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Review

Bone tissue engineering with scaffold-supported perfusion co-cultures of human stem cell-derived osteoblasts and cell line-derived osteoclasts

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ABSTRACT

The aim of this study was to investigate the effects of perfusion co-culture on bone tissue regeneration *in vitro*. Human mesenchymal stem cell (hMSC)-derived osteoblasts and THP-1 human acute monocytic leukemia cell line-derived osteoclasts were dynamically co-cultured on the chitosan-hydroxyapatite (chitosan-HA) superporous hydrogel. In the perfusion bioreactor set-up, bidirectional recycled perfusion with 6 mL/h flow rate was applied and cell seeding was realized in two-steps with a preculture time of 12 days. Outcomes were compared to static cultures. Two-step cell seeding and long preculture ensured good adhesion of cells on the scaffold surface and minimized cell loss during perfusion. The perfusion bioreactor enhanced mass transfer throughout the scaffolds, thus increased cellularity and provided flow-induced mechanical stimulation for osteoblastic and osteoclastogenic differentiation. The results indicated that osteoblast and osteoclast co-cultures in perfusion bioreactors provide a one-step approach to *in vitro* bone tissue engineering and emphasized the significance of enhanced mass transfer and mechanical stimulation on cellular activity and differentiation.

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1. Introduction

Bone is a metabolically active tissue that constantly undergoes a highly coordinated remodeling process which serves to ensure structural integrity, to adjust bone architecture and to repair micro-damages through well-balanced resorption and formation processes. During bone remodeling, close communication between osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) is involved [1,2].

A promising strategy of bone tissue engineering is to repair bone defects using scaffolds that undergo remodeling [3,4]. Therefore the feasibility of tissue engineering scaffolds to reproduce organized bone-like matrix and co-cultures of osteoblasts and osteoclasts have been studied [3–8]. The long term effects of osteoblast and osteoclast co-culture on the remodeling of silk films have been investigated by Hayden et al. and the results demonstrated that extracellular matrix (ECM) deposition was significantly increased in co-cultures when compared to monocultures of osteoblasts and osteoclasts [9].

Although static co-culture studies have had useful results, it is still not clear how co-cultures respond to a dynamic environment. Static approaches neglect the role of mechanical stimulation arising from physiologically relevant conditions in normal development and homeostasis of tissues [10]. In bone tissue engineering studies, various types of bioreactors have been used to stimulate cells in a flow-dependent manner [11–14]. The most commonly used approach is the perfusion bioreactor, which generally consists of a pump and a chamber joined by tubing [15]. In perfusion systems, the scaffold is fixed in the chamber to hold its position across the flow, so that the medium can be perfused throughout the scaffold. Perfusion bioreactors overcome problems present in static culture, such as poor nutrient and waste transport, limited cell viability, and lack of ECM formation in the center of 3D scaffolds. Perfusion culture also provides biomechanical cues that regulate a wide range of cellular events especially required for correct cellular differentiation and function [13,16]. Biomechanical stimulation is regarded as an important part of bone remodeling, however, the literature related to the use of perfusion bioreactors for the co-culture of osteoblasts and osteoclasts is limited to only one study by Papadimitropoulos et al. as an *in vitro* model to mimic the process of bone turnover in a perfusion bioreactor by the use of a 3D osteoblastic-osteoclastic-endothelial cell co-culture system [8].

In the presented study, human mesenchymal stem cell (hMSC)-derived osteoblasts and THP-1-derived osteoclasts were co-cultured on chitosan-hydroxyapatite superporous hydrogel composites (chitosan-HA SPHCs) in a perfusion bioreactor which provided recycled bidirectional flow with 6 mL/h. The experiments were performed by using a two-step cell seeding strategy with a 12-day preculture time period. The tissue constructs were characterized with respect to cell viability, bone related ECM gene expressions, and morphology.

2. Material and methods

2.1. Materials

Chitosan (minimum 85% degree of deacetylation, medium molecular weight) was purchased from Sigma (Germany). Glyoxal (40%, v/v water solution, Aldrich, Germany) and $NaHCO_3$ (Fisher Scientific, USA) were used as crosslinker and gas-blowing agent, respectively. Acetic acid, for pH-control, was purchased from Riedel de Haen (USA). Hydroxyapatite (HA) beads with particle size between 55 and 110 μm (Science Application Industries, France) were used both as bioactivation and reinforcing agents for bone tissue formation. In cell-culture studies, unless otherwise noted, all reagents were purchased from Life Technologies (Grand Island, NY).

2.2. Cell isolation and expansion

Human mesenchymal stem cells (hMSCs) were isolated from bone marrow aspirate (Lonza, Walkersville, MD) as described previously [17]. Aspirate from a male donor under 25 years old was mixed with proliferation medium including Minimum Essential Medium alpha modification (α-MEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) antibiotic/antimycotic (10,000 units/mL of penicillin, 10,000 μg/mL of streptomycin, and 25 μg/mL of amphotericin B), and 1% (v/v) non-essential amino acids (NEAA). The cells in flasks were cultured at 37 °C with 5% CO_2 in a humidified environment and in order to allow hMSCs to adhere, the flasks were rocked every day. Proliferation medium was added every three-four days until hMSCs reached 80% confluence. Human MSCs at passage two were tested for osteogenic and adipogenic differentiation potential and it was determined that they could differentiate into both osteoblasts and adipocytes (supplementary data). THP-1 human acute monocytic leukemia cell line cells (ATCC#TIB-202) were cultured in proliferation medium (RPMI 1640, 10% v/v FBS and 1% v/v antibiotic/antimycotic) at 37 °C with 5% CO_2 in a humidified environment. Cell density was not allowed to exceed 1×10^6 cells/mL and the medium was changed every two to three days.

2.3. Scaffold fabrication

Chitosan-HA SPHCs were fabricated as described previously [11]. Briefly, 2% (w/v) chitosan solution was prepared by dissolving chitosan in 0.1 M acetic acid. HA beads (100 mg/mL), glyoxal (2 μL/mL) and $NaHCO_3$ (3 mg/mL) were added to this solution and 2 mL of this mixture was poured into each well of a 24-well tissue culture polystyrene (TCPS) dish. Finally, cross-linking reaction and foaming took place at the same time in a microwave synthesis oven (Milestone, USA) with 90 W for 90 s. The samples were frozen at −20 °C overnight and lyophilized at −80 °C for two days (Christ, Germany). For stabilization, dried hydrogels were placed in ethanol overnight and dried again in a freeze-drier for another 24 h.

2.4. Perfusion bioreactor set-up

The schematic of the perfusion bioreactor is given in Fig. 1a. In the perfusion bioreactor set-up, P3D-6 chambers (EBERS Medical Technology SL, Spain), which are designed to hold porous cylindrical scaffolds, were utilized. These chambers provide several advantages regarding to the ease of scaffold insertion or removal, *i.e.* transparency for checking against contamination, luer connections for leak-proof working and ready to use sterile packing. In the perfusion bioreactor system, a multi-channel programmable syringe pump with eight channels (NE-1800, New Era, Farmingdale, NY) was used (Fig. 1b). The flow rate was adjusted as 6 mL/h and perfusion is set to be bidirectional with medium recycle in every