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Journal of Dermatology and Skin Care

RESEARCH ARTICLE

Skin Tissue Engineering: Principles and Advances

Dhasmana A[1*], Singh S[1], Kadian S[1] and Singh L[2]

[1]*Department of Polymer and Process Engineering, Indian Institute of Technology Roorkee, Saharanpur, Uttar Pradesh-247001, India*

[2]*Department of Pharmacology, Kharvel Subharti College of Pharmacy, Meerut, Uttar Pradesh, India*

[*]**Corresponding author:** Dhasmana A, Department of Polymer and Process Engineering, Indian Institute of Technology Roorkee, Saharanpur, Uttar Pradesh-247001, India, Tel: +91-9536885390, E-mail: archidpt@iitr.ac.in

Citation: Dhasmana A, Singh S, Kadian S, Singh L (2018) Skin Tissue Engineering: Principles and Advances. J Dermatol Skin 1: 101

Abstract

In today's world, there is a strong demand of skin substitute in the world because of large skin defects resulting from burns, trauma, genetic defects and other diseases that can lead to skin necrosis, ultimately became a major healthcare challenge. Skin is the first tissue-engineered the product and currently, we have many available skin substitutes out of which some are commercially available (e.g., Alloderm, Integra, Recell) and some are under clinical trials. There are different methods and materials (cell line, polymers, growth factors) used for skin-substitute fabrication but each of them has certain limitations. Therefore, from the commercial point of view, tissue-engineered skin substitutes are not very successful yet. Unfortunately, there is also currently no bioengineered skin that can completely simulate the complexity of human skin either in form or function. Thus, we have to find an ideal skin substitute that can mimic native skin's structure and function.

Keywords: Skin; Tissue Engineering; Wound; Acellular; Regeneration

Introduction

Skin (cutaneous membrane) consists of approx 7% of total body weight and total surface 2 m^2. It is a bilayered membrane consisting of an upper superficial epithelial tissue layer called epidermis and lower fibrous connective tissue layer called dermis (Figure 1) [1,2]. Below the dermal layer, areolar connective and adipose/fatty tissue layer called hypodermis present which helps to connect the skin with internal organ system [1]. The thickness of the skin is varied for facial and palm i.e., 1.5-4 mm. Several other accessory appendages (e.g. nails, glands, hairs) derived from epidermal cells are deep extending into the dermal layer. Along with accessory organs and hypodermis (subcutaneous fatty layer) consists the largest system called Integumentary system, 16% of body weight, which plays an essential role to maintain the homeostasis and protection of inner organs [3].

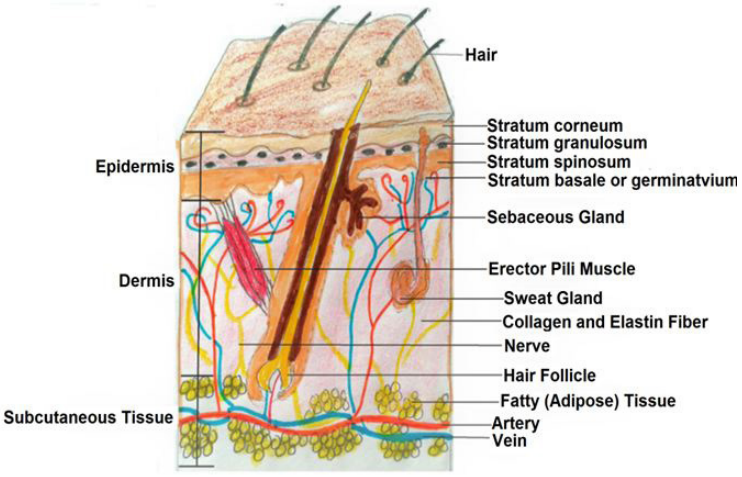

Figure 1: Anatomy of human skin

Skin acts as an anatomical protective barrier between the external environment and internal organ system, to provide protection against pathogens, regulate body temperature, provide sensation and synthesize Vitamin D etc. Although, the damage of skin tissue results in infection, losses of tissue function and scar formation which ultimately becomes a major healthcare challenge [3]. There is a different type of skin wounds, some are traumatic-burn abrasion, puncture, blister, incision, laceration, avulsion, contusion, pressure ulcer and genetic disorders or diseases e.g. MRSA, diabetic ulcer, cancer [3,4].

Wound healing is a complex process divided into different stages – *hemostasis; inflammation; proliferation and remodelling of the regenerated tissue*, includes the chemotaxis or signalling, phagocytosis, neo-collagenesis and remodelling of collagen matrix [5]. Although these all phases, there are different factors affecting the wound healing process categorized into two categories i.e., external and internal factors (Figure 2). Moreover, during wound healing, *regeneration* and *repairing* are two different important aspects of functional tissue/organ formation, without regeneration repairing of injured tissue results scar or fibrosis [6]`

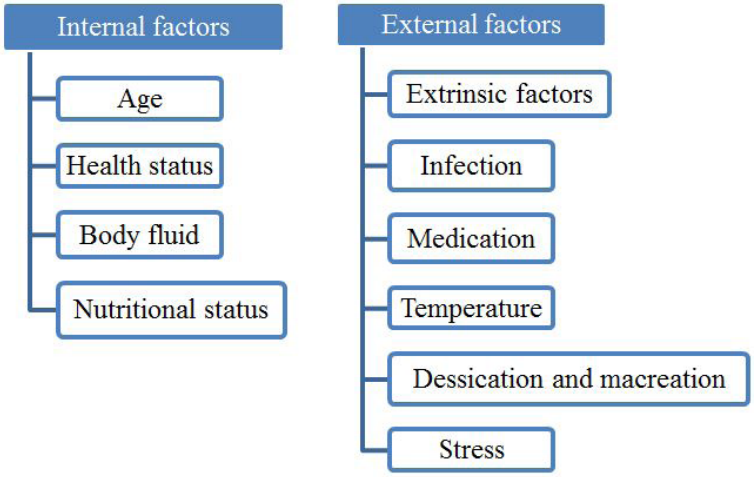

Figure 2: Factors affecting wound healing process

According to the WHO survey annually, over 300,000 deaths caused by burn injuries and 6.5 million individual suffer from chronic skin ulcers [7]. In India, over 10 lakh people are moderately or severely burnt every year. As per the 2012 data of the Union health ministry of India, 70 lakh burn injury cases annually of which 1.4 lakh people die every year [8,9].

Conventional Treatments

Naturally, the skin has regenerating capability and in the response to any injury, body healing or repairing mechanism comes in action to attempt the replacing of the damaged tissue with the regenerated functioning neo-tissue. Skin wounds are generally classified into different groups (i) *on the basis of injury*-surgical and non-surgical (traumatic); (ii) *on the basis of depth- superficial*, partial and full-thickness wound and (iii) *on the basis of healing*-acute and chronic [5,10]. In case of small wounds, surrounding skin tissue repair the damaged area by natural self-regeneration capacity but in cases of infectious, deep or non-healing wounds various types of conventional treatments [3]. Different types of drugs, biomolecules loaded ointments/creams e.g. topical antibiotics, herbal extract, silver and surgical dressing e.g., cotton gauze and bandage materials (Figure 3). However, when the wounds are of large size and deeper below the dermis, skin grafts are required to aid repair and regeneration for the restoration of normal skin function [11]. Different types of basic wound dressing material are classified into different categories.

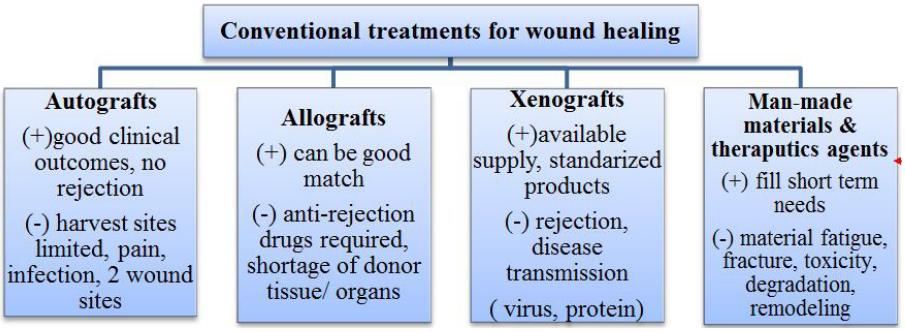

Figure 3: Conventional treatments for tissue repairing and regeneration

Facial reconstruction surgery was invented in 600 B.C, before 150 years of Hippocrates by an ancient Indian physician *Sushruta,* who was the first surgeon, performed the plastic surgery in human [12]. Later Reverdin in 1871 introduces the various skin-grafting techniques which have been used successfully at clinically level [13]. Skin grafts provide immediate coverage to the wounds and provide the supporting matrix or bed for the faster tissue regeneration. Generally, autograft is used for grafting because they are

non-immunogenic but the limited availability of autologous skin especially in case of large area wounds, pain, scarring, infection and morbidity at donor site are the major issues still faced by surgeons. Clinically allograft and xenograft have been focused, but it is reported that these grafts have chances of immune-rejection and disease transmission (cattle to human, HIV etc.) from donor to receivers [14]. Therefore, to overcome all these problems, researchers have been focused on the fabrication of other skin substitutes by applying the tissue engineering principle.

Skin Tissue Engineering

Tissue-engineered skin substitutes are classified into three categories (i) *on the basis of material*: biological, synthetic and bio-synthetic; (ii) *on the basis of covering time:* temporary and permanent and (iii) *on the basis of the layer:* epidermal, dermal and bilayered skin substitutes.

The first attempt in the field of STE was done in 1974 by Rheinwald and Green by fabricating autograft (CEA) from the small piece of skin containing sufficient cultured healthy human keratinocytes [15]. Later in 1981, O'Conner and Gallico clinically used CEA for burn treatment [16]. These autologous cultured sheets were used subsequently by different groups although certain demerits such as graft stability, prolonged cultures time, the formation of fragile skin after healing and lack of dermal matrix support limit its application [13]. To overcome these limitations of CEA, Bell and co-workers, developed a skin-equivalent consist of a fibroblast-seeded collagen matrix having upper layer covering of keratinocyte and used this product clinically named as 'Apligraf' for chronic wound [17].

In 1979, Woodroof designed a composite dressing material 'Biobrane'-consist an outer layer of silicone membrane bonded to the inner layer of nylon mesh-porcine dermal collagen matrix; clinically used for burn wound treatment [18]. Later in 1980, Yannas and Burke, designed the similar bilayered composite burn wound dressing material 'Integra'- consisting of bovine collagen and shark GAGs protein matrix as a dermal template with an outer layer of Silicon membrane [19]. All these bilayered matrix products are commercially available as temporary skin substitutes only for wound dressing. Therefore, the efforts made by the above groups were far from the ultimate goal of replacing skin autografts for permanent coverage of deep or full thickness burn wounds [20].

After that many attempts have been done to fabricate ideal skin-substitutes by applying the tissue engineering principles and its triads' i.e. scaffold, cell-lines and growth factors. A number of approaches based on the choice of cell types (keratinocyte, fibroblast, stem cells), their source (autologous or allogeneic), choice of biomaterial for matrix formation (synthetic, natural, ECM based) have been made to improve tissue engineered skin-substitutes. The main aim of all these different techniques is to enhance cellular survival and physiological functioning of damaged tissue during regeneration and some are successful to some extent [21].

At international level, there are many tissue-engineered skin substitutes manufacturing companies e.g., Acelity, Smith & Nephew, Molnlycke, ConvaTec, Coloplast, Organogenesis, Integra Lifesciences Corporation, Medline Industries, 3M, Derma Sciences, Hollister Incorporated, Human Biosciences, Medtronic, Hartmann Group, B.Braun Melsungen, BSN Medical, Urgo Medical, Mimedx Group, Inc., Nitto Denko, Winner Medical Group. Among them, the top three companies are Acelity, Smith & Nephew and Molnlycke and they maintain a stronghold over revenue shares in market value. Their collective share in market value in 2014 was 51.4%. The global market for tissue-engineered skin substitutes is expected to generate revenue at a highly positive CAGR of 17.2% within a forecast period from 2015 to 2023 [22]. This market is expected to generate revenue of US$3.87 bn by 2023 and acellular skin substitutes are expected to generate revenue of US$2.29 bn by 2023 [23]. Different skin substitutes are commercially available but the cost factor is also a limitation, therefore, enormous research is still going on to make a cost-effective and efficient skin substitute [7,21,24].

Advances in STE

Majority of commercially available and clinically approved tissue engineered skin-substitutes are cellular therapy, acellular ECM constructs and composite cell-seeded matrix. Although, these products are not the perfect replacement of natural skin they may reach the need of skin grafts to some level by providing immediate protection to the wounds and improved quality of tissue regeneration after injury [25].

Autologous cell-based products showed positive outcomes during clinical studies with scar-less healing, but the long-time cell culturing procedure, complex and expensive method of cell-isolation, the short-life span of cell-sheet and inappropriateness for deep wounds and large burn (more than 80%) limits their applicability [26,27]. Similarly, other products used for the treatment of partial and full-thickness wound provides the protective barrier to the wound but the bio-functional properties such as sensation, thermoregulation, pigmentation etc., of the regenerated skin have not been achieved yet [28]. Therefore, to overcome the above-mentioned problem, researchers designed cell-seeded tissue engineered constructs for bio-functional recovery of the regenerated tissue. Combination of different type of skin cells-keratinocytes, fibroblast, hair-follicle cells, as well as stem cells, the adipose cell with biomaterial construct or matrix to create functional skin has been performed [9,29-34]. Scaffold or biomaterial matrix seeded with cells act as a house for cells, which plays a significant role in cellular interaction, migration, proliferation and the regeneration of fully vascularized functional tissue.

In this direction, many researchers and scientists developed some smart biomimetic hybrid material along with functionalized matrix to enhance the cellular interaction with matrix and restore the skin native properties after regeneration (Figure 4) [27,35-

37]. Potentially, some researchers introduced some novel design of skin substitutes using different biomaterial: keratin-collagen sponge; silk-fibroin-alginate matrix; bacterial cellulose matrix; collagen paste; nanofibrous membrane consist of PCL-collagen, PLGA-chitosan; PU microfibrous membrane etc. Currently, some groups are focusing on decellularized cadaveric tissue and come up with very promising material for scaffold fabrication due to their strong biocompatibility and preserved 3D biomimetic structure [38-47]. Scaffold fabricated from decellularized cadaveric human, porcine and bovine tissue are already in use nowadays but the risk of disease transmission (e.g., spongiform encephalopathy-cattle to human; HIV-human to human), limited availability (cadaveric human tissue) and ethical issues related the use of human/animal origin tissue, limits the use of tissue from these sources [48,49]. Still, a lot of research is going on using cadaveric tissues from other animal sources including cadaveric fish and goat tissue, which is easily available, less-immunogenic and has no chances of disease transmission from animal to human [50-52]. Therefore, the selection of material for skin-substitute is an important aspect for designing the biocompatible matrix for bio-functional tissue regeneration. The biomechanical characteristics of the skin-substitute are also important to attempt the following changes occurs in the matrix after the repopulation of cells and subsequent degradation of the matrix with the progression of time [53-55].

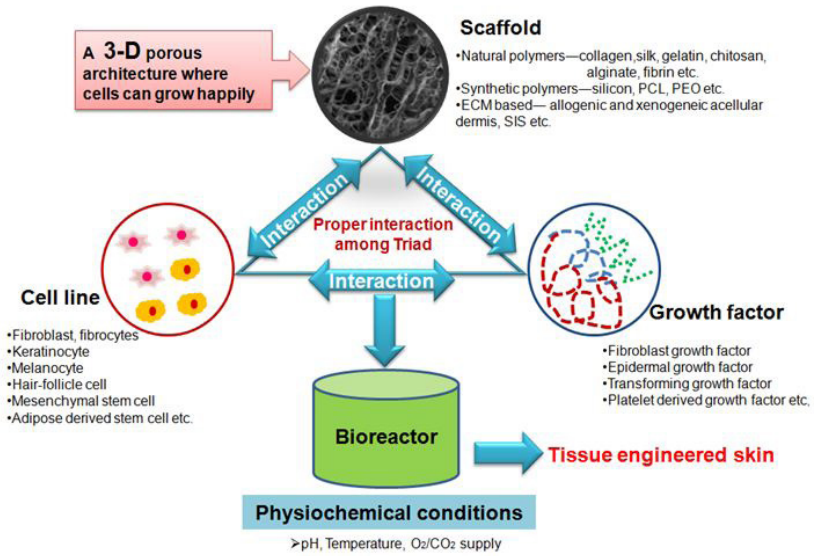

Figure 4: Triads of tissue engineering for the regeneration of bio-functional skin tissue

Another approach for the functional tissue regeneration and scar-less healing as similar to the fetal wound healing has been done by incorporation of growth-factor in skin-substitute. Incorporation of specific growth-factors e.g. TGF-β results in scar-less healing, better signaling at a molecular level for proper alignment of fibril matrix and inhibits the chance of fibrosis. However, growth-factor beneficial for regeneration of tissue but the dose-concentration, mechanism or mode of action and knowledge of clinical safety is another important aspect [56-58]. The challenge of higher cost and quality control is still along with the increased complexity of the tissue engineered product. Three-dimensional (3D) or ink-jet printing is the latest advanced technology, in which bio-functional tissue formed by printing the matrix contains specific cell in controlled shape and depth of the wound [59,60]. Different types of bioprinting techniques include magnetic bioprinting, stereolithography, photolithography, and direct cell extrusion has been used for designing complex 3D architectures, which provides a microenvironment for integrated cells to mimic natural ECM of a particular tissue [61]. This technology provides a platform for designing artificial skin by using autologous or allogeneic cell line, biomolecules and suitable biomaterials with the help of computer aided designing software [62-64]. However, lack of the compatibility and bio-elasticity of bio-ink (biomaterial/polymer) used for the printing, and the number of cell seeding and their viability are the main issues still faced by the researchers for the fabrication of complex architecture of the skin tissue [65,66].

Till date, in the market, a lot of tissue engineered products (scaffolds) are available: Integra and Biobrane (bio-synthetic); Alloderm, SureDerm and GraftJacket (allogeneic); OrCel, Apligraf, Matriderm, Permacol and Oasis (xenogeneic). The tissue sources which are used worldwide for fabricating ECM based scaffold, includes Human skin (allograft), Porcine (small intestine, dermis) and Bovine (pericardium, fetal dermis) tissues (xenograft). The potential and pitfall of some commercially available skin substitutes are explained below:

Cellular skin-substitutes

EPIBASE®: EPIBASE® (Genverier Lab, Sophia-Antipolis, France) having confluent stage autologous keratinocytes cells, which spray over the wound site to provide outer cell layer covering especially in the case of extensive burn and cutaneous calciphylaxis. But the higher cost of treatment i.e, $53/cm^2 wound area, long culture time, difficulties in handling and short life are the certain demerits of this product [67,68].

Recell®: Recell® (Avita Medical Ltd.US) is cell spraying technology consists of subsequently cultured keratinocyte and melanocyte isolated from the patient body. After sufficient cells proliferation up to 3 weeks, cell suspension spray over the wound site for the

permanent wound coverage. The cost of treatment is approximately $3/cm^2$ wound area. In this treatment isolated melanocyte helps to restore skin colour, overcomes the problem of scar and hypopigmentation after wound healing. But limited to recover <2% of total body surface area burn in adults and 4% in children [69].

Non-cellular skin substitute

Scaffold (3D polymeric matrix) is one of the most important elements of tissue engineering for regeneration of bio-functional neotissues. It provides a template for cellular adhesions and their proliferation for tissue regeneration. Therefore, many researchers have been focusing on the fabrication of biomimetic matrix, which can be used as temporary (dressing material) and permanent substitute for the repairing, regeneration of damaged skin tissue and subsequently restore the functionality. In this attempt, different type non-cellular matrices have been designed by researchers, which are commercially available in the market.

Biobrane®: In 1979, Biobrane® (Bertek Pharmaceuticals, Morgantown, WV, US), is the first commercially available bio-synthetic composite dressing material. It is a bilayer matrix consists of a porous nylon mesh with porcine-derived collagen adsorbed onto the "inner" surface and silicone adsorbed onto the "outer" surface. The outer thin semi-permeable silicone membrane work as epidermis bonded to the inner nylon-collagen fabric, which acts as dermis part of the skin. The matrix has a lower cost i.e., $1.30 per cm^2 sheet and provides immediate coverage for the partial-thickness excised wounds until skin graft material is available [70]. Although this material has good wound healing rate with lower pain and morbidity at the wound site, it has certain demerits-requirement split-thickness skin grafting (STSG), the risk of infection and toxic shock syndrome due to the accumulation of exudates underneath the graft [70,71].

Integra®: In 1980, Yannas and Bruke designed bilayered skin substitute-Integra® (Integra Life Sciences, Plainsboro, NJ, US), consist of an inner biodegradable porous dermal layer of bovine collagen-chondroitin-6-sulfate and an outer temporary pseudo-epidermal layer of synthetic silicone polymer. In 1996, FDA approved the Integra as an artificial skin for the treatment of deep partial- or full-thickness burns. It has a long shelf life up to 2 years, provides immediate availability which allows time for the neo-dermis formation, and good aesthetic results [72]. The cost of per cm^2 sheet is $6.15. However, the disadvantage is its high cost and two-step procedure i.e., after re-epithelization epidermal layer (outer silicon layer) replaced by autograft (STSG). Clinically studies reported that dermal tissue form without scarring but the accumulation of exudate underneath occur at the wound site that leads to infection [72-74].

Pelnac™: Pelnac™ Standard/Pelnac Fortified (Gunze Ltd, Medical Materials Center, Japan) is a bilayer matrix consists of the inner porcine tendon collagen spongy layer with outer non-adhesive silicone film. It has long shelf-life and applicability for the wide range of full-thickness skin defects, e.g., traumatic, surgical wounds, deep burn, and cancer [75]. Although it is safe and shows excellent long-term effect; it requires secondary post-operative surgery (STSG) for the wound coverage after the removal of the upper silicon film. Clinical studies reported that Pelnac treated full-thickness wounds along with STSG results significant recovery (in 90% cases) [76].

Suprathel®: Suprathel® (BioMed Sciences, Allentown, PA, USA) is a synthetic- absorbable wound dressing material consisting of polymer lactic acid [77]. It is useful for the treatment of partial-thickness burns and acute surgical wounds. The approximate cost of per cm^2 sheet is $1 only. It has a long shelf life, antisepsis property and causes less bleeding compared with other product. However, in the case of deeper wounds, the therapeutic effects are not so significant and delayed treatment timing [78,79].

Terudermis®: Terudermis® (Olympus Terumo Biomaterial Corporation, Japan) is a bio-synthetic skin-substitute as similar to the Integra. It is a bilayer skin substitute consists of inner lyophilized dehydrothermally cross-linked bovine collagen sponge bonded with the outer silicon membrane, which acts as a barrier to the outer environment. The cost of per cm^2 sheet is $6.52, similar to of Integra. Clinically this product requires STSG (after the removal of the Silicon layer) for the treatment of deep burns, chronic ulcer wound, post-traumatic deformity injury [80-82].

Hyalomatrix PA®: Hyalomatrix PA® (Fidia Advanced Biopolymers, Italy), is a bilayer matrix consist of benzyl alcohol esterified hyaluronic acid-HYAFF covered by a temporary silicone layer serving as the epidermis. It acts as a temporary substitute only for the treatment of partial-thickness wounds. Clinically, Hyalomatrix PA is not beneficial for the treatment of chronic wounds and causes infection at the wound site [83,84].

Decellularized acellular graft

Allogeneic acellular graft of cadaver human tissue: Cadaver skin obtained from the genetically similar species is used for the fabrication of the acellular graft, which is used for the treatment of skin wounds and soft tissue reconstruction. Clinically, these products are non-immunogenic, biocompatible and have a better-wound healing rate. Besides that, they have certain limitations-high cost, ethical restriction, limited availability of material etc. Some of the commercially allogeneic products are described below:

SureDerm®: SureDerm® (HANS BIOMED Corporation, Seoul, Korea) is an acellular graft made up of the lyophilized human pre-meshed dermis. It is used for soft tissue reconstruction and burns wound treatment. This product has long shelf-life up to 2 years, and permanently incorporated at the wound site and acts a basement for the subsequent STSG [85-87].

Alloderm®: Alloderm® (LifeCell Corporation, Branchburg, NJ, US, 1992) is processed cadaveric human acellular lyophilized

dermis with the intact basement membrane. The cost per cm^2 sheet is approximately $6.45. The acellular dermal graft has been clinically used for the treatment of different types of wounds including burn, surgical, reconstructive surgery and reconstruction of soft tissue damages [88-90]. However, in the case of full-thickness or deep wounds, thin STSG should be required along with the acellular dermal graft as a single step treatment [91].

GraftJacket®: GraftJacket® (Wright Medical Technology, Inc., USA) is an allogeneic acellular pre-meshed dermis fabricated from cadaver human skin. The thickness of the graft is up to 0.4-0.8 mm thick only, which induce better vascularization. It is clinically used for the treatment of partial and deep thickness wounds. But the higher cost of the product limits its applicability [92-94].

Xenogeneic acellular graft of cadaver porcine and bovine tissue

To overcome the limitations of allogeneic graft or skin-substitutes, researchers have been focusing on xenogeneic cadaveric bovine and porcine tissue. Xenografts are widely available cheaper material for wound healing and tissue engineering application. But the ethical issues and the risk of immunogenic response limits the applicability of xenografts. Clinically acellular xenogeneic grafts which are available in market discussed below.

Permacol™: Permacol™ Surgical Implant (Tissue Science Laboratories plc, UK), is the acellular porcine dermal tissue matrix, mainly consist of collagen and elastin protein and cross-linked by diisocyante [95]. It acts as a permanent substitute for the reconstruction of abdominal wall such as in the case of a hernia [96]. Although in the certain case, it is used for dermal reconstruction along with subsequent overlying STSG. But due to the lack of vascularization and high cost $17/$cm^2$ of the product, it gets less attention [97].

OASIS®: OASIS® (Cook Biotech In, West Lafayette, US), is another porcine tissue acellular graft, first invented in 1989. It is consisting of lyophilized porcine small intestine submucosa (SIS) and clinically applied for the chronic wound healing. Clinically, it was reported that the healing rate was good in case of ulcer wounds but the applicability of matrix for full-thickness wound healing was inefficient and the cost of the product is high having cost $11.2/ cm^2 [98-100].

Pri-matrix®: Pri-matrix® (TEI Biosciences Inc, South Boston, MA, USA) is derived from the fetal bovine dermis. Acellular dermal matrix provides an environment to support cellular repopulation and revascularization for better wound healing. It is rich in Type III collagen, a collagen found in the fetal dermis that is active in developing and healing tissues. It is used for the treatment of different wounds types-ulcers, traumatic, second-degree burn and surgical [101-103].

Matriderm®: Matriderm® (Medskin solutions, Dr Suwelack AG, Billerbeck, Germany) is a lyophilized bovine dermis of 1mm thickness and coated with elastin hydrolysate, used in the single-stage surgical intervention. It is used in necrotizing fasciitis defects and for the management of exposure of Achilles tendon in the cases of secondary burn injury. It requires additional STSG for coverage of wound [104,105].

Composite skin substitute

Epicel®: Epicel® (Genzyme Biosurgery, Cambridge, MA, USA) consists of confluent auto keratinocytes cultured on Petroleum gauze backing. It was FDA approved in 2007 for the severe deep dermal or full-thickness burns treatment. It provides permanent wound coverage of large area wound from the small amount of skin harvested at cost of $15.15/$cm^2$. Here the use of autologous cells prevents immune-rejection and provides permanent large area wound coverage, especially in extensive burns. Clinically, it was reported that cell-sheet implant takes at least 3 weeks for obtaining epidermis but lacks a dermal component. It has certain limitations-long culture time (2-3 weeks), variable take rate, poor long-term results, 1-day shelf life, expensive, the risk of blistering, contractures, and infection [106,107].

Epidex™: Epidex™ (Euroderm GmbH, Leipzig, Germany) is a permanent epidermal skin substitute consisting of the autologous outer root sheath hair follicle cells cultured on silicon membrane. Stem cells obtained from hair-follicle differentiated into keratinocytes and form cell-sheet, which is reinforced onto Silicon membrane and finally grafted at the wound site. The cost of the product is same as of Epicel, $15.05/$cm^2$. It is used for the treatment of chronic ulcers but difficult to handle or fragile and long fabrication time (almost 6 weeks) are the major disadvantage of it [108,109].

Apligraf®: Apligraf® (Graftskin) (Organogenesis/ Novartis, Canton, MA, US) is a bilayer bovine collagen gel scaffold seeded with live allogeneic neonatal foreskin fibroblasts and keratinocytes, developed by Eugene Bell in 1981. In 1998, FDA commercially approved it for the treatment of venous leg ulcers, and in 2002 approved for diabetic neuropathic ulcers; burn wounds. Clinical studies showed that it can be used as a temporary dressing for the treatment of acute surgical wounds, chronic ulcers and epidermolysis bullosa (EB), and required STSG for better healing. Meanwhile, it requires repeated applications, minimum *in vivo* of cells survival rate, short shelf life, difficult in handling, the risk of disease transfer, high cost-$30/$cm^2$, large-scale clinical trials are still needed for the same [110-112].

OrCel®: OrCel® (Ortec International, New York, USA) is a bilayer graft consists of viable allogeneic neonatal foreskin fibroblasts and keratinocytes cultured in bovine collagen sponge, developed in 1971 by Mark Eisenberg. It acts as a bio-absorbable matrix and provides a porous matrix for the migration of cell. In 2001/2008, FDA approved its application for the treatment of split-thickness donor sites in patients with burn and acute surgical wounds in EB. Its cost $6.32 for the coverage of wound of per cm^2. Clinically,

it showed reduced scarring and faster healing of wounds. However, it has the risk of rejection and uses only for biological dressing rather than a permanent skin substitute [113,114].

Laserskin®: Laserskin®/Vivoderm (Fidia Advanced Biopolymers, Padua, Italy) is a recombinant matrix comprising of patient own keratinocytes cultured over the laser-microperforated hyaluronic acid ester matrix for cell migration. Clinically, it was showed that the graft is biocompatibility, less-immunogenicity and results in scar less wound healing. But, it has a short life span, expensive-$129/cm^2 and applicable only for the treatment of partial thickness (epidermal) wound [115,116].

Bioseed-S: Bioseed-S (BioTissue Technologies GmbH, Freiburg, Germany) a composite matrix contains patient own keratinocytes seeded on a fibrin sealant. It is used as an epidermal substitute for the treatment of chronic leg ulcers and cost-effective (cost-$0.5/cm^2). Clinical studies reported its efficiency for the treatment of recalcitrant venous ulcers, almost 50% increase in wound-healing efficiency compared with standard treatment but not applicable for infectious chronic wounds [117,118].

CryoSkin™: CryoSkin (Altrika Ltd. UK) is a cryopreserved monolayer of non-cultured allogeneic keratinocytes on the silicon backing. The cost of per cm^2 sheet is $5.32. Clinically it was reported that it is effective for the treatment of leg ulcers. But the repeated application, more healing time (24 weeks) and the chances of infection limits its applicability for the treatment of chronic wounds [119].

Hyalograft 3D®: Hyalograft 3D® (Fidia Advanced Biopolymers, Italy) fibroblast seeded over the esterified hyaluronic acid fibrous matrix. It is mainly used for articular cartilage tissue regeneration. In certain clinical studies, it showed effectiveness in diabetic ulcer and used as permanent skin substitute for the deep lesion wound [120,121]. It enhances the basement membrane formation but used only for temporary dressing [84].

Transcyte® (DermagraftTC): Transcyte® (DermagraftTC) (Advanced BioHealing, Inc., USA) is a bilayer graft consists of nylon mesh coated with porcine dermal collagen and bonded to a silicon membrane seeded with viable neonatal human dermal fibroblast. In 1997 & 2001, FDA approved it as a temporary dressing material for burn treatment. The cost of per cm^2 sheet is $15. It has been used for the treatment of second and third-degree burn and has long shelf-life. But requires secondary surgical procedure STSG and also causes immune rejection of graft because of allogeneic cell line [111,122].

Permaderm™: Permaderm™ (Regenecin Inc. NJ, US) consists of autologous keratinocytes and fibroblast cultured on an absorbable bovine collagen matrix. It helps to reduce the morbidity and mortality in extensive deep burn; shows better outcomes compared with meshed grafts with shorter wound closure time and cheaper in cost $1/cm^2 sheet. It was reported that in certain cases the graft-induced the immunogenic response. Permaderm has limited availability and requires frequent regrafting process [26,123].

PolyActive®: PolyActive® (Holland Composite Implants B.V., The Netherlands) consists of elastomeric & biodegradable polyethene oxide terephthalate/ Polybutylene terephthalate copolymeric matrix seeded with autologous keratinocytes and fibroblast. It is a temporary substitute, use for the partial-thickness wound coverage because of non-biodegradability. It does not induce any immune-rejection but higher in cost ($26/cm^2) as compared to other allogeneic products [124-126].

TissueTech Autograft System: TissueTech Autograft System (Fidia Advanced Biopolymer, AT, Italy) is a recombinant system consist of Laserskin (dermis) & Hyalograft 3D (epidermis). Clinical studies reported that more than 85% of cases of full-thickness ulcers wounds with an area >5cm^2 were completely healed and confirms its applicability for the diabetic ulcers wound. Although the wound healing rate is good but it is not categorized as true skin, because it requires two products for grafting which is a complicated process [127,128].

Challenges and Future Perception

In this review, we describe the recent development in the field of skin tissue engineering as well as the current status of the commercially available skin substitutes. Extensive research has been done on the fabrication of bioengineered skin and to overcome the limitation of the commercially available products. Skin tissue engineering is an emerging field of biotechnology and biomedical engineering. Over the last two decades, significant improvement has been done for the development of bio-engineered skin and various bio-engineered skin substitutes are commercially available for clinical application. However, the selection of suitable bio-material, fabrication technique, identification of cell-lines and physiological condition for the regeneration of neo-skin tissue is an active area of research. All these approaches not only focused on its applicability for wound healing or tissue regeneration but also for the development of products for the study of the drug-delivery system to replace the animal model system.

3D printed graft or scaffold improves the quality of regenerated skin tissue by providing an interconnected porous structure for the better vascularization, nutrient diffusion, cell migration and tissue regeneration. Another important aspect is the selection of specific cell lines and reprogramming of cell cycle pathway e.g. conversion of fibroblast to pluripotent for bio-functional tissue regeneration. These reprogrammed cell lines have similar characteristics to the embryonic stem cells and the combination of these cell lines with 3D scaffold improves the tissue regeneration and repairing properties. Although, 3D printed grafts have complex tissue architecture but still bio-printed skin lacks formation of skin appendages e.g., hair follicles, sweat glands, which limits its applicability as true skin.

Despite that, there are many bio-engineered skin-substitutes available on the market and some are the under-clinical trial. But still we don't have an ideal skin substitute to overcome the problems of repairing and regenerating the biofunctional tissue and to analyses the drug/bimolecular delivery in *in vivo* system study [129]. As well as, the limited availability of experimental data/ clinical study and the higher cost of the products limit their applicability [130]. Therefore, the cost of bio-medical procedure get reduces and provides the superior medical facilities at lower cost with higher throughput.

In future, the researchers should focus on try to recapitulate the properties of *in vivo* skin, which may provide the fast service and better recovery. Many researchers focus on designing bio-sensors, which are soft, compatible electronic devices to measure the wound healing rate and the microenvironment of the wound area. These novel approaches provide the platform for better regeneration of tissue; control the therapeutic effects and continuously monitoring the tissue healing. Bio-sensor and 3D printing technology provides advanced competence and better fidelity for the translation study from the lab testing to clinical applications. Therefore not only the biological principles but the multidisciplinary concepts (e.g., chemical engineering, biophysics and electronics) of engineering should be applicable to fabricate the bio-engineered skin for improving the quality of life.

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