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Decellularized adipose tissue microcarriers as a dynamic culture platform for human adipose-derived stem/stromal cell expansion

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ABSTRACT

With the goal of designing a clinically-relevant expansion strategy for human adipose-derived stem/stromal cells (ASCs), methods were developed to synthesize porous microcarriers derived purely from human decellularized adipose tissue (DAT). An electrospraying approach was applied to generate spherical DAT microcarriers with an average diameter of 428 ± 41 μm, which were soft, compliant, and stable in long-term culture without chemical crosslinking. Human ASCs demonstrated enhanced proliferation on the DAT microcarriers relative to commercially-sourced Cultispher-S microcarriers within a spinner culture system over 1 month. ASC immunophenotype was maintained post expansion, with a trend for reduced expression of the cell adhesion receptors CD73, CD105, and CD29 under dynamic conditions. Upregulation of the early lineage-specific genes *PPARγ*, *LPL*, and *COMP* was observed in the ASCs expanded on the DAT microcarriers, but the cells retained their multilineage differentiation capacity. Comparison of adipogenic and osteogenic differentiation in 2-D cultures prepared with ASCs pre-expanded on the DAT microcarriers or Cultispher-S microcarriers revealed similar adipogenic and enhanced osteogenic marker expression in the DAT microcarrier group, which had undergone a higher population fold change. Further, histological staining results suggested a more homogeneous differentiation response in the ASCs expanded on the DAT microcarriers as compared to either Cultispher-S microcarriers or tissue culture polystyrene. A pilot chondrogenesis study revealed higher levels of chondrogenic gene and protein expression in the ASCs expanded on the DAT microcarriers relative to all other groups, including the baseline controls. Overall, this study demonstrates the promise of applying dynamic culture with tissue-specific DAT microcarriers as a means of deriving regenerative cell populations.

1. Introduction

Adipose tissue is an accessible and uniquely expendable source of regenerative cells, termed adipose-derived stem/stromal cells (ASCs), which are currently being investigated in pre-clinical trials for a broad range of applications in tissue engineering and regenerative medicine [1,2]. In addition to demonstrated multipotency towards the adipogenic, osteogenic and chondrogenic lineages, ASCs can also secrete an array of beneficial paracrine factors that can help to establish a more pro-regenerative microenvironment within host tissues [3]. However, to advance towards effective cell therapy in humans, there is a critical need to develop robust cultivation methods that can reproducibly supply the 1–5 million cells/kg body weight estimated to be required per session for clinical applications, while maintaining the stem cell phenotype and multilineage differentiation capacity [4,5]. In this respect, the ability to produce large ASC populations from small patient biopsies would represent a clinical advantage for the translation of innovative autologous or allogeneic therapies from the 'benchtop to the bedside'.

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Standard cultivation practices typically involve monolayer culture on tissue culture flasks for small-scale laboratory operations or larger-scale multilayer stacked trays that can yield up to 50 million cells per 40-layer stack via automated or manual handling [6]. Although convenient, these systems do not possess the complex biochemical, biophysical, and biomechanical properties of the native stem cell niche within tissues, which may contribute to the reduced ASC proliferation and multilineage differentiation capacities reported upon extended culturing [7–9]. Furthermore, other concerns regarding scale-up include the low surface area-to-volume ratio of 2-D culture flasks, material costs, labour intensiveness, the risk of contamination with repeated passaging, and the lack of control over culture parameters and monitoring of the system [4].

To address the limitations of conventional static culture methods, various stirred or perfusion bioreactor systems have been explored to efficiently expand and deliver high-quality stem cells [10–13]. In particular, suspension culture strategies involving microcarriers are an attractive approach due to the potential for continuous rapid expansion of adherent cell populations and simple collection through gravity settling. Depending on the materials selected, these microcarriers may also serve as direct injectable cell delivery vehicles. For example, bioresorpable microcarriers have been fabricated from collagen I, poly(lactic-co-glycolic acid) (PLGA), chitosan, and decellularized tissues [14–19]. The latter approach using decellularized matrices may retain complex biochemical and biophysical elements of the extracellular matrix (ECM), which have been shown to modulate cellular behaviour and promote constructive tissue regeneration [20–22]. The favourable biological characteristics of decellularized ECM provide a strong rationale for its use as a natural bioactive substrate in microcarrier development.

Previously, we demonstrated that photo-crosslinked microcarriers derived from pepsin-digested decellularized adipose tissue (DAT) supported the proliferation of human ASCs within a spinner flask system, highlighting the potential use of tissue-specific ECM to facilitate cell expansion [23]. While these first-generation DAT microcarriers served as promising cell supportive and adipoinductive substrates [19], the previous fabrication methods had some limitations. In particular, digesting the DAT with the proteolytic enzyme pepsin made it necessary to incorporate alginate as an intermediate stabilizer to be able to synthesize DAT microcarriers that were structurally robust following crosslinking with rose bengal, riboflavin or glutaraldehyde [19,23]. However, the microcarriers generated with this approach had low porosity and poor stability below a size range of 900–950 μm, restricting the flexibility of the design. Moreover, it was recognized that the chemical crosslinking could potentially alter the bioactivity of the ECM. In the current work, we sought to address these limitations by developing a novel electrospraying method to generate pure DAT microcarriers that were stable without the need for chemical crosslinking. More specifically, our goal was to synthesize DAT microcarriers within a target size range of 350–500 μm for use as a dynamic cell culture platform for human ASC expansion [20].

Following methods development and microcarrier characterization, ASC proliferation on the refined DAT microcarriers was investigated within a low-shear spinner culture system in comparison to commercially-available Cultispher-S microcarriers. Cultispher-S microcarriers were selected for this study based on their natural composition (crosslinked porcine gelatin), macroporous architecture, and established history of use for mesenchymal stem cell (MSC) cultivation [11,24–26]. Subsequent analyses explored changes in the ASC immunophenotype and multilineage differentiation capacity following expansion on the DAT or Cultispher-S microcarriers relative to static 2-D tissue culture polystyrene (TCPS) controls. Initial trials focused on the adipogenic and osteogenic lineages, which have more well-established culture conditions for human ASCs, and the final trial was extended to include a pilot study assessing the chondrogenic potential of the ASCs expanded on the various substrates. An overview of the project scope is provided in Fig. 1.

2. Methods

2.1. Materials

Unless otherwise indicated, all chemical reagents for this work were obtained from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) and were used as received. Cultispher-S microcarriers were purchased from Percell Biolytica AB (Åstorp, Sweden).

2.2. Adipose tissue procurement and decellularization

Excised subcutaneous human adipose tissue was acquired from female patients undergoing routine breast and abdominal reduction surgeries at the Kingston General Hospital and Hotel Dieu Hospital in Kingston, ON, Canada or from the University Hospital in London, ON, Canada. All studies were reviewed and approved by the Research Ethics Boards at Queen's University (REB # CHEM-002-07) or The University of Western Ontario (REB # 105426). ASC isolation was performed within 2 h of collection following established methods, while adipose tissue to be further processed for decellularization was stored at −80 °C in a hypotonic buffer solution [27].

2.3. Non-chemically crosslinked DAT microcarrier fabrication

Human adipose tissue was decellularized using published protocols and stored in 70% ethanol at 4 °C [27]. In brief, the tissue was treated with a 5-day detergent-free extraction protocol involving freeze-thaw cell lysis in hypotonic buffer, multiple extractions in isopropanol to remove lipids, and enzymatic digestion with trypsin-EDTA, as well as DNase, RNase and lipase. For microcarrier fabrication, DAT was pooled from a minimum of 5 donors and lyophilized for 48 h. Following lyophilization, the DAT was finely minced (1–2 mm^3 pieces) and cryomilled using a Retsch® MM400 ball mill system (Haan, Germany). In brief, the minced DAT was transferred into a 25 mL milling chamber with two 10-mm stainless steel milling balls. The chamber was submerged in liquid nitrogen for 3 min and then milled continuously for 3 min at 1800 rpm to yield a fine DAT powder.

The milled DAT was subsequently digested with α-amylase and used to generate a homogeneous DAT suspension following methods adapted from Steven et al. [28,29]. In contrast to the small collagen fragments produced following proteolytic digestion with pepsin, the glycosidic enzyme α-amylase is postulated to cleave carbohydrate groups in the telopeptide regions of the collagen. This approach enables the preparation of a suspension of collagen fibrils in dilute acetic acid that can be used to synthesize stable microcarriers through electrospraying [28,30]. Briefly, 250 mg of cryomilled DAT was placed into a 15 mL conical tube and 5 mL of 0.22 M NaH_2PO_4 (pH 5.3) supplemented with 0.3% (w/w of dry tissue) α-amylase (Sigma, Cat. # 10065) was added. The DAT was digested at room temperature for 72 h under continuous agitation at 300 rpm, followed by centrifugation at 1500×*g* for 10 min. The processed DAT was rinsed twice with 10 mL of 5% NaCl solution and once with distilled water under vigorous agitation at room temperature for 10 min, with centrifuging at 1500×*g* for 10 min between each rinse. Finally, 0.2 M acetic acid was added to obtain a total volume of 5 mL, yielding a 50 mg/mL DAT suspension stock. The DAT suspension was agitated at 120 rpm and 37 °C overnight