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Anatomically shaped osteochondral constructs for articular cartilage repair

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Abstract

Few successful treatment modalities exist for surface-wide, full-thickness lesions of articular cartilage. Functional tissue engineering offers a great potential for the clinical management of such lesions. Our long-term hypothesis is that anatomically shaped tissue constructs of entire articular layers can be engineered in vitro on a bony substrate, for subsequent implantation. To determine the feasibility, this study investigated the development of bilayered scaffolds of chondrocyte-seeded agarose on natural trabecular bone. In a series of three experiments, bovine chondrocytes were seeded in (1) cylindrical bilayered constructs of agarose and bovine trabecular bone, 0.53 cm^2 in surface area and 3.2 mm thick, and were cultured for up to 6 weeks; (2) chondrocyte-seeded anatomically shaped agarose constructs reproducing the human patellar articular layer (area = 11.7 cm^2, mean thickness = 3.4 mm), cultured for up to 6 weeks; and (3) chondrocyte-seeded anatomically shaped agarose constructs of the patella (same as above) integrated into a corresponding anatomically shaped trabecular bone substrate, cultured for up to 2 weeks. Articular layer geometry, previously acquired from human cadaver joints, was used in conjunction with computer-aided design and manufacturing technology to create these anatomically accurate molds. In all experiments, chondrocytes remained viable over the entire culture period, with the agarose maintaining its shape while remaining firmly attached to the underlying bony substrate (when present). With culture time, the constructs exhibited positive type II collagen staining as well as increased matrix elaboration (Safranin O staining for glycosaminoglycans) and material properties (Young's modulus and aggregate modulus). Despite the use of relatively large agarose constructs partially integrated with trabecular bone, no adverse diffusion limitation effects were observed. Anatomically shaped constructs on a bony substrate may represent a new paradigm in the design of a functional articular cartilage tissue replacement.

Keywords: Anatomic molds; Cartilage; Tissue Engineering; Osteochondral; Agarose

1. Introduction

Damage to articular cartilage is a common condition affecting the joints of millions of people in the US alone. This damage is complicated by the poor regenerative capacity of adult articular cartilage and the disability and pain that accompanies these injuries (Mankin, 1982). Nine percent of the US population aged 30 and older has clinical osteoarthritis (OA) of the hip or knee, with total direct costs estimated at $28.6 billion dollars per year (Felson, 2000). There exists a range of clinical options (short of total joint replacement) with variable degrees of success, for the repair of focal lesions and damage to the articular surface. These approaches include tissue adhesives (Ahsan et al., 1999; Harper, 1988), enzymatic treatments (Caplan et al., 1997), laser solder welding (Zuger et al., 2001), autograft cell/tissue transfer via periosteal grafts (O'Driscoll and Salter, 1986), autologous osteochondral grafting such as mosaicplasty (Hangody et al., 1996) and the Carticel method (Brittberg et al., 1996). Additionally, there have been significant research efforts in the past decade to develop cell-based therapies for cartilage repair,

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including tissue engineered constructs of cultured cells on three-dimensional scaffolds, (Pazzano et al., 2000; Vunjak-Novakovic et al., 1999).

While the above methods may provide temporary relief of symptoms, several long-term questions remain. Harvest of tissue from the joint, whether for autologous osteochondral grafting or cell-based therapies is limited by the amount of healthy cartilage remaining in a severely eroded joint. Furthermore, the harvesting procedure itself can cause joint-wide changes in tissue properties and may create significant donor site morbidity (Lee et al., 2000a–c). In the case of autologous osteochondral grafting, tissue harvested from non-load bearing regions may provide tissue with sub-optimal material properties or topography for use in contact regions (Ahmad et al., 2001). Furthermore, incongruities in the articular surface that arise at the graft edge can create stress concentrations on the articular surface, and possibly lead to graft loosening (Ahmad et al., 2001). A further challenge to cartilage repair arises from the absence of structural bonding of the graft with the host tissue in the defect, resulting in poor cartilage–cartilage tissue integration (Ahsan and Sah, 1999; Bobic, 1999; Hunziker, 1999). To create constructs having more favorable integrative properties upon implantation, several researchers are exploring the development of tissue engineered osteochondral composite constructs (e.g., Kreklau et al., 1999; Schaefer et al., 2000; Sherwood et al., 2002; van Susante et al., 1998), and have investigated enzymatic treatments and chemical alterations coupled with in situ polymerization for increased graft adherence (Obradovic et al., 2001; Williams et al., 2002). In order for such tissue engineered constructs to provide a better clinical option than autologous transplants, a suitable cell source (e.g., stem cells, Caplan et al., 1991, 1993; Gao et al., 2002; Erickson et al., 2002; Mauck et al., 2003a; converted cell types, Nicoll et al., 2001, etc.) would likely have to be incorporated into the construct design.

The long-term hypothesis of this study is that a potential solution to the problems of integration and congruence is to create constructs with native material properties and surface topology in vitro, for subsequent implantation. This study explores a novel development of anatomically shaped osteochondral constructs incorporating a bony layer and an agarose gel layer (seeded with chondrocytes) to replicate the surface articular layer. A premise of this strategy is that a tissue engineered graft which encompasses the entire articular layer need only integrate with the underlying bone, thus overcoming the challenge of graft-to-native cartilage integration. Furthermore, by elaborating the construct in vitro, it is possible to carefully control its environment to optimize its functional properties prior to implantation.

The current study describes several experiments conducted in order to investigate the feasibility of this long-term goal. The first experiment (Experiment 1) consists of creating engineered cylindrical osteochondral constructs (8.2 mm diameter, 3.2 mm thickness), which demonstrate that such bilayered constructs can be produced with relative simplicity and can maintain chondrocyte viability and biosynthetic activity. The next experiment (Experiment 2) explores the feasibility of creating whole articular layers from chondrocyte-seeded agarose gels, with anatomically shaped articular and subchondral surfaces. Such constructs have considerably larger surface area and volume than most tissue engineered constructs described in the literature, and the objective of this experiment is to investigate whether cell viability, biosynthetic activity, and tissue development can be maintained in these larger constructs. In the final experiment (Experiment 3), these two technologies are combined to generate anatomically shaped osteochondral constructs for the potential replacement of an entire damaged articular joint surface.

2. Materials and methods

In this study, constructs seeded at 10 million cells/ml were first investigated (cylindrical osteochondral constructs and patella-shaped gel-alone constructs) followed by a second set of studies using constructs seeded at 60 million cells/ml (cylindrical and patella-shaped osteochondral constructs). The constructs at 10 million cells/ml reflect the cell density that we demonstrated previously to respond to applied deformational loading in our efforts toward functional tissue engineering of articular cartilage (Mauck et al., 2000). The higher cell seeding density was chosen in light of more recent work in our laboratory aimed at optimizing this loading response, which demonstrated a significant enhancement in matrix elaboration in free-swelling (Mauck et al., 2002) and with applied deformational loading (Mauck et al., 2003d) concurrent with an increased cell seeding density. As the experiments were not performed in parallel, some aspects of the studies were not performed identically. Rather than to discuss these studies chronologically, we have chosen to present them in a manner that is intended to more effectively convey our overall goal to explore the inclusion of an underlying bony substrate to produce chondrocyte-seeded gel–bone constructs and the production of anatomically shaped molds (with and without a bony substrate).

2.1. Cell culture

Articular cartilage was harvested from 5 to 10 carpometacarpal joints from freshly slaughtered 4–6 month

old bovine calves. Cartilage was then rinsed in fully supplemented DMEM with 10% FBS as described previously (Mauck et al., 2000). The cartilage pieces were combined and digested in DMEM (5 ml/g tissue) with 2.5 mg/ml pronase (Calbiochem, San Diego, CA) for 1 h at 37°C with stirring, followed by 0.5 mg/ml collagenase type II (Sigma Chemicals, St. Louis, MO) for 4 h at 37°C with stirring. For the preparation of chondrocyte/agarose constructs, one volume of chondrocyte suspension (at 2.0×10^7 or 1.2×10^8 cells/ml) was mixed with an equal volume of 4% low-melt agarose (Type VII, Sigma) in phosphate buffered saline (PBS) at 37°C to yield a final cell concentration of 1.0 or 6.0×10^7 cells/ml in 2% agarose. After mixing, the chondrocyte/agarose mixture was poured into sterile molds (described below). Constructs were maintained in culture for up to 42 days, with thrice weekly changes of growth medium. The growth medium consisted of DMEM supplemented as indicated above with 50 μg/ml of fresh ascorbic acid (Sigma Chemicals, St. Louis, MO).

2.2. Mechanical testing

Constructs were tested in either unconfined or confined compression using a custom computer-controlled testing system (Soltz and Ateshian, 1998). At equilibration, under a tare load of 2 g, stress-relaxation tests were conducted to 10% strain at a ramp rate of 1 μm/s. The Young's modulus (in unconfined compression) or aggregate modulus (in confined compression) of the construct was calculated from the equilibrium stress and initial cross-sectional area. For bilayered constructs, the magnitude of strain was calculated based on the gel region only, as the bony region is much stiffer and has negligible deformation under these conditions.

2.3. Biochemistry and histology

Samples were analyzed for water content followed by papain digestion for further biochemical analysis (Mauck et al., 2000, 2002). Glycosaminoglycan (GAG) content was determined using the 1,9-dimethylmethylene blue dye-binding assay (Farndale et al., 1982). Construct viability was visualized through the cross-section of halved constructs stained with LIVE/DEAD stain (Molecular Probes, Eugene, OR). For histology, samples were fixed overnight at 4°C in acid formalin ethanol, and decalcified (when a bone region was present) in 22.5% formic acid, 0.68 M sodium citrate for 1–4 days. Samples were then embedded in paraffin and sectioned on a rotary microtome. Staining with H&E and Safranin O, and immunohistochemistry for type II collagen was performed on 8 μm thick sections (Mauck et al., 2003c). Images were taken at the center of each construct from the gel region, bone region, and interface region of constructs at a magnification of either 10× (for Safranin O staining) or 20× (for type II collagen staining).

2.4. Design and production of anatomic molds

Using a computer-aided design (CAD) system (I-DEAS, EDS, Plano, TX), molds were produced based on the anatomy of representative human joints obtained from stereophotogrammetry (Ateshian et al., 1991; Ateshian et al., 1992a, b). Molding surfaces were created for the human retropatellar articular layer of a patellofemoral joint. These molds were designed to reproduce the negative projection of the articular surface and the positive projection of the underlying subchondral bone. G-code was generated from the CAD models to drive a CNC milling machine (Fadal Vertical Machining Center, Model #VMC14-CNC88HS, Chatsworth, CA), and surface molds were milled from stainless steel. Two halves of the mold (having the specified articular topography of the articular surface and subchondral bone surface) were separated by a custom collar that defines the thickness of the scaffold construct and serves to create an enclosed volume of the desired shape. After machining, molds were sanded, polished, and subsequently sterilized with absolute alcohol for each use. Using the same technique, molds recreating the surface geometry of the trapeziocarpal articular layer of the thumb joint were also created.

2.5. Experiment 1: osteochondral constructs

Devitalized trabecular bone disks were prepared by coring specimens ($d = 8.2$ mm) from bovine tibia. Disks were cut with a diamond blade saw to a thickness of 1.6 mm, cleaned of marrow with a water pick, and sterilized in 70% ethanol for 4 h. The cell–agarose suspension, at either 10 or 60×10^6 cells/ml, was loaded onto the bone disks in a custom mold that produced a final construct thickness of ~3.2 mm. To cast disks, molds were placed on a rigid surface overlaid with filter paper wetted with sterile PBS. Bone cylinders were placed at the bottom of the mold (on the filter paper) and molten cell-laden agarose was poured to fill the mold. Using this technique, agarose was found to fully penetrate the underlying bone. After gelling for 15 min at room temperature, constructs were cultured in free-swelling conditions at 37°C and 5% CO_2 for up to 42 days. Media (30 ml for 20 constructs) was supplemented with 50 μg/ml ascorbic acid and changed every other day. To assess construct development in culture, mechanical testing was carried out in confined compression, as described above. Each disk was then halved, and one half used for biochemical analysis and the other half for histology. Three total disks (unless otherwise noted in the text) were studied at each time point. For

constructs seeded at 10 million cells/ml, biochemical and histological assays were performed on days 7, 14, 21, and 28, and mechanical testing was performed for days 14, 28 ($n = 5$) and 42. In a subsequent study, three total constructs seeded at 60 million cells/ml were harvested on days 0, 7, 25, and 30 for biochemical and histological analysis and mechanical testing.

2.6. Experiment 2: anatomic shaped gel constructs

Anatomically shaped patellar gel constructs were created by pouring chondrocyte-laden agarose (at 10×10^6) between the molding surfaces and allowing the construct to gel for 15 min at room temperature. After gelling, constructs were removed from molds and cultured in fully supplemented DMEM (50 ml per patellar construct). In these studies, one gel-alone patella was studied for each time point. To prepare specimens for biochemical and mechanical analysis, three cores (4.76 mm diameter) were extracted from the central region of each patella, with each core further sectioned by depth into three equal 1.6 mm layers (top-articular, middle, bottom-bone). Each sectioned layer was tested in confined compression (as described above). After testing, samples were assessed for GAG content and cross-sections representing the full-thickness of the midline short axis taken and processed for histology.

2.7. Experiment 3: anatomically shaped osteochondral constructs

To create anatomically shaped osteochondral constructs, trabecular bone blocks were obtained from the distal end of 1–2 year old bovine femurs using a bandsaw and demarrowed. Blocks were then contoured down to the proper surface topography with CNC milling, using the same G-code used to create the subchondral component of the stainless steel patella mold. After milling, bone blocks were cleaned and sterilized in absolute ethanol. The articular surface of the patellar mold and collar were assembled and positioned with the articular molding surface facing up. Cell-laden agarose (15 ml) was poured onto the articular surface molding piece, and the contoured trabecular bone surface was placed on top to permit controlled interpenetration of gel into the bony region. Three patellar osteochondral constructs were cultured for 2 weeks in fully supplemented DMEM (75 ml) with ascorbic acid, with thrice-weekly changes of growth medium.

2.8. Statistical analysis

Statistics were performed using ANOVA with Fisher's Least Significant Difference post-hoc tests or unpaired t-tests using the STATISTICA software package (Statsoft, Tulsa, OK), with $\alpha = 0.05$. Means and standard deviations for $n = 3$ constructs (unless otherwise noted) are provided in the graphs.

3. Results

3.1. Experiment 1: osteochondral constructs

For cylindrical osteochondral constructs, agarose was observed to fully infiltrate the trabecular bone, with both halves of the construct remaining interconnected over the entire culture period. Vital staining showed that chondrocytes remained viable in both halves of the construct (Fig. 1). Chondrocytes assumed a round morphology within both layers, with some cells near the bony layer beginning to flatten out after 28 days in culture, indicative of cell attachment to bone. Osteochondral constructs became increasingly opaque with

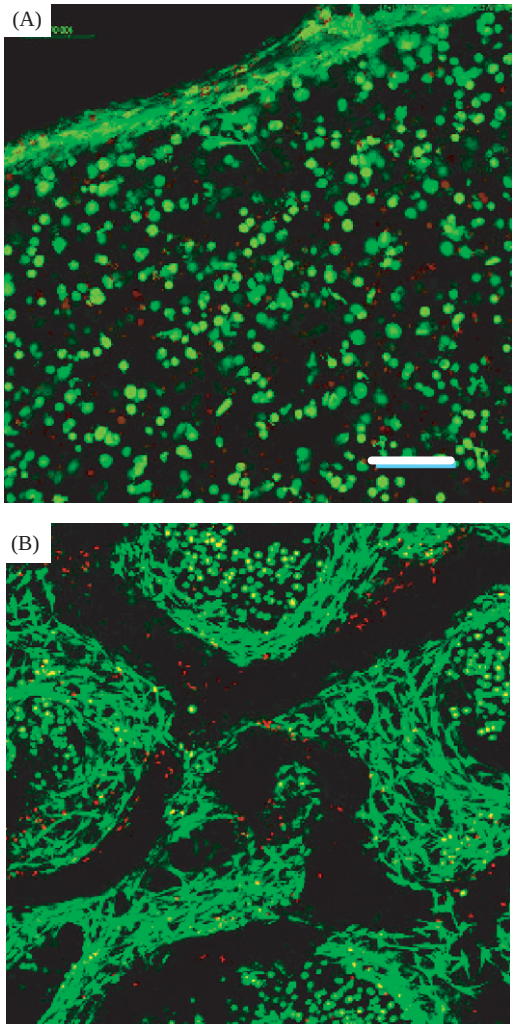

Fig. 1. Viability staining in (A) the gel region and (B) the bony region of an osteochondral construct on day 28. Scale bar = 100 μm.

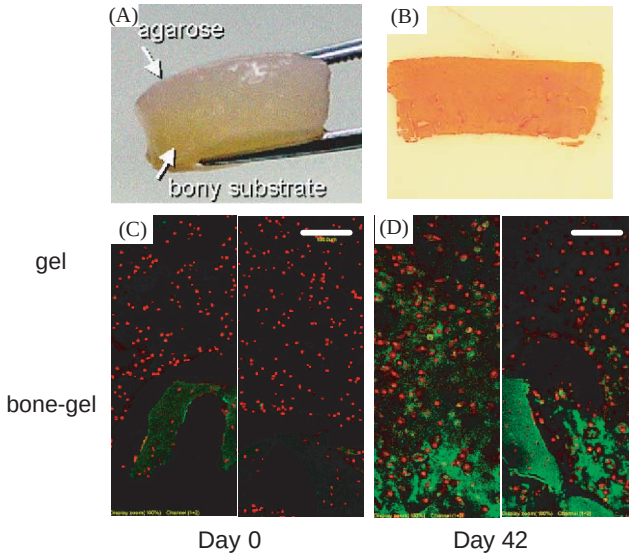

Fig. 2. (A) Cylindrical osteochondral construct of agarose hydrogel cast through the full-thickness of a natural trabecular bone substrate (diameter of 8.2 mm and thickness of 3.2 mm; cell seeding density of 60×10^6 cells/ml) on day 30. (B) Safranin O staining of construct cultured for 30 days, with staining evident in both halves of the bilayer construct. (C) Type II collagen staining (green) and propidium iodide nuclear staining (red) of two constructs cultured for 0 days and (D) two constructs cultured for 30 days. Note that the staining for Type II collagen increases with culture time, perhaps more intensely in the bony region. Scale bar = 100 μm.

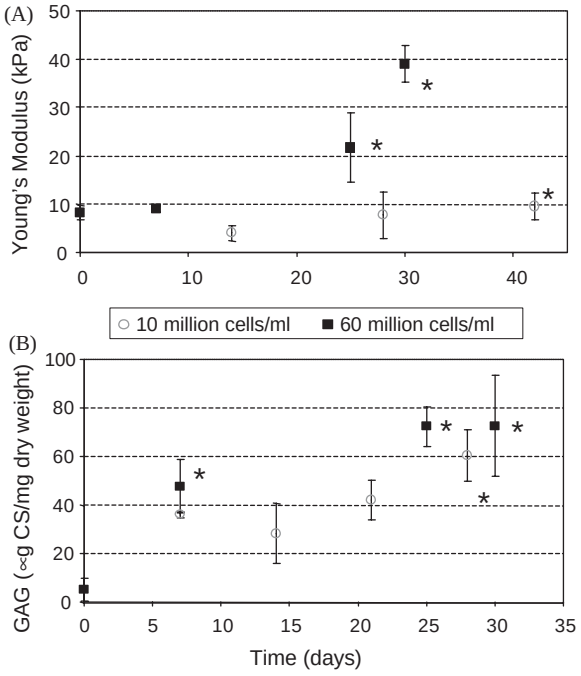

Fig. 3. (A) Graph of the Young's modulus versus culture time for cylindrical osteochondral constructs, seeded at 10 and 60×10^6 cells/ml (cells derived from different isolations). * represents significant difference from earliest time point measured, $p < 0.05$. (B) Corresponding GAG content (normalized to dry weight) for cylindrical osteochondral constructs showing increasing matrix elaboration with culture time. Note that GAG content for 10×10^6 cells/ml constructs was derived from the upper gel-only portion of the construct, where as GAG for the 60×10^6 cells/ml constructs was derived from the whole construct.

time in culture (Fig. 2A), and staining for proteoglycans (Fig. 2B) and type II collagen (Figs. 2C and D) revealed cartilage-like matrix deposition in both halves for disks seeded at 60×10^6 cells/ml. For some confocal microscopy sections, type II collagen staining after 42 days in culture appeared more intense in gel regions associated with the bony regions of the osteochondral constructs (Fig. 2D).

Mechanical properties of osteochondral constructs increased with time in culture. Constructs seeded at 10 million cells/ml exhibited a two-fold increase in Young's modulus during a 42 day culture period (reaching 9.5 kPa, $p < 0.05$ versus day 14), whereas constructs seeded at 60 million cells/ml increased nearly five-fold over a 30 day culture period (reaching 39 kPa, $p < 0.05$ versus day 0) (Fig. 3A). Construct stiffness was accompanied by steadily increasing GAG levels over the respective culture periods (Fig. 3B). Note that the GAG data for the 10 million cells/ml were determined from the gel-only portion of the osteochondral construct, whereas for 60 million cells/ml constructs, GAG was measured from both halves of the construct.

3.2. Experiment 2: anatomic shaped gel constructs

Anatomic molds based on joint specific surface topography data derived from stereophotogrammetry (Fig. 4A) were created using CAD (Fig. 4B). These anatomic molds were then fashioned out of either stainless steel or native trabecular bone. Finished machined molds were found to duplicate many of the features of the measured native surfaces. Patellar molds had a surface area of 11.7 cm^2, and an anatomic cartilage layer volume of 4.02 cm^3 with an average thickness of 3.4 mm (Fig. 5A). Trapeziometacarpal molds had a surface area of 0.90 cm^2 and a cartilage layer volume of 0.21 cm^3 with an average thickness of 2.3 mm (Fig. 5B).

Chondrocyte-seeded agarose (2% wt/vol, 10×10^6 cells/ml) was poured into the molds to form anatomically shaped constructs (see Figs. 5C and D). These constructs maintained their gross morphology with time in culture (Fig. 6A). For patella constructs, Safranin O staining was evident throughout the constructs, with the spatial distribution of PG greater at the periphery (Fig. 6B). A similar pattern of type II collagen was observed (data not shown).

GAG content for the cylindrical cores derived from the top (articular surface), middle and bottom regions of the patella construct (five total patellae studied)

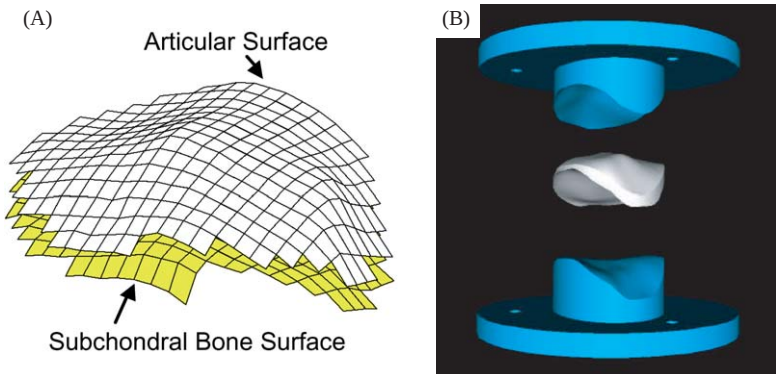

Fig. 4. (A) Surface mapping of articular (white) and bone (yellow) surfaces of patellar articular cartilage layer derived from stereophotogrammetry (SPG) data. (B) CAD model of patella mold with virtual construct created from SPG surfaces.

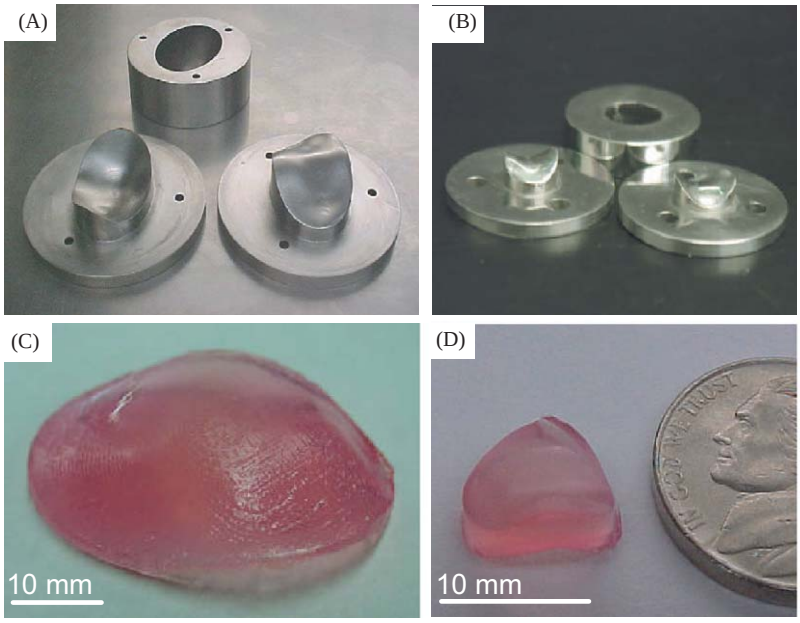

Fig. 5. Stainless steel molds (and collars) machined using a computer-controlled milling machine and G-code generated from CAD models for producing (A) human retropatellar and (B) trapeziometacarpal articular cartilage constructs. Cast cell-free agarose molds of (C) retropatellar and (D) trapeziometacarpal articular cartilage constructs.

increased during the first 2 weeks of culture and then appeared to plateau (Fig. 7B). In general, the corresponding aggregate modulus (H_A) of cylindrical cores taken through the depth of the tissue exhibited a temporal increase with culture time (Fig. 7A). Further evidence for the ability of these constructs to grow in culture was provided by a second independent study that demonstrated significant increase in material properties for the middle section of a day 35 patella construct ($H_A = 19.9 \pm 1.5$ kPa) compared to that of a day 21 construct ($H_A = 11.4 \pm 1.9$ kPa), $p = 0.0005$ ($n = 3$ cores for a single patella at each time point). Overall, for these anatomically shaped gel-alone constructs (10 million cells/ml), biochemical and material property measurements demonstrated significant growth (GAG content or aggregate modulus) for all patella constructs cultured relative to the initial time point measured.

3.3. Experiment 3: anatomically shaped osteochondral constructs

To investigate the feasibility of growing an anatomically shaped osteochondral construct, chondrocyte-seeded agarose (at 60×10^6 cells/ml) was poured into a mold comprised of the stainless steel collar (spacer), the upper stainless steel molding piece having the articular surface topography and a lower molding piece,

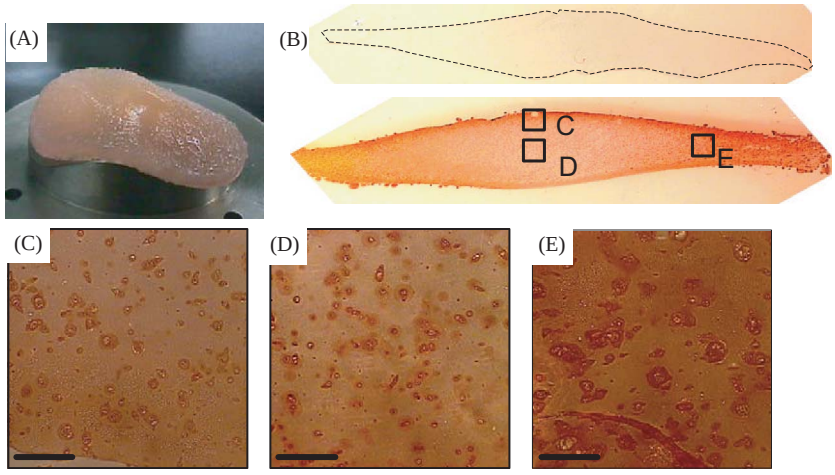

Fig. 6. (A) Day 35 patella construct. (B) Safranin O staining of a short axis cross-section from the middle of a patella construct (seeded at 10×10^6 cells/ml) cultured for 0 (top) or 35 (bottom) days. (C) Subregions 1, 2, and 3 indicate spatial variations in GAG deposition that may result from the variable thickness of the patella construct. Scale bar = 200 μm.

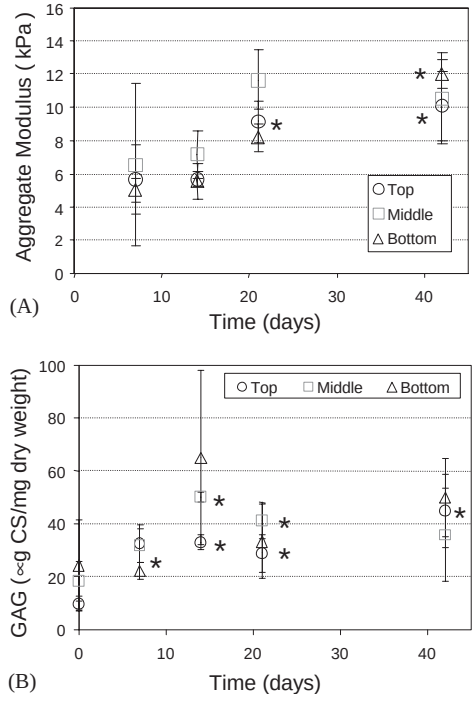

Fig. 7. (A) Aggregate modulus of regions of variable depth from chondrocyte-seeded agarose patellar constructs cultured for up to 6 weeks. Testing was performed on cylindrical cores derived from the top (articular surface), middle, and bottom regions of each construct. (B) Corresponding GAG content for the patella cores. * represents significant difference from earliest time point measured.

machined out of bovine trabecular bone, having the topography of the subchondral bone surface. The latter served subsequently as the subchondral bony substrate of the construct (Fig. 8A). Similar to the smaller cylindrical osteochondral constructs, the patella-shaped osteochondral constructs (three total constructs studied) exhibited agarose penetration through the desired depth (e.g., partial thickness) in the underlying bony substrate (Fig. 8B) and increasing opacity (Fig. 8C) and matrix deposition (Fig. 8D) throughout the 14 day culture period.

4. Discussion

The natural response of articular cartilage to injury is variable and, at best, unsatisfactory (Mankin, 1982; Ratcliffe and Mow, 1990). It is well known that partial-thickness defects of a certain size in the articular cartilage do not heal spontaneously (Bobic, 1999). If the cartilage injury does not penetrate the subchondral bone, it will not heal and can progress to the degeneration of the articular layer (Hunziker, 1999). Injuries that do penetrate the subchondral bone undergo a repair process characterized by the formation of a transient fibrous cartilaginous tissue with material properties lower than those of native tissue, which degrades within 1 year of injury (Hunziker, 1999). With the goal of attaining adequate graft fixation with full incorporation in the adjacent articular cartilage and the subchondral bone (van Susante et al., 1998), this study has explored the development of cell-seeded agarose hydrogel constructs grown on an underlying bony substrate. Chondrocytes seeded in agarose have been shown to elaborate a functional extracellular matrix in vitro (Benya and Shaffer, 1982; Buschmann et al., 1995; Lee et al., 2000a–c) and has also been adopted for cartilage tissue engineering (Mauck et al., 2000; Rahfoth et al., 1998; Sittinger et al., 1994; Weisser et al., 2001). Our findings for both cell seeding densities examined provide supporting evidence that integration of a bony substrate to chondrocyte-seeded agarose constructs

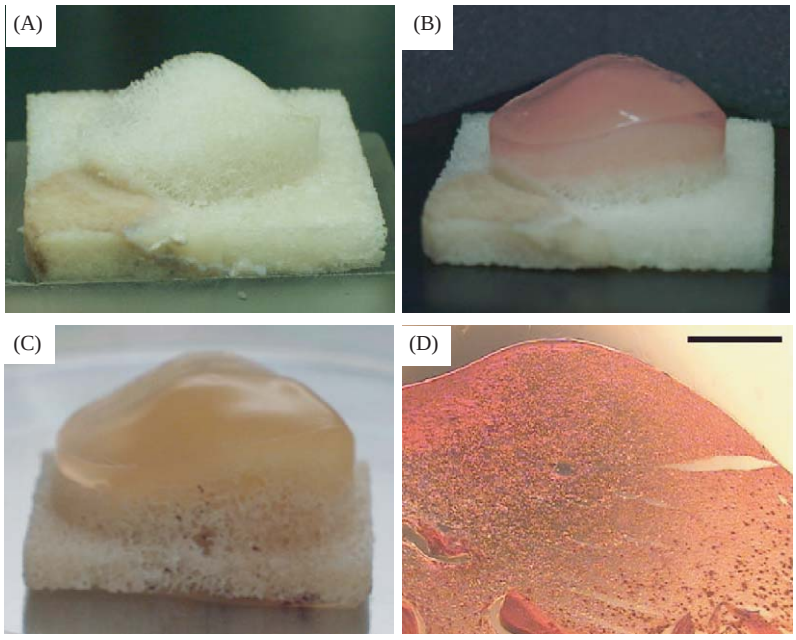

Fig. 8. (A) Bony substrate having the subchondral shape of the patella machined from natural bovine trabecular bone. (B) Chondrocyte-seeded osteochondral patella construct (60×10^6 cells/ml) with gel penetration through the partial thickness on day 0. (C) Patellar osteochondral construct on day 14. (D) Magnified region from upper gel-alone articular surface of day 14 construct stained with Safranin O. Scale bar = 500 μm.

continues to support tissue elaboration (maintenance of the chondrocyte phenotype, biochemical content and material properties). In this study, cylindrical-shaped, bilayered constructs of chondrocyte-seeded agarose and natural bone were observed to remain viable in culture. Chondrocytes exhibited a spherical morphology in the entire upper gel-alone areas and in regions of the lower gel–bone construct. With regard to the latter, some chondrocytes appeared to migrate from the gel onto the surface of the trabecular bone, spreading and assuming a spindle-like appearance. While this flattened morphology may indicate a shifting phenotype in some cells (Benya and Shaffer, 1982), type II collagen staining was evident in the entire construct. Constructs exhibited increasing proteoglycan deposition and development of material properties that reflected initial cell seeding density and culture duration (Fig. 3). These findings suggest that the trabecular bone did not adversely affect chondrocyte phenotypic stability in the gel-alone articular region. The possibility of dedifferentiation of some cells in the gel–bone region may prove to be beneficial for fostering graft integration, but remains to be further studied.

In the osteochondral studies, agarose was cast through the entire bony thickness (Fig. 2A) or partial thickness (Fig. 8B), with the degree of the interfacial region controlled by the volume of cell–agarose solution added to the mold. The selective penetration of agarose into the bony scaffold may help to facilitate cell migration from host tissue into the construct. When not using an interpenetrating hydrogel-like agarose as the upper scaffold, a biocompatible adhesives (Brittberg et al., 1997) or suturing (Schaefer et al., 2000) may be employed to join the layers. A natural bone substrate was chosen in this study for its intrinsic material properties and its established use in bone repair (Horowitz and Friedlaender, 1991; Pelker and Friedlaender, 1991). Although not performed in the current study, the underlying bony construct could be pre-seeded with cells of the same or of a different type (Brown et al., 2000) than the gel-alone region. Other materials explored for bone tissue engineering applications could also serve as the underlying bony substrate (El-Amin et al., 2002; Fisher et al., 2002; Haddock et al., 1999; Livingston et al., 2002; Ma et al., 2001; van Susante et al., 1998). The substrate porosity will influence diffusion into the scaffolds as well as the material properties of the bony region.

While resembling native tissue grafts used in autologous osteochondral grafting procedures, cylindrical constructs were initially studied to assess interaction of chondrocyte-seeded agarose hydrogels and trabecular bone, and to facilitate construct material testing and comparison with our earlier studies (Mauck et al., 2000). Encouraged by the results of the cylindrical construct study, we then focused on extending this approach to address our longer term objective for the treatment of traumatic injury or severe OA, where replacement of a substantial portion or even the entirety of the articular surface may be necessary (Hunziker, 1999). The design of constructs that possess the functional material properties (Guilak et al., 2001) as well as anatomic

shape can provide tissue substitutes that recapitulate the normal contact geometry of the articulating surfaces and normal load distribution across the joint when implanted (Ateshian et al., 1992a, b; Brown and Shaw, 1983; Cooney and Chao, 1977; Eberhardt et al., 1990; Huberti and Hayes, 1984). The latter may be an important contributing factor to the development of a successful tissue engineered articular cartilage substitute (Mow et al., 1999). Earlier studies in the literature have explored matrix development of thick hydrogel constructs for cartilage tissue engineering (Bryant and Anseth, 2001). Our anatomically shaped patellar constructs, while nine-fold larger in volume than cylindrical constructs, exhibited matrix elaboration and increased material properties over the entire culture period. The relatively large construct size did not appear to adversely affect chondrocyte viability or matrix elaboration in a total of seven patella constructs examined over the course of two studies, although some evidence of tissue inhomogeneity was observed (Fig. 6B).

In recent studies, MRI has been used to construct stereolithographic models of the temporomandibular (TMJ) for anatomic studies (Undt et al., 2000) and an image-based approach has been described for the design and manufacture of a biomimetic tissue engineered TMJ condylar prosthesis (Feinberg et al., 2001). CAD and rapid prototyping techniques have also been utilized in the design and fabrication of 3D microstructure of synthetic polymeric scaffolds (Hutmacher, 2001). In vitro growth of pre-shaped cartilaginous implants for craniofacial reconstruction have been described in the literature (e.g., Cao et al., 1997; Chang et al., 2001; Feinberg et al., 2001; Poshusta and Anseth, 2001). However, to our knowledge, no studies have assessed the feasibility of developing an anatomically shaped, tissue engineered articular construct for cartilage repair in large diarthrodial joints. We chose to reproduce the anatomic shape of the thumb joint due to its intricate saddle-shaped surface contour whereas the patella was chosen for its relatively large size and because it is among the thickest articular layers found in the human body. Additionally, both joints are clinically relevant sites of osteoarthritis (Buckwalter and Mankin, 1998; Kelsey, 1982), affecting the mobility and dexterity of significant portions of the population. For clinical applications, the anatomic shape of the scaffold may be based on data obtained through imaging of the patient's healthy contralateral joint surfaces, mirroring and optionally modifying it to provide a more functional, patient-specific surface topography (Ateshian et al., 1991; Cohen et al., 1999). Alternatively, the anatomic shape of the mold may be based on a database of joint surface measurements, where selection is based on the physical characteristics of the patient.

While not assessed in this study, a potentially significant factor for the development of a functional anatomically shaped tissue engineered construct is the ability of the initial construct geometry to be maintained during growth in culture. Whereas constructs comprised of certain materials, such as polyglycolic acid, may exhibit a distorted appearance or gross dimensional changes with time in culture (e.g., Schaefer et al., 2000), hydrogel constructs appear to better maintain their shape in culture (Chang et al., 2001), with only small amounts of swelling (on the order of 10%, Mauck et al., 2002). The spatial uniformity of cells after seeding (compared to seeded fibrous scaffolds) has also been cited as an additional advantage of hydrogel systems (Bryant and Anseth, 2001). Application of physiologic deformational loading for functional cartilage tissue engineering (Butler et al., 2000; Guilak et al., 2001) has been reported by our laboratory to enhance the development of material properties in chondrocyte-seeded agarose constructs as well as better maintain construct geometry (Mauck et al., 2000, 2002, 2003c). Using the molding surfaces described in this study as loading platens that conform to all or part of the articular surface, future studies will apply physiologic loading to mechanically pre-condition these anatomically shaped constructs.

This study demonstrates the ability to cultivate anatomically shaped tissue constructs aimed at the eventual replacement of the entire articular surface of a diarthrodial joint. Such an approach for cartilage repair will require the constructs to have functional properties similar to the native tissue ($\sim$270 kPa for young bovine tissue). While the peak material properties of the constructs achieved in this study remain well below that of the native tissue (one-eighth), concurrent studies on cylindrical gel-alone agarose constructs using more optimal mechano-chemical conditions have yielded engineered tissue constructs that approach two-thirds of the native cartilage material properties (Mauck et al., 2003d). Our functional tissue engineering efforts will undoubtedly be aided by knowledge from basic science studies concerning cartilage tissue healing and repair (e.g., DiMicco and Sah, 2001; Lee et al., 2000a–c; Reindel et al., 1995; Schaefer et al., 2000). The combination of physiologic loading with the use of different cell populations (Caplan et al., 1993; Klein et al., 2002), and growth factors (Martin et al., 1998; Mauck et al., 2003b; Sah et al., 1994), may also permit further design of local and bulk tissue properties. Nutrient gradients (Mauck et al., 2003d; Obradovic et al., 2000) and applied physical environmental conditions (e.g., Mauck et al., 2003d; Vunjak-Novakovic et al., 1999) may further serve as a means of controlling the development of inhomogeneous (Guilak et al., 1995; Schinagl et al., 1997; Wang et al., 2000; Wang et al., 2002) within growing cartilaginous and osteochondral constructs.

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