



Novel strategies for designing regenerative skin products for accelerated wound healing

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Abstract

Healthy skin protects from pathogens, water loss, ultraviolet rays, and also maintains homeostasis conditions along with sensory perceptions in normal circumstances. Skin wound healing mechanism is a multi-phased biodynamic process that ultimately triggers intercellular and intracellular mechanisms. Failure to implement the normal and effective healing process may result in chronic injuries and aberrant scarring. Chronic wounds lead to substantial rising healthcare expenditure, and innovative methods to diagnose and control severe consequences are urgently needed. Skin tissue engineering (STE) has achieved several therapeutic accomplishments during the last few decades, demonstrating tremendous development. The engineered skin substitutes provide instant coverage for extensive wounds and facilitate the prevention of microbial infections and fluid loss; furthermore, they help in fighting inflammation and allow rapid neo-tissue formation. The current review primarily focused on the wound recovery and restoration process and the current conditions of STE with various advancements and complexities associated with different strategies such as cell sources, biopolymers, innovative fabrication techniques, and growth factors delivery systems.

Keywords Skin · Chronic wounds · Engineered skin substitutes · Wound healing · Cell sources · Fabrication techniques · Growth factor delivery systems

Abbreviations

3D	Three dimensional
3R's	Regeneration, repair, and replace
3T3 fibroblasts	3-Day transfer, inoculum 3×10^5 fibroblast cells
ADSCs	Adipose-derived stem cells
AgSD	Silver sulfadiazine
ATMP	Advanced therapy medical product
BMSCs	Bone marrow stem cells

BC	Bacterial cellulose
CAM	Computer-aided manufacturing
CAD	Computer-aided design
CEA	Cultured epithelial autografts
Ceffe	Cell-free fat extract
Ch	Chitosan
CM	Cortex Moutan
CNCs	Cellulose nanocrystals
Col	Collagen
CS	Chondroitin sulphate
dECM	Decellularized extracellular matrix
DEJ	Dermal–epidermal junction
DFUs	Diabetic foot ulcers
ECM	Extracellular matrix
EE%	Encapsulation efficiency percentage
EMA	European medicines agency
EUP3	Polysaccharide— <i>Eucommia ulmoides</i>
FDA	Food and Drug Administration
GAGs	Glycosaminoglycans
GFs	Growth factors
hiPSCs	Human-induced pluripotent stem cells
hBMSCs	Human bone marrow derived mesenchymal stem cells

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HLA	Human leukocyte antigen
HeCaT	Human epidermal keratinocyte cell line
HFF	Human foreskin fibroblast
HDF	Human dermal fibroblasts
HUVECs	Human umbilical vein endothelial cells
HEKs	Human epidermal keratinocytes
HA	Hyaluronic acid
hKCs	Primary human keratinocytes
hFBs	Primary human fibroblasts
IGF-1	Insulin growth factor-1
KCNH-1	Potassium voltage-gated channel sub-family H member-1
Kef	Kefiran
KGF	Keratinocyte growth factor
L-929	NCTC clone 929 Clone of strain L fibroblast cell line
MSCs	Mesenchymal stem cells
MDA-MB-231	Epithelial, human breast cancer cell line
MCF7	Breast cancer cell lines
M Φ	Macrophages
NIH3T3	Mouse embryonic fibroblast cells
NHDF-neo cells	Human dermal fibroblasts-neonatal cells
PLA	Poly(lactic acid)
PCL	Poly(ϵ -caprolactone)
PLGA	Poly D,L-lactic-co-glycolic acid
PDGF	Platelet-derived growth factor
PVA	Poly(vinyl acetate)
PGA	Poly(glycolic acid)
PEO	Poly(ethylene oxide)
PU	Poly(urethane)
PEG	Poly(ethylene glycol)
PMNs	Polymorphonuclear neutrophils
rhVEGF	Recombinant human vesicular endothelial growth factor
SIS	Small intestine submucosa
STE	Skin tissue engineering
STSGs	Split-thickness skin grafts
TE	Tissue engineering
TESS	Tissue-engineered skin substitutes
TERM	Tissue engineering and regenerative medicine
TGF- α,β	Transforming growth factor- α,β

Introduction

The human skin is the major and the most complex multilayered sensory organ, encompassing 1–1.5 m² of total exposed surface area and also accounts for one-tenth of overall body mass (Moore and Chien 1988). It shields the body's internal vital organs from the hostile environment. Skin tissue is

subdivided into three layers, called: the epidermis (upper layer), dermis (middle layer), and subcutaneous tissues or hypodermis (bottom layer), as shown in Fig. 1 (Kanitakis 2002; Abdo et al. 2020). The outermost waterproof epidermal layer is mainly composed of 90% of viable Keratinocyte cells along with melanocytes (containing skin color pigment-protein called melanin providing color to the skin and acts as a harmful UV ray barrier), Merkel cells (which mediate sensations and help in pressure detection on the skin), and Langerhans cells (provides immunity by recognizing antigens and presenting them to T-helper cells which further activates the immune responses) (Brohem et al. 2011; Yousef and Sharma 2018). In-between both the epidermis and dermis layers, a dermal–epidermal junction (DEJ) is found, called Rete ridges. It provides mechanical strength to the epidermis and also allows the diffusion of soluble molecules within the layers (Butcher and White 2005).

The dermis, or middle stratum of the skin, is positioned between the epidermal and subcutaneous fatty layers. It is an integrated connective tissue system comprising ECM components (including collagen, elastin, reticulin, polysaccharides), blood vessels, fibroblasts, mast cells, hair follicles, sweat glands, nerve endings, and lymphatic systems (Brohem et al. 2011; Roig-Rosello and Rousselle 2020). This layer provides elasticity, tensile strength, and pliability to the skin, thus protecting the body from abrasions and helping in wound healing (Roig-Rosello and Rousselle 2020).

The subcutaneous fatty layer or hypodermis is the bottom-most part of the skin tissue that acts as a storehouse of energy and exclusively comprises fat cells, fibroblasts, nerves, and blood vessels (Brohem et al. 2011). Its major role is to provide cushioning and heat to the body (Lawton 2019; Watt 2014). Overall, each layer of the skin has distinct functions and performs multiple active roles such as thermoregulation, body homeostasis, sensation, and body structure confinement. Additionally, it provides protection against physical, chemical, and biological influences caused by the external environment. Despite the above key roles, the skin also has a pivotal role in vitamin D synthesis and storage (Barry 1983; Park 2015; Lawton 2019).

Skin injuries can occur for various reasons, including thermal, mechanical, physiochemical, and biological damages. Depending on nature, type of damage, and its ability to cure, they fall into two categories: acute and chronic wounds (Boateng et al. 2008; Augustine et al. 2014). Acute wounds heal completely within 8–12 weeks by undergoing normal stages of wound healing. However, chronic wounds heal slowly, taking more than 12 weeks and leaving abnormal scars. It has been seen that chronic wounds reappear after recovery because of several factors such as poor blood circulation, edema, repetitive trauma, extensive wounding, infection, aging, obesity, and diseases such as diabetes and autoimmunity (Boateng et al. 2008; Cole-King and Harding

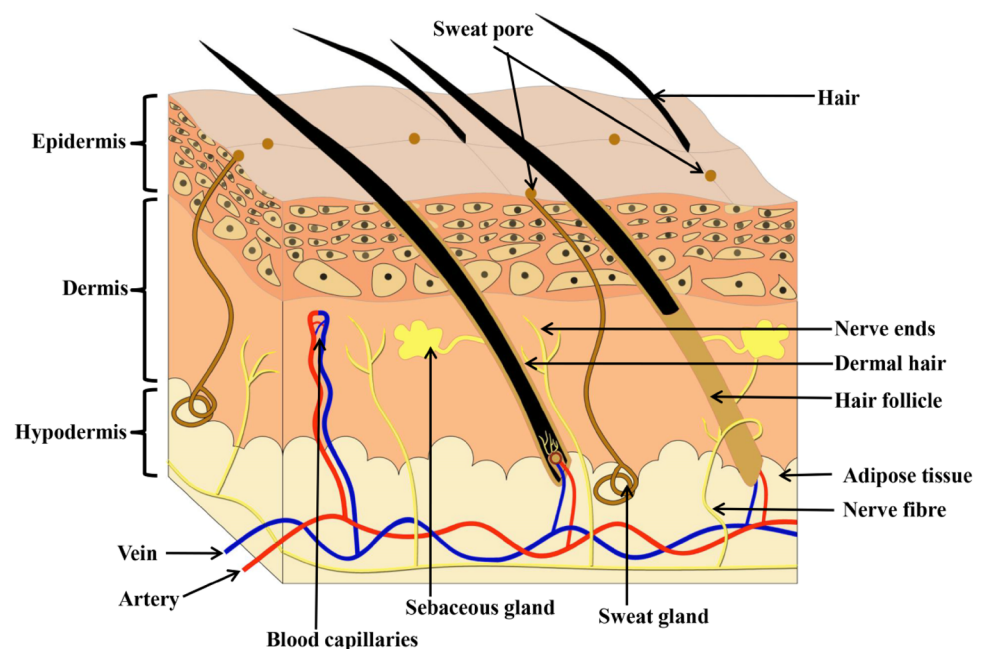
2001). Although precise observational studies on impaired chronic wounds are tough to obtain realistic data. However, research conducted in the United States proclaimed that chronic wound sickness affects 6–6.5 million individuals worldwide, costing \$20 billion in the United States each year (Sen et al. 2009). As a result, wounds have a high incidence of severity and fatalities, as well as a significant psychological, psychosocial, and financial influence on individuals and substantial expenditures for the health system, making them a critical public health issue.

Skin wound healing is the inherent and highly complex process of the human body which involves several sequential phases, including hemostasis (blood clotting at the wound site), inflammation (immune cells infiltration that cleanses out the dead cell debris), re-epithelialization (keratinocyte's migration from the periphery to the center at a wound site), granular tissue formation (multiplication and migration of fibroblast cells and endothelial cells that secretes ECM components), vascular tissue regeneration and dermal remodeling (collagen remodeling and wound size reduction) (Demidova-Rice et al. 2012; Rousselle et al. 2019). Different external and internal factors significantly affect the tissue healing process, including body fluid, intrinsic and extrinsic factors, temperature, stress, oxygenation, medications, infections, diabetes, obesity, and nutrition. A healing process also varies with age; fetal wound healing happens rapidly, leading to scarless tissue formation, whereas the healing process slows down and leads to scarring in adults (Guo and Di Pietro 2010; Ding et al. 2021). The sequential regulated cycles that occur just after skin tissue damage and scar development are remarkably equivalent to those that occur after myocardial infarction and many other tissue injuries (Evans et al. 2013). In this respect, particularly

owing to its availability, skin is among the greatest models for studying tissue regeneration mechanisms and developing innovative biomedical approaches.

Tissue engineering (TE), a crucial rising topic in therapeutic medical science and biological engineering, has shown tremendous promise in designing and constructing biosynthetic alternatives for cadaver tissues or organs, implants, and prosthetics to reduce patient suffering and mortality rates. Tissue-engineered skin substitutes (TESSs) provide an alternative therapeutic medication for major skin tissue injuries and burn wounds. Since the successful isolation and expansion of human epidermal cells and keratinocytes in 1975, TESSs have evolved from epidermal replacements to full-thickness grafts, including multiple skin seeded cells (Kim and Evans 2005). Recently, an incredibly innovative advancement in designing and constructing skin substitutes has been clinically reported in the domain of STE. Further, the skin tissue construct should meet the following attributes: clinical effectuality, biodegradability, durability, elasticity, biocompatibility, low toxicity, cytocompatibility, and medical stability. Although skin tissue engineering is one of the prominent therapies where translational investigations are at the frontline, the need to consider several important factors while designing and constructing a skin tissue scaffold, such as physicochemical properties, excellent interconnectivity, standard pore size, permeability, etc., makes it much more challenging. For decades, split-thickness skin grafts (STSGs), blended and clinically grown skin autografts have been the global method of treatment for extensive burn wounds and are still the method of choice for wound closure (Liu et al. 2019). Commercially available acellular dermal substitutes like Integra[®], Renoskin, Hyalomatrix, and Terudermis, comprising dermal and epidermal components, are

Fig. 1 Human skin structure



extensively used by surgeons to achieve wound contraction and healing. However, these substitutes have certain limitations due to high economic costs and vulnerability to infection (Chocarro-Wrona et al. 2019).

Various innovative advancements in designing and constructing skin substitutes have recently been clinically reported in the STE domain. Significant advancement has been achieved in designing and fabricating grafts for regenerative and biomedical purposes during the last few decades. However, a skin substitute that replaces or resembles the anatomy and physiology of the native healthy skin is yet to be achieved. The central purpose of this review article is to show the many approaches used in the research area of STE. It discusses the most up-to-date sophisticated techniques for creating perfect and effective skin replacements that can imitate the ECM structure of the skin tissue.

Overview of skin wound healing mechanism

The epidermis, dermis, and subcutaneous or fatty layers of skin serve as a defensive layer to shield pathogenic microbial infection and fluid loss (Yousef et al. 2017). A wound is developed due to the disturbance of layers of skin tissue. In superficial or minor wounds, the skin repairs to generate tissue nearly identical to the native skin. However, if the lesion is sufficiently deep or extensive, it will regenerate, leading to scarring. These injuries are regularly restored and regenerated in the human body through the inherent capability of the skin wound healing process. The complex and sequential skin repair process has been broadly grouped into four regulated overlapping stages: hemostasis (or blood clotting phase), inflammatory (activation and infiltration of immune cells phase), proliferative (multiplication and migration of skin cells), and tissue maturation/remodeling (extracellular matrix protein remodeling phase) (Zomer and Trentin 2018). The early stage in skin wound repair is hemostasis, and this phase lasts for seconds to hours. This phase includes many steps such as vasoconstriction, which decreases the flow of blood; platelet stimulation and recruitment at the site of damage which continues to produce numerous growth factors (GFs) that further promote cellular migration and multiplication; and lastly, the thrombus formation all around the blood clot (Rajendran et al. 2018).

After the clotting of blood at the injury site, within an hour, different inflammatory cells move into the injury and initiate the inflammation phase, which is marked by the successive infiltration of polymorphonuclear neutrophils (PMNs), monocyte differentiated macrophages (MΦ), and lymphocytes (Short et al. 2022). These inflammatory cells actively participate in phagocytosis, eliminating pathogenic microorganisms and cellular debris from the wound. Furthermore, they also secrete various GFs and cytokines,

which facilitate the activation of different cells from nearby tissues, such as fibroblasts which further differentiate into myofibroblasts, endothelial cells, and keratinocytes during the proliferation phase (Wallace et al. 2017). These cells continue to migrate and proliferate, synthesizing new ECM, promoting neo-vascularization and forming granular tissue, progressively establishing an obstruction between the wounded region and the environment, permitting wound closure (Armstrong and Meyr 2022). The remodeling phase of the wound repair process, which lasts weeks to months, co-occurs with the development of neo-granular tissue. During this phase, recruited cells from the injured site (fibroblasts, myofibroblasts, macrophages, endothelial cells, and keratinocytes) remodel the skin's ECM to form collagenous networks. These cells release matrix metalloproteinases that degrade unorganized collagen Type III of the granular tissue and start replacing it with highly organized collagen Type I matrix, which closely resembles the intact skin tissue (Wilkinson and Hardman 2020).

The conventional approach to wound healing

The skin tissue itself has the capability to restore the damaged tissue naturally through the process of re-epithelialization and helps in maintaining its integrity and tensile strength (Barry 1983; Swaney and Kalan 2021). It is also observed that the self-healing properties of the skin get weakened in some circumstances, such as deep chronic wounds, diabetes, and non-healing ulcers, which further increases the chances of infections and also affects the patient's daily life. Thus these wounds require wound dressings that can assist and strengthen the wound healing process at the desired pace (Watt 2014; Lee and Koehler 2021). Wound patches, including natural or synthetic dressings, cotton wool, lint, and gauzes, were once used to keep injuries dry and sterile by aiding wound exudates to evaporate and preventing harmful microorganisms from infecting the wound (Boateng et al. 2008; Pereira and Bartolo 2016). Since pre-modern times, conventional techniques have been used to treat damaged tissue because of their low cost, feasibility, simplicity, and effectiveness in the wound healing process.

The skin grafts provide instant coverage for extensive wounds and protect against infections and fluid loss. The consolidated role of the graft further reduces inflammation and allows rapid wound healing. Surgeons generally use autografts to treat more profound dermal injuries due to their non-immunogenic nature (Koller 2005; Luo et al. 2019). Various drawbacks, including limited donor availability, are associated with autografts. The above limitations can be sorted out using allografts, genetically engineered xenografts, and amnion membranes (Koller 2005;

Carter and Holmes 2016; Cooper et al. 2016; Liu et al. 2010). Clinically, it has been reported that most grafts may cause immune rejection and disease transmission during transplantation (Koller 2005; Liu et al. 2010). The placental amniotic layer in mammals is the thinnest, measuring up to 0.5 mm thick. (Murphy et al. 2017). Since the early twentieth century, amnion membranes have been employed extensively for wound and regenerative applications (John 2003). The human amniotic membrane is a safe, painless, and effective method to treat wounds and has been reported in numerous studies (Murphy et al. 2017; Farhadhosseini-Abadi et al. 2018). Irrespective of its benefits, it has significant drawbacks, such as low availability, quick biodegradation, handling issues, and high cost, all of which limit its use (Taghiabadi et al. 2015; Murphy et al. 2017). As a result, researchers in the discipline of tissue engineering and regenerative medicine (TERM) have been working to resolve the drawbacks of conventional established methods and develop a skin substitute that can mimic the functional skin tissue and be used to treat a broad range of skin problems, including acute and chronic wounds, as well as burns. Some of the current conventional graft implants that have been analyzed are tabulated below with their pros and cons as follows (Table 1).

Skin tissue engineering: principles

Tissue engineering, as described by Langer and Vacanti (Vacanti and Langer 1999), is an "inter-disciplinary field or a growing approach that uses the principles of basic biology, engineering and fundamental science for the restoration, improvement, and maintenance of damaged tissues, organ failure, and regeneration of the lost tissue. In recent times advancement in STE minimizes the need for organ transplantation, reducing graft rejection, pain, the need for solid immune suppressants, the transmission of diseases,

complications, and the cost of processing (Hutmacher et al. 2001; Cao et al. 2020).

In skin tissue engineering, an ideal skin substitute should possess some desirable properties such that they should mimic the local skin environment, speed up the healing process of wounds, cytocompatibility, non-toxicity, allows regulated and sustained drug/growth factor release at the target site without losing its bioactivity and so on (Halim et al. 2010; Lord et al. 2017; Chen et al. 2022). A modern era of tissue engineering was started in the 1980s with the ultimate goal of maintaining, improving, strengthening, and recovering damaged or lost tissues (Alrubaiy and Al-Rubaiy 2009). The basic intellection behind tissue restoration includes 3Rs: tissue regeneration, repair, and replacement. Regeneration or resurrection is basically where the tissue is replaced with tissue itself, thus, initiating the regeneration where it is usually not seen. In tissue repair, drugs or growth factors are externally delivered to the targeted site to enhance the repair rate.

Furthermore, replacement is the possible alternative where tissue regeneration and repair are not possible. This approach replaces the damaged or lost tissue with a substitute. The 3R's concept can be applied to all types of tissues; however, the degree of complexity would differ among the targeted tissues, for example, kidney, heart, bone, liver, etc. The fundamental goal of this approach is to overcome the certain limitations associated with conventional methodologies and provide an alternative treatment to patients suffering from different disabilities and ailments (Theoret 2009).

The approach of tissue engineering triad

The tissue engineering strategies basically involve the concept of using the living cells (autogenic, allogenic, xenogenic, syngenic, stem cells, and genetically engineered cells), natural or synthetic scaffolds (must be biocompatible and biodegradable), and signals (growth factors, drugs,

Table 1 Conventional methods of wound healing

Skin graft	Advantages	Disadvantages	References
1. Autograft (Isograft)	No graft rejection; satisfactory clinical findings; cost-effective; less or no immune responses	Limited donor sites; risk of cell morbidity; cause pain, infections; scarring; ethical issues	Koller (2005); Carter and Holmes (2016)
2. Allograft (Homograft)	Temporary graft; barrier against infections and fluid loss; reduces inflammation; abundant graft availability	Disease transmission; immune rejection; cost; strong immunosuppressants are required	Koller (2005)
3. Xenograft (Heterograft)	Ample graft availability; cost-effective; reduces pain	Graft rejection; inflammatory response; strong immunosuppressants are required; risk of infections; vascular barrier	Koller (2005); Cooper et al. (2016)
4. Amnion layer	Reduces fluid loss; prevents infections; reduces pain; it Hastens wound healing and closure	Limited donor availability; disease transmission; rapid degradation; handling issues; high cost	Liu et al. (2010); Murphy et al. (2017); Taghiabadi et al. (2015)

mechanical forces, and physiochemical signs) independently or in different combinations, to build a 3D construct. This concept further helps to regenerate the functional tissue-like structure under controlled laboratory conditions for other clinical applications (O'Brien 2011; Almouemen et al. 2019). These three factors are collectively known as the "Tissue Engineering Triad," as shown in Fig. 2.

Currently, there are different ways to develop a functional tissue or an organ by using scaffolds, cells, or signaling molecules that can be accustomed separately or in various combinations in the form of a triad (O'Brien 2011). Even though these factors have shown promising results when used independently, they can only help regenerate the target tissue to a certain extent. For instance, Cultured Epithelial Autografts (CEA) and Keratinocyte suspensions like Epicel, RECELL, and BioSeed have been used as clinical adjuncts with conventional treatment methods to accelerate wound re-epithelialization. However, they are still limited to minor injuries (Brockmann et al. 2018). Therefore, to achieve the comprehensive, a multi-functional skin substitute is required that can mechanically, micro-structurally, and functionally mimic the healthier tissue. The extraordinary advances in STE to recreate the functional skin substitute have been accomplished because of the developments in bio-fabrication methods, genetically engineered cell sources, and different ways of delivering the signaling molecules (Khademhosseini and Langer 2016; Bhardwaj et al. 2018). While all of these advancements are still being researched, there seems to be a substantial likelihood that many, not all but most, of the skin appendages, such as hair follicles, hair shafts, and sweat glands, will be included in future designs of skin replacements and medically accessible for the management of full-thickness or chronic injuries, including deep burns.

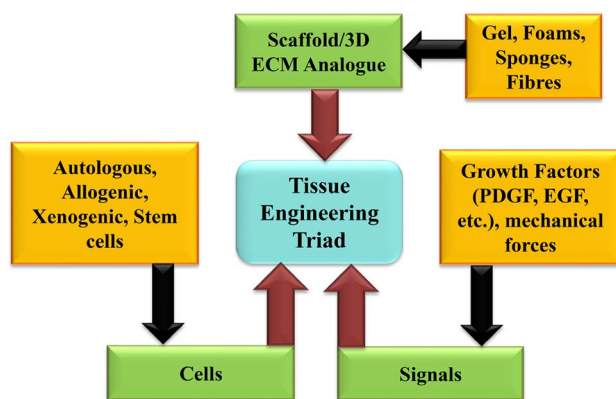


Fig. 2 Tissue engineering triad

Clinically and commercially available TESSs for wound healing

Skin replacements are used to restore skin's integrity and functionality after burns or many other severe skin problems. In situations where conventional therapeutic approaches are not applicable, skin substitutes offer a solution to standard injury covering. All engineered skin replacements must meet some critical areas: patient safety, clinical efficacy, user-friendly, ease of availability, long shelf life, non-fragile, and cost-effective (MacNeil 2007; Wang et al. 2019; Kharaziha et al. 2021). In the broad sense, such therapeutic biomaterials should be non-cytotoxic, non-allergic, or induce no inflammation and pain. Furthermore, they should be biocompatible and allow complete wound healing without posing any scarring, fluid loss, infections, and disease transmission (Yu et al. 2018). There are not presently commonly available engineered skin substitutes that offer all of the aforementioned attributes, nor can they entirely replicate the morphological and functional features of native skin tissue. The majority of the presently commercialized and therapeutically accessible TESS products were addressed in this review study based on the different classifications discussed below.

1. A layer of skin they restore:
2. epidermal
3. dermal
4. dermo-epidermal (or composites)
5. Duration of wound coverage:
6. temporary (these replacements are often used as a wound dressing for a limited period to safeguard the wounded region from possible environmental risks such as microbial infection or physical damage and also offer safer as well as hydrated conditions to support and assist the healing process and are normally replaced with an autogenous graft after 3 to 4 weeks)
7. semi-permanent (these are acellular replacements that may be left at the wounded location for many days and operate as secondary dressings to prevent fluid loss and microbial infections, as well as to heal and regenerate injured skin tissue, although they are ineffective for severely injured wounds)
8. permanent (these TESSs have been used to replace and restore the overall equivalent depth of skin layers and to remain permanently at the injured site to enhance the overall integrity of the skin tissue, thus reducing the need for donor skin sites)
9. Type and source of the biomaterial:
10. biological (auto-, allo-, and xenografts)
11. synthetic (biodegradable or non-biodegradable)

12. Classification of skin replacement in terms of cellular components:
13. cellular
14. acellular

There are several distinct classes of commercially available TESS products that are now capable of helping with tissue healing and regeneration (Snyder et al. 2020) and are summarized in Table 2. Each has its own range of qualities that make it acceptable to be used in different ailments (burns or chronic wounds), but only a few can completely heal regenerative native skin tissue.

A patent overview for skin tissue engineering (STE)

STE is a novel approach that tends to bring combined multidisciplinary teams to facilitate effective healthcare translation. Severe deeper wounds, including burn wounds and other serious skin loss problems, are clinical reasons where tissue-engineered skin replacements are being researched as an alternative that can restore the epidermal and dermal structural integrity of the skin tissues. In comparison to existing standards of treatment, the TESSs could minimize fatalities and disability, thus improving the quality of people's lives and fully functioning results. In the STE discipline, it is sometimes neglected and difficult to understand how TE-related fundamental experimentation may be transferred to practical and translational work, eventually seeking to produce and commercialize TE products (Al-Himdani et al. 2017). However, technological advancements such as the advent of automated bioreactors and bio-printing for fully functional skin tissue development may make healthcare products more accessible (Zhu et al. 2022).

Furthermore, all TE products must be approved by governmental authorities (Food and Drug Administration, FDA in the United Nations, etc.) to assure excellent quality, reliability, health safety, and verified efficiency (Haddad et al. 2017). Over the last few decades, various trustworthy STE patents have been researched; for example, RenovaCare, Inc. has recently established patented innovative technologies such as the CellMist™ and SkinGun™ systems spraying self-donated stem cells for functional skin tissue and other organ regeneration (Kareem et al. 2021). Other patented TESSs include Kerecis's fish-skin products; dCELL (decellularized dermal skin allograft); ReCell (device for extraction of autologous skin cells), and many more, which are used for healing and regeneration of a wide variety of skin wounds (Dai et al. 2020; Kirsner et al. 2020). In one research, *in vitro* examinations of decellularized fish skin revealed improved structural and mechanical qualities comparable to normal human skin for regeneration, as well as

improved cellular activities, indicating that it might be a viable choice for skin tissue regeneration (Kamalvand et al. 2021). Skin sprays are a potential approach for STE applications since they can transport cells and biocompatible hydrogels to extensive lesions with ease and safety. Several clinical studies are being conducted to investigate spray solutions for skin illnesses such as chronic wounds, diabetic foot ulcers (DFUs), psoriasis and others (Esteban-Vives et al. 2018; Chen et al. 2020). Decellularized ECM (dECM) derived from diverse human and animal tissues has been used for burn wound therapy and surgical restoration for the past few decades. Tissue decellularization is necessary for transplantation operations to minimize the activation of immunological responses and inflammatory responses that cells in the donor tissue may trigger, leading to transplant rejection. For instance, Singh et al. demonstrated that integrating curcumin into modified decellularized small intestine submucosa (SIS) membranes resulted in potent antimicrobial and free radical scavenging activity, making them effective in neutralizing the negative effect of oxidative stress and bacterial colony development in chronic skin wounds (Singh et al. 2022). Eventually, these approaches and advancements in STE will reduce or eliminate the requirement for skin autografts and allow for faster commercial translation of viable autologous engineered skin grafts ideal and safe for patient usage.

The current approaches in STE

An impactful TESSs, at its most fundamental aspect, optimally replicates the complexity of the original 3D anatomy and performs the mechanisms of biological skin tissue. Furthermore, these substitutes should promote pre-vascularization and offer supporting signals to cells in the surrounding environment. Finally, it should be able to successfully integrate into the recipient with minimum or no scarring while creating a minimal and regulated immune response if transplanted *in vivo*. Over the past few generations, a wide range of strategies has been developed and implemented in the STE field. The foundation of this area is the notion that efficient tissue growth requires the synergistic action of numerous cell types rather than the isolated implications of any one population. Currently, several approaches are investigated to design skin substitutes analogous to native healthy skin. Over the past three decades, TESS developed by Cincinnati, Ohio, researchers comprised of autologous cells, including keratinocytes and fibroblasts seeded in bovine-derived collagen-glycosaminoglycans (GAG) scaffold is currently considered to be the most clinically successful scaffold with skin structure analogy (Mohamad et al. 2019; Smiley et al. 2006). Yet another skin substitute based on bovine collagen is commercially available denovoSkin™,

Table 2 Commercially available TESS products for wound healing

Skin substitutes	Composition	Clinical application	Merits	Demerits	References
Temporary skin substitutes					
1. Dermagraft	Bioadsorbable skin substitute seeded with cryopreserved allogenic neonatal human foreskin fibroblasts	Burn, chronic wounds; diabetic foot, and venous ulcers	No handling issues; no immune rejection	Poor ECM structure; chances of infection; high cost	Marston et al. (2003)
2. TransCyte	Human fibroblast-derived; consist of polymer membrane and neonatal human fibroblast cells cultured on nylon mesh	Provides a temporary protective barrier	No chances of infectious disease transmission	At a later stage, autografts will be required to replace it; expensive; temporary	A mani et al. (2006)
3. Apligraf	Allo neonatal human fibroblasts in collagen gel + stratified allo-neonatal human keratinocytes	Diabetic foot ulcers (DFUs); Venous leg ulcers	No chances of infectious disease transmission; ease to use	Short shelf-life; high cost	Hu et al. (2006); Edmonds et al. (2009)
4. StrataGraft	Full thickness-skin substitute with dermal and fully differentiated epidermal cells	Traumatic skin wounds	Non-immunogenic	Temporary coverage; high cost	Centanni et al. (2011)
5. EZ Derm	Aldehyde cross-linked reconstituted porcine dermal collagen	Partial-thickness burns, chronic and surgical wounds, venous ulcers	Instant availability; prolonged storage life	Less clinical data; chances of disease transmission	Troy et al. (2013)
6. Biobrane	Bilaminated membrane formed by nylon mesh filled with type I porcine collagen plus keratinocytes	Full- and partial thickness wounds	Improves re-epithelialization; good dressing; diminishes pain; prevents wound dehydration; favors permeability to antibiotics; diminishes fibroblast capacity for wound contraction	Not used to treat caused by chemicals or petroleum-based products; no hemostasis; expensive; potential antigenicity; risk of unusual infection	Farroha et al. (2013); Schulz et al. (2016)
Semi-permanent skin substitutes					
7. Integra	Silicon coated bilaminated sheet of bovine collagen and chondroitin-sulfate	Burns, surgery	Non-immunogenic; scarless tissue formation; easily available; long shelf life; capable vascular tissue formation	Chances of infections; high cost; both dermal and epidermal layers can't be replaced; weak clinical evidence	Chang et al. (2019); Nguyen et al. (2010)
8. Hyalomatrix	The derivatized hyaluronic acid semi-permeable membrane	Partial- and full-thickness wounds	Simplifies monitoring; natural re-epithelialization; proper coverage; reduces pain; allows cellular invasion and capillary growth; retain the moisture	Risk of infection	Erbatur et al. (2012)
9. Terudermis	Heat-denatured collagen sponge consisting of silicone and bovine lyophilized crosslinked collagen	Burns and other post-traumatic and mucosal defects	Easy and quick grafting procedure; enhanced wound angiogenesis	7–14 days to grow vessels into the sponge	Park et al. (2018)

Table 2 (continued)

Skin substitutes	Composition	Clinical application	Merits	Demerits	References
Permanent skin substitutes					
10. AlloDerm	Acellular dermal allograft	Used mostly for deep partial- and full-thickness lesions, potentially allowing thinner STSGs to be used	Showed less scarring; easy to store and process; long shelf life up to 2 years	Risk of infectious disease transmission; costly	Sclafani et al. (2000)
11. Epicel	Autologous keratinocytes + murine keratinocytes	Deep partial- and full-thickness burns	Availability of autologous tissue; FDA approved; minimal portion of tissue from a donor is needed; ideal for patients with deep burn injuries	Expensive; time required for preparation; highly fragile; short availability	Carsin et al. (2000)
12. PermaDerm	Collagen-GAG's substrates contain autologous keratinocytes and fibroblasts	Burn wounds	Easy to use	Not yet approved by FDA for clinical trials	MacNeil (2007)
13. MySkin	Silicone support layer with a specially formulated surface coating with cultured keratinocytes	Superficial burns; Pressure and diabetic foot ulcer	Easy to use; reduce the time for cell culture; suitable for wounds of all sizes	Patient biopsy needed; slow healing properties; need for dermal support	Moustafa et al. (2007)
14. Matriderm	Bovine collagen and elastin	Burns, reconstructive surgery	Reduces wound contracture; acts as a physical barrier; accelerates wound closure; restores skin elasticity	Weak effect on decreasing redness; limited effect on improved skin humidification	Min et al. (2014)
15. OASIS Wound Matrix	Porcine acellular lyophilized small intestine submucosa	Lesions, chronic, and burn wounds	Immediate availability; prolonged shelf-life; provide up to 90% wound reduction	Weak clinical data	Brown-Etris et al. (2019)

made up of collagen hydrogel, fibroblast, and keratinocytes. DenovoSkin™ has received FDA and EMA Orphan status to treat burns under Advanced therapy Medical Product (ATMP) (Schiestl et al. 2021). However, these skin tissue substitutes have certain limitations owing to their xenogenic components, raising the risk of an immune response and high cost of production. Few approaches have shown significant clinical effectiveness, using fibroblast alone or with a subsequent seeding density of keratinocytes along with fibroblasts. However, the constrain of the long production time (about 9 weeks) still remain a challenge. Furthermore, researchers use autologous, allogenic, xenogenic, and stem cells as a cell therapy to create skin analogs that closely resemble native tissue architecture (Sierra-Sánchez et al. 2021). The creation of stratified constructions matching the bilayered organization of the epidermis and dermis has dominated skin restoration in tissue regeneration for the most part. An ideal engineered skin construct must allow proper cell adherence, regulated and sustainable delivery of growth factors/drugs, and the re-growth of the tissue in the injured region after its implantation. Currently, three major approaches are being used to create the best possible

skin substitutes: scaffold-based, cell-based, and drug/signals delivery-based approaches, as briefly discussed below and schematically represented in Fig. 3.

Scaffolds: different biomaterials and fabrication methods

The "Scaffolds" play a significant supportive role in the domain of TERM because they tend to reproduce the structural and functional properties of ECM, as well as aid in enhancing the cellular activities, growth factor delivery, and neo-vascularization in vivo to improve healing and regeneration of the worn-out tissue (Shafiee and Atala 2017; Dutta and Dutta 2009). The primary purpose of an ideal scaffold is to offer a three-dimensional habitat for cells to grow and synthesize their matrix, which will eventually replace the scaffold, resulting in a three-dimensional structure for the injured tissue (Yu et al. 2019). Its framework determines the final structural layout of newly formed soft or hard tissue. The synthesis of cellular matrix and breakdown of scaffold framework should be coordinated so that one process does not overtake the other (Naderi et al. 2011; Afjoul et al.

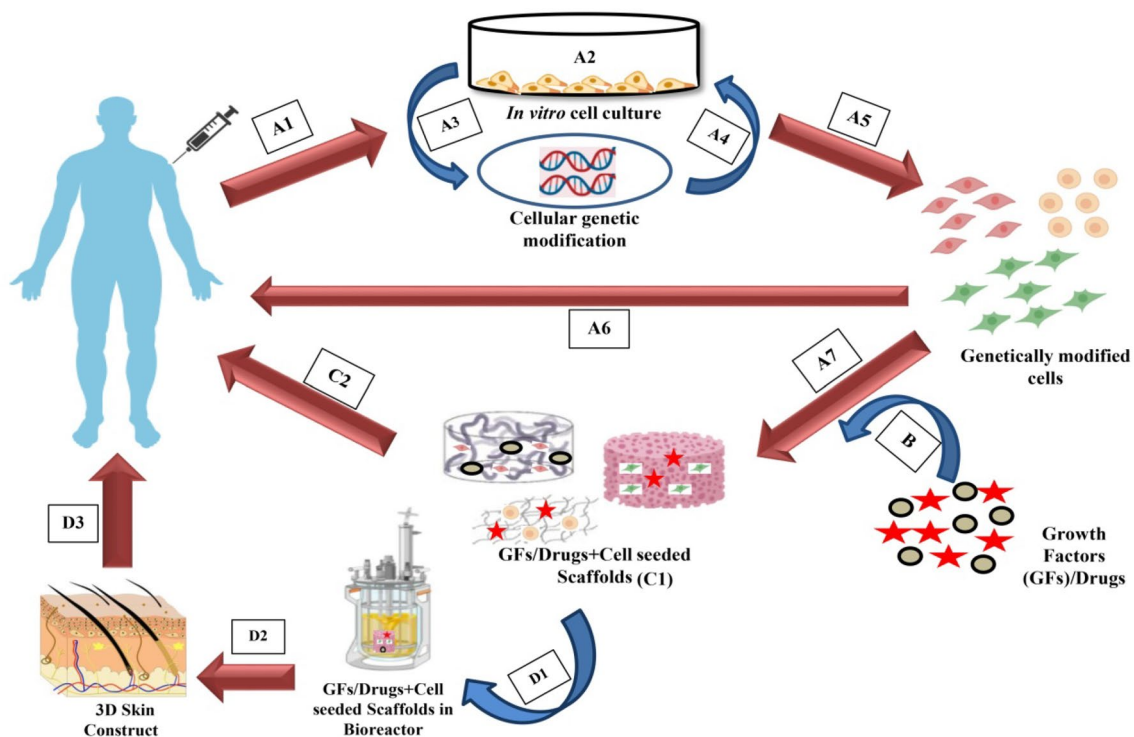


Fig. 3 Diagrammatic representation illustrating different strategies of STE. STE Approach I: cells isolated from an individual (A1), isolated cells are further cultured under controlled in vitro conditions (A2) to differentiate and proliferate, eventually the cultured cells are genetically modified (A3 and A4) and expanded under specified lab conditions (A5) preceding to potentially being re-implanted into the same individual's body (A6). STE Approach II: explanted genetically

altered cells (A7) along with growth factors/drugs (B) could be added to the polymeric scaffolds (C1) before implantation onto the target site for tissue restoration (C2); Approach III: Pre-implantation, cell-seeded or GFs/drug-loaded constructs (D1) can be cultivated in a bioreactor to develop an artificial skin tissue (D2) and then transplanted at the target site of an individual (D3)

2020). In addition, a variety of factors determine the scaffold's selection and functionality (Auger et al. 2013; Atala 2012; Solovieva et al. 2018; Ghosal et al. 2017; Chang and Wang 2011; Mndlovu et al. 2020), as shown schematically in Fig. 4. The scaffold can be employed as a delivery system to supply growth factors/drugs/signals to the affected area to guide and promote cellular proliferation and differentiation for controlled tissue growth (Naderi et al. 2011; Chen et al. 2022; Sharma et al. 2020). A large variety of biomaterials with natural, synthetic sources and their blends, as well as innovative manufacturing processes, have been suggested in recent decades and are briefly outlined hereunder.

Scaffolding materials: natural, synthetic, and composites

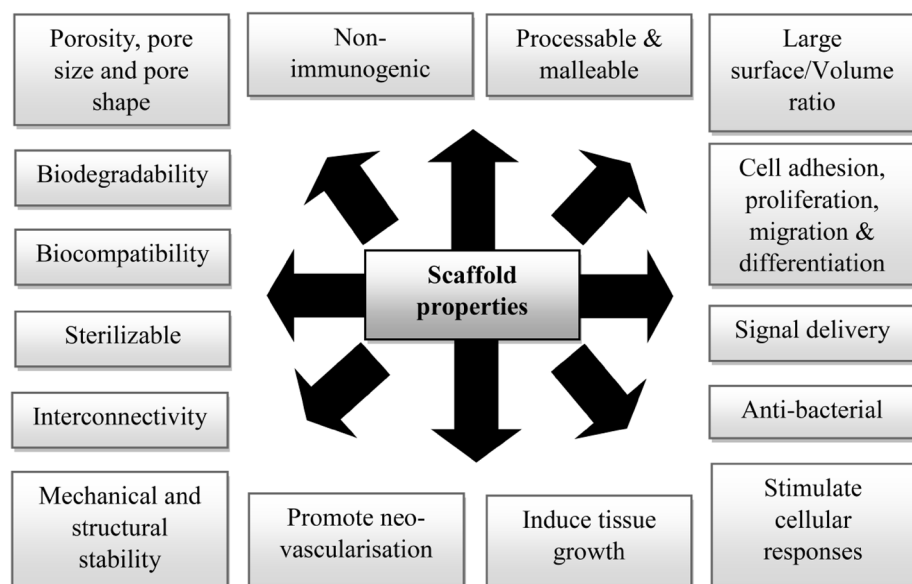
Tissue-designed skin substitutes have been in use for decades, and the biomaterial used for scaffolding must be customized to the specific requirements of the target tissue. Numerous research work has demonstrated the positive impacts of biomaterial modification on skin tissue engineering scaffolds qualities such as pro-angiogenesis, excellent physiochemical properties, enhanced cellular responses, and many other superior attributes (Shi et al. 2018; Zhang et al. 2017b; Zehra et al. 2020; Yin et al. 2021; Eskandarinia et al. 2020; Olad and Hagh 2019; Martins et al. 2018; Chandra et al. 2020). The development of "smart or novel biomaterials" capable of regulating cellular activities and/or increasing tissue functioning is now the subject of study (Furth and Atala 2014; Govindharaj et al. 2019). The interaction between the scaffold and cells depends on the properties of the selected material, either natural or synthetic, applied for the specific tissue. In producing engineered skin substitutes, three distinct biomaterials are most often used, including

synthetic polymers, natural polymers, and their blends, as summarized in Table 2.

Natural polymers, often known as biopolymers, are organic compounds produced by biological entities. They are further sub-divided into two categories, including protein-based materials (collagen, fibrin, silk fibroin, keratin, and gelatin) and polysaccharide-based materials (hyaluronan, cellulose, alginate, chondroitin, and chitosan) (Sahana and Rekha 2018). The freeze-drying approach was used to generate an entirely novel ciprofloxacin-loaded collagen–chitosan matrix for wound therapy (Tripathi et al. 2021b). In current history, 3D printed chitosan (Ch) structures with enhanced cytocompatibility and biocompatibility were already viewed as a useful and convenient biomaterial for wound repair (Intini et al. 2018). Several prior studies have shown that blending gelatin with a variety of other natural biopolymers promotes human dermal fibroblasts (HDF) growth and division, which further enhances the rate of wound healing (Vatankhah et al. 2014; Yuan et al. 2018). Multiple studies have shown that different natural polymers and their combinations could construct scaffolds for wound healing applications (Sajjad et al. 2020; Afjoul et al. 2020; Zhang et al. 2017a).

Synthetic polymers, such as polylactic acid (PLA), polyvinyl alcohol (PVA), polycaprolactone (PCL), polyglycolic acid (PGA), and their copolymers (e.g., PLGA, etc.) are extensively implemented. The FDA authorized biomedical skin applications because of their non-toxic by-products and tunable physiochemical characteristics, processability, and malleability (Pina et al. 2019). The biodegradability and biocompatibility of Ciprofloxacin-loaded electrospun PLGA nanofibrous mats treated with sodium alginate microparticles as skin replacements were studied by Liu et al. (2018b).

Fig. 4 The crucial desirable properties of an ideal scaffold



In one research, composite scaffolds made of PCL, Zein, and Gum Arabic exhibited increased cellular activity, porosity, and hydrophilicity (Sharifi et al. 2020). In vitro and in vivo investigation exhibited cytocompatibility, remarkable tissue development, and neovascularization, confirming its promising applications for skin tissue engineering (Zhang et al. 2018). A dual system of PVA and sodium alginate (SA) hydrogel integrating bFGF-loaded PCL microspheres exhibited enhanced cell-induced wound healing in vitro and in vivo (Bahadoran et al. 2020).

Almost all of the above-discussed biomaterial has its own set of benefits and, unsurprisingly, drawbacks. Composite scaffolds derived from natural sources and/or synthetic materials combine the benefits of each polymer's specific chemical, mechanical and biological qualities to surpass each polymer's limits and allow the construction of an ideal as well as a functional skin substitute. Electrospun hybrid nanofibrous mats (PVA/Chitosan/Starch) were shown to be non-cytotoxic and antibacterial, allowing for faster wound healing (Adeli et al. 2019). The fibroblast-loaded composite nanofibrous scaffold (PCL/gelatin/collagen type I) could be a possible future strategy for repairing and regenerating skin tissues (Gomes et al. 2017). The recombinant collagen-hyaluronic acid composites had outstanding physical characteristics and bio-compatibility, suggesting that they might be utilized for wound repair or biomedical applications (He et al. 2020). In current history, the results of cross-linked nanofibrous bilayer scaffolds (Fish collagen-PCL) demonstrated improved healing of the target tissue (Chandika et al. 2021). In vitro and in vivo investigations with curcumin-loaded Cellulose-PLLA-nanosilica showed improved cell proliferation, granular tissue formation, neovascularization, wound healing, and full-thickness tissue restoration (Ramphul et al. 2020).

Scaffold fabrication strategies for STE

Modern TERM methods have primarily focused on 3D porous hydrogels or composite scaffolds generated in nano-structured frameworks featuring regulated breakdown efficiency and permeability for gas, nutrition, and growth regulatory component transfer. 3D biocompatible scaffolds in TE serve as cell and GF/drug mediators, providing a favorable environment for cell expansion and controlled GF release. The usage of TE constructs can alleviate the scarcity of tissues, including skin, bone, cartilage, and so forth, minimizing the need for animal models while increasing the dependability of observational data. Various fabricating procedures, as described in Fig. 5, are often used to create 3D polymeric constructs with excellent porosity, interconnectivity, uniform pore size, and surface area using biodegradable and biocompatible natural/synthetic materials. Traditional and advanced rapid prototyping are two methods for fabricating

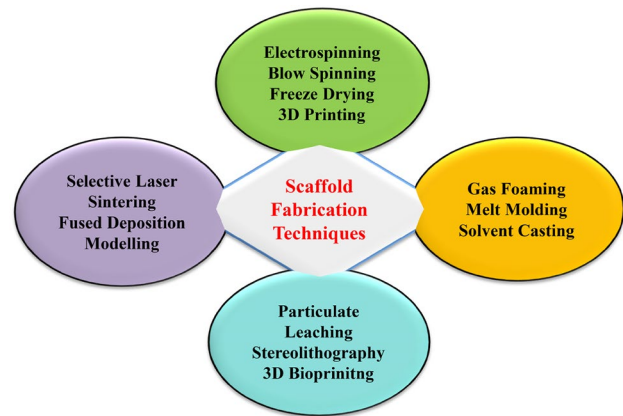


Fig. 5 A multitude of fabrication approaches used for STE

functionalized 3D constructs with suitable designs. Conventional fabrication methods (e.g., porogen leaching (Yin et al. 2016), phase separation (Mi et al. 2003), gas foaming (Ji et al. 2011), lyophilizer (Afjoul et al. 2020; Bahadoran et al. 2020; Niu et al. 2020), and many more), are inexpensive, easy to scale up and allow to construct of a scaffold of the desired shape. Still, they frequently fail to offer appropriate strength characteristics, and as a result, such scaffolds distort due to cellular movements. On the other hand, the prototypic method (e.g., electrospinning Atila et al. 2015; Fang et al. 2019; Lopresti et al. 2021), blow spinning (Singh et al. 2018, 2019), 3D bioprinting (Liu et al. 2018a; Seol et al. 2018; Michael et al. 2013; Kim et al. 2017), melt-blowing, etc.) seems to have no drawbacks and could offer additional necessary qualities, including higher porosity, physiochemical properties as well as enhanced cellular activity to TESS (Table 3).

The current advanced strategy to fabricate scaffolds includes 3D bioprinting. It's indeed a CAD-based approach in which live cells and structural material or hydrogel are deposited in a temporal and spatially structured way using a printer-based dispensing mechanism (Vijayavenkataraman et al. 2016). The polymeric solution containing cells (or bioinks) are printed in a 3D framework using a range of processes, including extrusion-, laser- and inkjet-based methods (Mahendiran et al. 2021; Liu et al. 2018a; Seol et al. 2018; Michael et al. 2013; Kim et al. 2017), and the entire procedure is illustrated in Fig. 6 (Augustine 2018). 3D bioprinting allows for accurate cell communication inside a 3-dimensional space by allowing the architecture to be controlled at both the micro- and nano-scale (Michael et al. 2013). Therefore, it is conceivable to create human skin, cartilage, blood vessels, and bone in their native form. A 3D printing approach combining two or more popular scaffold manufacturing technologies is currently being developed. Kim et al. demonstrated the effects of integrating the two

Table 3 Scaffolding polymers and their significant role in wound healing

Polymers	Fabrication method	Biological model	Findings	References
Natural polymers				
1. Silk Fibroin + Hyaluronic Acid	Freeze drying	L929 cells; Sprague–Dawley rats	Blend scaffolds possess a leaf-like pore shape with a pore radius of 32.22 μm to 290.76 μm and porosity approx. 74.1% to 91.15%; higher interconnectivity; good cytocompatibility and different experimental tests showed that the scaffolds prepared were effective in promoting L929 fibroblast adhesion and proliferation, as well as improving the creation of neo-vascularization, which has wound repair potency	Zhang et al. (2017b)
2. Chitosan	3D printing	Human fibroblast (Nhdf); HeCaT cells; Female Wistar rats	Highly porous structure; biocompatible and non-toxic; enhanced cell growth; and during the animal study, it was revealed that wound healing was hastened in the treated group when compared to a control group	Intini et al. (2018)
3. Chondroitin sulfate-incorporated collagen (epidermal layer) + sodium carboxymethyl cellulose (dermal layer)	Freeze drying	Detroit 551 (human fibroblasts); HS2 (human keratinocytes)	Bilayered scaffolds exhibited interconnected porosity, sufficient water-holding capacity, adequate durability, and elastic moduli. It also revealed increased cellular activity	Kilic Bektas et al. (2018)
4. Gelatin Type B	Emulsion template method	L929 cells; L02 cells	Interconnected micro- and macroporous structure; hemocompatibility; cytocompatibility	Yuan et al. (2018)
5. Curcumin-loaded bacterial cellulose (BC)	Freeze drying	NIH 3T3 cells; mouse burn model	The BC-Cur nanocomposite demonstrated biocompatible and non-toxic; possesses antimicrobial properties. Furthermore in an animal study scaffolds showed a faster healing process, tissue regeneration, as well as wound contraction	Sajjad et al. (2020)

Table 3 (continued)

Polymers	Fabrication method	Biological model	Findings	References
6. Alginate + gelatin	Freeze gelation method	L929 cells; Wistar rats	Improved tensile strength; suitable degradation and swelling behavior; excellent biocompatibility; interconnected porous structure favoring cell adhesion and proliferation; and in an <i>in vivo</i> investigation, cell-loaded scaffolds resulted in enhanced epidermal layer development, decreased inflammation, and regeneration of skin appendages	Afjoul et al. (2020)
7. Collagen + Chitosan loaded with ciprofloxacin	Freeze drying	Fibroblasts; albino mice	Interconnected micro- and macropores; highly porous structure with pore sizes of roughly 125 microns; hemocompatible; antibacterial; improved granulation, angiogenesis, and remodeling of the extracellular matrix, as well as epidermal and dermal layer skin regeneration	Tripathi et al. (2021b)
8. PGA	Melt foaming	Fibroblasts; Sprague–Dawley rats	Polymeric constructs showed homogenous porous structure; 92% interconnectivity; enough mechanical strength; excellent biocompatibility; promotes cell adhesion and proliferation	Zhang et al. (2018)
9. Ciprofloxacin + alginate particles loaded-PLGA nanofibers	Electrospinning	-	Fibrous mats exhibited uniform diameter; improved wettability; antimicrobial activity and possessed adequate mechanical assistance for the injured site	Liu et al. (2018b)
10. bFGF-PCL microspheres-loaded PVA + sodium alginate (SA) hydrogel	Freeze-thawing method	L929 cells; Wistar rats	Microspheres loaded hydrogels demonstrated boosted porosity; higher water retention ability; increased content of SA further increases the degradation rate and reduced mechanical strength; sustained drug release; antibacterial activity; non-cytotoxic with an approx. 70% cell viability; stimulates cell attachment, migration, and proliferation; 95% wound contraction rate	Bahadoran et al. (2020)

Table 3 (continued)

Polymers	Fabrication method	Biological model	Findings	References
11. <i>Nigella sativa</i> extract-loaded PCL-PLA nanofibers	Double-nozzle electrospinning	hMSCs cells	The nanofibrous construct showed beadless and smooth nanofibers were obtained with an average diameter of 638 ± 69 nm; improved hydrophilic properties due to the presence of drug which further enhanced the cell adherence and proliferation; antibacterial behavior; the cellular toxicity was minimized by increasing the quantity of herbal extract in the nanofibers; increased cellular viability	Sharifi et al. (2020)
Composite polymers				
12. Chitosan + PCL + Gelatin	Electrospinning	Human fetal fibroblasts (cell line HFFF2)	Electrospun nanofibers demonstrated boosted porosity; highly hydrophilic scaffolds with 0° contact angle; higher elastic modulus; excellent cell adhesion and proliferation rate; allowing sufficient ECM production	Gomes et al. (2017)
13. PVA + Chitosan + Starch	Electrospinning	L929 cells	Nanofibrous constructs showed interconnected uniform porous structures with an average porosity of $\geq 90\%$; higher water retention ability; good mechanical strength and higher durability; showed anti-bacterial activity; improved cell attachment and proliferation; non-cytotoxic with maximum cell viability	Adeli et al. (2019)
14. Curcumin-loaded Cellulose-PLL-A-Nanosilica	Electrospinning	L929 cells; HeCaT cells; endothelial cells; macrophages; Wistar rats	Electrospun cellulose-based constructs showed biocompatibility is improved because the amount of macrophage fusions is decreased; possess anti-inflammatory, antioxidant, and antibacterial properties; and the tissue's histopathology revealed collagen fibers, as well as a limited number of mononuclear inflammatory cells and a significant level of neovascularization	Ramphul et al. (2020)

Table 3 (continued)

Polymers	Fabrication method	Biological model	Findings	References
15. Recombinant collagen (rCOL) + hyaluronic acid (HA)	Freeze drying	L929 cells	In this study, the construct showed a highly porous structure (> 90%) along with uniform pore size (100 to 250 µm) and distribution; improved swelling ability; lower degradation rate (20% degradation after 48 h); increased mechanical strength; high biocompatibility; promotes cellular adhesion, migration, and proliferation	He et al. (2020)
16. Collagen (COL) and poly(ε-caprolactone) (PCL)	Electrospinning	NHDF-neo cells; HaCaT cells; ICR mice	Bilayered nanofibrous construct showed interconnected fibrous structure; higher water retention ability; promotion and modulating adhesion, migration, and proliferation for both cell types; improved mechanical properties; wound site showed signs of fast healing, re-epithelialization, and contraction	Chandika et al. (2021)

3D printing techniques on wound healing and regeneration (Kim et al. 2017). Despite the many advantages of 3D printing, fabricated scaffolds may well not encourage biologically important responses (Tamay et al. 2019). 4D bioprinting could be utilized to resolve the challenges associated with 3D printed technology in which 3D printed frameworks are designed to change over time in response to external or internal stimuli (physiochemical and biological responses) (Mota et al. 2020; Qasim et al. 2019). The same rapid prototyping processes and technologies used in three-dimensional printing are used in four-dimensional printing. Table 4 summarizes ongoing cellular and acellular research for STE that used various scaffolding methods.

Cell sources and their application in STE

Cells are key constituents of skin because they play a multitude of activities in maintaining natural skin homeostasis and physiological functions. Cells including keratinocytes, endothelial cells, melanocytes, and dermal fibroblasts are often used in skin injury and burn therapeutic products (Shevchenko et al. 2010; De Pieri et al. 2021). Tissue regeneration advancements have improved the potency and application of cell-based treatments. There are several methods for strengthening cellular skin therapies. This would include cell type selection, cell source (autologous vs. allogenic vs. xenogenic), and strategies for increasing cellular survivability and biological function after seeding. The first methodology is to use cells as a therapeutic agent for the restoration of functional tissue (De Pieri et al. 2021). This method entails isolating various types of skin cells, including autologous, allogenic, and xenogenic, growing them under suitable growth conditions, and then implanting them back to the target site for proper tissue functioning and restoration. Furthermore, the second approach involves isolating and seeding multiple types of skin cells onto scaffolds from diverse sources, subsequently implanting them back into the target site for appropriate tissue functioning and restoration (Sierra-Sánchez et al. 2021; Goyer et al. 2019).

The use of autologous cell sources in TE is of significant clinical interest because of their cost-effectiveness and non-immunogenic nature, reducing the intake of solid immunosuppressants and their ill effects. Still, their limited availability, long culturing periods, handling issues, and scarring limit their usage for clinical purposes (Cozzolino et al. 1999; Olender et al. 2011; Goyer et al. 2019; Sierra-Sánchez et al. 2021). The other cell sources, such as—allogenic and xenogenic, provide an alternative to autologous cell sources with the advantage of their ample availability, ease to use, painless grafting, and scar-free tissue formation (Olender et al. 2011; Salgado et al. 2017). However, autologous cells source is clinically preferred over allo- and xenogenic cell sources because later one has certain limitations such as

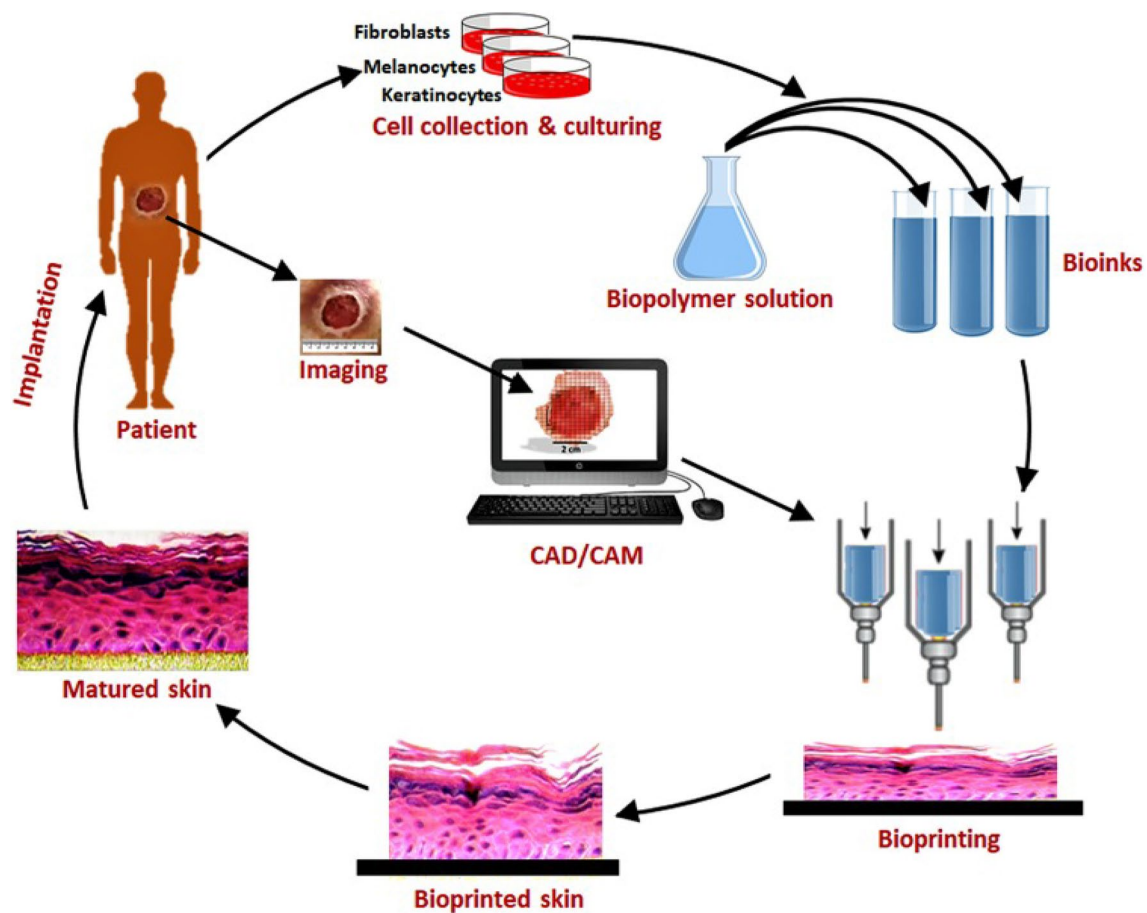


Fig. 6 “Steps in the fabrication of bioprinted skin. Various cells such as keratinocytes, fibroblasts and melanocytes would be collected from the patient and grow and multiply in cell culture system. A suitable biopolymer is mixed with the cells and the formed bioink is fed to the bioprinting system. Features of the wound are captured and a 3D

structure is reconstructed using CAD/CAM approaches. According to the 3D pattern, wound tissue will be reconstructed, allowed for maturation in vitro and implanted back to the patient” by (Augustine 2018), licensed under CC by 4.0

their immunogenic nature, intake of solid immunosuppressants, high risk of disease transmission, and ethical issues (Lu et al. 2011; Tomford 1995; Haldar et al. 2019).

In the current scenario, a wide range of cell sources, including genetically modified stem cells as shown in Fig. 7 (Kwon et al. 2018), and cell lines have been explored and are manipulated, providing an alternative option to the above-discussed cell sources in the field of skin tissue regeneration and are extensively discussed in Table 5. Clinically, the most common and actively used stem cell sources for STE are discussed below.

Embryonic stem cells (ESCs), derived or extracted from the blastocyst's inner cell mass, are the most appropriate cell source that can be used for the origin of various differentiated cell populations under controlled culture conditions (Kitsberg 2007). Even though ESC have remarkable advantages such as abundancy, self-renewal potency, and pluripotency, their usage is limited at clinical levels because of their immunogenic nature, ethical issues, the transmission of

inherited diseases or chromosomal aberrations (Kwon et al. 2018), and the formation of teratomas during the differentiation of ESCs into a particular lineage (Spits et al. 2008; Khademhosseini et al. 2020). The hESCs and iPSCs bank typed with human leukocyte antigen (HLA) have recently been tested in clinical trials to overcome the immunological rejection problem in allogenic patients associated with these stem cell therapies (Taylor et al. 2011).

Adipose-derived stem cells (ADSCs) are mesenchymal-derived adult stem cells that can be found in any white animal tissue, connective tissue, and omental fat. These stem cells are younger and have the potential to regenerate themselves with multipotential cellular differentiation (mostly cartilage, bone, tendon, and fat), and are easy to harvest in large quantities via the liposuction process under optimal culture conditions (Estes et al. 2006; Li et al. 2014). One study of an albino rat with a full-thickness defect treated with a bioscaffold composed of adipose-derived stem cells showed minimal wound contraction, ECM remodeling,

Table 4 Principle scaffold fabrication methods for STE

Fabrication method	Polymer composition	Biological model	Type of matrix	Significance	References
Conventional fabrication approaches					
1. Phase separation	Chitosan + Silver sulfadiazine (AgSD)	3T3 fibroblasts	Sponge-like porous membrane	Simple and cost-effective approach; good pore size, porosity, and interconnectivity; prolonged antibacterial activity; promotes wound healing	Mi et al. (2003)
2. Gas foaming	Chitosan	Human skin fibroblast cells	Porous hydrogel	Homogenous pore formation; enhanced cellular activities; boosted porosity	Ji et al. (2011)
3. Lyophilization	Gelatin + Sodium Alginate PVA + Sodium alginate incorporated with PCL loaded with bFGF + BSA Ag-loaded chitosan	L929 fibroblast cell line L929 fibroblast cell line Mouse fibroblast cell (L929)	3D porous scaffold Hydrogel 3D porous scaffold	Homogenous pore formation; high porosity; good biocompatibility; improved mechanical properties; increased cell infiltration	Afjoul et al. (2020); Bahadoran et al. (2020); Niu et al. (2020)
4. Porogen leaching	PLA + PEG	Rat bone marrow stem cells (BMSCs)	Porous sponge-like scaffold	Simple and low-cost approach; good control over pore size and porosity; interconnected pores; boosted porosity; enhanced cellular activities	Yin et al. (2021)
Advance fabrication approaches					
5. Laser-based technique	Matrigel	NIH3T3 fibroblasts; HaCaT keratinocytes	3D-layered constructs	Enhanced cellular activities; maintain structural integrity; promote vascularization; rapid wound healing; multi-layered tissue growth	Michael et al. (2013)
6. Electrospinning	Cellulose acetate + Pullulan PLA and γ -PGA loaded with rhodamine and coumarin-6 PLA + kefiran (Kef) coated	L929 fibroblast cell line Mouse fibroblast cells (NIH/3T3) NIH3T3	Nano-fibrous mesh Core-shell nano-fibers Core-shell nano-fibers	Large surface to volume ratio; increased porosity; enhanced cellular activities; 90% re-epithelialization; possesses tunable properties; controlled degradation rate; cytocompatible	Atila et al. (2015); Fang et al. (2019); Lopresti et al. (2021)
7. 3D bioprinting	Fibrin + Human plasma	Primary human keratinocytes (hKCs) and human fibroblasts (hFBs); immunodeficient mice	3D-bilayered construct	Promote proper growth and spreading of cells; effective tissue regeneration; 3D-construct mimics native skin tissue; simpler and robust	Cubo et al. (2016)
8. Hybrid 3D-printing technique (Extrusion-Inkjet method)	Collagen + PCL	Human epidermal keratinocytes (HEKs); dermal fibroblasts (HDFs)	3D-layered mesh	Promote cell infiltration; increased epidermal thickness; effective tissue regeneration; simpler and robust	Kim et al. (2017)

Table 4 (continued)

Fabrication method	Polymer composition	Biological model	Type of matrix	Significance	References
9. Extrusion-based printing	GelMA + Alginate HA + Gelatin + Glycerol + Fibrinogen + PU	HUVECs; NIH3T; MDA-MB-231; and MCF7 cell lines Human fibroblasts, keratinocytes	3D-microfibrous constructs Hydrogel	Enhanced cellular activities; higher porosity; superior biocompatibility; biostability; rapid wound healing & tissue growth	Liu et al. (2018a); Seol et al. (2018)
10. Solution Blow Spinning (SBS)	Gelatin Core (PEO + PDLLA + PCL) and shell (PCL + PEO) loaded with bFGF	Human bone marrow-derived mesenchymal stem cells (hBMSCs) hBMSCs	Nano-fibrous mesh Core – shell nano-fibrous mat	Large surface to volume ratio; easy and low-cost method; excellent biocompatibility; increased porosity; superior biodegradability; facilitates the cell growth; imitates a dynamic in vivo conditions	Singh et al. (2018, 2019)

epithelialization, and promoted angiogenesis (Ozpur et al. 2016). In another study, the 3D-printed ADSC-loaded scaffolds showed an enhanced wound healing and angiogenesis rate, thus providing an ultimately engineered skin graft (Roshangar et al. 2021). These cells are still in clinical trials to monitor their cellular properties and efficacy (Aso et al. 2016).

Mesenchymal stem cells (MSCs), similar to ADSCs, are adult stem cells with the capacity to distinguish between nearly all distinct types of cellular lineages, such as osteoblasts, chondrocytes, muscles, and adipocytes (Tong et al. 2015). A growing body of research indicates that MSCs (mesenchymal stromal cells) could be helpful in the treatment of cutaneous wound tissue regeneration. In a research of a pig partial-thickness severe burn wound model, it was discovered that MSCs could develop into dermal cells (Li et al. 2006), allowing for a faster wound healing process, keratinization, and wound contraction, as well as increased vascularization. In one survey, MSCs were found to facilitate the functionality of rejuvenated skin, regulate collagen accumulation, augment re-epithelization, significantly improve neo-angiogenesis, and encourage restoration of skin appendages by seeding them well within the scaffolding matrix (Formigli et al. 2015). Besides this, MSCs possess immune-modulatory, pro-angiogenic, and scarless wound healing properties (Li et al. 2019; Martinello et al. 2018). MSCs provide an ultimate auto- or allogenic source for the treatment of moderate to severe wounds/burns and relatively lacking ethical issues in comparison to ESCs (Pittenger et al. 1999; Maxson et al. 2012). Several MSC-based cellular therapeutics are clinically approved and available at the commercial level. However, their stability and efficacy are still in discussion for several reasons, including their limited proliferation rate, reduced secretion of growth factors, and the loss of cellular differentiation (Josson et al. 2018; Zhang et al. 2012).

Another stem cell source known as induced pluripotent stem cells (iPSCs) has been managed to be produced from the differentiated adult stem cells by artificially reprogramming the main four transcription factors (Oct-4, Sox-2, Klf-4, and c-Myc) (Takahashi and Yamanaka 2006; Takahashi et al. 2007). The iPSCs, in their potency, are very similar to embryonic stem cells (ESCs) and are the inexhaustible and renewable resource of autologous cells (Park et al. 2008). One study showed that the potential proliferation and survival rate of iPSCs-derived MSCs is better than that of bone marrow-derived MSCs because of the higher expression of the KCNH1 ion channels. However, the functional role of these ion channels on the proliferation rate of iPSCs-MSCs is still not fully understood (Zhang et al. 2012). Furthermore, iPSCs-derived human melanocytes are being investigated to treat the skin discoloration disorders such as albinism, vitiligo, or melasma. They can also be used as a cell source

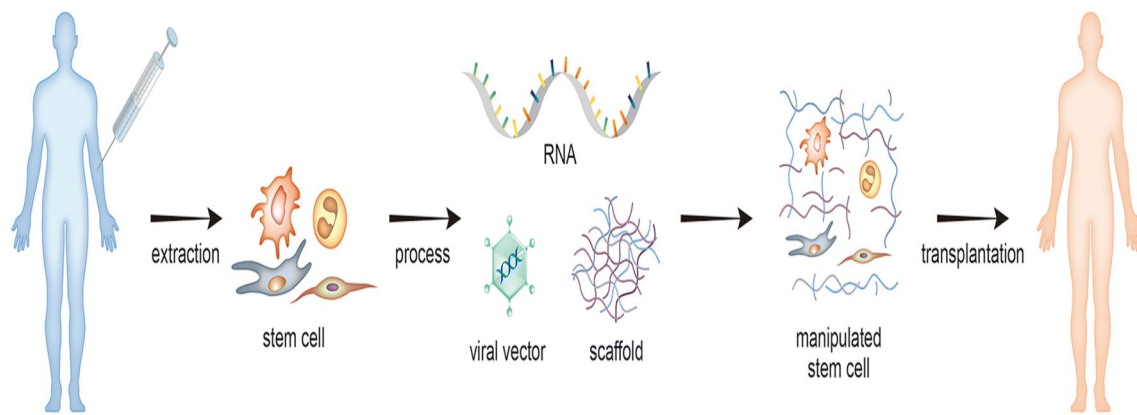


Fig. 7 “Stem cell engineering strategy” by (Kwon et al. 2018), licensed under CC by 4.0

for the development of TESSs (Ohta et al. 2011; Gledhill et al. 2015). According to a recent study, an organoid culture technique could be used to build multilayered skin from the human-induced pluripotent stem (Lee et al. 2020). Itoh and his co-workers have created in vitro 3-D skin analogs made entirely of hiPSC-derived skin cells, mainly keratinocytes and fibroblasts (Itoh et al. 2013). However, iPSCs are clinically prohibited because of their immunogenicity, tumorigenic nature (caused by genetic instability and active reprogramming factors), and heterogeneity (Garreta et al. 2018; Yamanaka 2020; Doss and Sachinidis 2019). As a consequence, researchers are concentrating their efforts on establishing alternative approaches to iPSC-related complexities. For example, tumorigenic testing kits are accessible to anticipate the presence of tumors, HLA-typed hiPSCs, as well as HLA or immunocloaking practices, are in clinical trials to decrease immunogenicity and the generation of naive human iPSCs, or the employment of diverse attributes to combat heterogeneity (Sato et al. 2019; Taylor et al. 2011; Gornalusse et al. 2017; Rohani et al. 2020; Kunitomi et al. 2016).

On the other hand, cell lines are a viable alternative to the previously mentioned cell sources due to their robustness, immortality, and ease of culture. HeCaT (human keratinocyte cell line) (Intini et al. 2018), HFF (human foreskin fibroblast cell line) (de Torre et al. 2018), L-929 (murine fibroblast cell line) (Bahadoran et al. 2020), NIH3T3 (fibroblast cell line) (Fang et al. 2019), and other cell lines are currently being utilized in clinical practices to develop a bio-compatible skin substitute. Above mentioned cell lines can also be used as a feeder layer to nourish and support grown cells (Unger et al. 2009). Instead of being robust and immortal, they may become genetically modified or have significantly altered cellular properties, resulting in differences in

the outcomes, and thus are not very dependable (Seo et al. 2012).

Growth factor/drug delivery strategy and their role in STE

Tissue restoration and re-growth are a crucial part of the healing process and are thus essential for all species' proper functioning and survivability. Growth factors/bioactive/drugs were already well understood as a crucial aspect of the wound recovery process, whose involvement begins when damage occurs and persists until the injury is entirely cured. Growth factors are either produced immediately by cells within the body or become incorporated into the matrix and delivered in a regulated way. Many composites can provide crucial structural reinforcement and adhesion sites, but they cannot influence cellular phenotype as effectively as signaling molecules. These factors are the primary source of chemical and biological signals in biomedical applications by guiding the ultimate destiny of cells and permitting the regeneration of target tissue (Ladewig 2011; Demidova-Rice et al. 2012; Atienza-Roca et al. 2018). The characteristics of the GFs/drugs and polymers utilized to enable effective restoration of wounds and tissue regeneration are just as essential as the delivery methods adopted. Targeted and sustained administration of wound healing agents has excellent promise for critical patient wound treatment, especially as the proportion of individuals diagnosed with incurable chronic infections grows across the world (Whittam et al. 2016).

Exogenous GF's/drugs are a viable therapeutic option for many chronic wound conditions. A variety of GFs, such as PDGF, VEGF, EGF, FGF, KGF, and TGF- α , β have been extensively established in studies for their ability to hasten wound healing (Atienza-Roca et al. 2018; Martí-Carvajal

Table 5 Different cell choices for STE

Cell type	Source	Advantages	Disadvantages	References
A. Autologous	Cells isolated from the patient's own body	Cost-effectiveness; non-immunogenic nature; significant clinical interest; no ill-effects	Limited availability; scarring; handling issues; long culturing periods; painful procedure	Sierra-Sánchez et al. (2021); Goyer et al. (2019)
B. Allogenic cells	Isolation of cells from the genetically different donor	Ample availability; ease to use; less expensive; painless grafting; scar-less tissue formation	Immunogenic in nature; intake of strong immune suppressants; high risk of disease transmission; ethical issues	Olender et al. (2011); Kouhbananinejad et al. (2019)
C. Xenogenic cells	Isolation of cells from other species	Ample availability; ease to use; inexpensive; painless grafting; scar-free tissue formation	Immunogenic in nature; intake of strong immune suppressants; high risk of disease transmission; ethical issues	Salgado et al. (2017)
D. Stem cells				
a. Embryonic stem cells (ESCs)	Inner cell mass of the blastocyst	Abundance source; self-renewal capability; pluripotency; easy to harvest; low cost	Immunogenic nature; ethical problems; teratomas formation; disease transmission	Kitsberg (2007); Kwon et al. (2018); Spits et al. (2008); Khademhosseini et al. (2020)
b. Adipose-derived stem cells (ADSCs)	Adipose tissue	Abundance; easy to harvest; self-renewal capability; multipotent cellular differentiation; low antigenicity; reduced inflammatory responses; possess immuno-modulatory properties; enhanced wound healing and neo-angiogenesis; minimal invasiveness	Lack of standard protocols; clinically not approved	Ozpur et al. (2016); Roshangar et al. (2021); Aso et al. (2016)
c. Mesenchymal stem cells (MSCs)	Bone marrow; fat; peripheral blood; amniotic fluid; adipose tissue; dermis	Clinically approved; allogenic source; self-renewal capability; multipotent cellular differentiation; possess immuno-modulatory properties; enhanced wound healing and neo-angiogenesis; abundant source; low cost	Stability and efficacy are under discussion; low proliferation rate; reduced GF secretion; loss of cellular differentiation; difficulty to harvest	Tong et al. (2015); Li et al. (2006), (2019); Formigli et al. (2015); Martinello et al. (2018); Maxson et al. (2012); Zhang et al. (2012)
d. Induced pluripotent stem cells (iPSCs)	Reprogrammed adult cells	Pluripotent; no ethical issues; autologous source; high proliferation rate; enables in vitro disease investigation; allows drug development	Immunogenicity; tumorigenic nature; heterogeneity; the need for standard protocols; genetic instability; uncontrolled growth and differentiation	Takahashi et al. (2007); Park et al. (2008); Ohta et al. (2011); Gledhill et al. (2015); Sato et al. (2019)
E. Cell lines	HeCaT; HFF; L-929; NIH3T3	Robust cell source; immortality; easy to harvest	Genetic instability; altered cellular properties; costly	Intini et al. (2018); de Torre et al. (2018); Bahadoran et al. (2020); Fang et al. (2019)

et al. 2015). Growth factors or drug delivery methods have recently been studied in wound healing and skin re-growth and are summarized in Table 6. The potential of fabricating method and biomaterial framework to retain and allow for customized release kinetics (Wang et al. 2017) (e.g., burst, sustained, prolonged, mixed, and pre-programmed) of relevant dosages of GF's or drugs for an extended period well within the specified site is driving attention in their advancement towards their treatment delivery (Wang et al. 2015, 2016; Geer et al. 2005; Goh et al. 2016; Garcia-Orue et al. 2017; Choi et al. 2017; Değim et al. 2011; Gainza et al. 2014; Mizuno et al. 2003; Niiyama and Kuroyanagi 2014; Li et al. 2017; Xu et al. 2020; Cao et al. 2015; Shi et al. 2019; Tripathi et al. 2021b; Mo et al. 2017; Ghaseminezhad et al. 2020). For example, a regulated and prolonged release of EGF is necessary to boost cell proliferation along with wound healing and neo-tissue development (Johnson and Wang 2013). In addition, these delivery systems should preserve biostability, restrict burst release, reduce immune reactions, enhance cellular activity, favor neo-angiogenesis, permit effective chronic wound healing, and allow for neo-tissue regeneration (Ladewig 2011; Demidova-Rice et al. 2012; Atienza-Roca et al. 2018; Whittam et al. 2016; Wang et al. 2017; Martí-Carvajal et al. 2015).

Conclusion and future direction

As stated earlier, distinct layers of skin tissue have prominent non-homogenous properties that must be considered while evaluating and developing constructs for skin tissue engineering purposes. The worldwide cost of skin wounds has significant public health care consequences, accounting for almost half of the globe's yearly medical expenditure. The rising area of skin tissue engineering (STE) offers hope to sufferers who urgently demand skin grafts in a period when tissues seem to be less available for transplantation, and there is a significant need for viable and functional alternatives. While assessing the skin regenerative characteristics of tissue-engineered implants, wider and complex injuries have a number of difficulties that must be taken into account. To present, potential therapeutic findings have been reported using TESSs based on cells and drug delivery systems in terms of effective transplant (60–90% in most investigations), durability (minor negative effects in certain case scenarios), re-epithelialization, and injury recovery rates.

In general, the translation of such TE skin grafts utilizing the innovative technologies discussed above demands model-based test techniques as well as relevant norms and standard safety guidelines in order to prove the transplants'

durability, stability, and fully operational dependability. However, after achieving significant clinical achievements with widely accessible commercial skin substitutes, the desire for an ideal full-thickness graft that precisely replicates the host's native tissue in terms of multilayer complexity, thermoregulation, sensations, appendage regeneration, Ultraviolet rays shielding, pigmentation, impaired angiogenesis, and reduced scarring continues to be unfulfilled. Angiogenesis is critical for the effectiveness of engineered skin grafts, resulting in a longer life period and improved compatibility with the host's native skin. By integrating cells including endothelial cells, MSCs, and ADSCs into the scaffold framework as well as utilizing angiogenic active biomolecules, and modifying the microstructural and functional features of the engineered grafts, in vivo neo-angiogenesis of the grafts may be encouraged (Hashemi et al. 2021). It is feasible to promote the migration and recruitment of cells, either stem cells or progenitor cells, at the injury site by developing bioactive smart scaffolding materials in vitro with appropriate 3D frameworks. Additional strategies for preserving artificial tissues are required for effective transportation from the manufacturing location to the implantation site still has to be improved.

The following are some of the most significant challenges and the active areas of future research:

- (i) selection and designing of different composites that can stimulate cellular activities, promote pre-vascularization and tissue re-growth;
- (ii) choosing a manufacturing technique capable of creating a scaffold with suitable skin tissue-specific properties;
- (iii) identifying the type and origin of cell source, along with growth factors, in perspective of diversity throughout the layers of the skin tissue and;
- (iv) development and optimization of innovative GFs/drug delivery methods to maintain and strengthen the therapeutic effectiveness of GFs/drugs on wound healing by exerting temporal and spatial command throughout their delivery.

To summarize, these many techniques to develop advanced skin replacements, such as the utilization of stem cells, smart biomaterial, rapid prototyping fabrication techniques, and novel drug delivery systems, provide new optimism that the functional and ideal skin constructs may indeed be produced soon and will be easily accessible in adequate quantities and cost-effective. Latest advancements, particularly in the engineering of biomaterials allowing integration into skin replacements, along with stem cell therapy, suggest that more successful methods may be possible shortly.

Table 6 Growth factors delivery systems implicated in wound healing

Delivery system	Growth factor/drugs	Biomaterial	Method	Significance	References
1. Films	bFGF	Hydroxypropyl-chitosan	Freeze drying	Sustained release; enhance wound healing; increase cell proliferation and neo-angiogenesis	Mizuno et al. (2003)
2. Hydrogel	hEGF KGF Cortex Moutan (CM) aq. extract	StarPEG-heparin Thiolated heparin-diarylated PEG Poloxamer 407/ Carboxymethyl cellulose sodium (P407/CMCs)	Mixing and cross-linking Mixing and photo-polymerization Cold method	Sustained release; improved tissue regeneration; bioactivity maintenance; helps in neo-vascularization; possesses re-epithelialization and wound healing capability; excellent biocompatible properties; high moisture retention potential	Geer et al. (2005); Goh et al. (2016); Wang et al. (2016)
3. Liposome	EGF + IGF-1 + PDGF-A EGF Quercetin	HA + Protamine Chitosan gel PLGA-Gelatin liposomal hydrogel	Cationic elastic liposome Film hydration method Double-emulsion solvent evaporation technique	Sustained release; enhances wound healing; higher cell proliferation; bioactivity maintenance; hemocompatible; promotes re-epithelialization; rapid tissue regeneration and restoration	Choi et al. (2017); Değim et al. (2011); Jangde et al. (2018)
4. Nano-particles	EGF Ciprofloxacin	Glyceryl Palmitostearate PCL and PLGA	Emulsification-ultrasonication Nano-precipitation method	High encapsulation efficiency (EE%); controlled burst-sustained release; effective and enhance wound healing; higher cell proliferation; bioactivity maintenance	Gainza et al. (2014); Günday et al. (2020)
5. Sponge	PDGF FGF + FGF + Vitamin C	EUP3 + Gelatin HA + Collagen	Electrospinning Freeze drying	Sustained release; enhance wound healing; bioactivity maintenance; promotes neo-tissue regeneration	Li et al. (2017); Niyyama and Kuroyanagi (2014)
6. Porous scaffold	rhVEGF + Gentamycin Ciprofloxacin	PLGA + Col + Ch Col + Ch + PCL + Calcium peroxide	Melt molding Freeze drying	Controlled release; facilitate cellular activities; anti-bacterial effect; neo-tissue regeneration; neo-vascularization; enhance wound healing	Wang et al. (2015); Tripathi et al. (2021a)
7. Liposome	EGF + IGF-1 + PDGF-A EGF Quercetin	HA + Protamine Chitosan gel PLGA-Gelatin liposomal hydrogel	Cationic elastic liposome Film hydration method Double-emulsion solvent evaporation technique	Sustained release; enhances wound healing; higher cell proliferation; bioactivity maintenance; hemocompatible; promotes re-epithelialization; rapid tissue regeneration and restoration	Choi et al. (2017); Değim et al. (2011); Jangde et al. (2018)

Table 6 (continued)

Delivery system	Growth factor/drugs	Biomaterial	Method	Significance	References
8. Micro-particles	bFGF + Rifamycin	Alginate-CaCO ₃	Microfluidic method	Sustained release; promote cellular activities; neo-angiogenesis; promote wound healing; ECM production and neo-tissue formation; good biocompatibility & biodegradability	Shi et al. (2019)
9. Microspheres	FGF10 bFGF Ag ⁺	PLGA Col+CH+CS + PLGA Chitosan	Compound emulsion Freeze drying Emulsified cross-linking method	Sustained release; accelerated wound healing and closure; promote cellular proliferation; ECM production and neo-tissue formation; neo-angiogenesis; bioactivity maintenance	Xu et al. (2020); Cao et al. (2015); Gunday et al. (2020)

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