At Organogenesis, we have created a skin construct, Apligraf, that is unique in that it is made up of the two layers that constitute human skin, the dermis (inner layer) and the epidermis (outer layer). In May 1998 Apligraf was approved as a biomedical device by the U.S. Food and Drug Administration. It became the first device containing living human cells to win such approval.

During the development of Apligraf, we at Organogenesis had to decide whether to attempt to win regulatory approval for products that were, in effect, precursors to Apligraf—either dermis or epidermis by itself—or to trust that we could develop our product before other companies beat us to it. We bet on two-layered skin because it was closer to true skin, and grafts of true skin clearly worked. In addition, the dermal substrate would enhance the epidermal layer’s survival. Our gamble paid off.

The idea for Apligraf dates back almost two decades. While at the Massachusetts Institute of Technology, Eugene Bell noted that fibroblasts, the cells that form the dermis, could infiltrate a collagen gel and turn it into a fibrous, living matrix. Collagen is a fundamental part of the extracellular matrix, the biological “glue” that holds cells in place. In 1981 he found that keratinocytes, the cells of the epidermal layer, would grow on that dermal substrate, forming a primitive skin equivalent. He also determined that the construct could be grafted onto rats. Organogenesis was founded in 1985 to commercialize Bell’s technology. I brought my background in keratinocyte biology to the company in 1986.

We were confident that artificial, bilayered skin would have clinical benefits. A temporary skin substitute made of collagen and another extracellular matrix constituent was created by John F. Burke, then at Shriners’ Hospital in Boston, and Ioannis V. Yannas of M.I.T. It had helped burn patients in clinical trials by preventing water loss and promoting dermal healing. In addition, Howard Green of Harvard Medical School had devised a method for growing sheets of epidermal cells for burn patients.

An initial obstacle to developing Apligraf was obtaining a supply of collagen to support the growth of the cells. Suppliers could not guarantee us a sufficiently pure form of collagen with the correct properties. To overcome this, Paul Kemp of our company and his colleagues developed a way to derive collagen from bovine tendons. They also came up with a cold chemical sterilization technique that destroyed any contaminants without disrupting the collagen.

My colleagues and I then set out to find the culture conditions that would provide the optimum number of living human keratinocytes. At the time, however, all known methods for culturing keratinocytes were covered by patents held by other companies, and some aspects of those techniques were undesirable for our purposes. Accordingly, we set out to develop our own, unique keratinocyte culture systems. In doing so, we gained a deeper understanding of keratinocyte growth that helped us develop our subsequent production procedures.

We looked to newborn human foreskins collected from circumcisions as a source for fibroblasts and
keratinocytes because of those cells’ tremendous proliferative potential and their ready supply. We had learned how to grow a dermal layer and to seed the top with the epidermal cells, but a great challenge was maintaining that two-sidedness. Real skin migrates to cover wounds, and, unchecked, an epidermal layer will simply continue to grow around anything, forming a cyst.

We found a solution to this problem serendipitously. One day Kemp cast a collagen lattice into a transwell, a small cup on a plate of many such cups that is used to grow cell cultures. The bottoms of transwell cups are porous, and the sides usually carry slight electrical charges, which impels cells to stick to them. But the plate Kemp used was old and had lost its charge, so the collagen stuck only to the porous bottom. It pulled down and away from the sides of the cup, forming an almost level top rather than the usual curved shape. This particular conformation turned out to be perfect for supporting the growth of a controlled layer of epidermis on top of the collagen-fed dermal layer.

At this point, we could have developed either layer separately and attempted to win FDA approval. But we decided to risk the wait and go for bilayered skin. From 1990 to 1992 we marketed a version of our product that was used as an alternative to animals in toxicological and pharmacological studies. At that point, Michael Sabolinski of our company set out to determine the most appropriate first clinical application of our technology so we could design a clinical trial that stood the best chance of passing muster with the FDA. Apligraf is a device, but because it is alive it also has biologic activity. We therefore worked with FDA officials to determine the standards of approval, safety testing and manufacturing by which we would be judged.

We chose venous ulcers, skin lesions resulting from leaky veins caused by faulty valves in the leg, as Apligraf’s test wound. In our trial, Apligraf revealed multiple mechanisms of action: it worked as a simple graft in some cases; in others, it directly stimulated wound repair through its own natural contingent of growth factors and other proteins. The most difficult ulcers, those that had existed for at least a year, showed the most striking healing. After 24 weeks, 47 percent of the hardest-to-heal wounds were completely closed with Apligraf, compared with only 19 percent with conventional therapy, which consists basically of applying pressure and keeping the wound moist. These results convinced the FDA to approve Apligraf for this use.

Apligraf is now commercially available in the U.S. and Canada, marketed by Novartis Pharmaceuticals. It is delivered “fresh” and has a five-day shelf life at room temperature. Studies in patients with burns or diabetic ulcers and in those undergoing dermatological surgery are either completed, near complete or under way.

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product is also frozen, for easy shipment and storage, but in a manner that leaves the cells alive. Following cryopreservation, the product is shipped and stored at –70 degrees Celsius (–94 degrees Fahrenheit); it is thawed before use and cut to the exact shape and size of the wound.

Dermagraft’s odyssey through the regulatory process thus far has been both instructive and, at times, frustrating. At the start, no cookbook for tissue manufacturing existed, and little was known about cryopreservation. We developed production procedures and learned much about the effects of freezing on tissue function. During our pivotal trial for the treatment of hard-to-heal diabetic foot ulcers, we learned that 50 percent of the cells in Dermagraft need to survive freezing for the product to function optimally. Fifteen percent of diabetics develop these ulcers, as their prematurely aging cells fail to produce normal collagens and matrix proteins.

Those who received Dermagraft with at least 50 percent living cells improved greatly; 50.8 percent healed in 12 weeks. In contrast, the ulcers of only 31.7 percent of patients treated using conventional methods healed during the same time frame. Patients who received low-activity Dermagraft, with too few live cells, did no better than controls.

A supplemental, uncontrolled trial of the active version of Dermagraft again showed excellent healing, confirming the importance of a specific number of live cells in the implant. Based on these data, a panel of outside experts convened by the FDA recommended in January 1998 that Dermagraft be approved for the treatment of diabetic foot ulcers, contingent on an additional clinical trial after the product was released. The FDA ordinarily agrees with such panel recommendations. In this case, however, the FDA asked that the additional trial take place before approval.

We have since embarked on a fully controlled 30-center trial of the metabolically active version of Dermagraft and expect FDA approval at its successful conclusion. The new world of tissue-engineered products presents the FDA with unique challenges. In parts of Europe, our products are considered pharmaceuticals. In the U.S., they are devices. Regulations that cover all circumstances simply have not yet been fully defined for devices that have pharmacological activity, such as Dermagraft. We thus understand the FDA’s conservatism in this area. In the meantime, the agency has recognized Dermagraft’s value by granting it an Investigational Device Exemption, or IDE. This exemption basically allows Dermagraft to be available while it is still wending its way through the regulatory process.

Following approval for diabetic foot ulcers, Dermagraft should find roles in the treatment of venous ulcers, pressure ulcers (bedsores) and other chronic wounds. Knowledge gained from this enterprise has helped us create a “recipe” for frozen tissues with long shelf lives. That knowledge is being incorporated into other products in development, such as cartilage and blood vessels.

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