Tissue and cellular approaches to wound repair

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Tissue engineering can be defined as the use of biomaterials with or without small molecules, cells, genes, or gene products to maintain, replace, or repair organ function with the objective of correcting the underlying pathology. This new field of medicine is based on the use of engineered cells, tissues, and synthetic materials that can potentially extend and improve a patient’s life. At the present time, treatments for organ or tissue loss include organ transplants, surgical reconstruction, the use of mechanical devices [1,2], and, recently, cellular therapy.

The research and development of tissues and cell-based products have taken many approaches to advance the field of regenerative medicine. This involves the use of cell lines and the inclusion of an extracellular matrix, thus forming a tissue architecture, which allows communication through cell-to-cell interactions and cell-to-matrix interactions. Such interactions are essential for providing tissue support and functionality through the release of a variety of cytokines and growth factors.

Therapeutic approaches for cellular and tissue therapy

Three general strategies are currently used in the development of tissue-engineered products. The first strategy involves the use of cells without matrix. When transplanted, these cells restore function to an injured organ or organ system. Stem cell therapy and autologous cell transplant are examples of this approach. The second strategy involves the development of synthetic polymers or biomaterials that act to restore organ system function when used alone (i.e., synthetic scaffold materials) or with the addition of proteins, such as growth factors and cytokines, which may be released in a controlled fashion. The last therapeutic approach involves the use of cells within a 3-dimensional matrix. For example, bilayered skin substitutes composed of keratinocytes and fibroblasts incorporate into biologic collagen matrices or synthetic bioabsorbable scaffolds before implantation and serve to enhance or promote tissue repair processes.

The stem cell concept

A stem cell is an undifferentiated cell with the capacity for self-renewal that gives rise to ≥1 highly differentiated cell type [3]. Stem cells are derived from either embryonic cells, referred to as pluripotent stem cells, or from fetal and adult tissue, both referred to as multipotent stem cells [4]. Embryo-derived stem cells are pluripotent, capable of giving rise to most tissues. They can be derived from the inner cell mass of the preimplantation blastocyst or primordial germ cells of embryos and are used for the development of many cell-derived products because of their unique ability to divide for an indefinite period of time in cell culture and to give rise to many lines of specialized cells.

Fetal stem cells, like adult-derived stem cells, are multipotent stem cells derived from either fetal or adult tissues, which possess a high degree of plasticity. These cells can be obtained from mesodermal tissues, such as muscle and bone marrow, including hematopoietic, mesenchymal, and endothelial stem cells, as well as ectodermal and endodermal tissues, including neural, epidermal, intestinal, liver, and pancreatic stem cells [4]. Multipotent stem cells are the result of further specialization of pluripotent cells into particular cell lines, such as blood and skin. Both fetal and adult-derived stem cells are being successfully developed for multiple indications, including cardiomyocyte replacement for heart conditions, chondrocyte replacement for osteoarthritis, and neuronal replacement, which has led to symptom reversal for Parkinson and Huntington diseases [5]. Recently, multiple studies have demonstrated that adult stem cells possess a broader developmental capacity than was previously thought. The plasticity of adult-derived stem cells was illustrated when brain-derived adult neural stem cells from mice contributed to the formation of a chimeric chick and mouse embryo and gave rise to all germ layers.
Although adult-derived stem cells have shown this broad developmental capacity, additional studies are needed to confirm the in vitro and in vivo fate of these cells.

The bone marrow contains a population of stem cells. These cells include hematopoietic stem cells, which give rise to blood and lymphoid cells; mesenchymal stem cells, or marrow stromal cells, which regenerate bone and marrow cells (adipocytes, reticular cells); and endothelial stem cells [6]. Bone marrow–derived mesenchymal stem cells have been used to derive a range of cell types, including hepatocytes and neurons [8]. Thus, although mesenchymal stem cells are potentially less plastic than embryonic stem cells and possess a predetermined differentiation capability, they may be reprogrammed to derive new cell types through extracellular signaling processes that revert precursor cells to multipotent stem cells. For example, oligodendrocyte precursor cells can be reprogrammed to revert to multipotent neural stem cells and give rise to various neural tissues, including neurons, astrocytes, and mature oligodendrocytes [9].

Advancements in new cell culture techniques and a growing knowledge about optimal oxygen levels are enhancing the development of commercial quantities of stem cells. Lowered oxygen levels (3%) in cell culture have been shown to reduce apoptosis, increase the number of central nervous system precursors, and increase differentiation rates, all of which have resulted in a significant increase in cell yield [10].

The potential therapeutic uses of stem cells in the advancing field of regenerative medicine currently under development include cell transplantation, the development of bioartificial tissues, and the induction of resident stem cell proliferation and differentiation [4]. There are, however, many safety and ethical issues associated with the use of stem cells. Uncontrolled plasticity and proliferation with the potential for teratocarcinomatous formation and epigenetic instability [11], along with immunorejection and contamination, are some of the disadvantages associated with this approach [12]. At the present time, only differentiated somatic cells are available for commercial use, including allogeneic-derived keratinocytes and fibroblasts (Apligraf for wound healing; Novartis, East Hanover, NJ), autologous-derived keratinocytes (EpiDex [MODEX Thérapeutiques SA, Lausanne, Switzerland] and Epicel [Genzyme Biosurgery, Cambridge, MA] for epidermal repair), and chondrocytes (Carticel for osteoarthritis; Genzyme Biosurgery).

**Development of cell-based products**

The development of cell- and tissue-based products is complex and requires some unique considerations that differ from traditional therapeutic approaches to wound repair involving small molecules, proteins, and antibodies. Safety assessment and characterization of the cell line under development are aspects requiring special attention. A screening donor program must be in place to minimize the potential for blood-borne pathogens, along with a system to detect and eliminate contaminants, such as latent viruses. Full characterization of cell lines by establishing identity, purity, and potency must be performed while establishing cellular proliferation and differentiation patterns, which are fundamental to understanding and minimizing oncogenic potential and establishing the correct cell phenotype. The potential for rejection of allogeneic cells by the recipient’s immune response and the use of immunosuppression therapy must also be addressed early in the development process. Finally, manufacturing and storage of the final product, by cryopreservation or other techniques that maintain cell viability and efficacy, must be in place. The final goal in the production process is to obtain a highly controlled and robust manufacturing process, meeting regulatory guidance.

Cellular-derived tissue-engineered products may consist of single cell lines (eg, chondrocytes, keratinocytes, or dopaminergic neurons) or multiple cell lines. These products consist of fully mature cellular structures, such as keratinocytes or fibroblasts within an acellular matrix. These cells are capable of cross-talk between cell lines, which is vital to...
the therapeutic response leading to the regulation of gene expression and subsequent growth factor or cytokine release. As depicted in Table 1 [23–48], both keratinocyte and fibroblast cell lines used as biologic drug delivery systems secrete a number of potentially beneficial molecules for skin repair. In addition, modified cells may be constructed by inserting particular genes to produce either a desired agent or to control the duration and level of biologic effect required to achieve a therapeutic response. For example, ex vivo modification of keratinocytes to overexpress platelet-derived growth factor (PDGF)–A has been shown to enhance healing [49], and transplants of genetically modified fibroblasts secreting brain-derived nerve growth factor in an animal model of spinal cord injury have been shown to survive, cover the lesion, and promote the ingrowth of axons [50]. Transduction levels of gene expression vary according to the vector system used. Retroviral transduction in keratinocytes, for example, has a transfection efficiency of 40% to 80% [51,52], depending on the presence of stem cells in cutaneous epithelium [53], which leads to long-term or permanent incorporation of the virus into the human chromosome(s). Alternatively, adenoviral gene transfer, which has an improved 95% transfection efficiency, maintains its episomal status, minimizing its integration into the human genome, maintaining stable gene expression for at least 2 to 6 weeks in vitro and at least 2 weeks in vivo [54–56].

### Tissue-engineered products for wound repair: a cellular approach

Tissue-engineered wound-healing products may be cellular or acellular. Acellular matrix products interact with the host by binding to the surrounding tissue, aggregating cells, and may act as a DNA delivery system by containing viral vectors or plasmids, which release growth factors that enhance repair processes. Acellular matrices have been shown to stimulate angiogenesis and modulate endogenous growth factor functions. Some examples of acellular components used in the manufacturing process include fibrin, fibronectin, hyaluronic acid, porcine tissue, bovine collagen, and decellularized cadaver dermis. Fibrin glue is often used in skin grafts and tissue-engineered skin replacement for multiple reasons, including hemostasis, which prevents bacterial proliferation in blood clots [57].

Acellular matrices may be used as DNA delivery systems. For example, type I collagen matrix, containing an adeno viral DNA vector or plasmid-encoding human PDGF-A and PDGF-B, have been found to increase granulation tissue formation, vascularization, and reepithelialization in animal models of tissue repair [58]. Poly(lactide-co-glycolide) matrix containing a plasmid encoding PDGF-BB has also been shown to enhance granulation tissue formation and neovascularization [59]. In addition, a collagen matrix containing a plasmid-encoding parathyroid hormone has been used in bone defects with positive outcomes [60]. Examples of unilaminar matrix products in this category include E-Matrix (Encelle, Inc, Raleigh, NC), a biosynthetic acellular xenograft; Oasis (Cook Biotech, Lafayette, IN), a porcine small intestinal submucosa acellular collagen matrix [46]; a human cryopreserved decellularized dermis (LifeCell Corporation, Branchburg, NJ); Integra (Integra Life Sciences Corporation, Plainsboro, NJ), a bovine collagen and chondroitin-6-sulfate with silicone; and EZ Derm (Brennan Medical, Inc, St. Paul, MN), an acellular porcine xenogeneic collagen matrix. Bilaminar matrix products, including Biobrane (Bertek Pharmaceuticals Inc., Research Triangle
Cellular approaches to wound repair

Cellular-derived products for wound healing contain living cells, such as keratinocytes and fibroblasts, within a collagen or polylactin mesh matrix. Autologous human skin, such as split-thickness skin grafts used in burn wounds, is considered the “gold standard” for the treatment of burns. However, successful outcome is limited by tissue availability and the lack of controlled clinical trials on the effectiveness of skin grafting [61]. In addition, donor-site wound complications, including delayed healing, scarring, pain, and risk of infection, are associated with autografts. Ideally, a bioengineered skin substitute should restore the functional properties of both the epidermis, a product of terminal keratinocyte differentiation, which reestablishes a barrier function, and the dermis, which allows for extracellular matrix synthesis, remodeling, and keratinocyte growth and differentiation. Autologous-derived cellular products include, among others, the use of cultured epithelial grafts through the Green technique [62], EpiDex, and Epicel. Cultured epithelial grafts provide an epithelial covering for extensive burn wounds within 2 weeks. While the cultured epithelial graft is prepared, the patient’s wounds are covered with allogeneic cryopreserved cadaver skin. Autologous keratinocytes are obtained through a biopsy specimen and cultured on a layer of feeder fibroblasts. A functional dermis is obtained after 3 to 5 years [63,64]. EpiDex consists of autologous skin cells that are derived from the outer root sheath of plucked anagen human hair follicles to produce keratinocyte sheets [65]. EpiDex is a product based on the finding that adult pluripotent stem cells are located in the bulge of hair follicles of humans and may be used to promote wound-healing processes [66]. Epicel is derived from autologous skin after biopsy that is expanded in culture for 2 to 3 weeks to obtain epidermal cells. Both Epicel and EpiDex are permanent epidermal skin grafts that provide wound coverage and promote formation of granulation tissue. Other approaches provide a dermal equivalent that increases the viability of the epidermal layer through dynamic dermal–epidermal interactions, allowing for extracellular matrix synthesis, remodeling, and keratinocyte growth and differentiation.

Allogeneic products include, among others, Apligraf, OrCel (Ortec International, New York, NY), and Dermagraft (Smith and Nephew/Advanced Tissue Sciences, London, United Kingdom). They are available without the need of a biopsy. This type of product, using living cells within a matrix to cover the wound bed, has been defined by the United States Food and Drug Administration (FDA) as an interactive wound dressing. OrCel is a bilayered, allogeneic product consisting of both dermal and epidermal layers supported within a bilaminar bovine type I porous collagen matrix. This promising technology is advantageous because it contains 2 cell types (keratinocytes and fibroblasts). OrCel is currently indicated only for the treatment of epidermolysis bullosa and skin donor site healing, whereas clinical trials are underway for possible application in chronic wounds. Dermagraft is a 3-dimensional, allogeneic, cryopreserved human fibroblast–derived dermal substitute. It is approved only for diabetic foot ulcers. It is composed of living fibroblasts derived from neonatal foreskin, extracellular matrix, and a biodegradable scaffold [67,68]. The fibroblasts contained in Dermagraft secrete growth factors including vascular endothelial growth factor (VEGF), PDGF-A, insulinlike growth factor (IGF)–1, granulocyte/macrophage colony-stimulating factor (GM-CSF), interleukin (IL)–8, IL–6, tumor necrosis factor (TNF)–α, transforming growth factor (TGF)–β1, and matrix proteins involved in the wound-healing process [25,67,69]. Currently, it is approved for diabetic foot ulcers. However, because of the limited clinical trial data available, its real clinical effectiveness needs to be elucidated.

Apligraf is a living bilayered skin construct. Similar to normal skin, it consists of an epidermis and a dermis. The epidermis of Apligraf consists of a basal and all suprabasal keratinocyte layers. Basal keratinocytes are shown to divide with a mitotic rate similar to human skin. The suprabasal epidermis displays keratinocyte morphology similar to that of normal skin, with well-defined spinous and granular layers and a cornified layer containing basket-weave keratinocytes [70]. The dermal layer contains human fibroblasts supported by an extracellular collagen matrix composed of both bovine and human collagen. It does not contain melanocytes, macrophages, lymphocytes, Langerhans cells, blood vessels, hair follicles, or sweat glands. Apligraf has FDA approval for the treatment of venous stasis ulcers and diabetic foot ulcers. In addition to chronic wounds, this product has also been used to treat a variety of acute wounds, including donor sites, surgical excisional wounds, and epidermolysis bullosa [71–74].

Although the mechanism of action of cellular bilayered skin products remains to be elucidated, it is believed that Apligraf creates a microenvironment that stimulates healing in chronic wounds. This means that it provides a physical and biological barrier against wound infection and behaves as cellular therapy through the expression of proteins that are known to be induced in response to injury and to have a function in wound healing.

Immunostaining for matrix metalloproteinases (MMPs) indicates that Apligraf keratinocytes produce MMP-2 and MMP-9, which are involved in regulating keratinocyte migration [75] and may contribute to the elimination of nonviable tissue. The presence of tissue inhibitory factors of MMP, such as tissue inhibitor of MMP-2 (TIMP-2) has also been demonstrated in Apligraf dermis, thereby suggesting...
regulatory activity between MMPs and TIMPs. Separation of the Apligraf dermis from the epidermis results in enhanced production and activation of MMP-9 by the epidermal layer, and secretion of latent and active MMP-2 by the dermal layer. Moreover, the incubation media of the separated epidermis demonstrates stronger MMP activity than intact Apligraf or its dermal component. These observations suggest that epidermal–dermal interactions suppress epidermal gelatinase activity. Because TIMPs and fibronectin are expressed in the Apligraf dermis, the presence of an MMP regulation system within the tissue-engineered product is strongly suggested [76].

One potential advantage of a product that contains fully differentiated cells is the ability to self-heal when injured. When a wound is created on the epidermal surface of Apligraf, the product achieves complete healing with reepithelialization and granulation tissue deposition 5 days after wounding. The proliferative potential of Apligraf keratinocytes is greater than that of normal adult skin, as suggested by the immunostaining of the cell proliferation marker Ki67 antibody. The increased regenerative capability observed in Apligraf keratinocytes may be related to the origin of the cells from human foreskin, which has been reported to contain keratinocyte stem cells [77]. Similar to normal skin, Apligraf epidermis showed Ki67-positive cells in the basal layer of keratinocytes; however, the intensity was significantly increased in Apligraf. This finding suggests that although skin constructs such as Apligraf may have a hist-

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Fig. 1. Potential mechanism of action during initial phase of wound healing in chronic wounds with Apligraf. bFGF = basic fibroblast growth factor; FGF = fibroblast growth factor; GM-CSF = granulocyte/monocyte colony-stimulating factor; IGF = insulinlike growth factor; IL = interleukin; MMPs = matrix metalloproteinases; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.
tologic appearance similar to normal skin, the metabolic pathways and cellular activities are highly enhanced in Apligraf.

Stimulators of extracellular matrix formation, including TGF-β and PDGF, are expressed in Apligraf. The level of expression is similar to that seen in newly formed granulation tissue.

Gene expression (messenger RNA) profiling using gene chips can be used to study the differences in gene expression in cellular-based products. This profiling may help to further elucidate Apligraf’s mechanism of action through the discovery of gene patterns and the proteins expressed, which may in turn serve as markers of both efficacy and toxicity. For example, high expression levels of keratin-14 (involved in epidermolysis bullosa simplex) and β-defensin-2 (natural antibiotic) have been recently demonstrated in Apligraf using this approach [78].

**Mechanism of action of cell-based wound-healing products**

The mechanism of action of cell-based wound-healing products in chronic wounds is poorly understood. The cells contained in the Apligraf grow and proliferate, producing growth factors, collagens, and extracellular matrix proteins, which stimulate reepithelialization, formation of granulation tissue, angiogenesis, and neutrophil and monocyte chemotaxis. Extensive clinical experience suggests that Apli-
Apligraf acts as a potent cellular therapy that can provide an adaptable response and may act differently in acute and chronic wounds. After Apligraf application in chronic wounds, outgrowth of previously dormant keratinocytes at the wound edge is observed, suggesting that Apligraf releases factors that activate keratinocytes and stimulate migration and reepithelialization [79–81]. It is believed that Apligraf stimulates wound healing in 2 phases. During the initial phase, the release of growth factors from Apligraf cells results in healing from the indolent skin (edge effect). Apligraf both covers the wound, providing a barrier, and interacts with the wound bed [82]. This interaction alters the nonhealing status of the wound to an actively healing status. There is cross-talk between the tissue-engineered product and the host tissue through an endogenous cellular response from the skin cells and other cells attracted to the wound site with the subsequent release of wound-healing–related growth factors and cytokines (Fig. 1). This type of product is not a graft; it provides a temporally dynamic relationship between the product and the underlying wound. Apligraf persists in the wound bed of a chronic wound for only a limited period (6 to 8 weeks). During this time, cells stimulate wound healing through the orderly release of important wound-healing factors.

The need for an external stimulus to initiate the healing response in chronic wounds is demonstrated by the decrease in proliferative potential and the presence of cellular senescence in fibroblasts, which are isolated within venous stasis or diabetic foot ulcers [23], whereas macrophages, present at the wound edge of chronic leg ulcers, fail to show activation markers [31]. It is believed that Apligraf helps to restore the healing environment, in part by providing this external stimulus through the release of multiple growth factors, inflammatory cytokines, and angiogenic factors. These proteins are expressed by the living cells of Apligraf, which are involved in the regulation of growth differentiation, remodeling, and extracellular matrix deposition [83,84]. Remnants of Apligraf are still detected on the actively healing wound bed during the late phase of wound healing. During this phase, a yellow exudatellite material is found at the application site. Thickened and swollen-appearing Apligraf dermis has been observed in the residual tissue after Apligraf application. The exudatellite material is composed of mucin, which is observed in both patient and Apligraf dermis, indicating production of ground substance [85] (Fig. 2).

**Conclusion**

Tissue and cellular approaches to wound repair are part of a rapidly evolving field. Although the mechanism of action of cell-based products is not fully understood, the use of therapies containing living cells has proved valuable in the treatment of chronic wounds.

References


