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Review Article

Skin Substitutes; an Updated Review of Products from Year 1980 to 2017

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Abstract

Skin substitutes help skin repair and regeneration, and restore the efficient properties of skin in the time of acute burn injuries or other chronic skin lesions. They can act as permanent skin replacements or temporary wound covers, depending on their composition and design. Recent studies have overcome some obstacles, but till today no ideal skin substitute has been developed. The aim of this study is to introduce some commercially available and under development products and also to provide information about these substitutes and their limitations in order to use native-like skin substitute design and production. Currently the accessible skin substitutes have several limitations such as infection risk, reduced vascularization and lack of integration to host tissue. The absence of various cells which are responsible for temperature control and insulation, pigmentation, immune regulation and nerve supply is among the mentioned limitations. Further researches will be required to resolve different issues and suggest practical solutions toward a true skin substitute with excellent engraftment and durable viability. In addition, availability and awareness of these skin substitutes in developing countries is not adequate in spite of the number of cases requiring this kind of treatment, therefore, it is needed to develop indigenous economical technology to promote available treatments in hopes of achieving substitutes with higher quality and reasonable cost available to a greater percentage of patients.

Keywords: Tissue-engineered skin; skin substitute; wound healing; Commercial products; USFDA

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Introduction

Skin plays a vital role in protecting the body from mechanical damages such as wounding. In time of severe skin injury such as acute burn wounds or chronic wounds, the skin needs instant coverage to facilitate regeneration and repair [1]. The treatment of full-thickness skin defects represents an important and common clinical problem worldwide. Today, the most autologous skin grafting techniques are based on transplanting split-thickness skin from a donor site to the area of defect. Limited donor sites for harvesting split-thickness skin are often a significant problem especially when a large area of defects has to be covered. The lack of dermal tissue within the wound area is an additional drawback of the split-thickness skin which frequently leads to significant scarring and wound contraction. Therefore, split-thickness skin is often used in combination with a dermal template [2, 3]. Also, the presence of a large number of cells in bio-engineered autologous skin substitute facilitates rapid regeneration of native-like skin in the wound area [1].Tissue engineering of the skin was started through the concurrent works of two groups forty-two years ago in the United States. In 1975, Rheinwald *et al*., reported the in-vitro sequential cultivation of human epidermal keratinocytes. The expansion of these cells into epithelia became possible and they were suitable for grafting [4, 5]. Simultaneously, Yannas *et al*., reported the in vitro and in vivo characterization of collagen degradation rate, which was the beginning of the designation of artificial biological dermal substitute, which led to "tissue engineering of the skin dermis". In 1981, both groups reported clinical use of their tissue-engineered substitutes in treating extensive and severe burns but with different approaches [6, 7]. O'Connor *et al.*, reported the world's first successful grafting in extensive burns with cultured epidermal autografts (CEA) [8, 9]. At the same time, Burke *et al.*, reported the use of an artificial dermis for the extensive burn treatment with full thickness component which is now known as Integra® Dermal Regeneration Template and it is considered the "gold standard" status for the treatment of full-thickness burn injuries [10, 11]. In the mid-1980s, Cuono *et al.*, have proven the importance of dermal layer in substitutes by reporting a good graft take of CEA laid on vascularized allogeneic dermis in the wound bed [12, 13]. The Indiana University reported a final graft take of 72.7 % with a 91 % overall survival rate in severe burn patients and since 1990s, the allodermis/cultured autograft technique has been used by various centers [14].The application of a living human dermal skin substitute delivers some vital regulatory proteins and cytokines that stimulate keratinocyte proliferation, fibroblast migration and angiogenesis to accelerate wound healing process [15]. Different skin substitutes including single layer with keratinocytes, single layer with fibroblast or bilayer with both keratinocytes and fibroblasts secrete various mediators after transplantation which promote wound repair. Keratinocytes are the main cells of epidermal layer and form a stratified epithelium. Fibroblasts, the main dermal cell type, produce remodeling enzymes such as

collagenases and proteases which play important roles in the wound healing process. Different studies showed that bilayer substitutes secrete significantly higher amounts of chemokine (C-X-C motif) ligand 1 (CXCL1), chemokine (C-X-C motif) ligand5 (CXCL5), granulocyte-colony stimulating factor (GCSF) and IL-6. In contrast, the single layer substitute with keratinocytes secretes VCAM-1 more than other substitutes [16]. Containing only two cell types, fibroblasts and keratinocytes are considered the major limitations of currently available skin substitutes, however, recent studies showed that it is possible to incorporate different cell types into tissue engineered skin, including melanocytes, Langerhans cells, hair follicles (17, 18, 19) and adipose tissue (due to the ease of isolation and abundance of endothelial and mesenchymal cell lineages) [20]. In recent studies, scientists introduced $LGR6^+$ stem cells with the ability to undergo proliferation, differentiation, migration, and inducing epithelialization, hair growth and angiogenesis within the wound beds; it was the first applicable stem cell–based substitute capable of repairing full-thickness wounds and regenerating hair cells [21].

The aim of this review is introducing various products including the products that are already commercially available for clinical use and have been studied extensively in randomized controlled trials. We have also tried to give a brief insight into those under development. The current study seeks to provide skin tissue engineering with the information regarding the limitations of currently available products in making future research suggestions to solve the problems and to achieve more functional and applicable substitutes with a wide range of surgical options. Finally, it can contribute to move towards attaining the final goal of a complete full-thickness skin substitute.

Skin substitutes

Skin substitutes are artificial skin replacements that provide skin protective barrier when placed over acute and extensive burn injuries or chronic skin wounds. The main objective of tissue engineered products is to work as skin equivalents, restore the functional properties of skin and facilitate repair and regeneration. Several skin substitutes in forms of one or two replacement layers of the skin can act as temporary wound covers or permanent skin replacements, depending on their composition and design. They remove or reduce inhibitory factors, and help to provide a safe and rapid coverage. Skin substitutes also reduce mortality and morbidity from scarring (both at donor and treatment sites) and decrease the patient's risk of infection. More importantly, they reduce the total number of required surgical procedures and hospitalization time [1]. Both cellular and acellular substitutes provide cells and other key elements that promote re-epithelialization and revascularization of the wound bed while preventing degradation of the ECM, which are useful in the management of a variety of chronic wounds in combination with standard wound care [22]. Although each of these substitutes have their own advantages and applications in burn and wound treatment, none of them can fully simulate native skin.

Characteristics of an ideal skin substitute:

- Ability to resist infection and no antigenicity
- It is with both epidermal and dermal components
- withstand wound hypoxia
- Easy to prepare, store and use
- Cost efficient and Widely available
- Long-term stability [23]

Types of skin substitutes (Classification)

There are many different classifications of currently available skin substitutes [24]:

A) Anatomical structure

- Epidermal: those that consist of cultured epidermal cells with no dermal components.
- Dermal: those with only dermal components (with or without cells)
- Dermo-epidermal (composite): a bilayer containing both dermal and epidermal components. Dermal fibroblasts in static culture can assemble a native extracellular matrix (ECM) that is termed self-assembly. The dermis generated by this process can be seeded with keratinocytes to produce a bilayer construct structurally more similar to skin [25].

B) Type of the biomaterial

- *Biological (natural or tissue engineered skin)*

i) Autograft: permanent covers which use the skin from different parts of individual's body. Compared to autografts, they have the gold standard for skin coverage. They are generally divided into three main categories:

1- Split-thickness skin grafts (STSGs): They contain the epidermis and a variable thickness of the upper layers of dermis. The remaining layers of dermis heal wound by secondary epithelialization from the wound edges and keratinocytes of the deeper dermis. These types of autografts are most commonly used to repair large wounds.

2- Full-thickness skin grafts (FTSGs): They comprise the epidermis and the entire dermis. These grafts are preferred in areas where contracture of the grafts has harmful aesthetic or functional consequences.

3- Cultured autologous skin: Patient's skin cells are multiplied in the laboratory and then implanted onto various scaffolds to be used as skin substitutes. These are mostly known by the name of the manufacturer [1].

ii) *Allograft*: uses skin from other individuals (e.g., cadaver). A fresh allograft is difficult to obtain in cases of emergent requirements and it is more antigenic than a processed allograft with higher risk of transmission of infective diseases like hepatitis B and C and HIV. Therefore, skin banks have vital role in treating allografts through various methods such as cryopreservation and chemical treatment with glycerol, in order to preserve skin grafts for a longer time and also reduce antigenicity [26].

iii) *Xenograft*: uses skin from other species (e.g., porcine or bovine). All these grafts are temporary and eventually rejected by host immune system. Therefore, they have to be replaced by autografts or other substitutes.

- *Synthetic*

i) Biodegradable

ii) Non-biodegradable

- *Biosynthetic*

C) Skin substitute composition regarding cellular component:

- *Cellular*
- *Acellular*

D) Duration of the cover depending on its design and composition [27]:

- *Permanent* (Table 1)
- *Semi-permanent* (Table 2)
- *Temporary* (Table 3)

Some of the newly reported products are presented in Table 4 as in development products.

Table 1. Permanent skin substitutes; HCT/Ps: Human cells, tissues, or cellular-based products, *Commercially available products, 510(k): Premarket notification process

Brand name	Manufacturer/ year	FDA/ Status	Source (cell/scaffold)	Indication	Ref.
Epidermal substitutes					
Epicel™	Genzyme Biosurgery, Cambridge, MA, USA, 2000	HCT/Ps	Cultured autologous keratinocytes attached to petrolatum gauze support	Deep partial thickness and full-thickness burns	1, 28
EpiDex (Euroderm AG)	Modex Therapeutics, Lausanne, Switzerland, 2003	No FDA designation	Cultured autologous keratinocytes	Heal up to three-quarters of recal-citrant chronic leg ulcers	29
MySkin	CellTran Ltd, Sheffield, UK, 2004	-	Silicone support layer coating cultured auto-logous keratinocytes	Neuropathic, pressure and di-abetic foot ulcers, superficial burns	24
Laserskin or Vivoderm	Fidia Advanced Biopolymers, Padua, Italy, 2002	510(k)	Benzyl esterified hya-luronic acid derivative withautologous kera-tinocytes	Scarless fetal wound healing	24
Bioseed-S	BioTissue Technologies GmbH, Freiburg, Germany, 2002	-	Cultured autologous keratinocytes	Used to treat ther-apy-resistant chronic venous leg ulcers	24
ReCell CellSpray	Avita Medical Europe Ltd, Melbourn,UK, 2010	-	Autologous keratino-cytes in their most active proliferating state	Reducing the need of donor sites in deep dermal inju-ries, for the cor-rection of pigment disorders	30
Celladerm	Celladermceldon science LLC. Brooklins Mass, 2008	HCT/Ps	Living foreskin de-rived allogenic kerati-nocyte	Partial and full thickness burns; venous ulcers	31
Dermal substitutes					
Alloderm™	LifeCell Corporation, Branchburg, NJ, USA, 1999	HCT/Ps*	Human acellular lyo-philized dermis	Burns and full thickness wounds	1,32
SureDerm®	HANS BIOMED Corporation, Seoul, Korea, 2003	HCT/Ps	Human acellular lyo-philized dermis	Hypertrophic scar revision and burn wounds	24
GraftJacket®	Wright Medical Technology, Inc., Arlington, TN, USA, 2006	HCT/Ps*	Human acellular pre-meshed dermis	As a foundation for revasculariza-tion and cellular repopulation, reduces inflam-mation	15, 33
Matriderm®	DrSuwelack Skin and HealthCare AG, Billerbeck, Germany, 2000	510(K)*	Bovine acellular der-mal Substitute	To treat full-thickness burns	34
Bard® CoilaMend™ (Permacol)	Surgical Implant Tissue Science Laboratories plc, Aldershot, UK, 2006	510(K)*	Porcine acellular dermis	For abdominal wall hernia, its use for dermal reconstruction is limited	24, 35
Allopatch HD™	Musculoskeletal Transplant Foundation	HCT/Ps	Human acellular der-mis (cryopreserved human cadaver skin)	Acute and chronic wounds.	36
Hyalograft 3D	Fidia Advanced Biopolymers, Abano Terme, Italy, 2003	510(k)	Based on hyaluronic acid derivate with cultured fibroblasts	Deep burns treat-ment, healing wound with growth factors and cytokines	24

Oasis ® Wound Matrix	Cook Biotech Inc., West Lafayette, IN, USA, 2006	510(k)*	Porcine acellular lyophilized small intestine submucosa ECM	Acute, chronic and burns wounds. It delivers growth factors to stimulate and cell migration angiogenesis	15, 37
Cymetra®	LifeCell, KCI	HCT/Ps	Injectable form of AlloDerm Regenerative Tissue Matrix	Burns and full thickness wounds	38
Dermal-epidermal substitutes					
TissueTech Autograft System	(Laserskin and Hyalograft 3D) Fidia Advanced Biopolymers, Abano Terme, Italy, 2003	*	Hyaluronic acid with cultured autologous keratinocytesand fibroblasts	Diabetic foot ulcers	24
Permaderm™	Amarantus BioSciences, USA	-	Autologous fibroblasts and keratinocytes in collagen matrix consisting of epidermal and dermal cells	Severe burns	1

Table 2. Semi-permanent skin substitutes; PMA: Premarket approval, *Commercially available products, 510(k): Premarket notification process,

Brand name	Manufacturer/year	FDA/ Status	Source (cell/scaffold)	Indication	Ref.
Dermal substitutes					
Integra TM Dermal Regeneration Template	Integra Life Sciences Corp., NJ, USA, 2016 Initially designed by Yannas & Burke,1980	PMA (1996) 510 K (2002)*	Acellular Bovine type I collagen and chondroitin-6-sulfate copolymer coated with a thin silicone elastomer	Deep partial thickness and full thickness burns	1, 15
Terudermis	Olympus Terumo Biomaterial Corp., Tokyo, Japan, 1999	-	Acellular bovine collagen sponge	Deep burns treatment, skin flap donor site regeneration, post-traumatic deformity corrections	24
Pelnac Standard/Pelnac Fortified	Gunze Ltd, Medical Materials Center, Kyoto, Japan, 2000	-	Acellular silicone (silicone and collagen derived from pig tendon)	Third-grade burn injuries, full-thickness skin defects (tumor, naevus, scar or skin ulcer removal)	24
Hyalomatrix®	Fidia Advanced Biopolymers, AbanoTerme, Italy, 2007	510(k)	Acellular non-woven pad of benzyl ester of hyaluronic acid and a silicone membrane	Cellular invasion and capillary growth	15

Table 3. Temporary skin substitutes; HCT/Ps: Human cells, tissues, or cellular-based products, *Commercially available products, 510(k): Premarket notification process, PMA: Premarket approval

Brand name	Manufacturer/year	FDA/ Status	Source (cell/ scaffold)	Indication	Ref.
Epidermal substitutes					
Epifix®	MiMedx, 2013	HCT/Ps*	Dehydrated allograft, amniotic and chorionic membranes	Acute and chronic wound care	15, 39

Matristem®	ACell, Inc., 2009	510(k)	A porcine-derived, lyophilized extracellular matrix sheet (ECM)	Partial and full-thickness wounds, pressure ulcers, venous ulcers, diabetic ulcers	40
Unite® Biomatrix	Synovis Orthopedic and Woundcare, Inc., 2007	510(k)	A decellularized equine pericardial extracellular matrix	Draining wounds, pressure sores or ulcers, venous ulcers, chronic vascular ulcers, diabetic ulcers, trauma and surgical wounds	41
Dermal substitutes					
Glyaderm®	Euro Skin Bank (ESB), Beverwijk, The Netherlands, 2011	-	Acellular human dermis	Acute burn wound surgery	42 43
TransCyte™ (earlier name, Dermagraft-TC)	Advanced BioHealing, Inc., New York, NY and La Jolla, CA, USA, 2011	PMA (1998)*	Nylon mesh seeded and porcine dermal collagen with cultured neonatal human foreskin fibroblasts	Full and partial thickness burns	44
Dermagraft™	Advanced BioHealing, Inc., New York, NY and La Jolla, CA, USA, 2001	PMA (2001)*	Human cultured neonatal fibroblasts seeded on polyglactin scaffold	Treatment of diabetic foot ulcers, secondary to epidermolysisbullosa	1, 15, 45
EZ Derm ™	Brennen Medical, Inc., MN, USA, 1994	510(k)	acellular porcine dermal collagen	partial-thickness burns	46
DermACELL®	LifeNet Health, Inc. 2011	HCT/Ps	Decellularized human dermis allograft	Chronic nonhealing wounds	15, 47
Grafix®Core	Osiris Therapeutics, Inc. 2013	*	Placental membrane comprised of an extracellular matrix and epithelial cells native to the tissue.	Acute and chronic wounds	48, 49
Promogran™	Acelity and KCI Headquarters San Antonio, TX, US, 2002	510(k)*	Composite of collagen and oxidized regenerated cellulose	Granulation tissue formation, epithelization, optimal wound healing and a scaffold for cellular migration	15, 50
Talymed®	Marine Polymer Technologies, Inc, 2010	510(k)	shortened fibers of N-acetyl glucosamine isolated from microalgae. equivalent to Oasis® Wound Matrix	diabetic foot ulcers, venous stasis ulcers, pressure wounds, full and partial thickness wounds	15, 51
XenoMem™ Wound Matrix	Viscus Biologics LLC, Dayton, OH 45402, USA, 2015	510(k), Pending	Acellular, porcine peritoneal matrix, substantially equivalent to Oasis® Wound Matrix	Partial and full-thickness wounds; venous ulcers, diabetic ulcers, chronic vascular ulcers, Trauma wounds	52

Biobrane™	UDL Laboratories, Inc., Rockford, IL, USA, 2008	510(k)*	Acellular ultrathin silicone film and 3D nylon filament with type I collagen peptides	Partial-thickness burns in children; toxic epidermal-necrolysis, paraneoplastic pemphigus and chronic wounds	1, 53
Dermal- epidermal substitutes					
StrataGraft™	Stratatech Corp. (Madison, WI, USA)	An orphan drug	Immortalized keratinocyte cell line, contains two different cell types	Partial and full-thickness burns	1, 54
Karoskin	Karocell Tissue Engineering AB, Karolinska, Sweden	*	Native human cadaver skin with allogenic dermal and epidermal cells	Deep wound	24
Apligraf (Graftskin)	Organogenesis Inc., Canton, Massachusetts, CA, USA, 1998	PMA*	Bovine type I collagen seeded with human allogeneic neonatal cultured fibroblasts and keratinocytes	Treatment of various forms of epidermolysisbullosa	55
OrCel	Ortec International, Inc., New York, NY, USA, 2001	PMA (1998)*	Type I bovine collagen matrix seeded with allogeneic cultured neonatal foreskin fibroblasts and keratinocytes	Epidermolysisbullosa, mimics cytokine expression of healing skin	56
GammaGraft™	Promethean LifeSciences, Inc.	HCT/Ps	Irradiated cadaveric human skin allograft	Burns, venous stasis ulcers, diabetic foot ulcers	57
TheraSkin®	Soluble Systems, LLC, 2011	HCT/Ps	Human skin allograft harvested from cadavers and extracellular matrix	Provides growth factors, cytokines and human collagen for wound healing	15, 58
Alloskin™	AlloSource Inc. Centennial, CO. 2011	HCT/Ps	Derived from epidermal and dermal cadaveric tissue	Protect the wound and provide biologic factors native to human skin	59
PolyActive	HC Implants BV, Leiden, The Netherlands, 1999	*	Polyethylene oxide or poly butylene terephthalate with cultured keratinocytes and fibroblasts	Partial-thickness wounds and skin graft donor sites	24

Table 4. In development skin substitutes.

brand name	Manufacturer/year	Source (cell/ scaffold)	Indication	Ref.
Dermal substitutes				
DenovoDerm™	Tissue Biology Research Unit, University of Zurich, Switzerland	Fibroblasts in collagen hydrogel skin defects	In split-thickness skin grafts	1, 60

Dermal- epidermal substitutes				
FirstCover	Elanix Biotechnologies AG, Switzerland	Fetal fibroblasts and keratinocytes in matrix	Acute skin wound care	2, 61
DenovoSkin™	Tissue Biology Research Unit, University of Zurich, Switzerland	Fibroblasts in collagen hydrogel and keratinocytes	For the treatment of burns and skin defects	1, 62
Tiscover™ (A-Skin)	A-Skin, The Netherlands CHU de Québec,Université Laval, Canada	Self-assembled autologous skin substitute with fibroblasts and keratinocytes	Healing of chronic, therapy-resistant wounds, ulcers, large burns	1, 63

Regulatory status of FDA

- Human cells, tissues or cellular-based products (HCT/Ps): it is not considered a medical device, and does not require PMA or 510(k) approval.
- Premarket approval (PMA) (class III): for interactive wound and burn dressing which directly or indirectly interact with the body tissues.
- 510(k) premarket notification process (Class II): Animal-derived products
- Acellular human tissue products do not require any FDA clearance or approval and are intended for homologous use only [46].

Limitations of skin substitutes

The currently available skin substitutes have several limitations, as follows:

a) They are time consuming because of extensive cell culture procedures and production time.

b) Their reduced vascularization due to their long-term survival. Even though some of the commercially available skin substitutes allow angiogenesis, the extent of vascularization is mostly insufficient and requires further improvements [1].

c) Their incapability of providing adequate temperature control, insulation, pigmentation, immune regulation and pressure sensation [27].

d) Failure to integrate into host tissue and problem of immune rejection.

e) Scar development at the graft margins after grafting and diverse functional, mechanical and aesthetic problems.

f) Remained risk of infective agent transmission in spite of rigorous screening for viral diseases and standardized sterilization techniques [24].

g) The costs of the applied products, the insurance coverage of such therapies, and the intricate and costly process of such technologies require approval [64].

h) They comprise only two cell types: fibroblasts and keratinocytes, and therefore, they lack the ability to form a differentiated structure such as sweat and sebaceous glands, hair follicles [1].

Conclusion

Skin substitutes represent a significant aid in the healing process of chronic wounds including venous, diabetic, surgical wounds and other conditions such as burns, epidermolysisbullosa. They provide cells and other vital elements that promote re-epithelialization and revascularization of the wound bed while inhibiting degradation of ECM. Due to various microenvironment of each wound type, diverse types of skin substitutes have been developed.

The need for clinically applicable skin substitutes continues to be of importance. An ideal substitute would be a durable bilayer construct that is biochemically, functionally and morphologically similar to native skin [25].

Further research will address various issues and unanswered questions and would suggest practical solutions toward a true skin substitute with excellent engraftment and long-term viability and would also be able to remove the barriers of streamlining the manufacturing process. Although their use in developing countries is still far off, these newer skin substitutes will propose even more options to the plastic surgeon.

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