17 - Title: Better Healing in Skin Grafting: Effects of Vascular Network Stability and Mural-Like Cell Support

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Background: The success of tissue-engineered skin substitutes relies on rapid and stable vascular integration to ensure graft survival and support functional wound healing. While pre-vascularized constructs contain capillary-like networks before implantation, their ability to inosculate with host vasculature and remain viable post-implantation remains a major challenge. Dental Pulp Stem Cells (DPSCs) secrete key growth factors, including Vascular Endothelial Growth Factor (VEGF) and Platelet-Derived Growth Factor (PDGF), which may aid in stabilizing these networks and promoting vascular remodeling (1). This study investigates the in vivo maturation of pre-formed vascular networks and evaluates the impact of DPSC inclusion in tissue engineered skin constructs.

Methods/Research Design. Pre-vascularized skin constructs were generated using human keratinocytes (KCs), fibroblasts (FBs), and Green Fluorescent Protein (GFP)-labeled human umbilical vein endothelial cells (HUVECs), with and without DPSCs. Full-thickness wounds (8 mm in diameter) were created in 10- to 11-weeks-old athymic mice, and the constructs were implanted into the defect sites (Figure 1). Wounds were allowed to heal for 4 and 7 days before tissue harvest. Fluorescence microscopy was used to visualize and quantify vascular networks. Key parameters, including network connectivity, vascular density, and inosculation efficiency, were compared between DPSC+ and DPSC− constructs.

Results (or Preliminary Results, as applicable for a project in progress): Fluorescence imaging of pre-vascularized skin constructs before implantation confirmed the successful formation of GFP-labeled HUVEC vascular networks in both groups. However, networks in DPSC+ constructs appeared more interconnected compared to the no DPSC control group. Post-implantation analysis at Day 4 revealed key differences. In DPSC+ constructs, GFP-positive vessels were visible, with early anastomosis occurring between graft-derived capillaries and host vasculature. In contrast, control constructs exhibited fewer and smaller GFP-positive vessels, suggesting reduced inosculation efficiency or early vessel regression in the absence of DPSCs. By Day 7, DPSC+ constructs maintained prominent GFP-positive vessels, indicating sustained vascular integration. In control constructs, GFP-positive vessels were larger but fewer, and disintegrated networks exhibited poor circulation, leading to blood accumulation in the wound bed. The lack of an established circulatory system in the DPSC− group resulted in blood pooling and potential hematoma formation, highlighting inefficient vascular perfusion. While both conditions supported some degree of vascularization, DPSCs appeared to enhance vascular retention and stability over time (Figure 2).

Conclusion (or Preliminary Conclusion, as applicable for a project in progress): Comparative analysis between Day 4 and Day 7 revealed that DPSC+ constructs maintained a more stable vascular network, whereas control constructs exhibited greater vessel regression over time. The findings suggest that DPSCs support long-term vascular persistence, likely by promoting endothelial stabilization and enhancing inosculation efficiency.
These insights will help refine next-generation tissue-engineered skin substitutes for chronic wound treatment and reconstructive surgery. Future studies will focus on optimizing blood vessel size and improving vascular network connectivity to enhance circulation efficiency within engineered constructs. Additionally, incorporating preadipocytes will further stabilize vascular networks and enhance overall perfusion, promoting better tissue integration and long-term graft viability.