Enhancing Tissue-Engineered Skin by Genetic Modification

Chronic ulcers of the skin are a significant medical problem in need of new and more effective treatments. Each year in the United States, an estimated 800,000 diabetic ulcers occur, which lead to about 80,000 amputations, and of the 1 million venous ulcers, only 40 to 50% are healed by standard therapies.

Some of the first efforts in tissue engineering focused on developing skin replacements or bioengineered skin to treat large-surface-area burns and chronic ulcers of the skin. In fact, several of the pioneering research efforts have moved successfully from bench to bedside and shown some success in the clinic. Presently, several tissue-engineered products are available commercially for the treatment of skin defects, yet the medical problem is still significant.

Central to the production of living bioengineered skin is the ability to culture large numbers of human epidermal keratinocytes as well as dermal fibroblasts. For keratinocytes, it has been estimated that a 1-cm^2 biopsy can produce approximately 1 m^2 of skin within about 25 days of culture, and with continued serial culture, it is possible to produce over 1000 m^2 of skin from that same biopsy.

Discarded human foreskin tissue is a ready source of allogeneic cells that are used for the large-scale production of bioengineered skin. Rather than close a chronic ulcer as a permanent graft, the allogeneic bioengineered skin is used as a temporary graft, which is believed to promote healing of the ulcer by the local secretion of factors that stimulate the host's own wound-healing response. Often, repeated applications of the allogeneic bioengineered skin are needed to close the wound.

Our laboratory has been exploring the possibility of enhancing the function, and thus performance, of bioengineered skin by genetically modifying the skin cells. This combined approach of gene therapy and tissue engineering has several distinct challenges. Unlike gene therapy for inherited diseases, in which the gene to be delivered is usually known, it is unclear which gene or gene product has significant effects on the performance of bioengineered skin.

Because one theory holds that normal bioengineered skin promotes healing of ulcers by the local synthesis of wound-healing growth factors, interest has focused on the use of genetic modification to program cells to secrete higher levels of growth factors. However, there is a very long list of wound-healing growth factors synthesized and secreted by a myriad of cells involved in the healing of skin, including platelets, macrophages, neutrophils, endothelial cells, keratinocytes, and fibroblasts.

Moreover, the extent and duration of the synthesis of these factors varies significantly as a wound progresses through the inflammatory, proliferative, and remodeling phases of healing. And lastly, although most activities of growth factors have been well characterized in vitro, their precise function in vivo and their role in the healing events during the engraftment of living bioengineered skin are largely unknown. Thus, it is nearly impossible to predict from our current knowledge which growth factor or factors may be rate-limiting in the wound-healing process.

Prior work from our laboratory as well as others has produced genetically modified bioengineered skin that secretes high levels of several wound-healing growth factors, including insulin-like growth factor-1, platelet-derived growth factor A and B chain, vascular endothelial growth factor, hepatocyte growth factor, and epidermal growth factor. These studies have shown the ease with which bioengineered skin can be genetically manipulated and have indicated that growth factor overproduction can influence at least one facet of the performance of bioengineered skin.

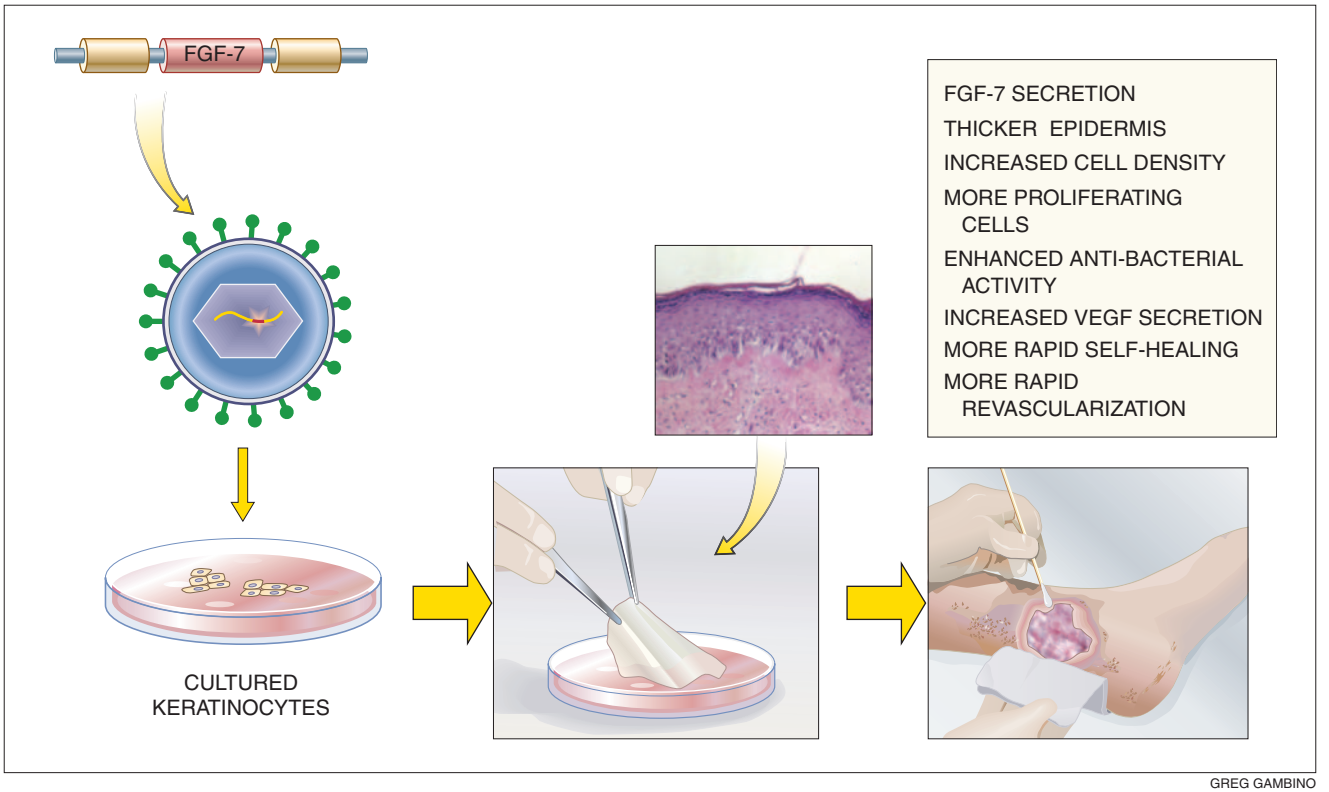
In a recent report, we showed that the genetic modification of the keratinocytes of bioengineered skin to produce fibroblast growth factor-7 (FGF-7) had a profound effect on multiple functions of bioengineered skin and dramatically enhanced its performance in several key areas of wound healing.

FGF-7, also known as keratinocyte growth factor, is a member of the family of structurally related growth factors known as fibroblast growth factors (FGFs). Most FGFs stimulate the proliferation of a broad range of cells of mesodermal, ectodermal, and endodermal origin; however, FGF-7 is highly specific for epithelial cells, including epidermal keratinocytes.

FGF-7 is a small polypeptide of molecular weight 26 to 28 that is normally produced by dermal fibroblasts, but not by epidermal keratinocytes. However, keratino-

"Tissue Engineering" is edited by Jeffrey R. Morgan of the Division of Biology and Medicine, Brown University, and Martin L. Yarmush of the Center for Engineering in Medicine, Massachusetts General Hospital and Harvard Medical School.

Jeffrey_Morgan@Brown.edu



GREG GAMBINO

To produce gene-modified bioengineered skin, a recombinant retrovirus encoding FGF-7 is used to genetically modify cultured allogeneic human keratinocytes. Modified cells are seeded on an analog of the dermis to produce a bioengineered skin with enhanced properties useful for the treatment of chronic ulcers of the skin.

cytes express FGFR2-IIIb, the only known high-affinity receptor for FGF-7, and FGF-7 is known to stimulate the proliferation and migration of keratinocytes in vitro. Interestingly, in normal wound healing, expression of FGF-7 by dermal fibroblasts is greatly upregulated, suggesting that FGF-7 has an important role in wound healing.

Using a recombinant retrovirus, we genetically modified cultured human keratinocytes to express and secrete FGF-7, a growth factor they normally do not synthesize. To form a bioengineered skin, we seeded the gene-modified cells onto the papillary surface of acellular human dermis, a complex matrix of collagen, elastin, and other extracellular matrix proteins derived from normal skin. The cell-seeded construct was raised to the air/liquid interface for 7 days to stimulate the keratinocytes to form a stratified and differentiated epidermis.

When compared to the control epidermis formed by unmodified cells, the epidermis formed by the cells expressing FGF-7 had several notable changes: First, the FGF-7 transgenic epidermis was thicker than control epidermis and had a higher density of small keratinocytes along the basement membrane as well as a 4- to 5-fold increase in the total number of cells per cm^2 of skin. Thus, the keratinocytes in the FGF-7-expressing epidermis were more densely packed.

Second, when stained for proliferating cells, the epidermis expressing FGF-7 had a dramatic increase in proliferating cells on the basal layer and numerous proliferating cells in the suprabasal layer, a hallmark of hyperproliferation.

Third, integrin expression was upregulated, and the integrins were preferentially localized to the basement membrane, suggesting increased adhesion. Finally, when stained for markers of differentiation, the epidermis expressing FGF-7 showed a delay in differentiation consistent with the increase in the proportion of proliferating cells.

None of these changes altered the ability of the FGF-7 epidermis to form granular or cornified layers,

the two uppermost differentiated layers of the epidermal barrier, and barrier function was comparable between control and FGF-7 epidermis.

The thicker and more proliferative FGF-7 epidermis resulted in several other significant changes. Normal keratinocytes synthesize and secrete numerous trophic factors involved in wound healing, and one critical factor is vascular endothelial growth factor (VEGF). VEGF is a potent mitogen for endothelial cells, and its secretion by keratinocytes is thought to help promote angiogenesis and vascularization in the underlying dermis.

When assayed for levels of secreted VEGF, FGF-7-expressing bioengineered skin produced five times more than control skin, presumably because its epidermis was thicker and had 4 to 5 more cells per cm^2. Another change attributable to the thicker, more proliferative epidermis was the increased speed with which the FGF-7-expressing epidermis healed itself. After wounding with a biopsy punch, the FGF-7-bioengineered skin self-healed about 2 days faster than control bioengineered skin.

One surprising change due to FGF-7 expression was an increase in the antibacterial properties of the bioengineered skin. Previously, we had shown that bioengineered skin can mount an active antibacterial response if stimulated with selected cytokines such as interleukins-1 and -6, well-known upregulators of the innate immune response.

To determine if FGF-7 expression altered the antimicrobial properties of bioengineered skin, we inoculated control and FGF-7-expressing skin with *Escherichia coli*, *Staphylococcus aureus*, or *Pseudomonas aeruginosa*. After 24 hours of incubation, the bacterial load of both gram-negative and gram-positive bacteria was 100 to 1000 times less in skin expressing FGF-7 compared to control skin.

Finally, the FGF-7 bioengineered skin was tested in vivo by transplantation to athymic mice. The FGF-7 skin formed a stratified and differentiated epidermis that was thicker and significantly more proliferative than control bioengineered skin. Of special importance was the observation that the acellular dermis of the FGF-7 bioengineered skin became revascularized significantly faster than control skin, presumably due to the increased levels of VEGF and other trophic factors secreted by the high density of keratinocytes in the FGF-7 epidermis.

Thus, genetic modification resulting in FGF-7 expression produced profound changes to the structure and function of tissue-engineered skin, and many changes resembled the activated state seen in the epidermis of healing wounds. Bioengineered skin with a thick, hyperproliferative epidermis with a high cell density had enhanced self-healing and upregulated antibacterial activity, and because it is more rapidly revascularized, it may be more effective at closing the wound and initiating the healing process of a chronic ulcer. Stable genetic modification using a recombinant retrovirus will facilitate the large-scale production of gene-modified bioengineered skin for clinical evaluation.

JEFFREY R. MORGAN

Center for Biomedical Engineering
Brown University
Providence, RI

Jeffrey_Morgan@Brown.edu

REFERENCE

Erdag G, Medalie DA, Rakhorst H, et al: FGF-7 expression enhances the performance of bioengineered skin. *Molecular Therapy* 10:76-85, July 2004.