

Tissue engineering in burn wound healing: current modalities and future directions

Abstract

Current advances in tissue engineering and development of novel skin tissue substitutes provide a well opportunity to treat burn patients especially in the case of large burn wounds. Although the ability to culture autologous epidermal cells in-vitro from a small skin sample into multiple-layer sheets of epithelial cells alleviate the limitation of inadequate donor site in extensive burn wounds, many burn centers still face some challenges such as insufficient skin allografts which are used as wound coverage after surgery. Today, skin substitutes offered by TERM technology to meet emergency claims have been used fairly successfully. Current options including many commercially available or marketed skin-like tissue products is facing serious problems such as very high price, sub-optimal skin tissue microstructure and inconsistent transplantation, especially in full thickness burns. The TERM scientists and researchers conducted different experiments to reach novel tissue-engineered skin substitutes, as adjuncts to accelerate re-epithelialization for wound closure and to improve the functional and cosmetic results and thus ameliorate life quality. This paper reviews the history of skin tissue engineering, explains some of the commercial products developed to treat extensive burn wounds and discusses the future direction in utilization of stem cells in the hope to improve the current practices.

Keywords: tissue engineering, burn; wound healing, regenerative medicine, skin substitute

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Abbreviations: TERM, tissue engineering and regenerative medicine; GAG, glycosaminoglycans; CEA, cultured epithelial autograft; SCS, stem cells; MSCS, mesenchymal stem cells, BMMSCS, bone marrow-derived mesenchymal stem cells; ADSCS, adipose -derived mesenchymal stem cells; ECM, extracellular matrix; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; IGF-1, insulin-like growth factor I; CSF, colony stimulating factors; ILS, interleukins; TNF, tumor necrosis factor; TGF-B, transforming growth factor B; MMPs, matrix metalloproteinases

Introduction

Acute thermal injuries or burn wounds requiring special medical treatment affect millions of people worldwide, with approximately 265,000 deaths annually.¹ The survival rate in admitted burn cases has been improved consistently over the past 40 years. This can be largely attributed to improvements in burn wound care that has been achieved by several researches in burn wounds over recent decades.² This progress has been made by significant improvements in early revival; infection management, wound debridement and coverage, and body fluid management which helped in reducing the mortality rate.³ Since ancient times, reconstructive surgery has been performed to improve the quality of human life, especially in some conditions such as natural disasters and wars which frequently challenge humanity to innovate new modalities.^{4,5} Nowadays, most cases with extensive burns will survive by application of reconstructive surgery. The autologous skin graft including an epidermal graft with only a thin dermal layer became as the gold standard method in burn surgery because of donor site availability and minimizing rejection rate of the transplanted tissue.⁶ However, in circumstances such as extensive burn

wounds, the conventional treatment options have not been shown to be effective and new modalities with better efficacy, availability and compatibility have been required.⁷

Tissue engineering and wound healing

Tissue engineering and regenerative medicine (TERM) can be considered a multidisciplinary and emerging field in technology, used to regenerate damaged organs, produce complex tissues and provide the maintenance of normal cell homeostasis. One of the most important goals in TERM is designing new tissue and organ replacements which closely mimic a normal physiological environment for cells.⁸ TERM wound healing products can be classified into two major categories: 1. cell-based skin constructs and 2. Acellular productions. These products include a wide variety of materials which have been categorized as epidermal and dermal substitutes.⁹

The dermis is composed of dense irregular connective tissue containing collagen fibers, elastic fibers, and extracellular matrix, anchoring the dermis to the epidermis. The superficial area (upper layer) of the dermis, the papillary region, is a cell-rich layer containing macrophages and fibroblasts allowing dermis-epidermis interactions. Synthesis of ECM components which is induced by this interaction can stimulate the differentiation and growth of keratinocytes.¹⁰ Some allogeneic and xenogenic tissue-engineered skin substitutes, in most instances carry the risk of graft rejection, have been developed so far. In the 1970s, a gap of artificial skin substitutes for transplantation of large skin defects was concluded.¹¹ This challenged the plastic surgeons and researchers to fabricate dermal like tissues in the lab with the help of tissue engineering. Since then, many studies have been conducted on dermal tissue substitute production¹²⁻¹⁴ and some

of them have become commercially available and were approved by FDA to be a valuable skin substitute for large burn wounds treatment. Table 1 provides some of the commercially available acellular skin constructs.

Over the past decades, many types of cell-based skin constructs have been developed for full-thickness skin defects. For the first time in 1975, keratinocyte colonies were cultured from single-cell suspensions of human epidermal cells.¹⁵ This method has been used as a possibility for the closure of large burn wounds and a potential life-saving approach, especially when autologous skin graft was limited. Since then, several clinical case reports have been published describing the use of CEA as confluent sheets to provide burn wounds

coverage. However, poor survival rate of the keratinocytes in cell sheets has been a major concern in this regard.¹⁶ The innovation of novel biocompatible carriers for CEA allowed grafting at earlier culturing stages of keratinocytes. The colonies of keratinocytes have also been used to graft onto the burn wounds site before they form a single-cell layer sheet. There are only a few products commercially available as cell-based skin substitutes, consisting exclusively of human allogeneic skin cells, as yet. For instance, Apligraf® (Organogenesis Inc., MA, USA) is a FDA approved skin substitute which consists of human allogeneic keratinocytes and fibroblasts derived from neonatal foreskins seeded onto a bovine type I collagen gel [78,79,80,81]. Table 2 shows some of the commercially available cell-based skin substitutes.

Table 1 A selection of commercially available acellular skin substitutes

Skin substitute	Composition	Company
EZ Derm®	Porcine collagen dermis	Mölnlycke Health Care AB, Gothenburg, Sweden
Permacol™	Acellular porcine dermis	Covedien, Mansfield, MA, USA
Graftjacket®	Acellular human dermis	Wright Medical technology Inc., Memphis, TN, USA
Matriderm®	Acellular bovine dermis	Med Skin Solutions Dr. Suwelack AG, Billerbeck, Germany
Alloderm®	Acellular human dermis	Life Cell Corporation, Bridgewater, NJ, USA
Integra®	Bovine collagen and GAG with silicone top layer	Integra Life Sciences Corp., NJ, USA
Glyaderm®	Acellular human dermis	Euro Skin Bank (ESB), Beverwijk, The Netherlands.
Oasis®	Porcine small intestine submucosa	Smith & Nephew Inc. Fort Worth, TX, USA

Table 2 A selection of commercially available cell-based skin substitutes

Skin substitute	Cell type	Carrier	Company
OrCel™	Allogeneic fibroblasts and keratinocytes	Bovine collagen type I sponge	Forticell Bioscience Inc., NY, USA
Laserskin®	Autologous keratinocytes	Benzyl esterified hyaluronic acid membrane	Fidia Advanced Biopolymers, Abano Terme, Italy
Epicel®	Autologous keratinocytes with murine fibroblasts (sheet)	-	Genzyme Corporation, Cambridge MA, USA
Dermagraft®	Allogeneic human dermal fibroblasts	Polyglactin mesh scaffold	Organogenesis Inc., MA, USA
TransCyte®	Allogeneic human dermal fibroblasts	Silicon mesh-like material	Organogenesis Inc., MA, USA
Apligraf®	Allogeneic human keratinocytes and fibroblasts	Bovine collagen type I gel	Organogenesis Inc., MA, USA
ICX-SKN®	Allogeneic human dermal fibroblasts	Human collagen matrix	Intercytex Ltd. MCR, UK

A very interesting TERM modality in managing the burn wounds is to prepare an epidermal cell spray directly applied on the defect site during surgical operation.¹⁷ For this purpose, some commercially available kits such as ReCell® (Avita Medical Europe Ltd, Melbourn, UK,) have been developed. ReCell® is a special kit that enables medical professionals to harvest epidermal cells from a small sample of a patient's own skin to create a cellular suspension.¹⁸ Usually a thin split skin sample is collected through isolation of epidermal cells by this kit, because it allows better separation of the epidermal layer from the dermal layer, facilitating the isolation process. This method has an indication for small burns where no cell culturing is required. Gravante et al.¹⁸ compared the ReCell® system of epidermal cells delivery with the standard transplantation of autologous skin

grafting for the treatment of deep partial thickness burns.¹⁸ They concluded that the ReCell® procedure is a feasible, simple and safe technique giving similar results to skin grafting with smaller donor site size biopsy areas and less postoperative pain than the classic grafting method. However, this practice took more time and is also more expensive. Another recent published study compared ReCell® procedure versus autologous meshed split thickness skin graft for the treatment of acute burn injuries.¹⁹ The results showed that the ReCell® procedure was more beneficial because it resulted in reduction of the size of the donor site with comparable outcome. Today, correction of skin pigment disorders such as vitiligo has been experimentally made by cell spray approach, as melanocytes are also included in the cell suspension that is sprayed onto the area of skin lesions.^{17,20} Co-

delivery of epidermal cells with fibroblast (Apligraf® and OrCell®) has been used to increase the efficacy of cell-based therapy. Fibroblasts incorporated with the commercial cell-based skin substitutes secreted several growth promotive factors such as VEGF, PDGF, IGF-I, CSF, ILs, TNF, and TGF-β. Dermagraft® and TransCyte® are laboratory-grown ECM of allogeneic human dermal fibroblasts which were seeded on mesh like material/scaffold. One study analyzed the safety and efficacy of TransCyte® in treatment of partial-thickness burns²¹ and it was demonstrated that the wounds treated with TransCyte® healed faster, with no infection and less hypertrophic scarring than the site treated with silver sulfadiazine (positive control).

Over the last few years, the discovery of the availability of SCs has opened up new horizon in the field of TERM.²² This discovery has attracted great interest in the community of TERM by introducing SC therapy at the front line of this field for human treatment.²³ SCs can differentiate into a wide variety of cell types and have a great capacity to modulate immune responses, which make them ideal for TERM purposes.²⁴ It has been shown that SCs could improve burn wound healing as well as the degeneration of skin appendages.²⁵ Until now, BMMSCs are the most favored cell type presently under clinical trial, but the use of ADSCs is increasing because of more advantage in comparison to BMMSCs. This exciting field is associated with an increasingly amount of *in vitro* and *in vivo* experiments and clinical trials.^{26,27} However, the safety of SC therapy for human use is not fully elucidated yet, and the process of this therapy was very expensive.²⁶ Therefore, treatment of burn patients by SCs is a very promising option but not a common modality nowadays.

Future directions

During the past decade, MSCs harvesting from different sources rapidly moved from *in-vitro* and *in-vivo* studies into human clinical trials as a therapeutic option for a diverse range of clinical applications.²⁸ MSCs therapy could be the future direction of treatment for extensive burn wounds in addition to existing therapeutic modalities. MSCs have demonstrated a number of properties *in-vitro* that can promote skin tissue repair, including the ability to recruit a wide variety of cells such as keratinocytes, fibroblast and other stem cells, in addition to production of several growth factors, cytokines, collagens, and MMPs.²⁹ It has been shown that MSCs can increase wound healing through differentiation and angiogenesis.³⁰ Till now, some clinical cases on the use of cultured BMMSCs for localized treatment of chronic burn wounds have been reported. Yoshikawa et al. treated 20 patients with various non-healing wounds (i.e., burns, pressure ulcers) using autologous BMMSCs culture expanded and with or without autologous skin graft.³¹ The results illustrated that the wounds in 90% patients healed completely with the cell-based graft transplant with the delivery of MSCs facilitated regeneration of the native tissue by histological examination. The immunomodulatory effect of MSCs on immune cells plays a key role in utilization of these cells for rapid treatment of extensive burn wounds. MSCs can modulate both innate and adaptive immune responses and it is clear that the local microenvironment is important for activation of MSCs to become immunosuppressive.³²

Today, the first universal clinical trial is in progress in Argentina which used allogeneic BMMSCs to treat ten patients with large severe deep burns. This clinical trial uses BMMSCs seeded on acellular dermal biological matrix.³³ Future research in MSCs and its

inflammation control, infection, route of transplantation, biomarkers production, and rehabilitation will create new treatment options for burn wound healing.

Conclusion

Various clinical limitations in treatment of acute thermal injuries and extensive burn wounds include many systemic and topical bio factors that affect time of wound healing, time of wound closure, the risk of infection, and the overall time to complete recovery of functionality. Remarkable advancements have been made in care of burn patient, including tracking wound healing process, developing novel skin substitutes and coverage modalities, controlling infection and inflammation, optimizing mineral and dietary needs, and analyzing unique pharmacological interventions. As a result of these attempts, the patient survival rate has improved along with an attendant decrease in the length of hospital care, which in turn resulted in increased patients satisfaction and a decreased cost to the medical providers.³⁴

No single treatment can be recommended in the management of burn wound healing according to the current and emerging therapeutic options. However, based on the existing knowledge, related technology and available products for skin substitute or rapid coverage of large burn wounds- acellular skin substitute, cell-based skin constructs and cell therapy (MSCs)-seem to be of the most impressive and cost-effective management approaches. Tissue-engineered skin substitutes can be used in order to reduce scars and lesion contractures leading to improve the quality of life of the patients. Before the TERM scientists can fabricate and introduce a functional skin substitute with a logical and reasonable cost, the need for human skin allograft tissue banks for treating the extensive burn wounds cannot be overstated.³⁵ In addition, an autologous epithelial cell culture laboratory and a stem cell research center should be accompanied by the tissue bank for skin tissue engineers, scientists and plastic surgeons. Finally, to fully understand all requirements for patients, working closely of TERM scientists with clinicians can navigate this pool of personnel innovate, harness emerging technologies, and manage cost for skin-based tissue engineering.

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Conflict of interest

The author declares no conflict of interest.

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