In Vitro Expanded Living Skin Matrices for Reconstructive Procedures

Mitchell R. Ladd, Sang Jin Lee, Anthony Atala, James J. Yoo.
Wake Forest Institute for Regenerative Medicine, VT/WFU School of Biomedical Engineering and Sciences

Statement of Purpose: Numerous reconstructive procedures result in wounds that require skin grafting. However, the amount of autologous tissue available from donor sites is often limited and large wounds remain difficult to manage [1]. *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. Moreover, discomfort associated with the increasing expander volume and frequent tissue fibrosis are among the limitations [2]. In this study we investigated whether these limitations could be solved by increasing the surface area of skin tissue *in vitro* while maintaining tissue viability.

Methods: Human foreskin was incrementally expanded over 6 days to increase its surface dimensions in a computer-controlled bioreactor system under tissue culture conditions. Morphological, structural and mechanical properties of the foreskin were evaluated before and after expansion using histology, scanning electron microscopy (SEM), and tensile testing. Stains performed were hematoxylin and eosin (H&E), Masson's Trichrome, and immunohistochemistry for proliferating cell nuclear antigen (PCNA). Samples for SEM were fixed in 2.5% glutaraldehyde, dehydrated via freeze drying, and then sputter coated with gold. Tensile testing was performed at a strain rate of 10.6 mm/min[3] as cited in the literature.

Results: The surface area of the expanded skin matrices was increased by 83%.

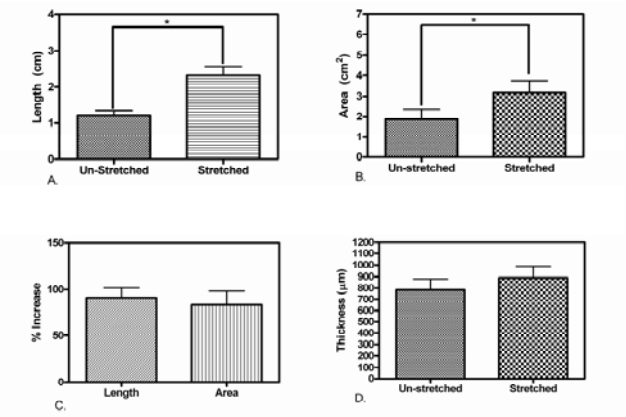

Figure 1. Dimensional changes of expanded skin matrices. **A.** Length, **B.** Area, **C.** % Increase of length and area from initial, **D.** Thickness.

Histological evaluation displayed the maintenance of cell viability and proliferative potential. Histomorphological and ultrastructural analyses showed that dermal structural integrity was preserved. Young's modulus and ultimate tensile strength were increased in expanded skin matrices due to collagen fiber alignment. Strain at failure was decreased after expansion but no significant difference was found. Despite these changes, the mechanical properties of the skin tissue after expansion were adequate in terms of Young's modulus, ultimate tensile strength, and strain at failure.

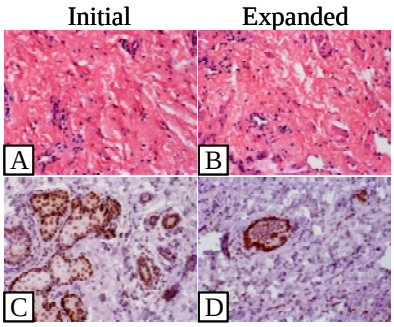

Figure 2. Histological evaluation. **A, B.** H&E staining of initial and expanded skin matrices respectively, **C, D.** immunohistochemical staining for PCNA of initial and expanded skin matrices respectively. (Magnification 200X)

Conclusions: These findings show that expansion of living skin matrices can be achieved using a bioreactor system. This technique provides an opportunity to generate large amounts of skin for reconstructive procedures and may overcome the current limitations of the *in vivo* tissue expander system.

References:

1. Atiyeh, BS et. al. *New technologies for burn wound closure and healing-review of the literature*. Burns. 2005. **31(**8): p. 944-956.
2. LoGiudice J, Gosain AK. Pediatric tissue expansion: indications and complications. J Craniofac Surg. 2003 Nov;14(6):866-72.
3. Edsberg, LE et. al. *Mechanical characteristics of human skin subjected to static versus cyclic normal pressures*. J Rehabil Res Dev. 1999. **36**(2): p. 133-141.