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The effect of devitalized trabecular bone on the formation of osteochondral tissue-engineered constructs

Eric G. Lima [a,d], Pen-hsiu Grace Chao [b], Gerard A. Ateshian [a,e], B. Sonny Bal [c], James L. Cook [c], Gordana Vunjak-Novakovic [a], Clark T. Hung [a,*]

[a] Department of Biomedical Engineering, Columbia University, 351 Engineering Terrace MC8904, 1210 Amsterdam Avenue, New York, NY 10027, United States
[b] Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan
[c] Comparative Orthopaedic Laboratory, University of Missouri, Columbia, MO, United States
[d] Materials Characterization Laboratory, Cooper Union, 51 Astor Place, New York NY, 10003, United States
[e] Department of Mechanical Engineering, Columbia University, Department of Mechanical Engineering, 500 West 120th Street, Mail Code 4703, 220 S.W. Mudd

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ABSTRACT

In the current study, evidence is presented demonstrating that devitalized trabecular bone has an inhibitory effect on *in vitro* chondral tissue development when used as a base material for the tissue-engineering of osteochondral constructs for cartilage repair. Chondrocyte-seeded agarose hydrogel constructs were cultured alone or attached to an underlying bony base in a chemically defined medium formulation that has been shown to yield engineered cartilaginous tissue with native Young's modulus (E_Y) and glycosaminoglycan (GAG) content. By day 42 in culture the incorporation of a bony base significantly reduced these properties ($E_Y = 87 \pm 12$ kPa, GAG $= 1.9 \pm 0.8$%ww) compared to the gel-alone group ($E_Y = 642 \pm 97$ kPa, GAG $= 4.6 \pm 1.4$%ww). Similarly, the mechanical and biochemical properties of chondrocyte-seeded agarose constructs were inhibited when co-cultured adjacent to bone (unattached), suggesting that soluble factors rather than direct cell–bone interactions mediate the chondro-inhibitory bone effects. Altering the method of bone preparation, including demineralization, or the timing of bone introduction in co-culture did not ameliorate the effects. In contrast, osteochondral constructs with native cartilage properties ($E_Y = 730 \pm 65$ kPa, GAG $= 5.2 \pm 0.9$%ww) were achieved when a porous tantalum metal base material was adopted instead of bone. This work suggests that devitalized bone may not be a suitable substrate for long-term cultivation of osteochondral grafts.

1. Introduction

Articular cartilage is a specialized connective tissue that bears load and reduces friction across moving joints. It is composed of an extracellular matrix that contains no nerves or blood vessels and relatively few cells (5% volume). When damaged, articular cartilage does not heal, but instead often degenerates further, leading to pain and loss of function [1]. Due to the prevalence of osteoarthritis (OA) and damage to articular cartilage, coupled with this poor intrinsic healing response, there is a great demand for clinical intervention, usually in the form of a highly invasive non-biological prosthetic (such as total joint arthroplasty). Currently, the most common biological alternative to arthroplasty entails the transplantation of healthy osteochondral autografts (cartilage along with some of the underlying bone) from a non-load bearing region to the affected site [1–5]. Osteochondral grafts are better anchored than chondral-only grafts and are less likely to be displaced by shearing forces within the joint. While these autologous grafting procedures are promising, they are limited both by the amount of tissue available and donor-site morbidity associated with its harvest. Tissue-engineering strategies, if successful, would alleviate these problems by creating replacement tissues of the proper size and shape without concurrent damage to other regions of the patient's body.

Osteochondral constructs are designed to be press-fit into pre-drilled cavities in the damaged joint, replacing the host cartilage above while anchoring to the bone below. There are a great variety of tissue-engineering approaches to form osteochondral constructs, but typically they all entail the use of some form of tissue-scaffold.

The most important characteristics of the scaffold are its mechanical properties, its porosity, and its biocompatibility. Specifically it must be able to properly bear and transfer loads to the surrounding tissue without being crushed. Since this environment also includes shear forces, the interface strength between the cartilage and bone regions should also be considered. The scaffolds must also have the proper porosity to allow for cell infiltration and nutrient transport and be biocompatible to mitigate immunogenic

* Corresponding author.
E-mail address: cth6@columbia.edu (C.T. Hung).

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issues while allowing engineered tissue to develop functional properties. The two regions of an osteochondral construct can be formed from a single continuous scaffold [6–8] or from two independent scaffolds that have been joined together [9] with each region specialized to promote chondrogenic or osteogenic development.

Although this development process would ideally take place entirely within the defect site [10–15], there is evidence that some *in vitro* pre-culture may be necessary to have enough fortitude to survive the harsh mix of inflammatory cytokines and concentrated loads that are common in an injured joint [16,17]. The amount of time dedicated to *in vitro* pre-culture must be considered in the choice of engineered scaffolds, especially if the scaffold material is designed to degrade over time. We have recently developed a tissue culture protocol using chondrocyte-seeded agarose hydrogel and the temporal addition of growth factors that yields engineered tissue with native Young's modulus (E_Y) and glycosaminoglycan (GAG) content [18]. In Study 1 of the current set of experiments we expand this system to include osteochondral constructs formed using a devitalized trabecular bone as a base scaffold.

Among its many benefits, trabecular bone represents the gold standard in terms of mechanical properties, and unlike degradable substrates these properties do not change substantially over time in culture, allowing for extensive *in vitro* cultivation. Bone also has an interconnected porosity that is ideal for gelling chondrocyte-laden agarose or other hydrogels. It is abundantly available for research use and easily machined into a multitude of forms. Devitalized and demineralized bone is already approved by the FDA for clinical use as a scaffold to promote bone growth, as a source of osteoinductive factors, and as allografts [19]. As such it is an alluring choice for an osteochondral scaffold, however, the results of preliminary trials [20] using devitalized trabecular bone suggest there are unanticipated inhibitory effects on chondral development.

Study 1 extends these preliminary results, by examining the effect of trabecular bone on chondral development when used both directly in the formation of multi-phase osteochondral constructs and indirectly when included in the culture medium unattached to the developing gels.

An alternate approach to forming osteochondral constructs is to culture the two phases separately and join them together after certain engineering milestones have been met (i.e., mechanical or chemical fortitude, etc.). Study 2 was designed to examine the feasibility of this approach by delaying the introduction of bone to the culture medium to allow for matrix development. Finally, in Study 3 we examine porous tantalum metal, a highly promising synthetic alternative to bone, as a tissue-engineering scaffold for the formation of osteochondral constructs with functional mechanical properties.

2. Materials and methods

2.1. Experimental design

Three studies were carried out in this manuscript (Fig. 1). In Study 1 the development of chondrocyte-seeded agarose hydrogel constructs (Study 1, *Gel*) and osteochondral constructs (Study 1, *OC(bone)*) were directly compared using the same tissue-engineering protocol. To assess the effects of soluble factors released by bone, *Gel* constructs were also co-cultured adjacent (but unattached) to devitalized bone (Study 1, *Co-culture(bone)*). To exclude the effect of soluble minerals *Gel* constructs were also cultured adjacent to demineralized bone (Study 1, *Co-culture-(demin)*). Finally, to test for the possibility that the inhibitory effect of bone is not related to soluble factors, but rather due to a decrease in the availability of growth factors through the absorption into bone, *Gel* constructs were cultured in medium without TGF-β3 (Study 1, *No TGF*).

In Study 2 the possibility of forming functional osteochondral constructs after separate cultivation of the chondral region was examined by delaying the introduction of bone to day 14 of culture (Study 2, *Bone Introduced on day 14*) or on day 28 (Study 2, *Bone Introduced on day 28*). *Gel* constructs without any *Bone Introduced* served as controls (Study 2, *No Bone Introduced*).

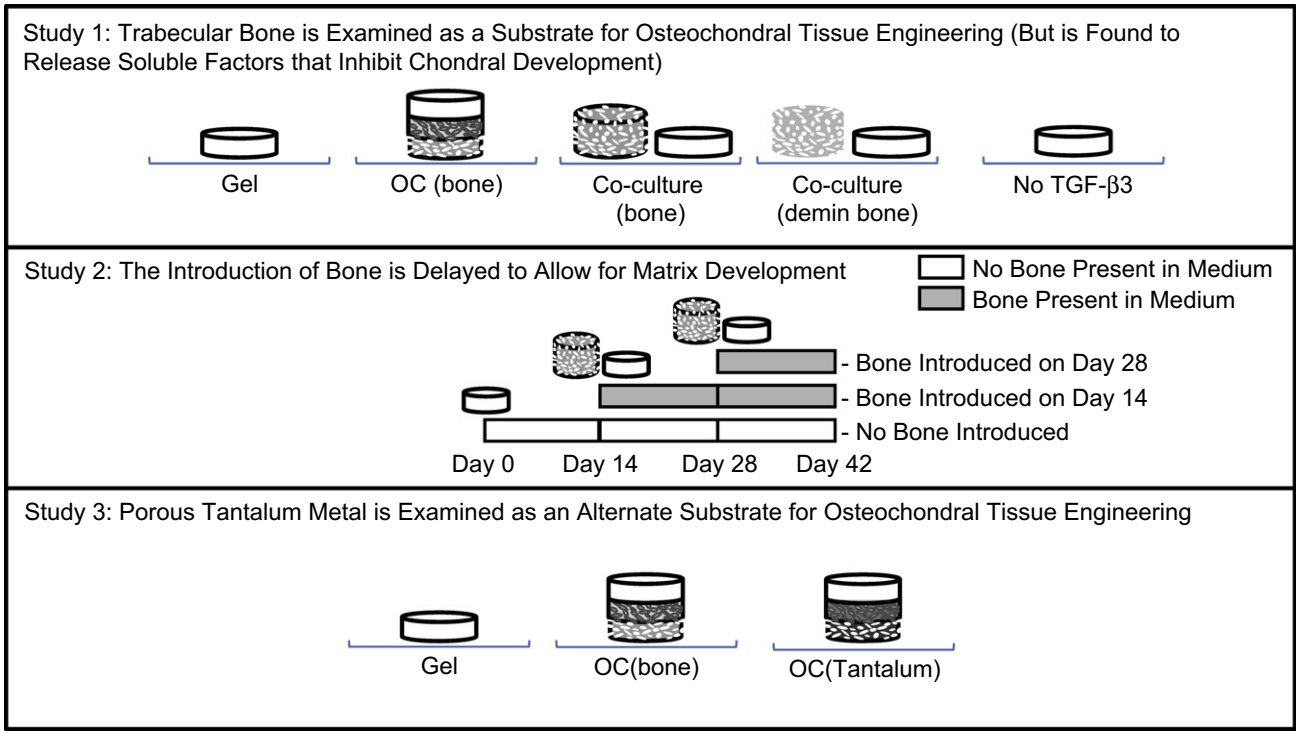

Fig. 1. Schematic of experimental design. Study 1: *Gel*: single-phase chondrocyte-seeded agarose constructs serve as controls, *OC(bone)*: multi-phase osteochondral constructs formed with a trabecular bone substrate, *Co-culture(bone)*: single-phase gel cultured adjacent to bone to test for the effect of soluble factors, *Co-culture(demin bone)*: *Gel* constructs cultured adjacent to demineralized bone to control for the effect of soluble minerals, *No TGF-β3*: *Gel* constructs cultured in medium without TGF-β3 to test for possible decrease in availability of growth factors through absorption into bone. Study 2: Bone is introduced to gel constructs at day 14 (*Bone Introduced on day 14*) or at day 28 (*Bone Introduced on day 28*) to examine the possibility of forming osteochondral constructs after gel is more developed. *Study 3*: *OC(Tantalum)*: Multi-Phase osteochondral constructs formed with tantalum substrate, *Gel* and *OC(bone)* serve as controls. Each study was carried out separately and all groups were cultured for 42 days.