highly suitable for skin cell growth. If we look at synthetic biomaterials, some nanomaterial-type examples like polyvinylpyrrolidone (PVP), polycaprolactone (PCL) [41], poly-ethylene-glycol (PEG), poly lactic acid (PLA) [31, 35], and others are good for enhancing the strength of the scaffold. This requires more research and efforts toward the direction of composite scaffolds. An extensive knowledge of all the facts listed above will lead to producing highly effective and suitable scaffolds for skin tissue regeneration. There are many subjects in tissue engineering that should be overcome though such as scaffold interaction with cells, cellular proliferation and differentiation speed, and vascularization of the engineered tissues [37–39]. Rapid advancement of organ-on-chip technology, which led to "skin-on-chip" technology, has cleared the way for generating engineered skin for wound healing and drug testing [12]. The development of perfused chip-based bioreactors offers improvement of culturing conditions for skin organ cultures, as well as variable mechanical stress. Another advancement to note is microfluidic technologies, which are developed to create perfused skin-equivalent cultures and show a promise in the field of applying various drug molecules that are associated with skin tissue and wound healing [14, 17, 32, 33].

In vitro skin prototypes are used for recognizing skin destructive or poisonous materials and have been established to be beneficial implements for the examination of rudimentary evolving progressions as well as for the documentation of compulsive circumstances. Even though the enterprise of the epidermal and dermal stratum imitating in vitro skin alternatives is almost ultramodern, there are obviously alterations among these replicas and natural in vivo skin [45]. One drawback of in vitro replacements is that they are not simply shaped, organized, or deposited. Practically, all in vitro skin is tailored and factory-made manually. A mechanized method would expressively decrease industrial charges and would propose

the comprehensive regulation of the procedure with a consistent result. The utilization of human keratinocyte derivative cell lines that are capable to cornify would support to lessen the charge of a skin replica even further and recover the expectedness of the last construct. Inopportunely, the mainstream of the presently obtainable cell lines is derivative of carcinoma cells that lack the capacity to arrange a corneous layer; only lately, a novel cornifying cell line was presented [46]. Additional factor that is restraining the accomplishment of skin alternatives is their short lifetime. One method to accomplish a prolonged usability is the expansion of appropriate skin substitute conservation procedures as it had been done for dermal skin grafts [47].

The sustained development of iPS cell reprogramming widens a perspective to patient with precise stem cell foundations for tissue substitution. The competence of iPS production was lately established to be enriched with the utilization of keratinocytes [48]. Moreover, iPS can be produced from keratinocytes obtained from hair. Therefore, pulling a single hair from a person delivers only the initial substantial required to make iPS cells that may afterward be differentiated to custom tissue-related stem cells [49]. In different words, it can be thinkable for keratinocytes isolated from hair to be unswervingly reprogrammed to many skin stem cells deprived of an in-between iPS state. The aptitude to generate different skin stem cells which can be integrated into an engineered skin tissue will allow renewal of all of the complex cell types within the skin.

Recently identified human newborn foreskin stem cells also carry an enormous potential to differentiate into different cell types which may also eliminate the risk factors of gene transfections in order to obtain stem cell properties. These cells have found to carry mesenchymal stem cell markers and some of the hematopoietic stem cell makers. Specific study of ours proved that they may be differentiated into chondrogenic, adipogenic, osteogenic, neurogenic, epithelial, and myogenic cells. In future prospects, they carry a potential to be used in tissue engineering models [10].

13. Future prospects

Future advances in in vitro skin alternatives should cover skin additions. The combination of sweat glands and hair follicles will assist to imitate a more accurate in vivo state and consequently involve a more precise experimental format. Many developments have been completed in the last decades for creating vascularized full-thickness in vitro skin models; nevertheless, the vascular-like structures could not be combined with an external vascular system wherein physiological shear circumstances are continued; thus, the growth of the in vitro vasculature was disallowed. The usage of more progressive biological scaffolds or synthetic vasculature imitating structures might assist to eliminate these problems.

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