Tube Formation by Human Microvascular Endothelial Cells after Addition to Cultured Skin Substitutes and Transplantation to Athymic Mice

Nikkiya M. Koval, B.S., and Steven Boyce, Ph.D.
Department of Surgery, University of Cincinnati and Shriners Burns Hospital

Abstract
The speed and efficacy of wound healing by cultured skin substitutes (CSS) is limited by their lack of a vascular plexus. A potential substitute for a capillary network could be cultured human microvascular endothelial cells (HMVEC) which organize into multicellular tubules *in vitro* when cultured in CSS. Vascular endothelial growth factor (VEGF), an angiogenic growth factor released by keratinocytes, could also promote the organization and survival of HMVEC in CSS if added to the culture medium prior to grafting. To test this hypothesis, CSS were prepared from fibroblasts ± HMVEC and keratinocytes inoculated sequentially onto collagen-glycosaminoglycan substrates, and incubated in control medium and medium containing 10 ng/ml VEGF. After 4, 10, 18, 21, and 28 days incubation including air exposure, CSS samples were labeled by immunohistochemistry for platelet-endothelial cell adhesion molecule (PECAM-1/CD3 1), a cell marker specific for endothelial cells. Positive-staining structures were photographed and scored using MetaMorph image analysis software. The CSS incubated in VEGF-containing medium exhibited increased amounts of HMVEC structures, indicating that VEGF promotes survival and organization of HMVEC *in vitro*.

To assess the effect of HMVEC in CSS *in vivo,* CSS containing HMVEC and incubated in VEGF-containing medium were grafted to full-thickness wounds on the flanks of athymic mice. For comparison, CSS without HMVEC and autografts were also grafted. At post-operative days 3, 5, 7, and 10, 2 mice from each condition were sacrificed and injected with India ink/0.l% gelatin. The grafts were excised and scored for ink perfusion and immunohistochemistry. Immunohistochemistry with antibodies to PECAM-1 demonstrated that HMVEC tubules persisted in the CSS up to 10 days after grafting, condensing into fewer, larger, tubes over time. Some evidence of branching was also present. Image analysis of India ink perfusion revealed that the CSS containing HMVEC had ink-perfused vessels closer to the dermal-epidermal junction in comparison to the control CSS. The results suggested that HMVEC tubules survive transplantion and vascularization in CSS, and that these structures may promote more rapid vascularization and wound closure. Further study will be required to investigate whether HMVEC tubes in CSS anastomose with donor vasculature, and to investigate whether revascularization time is shortened in CSS containing HMVEC.