INTRODUCTION: Since partial defects in articular cartilage do not heal spontaneously, whereas injuries that penetrate the subchondral bone undergo some repair [1], we have begun to consider the development of tissue engineered osteochondral constructs consisting of a hybrid scaffold of chondrocyte-seeded hydrogel and devitalized trabecular bone; such a scaffold may improve cartilage repair and graft-host tissue integration in vivo. The objective of the current study is to develop a methodology for assembling osteochondral constructs and to investigate their cellular response, where: chondrocyte seeded agarose gel forms the articular surface and extends through the full depth of the bony substrate (group 1); chondrocyte seeded agarose gel forms the articular surface and extends only partially through an unseeded (group 2) or osteoblast seeded bony substrate (group 3).

METHODS: Core Preparation: Cylindrical trabecular bone cores, Ø8.2 x 1.5 mm (group 1) or Ø5 x 4 mm (groups 2 & 3), were harvested from the epiphysis of metatarsal bones of 3 month-old calves using a coring tool and an isomet low-speed saw. The cores were cleaned of bone marrow, devitalized, and sterilized in 70% ethanol. Cell Culture: Bovine chondrocytes were suspended in 2% agarose (Type VII, Sigma) at 60 x 10⁶ cells/ml [2]. Bone cores were prepared and embedded in the hydrogel using a custom mold. Group 1 constructs were composed of two regions: a protruding upper ~1.5mm gel-only region acting as the articular surface, and a bone/gel interface region through the remainder of the construct. Group 2 & 3 constructs consisted of three regions: a ~2 mm gel-only region, a ~1 mm bone/gel interface region, and a ~3 mm bone-only region. For group 3, osteoblasts obtained from explant migration from trabecular bone chips of bovine metatarsals (passage 1) were pre-seeded onto the bone for 17 days and cultured in α-MEM supplemented with 10% FBS with 5 mM β-glycerophosphate prior to gel incorporation. All constructs were cultured in α-MEM supplemented with 10% FBS and 50 µg/ml ascorbic acid for the duration of the experiment.  Mechanical Testing: Three constructs were tested in unconfined compression at each time point using a stress-relaxation test to 10% strain (of the upper gel thickness). Biochemistry and Histology: At each time point (n=3), tissue slices of ~400 µm thick were cut and stained using live/dead cell viability stain (Molecular Probes, OR), and imaged with a confocal microscope (Olympus, NY). Specimens were fixed in neutral buffered formalin, decalcified, embedded in paraffin and sectioned. All sections were stained with Saffranin O; and type II collagen or alkaline phosphatase and counterstained with eosin.

RESULTS: Group 1 constructs elaborated a matrix that increased in stiffness over the culture period. While confirming our earlier findings [3], the temporal development of construct stiffness was greatly enhanced by a six-fold increase in chondrocyte seeding density adopted here. For all groups, positive Saffranin-O staining of constructs indicated a GAG-rich matrix, and positive type II collagen staining (not shown) showed chondrocyte phenotypic maintenance through the gel thickness (Fig. 2). Live-Dead staining indicated that cells remained viable for the duration of the culture period. Spherical, encapsulated chondrocytes migrated in some instances onto the bone, exhibiting a more spindle-morphology while staining positively for type II collagen. Osteoblasts exhibited alkaline phosphatase staining (not shown).

DISCUSSION: Chondrocytes grown in agarose maintain their phenotype [4] and have been found to develop a functional extracellular matrix in free-swelling and dynamically loaded cultures [2,3,5]. Interestingly, we observed that chondrocytes migrated into the bony substrate in both gel and gel-free areas (Fig.3). Thus chondrocytes may play a significant role in the eventual integration of the graft with host subchondral bone. While agarose-bone constructs demonstrate development of a functional tissue and maintain a chondrogenic phenotype, overall graft integration may be facilitated by the migration of host bone cells and marrow cells into the bony substrate. To this end we varied the degree of agarose gel penetration into the trabecular cores, creating gel-free regions open to cellular invasion. This configuration also permits future investigation of chondrocyte interactions with osteoblasts and progenitor cells. Successful graft-host integration may benefit from pre-seeding the bony substrate with bone cells, and chondrocyte-osteoblast interactions in culture can be optimized for this purpose. These results contribute to our current research efforts in the development of a tissue-engineered osteochondral construct.


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