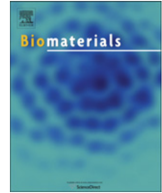
Biomaterials 34 (2013) 1004–1017

Contents lists available at SciVerse ScienceDirect

Biomaterials

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



Bone grafts engineered from human adipose-derived stem cells in dynamic 3D-environments

Heidi A. Declercq [a], Tamara De Caluwé [a], Olga Krysko [b], Claus Bachert [b], Maria J. Cornelissen [a,*]

[a] Tissue Engineering Group, Department of Basic Medical Sciences, Ghent University, De Pintelaan 185 (6B3), 9000 Ghent, Belgium
[b] Upper Airways Research Laboratory, University Hospital Ghent, De Pintelaan 185 (Medical Research Building), 9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 31 August 2012
Accepted 22 October 2012
Available online 10 November 2012

Keywords:
Tissue engineering
Adipose tissue derived stem cell
Microcarrier
Scaffold
Top-down
Bottom-up

ABSTRACT

Modular tissue engineering (TE) is a promising alternative to overcome the limits in traditional TE. In the present study, adipose tissue derived stem cells (ADSC)-laden microcarriers are used as building blocks (microtissues) that self-assemble into macrotissues in a bottom-up approach. These bone grafts were compared with a classical top-down approach (scaffolds). This concept was compared with bone marrow derived stem cells (BMSC) as cell source. Cells were immunophenotypically analyzed, followed by 2D/3D osteogenic differentiation in static/dynamic conditions. The bone graft quality was evaluated by (immuno) histochemistry and gene expression. After 6 weeks of dynamic culturing, scaffolds were highly colonized although not in the center and the osteogenic gene expression was higher in contrast to static cultures. A cell-to-microcarrier ratio of 5×10^6 cells-0.09 g microcarriers leaded to aggregate formation resulting in microtissues with subsequent macrotissue formation. ADSC/BMSC on scaffolds showed a downregulation of Runx2 and collagen I, demonstrating the end-stage, in contrary to microcarriers, where an upregulation of Runx2, collagen I together with BSP and osteocalcin was observed. This paper showed that high quality bone grafts (2 cm^3) can be engineered in a bottom-up approach with cell-laden microcarriers.
© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Since Langer and Vacanti introduced tissue engineering (TE) as a multidisciplinary field applying the principles of biology and engineering to the development of functional substitutes for damaged tissue, this multidisciplinary science has been evolved [1]. Traditional TE strategies typically employ a "top-down" approach by seeding cells onto pre-fabricated three-dimensional (3D) porous scaffolds followed by *in vitro* cultivation. In top-down approaches, the cells are expected to populate the scaffold and create the appropriate extracellular matrix (ECM) [2]. However, a concentration gradient of substrate molecules across the outer surface and inner center of the scaffold exists due to an intrinsic slow diffusion process. Preferential cell proliferation and ECM deposition on the outer region of engineered constructs became apparent. The presence of this thin tissue layer at the surface further exacerbates the situation of mass transfer limit, leading to nutrition deprivation and eventually cell death at the core [3]. Several strategies aiming to address these issues are under investigation.

Dynamic culturing in bioreactors can overcome the diffusion limitation to some extent by generating a continuous fluid flux to promote the mass transport into tissue constructs. Several bioreactor-based systems have been tested extensively and have been reported to successfully improve the dimension, colonization and cellular differentiation of tissue constructs [4]. However, despite diverse bioreactors, most of the engineered constructs are still limited within several millimeters to 1 cm in dimension [5,6]. As a result, these engineered tissue constructs have been largely restricted representing a hurdle for repairing macroscopic tissue defects, such as in bone injury [3].

Moreover, upon implantation into the patient, the grafts' survival depends on invasion by the recipient's blood vessels. As the generation of clinically relevant-sized constructs is critically hampered by limited vascularization, several approaches have been explored to stimulate vascularization of engineered tissues [7]. One challenging approach is by printing tissues with multiple cell types [8].

There is a growing awareness of tissue engineers that unless *in vivo* developmental processes are recapitulated *in vitro* [9], the quality of tissue engineered constructs is inadequate. How close tissue engineers can recapitulate and capture the most essential structure–function features of normal natural human tissues and organs, and how far they must try to imitate developmental histogenesis, morphogenesis and organogenesis, is still under debate [10]. Nevertheless, the biomimetics of *in vivo* development has been proposed as a new direction of TE [11,12].

* Corresponding author.
E-mail address: ria.cornelissen@ugent.be (M.J. Cornelissen).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.10.051

Most living tissues are composed of repeating units on the scale of hundreds of microns, which are ensembles of different cell types with well defined 3D microarchitectures and tissue-specific, functional properties [13]. Tissue and organs are self-organizing systems: cells and microtissues can undergo biological self-assembly and self-organization without human intervention [10].

With the conception that *in vivo* developmental processes should be recapitulated *in vitro* and the desire to create more complex tissues, TE techniques are beginning to focus on building modular microtissues with repeated functional units [2,9,14]. This bottom-up approach by assembling modular tissues aims to generate 3D constructs with no limitation in size [3]. Small building blocks (microtissues) are prepared to maintain high cell viability and subsequently, (self-)assemble into large-scale constructs (macrotissue). Macrotissues are envisaged to be permissive for vascularization *in vivo* due to the macroporous structures generated during the assembling process enabling cell infiltration and ensuring long-term tissue survival [6].

A bottom-up approach relies on the self-assembly or directed assembly of a scaffold from smaller components or modules [15,16]. Although formation of microtissues may also be performed by co-culturing pre-formed cell aggregates [16], it is reported that scaffold-free aggregates can only reach millimeter scale [6].

To prepare modular tissues, microcarriers are representing promising building blocks. Microcarriers were originally used as carriers for *in vitro* cell expansion [17–19] and recently serve as cell delivery systems to regenerate tissue at the site of trauma [20]. Microcarriers have been applicated across a wide range of disciplines within cartilage [21,22], adipose [23,24], skin [13,25] and bone [3,22,24,26–32] TE. Mature cells (fibroblasts [6,22], osteoblasts [26]) and stem cells from different origins (bone marrow [17,26,29], amnion [3], adipose tissue [24,31,32], embryonic stem cells [27,33]) have been expanded and differentiated successfully on microcarriers. Cell-laden microcarriers can either be directly injected [23], incorporated into a larger biomaterial [22,24,26–30,32] or assembled in a bottom-up approach [3,6,13,25] and subsequently transplanted at the site of trauma. Most importantly, small volume, high quality tissue constructs should be engineered *in vitro* before the assembling *in vitro* or *in vivo* into larger tissue constructs upon implantation. However, the quality of the bone grafts formed in a bottom-up approach are never compared with classical top-down approaches.

Human adipose tissue derived stem cells (ADSC) have been shown to have multilineage potential and were selected because of the ease of access of adipose tissue, high cell yield per unit tissue volume, high cell proliferation and the possibility of autologous use [34–37]. Moreover, the frequency of CFU-alkaline phosphatase in digested adipose tissue is 500 fold more than found in bone marrow [38].

In the present study, we hypothesized that the engineered bone grafts by a bottom-up approach have an equal/superior quality than engineered bone grafts by a top-down approach in static or dynamic culture conditions. ADSC were seeded onto macroporous CultiSpher-S microcarriers, followed by osteogenic differentiation leading to microtissues and subsequent macrotissue formation by self-assembly in a spinner flask. The optimum cell-to-microcarrier ratio was determined necessary to induce osteogenic differentiation. This bottom-up approach was compared with ADSC seeded on 3D scaffolds in a top-down approach and cultured statically or dynamically. In addition, we compared the osteogenic capacity of ADSC with the golden standard BMSC in both approaches.

2. Materials and methods

2.1. Culture and expansion of human adipose- and bone marrow derived stem cells

Human adipose derived stem cells (ADSC) were isolated from lipoaspirates according to the manufacturer (CryoSave, Belgium). Human bone marrow derived stem cells (BMSC) were purchased from Lonza (PT-2501) (Belgium). The cells were plated at a density of 5000 cells/cm^2 in MesenPRO RS™ (Gibco, Invitrogen) and expanded until P3-6 that were used for all experiments performed in our study.

2.2. Flow cytometry

After trypsinization, cells (up to 1×10^6) were washed in PBS (pH 7.2) containing 0.5% bovine serum albumin (Roche, Cat. 10735086001) and 2 mM EDTA (Promega, Cat. V4231) (buffer) and resuspended in 100 µl buffer. Cells were then incubated for 10 min in the dark (4 °C) with the MSC Phenotyping Cocktail (CD73-APC, CD90-FITC, CD105-PE, CD14-PerCP, CD20-PerCP, CD34-PerCP and CD45-PerCP) or with the Isotype Control Cocktail (Miltenyi Biotec B.V.). Dead cells were excluded from the analysis. The fractions were analyzed by flow cytometry using the FACS Canto II (Becton Dickinson). All data were corrected for autofluorescence and for unspecific bindings using isotype controls.

2.3. 2D osteogenic differentiation

To test their osteogenic capacity, ADSC and BMSC were plated at a concentration of 3000 cells/cm^2 (LD) and 23,000 cells/cm^2 (HD) on Thermanox® coverslips (Nunc, Thermo Scientific) in 24-well culture plates (Greiner, Bio-One) and cultured in osteogenic medium consisting of α-MEM (Gibco, Invitrogen), fetal bovine serum (Gibco, Invitrogen) (10%), dexamethasone (Sigma–Aldrich) (100 nM), L-ascorbic acid 2-phosphate (Sigma–Aldrich) (100 µM), β-glycerophosphate (Sigma–Aldrich) (10 mM) and penicillin–streptomycin (10.000 U/ml–10.000 µg/ml) (Gibco, Invitrogen) (0.5%). After 1–4 weeks of culture, the cells were visualized by phase-contrast microscopy and assessed for alkaline phosphatase and Von Kossa staining.

2.4. 3D colonization and osteogenic differentiation in a top-down versus bottom-up approach

Fig. 1 depicts the overall experimental procedure. In Table 1, the characteristics of the scaffolds and microcarriers are given.

2.4.1. Top-down approach: scaffold seeding and colonization

Before cell seeding, the collagen scaffolds (BD™ Three Dimensional Collagen Composite Scaffold, Cat No. 354613, BD Biosciences) were immersed in serum-free α-MEM medium in Eppendorf tubes. Air was removed from their pores by generating vacuum with a 20 ml syringe equipped with an 18-gauge needle. The scaffolds were left in medium on a gyratory shaker (37 °C, 70 rpm). After 24 h, the scaffolds were placed into 96-well tissue culture dishes (for suspension culture) (Greiner, Bio-One).

The scaffolds were seeded with 1×10^6 ADSC respectively BMSC/40 µl/scaffold and incubated for 4 h. Medium (160 µl) was added to each well and the seeded scaffolds were further incubated overnight to allow cell attachment. After 24 h, scaffolds were placed in a 12 well plate (static culture) or on a needle (3 scaffolds/needle) in a 5 ml Erlenmeyer flask (VEL) (dynamic culture) (Fig. 1). Osteogenic culture medium (3 ml) was added and the cell/scaffold constructs were cultured for 40 days on a gyratory shaker at a constant rate of 70 rpm (5% CO_2/95% air, 37 °C). A variation on cell seeding was performed by seeding 0.6×10^6 respectively 1.2×10^6 cells/scaffold.

2.4.2. Bottom-up approach: microcarrier seeding and colonization

0.09 g of dry macroporous gelatin-based CultiSpher-S microcarrier beads (diameter 130–380 µm) (Percell Biolytica) were hydrated in calcium and magnesium-free PBS (pH 7,4) for 1 h at room temperature and autoclaved. The PBS was replaced by culture medium (α-MEM supplemented with 10% FBS). The CultiSpher-S microcarriers were divided over 6 wells of a 12-well suspension culture plate (Greiner BioOne), and 166 000 respectively 833 333 cells were placed into each microcarrier-containing well (until a final concentration of 1×10^6 respectively 5×10^6 cells/0.09 g microcarriers; low density (LD) respectively high density (HD)). Cells were allowed to attach to the microcarriers in static conditions for 3 days in a humidified 5% CO_2 incubator, without medium renewal (Fig. 1).

To allow cell ingrowth and efficient colonization, the cell-loaded microcarriers were transferred to a 50-ml Erlenmeyer flask (Schott-Duran) (working volume 15 ml) and placed on a gyratory shaker (Gerhardt, Laboshake) to culture under dynamic conditions (stirring speed 70 rpm) in a 5% CO_2 atmosphere at 37 °C (Fig. 1). The cells were cultured in osteogenic medium on the microcarriers for 40 days.

2.5. Analysis

2.5.1. Alkaline phosphatase staining

Cultures grown on Thermanox coverslips were fixed with acetone (−20 °C, 5 min), washed with distilled water and let to dry (30 min). The cultures were incubated (30 min, room temperature) with BCIP/NBT liquid substrate system (Sigma–Aldrich), washed, dehydrated and mounted with DPX (Fluka Biochemica, Sigma–Aldrich).

Download English Version:

https://daneshyari.com/en/article/10229285

Download Persian Version:

https://daneshyari.com/article/10229285

Daneshyari.com