Recent Advances in Medical Grafting Involving the Transplantation of Skin

Meda Cosma, MD,

Abstract—Current therapies aim to restore the cutaneous barrier to fluid loss and infection. This is hard to achieve in large acute wounds or non-healing chronic wounds as patients have an urgent need of epidermal barrier. Large acute wounds are mainly treated focusing on coverage, while chronic wounds require coverage and convert a non-healing wound bed to an environment conducive to healing. The challenges in restoring skin have restricted treatments for large acute wounds to significantly change in 30 years, and treatments for chronic wounds have only developed in the past 10–15 years.

Keywords—tissue regeneration, skin grafting, keratinocytes

I. SKIN GRAFTING

Nowadays, the gold standard for wound coverage is the splitthickness autograft[1], impossible to be readily and completely carried out in patients with limited skin for donor sites.[2] Collateral therapies include Integra® (Integra Life Sciences, Plainsboro, NJ) and cultured epithelial autografts, commercially available since the 1990s, but unable to achieve widespread use. These treatments which are available today can restore the epidermal barrier with clinically satisfactory cosmetic outcomes. But clinically satisfactory cosmetic outcomes do not automatically include adnexal skin structures such as hair and pigment, which are crucial for normal skin functions.[3] Thus, the ultimate purpose of RM for the integumentary system is the restoration of fully functional skin which should be physiologically and cosmetically similar to the normal skin of the patient.

The most promising regenerative therapies for skin restoration can be divided into 2 main categories: artificial skin substitutes and cell-based therapies. Artificial skin substitutes mainly employ biomaterials for skin restoration, while cell-based therapies employ the healing response of skin cells. Novel therapies include new formulations of naturally occurring extracellular matrix (ECM) or in situ delivery of stem cells (SC). It must be noted that even if many potential skin therapies are successful in rodent models they might not be applied to humans because of major differences in wound healing mechanisms in thin-skinned versus thick-skinned animals. Future skin regeneration therapies should include biomaterials in combination with cell therapy.

The prototypical biomaterial approach to skin restoration is provided by Integra® is. The bilayered construct consists of type I bovine collagen combined with chondroitin-6-sulfate and an overlying silicone membrane.[4] The collagen/chondroitin layer manages the growth of “neo-dermis”, while the silicone membrane is a temporary epidermal barrier until the construct is finally covered with an autograft. Integra® has been mostly used in large burn wounds, being a front-line treatment at this medical department.[5] Many treatments have simulated its dermal and epidermal structure and all the new approaches must compete with these existing mechanisms.[6] Future skin regeneration approaches employing biomaterials are to manipulate the healing properties of the natural ECM. Collagen, fibrin and hyaluronic acid are ECM hydrogels produced at specific intervals in the normal wound healing process to stimulate migration and proliferation of skin cells.[7, 8] The knowledge of ECM-skin cell interactions is crucial when employing ECM as a regenerative therapy for wounds. Control of ECM composition by fibroblasts and keratinocytes has a major impact on scarring and wound contraction.[9] Cell migration facilitation is a well known feature of collagen and it is the ground for many of the currently existing dermal substitutes, such as Integra®. The initial sealant and scaffolding promoter in normal wound healing is fibrin, which reduces wound contraction if combined with skin grafts.[10] Consequently, fibrin is mostly used as a delivery vehicle for applying skin cells to a wound.[11] Despite the wide use of collagen and fibrin in new skin therapies, hyaluronic acid is a potential component of many future therapies. Hyaluronic acid is the major component of the ECM in fetal wounds that heal without scarring[12], thus having implications for adult healing.[13] The choice of biomaterial for skin regeneration clearly influences the clinical outcome. Future skin regeneration therapies should incorporate more natural scaffoldings in order to ease wound healing.

Biomaterials possess significant wound healing properties, but they lack the full wound healing potential of skin cells. Cells
close a wound and create the skin function structures. In the USA, cultured epithelial autografts (CEA) are the prototypical cell-based therapy,[14] where keratinocytes are grown in a sheet and applied on a wound[15] CEA can be cultured from autologous or allogeneic keratinocytes and can reconstruct the epidermal barrier with clinically satisfactory cosmetic outcomes.[14, 16] Nevertheless, cell-based therapies are potentially restricted by cell survival and the decreased storage abilities of living biological constructs.[17] However, these limitations have not constituted a drawback for several cell-based therapies, such as Apligraf® (Organogenesis Inc., Canton, MA), in becoming commercially applicable treatments for chronic wounds. [18]

CEA are produced in cell sheets grown in culture and applied to wounds as sheets, which can be very fragile and difficult to handle. Therefore, researchers and clinicians have analyzed the use of cell spraying to provide the same skin cells without handling a fragile cell sheet.[19, 20] This method can basically deliver any type of skin cell in a vehicle, mainly a fibrin hydrogel. Cell sprays have been further used in non-cultured autologous skin cells isolated in the operating room and directly applied to a wound.[21] In the clinical trials in the United States, this technology employed in burns, called ReCell® (Avita Medical, Woburn, MA), is part of the Department of Defense funded Armed Forces Institute of Regenerative Medicine.

Cell therapies have been promising wound treatments for the delivery of fibroblasts and keratinocytes. Nevertheless, fibroblasts and keratinocytes do not accomplish all skin functions, probably requiring a source of stem cells (SC) to fully restore all integumentary structures in a major wound. There are too many different cell types in fully functional skin to allow for a realistic delivery of each different cell type in specified locations within a wound. Skin SC sources have been identified in the epidermis and hair follicles.[19] These highly proliferative cells are crucial contributors to normal wound healing, showing increased wound healing after culturing, possibly due to proliferative progenitor selection throughout the culturing process.[22] Non-scarring fetal wounds might owe some of their unique properties to a high number of SCs.[23] Various SC types, including bone-marrow mesenchymal SC (MSC), adipose-derived SC and embryonic SC (ESC), have been investigated for the treatment of cutaneous wounds.[24] These cells can generate dermis and epidermis, but there is need for more research before turning SC therapy into a viable approach in the management of acute or chronic skin wounds.

As indicated before, the future of skin regeneration lies in the successful development of an approach combining the biomaterial with cell-based therapy. Stratagraft® (Stratatech Corporation, Madison, WI), one of the most promising options [25, 26], is a full-thickness skin substitute with a fully-stratified epidermal layer consisting of NIKS® cells grown atop a dermal layer consisting of human fibroblasts embedded in a collagen matrix. In 2011, StrataGraft® is going to enter a Phase I/II burn clinical trial.

II. THE NEED FOR TISSUE-ENGINEERED SKIN SUBSTITUTES

Patients with 50% total body surface area (TBSA) full-thickness wounds have only 50% of undamaged skin left to be used for split-thickness skin harvesting. The rest would be obtained from donor sites and result in 100% wound area covering. The combination between damaged epidermal barrier and poorer immunity of heavily burned patients can lead to bacterial sepsis, one of the main complications in deep extensive burns. [27] The healing of donor sites can occur with some scarring and can be quite painful. Therefore, there is need for an additional analgesic pharmacological load. Additionally, based on dermis thickness, it is only possible to harvest three to four split-thickness skin samples from the same site and in order to repeat this procedure, it is necessary to wait until re-epithelialization. [28]

When the wound is more extensive and there are limited donor sites, meshing techniques can be used, with a uniform perforation and stretching of grafted skin to cover a larger wound area. Even if this technique helps covering a larger area and results in lower mortality rates, the cosmetic and functional results are poorer than those of conventional split skin grafts (SSG). This is due to dermis absence in the interstices of the stretched meshed skin graft and slow epithelialization from graft margins across interstices, determining greater graft contraction, delayed healing, scar tissue formation and obvious ‘crocodile skin’ scar appearance. Even meshing techniques can be incapable in near-total full-thickness skin wounds due to unavailable donor sites. In this situation, wounds are covered with temporary dressings or cadaver skin to form a mechanical barrier to restrict fluid loss and microbial contamination. The only solution to heal injured skin in these cases is delayed serial autologous split skin grafting.[29, 29] These wounds are left unhealed for a long period of time throughout the treatment, waiting for epithelial regeneration, and severe complications may occur, possibly resulting in death.

For the treatment of extensive full-thickness wounds lacking donor sites for SSG harvesting, alternative life-saving approaches include the use of cultured autologous keratinocytes and/or bioengineered skin substitutes. Recently, the development and clinical use of these products has known a significant progress.[30-32] These approaches might be used for the treatment of extensive deep injuries due to their ‘off-the-shelf’ availability and quick production of adequate quantities of epithelium able to permanently close the wound.

The great significance and request for skin-substitution products has been the focus of many research groups around the world, all trying to create biomaterials for skin replacement. These biomaterials are sometimes confusingly termed. They are referred to as bioengineered skin equivalents, tissue-engineered skin, tissue-engineered skin constructs, biological skin substitutes, bioengineered skin substitutes, skin substitute bioconstructs, living skin replacements and bioengineered alternative tissue.[33] Despite the slight
difference from each other, most researchers consider these biomaterials to be equal and interchangeable. Therefore, our review will use these definitions to describe any skin substitute products, produced or modified artificially in any way, including modifications of naturally occurring substances, such as dermis, to fully or partially, temporary or permanently replace damaged skin, having some anatomical and functional similarities with human skin.

There are three major requirements that all tissue-engineered skin substitute biomaterials are required to comply with. They must be safe for the patient, clinically effective and easy to handle and apply. Several authors[34] have recently reviewed the properties of the ‘ideal’ skin substitute for in vivo use. Generally, these biomaterials must be non-toxic, non-immunogenic and should not cause excessive inflammation, having no or low risk of transmissible disease. Skin reconstruction biomaterials should be biodegradable, repairable and capable to support the reconstruction of normal tissue, possessing similar physical and mechanical properties as the original skin. It should offer remedy against pain, prevent fluid and heat loss and protect the wound from infection. It should be cost-effective, readily available, user-friendly and long-lived.

Currently, there are no commercially available tissue-engineered skin replacement biomaterials possessing all the above-mentioned properties, able to completely replace the functional and anatomical properties of the skin they replace. However, there are several bioengineered skin-replacement products available to clinicians and used for wound-healing purposes. Generally, these tissue substitutions only partly address skin functional requirements and surgeons are likely to use different products to attain certain purposes.[35, 35] described four groups of functions that can be provided by bioengineered skin-replacement products: protection, through a mechanical barrier against micro-organisms and vapour loss; procrastination, early wound cover until achieving permanent wound closure by means of serial skin grafts or cultured autologous cell applications, particularly in extensive burns; promotion, delivery of dermal matrix components, cytokines and growth factors, to boost natural host wound-healing responses; provision, new structures (dermal collagen or cultured cells) are administered inside the wound, persisting during and/or after wound healing.

III. DERMEO-EPIDERMAL (COMPOSITE) SKIN SUBSTITUTES

The purpose of dermo-epidermal or composite skin substitutes is to imitate the histological structure of normal skin, with both epidermal and dermal layers. This similarity has some functional resemblance to normal skin. In terms of epidermal and dermal substitutes, these are the most advanced and sophisticated products, as well as the most expensive tissue-engineered biological constructs for tissue repair. [36] The majority of these products is based on allogeneic skin cells incorporated into a dermal scaffold. This method favors the manufacture of large quantities of relatively ‘off-the-shelf’ available uniform product batches. On the other hand, these biomaterials are mainly temporary biologically active wound sheets[37], providing growth factors, cytokines and ECM for host cells and stimulating and mediating wound healing. Host immunogenic tolerance to allogeneic fibroblasts has been reported in some reviews[38], as well as their survival in the host for up to three weeks [39]. There have also been reports regarding the preservation of allogeneic fibroblasts and their proliferation for up to two months in the host, without indications of immune rejection. [40-43] Nevertheless, allogeneic fibroblast survival for more than 7 days could not be confirmed in porcine studies[44], or in clinical studies transplanting allogeneic fibroblasts onto burn wounds.[45]

On the one hand, allogeneic keratinocytes are effective pain relievers, accelerating wound healing, but on the other hand, they do not survive for more than a few weeks when applied to the wound, as they face host rejection,[45, 46] The expression of the human leukocyte antigen (HLA) complex might be different in fibroblasts and keratinocytes, thus subjecting allogeneic fibroblasts to tissue rejection determined by HLA. Fibroblasts are not able to induce T-cell proliferation through cytokine production when involving HLA class II molecules.[47] Some of the suggestions to study the immunologic tolerance to allogeneic fibroblasts in the host have been in vivo models examining acute graft-versus-host disease.[48] In conclusion, both allogeneic or autologous fibroblasts can be used to obtain permanent dermo-epidermal skin substitutes, but only the use of autologous keratinocytes can help provide permanent wound closure.

IV. EPIDERMAL SUBSTITUTES

The possible serial culture of human keratinocytes in vitro[49] increased the number of patient keratinocytes ex vivo, quickly transferring this technology into clinical applications[50], determining an improvement in patient survival rates.[51] But there are still disputes regarding the benefits of cultured keratinocytes.

An essential step in the design and production of epidermal substitutes is represented by the isolation of keratinocytes from a donor, followed by in vitro cell culture to provide the required number of keratinocytes for therapeutic purposes. Different approaches in the production of epidermal substitutes are related to: cell culture techniques (submerged or air–liquid interface models), cell differentiation stage and epithelial organization (confluent sheets, subconfluent cell layers and suspensions), cell delivery methods (confluent sheets mounted onto support layer, subconfluent dispersed keratinocytes delivered by means of aerosol techniques or microcarrier beads), as well as the use of additional substrates to improve cell culture and delivery (both synthetic and biological).[52, 53]
Autologous cell culture is initiated by a skin biopsy of 2–5 cm², generally removed along with initial wound excision during patient presentation to the clinic. Epidermis and dermis are separated from each other and single keratinocytes are delivered from the sheet via exposure to enzymes. These keratinocytes are plated into tissue culture vessels where single cells divide to produce colonies in the presence of mitotically inactivated mouse fibroblasts and culture medium containing foetal calf serum and necessary supplements. Keratinocytes can be expanded in xenogeneic-free conditions avoiding murine fibroblasts and bovine serum[54], but with considerably reduced proliferative lifespan of these cells.[55] Single colonies of keratinocytes mingle and form stratified epithelial layers which can be enzymatically detached from the culture flask, fitted onto backing supports (e.g. paraffin gauze) to maintain basal–apical orientation, further applying the sheet to the wound. [56]

Cultured epithelial autografts (CEAs) have qualities which are related to clonal cellular composition[57], which supposedly influences graft survival and long-term performance in in vivo applications. Basal keratinocytes producing holoclones (in vitro colonies with the highest proliferative potential) are basic tools in providing long-term graft survival. Meroclones, containing transient amplifying cells, possess a fluctuating proliferation potential and are only able to provide temporary wound closure in in vivo applications. Paraclones, which are committed keratinocytes, represent most of the normal epithelial cell population but can only replicate a few times before differentiation and senescence. Thus, CEAs containing only paraclones are not able to serve as substrate for permanent wound closure. Holoclone preservation is possible in current culturing techniques if keratinocytes are grown in vitro for a extensive period of time.[55]

CEA sheets are produced by means of in vitro keratinocyte expansion techniques, being large enough to cover all the body surface area in three to four weeks using only a 3 cm² skin biopsy.[58] When keratinocyte culture is supplemented with support substances, such as a fibrin matrix, CEA area can be expanded in shorter periods of time. In their study, Ronfard et al.[59] achieved 4.1 m² of graftable epithelium from a 4.5 cm² skin biopsy cultured for 15 days on a fibrin matrix, compared to 1.4 m² when cultured on plastic matrices. The use of this technique has also improved material handling and basement membrane formation.

There is a huge difference in the outcome of clinical removal or integration of such cultured epithelial autografts in a sheet form,[60]. This might be a result of CEAs containing terminally differentiated keratinocytes with altered integrin expression, responsible for matrix attachment.[61] Other drawbacks of CEA sheets include: extended culture time, graft friability, difficult handling and application procedures. A more precise coordination between tissue culture facility and clinic is also required. One of the biggest drawbacks of sheet application is the uncertain clinical outcome, ranging between 15 to 85% take rate.[62] In patients treated with confluent CEAs, the weak keratinocyte attachment, determining blistering when exposed to minor shearing forces, was obvious for several months after grafting. [63]

V. CULTURING SKIN COMPONENTS IN THE LABORATORY

Highly injured full-thickness skin cannot spontaneously regenerate in mammals. A fully functional skin substitute can only be created after completing recapitulation of ontogenesis, which cannot be achieved in vitro.[64] Nevertheless, the phenotype expressed by human skin cells in culture is very similar to wound healing physiology[64, 65], including cytokinesis, morphogenesis and histogenesis, but excluding organogenesis. Cells should promote the wound healing process and boost the developmental regeneration process. Therefore, they might provide full function to an artificial skin substitute produced like this. Individual skin components have been successfully cultured and transplanted in many experimental studies, but not as part of a fully functional skin replacement process. Connective tissue cells are believed to recolonize grafts from the wound bed, but many skin substitutes consist of dermal fibroblasts facilitating such repair processes.[66] Melanocytes have also been employed to recolonize burn scars and to treat vitiligo, as well as to supplement various polymer scaffolds.[67] Nevertheless, full restoration of skin sensation could not be proved by either split thickness skin grafts or engineered skin, whereas sweat and sebaceous glands have only been transplanted experimentally.[68] These latter structures, together with nerves and blood vessels, might be the most difficult to achieve, as they are neither restored nor regenerated.

References


[31] Macri L, Clark R. Tissue engineering for cutaneous wounds: selecting the proper time and space for growth factors,


[54] Deshpande P, Notara M, Bullen N, Daniels JT, Haddock DB, MacNeil S. Development of a surface-modified contact lens for the transfer of cultured limbal epithelial cells to the
47


