CRITICAL REVIEW

Critical Issues in Successful Production of Skin Substitutes for Wound Healing

Hojjat Naderi-Meshkin[1*], Raheleh Amirkhah[2], Asieh Heirani-Tabasi[1], Muhammad-Irfan Maqsood[1, 3]

[1] Iranian Academic Center for Education, Culture Research (ACECR), Khorasan Razavi Branch, Mashhad, Iran.
[2] Reza Institute of Cancer Bioinformatics and Personalized Medicine, Mashhad, Iran.
[3] Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran.

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ABSTRACT

Novel findings on fabrication techniques for bioactive materials, discovering further basic knowledge about wound healing process, and availability of stem cells as alternative candidate for differentiated cells have highly encouraged scientists for developing new bioengineered skin substitutes (BSS) that offer an effective remedy for a specific wound type. However, technical, clinical, legislative and economic reasons hamper wide-spread commercialization and clinical translation of BSS. Among the various types of strategies that target skin repair and regeneration, tissue engineering with stem cells is most promising route. Tissue engineering by cooperation of several disciplines forms a context on which the commercial development of BSS is possible to provide benefits for patients who currently have limited or no cure options. The principles of tissue engineering are to initiate cell cultures *in vitro*, grow them in monolayer or on porous scaffolds and transplant the composite into a patient with a specific wound indication *in vivo*. The potential for creating of custom-designed biomaterials and availability of stem cells from either autologous or allogenic sources have helped to produce novel innovative BSS. Currently, wide range of skin substitutes are already being fabricated for clinical use in different wound indications but not yet definitively established. Therefore, many novel engineered constructs might be fabricated in the future. In this review, we describe the progress that has been made to date in the field of skin substitutes and the critical issues that are still hindering successful production and bench to bedside translation of BSS and restricting the availability of these innovative therapeutic constructs. Integrity of the science and technology, interdisciplinary expertise collaborations, and early interaction with regulatory entities such as Food and Drug Administration (FDA) and European Medicines Agency (EMA), together with other critical determinants, is vital to the successful commercialization of tissue engineering products into the marketplace/clinic.

Keywords: Regenerative medicine, Thick engineered skin substitutes, Angiogenesis, Stem cells sources, Biomaterials, Drug delivery systems, Clinical translation, Rules and regulations, Costs and business plan.

* **Correspondence**: 10

Email: nahojjat@gmail.com

1. Introduction

The spread of chronic wounds is worldwide and closely correlated to increasingly ageing population, obesity and diabetes [1, 2]. Despite achievements in wound management, many patients suffered from chronic wounds fail to heal or their ulcers relapse [3]. They are causing organ amputations, morbidity and mortality of many patients worldwide. In addition, chronic wounds impose enormous and rapidly growing costs for health care systems, besides psycho-social burden and the individual distress [4]. Therefore, treatment methods that are medically effective, safe and inexpensive are desperately needed. Although organ transplantation has become ordinary nowadays, however, it is limited not only by surgical technique but also by donor availability [5]. Tissue engineered organs can surmount the human affliction caused by the scarcity of donor organs. Furthermore, tissue engineered organs reduce or eliminate the need for drug and toxicity testing in animal and human subject [6].

Regenerative medicine is a rapidly evolving field of therapy offer innovative scientific solutions by integrating different scientific and technological areas, including cell biology, material sciences, chemo-physical engineering, computer modelling, and clinical medicine. Therefore, regenerative medicine create an interdisciplinary exchange of experience, knowledge, ideas, skills, technologies and efforts between basic and clinical research that contribute to development of engineered tissue therapies [4, 7, 8]. In spite of significant advances have been made particularly in skin tissue engineering, the field so far has failed to fulfil the expectations and is still need further development [7]. Tissue engineering as an important way for the treatment of damaged skin by employing a source of cells and a biomaterial on which the cells can grow, proliferate, and differentiate are take part in developing engineered tissues/organs [9]. The first tissue engineered organ, which has advanced from the lab bench to the bedside (patient care) has been skin [10].

The current trend of wound care has been shifted from solely achieving satisfactory survival rate to improvement in function of the healed wounds and quality of wound (being scar free) [11–13]. The change in the trend has demanded the emergence of various skin substitutes in the management of skin injury such as the acutely burned patients as well as diabetic foot ulcers [14]. Conventionally, autologous split or full-thickness skin graft have been recognized as the gold standard of burn wound treatment, however, it is constrained by the low availability of donor source, especially in vast and severe burns. Moreover, autograft application create additional wounds and scarring at the donor site [15, 16]. Thus, bioengineered skin substitutes (BSS) might represent artificial, off-the-shelf alternatives to the skin grafts with the benefits of less pain, less risk of cross-infection, and less/no need for graft harvesting [17, 18].

It is key to understand the regulatory path developed by government entities to move the tissue engineered products successfully into the marketplace. Although the science is now universal and regulatory needs are being developed in Europe and USA, among others, a harmonized international regulatory approach, like FDA, would be highly demanded [19–21]. The aim of regulatory pathway is to gain efficiency, safety and reduce the costs while improving and maintaining quality [22]. Therefore, business plan that addresses potential risks and outlines a path to marketplace and optimize the product development process parameters across scientific, technical, clinical, regulatory, financial and commercial perspectives is critical for success. In this review, we portray the progress made in the field of skin substitutes so far and the remaining challenges in the development of BSS making up from cells/stem cells, biomaterials and growth factors.

2. Scientific overview of skin substitute and classification

Skin has been composed of two specialized layers, epidermis and dermis [23]. Although it is structurally simple organ rather than other complex organs its repair and regeneration remains challenging when substantially injured. As the largest organ, the skin has high potential risk of diseases and injuries [24]. Many skin substitutes have been developed over the past decades in an attempt to decrease the need for skin autografts. These skin substitutes are very diverse, and a head-to-head comparison is not possible [14, 23]. Skin substitute are in high demand for the therapy of burns and various acute and chronic wounds, result in that the sponsors highly invest in production of skin tissue engineering. Skin tissue engineering employ biomaterials, cells/stem cells, growth factors and an established biological and pathophysiological knowledge of healing process in the various types of wounds [9, 16, 25]. The main purpose of skin tissue engineering is to produce an ideal skin substitute product for using in wound repair, especially in the full-thickness skin defects, without leaving a scar [13, 16, 25]. However, there are no such a complete skin substitutes that able to replace injured skin same as the native skin. Consequently, the field of skin tissue engineering need to be further developed. It is worth to mention that even with the most advanced therapeutic approaches, proper wound debridement and basic wound care remain critical [3, 26].

There are many commercial skin substitutes, permanent or temporary, available in the market that designed for an intended use with a specific clinical scenario [9, 20]; however, no perfect or ideal skin substitute exists yet and each type of product has own specific implications. The diversity is much great so that one can do a head-to-head comparison of all substitutes together, and various factors have to be take into account while choosing one of these various substitutes [14]. Hence, we have attempted here to summarize the all possible kinds of skin substitutes in use (Figure 1). Skin substitutes are heterogeneous therapeutic tools that vary in their biology, structure, composite and application. Although there is no perfect skin substitute, some characteristics should be considered when evaluating alternatives (Box 1). Biomaterials and cells/stem cells are vital components for successful skin tissue engineering [9, 27]. Ignoring one of these components may decrease the opportunity for tissue engineering of an ideal skin substitute to foster complete healing of wound. Cell-containing scaffold will be of particular interest and usefulness in clinical setting in which the bed of the wound cannot provide these cells. Optimum characteristic of such skin substitutes have been summarized in Box 1.The combination of stem cells with a custom-designed new biomaterials might result in development of more complex engineered skin for wound healing. Nonetheless, many latest studies have tried to establish simplified approaches like cell spray [28, 29] or using scaffolds alone [30], due to clinically ease of handling and use.

2.1. Cell sources for development of skin substitutes

Sources and types of cells are another critical issue to consider in the development of BSS. The characteristics of an optimal stem cell source have been summarized in Table 1.

Cell-based approaches to develop skin substitutes can involve differentiated cells or stem cells (adult, embryonic or induce pluripotent stem cells). Several different types of cells have been studied for wound healing in both preclinical and clinical settings such as human dermal fibroblasts [31], foreskin derived-keratinocytes [32], keratinocyte stem cells (KSCs) [33], hair follicle stem cells (HFSCs) [34, 35], angiogenic endothelial progenitor cells (EPCs) [36], bone marrow-derived mesenchymal stem cells (BM-MSCs) [3], and adipose tissue-derived mesenchymal stem cells (AT-MSCs) [37].

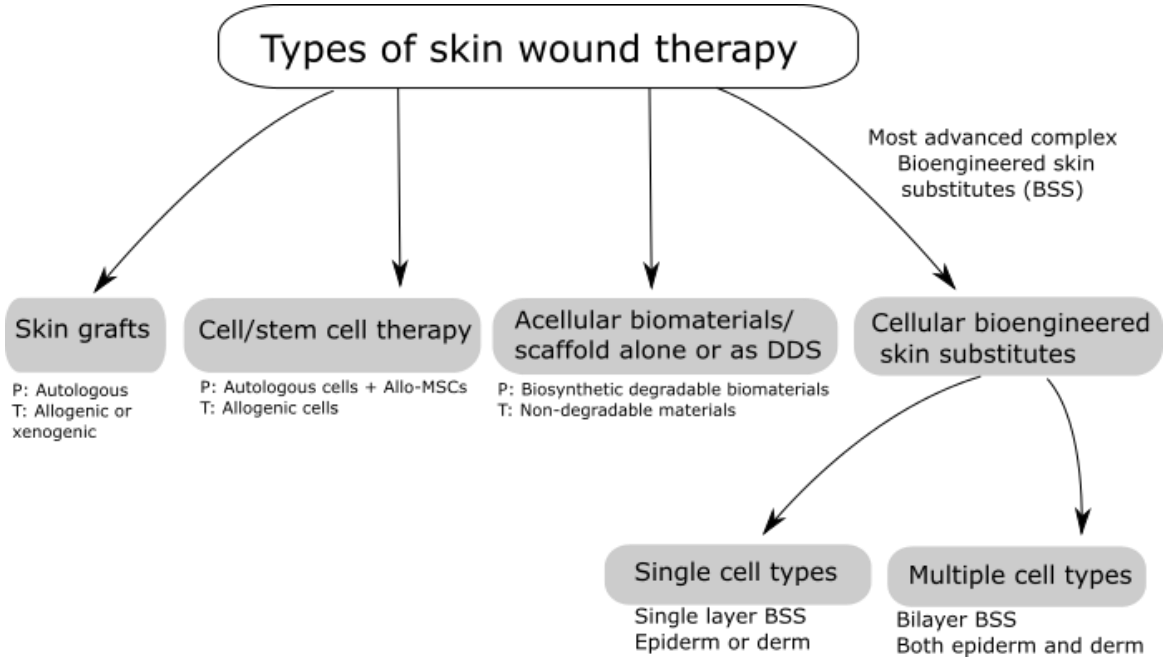

Figure 1: Different approaches for development of various kinds of skin substitutes for wound therapy. The classification has been proposed based on their composition from simple scaffold to much more complex multiple cells-containing bioengineered skin substitutes (BSS). Banked cadaveric skin, together with wide variety of biomaterials, natural or synthetic, or composite, are the primary used skin substitutes. Such substitutes have had limited success because they only provided a scaffold. In fact, these biomaterials gave good clinical results only when they were implanted in moderate and small wounds. Due to these reasons, novel approaches are currently explored, such as the combinatorial use of growth factors/cytokines and stem cells as potential alternatives. The cells can be applied either alone or in combination with a natural, synthetic or biosynthetic matrix. The combined approach is the most advanced of the three other alternatives. In such systems, cell populations are trapped within a matrix that might being functionalized with a biological signaling agent like growth factors. Cell maturation can be induced either before or after implantation. These skin substitutes can be just an epidermal layer or a dermal layer, or both of them (bi-layered) that keratinocytes and/or fibroblast or alternatively stem cells can be embedded into an acellular scaffold (a support for structure) forming a BSS. If stem cells are used, these may either be left to differentiate *in vivo* or induce towards a specific lineage *in vitro* prior to implantation. Additionally, growth factors and cytokine delivery by scaffolds might give rise to recruitment of the resident cells/stem cells for angiogenesis and neo-tissue formation. Bioengineered skin is designed permanently or temporarily to take over the functions of the epidermis and/or dermis until the patient's skin repairs/regenerates spontaneously or until certain skin replacement is possible. “P” denotes for permanent and “T” assigns for temporary/transient. DDS abbreviates for drug delivery systems.

Cultured normal human epithelial cells reconstitute keratinocytes sheets of stratified epithelia that retain biochemical and histological quality and features of the original donor site [38, 39]. Long-term maintenance of epithelial stem cells in culture [40–43] and a well-prepared receiving wound bed allow to appropriately regeneration of full-thickness wounds by means of *in vitro* constructed skin substitutes [39, 44]. In addition, cultured cells produce growth factors [45] and extracellular matrix (ECM) components with dynamic reciprocal effects that help resident cells to contribute to the wound-healing process [46, 47]. Skin is considered as a stem cells zoo due to have variety of resident stem cells that exert a crucial function in skin regeneration [9, 48]. Therefore, it is very important for scientists to develop a good understanding of this stem cells pool to engineer better skin substitutes in the future.

Box 1: Characteristics of an ideal cell-containing skin substitute [14, 23, 49].

- Low or no antigenicity.
- Long-term wound stability.
- Rheology comparable to the skin.
- Ability to resist shearing forces.
- Easy to prepare.
- Easy to store.
- Suitable cost/effectiveness.
- Easy to handle and use.
- Being permanent.
- Supply moist to wound environment.
- Able to secret cytokines and growth factors to stimulate tissue innate regeneration potential.
- Tolerant to hypoxic condition of wound.
- Rapid and sustained adherence to the wound bed.
- Impermeability to external bacteria and infection.
- Allows water vapor transmission similar to normal skin.
- Having inner surface structure that permits cell migration, proliferation and in-growth of new tissue.
- Flexible and pliable so it can conform to irregular wound surfaces.
- Included by epidermal and dermal components.
- Indefinite or long shelf-life.
- Minimal storage requirements.
- No local/systemic toxicity.
- Inexpensive and marketable.
- Widely and readily availability (off-the-shelf).

Many of commercial BSS constructed from sheets of cells derived from neonatal (allogenic) foreskin, for example, Apligraf™, Celaderm™, Dermagraft™, Trancyte™, and OrCel™ [9, 11, 20, 21, 49]. Neonatal foreskin is chosen because: it is a convenient source obtained from healthy babies be subjected to circumcision that provide off-the-shelf source by cell banking, supply a high content of keratinocyte stem cells with high proliferative capacity and low allogenic reactions rather than adult keratinocytes [12, 50]. Overall, stem cells characteristics have been extensively reported by many researchers [47, 51–55]. Stem cells, including both adult and embryonic stem cells (ESCs), have unique innate features that might represent an effective way to meet the challenge of skin replacement (Table 1). Furthermore, in comparison to fully differentiated keratinocytes in specific clinical settings, stem cells are available in a shorter time due to the higher proliferation capacity which is expected to contribute to a superior quality of wound healing and regeneration [56, 57]. More recently, MSCs with more than ten unique features [55, 58, 59], have been explored in the treatment of complex wounds. Several recent studies providing evidence that MSCs are immune privileged cells because the lack of MHC Class II, and low expression of co-stimulatory factors [58, 60, 61]. Therefore, due to their advantageous properties, MSCs as off-the-shelf product have robustly reinforced the tissue engineering field as ready component for an engineered bi-layered skin substitute [53]. MSCs can derive from various sources. Among these, adipose tissue as an abundant source of MSCs, have shown an improved outcome in wound healing studies [53, 62, 63]. AT-MSCs are pluripotent stem cells with the ability to differentiate into different lineages and to produce paracrine factors inducing tissue regeneration. The plentiful supply of fat tissue, extensive proliferative power of the derived-stem cells, and their ability to secrete angiogenic factors and cytokines make them so attractive for treatments of nonhealing wounds [53, 64, 65]. MSCs delivery to the wound by spray is a potential solution with minimal cell manipulation to facilitate tissue guided regeneration [66]. The spray technology is easy to use for clinicians. For example, Cryoskin™ is a cell spray-based skin replacement that is prepared upon request by clinicians [67].

Table 1: Classification of cell sources and their cons and pros [68, 69]. Generally, cell sources are fall into two categories; based on their origin (autologous or allogenic) and their differentiation potential (differentiated or stem cells).

Types of cells	**Advantages**	**Disadvantages**
Autologous	▪ No immunorejection. ▪ No graft-versus-host disease (GVHD). ▪ No risk of disease transmission. ▪ No need for stringent FDA regulations.	▪ Donor site morbidity. ▪ Limited availability. ▪ Decreased numbers with age. ▪ Variability from patient to patient. ▪ Limitations of harvesting in extremely short period of time in the acute clinical settings.
Allogenic	▪ Provide sufficient lag of time to complete appropriate compatibility, sterility, safety, consistency, efficacy and quality assurance analyses before product release, therefore, only cells with desirable characteristics and controlled critical parameters are selected and amplified. ▪ Broad availability because of cell banking opportunity. ▪ Possibility of providing off-the-shelf marketable product.	▪ Higher costs due to safety-testing and complying with cGMP criteria. ▪ Immunorejection. ▪ More heavily regulatory pathways.
Differentiated cells (e.g. keratinocyte, fibroblasts)	▪ Controlled cell proliferation rate. ▪ No risk of tumorigenicity. ▪ Highly committed and specialized cells.	▪ Scarcity of donor site. ▪ Low proliferation potential.
Stem cells (e.g. KSCs, HFSCs, MSCs)	▪ Unlimited source of donor material. ▪ High proliferative capacity, ease of isolation and *ex vivo* expansion. ▪ Opportunity to provide skin substitute in shorter time with potentially all appendages such as nerves, sweat glands, and blood vessels. ▪ Reproducibility and mass production. ▪ Freezing conditions do not affect their proliferation and differentiation potential. ▪ Good paracrine effects.	▪ Potential of tumor formation.

Recently, cell sheet (CS) have been constructed from AT-MSCs proposed to create 3D constructs to promote full-thickness skin wound regeneration, taking advantages of particular cell–cell and cell–ECM interactions. More stable human AT-MSCs CS construct were obtained within five days using thermoresponsive cell culture surfaces.

Human AT-MSCs-based constructs were made by superimposing three AT-MSCs-CSs and then transplanted into full-thickness excisional skin wound in mice. Their findings suggested that the transplanted AT-MSCs promote neotissue vascularization and extensively influence epidermal morphogenesis, mainly by paracrine effects on wound resident cells [62]. Furthermore, numerous preclinical animal studies and a few clinical studies in human wounds have shown that MSCs can augment wound closure [70, 71] and attenuation of scar formation [57]. Still, the contribution of MSCs to skin regeneration, detailed biological function and the long-term systemic effects of MSCs are yet to be determined. In addition, it needs to determine whether other types of stem/progenitor cells will be more effective. Therefore, more randomized controlled clinical trials need to be undertaken [3].

Although, autologous cells offer the opportunity of least risk concerning transmissible infective agents and do not reject by body, however, allograft in some situations can provide a better solution but rejection is a problem [68, 72]. Moreover, an allogenic approach allows sufficient retardation of time to complete appropriate compatibility and sterility analyses before product release [72]. In spite of all the privileges in the stem cells field, scientific obstacles including potential of tumorigenicity, unwanted differentiation, and functional characterization should not be ignored. Moreover, there are issues still need to be addressed when moving to cGMP environment and clinical applications [73]. Notwithstanding potential of off-the-shelf stem cell-based tissue engineered products, stem cell therapeutic potential is a work in progress to elucidate details of stem cell biology. The great consequence of innovative stem cell-based therapy is opening a new window to achieve full functional skin, including the construction of missing appendages, such as nerves, hair follicle, sweat glands, and blood vessels [13, 56]. To date, there are no FDA approved stem cell-incorporated skin substitutes. There are, however, many studies that assess various types of scaffolds together with MSCs as more competent cells for clinical setting [71, 74, 75].

2.2. Biomaterials for scaffolding

Three dimensional (3-D) scaffolds for engineering of skin should satisfy a number of criteria that have been listed in Box 2. One of the most vital aspects that should be considered is ability to early integration of BSS with surrounding tissue after implantation. Formation of functional vessels that supplies oxygen and nutrients to the skin constructs assure the early integration and survival of such skin constructs. This aspect is critical especially when the skin lesions are large and full-thickness, because nutrient diffusion is not effective in more than100 μm distance from the blood supply source [76, 77]. To fulfil this challenge successfully, induction of the vascular invasion has been attempted by improving the design of scaffolds and supplementing them with viable MSCs and/or growth factors (angiogenic) molecules [65, 78, 79]. Concerning the latter strategy, three approaches could be used to immobilize angiogenic molecules onto material surface while maintaining sustained release and bioactivity of the growth factors. These include; (i) covalent linkage via a chemical process; (ii) non covalent binding of growth factors via specific bioactive molecules (i.e., heparin-like molecules); and (iii) simple physical entrapment of bioactive factors into scaffold biomaterial as delivery-vehicles which release the angiogenic molecules based on degradation rate of the carrier biomaterial [80–82].

Furthermore, biomaterials delivery of angiogenic factors (such as VEGF) and chemokine (such as SDF1) could be utilized for *in situ* recruitment of local adult stem cells (EPCs, MSCs, KSCs and etc.) as strategy to successfully induce skin regeneration [83–85]. Approach of *in situ* regeneration have benefit of eliminating most elaborate regulatory requirements and related costs rather than when cells being included in the scaffold [83, 85].

Box 2: Characteristics of an ideal biomaterial for development of skin substitutes [9, 27, 49, 86]. All below parameters have to be taken into account when choosing material for skin-tissue engineering applications. However, it is hard to find such a complete scaffold.

- Being biocompatible, i.e., non-toxic and non-immunogenic.
- Biodegradable with rates of resorption corresponding to those of skin formation.
- Easy to manufacture and sterilize.
- Provides a permissive environment into which skin cells could migrate, differentiate, proliferate, and deposit skin ECM components.
- Supply surface chemistry, biochemical, and physicochemical as well as geometric aspects appropriate and instructive for skin cells.
- Retain the cell-cell and cell-biomaterial signaling, allowing the complete layer of skin to be engineered.
- Must be tolerated by the host, be retained permanently and later be able to degrade slowly over time.
- Communicate with the body's own repair mechanisms to stimulate angiogenesis, and remodeling to restore complete function of skin.
- Ability to absorb the nutrients for wound healing and the exudate of wound bed.
- Easy and safe to handle during grafting in the surgery room.
- Permissive to vascular network that supplies oxygen, nutrients transport, and waste removal which assure survival of thick skin constructs.
- Encourage recruitment of local adult stem cell pools.
- Successfully direct differentiation of stem cells.
- Good mechanical properties.
- Support cell growth and improve cell attachment in a similar manner as in the cells' original niche.
- Present multiple graded pore sizes allow the acceleration of tissue reconstruction by multiple cell types.
- Having the pores that are at least 100 μm in diameter that play a role in enhancing the ingrowth of cells and blood capillaries.
- Support uniform cell spreading into interconnected pores.
- Appropriate water uptake ration (WUR) which contributes to hydrophilicity and the maintenance of 3-D structure.
- Suitable water vapor permeability (WVP).
- Adoptable size and anatomical shape fit to the skin irregular defect.
- Inexpensive.
- Meet FDA approval.

Natural polymers due to their similarity to the ECM offer the benefit of well-recognition by cells because they maintain biological, chemical and physical features of native tissue which are instructive for cells and direct stem cells differentiation, growth and proliferation [39]. Collagen is the first natural biomaterial or skin replacement product that has been applied for tissue engineering of skin to reduce the use of allografts and autografts [9]. Cereceres *et al.* demonstrated that the successful modification of the $Scl2_{GFPGER}$ protein to engineered collagen ($eCol_{GFPGER}$), displayed enhanced stability and integrin interactions and provided a matrix with adaptive moisture technology, and optimal degradation rates. Thus, this modified type of collagen have potential for use in human wounds with ability of readily conform to irregular wounds [87]. However, natural and also synthetic biomaterials have their own benefits and drawbacks. Therefore, composite scaffold offer more beneficial properties rather than natural or synthetic alone (for review see [76, 88, 89]).

2.3. Importance of wound types and indications

Skin substitutes are a heterogeneous group of substances that aid in the temporary or permanent

coverage of many types of wounds; offer alternatives when standard therapies are not desirable [14].

Skin substitutes provide solutions that may be predominant to other available methods because they may increase the dermal component of the healed wound, decrease inhibitory factors and inflammatory response, provide rapid and safe coverage, and prevent or limit hypertrophic scar formation and pain [13]. It is of importance to note that usually in chronic full thickness skin defects, an anti-healing condition exists which is usually infected and prevents healing. Therefore, before applying a skin substitute, these microorganisms have to be removed by using classical surgical debridement techniques and the wound-bed has to be changed from anti-healing into regenerative state in order to successful treatment [4].

Chronic wounds are estimated to reach epidemic extent because of the aging population, obesity, and the increasing incidence of diabetes [87]. Chronic wounds are lesions that do not heal by themselves within a certain period of time. They may be treated in different ways such as surgery (i.e. using autologous skin grafts and flaps), specialized dressings, or the use of BSS [90]. Considering pathogenesis of chronic wounds, cell and molecular mechanism of action of repair and tissue regeneration are the critical in choice of appropriate skin substitutes.

Bioengineered skin substitutes can be used in patients with the following conditions: moderate to severe burns, diabetic foot ulcers, venous leg ulcers, ulcers resulting from peripheral arterial disease, pressure ulcers (bedsores), vasculitic ulcers, pyoderma gangrenosum, epidermolysis bullosa (EB), breast reconstruction surgery, systemic sclerosis, acute surgical wounds like those caused by excision of skin cancer and a variety of chronic wounds with the aim of faster healing and better cosmetic appearance [14, 20, 91]. Because no single product meets all criteria of an ideal skin substitute for variety of wounds [14], each patient case requires careful evaluation to ascertain the most proper solution. Although various chronic and acute wounds might benefit from a tailored multidisciplinary approach that utilizes one or more of the products, each patient should be evaluated for other possible therapies before use of skin substitutes with higher cost.

Indeed, diseased or injured skin may rule out a particular therapy based on wound depth. Each type of wounds, superficial, partial or full-thickness, need diverse skin substitutes that simultaneously function as a primary dressing [4]. Additionally, the choice of skin substitute depends on many factors including the normal skin anatomy, underlying medical condition, surgical comorbidities, amount of wound requiring repair/regeneration, and presence of contamination in wound. Other important factors are the amount vascularity of the wound bed, contour abnormalities, and aesthetic outcomes [4]. After all these clinical factors have been adequately taken into account, a strategy has to be planned with the aim of early wound healing, counteract the infection, permanent skin coverage, negligible or no donor site morbidities and early recover of normal function [23]. For example, exclusion criteria for using Apligraf™ are signs of infection, known allergy to bovine collagen, and patients with hypersensitivity to components in the shipping medium. Dermagraft™ is also contraindicated for using in ulcers that have infected, and patients with hypersensitivity to bovine products [14].

3. Technical issues

Development of enabling technologies for easy, fast, safe, effective manufacturing of BSS, their characterization, scale-up, storage and distribution have provided promising avenues for establishment of marketable products. However, there are many key technical obstacles in the development of bioengineered skin substitute that must be overcome (Figure 2).

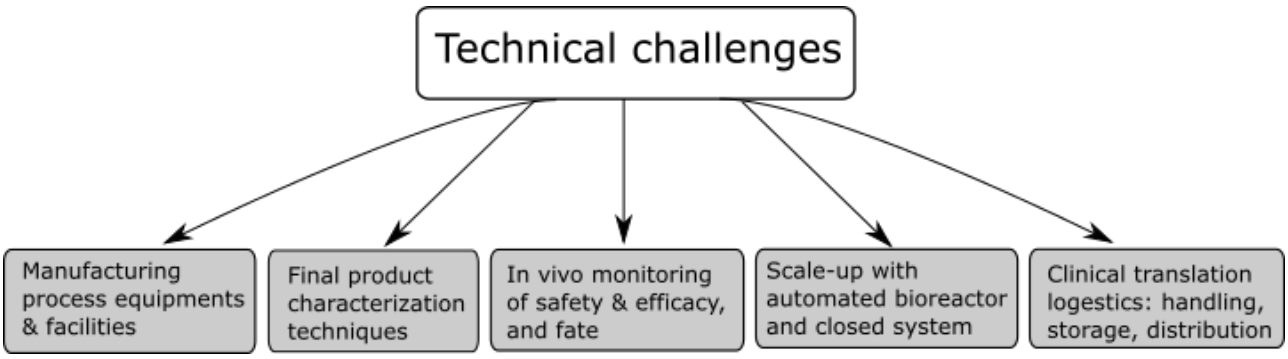

Figure 2: Technological issues need to be overcome for cGMP/GTP-adapted production and clinical translation of bioengineered skin substitutes. Many of the manufacturing facility, characterization testing, the infrastructure and systems are required for compliance with GMPs and GTPs.

Ongoing technical advances and innovative manufacturing will allow product manufacture to fulfil the clinical needs and be cost-efficient. Elements of the manufacturing facility have been well-explained by Burger *et al.* in four general categories: (i) cGMP manufacturing facility, with aseptic processing cleanroom, (ii) staffing (iii) manufacturing process equipment and (iv) analytical methods and devices [92]. Several reports have proposed outsourcing of manufacturing by contracting with service providers, particularly Contract Manufacturing Organizations (CMOs) or Contract Research Organization (CRO), capable of providing cell therapy manufacturing services quickly and relatively straightforward [21, 92, 93].

Experimental studies are primarily performed at a small-scale with cells that are cultured in tissue Flasks in various sizes as open systems. This approach is not an optimal practice for gaining the large quantities of cells generally needed for medical applications because it is extremely labor intensive and raise the cost of production of engineered construct. Robust processes for production of mass of cells must be produced in validated sterile devices, and high-throughput closed-systems for achieving cost-effective and safe products. In this regard, development of bioreactors that allow automation of fabrication process and scale-up in a GMP/GTP/GCP manner, is most desirable to drive down the costs, increase reproducibility, and permit more efficient regulation of anatomy and physiology of engineered skin substitute, and maintain and increase the quality assurance (QA) [74, 94, 95]. For example, the Kerator is a computer controlled bioreactor in which the exchange of medium and control of culture condition are fully automated during keratinocyte culture [96, 97]. This closed systems help to translate into better treatment outcomes for patients, greater availability of the product, and reduce costs and subsequently price of final product [97].

4. Clinical translation requirements for using skin substitutes

Advances in engineering and life sciences over the past decades have resulted in treatments by replacing, repairing, or regenerating of human tissue and organ function [16, 98]. However, precise consideration of the issues from bench to bedside is very important in maximizing the chances to translate a good idea into a good treatment [88]. The technological platforms for producing skin substitutes should be able to

navigate the scientific, regulatory paths, and business difficulties from research into the marketplace (Figure 3) [88]. Academia and industry collaboration and partnering must persist in the whole lifecycle of product to successfully translate scientific advances into safe and effective treatment for patients [99].

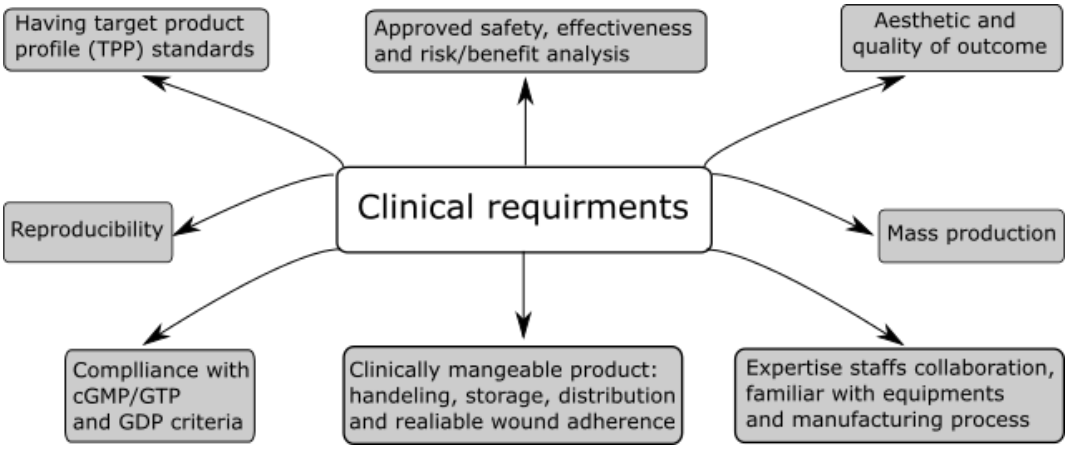

Figure 3: Various aspects that should be considered for clinical translation of skin substitutes. More importantly, task of staffing the facility will need individuals that are expertise and experienced in producing cell-based BSS comply with GMP and GTP manufacturing, Good Clinical Practice (GCP) and Good Distribution Practice (GDP) criteria.

Cell-based products should have information of the target product profile (TPP) including cells per product unit, product unit per dose, cell density, reserve stock, product container type/size, cryopreserved or fresh, Phase I–III specific lot size, commercial lot size, market size, cell stability, storage/shipping conditions, mode of administration [22, 93]. Additional requirements for commercial manufacturing also depend on whether the product is patient-specific. Inherently, most autologous cell therapy products are patient-specific, due to a need for immunologic compatibility, and to overcome current time-consuming clinical development rather than allogenic products [72, 92].

Recently, Dodson and Levine retrospectively examined the development of three autologous cell therapies including Epicel™ skin substitute, and four allogeneic cell therapies including Apligraf™ and Dermagraft™ skin substitute with the aim of identifying common challenges hampering attempts to bring new cell therapies to market. They identified several common challenges that cell therapy firms must address, have classified in three main categories, i.e. premarket, post-market, and manufacturing [21]. Generally, persevering through lengthy product development stages, navigating the respected regulatory pathway, securing suitable reimbursement, scaling up the production process, addressing distribution logistics and managing costs, and interactions among these various challenges have been mentioned as implications of commercialization for cell therapeutic products [21]. They have proposed early preparation for commercialization in the development process as a preliminary best practice. Coordination and communication between relevant offices of regulatory agencies should be considered as another key step at onset of product development and also during production process [21]. While many factors are critical to translating research into successful products, the

procedures of government entities for regulatory oversight (Figure 4) and respected criteria to compliance with cGMP/GTP (Table 2) are the keys.

4.1. FDA rules and regulation for engineered tissue products

Different regulations exist in different countries [100–104], but the USA policy is usually considered as the most comprehensive regulatory system and depend on regulating tissue engineered products. In fact, other countries consider the FDA and EMA regulation as a framework to develop their own rules and regulations corresponding to the policy and cultural believes. The FDA is a science-based regulatory agency in the US Public Health Service (PHS).

Table 2: Examples of cGMP (current good manufacturing practices) criteria for production of cellular engineered products. cGMPs, are intended to systematic monitoring of both the manufacturing processes and the final product in order to prevent disease transmission and product mixups, and include requirements for donor eligibility, and changeover practices to prevent contamination and cross-contamination, product tracking, and traceability.

Product testing and characterization	Biologics product testing purpose
Product characterization	• Define critical product attributes • Define and monitor/control all cell types present in the product • Establish proper specifications ➢ Ensure the safety and consistency of product lots
Sterility testing	• Microbiological testing – rapid release. • Absence of mycoplasma, adventitious viral agent and etc.
Identity testing	• Distinguish from other products processed in same facility • Ensure labeling is accurate • Phenotypic analysis
Purity testing	• Whether harmful residuals contaminants exist or not ➢ Reagents not intended to be in the product ➢ Unwanted cellular subsets ➢ Residual growth factors and serum proteins ➢ Pyrogenicity/endotoxin
Potency testing	• A quantitative biological assay
Stability testing	• Data generated at appropriate time and conditions ➢ Method, sampling times, temperature, assays • Shipping, storage, and holding of cells • Final formulation and dating period
Other tests	• General safety ➢ Cellular therapy products are exempt • Viability ➢ Generally >70% ➢ If not, data showing dead cells do not affect safety • Cell number/dose ➢ Minimum, maximum?

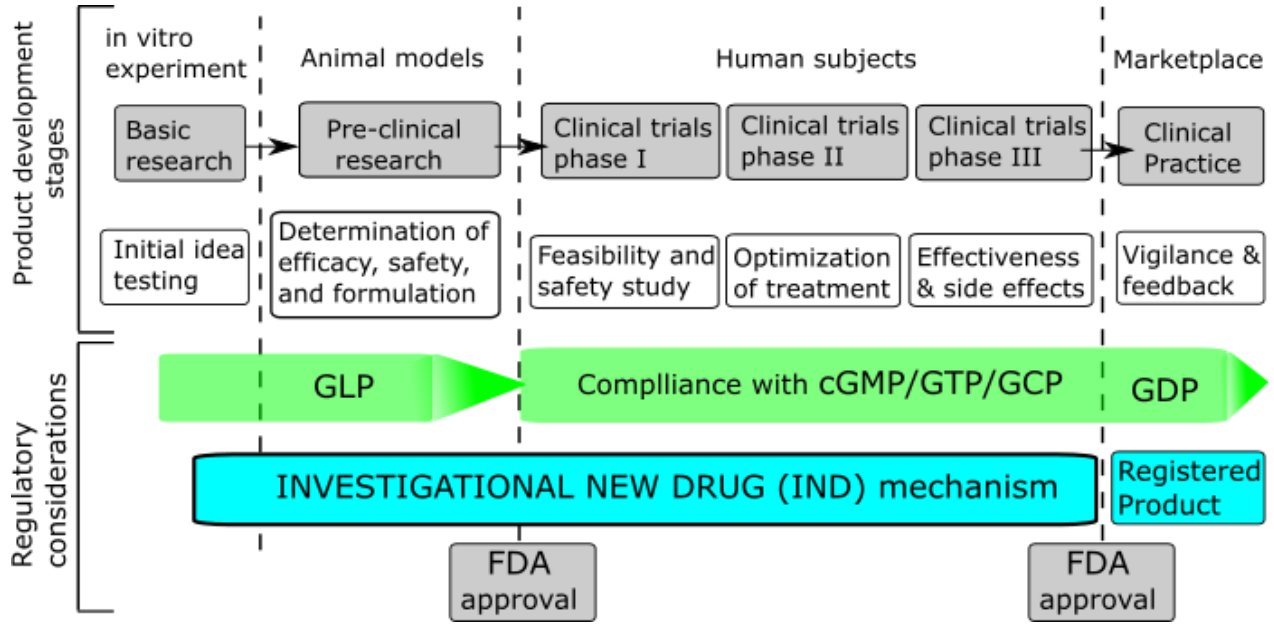

Figure 4: Development stages for bioengineered skin substitutes (when assigned as drugs or biologics) from science to the market. As FDA's expectations indicate, it is rarely possible that without clinical trials experience could develop effectively the manufacturing process and final product. Development of manufacturing processes continues through clinical trials phase I, II, and III. For those products requiring premarket review, the assessments of safety and effectiveness and the intended use (indication) of manufacturer's claim constitute the basic elements of the evaluation. Therefore, planning for commercial-scale production is based on the existing manufacturing process, materials, and technology, and a projection of the new commercial manufacturing process that all should be in compliance with FDA regulations. For all engineered cellular products, but not the "Minimally manipulated", safety and efficacy have to be established in clinical trials under IND, commercialization needs submission and approval of a Biologics License Application (BLA), and comply with Good Manufacturing Practices (GMPs) and Good Tissue Practices (GTPs).

The legislative authority of FDA for product oversight, premarket approval, and post market supervision and enforcement is taken principally from the Federal Food, Drug, and Cosmetic (FD&C) Act and the Public Health Service (PHS) Act. The intention of FDA Act is to: (i) control the spread of infectious disease; (ii) prevent manipulation that may harm tissue products; and (iii) ensure safety and efficacy of such products[105].

To this end, one of the main criteria that should be fulfilled is cGMP (Table 2). The FDA Agency has worked on developing appropriate strategies for the regulatory oversight of medical devices, drug, and biologic products (18). Most, if not all, bioengineered tissues fall into device and biologic categories (19), which have different regulatory pathways (Figure 5).

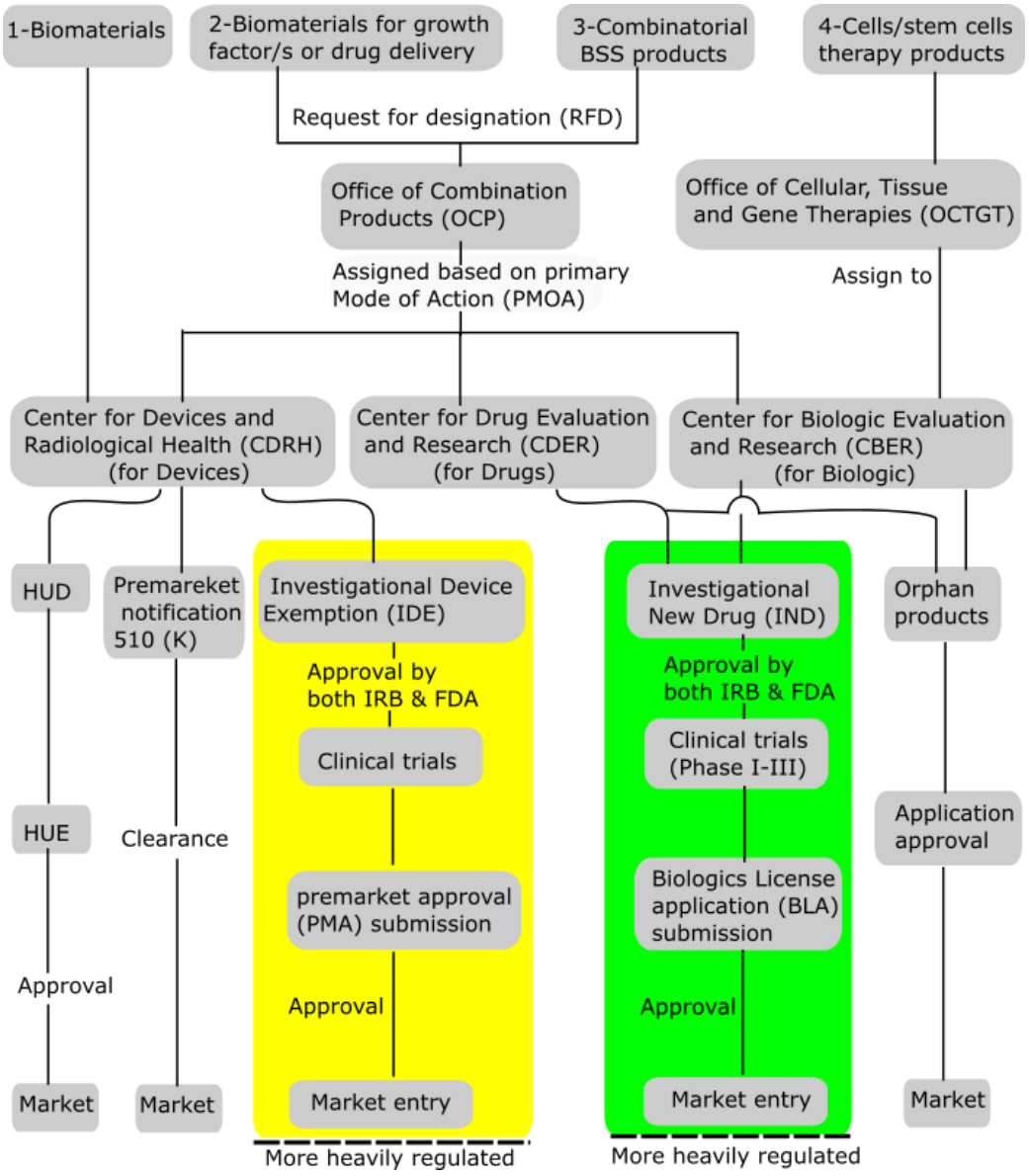

Figure 5: A flowchart depicting the overview of FDA regulatory pathways and considerations for various types of skin wound therapeutic products. The FDA assigns prospective therapies to one of three regulatory bodies: CDRH, CDER, and CBER that are in charge of regulations for Devices, Drugs, and Biologics, respectively. The product's classification determines the premarket review and approval process for demonstration of safety and effectiveness utilized by FDA centers. Under Federal Food, Drug, and Cosmetic Act (FD&C Act), a human medical product such as a skin substitute is classified as a drug, biologic, device, or combination of them. Of note is that most, if not all, engineered tissue products are combination products. Combination products first should interact with the OCP for assigning to one of the appropriate three main FDA centers by applying RFD; then are assigned based on PMOA. The choice of which FDA center to regulate products in tissue engineering has important financial consequences because the time and costs associated with approval via different centers can vary considerably. For those products requiring IND/BLA rules and regulations, the assessments

of safety and effectiveness and the intended use of manufacturer's claim build the basic elements of the evaluation (green Box). Likewise, an IDE also allows a device to be used in clinical trials to collect the safety and effectiveness data required for a PMA application (yellow Box). Clinical trials of devices with significant potential risks must be obtained approval and permission by both FDA and an IRB before the clinical study can begin. In addition to IND/BLA and IDE/PMA paths that regulate the respected products more heavily, there are less stringent subsidiary pathways for Devices, Drugs, and Biologics under some special conditions (see section 3.2 of text). Besides IDE, a device journey to the market can take one of two other pathways: (i) Humanitarian Device Exemption (HDE), and (ii) Premarket Notification (510(k)) that approved by the relatively straightforward 510(k) process. Also regarding Drug and Biologic products, in addition to IND/BLA, there is another path in specific situation called Orphan products that FDA authorizes to grant them. Detailed requirement for each of pathways have been clarified in Code of Federal Regulations Title 21. IRB: Institutional Review Board

The FDA have six centers from which three centers are responsible for review and regulatory oversight of human medical products including skin substitutes: (i) Center for Drug Evaluation and Research (CDER) in charge of drugs regulations; (ii) Center for Biologics Evaluation and Research (CBER) in charge of biological drug products; and (iii) the Center for Devices and Radiological Health (CDRH) in charge of regulations for medical devices. In addition to the centers, other offices such as the Office of Cellular, Tissue and Gene Therapy (OCTGT), Office of Combination Products (OCP) and Office of Orphan Products (OOP), help to the centers on regulatory procedures and facilitate inspections. OCTGT belongs to the CBER, which has scope over all cells, tissues, gene vectors, and tissue-engineered products. The OCP is in charge of the regulatory supervision of combination products and assigns appropriately to the one of FDA centers but not perform product reviews for market approval or clearance. Since many of engineered tissue products are combination products, OCP ensures timely and effective premarket review and appropriate post market regulation (Figure 5).

FDA requires that sponsors submit a Request for Designation (RFD) to OCP for identifying the primary mode of action (PMOA) product and recommend the lead center for product premarket review and regulation. Mode of action (MOA) of a product is identified as the means by which the therapeutic effect are obtained, i.e., drug, biologic, or device mode of action. Since combination products have more than one recognizable MOA, the PMOA is the single mode of action that provides the main therapeutic effect. There is PMOA Proposed Rule that describes an algorithm that the agency would use to assign a product to a center when it cannot determine with reasonable confidence which MOA provides the most important therapeutic effect. The Proposed Rule evaluate the product as a whole, its intended use and outcome; conformity with the application of similarly situated products; and safety and effectiveness issues [106]. This has major implications in product design because being regulated as a biologic in place of a device adds to the regulatory process (i.e. time) and cost. Getting PMA approval for a new medical device can cost low and typically takes less time, whereas getting BLA approval for a new medical drug or biologic through their relevant center can cost hundreds of millions of dollars and typically takes much more time [88, 107]. Thus, assigning to a regulatory pathway is extremely important in translating medical product such as skin substitutes into the clinic and should be considered at the beginning of system design [108].

Most advanced therapies in regenerative medicine will have to go through the CDER or CBER with more heavily rules and regulations (regulated under Section 361 AND biologic (IND/BLA) or device (IDE/PMA) regulations) [20]. It is of importance note that many of cellular products could consider as "Minimally manipulated", for example, banked human tissues or cells, hematopoietic stem cell transplantation and MNCs infusion, with relatively straightforward

regulations (regulated merely under Section 361 PHS Act) rather than "More than minimally manipulated" (Figure 6). The criteria for getting Tag of "Minimally manipulated" are: (i) cells or tissues must be intended for homologous use which means that have a similar function in the therapy, (ii) not be processed in a way that modifies the original respective characteristics; (iii) not have a systemic effect or be relay on metabolic effects for its primary action (except in homologous use or from close relatives); and (iv) not be combined with other drugs or devices. Minimal manipulation covers sterilization, preserving, or storage agent and techniques that sort out a specific cell population, like FACS, and
density gradient centrifugation, but does not cover any obvious alterations to the cells [20, 88, 105]. Therefore, any cell product that contains *in vitro* expanded stem cells will be considered as a "More than minimally manipulated" product and will require either IND or IDE clearance (Figure 6) [95].
Most, if not all, engineered tissues and regenerative medicine products are regulated by the FDA, as a science-based agency in the US Public Health Service (PHS), which has legislative authorization for premarket approval, and post-market vigilance and enforcement for a wide range of products in its regulatory preview [19, 20].

1.1. Special designation for some of engineered products

In case of rare diseases that comprising small size of the population (i.e. treatment or diagnosis of a disease that affects fewer than 4,000 individuals/year in the US), demand for new medical products may be prohibitive in view of cost of obtaining marketing approval; thus cost-benefit analysis might result in no available therapy. In such a situation, the FD&C Act allow the FDA to authorize special grant, consideration and exceptions to reduce the economic burdens for development of products for rare diseases. To grant these products by the FDA they should be recognized as a Humanitarian Device Exemption (HDE) for devices or as an Orphan drug for certain drugs or biologics [20]. A Humanitarian Use Device (HUD) exempts from the effectiveness assays but not safety requirements. Several bioengineered skin substitutes have been approved for market under the HUE designation [19].

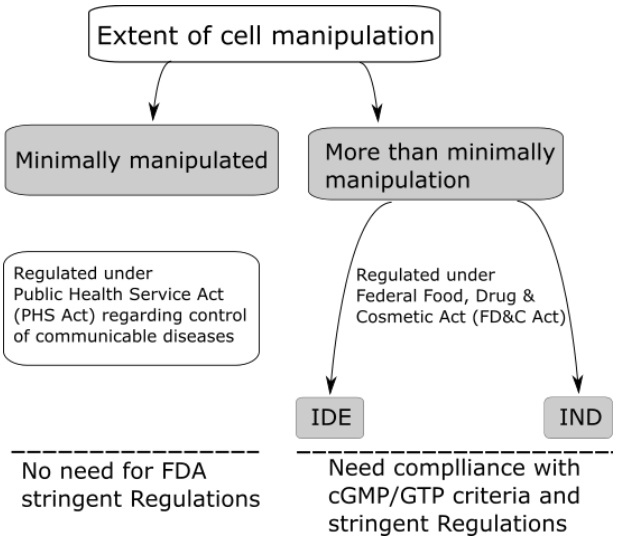

Figure 6: Extent of cell manipulation for FDA regulations. For cell-based therapeutic products, extent of cell manipulation determines regulatory status. "Minimally manipulated" products refer to cryopreserved cells which are not otherwise processed, e.g. CD34 positive cells selected by FACS). These kinds of products are regulated only by PHS Act. In contrast, "More than minimally manipulated" bioengineered products refer to any cell containing product that contains either *ex vivo* expansion or genetic manipulation. These kinds of products will require either Investigational New Drug (IND) or Investigational Device Exemption (IDE) clearance, and comply with both current Good Manufacturing Practices (cGMPs) and Good Tissue Practices (GTPs).

If a product regulated as a device, an IDE/PMA approval are required for demonstrating safety and effectiveness, or alternatively a premarket notification (510(k)) must obtain in relatively uncomplicated and easy premarket clearance; just for demonstrating substantial equivalence of the device to a legally marketed existing device.

Similarly, engineered products that regulated as biologics require review and approval of a IND/BLA for demonstrating the safety and effectiveness stringently before marketed commercially, or alternatively as an Orphan drugs intended to treat diseases or conditions affecting fewer than 200,000
individuals/year in the US, for which recovering the cost of product development and distribution from the sales of drug or biologics is not possible. The orphan drug designation is licensed under Section 351 of the PHS Act [20]. All these regulatory pathways have been depicted in Figure 5.

1.2. Costs and commercialization challenges

Perhaps the greatest challenge in the commercialization of the research into engineered cellular products is go through regulatory pathways to meet their requirements.
However, there is no doubt that for successful commercialization and keeping alive in the market, clinical value must go along with financial profit [21, 98].
A common characteristics of bioengineered products is that they are expensive to develop and produce, and to commercially become established in the clinical marketplace require substantial bankroll by their manufacturing firms [19, 8]. Therefore, it is important to have realistic financial predictions by writing a Business Plan (BP) and Business Model (BM) that include the developmental pipelines, approximate costs, and other critical details. Otherwise, profitability will not be gained and the product will not have durability [98].
Additionally, one of the key pre-market challenges is identifying and maintaining stable funding [21, 99], to move forward firms through lengthy developmental timelines (Figure 4) and regulatory processes (Figure 5).
The quickest and least costly regulatory pathway is a device designation with 510(k) notification [98].

2. Conclusion and future perspective

It seems that an ideal skin substitutes are constructed from biomaterial with combination of multiple cells of both epidermal and dermal layer in the future. Nevertheless, appropriate skin substitute have direct relationship with the type of wound. Additionally, skin substitutes that are off-the-shelf, inexpensive, less labor intensive, and permanently adhere to the wound bed, that produce an effective cosmetic effects and that do not contain animal or human serum will definitely be in high demand for skin tissue engineering goals.
To date, there is no complete skin substitute available. Acknowledging studies is ongoing to develop composite solutions, *in situ* guided regenerative solutions and the use of stem cells. It is of importance to mention that currently many ongoing researches on skin substitutes are under trial that might revolutionize the treatment of wounds in the next few years. Therefore, the list of skin substitutes is endless and skin substitutes being constructed from combination of stem cells and custom-designed biomaterials, remains as the most promising way for the future.
The keys for successful translation of research (product) into clinic (marketplace) have been summarized in Figure 7. On top, collaboration and partnering is the most important key that link the cell producing company, technology providers, clinician with the in-depth knowledge of the biology and pathophysiology and the healthcare systems, with the commercial teams to put the technology in the path of safe and effective implementation. By interdisciplinary collaboration and partnering there are real opportunities for successful production and clinical translation of bioengineered skin substitutes with high quality and cost-beneficial price into market.

Conflict of interest

Authors declared no conflict of interest.

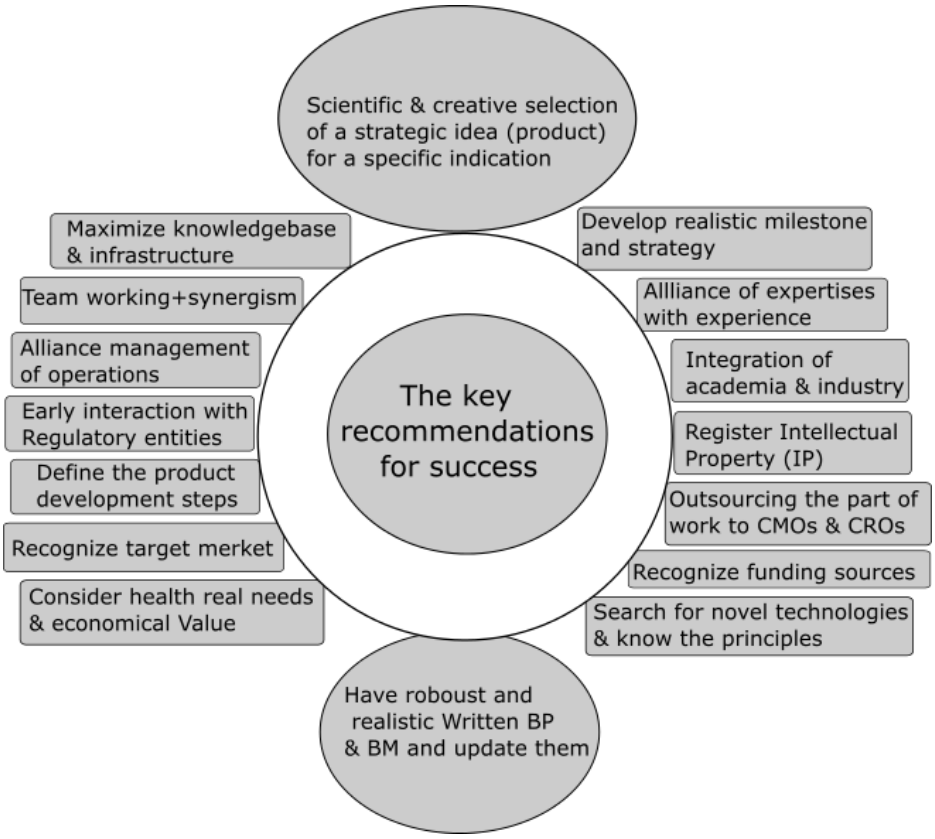

Figure 7: The keys for successful translation of research (product) into clinic (marketplace). In addition to deep knowledge of one field (being expertise), having smattering knowledge about other fields like novel developing technologies and their principles, regulatory pathways requirements, market and business model, and management will accelerate translation of the product into the market. For successful commercialization of an engineered product, it is vital that all parts of a plan works in coordination together like a whole body organs. CMOs: Contract Manufacturing Organizations, CROs: Contract Research Organization, BP: business plan, BM: business model.

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