Biomaterials 34 (2013) 3290–3302

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Porous decellularized adipose tissue foams for soft tissue regeneration

Claire Yu [a,b], Juares Bianco [a,b], Cody Brown [a,b], Lydia Fuetterer [a,b], John F. Watkins [c], Abbas Samani [d], Lauren E. Flynn [a,b,e,*]

[a] Department of Chemical Engineering, Queen's University, 19 Division St., Kingston, ON, Canada K7L 3N6
[b] Human Mobility Research Centre, Kingston General Hospital, 76 Stuart St., Kingston, ON, Canada K7L 2V7
[c] Division of Plastic Surgery, Department of Surgery, Queen's University, 166 Brock Street, Kingston, ON, Canada K7L 5G2
[d] Department of Electrical and Computer Engineering, Western University, Room 279, Thompson Engineering Building, London, ON, Canada N6A 5B9
[e] Department of Biomedical and Molecular Sciences, Queen's University, 80 Barrie St., Kingston, ON, Canada K7L 3N6

ARTICLE INFO

Article history:
Received 1 December 2012
Accepted 10 January 2013
Available online 4 February 2013

Keywords:
Adipose tissue engineering
ECM (extracellular matrix)
Collagen
Scaffold
Stem cell
Wound healing

ABSTRACT

To design tissue-specific bioscaffolds with well-defined properties and 3-D architecture, methods were developed for preparing porous foams from enzyme-solubilized human decellularized adipose tissue (DAT). Additionally, a technique was established for fabricating "bead foams" comprised of interconnected networks of porous DAT beads fused through a controlled freeze-thawing and lyophilization procedure. In characterization studies, the foams were stable without the need for chemical crosslinking, with properties that could be tuned by controlling the protein concentration and freezing rate during synthesis. Adipogenic differentiation studies with human adipose-derived stem cells (ASCs) suggested that stiffness influenced ASC adipogenesis on the foams. In support of our previous work with DAT scaffolds and microcarriers, the DAT foams and bead foams strongly supported adipogenesis and were also adipo-inductive, as demonstrated by glycerol-3-phosphate dehydrogenase (GPDH) enzyme activity, endpoint RT-PCR analysis of adipogenic gene expression, and intracellular lipid accumulation. Adipogenic differentiation was enhanced on the microporous DAT foams, potentially due to increased cell–cell interactions in this group. *In vivo* assessment in a subcutaneous Wistar rat model demonstrated that the DAT bioscaffolds were well tolerated and integrated into the host tissues, supporting angiogenesis and adipogenesis. The DAT-based foams induced a strong angiogenic response, promoted inflammatory cell migration and gradually resorbed over the course of 12 weeks, demonstrating potential as scaffolds for wound healing and soft tissue regeneration.

1. Introduction

To facilitate soft tissue regeneration and implant integration into the host tissues, bioscaffolds are typically designed to be highly porous to improve cellular infiltration and viability, to be structurally robust, and ideally, to promote normal cell signalling processes. In this context, recent studies have focused on natural collagenous foams as they have the potential to provide a cost-effective, biologically active and highly customizable base material that can be adapted for use in a variety of tissue engineering applications [1–4]. Standard foam fabrication methods often involve freezing acid-solubilized collagen in a preformed mould, followed by lyophilization to yield a homogenous porous matrix [5–7]. Typically, pore sizes larger than 100 μm are required to facilitate extensive cellular infiltration, which may not be easily achieved using this approach [6,8]. As an alternative, solid free form (SFF) technology has been investigated in which 3-D printers fabricate custom casting moulds to achieve deep channelling microarchitecture [9]. Similarly, other methods have been presented including a foaming/freeze drying technique or incorporating large ice particulates into the collagen solution before lyophilization [10,11].

To date, the majority of natural foams have been prepared using purified animal collagens or gelatin, which have limitations associated with their xenogenic sourcing [10,12,13]. Interestingly, the use of human extracellular matrix (ECM) has not yet been extensively studied for the fabrication of tissue-specific ECM-derived foams. Decellularized human tissues provide an interesting alternative for bioscaffold fabrication, as the complex microenvironment of the ECM mediates a wide range of cellular behaviour in

* Corresponding author. Department of Chemical Engineering, Queen's University, 19 Division St., Kingston, ON, Canada K7L 3N6. Tel.: +1 613 533 6000x79177; fax: +1 613 533 6637.
E-mail address: lauren.flynn@chee.queensu.ca (L.E. Flynn).

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http://dx.doi.org/10.1016/j.biomaterials.2013.01.056

a tissue-specific manner including survival, proliferation, migration, and differentiation, all of which are critical for tissue regeneration [14,15].

Recently, adipose tissue has emerged as an abundant and accessible source of human ECM that is enriched with basement membrane, which can be collected from either excised tissue blocks or from processed lipoaspirates through a range of decellularization techniques [1,16]. In our previous work with human decellularized adipose tissue (DAT), we have shown that both DAT and injectable DAT-based microcarriers provide an adipo-inductive substrate for human adipose-derived stem cells (ASCs) that hold great promise as bioscaffolds for adipose regeneration [16,17]. Other groups have investigated adipose-derived ECM in the form of powders, sheets and injectable gels, with positive results in terms of cell adhesion, proliferation, and integration following *in vivo* implantation [1,2,18,19].

Building on this previous work, in the current study we have developed techniques for fabricating porous DAT foams as bioscaffolds for soft tissue regeneration and wound healing. Moreover, we engineered an approach for synthesizing macro-porous "bead foams", comprised of a network of interconnected porous DAT microcarriers. When the DAT was enzymatically solubilized using α-amylase and prepared via freeze-drying techniques, the DAT-based foams were stable *in vitro* without the need for chemical crosslinking [20]. By varying the freezing temperatures and concentrations of the solubilized DAT, a range of foam and bead foam constructs were prepared and their physical properties were characterized. Subsequently, the *in vitro* adipogenic differentiation response of human ASCs seeded on the DAT-based foams was assessed in terms of adipogenic gene expression, enzymatic activity, and intracellular lipid accumulation. Finally, the *in vivo* response to the DAT-based foams was characterized in a subcutaneous Wistar rat model to evaluate scaffold biocompatibility, angiogenesis, adipogenesis and implant integration over 12 weeks.

2. Materials and methods

2.1. Materials

Unless otherwise indicated, chemical reagents for this study were obtained and used as received from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada).

2.2. Adipose tissue acquisition

In collaboration with surgeons from the Kingston General Hospital and Hotel Dieu Hospital (Kingston, ON, Canada), human adipose tissue samples were sourced from female patients undergoing elective lipo-reduction surgery of the breast or abdomen and transported to the lab on ice in sterile cation-free Dulbecco's phosphate buffered saline (D-PBS; Thermo Scientific HyClone, Cat. # SH30028, Fisher Scientific, Oakville, ON, Canada) supplemented with 20 mg/mL bovine serum albumin (BSA). The patient information was recorded (donor age, weight, height, and excision site) and the tissue was processed within 2 h for ASC isolation or decellularization, using published protocols [16]. Research ethics board approval for this study was obtained from Queen's University (REB# CHEM-002-07) and an experimental outline of the overall project scope is shown in Fig. 1.

2.3. Solubilization of DAT

DAT samples were pooled from multiple patients ($n = 5$) and solubilized using methods adapted from Stevens *et al.* [20]. Collective samples of DAT (2 g, wet weight) were repeatedly washed in 5% (w/v) NaCl solution followed by distilled water. The DAT was resuspended in 0.22 M NaH_2PO_4, the pH was adjusted to 5.4, and 0.3% (w/w) α-amylase was added to the tissue. The tissue digest solution was agitated continuously at room temperature for 72 h, after which the DAT was washed repeatedly with 5% (w/v) NaCl solution followed by distilled water. The DAT was then minced finely ($\sim$0.5 cm^3 fragments) and resuspended in 0.2 M acetic acid and allowed to agitate continuously at 37 °C for 48 h, with periodic homogenization (PowerGen Model 125 homogenizer, Fisher Scientific, Ottawa, ON, Canada). The liquefied DAT solution was centrifuged (12,000×*g*, 5 min, 4 °C) and the solubilized ECM-rich supernatant was collected. The insoluble DAT residue was subjected to an additional extraction with acetic acid and the supernatants were pooled.

The total protein content of the solubilized DAT (DATsol) was estimated based on the initial mass of the DAT and the volume of acetic acid added during the solubilization procedure.

2.4. Foam fabrication

The microporous DAT foams were prepared by pipetting 500 μL of DATsol into a cryomould (Tissue-Tek™ Biopsy Cryomold 10 × 10 × 5 mm; VWR, Cat. # 25608-922, Batavia, IL), freezing the scaffolds completely, and lyophilizing overnight. The combined effects of varying the DATsol concentration (25 mg/mL, 50 mg/mL, and 100 mg/mL) and freezing temperature (−20 °C, −80 °C) on the properties of the DAT foams were explored.

The "bead foams" were prepared using microporous DAT microcarriers fabricated using an electrospraying method adapted from Kim *et al.* [21]. Briefly, via a syringe pump set at a rate of 0.5 mL/min, the DAT beads were synthesized by electrospraying DATsol (25 mg/mL or 50 mg/mL) through a 25-gauge infusion needle (SURFLO®, Cat. # SV-25BLK, Terumo, Somerset, NJ) with an applied voltage of 10 kV into a bath of liquid nitrogen at a working distance of 15 cm. The frozen DAT microcarriers were collected and allowed to thaw completely at room temperature before they were transferred into the cryomould, followed by freezing (−20 °C, −80 °C) and lyophilization, to create a stable 3-D bead foam comprised of an interconnected network of packed DAT microcarriers.

2.5. Characterization of foam stability and structure

Characterization studies were conducted to assess the physical properties of a range of DAT-based foams as summarized in Table 1. Scanning electron microscopy (SEM) was used to visualize the micro-architecture of each foam formulation. To prepare the samples for SEM, the foams were fixed overnight in 2.5% glutaraldehyde, rinsed extensively in PBS and then freeze-fractured using liquid nitrogen to assess the internal microstructure. The samples were then subjected to an established chemical drying protocol using hexamethyldisilazane (HDMS) [16]. The dried samples were mounted and pulse coated with gold, and visualized with a JOEL JSM-840 microscope with an accelerating voltage of 10 kV and a working distance of 15 mm.

To measure the equilibrium water content (EWC), the dry weights (W_D) were recorded for all lyophilized foam samples prior to rehydration in deionized water. After 72 h, the foams were carefully blotted to remove excess liquid prior to recording the wet weight (W_W). The EWC was determined using the following formula:

$$\mathrm{EWC} = \left(\frac{W_W - W_D}{W_W}\right) \times 100\%$$

Macroscopic images of the foams were taken using a stereomicroscope to quantitatively assess changes in the dimensions of the foams using ImageJ analysis software. Using these measurements, a swelling ratio (SR) for each type of foam was calculated according to the following equation:

$$\mathrm{SR} = \left(\frac{A_W}{A_D}\right) \times 100\%$$

Where A_W represents the surface area of the foam in the hydrated state, and A_D represents the surface area in the lyophilized form.

In vitro stability over 14 days was confirmed through incubation in Ringer's simulated physiological fluid (8.6 mg/mL NaCl, 0.3 mg/mL KCl, and 0.33 mg/mL $CaCl_2$ in deionized water) at 37 °C, with protein release measured in triplicate at 72 h, 7 days and 14 days using the Bio-Rad Protein Assay [22].

2.6. Mechanical testing

Foam materials with various protein concentrations were prepared in cylindrical moulds with a diameter of 23 mm. To characterize the mechanical properties, the Young's modulus of each cylindrical foam sample was measured using an unconstrained indentation technique. In this technique, the cylindrical sample is indented using a small indenter while its wall is unconfined (Fig. 2a). Compared to a standard uniaxial test, this indentation test is advantageous as it is capable of assessing the homogeneity of the sample material. In this investigation, we used a custom-made indentation mechanical testing machine, as shown in Fig. 2a.

As described by Samani *et al.* [23], the indenter is connected to a linear LAL-30 servo motor (SMAC, Carlsbad, CA, USA) driven by a 6K2 motor controller device (Parker Hannifin Corporation, Rohnert Park, CA, USA) which has a motion range of 25 mm and resolution of 0.5 μm. The controller can be programmed to provide a user-defined indenter motion profile. To measure the indentation force profile corresponding to the applied user-defined indentation profile, a high precision LCL-113 model load cell (Omega, Quebec, Canada) with a capacity of 113 g was used. To enhance force measurement, the load cell data was sampled at 1000 samples/s. Each sample was carefully moved out of its mould and placed in the indentation machine's sample holder, which contained a thin layer of PBS to reduce sample friction with the holder and to prevent sample dehydration.