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ScienceDirect
Biomaterials 28 (2007) 3834–3842
Biomaterials
www.elsevier.com/locate/biomaterials

Adipose tissue engineering with naturally derived scaffolds and adipose-derived stem cells

Lauren Flynn[a,b], Glenn D. Prestwich[c], John L. Semple[d,e], Kimberly A. Woodhouse[a,b,*]

[a]*Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ont., Canada, M5S 3E5*
[b]*Institute of Biomaterials and Biomedical Engineering, University of Toronto, 4 Taddle Creek Road, Toronto, Ont., Canada, M5S 3G9*
[c]*Center for Therapeutic Biomaterials and Department of Medicinal Chemistry, University of Utah, 419 Wakara Way, Suite 205, Salt Lake City, Utah 84108-12, USA*
[d]*Division of Plastic Surgery, Department of Surgery, University of Toronto, 100 College Street, Toronto, Ont., Canada, M5G 1L5*
[e]*Women's College Hospital, 76 Grenville Street, Toronto, Ont., Canada, M5S 1B2*

Received 28 February 2007; accepted 4 May 2007
Available online 16 May 2007

Abstract

A tissue-engineered adipose substitute would have numerous applications in plastic and reconstructive surgery. This work involves the characterization of the in vitro cellular response of primary human adipose-derived stem cells (ASC) to three dimensional, naturally derived scaffolds. To establish a more thorough understanding of the influence of the scaffold environment on ASC, we have designed several different soft tissue scaffolds composed of decellularized human placenta and crosslinked hyaluronan (XLHA). The cellular organization within the scaffolds was characterized using confocal microscopy. Adipogenic differentiation was induced and the ASC response was characterized in terms of glycerol-3-phosphate dehydrogenase (GPDH) activity and intracellular lipid accumulation. The results indicate that the scaffold environment impacts the ASC response and that the adipogenic differentiation of the ASC was augmented in the non-adhesive XLHA gels.
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Keywords: Adipose tissue engineering; Scaffold; Extracellular matrix; Hyaluronan; Stem cells

1. Introduction

Soft tissue augmentation is a major challenge for plastic and reconstructive surgeons. Subcutaneous adipose tissue loss is associated with numerous conditions including traumatic injury, oncologic resection and congenital birth defects [1]. The resultant scar tissue formation can lead to a loss of contour, as well as functional impairment, particularly if the defect occurs in proximity to a joint [2]. In 2005, approximately 5.4 million people underwent reconstructive surgery in the United States, with 3.9 million cases associated with tumor removal [3]. In addition, over 10.2 million cosmetic procedures were performed, including various forms of soft tissue augmentation with biological or synthetic fillers and implants [3].

The current treatment strategies for soft tissue reconstruction primarily involve tissue transplantation, including composite tissue flaps, or synthetic substitutes. The use of autologous tissues is associated with the creation of a donor site defect and, potentially, the need for multiple, complex and costly surgical procedures [4]. Moreover, the clinical outcome of adipose tissue transplantation is unpredictable, with graft resorption due to a lack of vascularization resulting in poor cosmesis and impaired functionality [5]. Synthetic implants are associated with immune rejection, implant migration and resorption, and a failure to integrate into the host tissues [6].

A tissue-engineered adipose substitute, that would promote regeneration, rather than repair, would be invaluable to plastic and reconstructive surgeons. The substitute should incorporate a biocompatible scaffold that

*Corresponding author. Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ont., Canada, M5S 3E5. Tel.: +416 978 3060; fax: +416 978 8605.
E-mail address: kas@chem-eng.utoronto.ca (K.A. Woodhouse).

0142-9612/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2007.05.002

defines the appropriate three-dimensional tissue architecture and promotes host integration and implant vascularization [7]. Ultimately, the construct should degrade as it is replaced by healthy host soft tissue. A number of different synthetic scaffolds have been investigated for adipose tissue engineering applications including polyethylene glycol diacrylate (PEDGA) [8], polyglycolic acid (PGA) [9], polyethylene terephthalate (PET) [10], poly(lactic-co-glycolic acid) (PLGA) [11] and polytetrafluoroethylene (PTFE) [12]. Naturally derived materials such as collagen [13], derivatives of hyaluronic acid [14], matrigel [15] and fibrin [16] have also been studied. There are many factors to consider when designing a scaffold including the mechanical properties, degradation characteristics, immunogenicity, cellular response to the material, ease of handling in the clinic and cost [17].

Adipose-derived stem cells (ASC) may be an ideal autologous cell source for adipose tissue engineering [18]. ASC are much more resistant to mechanical damage and ischemic conditions than mature adipocytes [19]. The cells, which can be readily harvested from excised human subcutaneous fat or liposuction samples, have been shown to proliferate rapidly and differentiate into mature adipocytes both in vitro and in vivo [20–22]. The development of methods to maintain the ASC differentiation potential in culture, while obtaining sufficient cell populations for transplantation, will be critical to the clinical application of these cells [23]. Further, the optimization of the growth and differentiation conditions to maximize stable adipose tissue formation is required [24].

With a view to develop a tissue-engineered adipose substitute, we are investigating the response of human ASC to scaffolds comprised of placental decellular matrix (PDM) and crosslinked hyaluronan (XLHA). By investigating several different scaffolds, it is possible to obtain a more thorough understanding of the impact of the matrix environment on the ASC. We previously developed a detergent and enzymatic extraction protocol to fully decellularize human placenta [25]. We believe that the PDM holds promise as a scaffold for adipose tissue engineering applications. The placenta is a rich source of human extracellular matrix (ECM) components that can be harvested without harm to the donor. Constructs derived from the ECM may mimic the native environment of the body, promoting normal cellular organization and behavior. Natural materials also have advantages in terms of ease of processing, biodegradability and biocompatibility [26]. Hyaluronan (HA) is a highly conserved glycosaminoglycan (GAG) that functions in matrix stabilization, cell signaling, adhesion, migration, proliferation and differentiation [27]. Incorporation of XLHA into the PDM scaffolds may improve the construct bulking properties and may influence cellular infiltration, differentiation and wound healing [28].

The primary objective of this research was to develop effective seeding protocols and in vitro culture conditions for the ASC with the PDM and XLHA. The cellular organization was characterized in each of the scaffolds. The influence of the scaffold environment on the differentiation of the ASC was also investigated.

2. Materials and methods

2.1. Materials

All chemicals used in the experiments, unless otherwise stated, were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Canada) and were used as received. Water was distilled and deionized using a Millipore Milli-RO 10 Plus filtration system at 18 MΩ resistance.

2.2. Cell culture

Primary cultures of human ASC were established according to the methods of Flynn et al. [25]. The ASC were isolated from freshly excised subcutaneous abdominal adipose tissue from patients undergoing elective surgery at Women's College Hospital, Toronto, Canada, following the ethical guidelines of the University of Toronto. The cells were plated in a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F-12 nutrient mixture (DMEM:Ham's F-12), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 0.1 mg/mL streptomycin. The growth medium was changed every 2–3 days. To passage the cells, cultures at 90% confluence were trypsin-released (0.25% trypsin/0.1% EDTA, Gibco, Burlington, Canada), washed, counted and re-plated in new flasks at 30,000 cells/cm^2. Passage 2 cells were used for the seeding experimentation.

2.3. ASC differentiation

To induce adipogenic differentiation, the cells were cultured in serum-free DMEM:Ham's F-12 supplemented with 15 mM $NaHCO_3$, 15 mM HEPES, 33 μM biotin, 17 μM pantothenate, 10 μg/mL transferrin, 100 nM cortisol, 66 nM insulin, 1 nM triiodothyronine (T3), 100 U/mL penicillin and 0.1 mg/mL streptomycin. For the first 72 h of differentiation, 0.25 mM isobutylmethylxanthine (IBMX) and 1 μg/mL of troglitazone were added to the differentiation medium [29].

2.4. Preparation of the PDM

Placentas were obtained, with informed consent, from normal-term Caesarian-section deliveries at Women's College Hospital, Toronto, Canada and were decellularized as previously described [25]. In brief, the perfusive and diffusive decellularization protocol involved treatment with hypotonic and hypertonic solutions, enzymatic digestion, and multiple detergent extractions. Research ethics board approval for this study was obtained from Women's College Hospital, Toronto, Canada (REB # 9918). Histological analysis was conducted on representative sections of the processed tissues to confirm the effectiveness of the extraction protocol.

Following decellularization, the PDM was sectioned into samples by mass, with each scaffold consisting of a 300 mg portion of the villous tree network. The scaffolds were decontaminated by three 30 min rinses in ethanol, re-hydrated with three washes in sterile phosphate buffered saline (PBS) and stored at 4 °C in sterile PBS supplemented with 100 U/mL penicillin and 0.1 mg/mL streptomycin.

The PDM scaffolds that were used in the confocal analyses were labeled with an amine reactive Alexa Fluor® 350 carboxylic acid, succinimidyl ester (Molecular Probes, Burlington, Canada) to facilitate visualization. Briefly, the dye was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL. This stock solution was diluted with 0.15 M $NaHCO_3$ (pH 8.3) to obtain a working concentration of 0.3 mg/mL. The PDM scaffolds were agitated in the dye solution for 1 h at room

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