Engineering Vascularized Skin Flap with Decellularized Skin Flap Matrix Scaffold

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Statement of Purpose: Skin Flaps serve as workhorse in reconstructive microsurgery. However, the availability of qualitative autologous flaps and donor site morbidity significantly limit its application. Engineered skin flap may offer a clinically alternative to autologous flaps[1]. Several intermediate milestones have been reached by tissue engineers in heart, lung and liver regeneration with using decellularized whole organ matrix scaffolds.[2-4] We hypothesized that decellularized skin flaps (DSFs) that are composed of extracellular matrix (ECM) and intact vascular structures could provide an ideal bioscaffold system for skin flap engineering after recellularization. To test this hypothesis, we developed a DSF with a dominant vascular pedicle which was then repopulated with human adipose derived stem cells (hASCs) and human umbilical vein endothelial cells (HUVECs) for pre-vascularization to improve the vascularization and survival of engineered flap after transplantation.

Methods: *Preparation of DSFs* All procedures were approved by the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center and met all requirements of the Animal Welfare Act. Skin flaps (2cm x 3 cm) based on the superficial inferior epigastric artery (SIEA) were harvested from Lewis rats. The vascular pedicle included the SIEA and femoral artery. Harvested skin flaps underwent decellularization processing by a combination of physical, chemical, and enzymatic approaches. *Evaluation of DSF components* Native skip flaps and DSFs were fixed in 10% formalin, embedded in paraffin, and sectioned into 5-µm slices. Slides underwent histological and immunohistological stainings (hemoatoxylin and eosin (H&E), Masson Trichrome, DAPI, laminin, VEGF, MHC-1). Samples were imaged using an Olympus IX71 microscope. Cell removal was further quantified by measuring nucleic acid concentration with the Quant-iT™ PicoGreen® dsDNA assay kit. *Scanning electron microscopy (SEM)* Freezing-dried DSFs were coated with platinum alloy for a thickness of 25 nm, and examined in a JSM-5910 scanning electron microscope. *Cell culture* All procedures were conducted under institutional review board approval and in accordance with research guidelines at the University of Texas MD Anderson Cancer Center. Adipose tissue samples (subcutaneous adipose tissue in abdominal wall area) were collected from patients undergoing reconstructive surgery. Adipose tissues were minced and digested to obtain a cell pellet. Red blood cell lysis buffer was used to purify the cell pellet. HUVECs were purchased from Lonza and cultured as instructed. *Evaluation of hASCs and HUVEC integration with DSFs* hASCs and HUVECs within 4 passages were integrated with DSFs. Cells were stained with calcein AM or DAPI for observation. Samples were imaged with an Olympus IX81confocal fluorescence microscope.

Results: Skin flaps with a dominant vascular pedicle were decellularized. Well-retained vascular and nerve structures were observed in DSFs (Fig. 1). Cell removal in DSFs was proved by H&E and DAPI staining as well as DNA quantification (0.38 µg/mg DNA/dry weight in native skin, 0.04 µg/mg DNA/dry weight in decellularized skin, $P<0.05$; 0.35 µg/mg DNA/dry weight in native fat pad, 0.05 µg/mg DNA/dry weight in decellularized fat pad, $P<0.05$). Histological and immunohistochemical staining (Masson Trichrome, laminin, VEGF, and MHC-1) showed that DSFs were composed of collagen and laminin with well-preserved VEGF (Fig.2). Negative MHC-1 staining in DSF confirmed the removal of MHC-1 after decellularization. SEM evaluation showed nanofibrous structures were retained in DSFs (Fig.2). To apply DSFs for soft tissue repair, intact artery was successfully repopulationed with HUVECs; hASCs integrated in DSFs proliferated and formed 3D aggregates at day 7 of culture, indicating DSFs are promising stem cell carriers in stem cell-based therapy (Fig. 2).

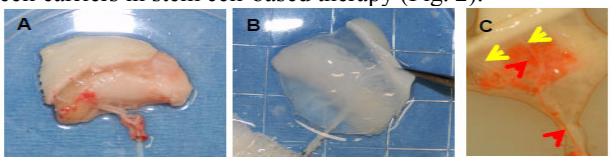

Fig. 1. Rat skin flap before (A) and after (B) decellularization. Vascular (red arrow) and nerve (yellow arrow) structures within DSF was preserved intact (C). Red dye was injected into artery (red) and filled the vascular structures.

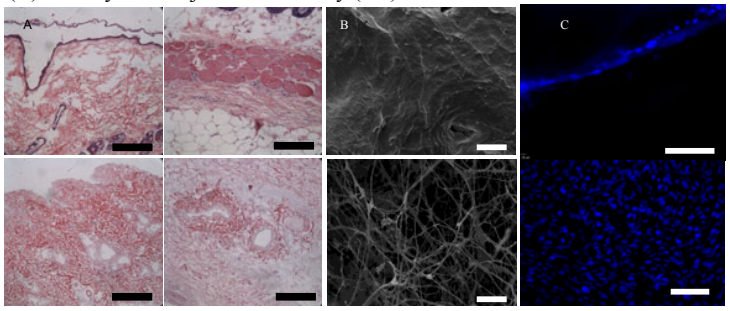

Fig. 2. (A) H&E staining for DSFs. (B) SEM of DSFs from skin (top) and subcutaneous (bottom) sides. (C) HUVECs (top) and hASCs (bottom) were integrated in DSFs. Scale bar = 200 µm in (A), 50 µm in (B), and 100 µm in (C).

Conclusions: This investigation engineered skin flap grafts by co-cultural of hASCs and HUVECs on DSFs to improve the vascularization and survival of constructs after transplantation. Combined with functional cells and ready-to-use vascular structure for conducting immediate blood supply in recellularized DSFs, this platform will address the donor shortage issue, dramatically decrease ischemia time in the graft and increases the success of transplantation with unlimited graft size.

References:

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