Evaluation of Adipo-inductive Foams Derived from Human Decellularized Adipose Tissue

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Introduction

A biomaterial with the ability to induce the differentiation of stem cells into adipocytes and with mechanical properties similar to native human adipose tissue (fat) would be of great clinical value for the repair of soft tissue defects in plastic and reconstructive surgery. Building on previous work that indicates that decellularized adipose tissue (DAT) is an adipo-inductive matrix [1], the objective of the current study was to investigate porous foams fabricated from enzyme-solubilized DAT (DATsol) as 3-D scaffolds for soft tissue regeneration with highly customizable shape and volume.

Materials & Methods

Foam Fabrication: Human adipose tissue was collected from routine lipo-reduction surgeries at Kingston General Hospital. Human Research Ethics Board approval for this study was obtained from Queen's University (CHEM-002-07). The tissue was decellularized using an established protocol [1] and the DAT was solubilized via enzymatic digestion with α-amylase under acidic conditions [2]. To fabricate 3-D DAT foams, a controlled freezing and lyophilization procedure was optimized using moulds to yield highly porous sponges . The foam ultrastructure was characterized by SEM and mechanical testing was conducted to measure the Young's moduli of the hydrated scaffolds. *In vitro* swelling and stability were analyzed in Ringer's physiological fluid over 14 days.

In Vitro Adipogenesis: The *in vitro* adipogenic differentiation of human adipose-derived stem cells (ASCs) seeded on the DAT foams and cultured in (i) proliferation medium or (ii) adipogenic differentiation medium [1] was assessed over 14 days in comparison to tissue culture polystyrene (TCPS) controls. Adipogenesis was analyzed in terms of adipogenic gene expression by RT-PCR, glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity, and intracellular lipid by oil red O staining. For all assays, ($n=3$, $N=3$) with statistical analysis by one-way ANOVA ($p<0.05$).

In Vivo Assessment: The *in vivo* response to a range of DAT foams (25 mg/mL DATsol, 50 mg/mL DATsol, and 100 mg/mL DATsol, frozen at -20 °C) was assessed over 12 weeks using a subcutaneous Wistar rat model. Prior to implantation, one set of foams was seeded with 1×10^6 rat ASCs collected from the epididymal fat pad of male Wistar rats. Triplicate samples (seeded and unseeded) were implanted into subcutaneous pockets on the dorsa of female Wistar rats, with non-solubilized DAT scaffolds implanted as a control group. The scaffolds were explanted at 1, 3, 8, and 12 weeks, fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 7 µm sections for histological and immunohistochemical characterization. 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

Using the developed foam fabrication methods, the porosity and mechanical properties of the DAT foams could be tuned by varying the DATsol concentration and freezing rate. DAT foams fabricated with 50 mg/mL DATsol at a freezing temperature of -20 °C demonstrated the greatest stability with enhanced porosity and an equilibrium water content of approximately 97%. Mechanical testing revealed that the majority of the DAT foams in the study had a Young's modulus

in the range of 3-4 kPa, similar to normal human adipose tissue [3]. Interestingly, the foam formulations within this range also exhibited the highest level of adipogenic differentiation.

In vitro analysis demonstrated that the DAT foams had adipo-conductive properties for the ASCs, promoting high levels of GPDH enzyme activity and adipogenic gene expression when cultured under differentiation conditions. The DAT foams were also found to exhibit adipo-inductive properties, with the non-induced foams cultured in proliferation medium exhibiting similar GPDH activities to the induced TCPS controls. Oil red O staining provided further evidence of adipogenesis with intracellular lipid accumulation in both induced and non-induced foams.

In vivo analysis using Masson's trichrome staining demonstrated that at early time points (1 week) the DAT foams retained their shape and volume and also stimulated a strong angiogenic response in the host tissues (Fig. 1), which was not observed with the intact DAT controls. At 3 weeks, the DAT foams were markedly reduced in volume (~ 50%) and significant cell infiltration was observed, which is consistent with a host inflammatory response. At 8 and 12 weeks, there was significant resorption of the foams and the collagen fibres within the scaffolds had become reorganized to align parallel to the surrounding host collagen fibres. In contrast, the intact DAT controls retained their volume over 12 weeks. Overall, the *in vivo* results demonstrated that all of the DAT-based scaffolds were biocompatible and bioactive materials that integrated well into the host tissues. In addition, functional blood vessels and mature adipocytes were observed demonstrating the adipo-inductive and angiogenic properties of the DAT (Fig. 2).

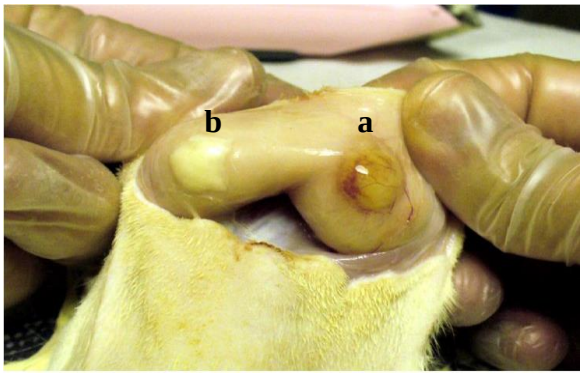

Figure 1: Angiogenesis can be observed around the (a) DAT foam implant in comparison with the (b) intact DAT control. Image was taken 72 h post-implantation.

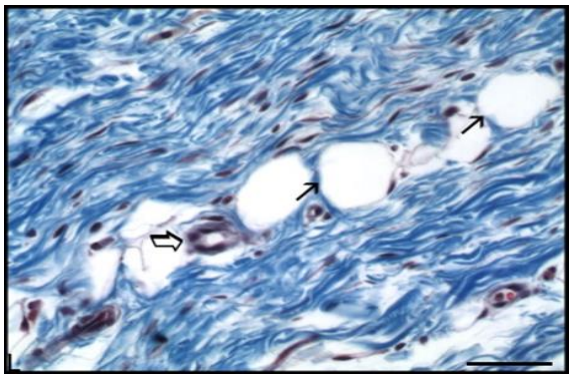

Figure 2: Masson's trichrome staining of DAT foam at 12 weeks *in vivo*. Mature adipocytes (black arrows) and blood vessels (white arrow) in the implant. Scale bar 250µm

Discussion

DAT foams are naturally adipo-inductive and adipo-conductive biomaterials that can be customized to create 3-D scaffolds with a defined shape and volume for use as soft tissue replacements. The DAT-based biomaterials provide an ideal microenvironment for ASC delivery. The differences in the foams and non-solubilized DAT scaffolds suggest differing applicability in reconstructive surgery. In particular, the angiogenic capacity and tissue integration characteristics of the foams could be useful in wound healing, such as for diabetic ulcers. In comparison to the foams, the intact DAT holds promise as a bioscaffold for use in 3-D volume augmentation and adipose replacement. Overall, decellularized human adipose tissue is a rich source of human ECM that can be used to fabricate a broad range of bioscaffolds that promote soft tissue regeneration.

References: [1] Flynn LE. *Biomaterials* 2010;31(17):4715. [2] Stevens FS. *Ann. rheum. Dis.* 1962;23:300. [3] Samani A, et al. *Phys Med Biol* 2007;52:1565.