Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 6167–6175
Biomaterials
www.elsevier.com/locate/biomaterials

# Matrix-mediated retention of adipogenic differentiation potential by human adult bone marrow-derived mesenchymal stem cells during ex vivo expansion

Joshua R. Mauney, Vladimir Volloch*, David L. Kaplan*

*Tufts University, Department of Biomedical Engineering & Chemical and Biological Engineering; Bioengineering & Biotechnology Center, 4 Colby Street, Medford, Massachusetts 02155, USA*

Received 3 January 2005; accepted 23 March 2005
Available online 23 May 2005

**Abstract**

Recently, cell-based approaches utilizing adipogenic progenitor cells for fat tissue engineering have been developed and reported to have success in promoting in vivo adipogenesis and the repair of defect sites. For autologous applications, human bone marrow-derived mesenchymal stem cells (MSCs) have been suggested as a potential cell source for adipose tissue engineering applications due to their ability to be isolated and ex vivo expanded from adult bone marrow aspirates and their versatility for pluripotent differentiation into various mesenchymal lineages including adipogenic. Due to the relatively low frequency of MSCs present within bone marrow, extensive ex vivo expansion of these cells is necessary to obtain therapeutic cell populations for tissue engineering strategies. Currently, utilization of MSCs for adipose tissue engineering is limited due to the attenuation of their adipogenic differentiation potential following extensive ex vivo expansion on conventional tissue culture plastic (TCP) substrates. In the present study, the ability of a denatured collagen type I (DC) matrix to preserve MSC adipogenic potential during ex vivo expansion was examined. Adipocyte-related markers and functions were examined in vitro in response to adipogenic culture conditions for 21 days in comparison to early passage MSCs and late passage MSCs ex vivo expanded on TCP. The results demonstrated significant preservation of the ability of late passage MSCs ex vivo expanded on the DC matrix to express adipogenic markers (fatty acid-binding protein-4, lipoprotein lipase, acyl-CoA synthetase, adipsin, facilitative glucose transporter-4, and accumulation of lipids) similar to the early passage cells and in contrast to late passage MSCs expanded on TCP. The ability of the DC matrix to preserve adipocyte-related markers and functions of MSCs following extensive ex vivo expansion represents a novel culture technique to expand functional adipogenic progenitors for tissue engineering applications.


*Keywords:* Mesenchymal stem cell; Adipose tissue engineering; Collagen

## 1. Introduction

Adipose tissue engineering represents an innovative approach for the development of novel clinical modalities for the repair and reconstruction of fat tissue defects. Each year a significant number of patients undergo a variety of medical procedures for both the repair of adipose tissue traumas including breast reconstructions following mastectomies [1,2] and cosmetic facial reconstructions of the cheek, chin, and jaw [3,4]. Currently, autologous adipose tissue represents a ubiquitous source of material for fat reconstructive therapies. However, this approach is restricted due to several limitations including a 40–60% reduction in graft volume following

*Corresponding authors. Tel.: +1 617 627 3251; fax: +1 617 627 3231.
*E-mail addresses:* vladimir.volloch@tufts.edu (V. Volloch), david.kaplan@tufts.edu (D.L. Kaplan).

0142-9612/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.biomaterials.2005.03.024

transplantation [5,6], limited proliferative capacity of mature adipocytes for ex vivo expansion [7], and extensive adipocyte damage encountered during harvest with conventional liposuction techniques [7].

Recently, cell-based approaches utilizing adipogenic progenitor cells for fat tissue engineering have been developed and reported to have considerable success in promoting in vivo adipogenesis and the repair of defect sites [8–14]. For autologous applications, human bone marrow-derived mesenchymal stem cells (MSCs) have been suggested as a potential cell source for adipose tissue engineering applications due to their ability to be isolated and ex vivo expanded from adult bone marrow aspirates and their versatility for pluripotent differentiation into various mesenchymal lineages including adipogenic [15–17], osteogenic [18,19] and chondrogenic [20]. However, despite the enormous potential of these cells to be used in fat tissue reconstruction, several limitations exist which hamper their utilization as a suitable cell source for adipose tissue regeneration.

Assays of colony forming units (CFU-f) of MSCs from human bone marrow aspirates routinely generate colony numbers at less than 0.1% of plated mononuclear cells (approximately $10^4$–$10^5$ cells per $cm^2$ of culture area), thus demonstrating the relatively low frequency of MSCs present within the marrow stroma [21–23] and the need for extensive population doublings to obtain substantial cell numbers for therapeutic applications. Previously, it has been reported that extensive ex vivo expansion of MSCs on conventional tissue culture plastic (TCP) substrates results in significant attenuation of their differentiation potential into various mesenchymal lineages including adipogenic [24–26]. Therefore, given the low level of MSCs isolated from human bone marrow aspirates and the gradual reduction of mesenchymal differentiation potentials during ex vivo expansion on conventional TCP, new methods of ex vivo expansion of MSCs which can preserve their function are needed.

Previously, we demonstrated the ability of a denatured collagen type I DC matrix to preserve functions associated with replicative cellular aging and in vitro osteogenic differentiation potential of human MSCs following ex vivo expansion in comparison to cultivation on TCP [24]. Based on this previous work and given the reported effects MSC–ECM interactions have on the development and differentiation of MSCs into other mesenchymal lineages [27–33], we investigated the hypothesis that ex vivo expansion of MSCs on a DC type I matrix would result in the preservation of their adipogenic differentiation potential in vitro in comparison to cultivation on TCP.

## 2. Materials and methods

### 2.1. Preparation of collagen matrices

Sterile rat tail-derived collagen type I (cat. #1179179, Roche, Indianapolis, IN) was dissolved at 0.5 mg/ml in 0.1% acetic acid and denatured by incubation at 50 °C for 12 h within a recirculating water bath. Conditions for complete denaturation of the type I collagen were chosen based on previous experiments where circular dichroism measurements demonstrated a thermal transition within the collagen at approximately 45 °C [34]. To prepare films, 1.5 ml of 0.5 mg/ml collagen solution was added to 9.6 $cm^2$ wells within a 6-well tissue culture dish (Nunclon, Rochester, NY) and dried under a laminar flow hood for 48 h. Tissue culture dishes in the absence of collagen were treated similarly but with a solution of 0.1% acetic acid alone. Both TCP and collagen coated dishes were washed with phosphate buffered saline (PBS) prior to cell seeding to remove any residual acetic acid solution.

### 2.2. MSC isolation and Ex vivo expansion

Human MSCs were obtained from commercially available bone marrow aspirates from a single male donor of 25 years of age (Clonetics-Poietics, Walkersville, MD) using methods previously reported [35]. Whole bone marrow aspirates were plated at 8–10 μl aspirate/$cm^2$ on 185 $cm^2$ tissue culture plates and cultivated until confluency (~12–14 days) in 40 ml of expansion medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin, 0.1 mM nonessential amino acids, and 1 ng/ml of basic fibroblast growth factor (bFGF) (Life Technologies, Rockville, MD). MSCs were maintained in a humidified tissue culture incubator at 37 °C with 5% carbon dioxide. MSCs were selected based on their ability to adhere to the TCP; non-adherent hematopoietic cells were removed during medium replacement after ~5 days in culture. Medium was changed twice per week thereafter. First passage (P1) MSCs were subsequently detached using 0.25% trypsin/1 mM EDTA, replated at $5 \times 10^3$ cells/$cm^2$, and cultured until confluency to generate second passage (P2) MSCs. P2 MSCs designated, "Early Passage Cells," were then frozen in liquid nitrogen in DMEM consisting of 10% FCS and 8% dimethyl sulfoxide or further expanded in expansion medium as described above on TCP to passage 8 (P8) or on the DC matrix to passage 10 (P10). Each passage corresponded to approximately 3 population doublings between P2 to P10 for expansion on both TCP and the DC matrix. MSCs cultivated on the DC matrix reached P10 at the same time MSCs on TCP had undergone 8 passages due to their higher proliferation