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Co-culture of osteoblasts and chondrocytes modulates cellular differentiation in vitro

Jie Jiang [a], Steven B. Nicoll [b], Helen H. Lu [a,*]

[a] *Biomaterials and Interface Tissue Engineering Laboratory, Department of Biomedical Engineering, Columbia University, New York, NY 10027, USA*
[b] *Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA*

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Abstract

Biological integration of cartilage grafts with subchondral bone remains a significant clinical challenge. We hypothesize that interaction between osteoblasts and chondrocytes is important in regenerating the osteochondral interface on tissue-engineered osteochondral grafts. We describe here a sequential co-culturing model which permits cell–cell contact and paracrine interaction between osteoblast and chondrocytes in 3-D culture. This model was used to determine the effects of co-culture on the phenotypic maintenance of osteoblasts and chondrocytes. It was found that while chondrocytes synthesized a type II collagen and glycosaminoglycan (GAG) matrix, GAG deposition was significantly lower in co-culture. Alkaline phosphatase activity was maintained in osteoblasts, but cell-mediated mineralization in co-culture was markedly lower compared to osteoblast controls. These results collectively suggest that interactions between osteoblasts and chondrocytes modulate cell phenotypes, and the importance of these interactions on osteochondral interface regeneration will be explored in future studies.

Keywords: Co-culture; Osteoblasts; Chondrocytes; Osteochondral; Micromass

Osteoarthritis is the most common form of arthritis, with over 20 million Americans suffering from this degenerative condition [1]. During routine physical activities, articular cartilage breaks down and its continued wear exposes the subchondral bone to the synovial environment. If left untreated, the sustained physiological loading results in symptomatic pain, swelling, and eventually the loss of joint motion. Due to the limited regenerative capacity of adult articular cartilage, surgical intervention is required to treat osteoarthritis [2].

Existing treatment options for the repair of focal lesions and damage to the articular surface have had limited success [2]. Traditional methods such as debridement, subchondral bone drilling, and microfracture typically result in fibrocartilage formation at the defect site [2]. Other methods, such as autologous cell or tissue transfer via periosteal grafts and tissue adhesives, have poor long-term outcomes and are associated with severe side effects [3]. Autologous chondrocyte implantation involves the isolation and expansion of chondrocytes in culture and injection of these cells under a periosteal flap at the defect site [3]. Wider application of this procedure is restricted by the lack of inter-patient consistency, as evidenced by a recent long-term in vivo animal study which revealed little difference from the control group [4]. None of the existing cartilage grafting methods have been able to demonstrate stable integration either with the surrounding cartilage tissue or with the subchondral bone. Thus, the regeneration of the native osteochondral interface remains a significant clinical challenge.

Additional treatments for cartilage focal lesions with promising initial results are autologous osteochondral grafting procedures such as Mosaicplasty and Osteochondral Autograft Transfer System. In these procedures, osteochondral columns from a non-load bearing region

* Corresponding author. Fax: +1 212 854 8725.
E-mail address: hl2052@columbia.edu (H.H. Lu).

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are surgically removed and transplanted into the defect site. While these grafts have shown good clinical results, they are limited by supply and donor site morbidity [2]. The ideal graft for the treatment of articular cartilage defects should be mechanically functional and able to structurally and functionally integrate with the host tissue [5]. Due to limitations with current treatment options for cartilage defects, there is a sustained interest in tissue-engineered solutions for cartilage repair and regeneration [6–15]. Several groups have reported on the development of tissue-engineered osteochondral grafts, which may have application in a clinical setting [16–23]. The long-term success of such grafts will depend largely on their ability to integrate with host tissue and to regenerate the osteochondral interface.

Currently, it is not well understood how the interface between bone and cartilage can be regenerated during cartilage repair. We hypothesize that the interaction between osteoblasts and chondrocytes is important in initiating the events which lead to interface regeneration. The potential of osteoblasts to regulate chondrocyte metabolism was first reported by Lacombe-Gleize et al. using a monolayer, trans-well insert co-culture model. It was found that osteoblasts up-regulated the mitotic potential of chondrocytes through TGF-β1 secretion [24]. Further interpretation of these results was limited by the fact that chondrocytes in this study were grown in monolayer instead of 3-D culture. A significant challenge in osteoblast–chondrocyte co-culture is that, unlike osteoblasts which are anchorage-dependent, chondrocytes de-differentiate and lose their phenotype in monolayer cultures [25].

Therefore, to test our hypothesis that chondrocytes and osteoblasts will modulate each other's phenotypic responses under physiological conditions, we have designed a simple and reliable 3-D co-culture model to study the interaction between osteoblasts and chondrocytes. In this co-culture model (Fig. 1), a sequential culturing protocol is used whereby chondrocytes are initially seeded in high density micromass cultures, after which osteoblasts are introduced and allowed to directly adhere to the chondrocyte micromasses. This novel co-culture model also mimics the initial point of contact between a tissue-engineered cartilage construct populated with chondrocytes and the subchondral bone upon implantation. The objectives of this study were (1) to validate the co-culture model; (2) to evaluate the effects of co-culture on the growth and phenotypic maintenance of osteoblasts and chondrocytes. It is anticipated that osteoblasts and chondrocytes can be co-cultured together in direct physical contact while maintaining their respective phenotype. Results of this study will support the efforts to elucidate the mechanism of regenerating the osteochondral interface on tissue-engineered grafts.

Materials and methods

Cells and cell culture. Primary bovine articular chondrocytes and osteoblasts were utilized in this study. Bovine articular chondrocytes were isolated from full-thickness articular cartilage of carpometacarpal joints of 2- to 6-month-old calves from a local abattoir (Green Village, NJ). Articular cartilage tissue was excised from the exposed carpometacarpal joint surface and minced into small pieces. Chondrocytes were obtained following serial enzymatic digestions of the cartilage tissue by modifying the method described by Mauck et al. [11]. The tissue was first digested for 1 h in a 0.25% protease (Calbiochem, San Diego, CA) solution dissolved in Dulbecco's modified Eagle's medium (DMEM, Cellgro-Mediatech, Herndon, VA), followed by 4 h of 0.05% collagenase (Sigma, St. Louis, MO) digestion. The isolated chondrocytes were maintained in fully supplemented medium, which consisted of DMEM with 10% fetal bovine serum (FBS, Cellgro-Mediatech), 1% non-essential amino acids, and antibiotics (100 U/mL penicillin, 100 mg/mL streptomycin, Cellgro-Mediatech).

Primary bovine osteoblast cultures were established from explant cultures of long bone fragments taken from the same joint used for

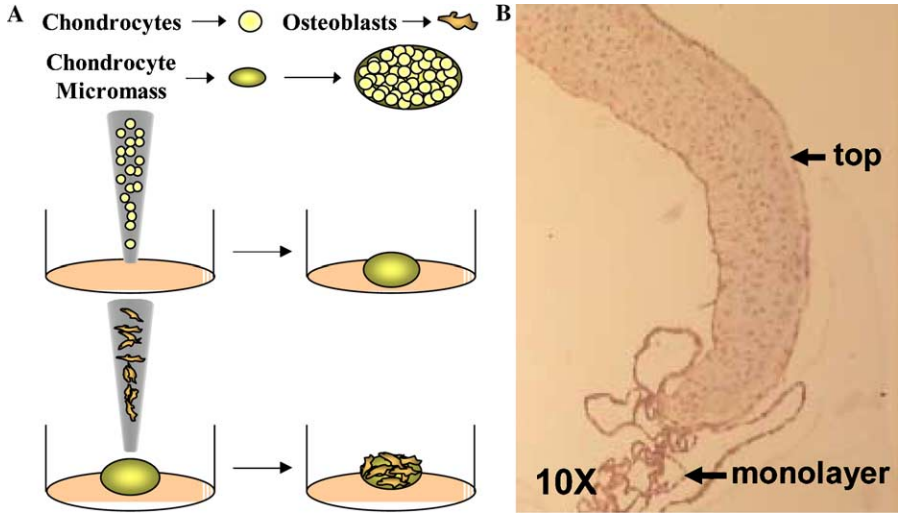

Fig. 1. (A) Schematic of the co-culture model of osteoblasts and chondrocytes. The chondrocyte micromass is first formed by plating a high density chondrocyte suspension (2×10^7 cells/mL) on pre-sterilized coverglass. The chondrocytes were allowed to adhere for 1 h, after which a drop of bovine osteoblast suspension (5×10^6 cells/mL) was added to the chondrocyte micromass. The osteoblasts were then allowed to adhere on the micromass for an another hour prior to the addition of media. (B) Cross-sectional H&E staining to visualize cell distribution.