

REVIEW

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# Tissue engineering of skin and regenerative medicine for wound care

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## Abstract

Engineering of biologic skin substitutes has progressed over time from individual applications of skin cells, or biopolymer scaffolds, to combinations of cells and scaffolds for treatment, healing, and closure of acute and chronic skin wounds. Skin substitutes may be categorized into three groups: acellular scaffolds, temporary substitutes containing allogeneic skin cells, and permanent substitutes containing autologous skin cells. Combined use of acellular dermal substitutes with permanent skin substitutes containing autologous cells has been shown to provide definitive wound closure in burns involving greater than 90% of the total body surface area. These advances have contributed to reduced morbidity and mortality from both acute and chronic wounds but, to date, have failed to replace all of the structures and functions of the skin. Among the remaining deficiencies in cellular or biologic skin substitutes are hypopigmentation, absence of stable vascular and lymphatic networks, absence of hair follicles, sebaceous and sweat glands, and incomplete innervation. Correction of these deficiencies depends on regulation of biologic pathways of embryonic and fetal development to restore the full anatomy and physiology of uninjured skin. Elucidation and integration of developmental biology into future models of biologic skin substitutes promises to restore complete anatomy and physiology, and further reduce morbidity from skin wounds and scar. This article offers a review of recent advances in skin cell therapies and discusses the future prospects in cutaneous regeneration.

**Keywords:** Burns, Cell therapy, Skin substitute, Tissue engineering, Wound closure, Scar, Regenerative medicine

## Background

Advances in burn care during the recent past have included improvements in fluid resuscitation, early wound excision, respiratory support and management of inhalation injury, improved nutrition and modulation of the hypermetabolic response, infection control and enhanced immune function, incorporation of aerobic exercise during recovery, and development of anti-scarring strategies [1]. These advances have led to significant reductions in mortality, hospital stay, and long-term morbidity. In addition to these comprehensive innovations, skin cell therapies have become part of the treatment plan for extensive burns. This review will summarize several of the most significant advances since 1980 and discuss prospects for further progress in cutaneous regeneration in the future.

## Review

### Medical needs

Cutaneous burns can generate a continuum of injuries with increasing depth into the skin. Partial-thickness burns often do not require grafting and, if debrided and treated with antimicrobial dressings, will heal spontaneously from regrowth of epithelial appendages (hair follicles, sebaceous and sweat glands) to cover the wounds. However, deep partial-thickness burns which do not heal within ~3 weeks and full-thickness burns require replacement of the epidermal barrier by transplantation of autologous keratinocytes. Transplantation can be accomplished by either conventional split-thickness skin grafts (STSG), applications of keratinocyte suspensions or sheets, or dermal-epidermal skin substitutes [2–5]. Autologous keratinocytes may persist indefinitely and provide permanent wound closure, whereas allogeneic keratinocytes will remain on the wound for a few days to weeks [6–8], delivering growth factors and extracellular matrix components to wounds that promote more

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rapid wound closure by autologous cells [9]. Combinations of widely meshed and expanded (i.e., 1:6) autografts or micrografts applied to excised, full-thickness burns and covered with allograft [10, 11] have been reported, but are slow to heal, allow granulation tissue to form, and tend to scar. Conversely, unmeshed sheet grafts applied as early as possible to critical areas (i.e., face, hands, feet, perineum) have been shown to reduce granulation tissue, minimize scar, and produce optimal functional and cosmetic outcomes [2, 12, 13].

**Biological requirements and current alternatives**

Wound closure after full-thickness burns requires reestablishment of stable epidermis as a minimum requirement. Stability of the epidermis depends on reformation of the basement membrane and vascularized connective tissues to anchor the outer skin to the body. Split-thickness skin satisfies these requirements but does not replace the epidermal adnexa (hair follicles, sebaceous glands, sweat glands) or regenerate a full complement of sensory or motor nerves. Table 1 summarizes the anatomic features of uninjured skin compared to STSG, engineered skin substitutes (ESS), and healed skin after grafting. It is important to note that split-thickness skin at the first harvest does not regenerate hair follicles, sebaceous glands, or sweat glands but does contain pigmented melanocytes and vascular and neural networks which the engineered skin does not. At the second and subsequent harvests of autografts, pigmentation becomes irregular and scar is more pronounced. Compared with autografts, autologous-ESS containing cultured keratinocytes and fibroblasts may also contain “passenger melanocytes” which may colonize the wound and generate focal, but incomplete, pigmentation [14–16]. Of these deficiencies, perhaps the absence

of sweat glands is most important to patients with large total body surface area (TBSA) burns because it impairs the ability to thermo-regulate properly.

Table 2 provides a partial list of acellular, temporary, and permanent skin substitutes that are either available commercially in the USA or in clinical trials. Acellular skin substitutes recruit fibro-vascular tissues from the wound bed and may consist of either biopolymers, such as collagen and chondroitin-sulfate or elastin (Integra® Dermal Regeneration Template [17]; MatriDerm®) [18], decellularized human dermis (AlloDerm™) [19], derivatized hyaluronic acid (Hyalomatrix®) [20], or polyurethane (BioTemporizing Matrix, “BTM”) [21, 22]. Each of these materials protects open wounds, promotes ingrowth of fibrovascular tissue, and may suppress granulation tissue and scar. However, the biologic materials (i.e., acellular dermis, collagen, hyaluronic acid) are prone to microbial contamination in the absence of antimicrobial agents due to their properties as biological ligands for bacteria and degradation by enzymatic activities [23, 24]. In comparison, synthetic polymers (i.e., polyurethane, poly-glycolic/poly-lactic acids) are often degraded by hydrolysis, have fewer microbial binding sites, and are less prone to microbial contamination. If used as dermal substitutes, the acellular materials may require 2 to 4 weeks to vascularize sufficiently to support a STSG. However, if used as a scaffold for cell transplantation, 2 weeks or longer for vascularization would negatively impact cell survival and reduce cellular engraftment and wound closure. Of the available acellular skin substitutes, Integra® currently has the broadest usage for extensive, life-threatening burns and burn scars in the USA [25] and has demonstrated very favorable outcomes [26, 27] since its introduction in 1996 [17]. Similar results have been described recently using

**Table 1** Comparisons of cell types in native, engineered and grafted skin (adapted from [99])

Tissue type	Cell type or structure	Uninjured skin	Split-thickness skin graft	Engineered skin substitutes	Healed skin after grafting
Epidermis	Keratinocytes	+	+	+	+
	Hair follicle	+	–	–	–
	Sebocytes	+	–	–	–
	Sweat gland	+	–	–	–
	Melanocytes	+	±	±	±
	Immune cells	+	+	–	+
	Nerve	+	+	–	±
Dermis	Fibroblasts	+	+	±	+
	Endothelial cells	+	+	–	+
	Extracellular matrix	+	±	±	±
	Smooth muscle	+	+	–	±
	Immune cells	+	+	–	+
	Nerve	+	+	–	±

**Table 2** Biologic skin substitutes, commercially available or in clinical trial (adapted from [100])

Model [references]	Composition	Indications for use
Acellular skin substitutes		
Integra Dermal Regeneration Template [17, 101]	Bovine collagen and chondroitin-sulfate coated with silicone	Burns, reconstructive surgery
AlloDerm [19, 102]	Decellularized human dermis	Repair or replacement of damaged or inadequate integumental tissue
MatriDerm [103, 104]	Bovine collagen and elastin	Burns, reconstructive surgery
Hyalomatrix [20, 105]	Derivatized hyaluronic acid	Partial- and full-thickness wounds
BioTemporizing Matrix [22, 106]	Bilaminar degradable polyurethane	Burns, reconstructive surgery
Temporary skin substitutes (dressings)		
Cadaver allograft [29, 107]	Split-thickness skin from human donors, unfrozen or cryopreserved	Burns, reconstructive surgery
Porcine xenograft [31, 32]	Split-thickness porcine skin, cryopreserved or lyophilized	Burns, reconstructive surgery
Apligraf® [108, 109]	Allo hF in collagen gel plus stratified allo hK	Diabetic foot ulcers
StrataGraft® [33, 110]	Allo hF in collagen gel plus stratified allo hK	Partial-thickness burns
DermaGraft® [111, 112]	Allo hF on poly-galactin mesh	Diabetic foot ulcers
Permanent skin substitutes (grafts)		
EpiCel® [34, 113]	Cultured auto hK multi-layer sheet	Full-thickness burns
ReCell® [3, 114]	Uncultured suspension of auto hK, delivered as a spray	Partial-thickness burns
Reconstructed skin [36, 115]	Auto hF on acellular scaffold of dermal extracellular matrix, plus stratified auto hK	Full-thickness burns, venous and mixed ulcers
Autologous engineered skin substitute [39, 116]	Auto hF on a collagen-GAG scaffold, plus stratified auto hK	Full-thickness burns

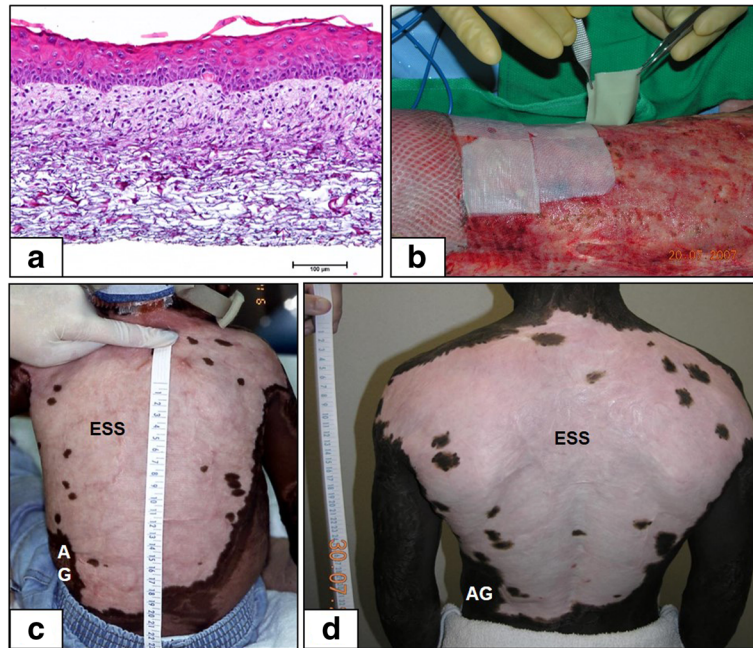
the BTM material which currently remains in clinical trial [22].

**Cellular skin substitutes**

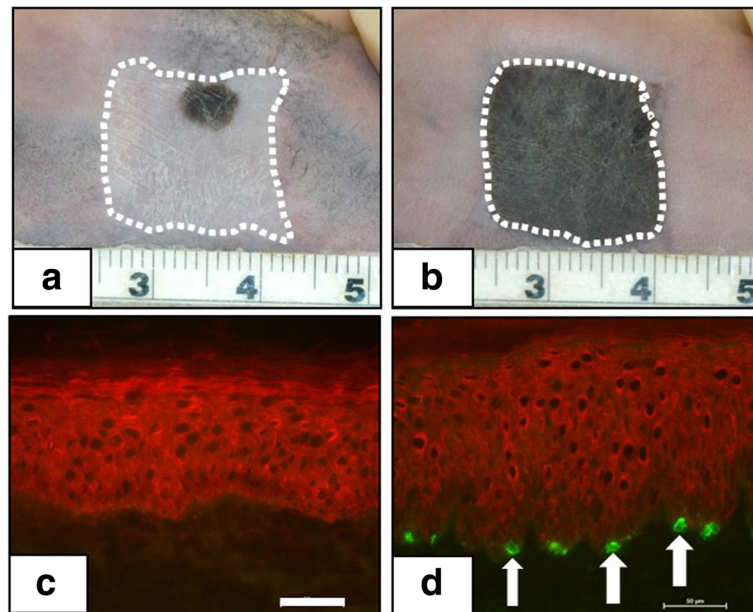
Transplantation of cellular skin substitutes has had wide-ranging results for temporary or permanent wound coverage. Temporary cellular dressings include direct harvest of split-thickness skin, available as either fresh or cryopreserved human cadaver skin [28, 29], or porcine skin with storage by chemical fixation or lyophilization

[30–32]. In addition, allogeneic human fibroblasts and/or keratinocytes have been combined with degradable scaffolds (i.e., partially denatured collagen (Apligraf™; StrataGraft™) [6, 33], poly-glycolic/poly-lactic acids (DermaGraft™)) which deliver growth factors and extracellular matrix to wounds to promote autologous healing but do not persist more than a few days to weeks. Autologous keratinocytes have been applied as cultured cell sheets (EpiCel™) [34], sprayed cell suspensions prepared during surgery (ReCell™) [35], with culture-expanded fibroblasts as the dermal component [36], or in combination with a polymeric dermal scaffold populated with autologous culture-expanded fibroblasts [37, 38]. Figure 1 shows the histologic organization of a dermal-epidermal skin substitute, surgical application, and results in a pediatric patient [39]. These approaches have helped to reduce mortality in large burns (> 40% TBSA), but they lack hair follicles and glands after transplantation. Limitations of keratinocyte sheets have included poor durability and ulceration [40, 41] and with sprayed keratinocyte suspensions a requirement for co-application with widely meshed skin autograft [42] which reduces the conservation of donor skin and increases scarring after wound closure.

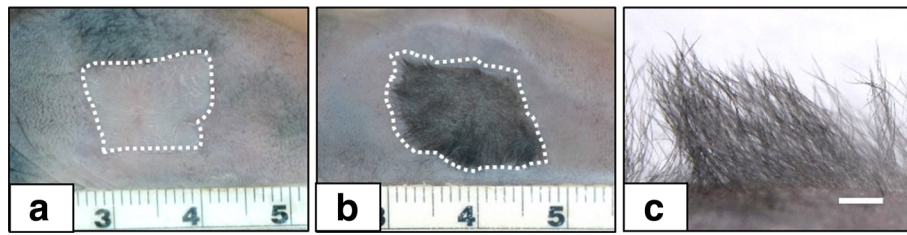
Preclinical investigations have reported more complex models that also include melanocytes [43–45], microvascular endothelial cells [46–48], mesenchymal stem cells [49–51], adipocyte stem cells [52], sensory nerve cells [53], hair follicle progenitor cells [54–56], or induced pluripotent stem cells (iPSCs) [57, 58]. Figure 2 shows restoration of natural skin color in human ESS with isogenic melanocytes grafted to immunodeficient mice [59] and localization of melanocytes to their normal anatomic location at the basement membrane. These kinds of models promote activation of biological signaling pathways which may stimulate more rapid and complete healing, or drive expression of additional phenotypes to correct anatomic deficiencies. The prospective benefits of progenitor cells may include generation of additional populations of differentiated parenchymal cells (e.g., hair, sweat glands, nerve) in engineered skin grafts. Figure 3 shows the expression of hair in engineered skin containing neonatal murine skin cells [54]. As biologic complexity increases and phenotypes are restored, engineered tissues gain structures and functions that do not result from mechanisms of wound healing. These added properties may derive from embryonic or fetal mechanisms that regulate tissue morphogenesis, in addition to the mechanisms of wound healing. Together, the combination of developmental biology, wound healing, and biomedical engineering constitute the emerging field of regenerative medicine.



**Fig. 1** Clinical application of autologous engineered skin substitutes (ESS). **a** Histology of ESS shows a collagen-based polymer scaffold populated with cultured dermal fibroblasts and epidermal keratinocytes. Scale bar = 0.1 mm. **b** Surgical application of ESS on prepared wounds can be performed using forceps and secured with staples. **c** An African-American subject treated with ESS at 3 years of age shows predominant hypopigmentation. **d** The same subject at 14 years of age has persistent hypopigmentation but has required no reconstruction of the ESS site. Scales in centimeters



**Fig. 2** Correction of pigmentation with cultured autologous melanocytes in preclinical studies. **a** Human engineered skin substitutes (ESS) on immunodeficient mice showing hypopigmentation at 12 weeks after grafting. **b** Correction of hypopigmentation after 12 weeks by addition of isogenic human melanocytes to ESS. Scales in centimeters. **c** Immunolabeling of epidermis with anti-cytokeratin (red) and the melanocyte-specific maker, tyrosinase-related protein-1 (TRP-1; negative). **d** Immunolabeling of ESS with added melanocytes shows epidermis (red), and TRP-1-positive melanocytes at the dermal-epidermal junction (white arrows) as in uninjured skin. Scale bars = 50 µm



**Fig. 3** Induction of hair follicles in vivo from neonatal dermal cells grafted to immunodeficient mice. **a** Human dermal fibroblasts and human epidermal keratinocytes express no hair. **b** Neonatal murine fibroblasts and human neonatal keratinocytes express chimeric hair at 4 weeks after grafting. Scales in cm. **c** Higher magnification showing density of regenerated hair is similar to that on positive control mice. Scale = 1 mm

**Contemporary research and regenerative medicine**

Although great progress has been made in reductions of morbidity and mortality in management of burn wounds, some of the most exciting advances remain ahead. These prospective advances include, but are not limited to, (a) complete restoration of skin anatomy and physiology, (b) gene therapies for specific applications, (c) automated and robotic fabrication of engineered tissues to increase efficiencies and reduce costs, and (d) quantification of wounds with non-invasive biophysical instruments.

**Table 3** Developmental pathways and regulatory factors for cutaneous phenotypes (adapted from [100])

Cutaneous structures and phenotypes	Regulatory pathways and factors
Epidermal barrier	Ca <sup>2+</sup> [117, 118] Transglutaminase, loricrin [119, 120] Essential fatty acids, stratum corneum lipids [121, 122]
Dermal-epidermal junction	Integrins [123, 124] FAK-Ras-MapK [60, 124] PKB/Akt-ERK1/2 [125]
Hair follicle genesis and cycling	Wnt/ $\beta$ -catenin/DKK4/BMPs [60, 61] EDA/EDAR [126, 127] Sox-2, -21 [128, 129]
Sebaceous glands	Rac1-Sox9-Lrig1 [61, 130] Wnt; Blimp1; IHH; c-myc [131] TGF $\beta$ -1 [132]
Sweat glands	Wnt/ $\beta$ -catenin [133] EDA/EDAR/NF- $\kappa$ B [134] DKK4, SHH [135]
Pigmentation	c-kit/SCF; [136] ET-3/EDNRB2 [137] Sox-10/Mitf; Eph/EphR; [138]
Sensory and motor innervation	c-jun [139] Sox-2,-10 [55] Oct-6; Krox-20; Pax 3 [140]
Cardiovascular system	Sox-7, -17, -18 [141] Mef2c/ $\beta$ -catenin [142] VEGF; HOXA9, VEZF1 [143]
Subcutaneous fat	PPAR $\gamma$ [144] Pref-1; Fabp4 [145] Myf5; Ebf2; Prdm16; Pgc-1 $\alpha$ [146]

EDA ectodysplasin, EDAR ectodysplasin receptor, TGF $\beta$ -1 transforming growth factor  $\beta$ -1, VEGF, vascular endothelial growth factor

Table 3 summarizes the anatomic and physiologic properties that may be missing from split-thickness skin autograft, ESS, or healed wounds after grafting. Among these phenotypes are epidermal barrier, dermal-epidermal junction, hair folliculogenesis and cycling, sebaceous glands, pigmentation, sensory and motor innervation, cardiovascular systems, and subcutaneous fat. Each of these phenotypes results from specific gene expression pathways that regulate its formation. Examples of these pathways are listed and referenced in the table. It is noteworthy that some of the phenotypes share regulatory pathways, such as hair follicles and sweat glands being regulated by wingless integration site of murine mammary tumor virus (Wnt),  $\beta$ -catenin, ectodysplasin (EDA), and its receptor (EDAR) [60, 61]. Similarly, there are members of the Sry-regulated HMG box (Sox) family of transcription factors that are expressed in formation of hair (Sox-2, -21), sebaceous glands (Sox-9), pigmentation (Sox-10), innervation (Sox-2, -10), and cardiovascular development (Sox-7, -17, -18). Despite these similarities, each pathway is expressed in a context of its microenvironment (e.g., stem cells, extracellular matrix) which also contributes to the genesis and stability of the phenotype. Undoubtedly, as continuing studies in developmental biology elucidate these pathways, greater capabilities to guide the anatomy and physiology of biologic skin substitutes will be gained.

Gene therapies for the skin have been studied extensively over the years and have met with limited success [62–64]. Risks from use of retrovirus-based expression systems suggest that lentiviral-mediated genetic modifications may have greater safety and efficacy in prospective studies [65, 66]. However, at least two examples of gene therapy in skin substitutes are currently active in the areas of innate antimicrobial peptides (e.g., cathelicidins,  $\beta$ -defensins) [67, 68] in allogeneic engineered skin to promote healing of chronic wounds and collagen VII for recessive dystrophic epidermolysis bullosa (RDEB) [69, 70]. These approaches to gene therapies require careful considerations for safety and efficacy in clinical applications. Constitutive overexpression of human beta defensin-3 with a non-viral plasmid DNA in an

allogeneic model of a skin substitute has been evaluated for microbial management of contaminated wounds and was not tumorigenic [71]. These kinds of approaches provide novel examples for wound management and correction of congenital skin diseases and open countless opportunities for future reductions of morbidity and mortality from skin wounds. The CRISPR/Cas9 system for gene editing [72] offers an alternative for genetic modification of cells without the associated risks of viral vectors [73, 74].

In addition to unique compositions of cells, gene expression, and scaffolds to construct analogs of skin, a critical and limiting factor to greater availability of skin substitutes is manual fabrication of these complex materials. To address this limitation, numerous methods for robotic fabrication of skin and other tissue substitutes have been described [75]. Many of these approaches are highly precise and involve extrusion of cell-populated matrices into specific shapes for transplantation. For skin models, techniques include multi-layering of multiple cell types [76], “ink-jet printing” [77], or transfer of cell-matrix droplets onto a culture substrate by actuation of a laser pulse [78]. Although these robotic systems accomplish physical transfers with relatively high efficiency, they may injure cells by transient exposures to high pressure, temperature, or chemical toxicities. Importantly, cells suspended in viscous scaffolds may be deprived of cellular attachments to cell surface receptors (e.g., integrins, cadherins), resulting in irreversible proliferative arrest and apoptosis [79]. Avoidance of these kinds of growth inhibitions will be essential to the eventual success of robotic systems. It is important to recognize that these kinds of attachment and signaling deprivations do not occur during fetal morphogenesis or wound healing. Therefore, providing tissue-specific ligands for cell surface receptors, or maintaining signaling pathways that regulate proliferation, will likely be required to optimize the mitotic rates of cells in engineered tissues. One approach to satisfying this requirement is formation of cellular organoids [75] which provide cell-cell attachments to preserve cell cycle signaling without attachment of cells to scaffolds or plastic vessels.

Assessments of skin wounds have progressed from subjective examinations by clinicians to more objective measures with non-invasive instruments for both diagnostic and prognostic evaluations. For diagnostic purposes, scanning laser Doppler flowmetry has been shown to provide accurate assessments of burn depth and color with simultaneous image capture [80–82]. Accuracy in determining the TBSA of burns has also been improved with computer software for digital mapping of skin injuries to better calculate critical interventions such as fluid resuscitation. Three-dimensional

photography and laser surface scanning [83, 84] provide topographic data that may be coupled with body mapping to generate virtual representations of patients that can be revised during the hospital course to construct a timeline of clinical progress. Non-invasive instruments for assessments of color, shape, surface texture, viscoelastic properties, blood flow, temperature, pH, surface hydration, and water vapor transmission have been adapted from applications in dermatology for more objective determinations of scars [85]. Although these kinds of instruments have high accuracy, they often provide assessments of individual points within fields of wounds or scars which must be considered in sampling plans for data interpretation. Because point measures typically do not represent heterogeneous wounds, data collection at multiple sites is needed to compensate for the subjective selection of individual points to measure within the treatment field. With these kinds of considerations, application of non-invasive instruments for wound assessments has been shown to correct for inter-rater variability in ordinal or observational evaluations of wounds and scars.

#### **Regulatory environments and requirements**

Safety and efficacy of skin substitutes are regulated in the USA by the US Food and Drug Administration (FDA). Biologic skin substitutes have increased in complexity from models that replace either dermis or epidermis, to dermal-epidermal models, to those that deliver combinations of biopolymer scaffolds, multiple cell types, or multiple cell sources, to those that express gene products for prospective improvements in wound healing. This spectrum of unprecedented materials presented questions regarding the regulatory framework within which each model would be evaluated for consideration of permission to market. Traditionally, the FDA has consisted of three centers for evaluation of human therapeutics: the Center for Devices and Radiologic Health (CDRH), the Center for Biologics Evaluation and Research (CBER), and the Center for Drug Evaluation and Research (CDER). Availability of cadaveric allograft has been provided under regulations for tissue banking, which are administered by CBER. As the spectrum of research models of skin substitutes broadened during the 1980s and 1990s, several investigative therapies had components that required consideration by multiple centers at FDA. The agency responded proactively with two initiatives that have contributed to greater clarity of the regulatory process and with Guidance for Industry [86, 87] on how to propose a path to market.

An early initiative was FDA's participation in establishment of definitions and standards for tissue-engineered medical products (TEMPs) through formation of a

Division IV of Committee F04 for medical devices through the American Society for Testing and Materials (ASTM) [88]. Beginning in 1997, this organization has had members from academics, government, and industry participating in a consensus process for composing definitions of materials and provision of methods for calibration and testing of the materials. With regard to skin substitutes, the ASTM process has resulted in a Standard Guide for Classification of Therapeutic Skin Substitutes [89], providing consensus definitions and nomenclature. The second initiative was FDA's establishment in 2002 of the Office of Combination Products, by which investigative therapies are reviewed initially for their primary mode of action [90]. This office confers with the Centers for Human Therapeutics to designate new therapies at a lead center at FDA with participation from other centers as appropriate. Together, these initiatives have added clarity to the assignment of novel therapeutics to a designated regulatory path [91]. In addition to providing a framework for innovative investigative therapies, FDA provides "expanded access" or "compassionate use" permissions for treatment of selected conditions that present high risks of mortality or morbidity to patients [92, 93].

More recently, the 21st Century Cures Act (Cures Act) was signed into law in the USA in December, 2016. As the name implies, this law is intended to facilitate and expedite the availability of novel therapies to patients with serious, or potentially life-threatening, conditions. The Cures Act provides for expedited therapeutic development programs including the Regenerative Medicine Advanced Therapy (RMAT) designation for eligible biologics products, and the Breakthrough Devices program which is designed to facilitate the review of certain innovative medical devices [94]. These new designations by FDA are in addition to previous expedited regulatory pathways of Fast Track development [95], Breakthrough Therapy designation [96], Accelerated Approval [97], and Priority Review designation for drugs [98]. Together, these alternative pathways to provisional or full marketing are likely to increase access to the most advanced therapies by patient populations with the greatest medical needs.

## Conclusions

Future prospects for biologic skin substitutes are extensive and diverse. Advances in use and regulation of stem cells in the skin are highly likely to lead to autologous skin substitutes with greater homology to uninjured skin by providing restoration of skin pigmentation, epidermal appendages (hair, sebaceous and sweat glands), a vascular plexus, and subcutaneous tissues. Genetic modification of autologous cells opens tremendous opportunities

for regulation of wound closure, reductions in scar formation, and correction of congenital diseases. As these advances in biologic skin substitutes translate into clinical care, it can be predicted with confidence that reductions in morbidity from acquired and congenital skin diseases will also be realized.

## Abbreviations

ASTM: American Society for Testing and Materials; BTM: BioTemporizing Matrix; CBER: Center for Biologics Evaluation and Research; CDER: Center for Drug Evaluation and Research; CDRH: Center for Devices and Radiologic Health; CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats; Cures Act: The 21st Century Cures Act; FDA: Food and Drug Administration; RMAT: Regenerative Medicine Advanced Therapy; STSG: Split-thickness skin graft; TEMPs: Tissue-engineered medical products; RDEB: recessive dystrophic epidermolysis bullosa

## Acknowledgements

The authors gratefully acknowledge the expert technical assistance of Christopher Lloyd and Mark Kleiner for the performance of the preclinical studies, Mary Rolles for the histology, Laura James for the statistical support, and Laura Fowler, RN, and Judy Nelson, RN, for the performance of the clinical research studies.

## Funding

Funding of the authors' studies has been provided by the National Institutes of Health (grant no. 1R21AR064341-01A1), the US Department of Defense (contract no. W81XWH-13-2-0052), and the Shriners Hospitals for Children (grant nos. 85200 and 84050).

## Availability of data and materials

Data and materials in this manuscript are referenced from previous publications and may be available from commercial suppliers or upon request from the reference laboratories.

## Authors' contributions

Both authors contributed to the content of this manuscript and provided consent for publication. Both authors read and approved the final manuscript.

## Ethics approval and consent to participate

All human subject research was performed with approval of the University of Cincinnati Institutional Review Board with regulatory oversight by the US Food and Drug Administration. All animal subject research was performed with approval of the University of Cincinnati Institutional Animal Care and Use Committee and the Animal Care and Use Research Office of the US Army Medical Research and Materiel Command.

## Consent for publication

An informed consent to publish de-identified data was obtained from the subjects.

## Competing interests

The authors declare that they have no competing interests.

Received: 18 September 2017 Accepted: 12 December 2017

Published online: 24 January 2018

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