Published in final edited form as:

J Knee Surg. 2012 July ; 25(3): 187–196.

Toward Engineering a Biological Joint Replacement

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Abstract

Osteoarthritis is a major cause of disability and pain for patients in the United States. Treatments for this degenerative disease represent a significant challenge considering the poor regenerative capacity of adult articular cartilage. Tissue-engineering techniques have advanced over the last two decades such that cartilage-like tissue can be cultivated in the laboratory for implantation. Even so, major challenges remain for creating fully functional tissue. This review article overviews some of these challenges, including overcoming limitations in nutrient supply to cartilage, improving in vitro collagen production, improving integration of engineered cartilage with native tissue, and exploring the potential for engineering full articular surface replacements.

Keywords

biological joint replacement; cartilage; tissue-engineering; nutrient diffusion; collagen production

Over 20% of the adults in the United States (25 years and above) have osteoarthritis (OA) of the hip or knee (>46 million Americans).¹ OA is ranked as one of the top three causes for disability and contributes more than \$185 billion dollars a year in related medical costs.^{2–4} More than 580,000 arthroplasty procedures are performed each year in the U.S.⁵ While joint replacement generally succeeds in decreasing or eliminating pain and restoring joint function, the lifespan of prostheses are limited due to wear, loosening, infection, and fracture of the implant or surrounding bone.^{6–9} Alternative treatments have not yet been successful in providing a viable long-term option for cartilage repair. For example, allografts are limited by donor tissue availability and graft viability,^{10,11} while autografts are limited by the availability of healthy tissue and donor site morbidity. Bio-engineered repair strategies that circumvent these limitations, while preserving the natural function of the joint and using

a procedure less invasive than total joint arthroplasty, may be optimal for treating younger OA patients.

Articular cartilage serves as the load-bearing material of joints and possesses excellent friction, lubrication, and wear characteristics.¹² Successful replacement of damaged or injured articular cartilage will hinge on the ability to recapitulate the mechanical and structural properties of the healthy native tissue before implantation. Over the past two decades, there has been a wide interest in developing functional engineered cartilage. To grow cartilage tissue, cells are cultured within a three-dimensional (3D) scaffold that provides an initial structure for the de novo tissue^{13–15}; alternatively, cells may be cultured using scaffold-less techniques.^{16,17} For example, autologous chondrocyte implantation (ACI) is a cell-based strategy where cells are injected directly into focal lesions and covered with a periosteal flap^{18,19} whereas CARTIPATCH¹⁸ uses a 3D agarose hydrogel scaffold to prevent leakage of cells, stabilize the chondrocyte phenotype, and promote a homogeneous distribution of cells.¹⁸ Although these techniques are designed for repair and regeneration of cartilage focal lesions, they can be scaled up to replace an entire articulating layer (Fig. 1). However, nutrient diffusion through the depth of these large scaffolds represents a major challenge facing the field.

There are two prevailing points of view regarding implantation of engineered cartilage constructs; one approach places the cell-scaffold construct immediately into the defect site and relies on the in situ biological and loading environment to foster construct development (e.g.,^{20–27}). Using this approach, poly ϵ -caprolactone (PCL) scaffolds have been designed to provide sufficient mechanical support upon implantation, while being porous enough to permit de novo tissue development.^{26,27} Lee et al demonstrated tissue growth in a full surface repair of a rabbit proximal humeral head following implantation of an acellular PCL scaffolds infused with transforming growth factors.²⁷ Scaffolds that provide sufficient mechanical support at implantation reduce the need for extended culture periods before the repair surgery. The regenerated tissue would develop under physiological loading conditions, which may ideally provide better functional tissue.

Another approach is to first precondition the cell-scaffold construct in vitro before implantation into the defect (e.g.,^{28–32}). In vitro cultivation provides a controlled nutrient supply and loading environment that may be optimized for matrix synthesis to produce stiff cartilage-like constructs that may ideally sustain physiological loading following implantation. The required mechanical properties of the engineered cartilage will be dictated by the extent of the damaged region and its mechanical demands. In this type of approach, studies have demonstrated that the most robust tissue properties may be achieved by optimizing the media formulation as well as the transport of solutes in the developing tissue. Applied dynamic deformational loading to cell-seeded hydrogel constructs provides physical cues to cells and enhanced solute transport leading to improved mechanical properties compared with free-swelling (unloaded) control.^{14,24,33–39}

Articular cartilage is a highly hydrated soft tissue whose solid organic matrix is comprised mostly of collagen fibrils (10 to 20% mass by wet weight) and proteoglycans (5 to 10% mass by wet weight).^{40–44} Chondrocytes comprise less than 10% of the tissue volume⁴⁵ and maintain the tissue by synthesizing and secreting extracellular matrix. Chondrocyte morphology, biochemical, and mechanical properties vary through the depth of the tissue. Near the articular surface cells are more elliptical and the tissue is softer than in the middle and deep zones.^{46–49} There have been many improvements in biological replacement strategies for cartilage; however, there are still some limitations and challenges that remain to be addressed for successful repair and regeneration.⁵⁰ The purpose of this review article is to summarize our advances in engineering cartilage and to identify approaches for scaling up

these strategies to engineer large constructs suitable for replacing entire articular surfaces in cases of traumatic injury and advanced joint degeneration.

Cell Sources for Cartilage Tissue Engineering

Previous studies have been successful in cultivating functional engineered cartilage using cells from juvenile bovine and adult canine cartilage.^{24,51,52} These studies have reported equilibrium compressive mechanical properties and glycosaminoglycan (GAG) content similar to native values.^{25,35,36,53} Cultivating functional tissue with adult chondrocytes shows promise for using these techniques as a clinical repair strategy, since OA is commonly observed in older patients. Autologous chondrocytes from a nearby healthy region of the joint is the ideal cell source for clinical applications of engineered cartilage. However, acquiring enough cells while minimizing donor site morbidity remains a major challenge. Donor cells can be expanded in culture before implantation, but the proliferation rate of human adult cells is generally low and would lengthen the time between surgeries.

Alternatively, stem cells have been investigated as a possible cell source for cartilage regeneration.^{26,30,51,54–58} Mesenchymal stem cells (MSCs) from adipose tissue, bone and synovium express surface markers also expressed by chondrocytes, suggesting that MSCs can potentially differentiate toward a chondrogenic lineage.^{55,59–61} Cells can be encouraged toward chondrogenesis by using a cocktail of growth factors to expand the cells in culture.^{54,55,57,62} Including growth factors, such as basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF) in the expansion culture medium improves the mechanical and biochemical properties of engineered cartilage in 3D culture.⁵⁵ Expansion of MSCs in vitro reduces the amount of tissue required to obtain a sufficient numbers of cells; mitigating damage to healthy tissue. Furthermore, encapsulation of MSCs in hydrogels and woven scaffolds demonstrates that these cells are capable of producing cartilage-like tissue with mechanical and biochemical properties tending toward native cartilage values (Fig. 2).^{26,30,55}

Previous studies that have used bone or synovium-derived MSCs have demonstrated that these cells can be passaged and expanded in monolayer culture with medium defined to promote chondrogenesis.^{51,55,62,63} In contrast, culturing chondrocytes on a hard surface, such as a glass plate or tissue culture flask, causes the cell to dedifferentiate and become more fibroblast-like. The change in cell behavior may be beneficial for increasing collagen production in vitro. A recent study by Anderson et al demonstrated that passaged chondrocytes can self-assemble into engineered cartilage plugs and that passaged cells produce 2× more GAG and 5× more collagen than primary chondrocytes.⁶⁴ However, most of the collagen produced with passaged cells was type I collagen, which is not ideal for recapitulating the native cartilage tissue composition.⁶⁴ In contrast, culturing differentiated stem cells in a 3D scaffold that promotes chondrogenesis (i.e., hydrogels) improves collagen type II production.⁶³ The loading environment experienced by the cell in two-dimensional (2D) culture significantly affects the matrix produced by the cells in 3D culture.^{55,64}

Strategies for Improving Nutrient Diffusion: Enhanced Transport of Nutrients

Closer examination of engineered cartilage constructs reveals that the mechanical properties and matrix distribution are spatially heterogeneous. These constructs typically exhibit mechanically stiffer regions and greater matrix deposition near their peripheral boundaries, whereas regions deeper inside the constructs are typically softer, showing less matrix deposition.⁶⁵ Nutrient diffusion deep into the construct is a challenge in the field that will be considerably amplified when thicker and wider constructs are cultured. Human articular

cartilage can be up to 7 mm thick^{66,67}; therefore, thicker constructs will be necessary for clinical applications.

Immature articular cartilage is well vascularized with canals that provide nutrients to the developing tissue and remove waste byproducts.^{68–71} Experimental studies have demonstrated that dynamic loading enhances the uptake of nutrients into agarose hydrogels and immature cartilage.^{72,73} For smaller macromolecules (i.e., 3 kDa), uptake by cartilage under dynamic loading conditions was threefold higher than uptake by passive diffusion. The effects of dynamic loading were found to be more pronounced with larger macromolecules. A 70 kDa molecule achieved a concentration nine times higher under dynamic loading than under passive diffusion into cartilage. This enhanced uptake of nutrients by the tissue was considerably facilitated by the network of cartilage canals, allowing for nutrients to transport deep into the tissue (Fig. 3). The increase in nutrient uptake was attributed solely to dynamic loading by demonstrating a return of the solute concentration to passive diffusion levels after termination of loading. Thus, loading provides an active solute pumping mechanism because the solid matrix of the immature cartilage can impart momentum to the solute at the tissue-bath interface, pulling it into the tissue.^{72,73}

Similarly, short-term dynamic loading of engineered cartilage constructs (i.e., for less than three hours) has demonstrated improved nutrient diffusion.^{33,34,74} Loading of anatomical size patellar constructs doubled the concentration of large molecules (70 kDa) in the constructs compared with constructs under free-swelling conditions (control; Fig. 4).³⁴ Dynamic loading has been shown to significantly improve mechanical properties of engineered cartilage; suggesting that the increased nutrient uptake during the 3 hours of daily loading influences matrix production and deposition.^{24,34,36,74,75} The loading type, duration, and frequency can greatly impact the mechanotransduction response of chondrocyte seeded-scaffolds.^{74–76} Long-duration loading protocols (six hours) results in a decrease in the nutrient diffusion into large constructs during loading, primarily due to decreased surface area available for free diffusion.³⁴ However, these constructs produce stiffer engineered cartilage than constructs cultured under free-swelling conditions.⁷⁵ In contrast to the beneficial effects of dynamic loading, static loading significantly decreases the nutrient uptake by engineered cartilage (Fig. 4). These findings in engineered cartilage are consistent with the observation that dynamic loading produces enhanced uptake of solutes into agarose and cartilage,^{72,73} considerably greater than under passive diffusion. However, other mechanotransduction pathways may also be at work when constructs are being loaded dynamically. Although the precise nature of these mechanisms is not completely understood, dynamic loading can be used to improve nutrient transport into large constructs designed to replicate entire articular layers such as the human retropatellar surface (Figs. 1 and 4).

Perfusion, a convective transport method, applies a biomimetic approach to provide nutrients into engineered constructs by mimicking the function of the vascular canals in developing cartilage (Fig. 4B, C). Currently, there are conflicting findings in the literature for the beneficial effects of perfusion, which may suggest further research is needed to determine the optimal flow rate and duration, and when perfusion should be applied over the tissue maturation period. Raimondi et al demonstrated that perfusion of chondrocyte-seeded constructs can potentially improve cell viability, GAG synthesis, and mechanical properties.^{77,78} Grayson et al demonstrated that perfusion of nutrients through the bone region of an osteochondral construct improves matrix production and distribution in the engineered cartilage.⁷⁹ In contrast, studies that have combined perfusion with dynamic loading have not observed additional nutritional benefits from perfusion.^{34,78,80} The findings of these studies suggest some potential benefits of using perfusion in the absence of mechanical loading stimuli to improve the compositional and mechanical properties of

immature osteochondral constructs. This will be especially important for large osteochondral constructs, because the bone/substrate interface will make limited nutrient diffusion into constructs an even greater challenge to overcome.

Strategies for Improving Nutrient Diffusion: Designing Multiscale Nutrient Pathways

Inspired by the anatomy and physiology of developing native tissue, microscopic and macroscopic channels have been incorporated in engineered cartilage constructs^{81–85} to provide pathways for improving nutrient transport. Large vascular-like canals can be incorporated at the macroscopic level by creating one or more channels through the thickness of the scaffold during fabrication. Adding a macroscopic channel (1 mm diameter) in the center of a cylindrical hydrogel construct (4 mm diameter) is a very effective method for decreasing the nutrient path length and improving the depth-dependent mechanical properties over time in culture (Fig. 5).⁸¹ Over time in culture, chondrocytes located in proximity to the channel deposit extracellular matrix that progressively fills it. Thus, even though channels may improve nutrient supply only initially, they may be most beneficial in the formative stages of large engineered constructs and may not be as critical for maintaining tissue properties following implantation into a joint. This nutrient channel method may be scaled up for larger scaffolds by adding more channels. In a study of 10 mm diameter constructs, the placement of three channels produced tissue with mechanical properties similar to native cartilage.⁸¹ As cartilage tissue engineering moves toward cultivating biological replacements for the entire articular surface, an array of channels may be critical for achieving adequate mechanical and biochemical properties.

Another approach that is being investigated is to incorporate lipid-shelled microbubbles or microtubes as a porogens for hydrogel scaffolds.^{85–88} Originally designed for drug delivery,^{89–91} these biocompatible porogens are utilized directly with cells during the hydrogel scaffold cross linking process. This allows engineers to create microlevel porosity in the superstructure of the hydrogel, while maintaining tight, nanolevel porosity in the scaffolding directly around the embedded cells. The porous superstructure creates pockets of fluid-fluid nutrient reservoirs that provide less resistance to solute diffusion (Fig. 6A).⁸⁸ Preliminary studies suggest that microporogens improve the homogeneity of mechanical properties through the depth of the construct.^{81,88} Constructs with a relatively low concentration of microporogens (0.2% wet by volume or 10% of the agarose hydrogel concentration) are more opaque and are 2× stiffer than control constructs without microporogens (Fig. 6B).^{85,88}

The gas-filled lipid microbubbles are incorporated into the scaffold filled with a stable gas. The size of the microbubble and the gas that is used to create them can be altered to control parameters such as the dispersion rate of the bubbles and the porosity of the hydrogel. Intriguingly, it may be possible to create microbubbles that maintain their gas-phase for extended periods of time. Under this scenario, it may be possible to purge the gas (and thereby create a fluid filled pocket) later in culture. This would allow both spatial and temporal control of hydrogel porosity. It may even allow platen-less dynamic deformational loading as the gas-phase of the bubbles are utilized in a hydrostatic pressure chamber.

Alternatively, lipid microtubes provide a hard tubular shell that may act as a nutrient channel on the microscopic scale (diameter = 0.5 μm , length = 40 μm).⁹² Similar to the lipid-microbubbles, preliminary data suggests that these porogens can be incorporated into hydrogels to improve nutrient diffusion into engineered constructs. Moreover, the length of the microtubes can be increased to provide larger fluid filled pockets. This approach combines the decreased nutrient path length provided by channels and the enzyme or

nutrient loading capability of microporogens. Since microtubes have a lipid wall between the cells and the open channel, it is not expected to fill with extracellular matrix with time in culture, providing long-term enhanced nutrient diffusion. Although these studies have shown promise for using microtubes to increase the scaffold porosity, nutrient diffusion, extracellular matrix production, and mechanical properties,^{85,87,88} future work is needed to confirm that the micropores are maintained with long-term culture and with physiological levels of loading.

Strategies for Improving Collagen Production

Collagen type II is a major constituent of the articular cartilage matrix. Although engineered cartilage is capable of producing native levels of GAG, recapitulating the collagen composition and structure remains a significant challenge for the field. Previous studies have described methods to increase in vitro collagen production by differentiating cells in monolayer culture before 3D encapsulation,⁶⁴ digesting the deposited GAGs or scaffold.^{53,85,93–95}

Digestion of deposited GAGs with chondroitinase ABC (chABC) is a counterintuitive approach, but has been a successful strategy for increasing the collagen content, since the depletion of GAGs may provide more space for cells to deposit collagen fibrils. In this strategy, ~90% of the GAGs are removed with chABC digestion of mature engineered constructs.⁵³ During the subsequent culture period, the GAG content recovers to the same level as undigested control samples within 4 weeks.^{53,85,95–97} Following GAG recovery, the tensile and compressive mechanical properties of digested constructs are significantly better than the undigested constructs.^{53,85,95–97} Multiple digestions with chABC can be applied throughout the culture period with additive increases in the collagen content and mechanical properties (Fig. 7).^{53,96} These intriguing results suggest that additional studies are needed to understand the interactions between GAG and collagen synthesis and deposition in engineered constructs.

An alternative but related approach is to digest the agarose hydrogel scaffold to increase the collagen content.^{53,94} In one such study, exposing mature constructs to agarase delivered through the culture media digested approximately half of the agarose content in 4 mm diameter constructs. The digestion decreased the compressive modulus by ~45%, leaving enough extracellular matrix to maintain construct integrity, while cell viability was unaffected.⁹⁴ After the digestion, the collagen content (normalized by wet weight) continued to increase throughout the culture period and achieved levels significantly greater than the undigested control.⁵³ After 8 weeks of digestion, (15 weeks in culture), the collagen content per wet weight was 6 to 7% for the agarose-treated constructs, which was 2.2× greater than the undigested control. The dynamic and equilibrium moduli, and the GAG content of the digested constructs recovered to the undigested control values within 7 weeks. The results of these studies suggest that biodegradable scaffolds designed to degrade within 4 to 6 weeks may provide ideal conditions for cultivating a fully bioengineered cartilage replacements.⁹⁸

In previous studies, chABC or agarase was added directly to the culture medium. The beneficial effects of the digestion were limited to the periphery of the construct, due to the diffusion-reaction gradient of enzyme toward the center of the construct (Fig. 6C).^{53,94} As described above, there is a growing interest to encapsulate biomaterials originally designed for drug delivery into hydrogels for cartilage tissue engineering.^{85–88} Furthermore, these biomaterials can be modified to encapsulate enzymes or growth factors. Lee et al developed a method for delivering thermostabilized chABC using sugar trehalose and hydrogel-microtubes for applications requiring extended enzyme release (~10 day release).⁹⁰ Encapsulation of microtubes during the fabrication process of engineered cartilage

constructs allows for uniform distribution of the microtubes throughout the scaffold (Fig. 6C). This concept has been tested in a preliminary study by encapsulating chABC-loaded microtubes in chondrocyte-seeded hydrogels.⁸⁵ This study demonstrated that early exposure of immature engineered cartilage to a low continual dose of chABC did not inhibit tissue growth or mechanical properties. As observed in previous studies that added chABC to the culture media, the collagen content of constructs with chABC-loaded microtubes was greater than control constructs at early culture time points. The improved collagen production during the first 2 weeks in culture resulted in improved compressive properties throughout the 7-week culture period. Furthermore, the uniform distribution of microtubes in the construct resulted in a more homogeneous distribution of GAG and collagen.⁸⁵ It is anticipated that chABC-loaded microtubes can improve collagen production in vitro, providing more functional engineered cartilage. The microporogens can be modified to alter the release rate or delay initial release of the enzyme, which would be beneficial in optimizing collagen production using enzymatic digestion.

These studies suggest that digestion of the scaffold or GAGs in mature constructs may provide a viable method for culturing engineered cartilage with near native collagen values. It is important to note that the culture time needed to create a functional engineered cartilage for implantation will increase with digestion, as full recovery of the GAGs may take up to 4 weeks. Clinical application of functional tissue engineering will need to balance the need for sufficient collagen content versus the cost of longer culture time.

Many studies have focused on increasing collagen production, but the collagen produced in vitro tends to be oriented randomly throughout the construct. In native cartilage, the collagen fibrils in the superficial layer are aligned tangential to the articular surface, whereas fibrils in the deep zone are oriented radially.⁹⁹ Collagen fibrils provide the tissue with tensile strength to help resist the lateral expansion of the tissue when subjected to elevated compressive and shear loads in situ.¹⁰⁰ Therefore, future work may need to focus on directing collagen fibril orientation during construct growth. Our previous work has suggested that applied deformational loading can influence fiber orientation in engineered cartilage, producing alignment perpendicular to the applied axial loading in unconfined compression of cylindrical constructs.⁶⁵ Engineering fibrocartilage tissue constructs may be achieved by using stiff fibers to provide a scaffold that can withstand the higher tensile stresses that these tissue experience under physiological loads. For example, microfibers may be fabricated to produce a prescribed nonlinear stress-strain response with a specific Young's modulus.¹⁰¹ Encapsulation of these micro- or nanoscaled fibers within a hydrogel scaffold may be important for providing a fiber network template.

Conclusion

Encouraging progress has been made in the field of cartilage functional tissue engineering over the last two decades, demonstrating that it is possible to engineer constructs from a variety of cell sources, while achieving native levels of GAG content and equilibrium compressive properties. Due to the competing effects of nutrient transport and consumption, engineering functional constructs is necessarily limited to small sizes relative to the overall dimensions of articular layers in human joints. Therefore, such constructs are currently more suitable for repairing focal defects only, and various strategies are needed to scale up these successful methodologies to produce full-size engineered articular layers. Several promising new strategies were reviewed above, including macro- or microporogens, placement of channels, and dynamic loading, all of which aim to enhance the supply of nutrients to chondrocytes.

Active methods for improving nutrient uptake include bioreactors that apply compression^{24,29,30,35,36,75,80} or rotation^{102,103} and perfusion devices.^{78,80,104,105} As discussed in the previous section, physiological levels of compressive dynamic loading (~10% strain, less than 6 hours) may be ideal for enhancing nutrient diffusion into engineered cartilage.³³ Future work will need to combine these techniques to understand whether the effects of increased porosity via micro- or macrochannels can be additive to the improved nutrient diffusion from dynamic loading (Fig. 8).

Another challenge confronting the field of cartilage tissue engineering is the requirement to produce collagen content levels and tensile mechanical properties that reproduce native values. For this challenge, a consistent observation emerging from recent tissue engineering studies is the apparent hindrance to higher collagen synthesis caused by the presence of hydrogel and the increasing levels of GAG in the constructs. Enzymatic digestion of either agarose or GAG has been utilized successfully to improve collagen content without sacrificing the mechanical integrity of the mature construct.

Although the studies summarized above have demonstrated marked improvements in collagen production and nutrient transport, the resulting constructs do not yet replicate the full complement of functional properties of native cartilage. Consequently, more investigations are needed to combine and refine these new methods, while continuing to develop alternative strategies to produce implantable, full-size articular layers that can withstand the harsh mechanical and biological environment of an osteoarthritic joint.

Acknowledgments

Funding was provided by the NIH (R01AR046568 and R01AR060361), an NIH diversity fellowship, and a FASEB postdoctoral fellowship.

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Figure 1. Engineered patella construct showing proteoglycan-rich matrix (red Safranin-O stain) limited to gel periphery indicating diffusion limitations. *interface between gel-bony substrate (adapted from Hung et al¹⁴).

Figure 2.

(A) Equilibrium modulus (E_Y) and (B) glycosaminoglycan content (GAG) normalized to percent wet weight (ww) for synovium-derived stem cell encapsulated hydrogels cultured with continuous growth factor (solid bar) or with transient release of growth factors at day 21 (white bar), mean $\pm$ SD, * $p < 0.05$ for continuous versus transient groups for $n = 5$ constructs/group (adapted from Sampat et al⁵⁵).

Figure 3.

Confocal images of fluorescently-labeled 70 kDa dextran solute in cartilage sections after testing under dynamic loading or passive diffusion conditions. These images demonstrate that solute pumping under dynamic loading occurs at cartilage canals as well as the outer boundaries of the explant, as evidenced from the narrow boundary layers of high solute concentrations at 30 seconds and 2 hours. Over time, solute concentration spreads out from the canals into the surrounding matrix (20 hours).

Figure 4.

(A) Human patella-shaped stainless steel molds for fabrication of patella constructs. (B) Schematic of loading platens for applying deformational loading to patella constructs with and without concomitant perfusion. (C) Solute transport study of engineered patella constructs using 70 kDa dextran analyzed at the peripheral edge or center region. The experimental groups are FS, free swelling; SL, static loading; SLP, static loading with perfusion; DL, dynamic loading; and DLP, dynamic loading with perfusion. Dextran concentration in engineered cartilage (normalized to bathing concentration) after 1.5, 3, and 6 hours (mean $\pm$ S.D. $N = 3$; $n = 8-16$ per group). * $p < 0.05$ relative to FS; ** $p < 0.05$ relative to DL and DLP.

Figure 5.

Schematic showing improved nutrient diffusion into (A) a hydrogel scaffold by adding a macroscopic channel (white circle) to the scaffold (B). Representative Young's modulus (E_Y) through the depth of a mature construct for a (C) control construct and (D) a construct with a channel in the center (adapted from Bian et al 2009⁸¹). Using a microscopy-based material testing device and digital image correlation, the modulus is determined and plotted for each construct, divided into five layers of equal thickness across the depth of the disks. * $p < 0.05$ versus the central region (2–4).

Figure 6.

(A) A schematic of solute diffusion in agarose hydrogel (ctrl). Microbubbles (pink circles) at varying concentrations can be incorporated into the hydrogel to increase the relative porosity of the scaffold upon their dissolution, thereby decreasing the nutrient path length to the center of the construct. Strategies for increasing collagen content of engineered cartilage include controlled enzymatic digestion of proteoglycans. (B) Gross image of an acellular hydrogel without (left) and with (right) microtubes. Bar = 1mm. (C) Schematic representing enzyme diffusion into the scaffold from the culture media bath (left) and from scaffold encapsulated lipid microtubes (right). The darker shading represents increased enzyme concentration.

Figure 7.

Digestion of mature engineered constructs with chondroitinase ABC (chABC) added to the culture media increases the collagen content. Multiple applications of chABC added to the media results in further increases in the collagen content. Control, undigested constructs; chABC 1T, single chABC digestion at day 35; chABC 2T, chABC digestions at day 35 and 58. * $p < 0.05$. Adapted from Bian et al.⁵³

Figure 8.

Schematic showing dynamic loading of an engineered construct molded in the shape of a full human patella surface. The scaffold porosity (AFM image, right column-top) can be improved by incorporating microporogens, such as microbubbles (right column—middle; with calcein-labeled chondrocytes in green), or macrochannels (right column—bottom; asterisk and dotted lines indicating channel lumen with calcein-labeled chondrocytes in green).