Skin substitute from stem cells

Tracey Baas, Associate Editor

Researchers at the **Institute for Stem Cell Therapy and Exploration of Monogenic Diseases** have generated epidermis from human embryonic stem cells that potentially could be used as a temporary skin substitute for burn victims waiting for grafts.¹ Safety issues such as tissue compatibility remain to be resolved, and manufacturing may be a challenge.

Improved temporary skin options for burn victims remain an unmet need. Standard treatment includes the use of cadaver skin, which is in short supply, or de-epidermized pig skin. Both treatments are ultimately rejected by the burn victim's immune system and thus only serve as biological wound dressings. Alternatives in development include inert, synthetic and biosynthetic matrices, but these substitutes may contain adult allogeneic (nonself) skin cells and bovine collagen. Both may cause graft rejection and could contain contaminants.

The Institute for Stem Cell Therapy and Exploration of Monogenic Diseases (I-STEM) team hypothesized that stem cell-produced skin could overcome supply and tolerability issues. Stem cells can be expanded to provide an unlimited resource for temporary skin, and stem cell-produced keratinocytes, in their early developmental stage, should be well tolerated by the recipient's immune system.

Using human embryonic stem cells (hESC) from two cell lines, Christine Baldeschi, associate professor and head of the genodermatosis team at I-STEM, and colleagues produced keratinocytes that formed an epidermal layer *in vitro*. They grafted the layer onto immunodeficient mice and observed the reconstitution of epidermis, which indicated that the cells were functional. After 12 weeks, the layer resembled the multilayered, differentiated epidermis of mature human skin.

The work was published in *The Lancet*.

Despite the success in a small animal model, Yves Poumay, professor of cell biology and histology at the **University of Namur**, told *SciBX* that treating bigger burn surfaces will require "a terribly large number of cells differentiated into keratinocytes. From a practical point of view, I wonder whether this very large number can be produced."

In 2004, Poumay and colleagues were the first to reconstruct human epidermis *in vitro* using cultured adult human keratinocytes.²

Norio Nakatsuji, director of the Institute for Integrated Cell-Material Sciences at **Kyoto University**, had a more positive view. He thinks that a ready-to-use, quality-controlled, temporary skin substitute would likely be faster and less expensive to produce than custom-made, patient-specific tissue products.

An imperfect match

Baldeschi's next step is to see exactly how long the stem cell-derived skin can provide benefit. The focal point will therefore be long-term immunogenicity, namely whether the major histocompatibility complex (MHC) antigen status of the hESC-derived epidermis is low enough to stay off the immune system's radar.

During the 12-week study, hESC-derived keratinocytes expressed little or no MHC antigens, which allow the immune system to distinguish self from nonself.

Baldeschi said those results suggest that the skin substitute would have low immunogenicity and should be well tolerated as a graft. The real test, however, will be what happens to the immune status in the longer term—whether MHC antigen expression increases as the graft matures.

In parallel with the work on stem cells, Baldeschi said her group will also use adult cells, which can be selected for MHC antigens that are likely to match patients, induced to pluripotency and then differentiated into keratinocytes.

Another way to avoid potential MHC immunogenicity would be to use banks of cell lines preselected to represent a majority of all possible MHC antigen combinations in a population. A cell line with the best-matching MHC antigen profiles for a specific patient, and therefore carrying a minimal risk of incompatibility with the recipient's MHC profile, could be induced to pluripotency for keratinocyte differentiation for implantation.

In 2008, a correspondence by Nakatsuji in *Nature Biotechnology* suggested that 50 such cell lines could generate skin for 9 out of 10 people in Japan,³ whereas a 2005 paper in *The Lancet* reported that 10 of the cell lines would be sufficient for 1 out of every 2 people in the U.K.⁴

Regardless of where the stem cell lines come from and whether they are embryonic stem cells or induced pluripotent stem cells, Lee Ann Laurent-Applegate thinks the I-STEM team will face a host of GMP issues.

Stem cells are an "utmost challenge because they need multiple growth factors to differentiate and feeder layers for cell growth, which tends to cause problems for consistency in the cell populations. These cells, once placed *in vivo*, would have a higher risk to dedifferentiate or become other tissue with the worst-case scenario being a tumor," said Laurent-Applegate, who is director of the cellular therapy unit at the **University Hospital of Lausanne** and a professor of musculoskeletal medicine.

Laurent-Applegate and colleagues are developing temporary skin coverings from fetal skin cells that stimulate production of the burn patient's own skin cells without the need for a secondary graft.⁵⁻⁷ The temporary covering can be prepared from off-the-shelf frozen vials of fetal skin cells, and the burns heal within about 14 days.

There is at least one marketed treatment for burn victims that does not require laboratory facilities—**Avita Medical Ltd.**'s ReCell device, which produces spray-on skin. The process involves tak-

ing a thin, 2 cm by 2 cm biopsy from a skin area that matches that of the burn. The biopsy is treated with an enzyme that promotes cell separation and allows the basal skin cells to be harvested and suspended in a solution. The patient's own cells are then sprayed over the wound, where they migrate and proliferate to form a new epithelium. The procedure is performed in the operating room and takes about an hour.

The product is approved in Europe, Australia, Canada, Mexico and China. John Geisel, SVP of business development, said Avita plans to begin clinical studies in the U.S. at the start of next year.

According to Baldeschi, I-STEM has filed a patent application covering the work published in *The Lancet*. The IP is available for licensing from I-STEM, which is a joint research unit of **Institut National de la Santé et de la Recherche Médicale** (INSERM) and the **University of Evry Val-d'Essonne**.

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REFERENCES

1. Guenou, H. *et al. Lancet*; published online Nov. 21, 2009; doi:10.1016/S0140-6736(09)61496-3
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2. Poumay, Y. *et al. Arch. Dermatol. Res.* **296**, 203–211 (2004)
3. Nakatsuji, N. *et al. Nat. Biotechnol.* **26**, 739–740 (2008)
4. Taylor, C. *et al. Lancet* **366**, 2019–2025 (2005)
5. Hohlfield, J. *et al. Lancet* **366**, 840–842 (2005)
6. Applegate, L. *et al. Skin Pharmacol. Physiol.* **22**, 63–73 (2009)
7. Ramelet, A. *et al. Exp. Gerontol.* **44**, 208–218 (2009)

COMPANIES AND INSTITUTIONS MENTIONED

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