Fabrication of Cellular Pre-vascularized Tissue Constructs from Autogenous Tissue


Abstract

Background: Although the use of decellularization techniques has made available acellular dermal matrices (ADMs) that may obviate the patient the donor site morbidity generally associated with reconstructive surgery, these resultant ADMs are avascular tissues, and as such they are dependent on neovascularization for their survival and incorporation. In order to overcome this problem, we sought to develop a bioengineered matrix with a dominant vascular pedicle that would serve as a platform for decellularization, re-seeding and ultimately direct donor-recipient anastomosis. This technique will then allow for immediate perfusion of our bioengineered replacement tissue upon implantation onto a suitable host, minimizing ischemia time and enhancing survival.

Methods: Fasciocutaneous flaps supplied by the superficial inferior epigastric artery (SIEA) were harvested from Sprague-Dawley rats. The femoral vessels were cannulated with a 25G cannula and a decellularization protocol was initiated via sequential perfusion with 4% sodium deoxycholate for 12 hours and DNAse I for 12 hours via peristaltic pump. Following decellularization, 6mm discs were obtained and equilibrated in standard culture medium (Dulbecco’s Modified Eagle Medium supplemented with fetal bovine serum and penicillin/streptomycin) for 48 hours. These acellular scaffolds were then topically seeded with 2x10^5 RFP-expressing Human Dermal Fibroblasts (HDFn) with media changes were performed daily. At 1, 3 and 7 days the scaffolds were fixed and processed for histology. Unseeded scaffolds served as the control.

Results: Histological analysis with H&E staining of unseeded scaffolds confirmed successful decellularization and removal of all cellular material. Cells were observed along the surface of seeded scaffolds and not on the unseeded controls. These cells were confirmed as HDFn via light and fluorescent microscopy as evidenced by fibroblast specific protein (fsp1) and RFP expression of cells along surface of the scaffold. Evidence of cellular invasion was evident at day 7 of culture, as cells were observed lining depilated hair follicles.

Conclusion: We have established a successful method for perfusion-based decellularization of a fasciocutaneous flap with preservation of the inherent micro and macrovasculature. These data demonstrate that biological scaffolds derived from fasciocutaneous tissues can support the adhesion and proliferation of vital dermal components, taking us one step closer in the development of an off-the-shelf replacement tissue construct.

Disclosure/Financial Support
None

None of the authors have any financial interest with any of the products, drugs or devices mentioned in this manuscript.