ENHANCED VASCULARIZATION OF A HUMAN SKIN SUBSTITUTE ENGINEERED TO SECRETE ELEVATED LEVELS OF ANGIOGENIC FACTORS

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Introduction: Rapid vascularization is essential to ensure the survival of split-thickness skin grafts. Current temporary wound dressings are not designed to promote angiogenesis and granulation. The goal of this study is to develop next generation skin substitutes that express elevated levels of angiogenic factors to promote robust vascularization of debrided burn wounds to enhance the survival of subsequent autografts.

Methods: The well-characterized NIKS keratinocyte cell line was stably transfected with non-viral plasmid vectors encoding either a single angiogenic factor (VEGF) or a transcriptional regulator that induces multiple angiogenic factors (HIF-1α). Genetically-modified skin substitutes were evaluated *in vitro* for secretion of angiogenic factors and stimulation of endothelial cell proliferation, as well as *in vivo* for the ability to stimulate vascularization after engraftment on nude or diabetic mice.

Results: Clonally-pure isolates of stably-transfected cells were identified that had the expression vector integrated at a single site. Skin substitutes modified to specifically express VEGF secreted 100 fold more VEGF than unmodified tissue. Tissue modified to express HIF-1α secreted elevated levels of several pro-angiogenic chemokines, including CXCL1, CXCL5, and CXCL6. Conditioned medium from tissue expressing VEGF stimulated the proliferation of human microvascular endothelial cells compared to medium from unmodified skin substitutes. *In vivo*, tissue expressing either VEGF or HIF-1α promoted enhanced vascularization compared to unmodified skin substitutes after grafting on nude mice. Skin substitutes expressing VEGF also enhanced the deposition of vascularized granulation tissue in diabetic mice.

Conclusions: Skin substitutes expressing elevated levels of angiogenic factors enhance vascularization in animal models of impaired wound healing. Uniform, non-viral genetic modification of NIKS keratinocytes offers safety and consistency advantages compared to heterogeneous modification of primary keratinocytes with viral vectors. The ability of skin substitutes expressing elevated levels of angiogenic factors to promote vascularization *in vivo* suggests that these substitutes may accelerate the vascularization and healing of traumatic wounds.

Applicability of Research to Practice: Strategies to stimulate the vascularization of debrided burn wounds have great potential to improve the success of autografting procedures in patients with large body surface area burns or in patients with underlying local or systemic impairments to wound healing. Clinical translation of this technology will provide an important new therapeutic option for severe or complicated burns.

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