A Bilayer Construct Controls Adipose Derived Stem Cell Differentiation

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Statement of purpose: Thermal injury accounts for approximately 5% of combat casualties and continue to be a significant source of morbidity. Combat burn injuries are often full-thickness burns, involving large total body surface areas (TBSA) of skin and are often compounded by multiple injuries. While a wide variety of dermal matrices have been developed for the treatment of burns injuries, the lack of a host cell source and the time involved for cell expansion have limited their clinical application. We hypothesize that autologous adipose derived stem cells (ASC) can be used to produce a clinically relevant tissue engineered dermal equivalent. ASCs possess a heterogeneous cell population with the potential to differentiate into endothelial and stromal cell lineages. In this study we describe the matrix-directed differentiation of ASCs into vascular and fibroblast lineages which can be used to develop tissue engineered wound healing treatments.

Methods: Our bilayer dermal equivalent comprises rat ASCs "sandwiched" in a bilayer hydrogel one layer of which is composed of rat type I collagen hydrogel and the other layer is composed of PEGylated fibrin.[1] Rat adipose mesenchymal stem cells (ASC) were isolated from rat adipose tissue using previous protocols. ASCs were seeded onto chitosan microspheres (CSM) which were prepared by water-in-oil (w/o) emulsification process along with an ionic coacervation technique. The PEGylated fibrin was prepared using BTC-PEG-BTC (benztriazole modified polyethylene glycol, 3400 Da, Sunbio, Inc.) which was added to fibrinogen (20 mg/ml in TBS pH 7.8, Sigma) at a molar ratio of 10:1. An equal volume solution of thrombin (200 U/ml in 40 mM $CaCl_2$, Sigma) was added to cause gelation. Collagen type I gels (Sigma) were formed using standard protocols. ASC-loaded chitosan microspheres were suspended in culture media and seeded onto the surface of the collagen gel prior to gelation of the PEGylated fibrin. Culture media was then removed and the PEGylated fibrin gel was then formed over the surface of the cells.

Results/Discussion: Adipose derived stem cells differentiated into two different morphologies depending on in the matrix layer. The differentiated ASC demonstrate a spindle-shaped fibroblast-like morphology when migrating in the collagen. (Figure 1A&B) A vasculature matrix mimic was formed as a result of modifying fibrinogen with a PEG derivative. (Figure 2A&B) We found by 7 days, ASC migrating into the PEGylated fibrin have formed vascular tube-like networks in the matrix in the absence of additional soluble cytokines. Similar to microvessels *in vivo*, the ASCs in the PEG-fibrin matrix express both endothelial (e.g. vWF, CD31) and pericyte (e.g. NG2, SMA) specific markers. (Figure 2)

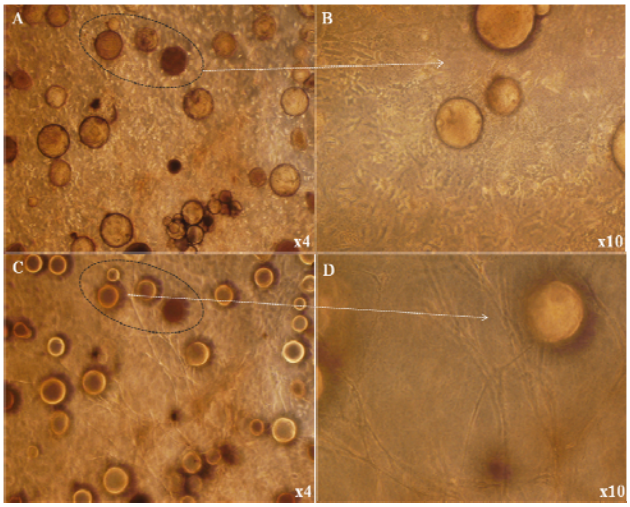

Figure 1: Phase contrast images of ASCs migrated from chitosan microspheres into collagen gel **(A, B)** and PEGylated fibrin gel **(C, D)**. ASCs released from the CSM into collagen gel were spindle-shaped in appearance **(A)**. ASCs that have migrated from CSM attached to the PEGylated fibrin gel shows classical sprouting **(C)**.

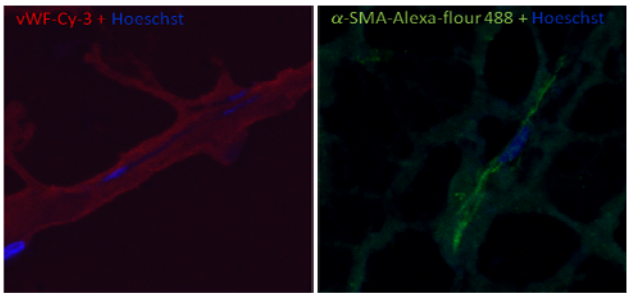

Figure 2: Immunofluorescence showed positive expression of both vascular (von Willebrand factor, left panel) and perivascular specific markers (alpha smooth muscle actin, right panel) to be expressed by the differentiated ASC in PEGylated fibrin gel after 7 days.

Conclusion: The ability of ASC to differentiate into both vascular and stromal cells provides a system for development of vascularized dermis, using appropriate conditions and biomaterials, for healing burn wounds. This phase involves the construction of a bilayered dermal equivalent. Failure of total skin equivalents after *in vivo* application results in large part due to the avascular nature of the construct. The dermal equivalent as described above has both a fibroblastic cell population combined with microvascular structures to overcome diffusional limitations of thicker constructs.

References:

1. G. Zhang, X. Wang, Z. Wang, J. Zhang, L. Suggs. Tissue Engineering, 2006, 12(1): 9-19.