**Abstract**

It remains a challenge to preserve stem and progenitor cells during *ex vivo* expansion of epidermal keratinocytes under serum-free and feeder-cell-free culture condition. This limitation greatly hinders the development of advanced autologous cell and gene therapeutics for inherited skin diseases such as epidermolysis bullosa and injuries such as severe burns. We have developed a serum-free and feeder-cell-free culture technology (EpiX™) that allows rapid generation of more than one-trillion epidermal keratinocytes while retaining the stem and progenitor cell population. In-depth whole genome sequencing and *in vivo* tumorigenicity studies demonstrated that the EpiX™-expanded cells maintain genetic stability and do not form tumors. The preservation of stem cell character is evidenced by repeated single cell cloning capability to enrich genetic engineered cells via CRISPR/Cas9-mediated gene knock-in into the AAVS1 safe harbor locus. EpiX™-expanded keratinocytes maintain a basal cell phenotype during *ex vivo* expansion and readily differentiate into stratified epidermis in organotypic culture on the air-liquid interface. When grafted into immunocompromised mice, human keratinocytes survived over several months *in vivo* and seamlessly integrated with wounded mouse skin. An improved manufacturing process allows us to make suturable clinical-sized (75 cm²) skin graft sheets with mesenchymal cell-populated dermis and stratified epidermis layers, thereby enabling the development of a range of gene engineered cellular therapeutics for diseases and injuries of the skin.

**Development of 75 cm² skin substitute sheets using EpiX™-expanded keratinocytes**

EpiX™-expanded keratinocytes were used in manufacturing suturable engineered skin substitutes in a clinically-relevant size, i.e. ~75 cm². The engineered skin substitutes had enough tensile strength to be handled easily.

**Workflow of producing skin substitutes for severe burn injuries**

- 200 µL viral keratinocytes from a 200x10⁶ Input
- Obtain ~500x10⁶ highly proliferative keratinocytes within 14 days.
- 31 st of engineered skin substitue ready for implantation within 25 days.

**Summary**

EpiX™ stem cell expansion technology allows for a trillion-fold expansion of primary epidermal keratinocytes in a short timeframe. During this rapid expansion phase, the cells remain genetically stable and do not become transformed *in vitro* or form tumors *in vivo*. EpiX™ technology enables genetic engineering and clone selection. After expansion, if the cells are placed into air-liquid interface culture conditions, they rapidly differentiate into a stratified epidermis structure *in vitro* that resembles the architecture of normal healthy skin. The cells can also generate a well-differentiated multilayer epithelium in a mouse wound healing model *in vivo*. Using keratinocytes expanded with EpiX™ technology, we have developed a process for making an engineered skin construct of sufficient size and mechanical strength to enable the manufacture of suturable skin substitutes. We believe these advances in creating a tissue engineered skin substitute will address multiple unmet medical needs including wound healing and curative treatments for inherited skin diseases such as Epidermolysis Bullosa.

**EpiX™ expansion technology enables ex vivo tissue engineering of skin using autologous patient-derived cells for regenerative medicine applications**

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