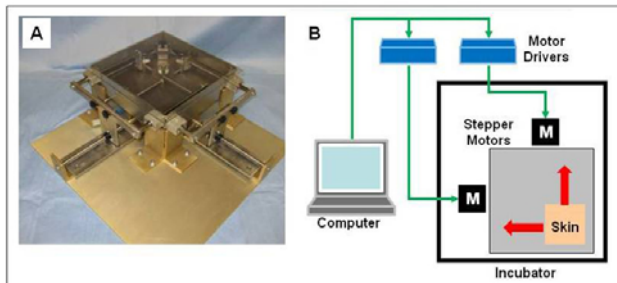


## In Vitro Expanded Living Skin for Burn Injuries

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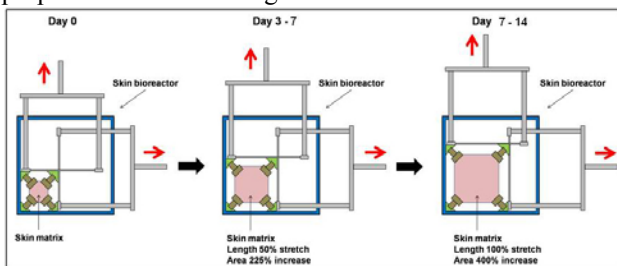
**Statement of Purpose:** *In vivo* tissue expanders have been used clinically to generate larger autologous skin. However, this method requires an additional surgical procedure for expander implantation and a long waiting time to obtain sufficient tissue for reconstruction. We previously showed that skin can be expanded rapidly *in vitro* by a controlled uniaxial strain for the treatment of burn [1]. However, this system is limited by the unidirectional expansion of skin. In this study, we have designed a biaxial skin expander system that provides the full potential to yield uniformly distributed expansion capability. We tested the feasibility of using the biaxial expansion system with porcine skin.

**Methods:** The new design of expander system consisted of a biaxial bioreactor in which the skin is clamped by its four corners and strained using two stepper motors (23L-102S-LW8, Anaheim Automation, Anaheim, CA) controlled by SMC60WIN software (Anaheim Automation) (Figure 1). Split thickness skin grafts from pigs were maintained under tissue culture conditions and expanded over a 4-12 day period. The expansion rate was determined by the initial area, setting an overall expansion goal in a set time frame, and calculating the amount needed to expand daily to reach the expansion goal. Digital imaging was obtained daily for area calculation.



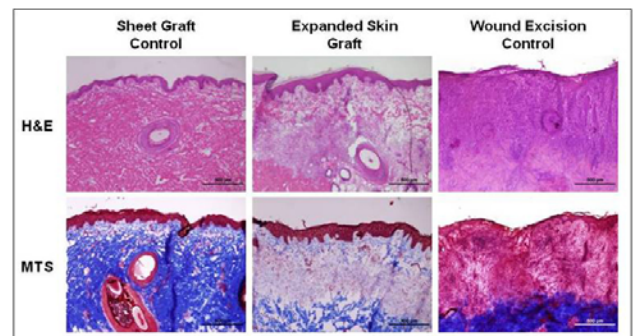
**Figure 1.** (A) Photograph of the biaxial skin bioreactor attached to a chassis. (B) Diagram of the entire system expander system which include a computer, controllers, motors, and bioreactor.

Fig. 2 shows a schematic diagram of a bioreactor for skin expansion and target percentage of expansion. Ideally, a skin sample can be theoretically expanded 400% of total area within 2 weeks in the bioreactor. It depends on the properties of initial skin graft.



**Figure 2.** Schematic diagram of a bioreactor for skin expansion.

**Results:** To investigate the feasibility of using the bioreactor expanded skin grafts for clinical application, a  $4 \times 4$  cm<sup>2</sup> partial thickness skin graft was taken from a pig at 0.0125 inches using a Padgett Electric Dermatome. The porcine skin was placed in a biaxial bioreactor system that incrementally exerted physical tensile stress. The bioreactor is filled with media to supply nutrients to the skin tissue for maintaining tissue viability. After 4 days of bioreactor application, the surface area of the expanded skin increased approximately 60%. The transplanted skin grafts were retrieved 6 days after grafting and embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E), Masson's trichrome (MTC), and immunohistochemistry for proliferating cell nucleus antigen (PCNA). Histological images showed that the epidermis in the expanded skin and normal skin grafts fully covered the excision sites and maintained typical dermal architecture (Figure 3).



**Figure 3.** Histological analyses of the skin grafts after 6 day-postoperation; (Left column) normal skin autograft, (Middle) expanded skin graft, and (Right) wound excision only.

**Conclusions:** We demonstrate that the biaxial bioreactor system is capable of controlled expansion of skin grafts and that optimal expansion can be achieved through a large initial expansion in the bioreactor, followed by smaller incremental increases over a period of days, although further optimization is needed.

### References:

[1] Ladd MR et al. Tissue Eng A. 2009;15:861-868.

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