Shaped, Stratified, Scaffold-free Grafts for Articular Cartilage Defects

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Abstract One goal of treatment for large articular cartilage defects is to restore the anatomic contour of the joint with tissue having a structure similar to native cartilage. Shaped and stratified cartilaginous tissue may be fabricated into a suitable graft to achieve such restoration. We asked if scaffold-free cartilaginous constructs, anatomically shaped and targeting spherically-shaped hips, can be created using a molding technique and if biomimetic stratification of the

shaped constructs can be achieved with appropriate superficial and middle/deep zone chondrocyte subpopulations. The shaped, scaffold-free constructs were formed from the alginate-released bovine calf chondrocytes with shaping on one (saucer), two (cup), or neither (disk) surfaces. The saucer and cup constructs had shapes distinguishable quantitatively (radius of curvature of 5.5 ± 0.1 mm for saucer and 2.8 ± 0.1 mm for cup) and had no adverse effects on the glycosaminoglycan and collagen contents and their distribution in the constructs as assessed by biochemical assays and histology, respectively. Biomimetic stratification of chondrocyte subpopulations in saucer- and cup-shaped constructs was confirmed and quantified using fluorescence microscopy and image analysis. This shaping method, combined with biomimetic stratification, has the potential to create anatomically contoured large cartilaginous constructs.

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Introduction

One aim of repairs for large defects in articular cartilage is to restore the anatomic contour of the joint [4]. Focal defects in cartilage are commonly observed during arthroscopy [12, 16] and have a limited capacity for self-repair [5]. Large defects and widespread joint degeneration, as found in osteoarthritis, impair normal joint function and are associated with pain [13]. Surface restoration is one of the goals of treatment with osteochondral allografts [6] and autografts or mosaicplasty [30], as well as with tissue engineering-based, chondral graft treatments, such as autologous cell implantation (ACI) [3] and matrix-guided autologous cell implantation (MACI) [11]. However, these treatments are limited by availability of donor tissue sources, donor site morbidity, and prolonged rehabilitation times.

Grafts that match the complex surface geometries of larger articular cartilage defects may facilitate healing. Second-generation tissue-engineered cartilaginous constructs that are in development have flat surfaces that do not address the highly contoured surfaces in large articular cartilage defects. Surface incongruity of osteochondral grafts to the surrounding native cartilage surface have been shown to result in local mechanical stresses that may be unfavorable for the success of the graft treatment [27]. Previous studies have examined shaped, tissue-engineered constructs for articular cartilage restoration or replacement created for patella [19], middle and distal phalanx [20, 35], and mandibular condyle [1]. Fabrication approaches for these shaped cartilaginous constructs are grouped into two main areas. In the scaffold prefabrication approach, a scaffold is shaped into the desired geometry, subsequently seeded with cells (chondrocytes, stem cells, etc.), and cultured *ex vivo* or subcutaneously [7, 22]. This approach is commonly used for osteochondral grafts, where most of the scaffold is used for the bony substance [35]. In the molding approach, a mixture of cell and scaffold is placed together into a mold where the scaffold is polymerized, and the shaped construct is cultured. Scaffolds for the molding method are commonly hydrogels, including agarose [19], alginate [8], and photopolymerizable polyethylene glycol derivative [1]. While the results of these studies are promising, the resultant constructs may change from the desired shape due to growth and remodeling, and the surface contours of these constructs have not been analyzed.

The hip has a high occurrence of osteoarthritis and fracture, is commonly treated by total joint replacement [15, 32], and is thus a potential site for biologic resurfacing using shaped cartilaginous constructs. The articulating surfaces of the hip contain two nearly spherical cartilage surfaces, concave on the acetabulum and convex on the femoral head [34]. Thus, a hemispherical cup-shaped graft may be useful as a replacement “cap” for a degenerate femoral head cartilage while a saucer-shaped graft with a hemispherical concave impression may be analogously useful for a degenerate acetabulum.

Biomimetic zonal stratification, another important structural feature of the articular cartilage, has been achieved in tissue-engineered chondral grafts. Cartilaginous constructs have been created with stratification of superficial and middle/deep zone subpopulations [26, 36] and with deep zone layer and calcified cartilage layer [2]. Such constructs maintain the zonal-specific characteristics, including the synthesis and secretion of proteoglycan 4 by the chondrocytes derived from the superficial zone [26], relatively high levels of matrix production by deep zone chondrocytes [36], and mineralization in the calcified cartilage layer [2]. This structural organization may be important since various cellular products are correlated

with inhomogeneous biochemical and mechanical properties of articular cartilage [28].

Thus, the objective of this study was to establish and validate a molding technique for fabrication of cartilaginous constructs that are anatomically shaped on one or two surfaces, targeting the spherically shaped hip, and biometrically stratified with superficial and middle/deep zone chondrocyte subpopulations.

Materials and Methods

Study I: Fabrication of Scaffold-free, Shaped Cartilaginous Constructs

We asked if scaffold-free cartilaginous constructs that were either flat (disk) or shaped on one (saucer) or two sides (cup) could be created. The surface contours of these constructs were assessed qualitatively and quantitatively in addition to their biochemical content (Fig. 1A).

To prepare for construct formation, the isolated chondrocytes were cultured in alginate beads for 7 days as described previously [25, 29]. We isolated the chondrocytes from the distal femoral condyles of bovine calf knees (1–3 weeks old) by sequential digestion in 0.2% pronase for 1 hour and 0.019% collagenase P for 16 hours. Then, the chondrocytes in 1.2% alginate at 4×10^6 cells/mL were expelled into 102 mmol/L calcium chloride using a 22-gauge needle and were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 with additives (100 U/mL penicillin, 100 µg/mL streptomycin, 0.25 µg/mL fungizone, 0.1 mmol/L minimum essential medium nonessential amino acids, 0.4 mmol/L L-proline, 2 mmol/L L-glutamine), 20% fetal bovine serum (FBS), and 25 µg/ml ascorbic acid at 1 mL/ 1×10^6 cells day. After 7 days, the chondrocytes with their cell-associated matrix were then released from alginate in 55 mmol/L sodium citrate in 150 mmol/L sodium chloride.

The shaped constructs were formed from the alginate-recovered chondrocytes (ARCs) with molds. The culture chambers for construct formation were machined from polysulfone and had a porous base of filter paper with 2% agarose to allow for nutrient transfer. The ARCs were seeded at 20×10^6 cells/mL into one of three culture chamber types: disk, saucer, and cup. Traditional disk constructs, with a flat base and no molding at the free surface, served as the control. For shaping of one cartilaginous surface, saucer-shaped constructs were created using polysulfone hemispherical molds (radius = 4.1 mm), which were placed on top of the chamber for the first 2 days of culture. For shaping of two cartilaginous surfaces, cup constructs were formed by seeding cells on hemispherical, concave agarose supports (radius = 4.1

Fig. 1A–B Schematic of methods for fabrication of shaped, scaffold-free cartilaginous constructs with (A) full-thickness chondrocytes and (B) zonal subpopulations with stratification are shown. Freshly isolated or expanded chondrocytes were cultured in alginate beads, released, and seeded into construct chambers with or without molds. (A) The shaped constructs in disk, saucer, and cup geometries were

analyzed for surface contour, biochemical content, and histology. (B) The stratified constructs were composed of chondrocytes from the superficial (S) and middle (M) layer sections of cartilage and were analyzed for stratification using fluorescence micrographs of cryosections. GAG = glycosaminoglycan; COL = collagen; RMS = root mean square.

mm) inside the culture chamber, again with application of hemispherical molds (radius = 3.2 mm) on top for initial culture. The disk and saucer constructs contained approximately 6×10^6 cells total, and the cup constructs contained about 1.6×10^6 cells total. All constructs were cultured in DMEM with additives, 10% FBS, and 25 μ g/mL ascorbic acid at 1 mL/ 1×10^6 cells-day for an additional 10 days, for a total of 17 days in culture. For the first 2 days of culture, we changed the medium every day to prevent the medium from flooding over the top of culture chamber and disturbing the coalescence of the ARCs into a cohesive construct. After 2 days, the molds were removed, and medium was added to submerge the culture chamber for the rest of the culture period. Medium was changed every 2 days after the first 2 days of construct culture. Two days was chosen as the molding time as a pilot study demonstrated this was sufficient time for molding.

After culture, the constructs were transferred to phosphate-buffered saline, weighed wet, and photographed for qualitative assessment of the construct shape.

To quantify surface contour, constructs were raster-scanned (ΔX of 0.2 mm and ΔY of 0.5 mm) with a non-contact laser displacement sensor (25- μ m resolution; Acuity AR200TM, Schmitt Industries, Portland, OR, USA). The three-dimensional surface measurements contain over 1,000 individual points for each of the disk and saucer-shaped constructs and over 600 points for each of the cup-

shaped constructs. We processed the measurements with MATLAB[®] (MathWorks, Natick, MA, USA) to fit a flat plane on top of the disk constructs or spherical surface on top of the saucer and cup constructs. From the fits, we computed the radii of curvature of the saucer and the inner surface of cup constructs. In addition, for each type of construct, we assessed the roughness of fit by root mean square (RMS) of error calculated from the fitted planes and spheres in comparison to the measured data.

The constructs were cut in half using a cutting guide with half-circle cutouts. One half of each construct was fixed in 4% paraformaldehyde at 4°C for 2 days, dehydrated, embedded in paraffin, sectioned at 5 μ m, and stained with safranin O to qualitatively assess glycosaminoglycan (GAG) distribution [33].

To quantitatively assess the biochemical contents, the other half of each construct was solubilized with proteinase K and assayed for DNA using PicoGreen[®] (Invitrogen, Carlsbad, CA, USA) [31], GAG using dimethylmethylene blue [14], and collagen using dimethylaminobenzaldehyde assay for hydroxyproline [40].

Data are expressed as mean $\pm$ standard error of the mean (SEM). The measured radii of curvature for saucer and cup constructs were compared to the mold radii as the hypothesized means by one-sample t test. The effect of construct shaping on RMS roughness and biochemical content were assessed by one-way analysis of variance with

Tukey post hoc test ($p < 0.05$). Power calculations were performed to determine the number of samples (n) sufficient for detecting the expected difference δ with $\alpha = 0.05$, and $1 - \beta = 0.8$. With the expected ratio of treatment effect for radius of curvature at about 3.6, $n = 3$ samples per group was appropriate. There was a total of 15 constructs (6 disks, 5 saucer-shaped, and 4 cup-shaped) that were cultured for a total of 17 days.

Study II: Fabrication of Stratified, Shaped, Scaffold-free Cartilaginous Constructs

We asked if shaped cartilaginous constructs in disk, saucer, and cup geometries could be fabricated with biomimetic stratification of superficial and middle/deep zone chondrocytes. We assessed the stratification using fluorescence microscopy (Fig. 1B).

Bovine calf chondrocytes from superficial zone (designated as S) (0–200 μm from the articular surface) and middle/deep zones (designated as M) (600–1600 μm) from patellofemoral grooves of multiple bovine calves were obtained using a microtome and isolated as described [25]. The S and M isolated chondrocytes were separately seeded in monolayer at 20,000 cells/ cm^2 , cultured with DMEM with additives, 10% FBS, and 25 $\mu\text{g}/\text{mL}$ ascorbate, and expanded until about 80% to 90% confluent (11 days). The chondrocytes were expanded in monolayer to obtain numbers required for construct formation. The S and M chondrocytes were then released into single cell suspensions using sequential digestion with 0.2% pronase for 5 minutes and 0.025% collagenase for 90 minutes. The S chondrocytes were labeled with 20 $\mu\text{mol}/\text{L}$ PKH26 (Sigma, St Louis, MO, USA) for 5 minutes at 25°C according to manufacturer's instructions [10]. S and M chondrocytes were then cultured for 9 and 7 days respectively in 1.2% alginate beads with DMEM/F12 with additives, 20% FBS, and 25 $\mu\text{g}/\text{mL}$ ascorbate as described above.

After 7 days in alginate bead culture, the M chondrocytes were released from alginate beads and labeled with 20 $\mu\text{mol}/\text{L}$ carboxyfluorescein diacetate succinimidyl ester (CFSE) (Invitrogen) for 15 minutes at 37°C according to manufacturer's instructions. These labeled M ARCs were then seeded into the three culture chamber types as described above. Two days after the initial seeding, the S chondrocytes were released from alginate beads and seeded on top of the M ARCs in the three culture chamber types at 25×10^6 cells/ mL with new molds with radii that are 0.8 mm smaller in the center (radius = 3.2 mm for saucer constructs and radius = 2.5 mm for cup constructs). The disk and saucer constructs contained approximately 1.6×10^6 S cells, and the cup constructs contained about 1×10^6 S cells. The molds were removed 2 days later, and

the constructs were cultured for 7 more days, for a total of 11 days (11 days with M chondrocytes and 9 days with S chondrocytes in culture chamber set up) in with DMEM with additives, 10% FBS, and 25 $\mu\text{g}/\text{mL}$ ascorbate at 1 $\text{mL}/1 \times 10^6$ cells-day. The chondrocytes were cultured for 29 days in total for expansion, preculture, and construct formation.

At the end of the culture period, these constructs were fixed for 2 days in 4% paraformaldehyde in the dark at 4°C and snap frozen with Tissue-Tek[®] O.C.T. (optimal cutting temperature) compound (Sakura Finetek, Torrance, CA, USA) in isopentane cooled with liquid nitrogen. We then cryosectioned these constructs vertically at 40- μm thickness and visualized them using epifluorescence and phase contrast microscopy with a 4x objective (Eclipse TE300, Nikon, Melville, NY, USA). Fluorescent images of PKH26-labeled S cells (red signal) and CFSE-labeled M cells (green signal) in the identical field were obtained separately and subsequently processed and merged.

The extent of stratification was quantified by two methods of analysis of images (field of view of $2.9 \times 2.2 \text{ mm}^2$) visualizing vertical cross-sections of the constructs. First, the area fraction, relative to the overall construct area, occupied by PKH26-labeled S chondrocytes was calculated from manual tracings. This area fraction occupied by the S chondrocytes was then compared to the theoretical percentage of S chondrocytes relative to all the cells seeded. Second, we analyzed three selected areas of width of 0.362 mm (200 pixels) and construct full-thickness from each fluorescent image to calculate red and green intensity profiles as a function of thickness-normalized distance from the construct surface. The profiles were averaged and plotted with respect to the distance from the surface of the constructs normalized to the construct thickness.

Data are expressed as mean $\pm$ SEM. We performed one-sample t tests after arcsine transformation to compare the measured S area fractions to the expected values based on S chondrocytes seeded compared to the total chondrocytes seeded (21% for disk and saucer constructs and 38% for cup constructs). We performed power calculations to determine the number of samples (n) sufficient for detecting the expected difference δ with $\alpha = 0.05$, and $1 - \beta = 0.8$. With the expected ratio of treatment effect for S area fraction approximately 2.8, $n = 4$ samples per group was appropriate. There was a total of 13 constructs (4 disks, 5 saucers, and 4 cups) that were cultured for a total of 29 days.

Results

Cartilaginous constructs, in disk, saucer, and cup geometries, were fabricated consistently. These constructs were

able to withstand manual handling and maintain their shapes. Qualitatively, such constructs had distinct surface contours, with top, both top and bottom, or neither surface molded and shaped for saucer, cup, and flat constructs, respectively (Fig. 2).

Quantitative analysis of surface contour measurements (Fig. 3) showed saucer and cup constructs had different radii of curvature from each other (5.5 ± 0.1 mm and 2.8 ± 0.1 mm), with the inner surface of cup constructs having smaller ($p = 0.000002$) radius of curvature than the saucer constructs (Table 1). The fitted planes and curves were relatively smooth, as evidenced by the RMS roughness values (< 0.3 mm) that were not different between groups ($p = 0.175$). The radius of curvature of the saucer-shaped contour was less than ($p = 0.0004$) that of the

corresponding mold, while that of the cup shaped contour was slightly less than ($p = 0.05$) that of its corresponding mold (Table 1).

Table 1. Quantification of shape of constructs by analysis of fitted planes and spheres

Measurement	Disk	Saucer	Cup
Radius of mold (mm)		4.1	3.2
Radius of curvature measured (mm)		$5.5 \pm 0.1^*$	$2.8 \pm 0.1^{\dagger, \ddagger}$
Root mean square roughness (mm)	0.20 ± 0.04	0.15 ± 0.01	0.26 ± 0.05

Values are expressed as mean $\pm$ SEM ($n = 3-6$); * $p < 0.001$ versus mold; $^{\dagger}p < 0.05$ versus mold; $^{\ddagger}p < 0.001$ versus saucer.

Fig. 2A–H Macroscopic images of cartilaginous constructs are shown: (A) top view of disk construct with no molding; (B) side view of disk construct with no molding; (C) top view of saucer construct with molding on top; (D) side view of saucer construct with molding on top; (E) top view of cup construct with molding on two surfaces; (F) side view of cup construct with molding on two surfaces; (G) top view of cup construct on top of saucer construct; (H) side view of cup construct on top of saucer construct.

Fig. 3A–F Representative three-dimensional surface contours of half of (A) disk, (B) saucer, and (C) cup constructs are shown. The surface contours were fit to plane and sphere for (D) disk, (E) saucer, and (F) cup constructs. The color scale indicates the heights in the z-axis in mm.

Fig. 4A–C The biochemical content of constructs is shown. (A) DNA, (B) GAG, and (C) collagen (COL) contents were normalized by wet weight (WW) for disk, saucer, and cup constructs. Bars represent mean $\pm$ SEM ($n = 4-6$). * = $p < 0.005$ versus disk and saucer.

Fig. 5A–C Safranin O histochemical sections of (A) disk, (B) saucer, and (C) cup constructs are shown. Distribution of chondrocytes and GAG appears uniform throughout the construct in all construct types.

Fig. 6A–F Merged fluorescence and phase micrographs of stratified constructs of (A, B) disk, (C, D) saucer, and (E, F) cup geometries are shown. S chondrocytes labeled with PKH26 dye, which fluoresces red, were seeded on top of M chondrocytes labeled with CFSE dye, which fluoresces green.

Constructs of all shapes had DNA, GAG, and collagen per wet weight that generally were similar (Fig. 4). The cup constructs had higher collagen content than the disk

($p = 0.003$) and saucer ($p = 0.003$). All three construct types contained GAG through the construct based on safranin O staining (Fig. 5).

Fig. 7A–D Quantification of stratification in (A, B) disk, (A, C) saucer, and (A, D) cup constructs is shown. (A) The bar graph shows measured and expected areas covered by S chondrocytes. The line graphs show the fluorescence intensity profiles of the PKH26-labeled S chondrocytes and CFSE-labeled M chondrocytes for (B) disk, (C) saucer, and (D) cup constructs, with error bars indicating average SEM values ($n = 4\text{--}5$). * = $p < 0.05$ versus expected.

Stratification by sequential seeding of two distinct chondrocyte subpopulations was achieved for cartilaginous constructs of all shapes (Fig. 6). The superficial zone chondrocyte layer, fluorescing red, was qualitatively distinct from the middle zone chondrocyte layer, fluorescing green, in all constructs as evidenced by fluorescence micrographs. A comparison of fluorescence micrographs and phase-contrast micrographs indicated the majority of the chondrocytes were positively labeled with only one of the dyes. The percent area occupied by S chondrocytes (S area fraction) in the shaped constructs (saucer and cup) was similar to the expected values based on seeded cells ($p > 0.7$) (Fig. 7A). The fluorescence intensity profiles indicated all three constructs achieved stratification of S atop M chondrocytes (Fig. 7B–D). The transitions from S to M chondrocytes occurred further from the surface in the shaped constructs than for the flat disk constructs, supporting the data from area fraction occupied by S chondrocytes.

Discussion

One treatment goal for large articular cartilage defects is the restoration of the anatomical contour of the joint with tissue having a structure similar to native cartilage. Since surface incongruities may limit graft success, shaped and stratified cartilaginous tissue may be a suitable graft to achieve such restoration. The objective of this study was to establish and validate a molding technique for fabrication of cartilaginous constructs that are anatomically shaped,

targeting the spherically shaped hip, and biomimetically stratified with superficial and middle/deep zone chondrocyte subpopulations. We present a molding technique for fabrication of shaped cartilaginous constructs using ARCs in saucer and cup shapes with one and two surfaces molded, respectively (Fig. 2). Qualitatively, the shaped constructs had surface contours different from those of the control disk constructs. Quantitatively, the saucer and cup constructs were distinct in their radii of curvature (Table 1). These results demonstrate molding fabrication can generate constructs that are contoured and fabricated from only chondrocytes and their biosynthetic products. The matrix products accumulated fairly similarly regardless of the shape of the construct, demonstrating the shaping does not adversely affect chondrocyte functions (Figs. 4–5). Additionally, this molding technique was adapted to create shaped cartilaginous constructs with biomimetic stratification (Fig. 6). Thus, in this study tissue-engineered cartilaginous constructs were designed to have, and analyzed for, anatomic three-dimensional contours and biomimetic stratification.

We note several limitations. Cell source is an important consideration since these constructs were formed solely from the chondrocytes and the pericellular matrix they produced. While this study was performed with immature calf chondrocytes, which are metabolically active, this shaping technology would likely be applicable to chondrocytes from mature articular cartilage since such chondrocytes can be used to form scaffold-free disk constructs [29]. Additionally, the cell source limitation may be circumvented by expansion of the cell source followed by three-dimensional alginate bead culture [9, 38].

Previous studies on shaped tissue engineering of cartilage have utilized molding or machining of the scaffold material to define the contour of the construct. These methods have been used for engineering of elastic and nonarticular cartilage such as auricle [7, 21, 41], nose [8, 21], and tympanic membrane [17]. Several shaped osteochondral constructs containing articular cartilaginous sections, for phalanx [20, 35] and mandibular condyle [1], for example, have been fabricated. Bone grafts also have been constructed in various shapes, including femoral head and mandible [23]. Many of these previous works rely on scaffolds to provide mechanical stability to the constructs and aid in the maintenance of their shapes. Like some of these previous studies, molding was used to shape the constructs in this study. However, here we sought to develop three dimensionally-shaped cartilaginous constructs supported only by the cells and its biosynthetic products, using a molding technique.

In previous studies, the shape of constructs changed qualitatively from the initial scaffold or mold shape due to growth and/or remodeling of the scaffold during culture

[7, 22, 35]. Additionally, many of the previous reports on shaped cartilaginous grafts did not quantify the construct shapes or contours. In the present study, we developed an analysis of the surface contour of the shaped constructs, from determining a method of accurate data acquisition and the development of the appropriate image processing protocol using MATLAB. Here, the measured radii of curvature of the shaped constructs differed from those of the hemispherical molds that were used (Table 1). However, contraction of scaffold-free constructs noted in a previous study with expanded chondrocytes cultured on agarose [18] was not observed here, possibly due to phenotype stabilization during alginate preculture. A longer shaping period with a permeable mold may allow for better retention of the initial shape and also adequate nutrient transfer. Also, reshaping by mechanical loading after the initial construct formation period may allow for finer control of construct shape [39]. This molding technique for shaping of tissue-engineered construct may also be applied to joints with geometries and contours that are more complex than those of the hip.

A biomimetic approach to cartilage tissue engineering in terms of construct shape and structural organization may be advantageous for clinical applications. The use of tissue-engineered grafts with flat surfaces may be sufficient to approximate the normal cartilage surface for smaller defects but not larger defects. Surface incongruity between osteochondral grafts and the surrounding native cartilage results in local mechanical stresses that may be unfavorable for the graft survival and treatment efficacy [27].

The applicability of this shaping technology to constructs with a biomimetic cellular organization suggests the possible application to tissue with multiple layers. Previously, stratified cartilaginous constructs with S chondrocytes atop M chondrocytes maintain the zonal characteristics typical of their source, such as proteoglycan 4 and differential matrix production [26, 36]. Such differential spatial characteristics result in inhomogeneous biochemical and biomechanical properties of native cartilage with respect to depth and may be important in the maturation and, ultimately, function of tissue-engineered cartilaginous constructs as well [24]. The stratification can be customized with different types and arrangements of cells and materials for various target application and location.

The biochemical content of the shaped constructs in this study is consistent with previously described constructs [26, 29, 38]. Like many tissue-engineered cartilaginous constructs, the constructs here had lower extracellular matrix content, especially collagen, compared to that of native cartilage. The slightly elevated deposition of collagen in cup-shaped constructs may be due to enhanced nutrient transport provided by the thinner cup constructs as compared to thicker disk and saucer-shaped constructs. It is

also possible the thicker agarose support on the bottom of cup constructs helped to retain more of the matrix products.

The shaping technique presented here has the potential to facilitate treatment of larger articular cartilage defects where recreation of surface contour is important. Such shaping methods may be coupled with noninvasive three-dimensional imaging to determine the surface contour of subchondral bone and/or of contralateral cartilage to appropriately tailor the shape of the construct [37]. Improved control over shaping and stratification would be useful in clinical translation of these shaped, stratified, scaffold-free grafts. Larger constructs that are needed for repair of large articular cartilage defects may become feasible with expansion and redifferentiation methods for the cells in conjunction with a bioreactor for improved construct growth and maturation. To attach such constructs to the surrounding native tissue, such shaped grafts may either be fixed in the defect area with fibrin glue, as with current tissue-engineered grafts like MACI [11], or be fabricated in vitro atop a boney substance, which can integrate into the surrounding native bone after implantation as with current allo- or autograft techniques. In vivo implantation of these grafts is needed to better assess the functionality and durability of such biomimetic, tissue-engineered grafts with appropriate shape and stratification.

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