Optimization, Scale-up and Biological Characterization of Decellularized Adipose Tissue Scaffolds for Soft Tissue Reconstruction

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Introduction

It would be a great advantage in reconstructive surgery to be able to provide an off-the-shelf biomaterial to promote regeneration and volume augmentation following soft tissue damage. Human adipose tissue (fat) is an abundant and accessible source of extracellular matrix (ECM). Previously, our group developed a 5-day detergent-free decellularization method that removes antigenic components while preserving the 3-D architecture of the ECM found in human adipose tissue [1].The main objective of the current project was to optimize and refine the decellularization process such that decellularized adipose tissue (DAT) can be economically produced on an industrial scale. Further, the key biological properties of the DAT were more fully explored to establish processing standards for the tissue bioscaffolds. Finally *in vivo* biocompatibility studies were conducted using an immunocompetent rat model and were followed by a pilot large animal study in a mini-pig model.

Material and Methods

Decellularization was carried out on human adipose tissue collected from liposuction or breast reduction surgeries at the Kingston General Hospital. Human Research Ethics Board approval for this study was obtained from Queen's University (CHEM-002-07). In order to optimize the DAT process, the sequence and timing of the decellularization treatment stages were systematically investigated, and a comparative analysis using a range of detergent-based approaches was conducted. The assessment also included determining the best strategy for processing large volume (1 - 2 L) tissue samples. At key intervals, the structure and composition of the DAT was assessed by histology, immunohistochemistry and scanning electron microscopy (SEM). Hydroxyproline and dimethylmethylene blue (DMMB) assays were conducted to quantify total collagen and glycosaminoglycan (GAG) content in the processed tissues. Picrosirius red staining with polarized light microscopy was used to assess collagen distribution and composition .The amount of residual DNA was quantified using a G-spin total DNA extraction kit, and visually confirmed through methyl green pyronine staining. The *in vitro* bioactivity of the scaffolds was tested by measuring the glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity of human adipose-derived stem cells (ASCs) seeded on the DAT and cultured for up to 14 days under either proliferation or adipogenic differentiation conditions. For all assays, (n=3, N=3) with statistical analysis by one-way ANOVA ($p < 0.05$). Based on the *in vitro* results, the biocompatibility of the DAT derived from the most promising protocols was tested in an established rat model. For each time point, triplicate scaffolds were subcutaneously implanted on the dorsa of female Wistar rats (12 weeks).At1, 3, 8, 12 and 16 weeks, the rats were sacrificed and the implants were excised in the surrounding tissues and fixed in 4% paraformaldehyde before paraffin-embedding and sectioning. Blinded histological analysis was conducted to assess the inflammatory response, fibrous capsule formation, angiogenesis and adipogenesis. Building on these results, a follow up study is currently being conducted using a male Gottingen mini-pig model, with assessment at 1 and 3 months. All *in vivo* work complied with the Canadian Council on Animal Care (CCAC) guidelines for the care and use of laboratory animals, and was approved by the University Animal Care Committee (UACC) at Queen's University

Results

Systematic investigation of the key treatment steps during the decellularization process significantly reduced the total processing time to less than 3 days and minimized the total costs associated with handling, supplies and reagents. The most effective protocols for adipose tissue involve a combination of mechanical processing, enzymatic treatment with trypsin-EDTA and polar solvent extraction. Testing of a range of solvents identified isopropanol as being the most effective solvent for lipid dissolution. Effective decellularization was confirmed histologically and was also demonstrated by the low residual DNA in the DAT produced according to the original protocol, as well as the optimized approach. Hydroxyproline assay results indicated that there was a high collagen content in the DAT, which was confirmed visually through picrosirius red staining (Fig. 1). Interestingly, when the DAT was processed with a standard detergent-based approach to decellularization including sodium dodecyl sulphate (SDS), there was macroscopic damage to the ECM architecture, combined with interference with the visualization of the collagen by polarized light microscopy (Fig. 1). An additional disadvantage was the difficulty in removing the residual detergent at the end of processing. The main results from the *in vitro* bioactivity analysis with ASCs cultured on the DAT are (i) that the GPDH enzyme activity was significantly higher on the DAT compared to the TCPS positive controls, (ii) there were no significant differences in GPDH activity for DAT from different donors, and (iii) the bioactivity of the DAT was reduced after long-term (6 months) storage in a hydrated state. In the *in vivo* studies, the implants were well tolerated in the rat model. There was no evidence of strong immune reaction towards the DAT decellularized using the original or optimized detergent-free approaches. The scaffold volume was well retained over the course of the study, with angiogenesis and adipogenesis observed at later time points (Fig. 2). In the pig model, there were no signs of adverse effects at 1 month and the implants integrated well into the surrounding tissues. A comparison of hydrated versus lyophilized DAT scaffolds indicated that the dried format was easier to handle in the clinic and rehydrated quickly within the implant site.

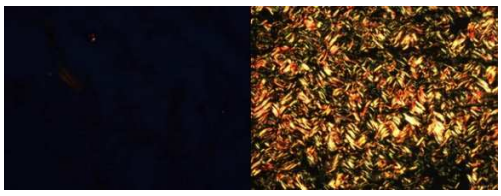

Figure 1: Polarization pictures of picrosirius red staining. On the left: DAT treated with the detergent sodium dodecyl sulphate (SDS). On the right: DAT according to original decellularization method.

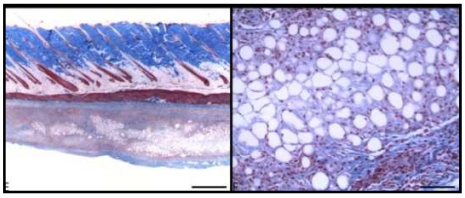

Figure 2: Masson's trichrome staining of DAT scaffold after 3 weeks *in vivo* showing formation of mature adipocytes

Discussion

This work represents an important step towards the clinical application of the DAT as an off-the-shelf biomaterial for soft tissue reconstruction. A comparative analysis of a range of protocols demonstrated that detergent-based approaches were not as effective at removing cellular components or lipid from the adipose tissue, and the detergents also negatively impacted the structure and composition of the ECM. Residual detergents also raise concerns about cytotoxicity and immune response following implantation. To facilitate clinical translation, the decellularization strategy must be cost effective, reproducible and scalable, all of which were achieved in the current approach. The *in vitro* and *in vivo* studies confirmed the biocompatible and adipogenic nature of the DAT and help to establish processing standards for producing DAT on an industrial scale. Ongoing studies are focused on more fully characterizing the response in the large animal model, identifying ideal handling and storage conditions, and validation of terminal sterilization approaches.

[1]Flynn LE. Biomaterials 2010; 31 (17):4715