Mentored Professional Enrichment Experience

Applicant:

Name of Project/Experience:

Examine the benefits of vascularized skin substitutes using Stro-1^+ adipocyte-derived adult stem cells and keratinocytes seeded onto MTF acellular dermal membrane

Location where Project/Experience will take place:

Department of Plastic Surgery, SIU School of Medicine, Springfield, IL, USA

Mentor Name and Contact Information:

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RATIONALE

Recovery for patients with traumatic skin injuries such as burn wounds presents a major challenge for physicians. Successful reconstruction requires skin grafts from others areas of the body to be transplanted to the injured site. The goal of skin graft surgery is to restore sensibility and improve functional utility of the injured area. Although transplant surgery has had considerable success, reconstructive surgery of larger injuries is limited because of the low availability of donor tissue. To resolve low donor tissue availability, commercial acellular dermal substitutes, such as FlexHD, have been used to provide scaffolding for cellular invasion and capillary growth from peripheral wound edges. Acellular layers, however, often require skin transplants such as split thickness skin grafts. Due to these limitations, there has been a growing interest in developing a tissue engineered skin graft that is highly vascularized, composed of surface epithelium, and sustainable for continuous growth.

Vascularization of newly transplanted skin grafts is essential for success rate and survival. Angiogenesis is a vital factor in promotion of wound healing, survival, and incorporation of skin grafts to patients. By lowering the time for vascularization, the likelihood for increased graft sizes, decreased infection susceptibility, and improved care becomes increasingly likely. To induce angiogenesis, factors must be manipulated in the cell. These include growth factors, proteins, elements of the extracellular matrix, and interactions of endothelial cells periocytes of the blood vessels (reviewed in (Folkman, 2003). Some examples include VEGF, FGF-2, TGF- β, and hyaluronan oligosaccharides.

Adult derived stem cells are multipotent progenitor cells that are capable of differentiation into a variety of diverse phenotypes including endothelial cells (Caplan, 1991; Haynesworth et al., 1992; Pittenger et al., 1999; Reyes and verfaillie, 2001). Typically, these cells are found in adult bone marrow. However, adipose tissue also contains adult stem cells and provides an option that results in less morbidity (Ringe et al., 2002; Yamada et al., 2003). Thus, adipocyte derived stem cells (ADSCs) are a perfect cell population to use in tissue engineering applications for generating blood vessels in prefabricated vascular tissue prior to transplantation (Huang et al., 2002; Tholpady et al., 2003; Zuk et al., 2001). This experiment will specifically examine ADSCs expressing the cell surface marker Stro-1. Stro-1^+ ADSCs have an enhanced ability to differentiate into numerous lineages, including $CD31^+$ endothelial cells (Simmons and Torok-Storb., 1991).

The proposed study will create a vascularized skin substitute using Stro-1^+ ADSCs and keratinocytes seeded on a MTF acellular dermal membrane. This engineered skin substitute will then be closely examined to determine how this construct recapitulates a typical epidermal layer. It is hypothesized that a tissue-engineered vascular skin substitute can be created with syngeneic keratinocytes, Stro-1^+ ADSCs, and an acellular dermal tissue layer.

GOALS

1. To engineer a skin substitute using Flex HD, Stro-1^+ ADSCs, and syngeneic expanded keratinocytes.
2. Examine the effectiveness of Stro-1^+ ADSCs on enhanced vascularization in skin substitutes.
3. Become familiarized with factors which induce angiogenesis.
4. Learn methods of immunohistochemistry, genetic labeling, and other laboratory techniques related to this project.
5. Collaborate with those in the department to assist their projects with the information I collect.

METHODS

Adipocyte tissue will be obtained from Lewis rats through bulk dissection of the inguinal fat pad. ADSCs will be isolated from differentiated adipocytes and the surrounding extracellular matrix by the use of collagenase (0.1% v/v final) and dispase (0.05% v/v final). The resulting solution will then be filtered through a 70 micron cell strainer to obtain single cells from undigested tissue. ADSCs will be isolated from this solution by centrifugation and then cultured in complete media (DMEM, 10% FBS, 1% Pen/Strep, 4mM L-Glutamine). Cells will be labeled with an anti-Stro-1 antibody then magnetically separated from Stro-1 negative cells to obtain a pure population of Stro-1^+ ADSCs.

After continued growth of ADSCs, these cells will be genetically labeled with green fluorescent protein (GFP) for continued examination. GFP will be introduced into cells through GFP encoding, replication-incompetent lentiviral particles. After GFP infection and continued growth for 1 week, ADSCs will be isolated based on GFP expression using FACSVantage (BD Biosciences) in the SIU SOM Flow Cytometry facility. Isolated cells will be expanded in media and stored on liquid nitrogen for later experimental use.

FlexHD, the acellular dermal layer, will be cut into six 2cm X 2cm squares and seeded with $1x10^5$ GFP positive Stro-1^+ ADSCs. The constructs will then be incubated in endothelial growth media (EGM2) to induce ADSCs to form vascular structures. To verify the formation of tube-like structures, immunohistochemistry will used to assess the presence of endothelial markers by GFP^+ ADSCs. Using specific antibodies and factors, resulting positive cells will be assessed by fluorescent microscopy.

Keratinocytes will be isolated from the epidermal layer of adult rats. The rats will be humanely euthanized and the dorsal skin layer will be removed. Then the epidermis will be carefully excised from the dermis. The resulting epidermis will be agitated in keratinocyte plating media (1:1 DMEM:Ham's F12, 10% FBS, 10ng/mL cholera toxin, 0.4 microg/mL hydrocortisone, 5 microg/mL insulin, 5 microg/mL transferrin, 1% antibiotic/antimycotic) and filtered to obtain single cells. The isolated keratinocytes will then be genetically labeled using red fluorescent protein (dsRed) to allow for continued examination; cells will be incubated overnight with dsRed encoding lentiviral particles and isolated by flow cytometry. The labeled keratinocytes will be selectively grown in media and used in future experiments as needed.

Finally, the labeled and expanded keratinocytes will be seeded onto the vascularized dermal membranes (~$2.5x10^5$ keratinocytes/2 cm^2 membrane) and allowed to sit in an air/liquid

interface to permit stratification. The final skin substitutes will be embedded in paraffin and processed by H&E staining and immunochemistry for epithelial markers (cytokeratin 1, 5 and loricrin). These analyses will be used to assess how closely this engineered skin substitute corresponds to a typical epidermal layer.
(Supplementary material on methods was received from Dr. Neumeister)

ANALYSIS

Stro-1$^+$ ADSCs will be genetically labeled with green fluorescent protein (GFP), seeded on the acellular membrane, grown in endothelial growth media, then assessed by confocal microscopy at days 1, 3, 7, 14, and 21 for the presence of tube-like structures. Captured Z-stack images will be combined to a single file to assess tube length and the number of branches by computer aided image analysis (MCID/M6; Imaging Research). This analysis will allow the determination of the optimal time point for vessel formation and this time point will then be used for establishing keratinocytes on the membrane.

Keratinocytes will be genetically labeled with red fluorescent protein to allow for assessment after the keratinocytes have been stratified to the vascularized dermal membrane. Once the keratinocytes have been embedded into the membrane, the skin substitutes will be processed for H&E staining and immunohistochemistry for epithelial markers (cytokeratin 1, 5, and loricrin). These analyses will be used to assess how closely this engineered skin substitute corresponds to a typical epidermal layer.

SUPPORT

1. Do you request support funds? Yes.
2. Would you be able to participate if a scholarship is not available? Yes.

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