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Characterization of Chondrocyte Scaffold Carriers for Cell-based Gene Therapy in Articular Cartilage Repair

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Abstract

Articular cartilage lesions in the knee are common injuries. Chondrocyte transplant represents a promising therapeutic modality for articular cartilage injuries. Here, we characterize the viability and transgene expression of articular chondrocytes cultured in 3-D scaffolds provided by four types of carriers. Articular chondrocytes are isolated from rabbit knees and cultured in four types of scaffolds: type I collagen sponge, fibrin glue, hyaluronan, and Open-cell PolyLactic Acid (OPLA). The cultured cells are transduced with adenovirus expressing green fluorescence protein (AdGFP) and luciferase (AdGL3-Luc). The viability and gene expression in the chondrocytes are determined with fluorescence microscopy and luciferase assay. Cartilage matrix production is assessed by Alcian blue staining. Rabbit articular chondrocytes are effectively infected by AdGFP and exhibited sustained GFP expression. All tested scaffolds support the survival and gene expression of the infected chondrocytes. However, the highest transgene expression is observed in the OPLA carrier. At four weeks, Alcian blue-positive matrix materials are readily detected in OPLA cultures. Thus, our results indicate that, while all tested carriers can support the survival of chondrocytes, OPLA supports the highest transgene expression and is the most conductive scaffold for matrix production, suggesting that OPLA may be a suitable scaffold for cell-based gene therapy of articular cartilage repairs.

Keywords

Articular cartilage; autologous chondrocyte implantation; chondrocyte; ex vivo gene therapy; Open-cell PolyLactic Acid (OPLA); scaffold carrier

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CONFLICT OF INTEREST The authors declare no conflicts of interest.

INTRODUCTION

Articular cartilage lesions in the knee are common injuries and have been shown to possess little intrinsic ability to heal. When patients fail conservative management, current surgical treatments include abrasion chondroplasty, osteochondral transplantation, and autologous chondrocyte implantation (ACI)^{1,2}. Clinical outcomes after treatment, while acceptable, are not optimal. These limited clinical results mandate that new biologic strategies may be a useful adjunct to further improve current clinical outcomes.

It has been reported that chondrocyte transplantation in monolayer culture results in chondrocytes assuming a fibroblastic dedifferentiated morphology³⁻⁵. These cells synthesize predominantly type I collagen with the subsequent loss of ability to produce matrix. This is analogous to the fibrocartilage, composed of type I collagen, found in healed full thickness cartilage defects⁶, and may account for the histologic and mechanical differences found between normal hyaline cartilage and repaired cartilage⁷. However, studies have demonstrated that the preservation of the chondrocyte phenotype can be achieved when seeded on a three-dimensional scaffold^{8,9}. These scaffolds provide a porous structure that facilitates cell adhesion and growth in a three-dimensional conformation, permitting high density cell suspensions and simulating normal cell architecture. This architecture also allows for better cell spacing with the subsequent formation of extracellular matrix. Furthermore, adhesion to a scaffold may prevent cell leakage from the osteochondral defect, and thereby cell loss, common with current ACI techniques. In order to augment the survival and growth of the implanted chondrocytes at the articular cartilage repair sites, various biologic substances have been used as cell scaffolds, including hyaluronan, collagen gels and sponges, fibrin glue, and synthetic materials¹⁰⁻¹³. Different carriers have achieved varying degrees of success. However, to the best of our knowledge, no comparison study has been carried out to determine how different scaffolds support cell growth, gene expression, and matrix production.

We hypothesize that optimal chondrocyte scaffolds enhance cell-based gene transfer in articular chondrocytes by providing necessary architecture for chondrocyte growth and matrix production in vitro. We choose commercially available biologic materials that have either received FDA approval or are biologically similar to FDA approved implants. These scaffold carriers include type I collagen sponge, fibrin glue, hyaluronan, and Open-cell PolyLactic Acid (OPLA)¹⁰⁻¹³. Type I collagen sponge represents a readily available biodegradable carrier with a significant body of prior research. Furthermore, it has been used in an intra-articular environment to deliver recombinant proteins¹⁴. Fibrin glue has multiple orthopaedic uses, including ACI^{13,15,16}, and has also been used as a chondrocyte carrier¹⁷. Hyaluronan has been extensively used intra-articularly, and can be manufactured to maintain any forms from a liquid to a viscous mixture to a solid sponge. Finally, polylactic acid exhibits multiple ideal material properties for use with cartilage regeneration due to its slow biodegradability, structural rigidity, and cell adhesion. Each of these scaffolds has unique material properties and has the potential to support and enhance chondrocyte growth and matrix production.

Here, we characterize and compare different scaffolds in relation to chondrocyte viability, level of transgene expression, and matrix production. We isolate articular chondrocytes from rabbit knees and culture them in the scaffolds. The cultured cells are transduced with adenovirus expressing green fluorescence protein (AdGFP) and luciferase (AdGL3-Luc). We find that all tested scaffolds support the survival and gene expression of the infected chondrocytes. However, the highest transgene expression is observed in the OPLA carrier. At four weeks, Alcian blue-positive matrix materials are readily detected in OPLA cultures. Thus, our results indicate that, while all tested carriers can support the survival of chondrocytes, OPLA supports the highest transgene expression and is the most conducive scaffold for matrix production, suggesting that OPLA may be a more suitable scaffold for cell-based gene therapy of articular cartilage repairs.

MATERIALS AND METHODS

Cell culture and chemicals

Human embryonic kidney cell line 293 (HEK 293) was purchased from the American Type Culture Collection (ATCC, Manassas, VA), and maintained in DMEM (Mediatech) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS, HyClone, Logan, UT) and streptomycin (100 µg/ml final concentration)/ penicillin (100 IU/ml final concentration) (Mediatech) at 37°C and 5% CO₂. Unless otherwise indicated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Isolation and culture of primary rabbit articular chondrocytes

The use of rabbits was approved by the Institutional Animal Care and Use Committee. The skeletally mature New Zealand White rabbits (male, 8 week old, Harlan Laboratories) were used. Approximately 3 × 3 mm pieces of articular cartilage were harvested from the knees. The specimens were rinsed twice with PBS buffer. Using a sterile surgical scalpel, the cartilage tissue was carefully minced and suspended in 30 ml of type I collagenase (1.0 mg/ml, Invitrogen, Carlsbad, CA) and deoxyribonuclease I (0.1 mg/mg, Sigma) containing complete DMEM/F-12 medium (Dulbecco's Modification of Eagle's Medium:F-12 Medium, 1:1) supplemented with 20% fetal calf serum (FCS), and incubated at 37°C for 5h in a spinner flask with continuous agitation at a rate of approximately 50 rpm. At the completion of digestion, centrifugation was applied to collect the dissociated cells. The cellular component was resuspended in DMEM/F-12 medium supplemented with 20% FCS, placed in 6-well plates and incubated overnight at 37°C in an atmosphere of 5% CO₂. On the following day, the media was changed to remove tissue and cell debris. The media was subsequently changed every 2-3 days. Once the cells approached 80-90% confluency, they were trypsinized and expanded in 25cm² cell culture flasks for seeding experiments using various carriers.

Construction and generation of recombinant adenoviral vectors AdGFP and AdGL3-Luc

We constructed recombinant adenoviral vectors that constitutively expressed green fluorescent protein (AdGFP) and firefly luciferase (AdGL3-Luc). These adenoviral vectors were constructed and amplified in HEK 293 cells according to our previously developed

AdEasy technology¹⁸. GFP expression in the infected cells was observed under a fluorescence microscope.

Implantation of adenovirus-transduced chondrocytes into various carriers

The cultured articular chondrocytes were infected with AdGFP and AdGL3-Luc to determine the optimal titer (multiplicity of infection, or MOI = 50). Exponentially growing primary rabbit articular chondrocytes were then seeded in 24-well cell culture plates and infected with AdGFP or AdGL3-Luc at this optimal titer. At 24 hours after infection, expression of GFP was verified with fluorescence microscopy. Approximately 2×10^6 cells in 50 μ l of complete DMEM/F-12 medium were used to seed each of the four carriers on 48-well plates.

Four types of commonly used biologic carriers were used: included type I collagen sponge (Sofamor Danek, Memphis, TN), OPLA (BD Bioscience, San Jose, CA), Tiseel Fibrin Glue (Baxter, Deerfield, IL), and Hyaluronan (HA, MW = 220 Kd, Lifecore, Chaska, MN). The type I collagen sponge was cut to measure approximately 0.04 cm³ (5×4×2mm), while the OPLA carriers were also approximately 0.04 cm³ (4mm diameter and 3mm height). 2×10^6 chondrocytes resuspended in 50 μ l of complete DMEM/F-12 medium were used for each seeding. 50 μ l of cells were directly pipetted onto type I collagen sponges and OPLA carriers. The cell mix was completely absorbed by the carriers, and no additional medium was added to the seeded wells of 48-well cell culture plates. The 1% hyaluronan was prepared in complete DMEM/F-12 medium and sterilized by filtration. 100 μ l of this 1% hyaluronan was mixed with 50 μ l of infected chondrocytes for a final concentration of 0.67%. Likewise, approximately 100 μ l of fibrin glue, which was prepared according to manufacturer's instructions, was mixed with the infected chondrocytes prior to clot formation. Non-seeded wells surrounding the carrier-containing wells were filled with sterilized PBS and the plates were loosely wrapped with Saran Wrap to prevent the carriers from desiccating. GFP expression of the seeded cells was examined regularly under a fluorescent microscope, and recorded at days 2, 5, and 10. In this study, the duration and extent of GFP expression in the infected articular chondrocytes was used as a measure of cell viability and transgene expression in the different growth environments (i.e., scaffold materials). Each assay condition was done in triplicate. Representative results from three independent experiments are reported.

Quantification of GFP signal and transgene expression

All images with GFP expression were captured and processed with NIH ImageJ software to quantify the amount and intensity of fluorescence and compare levels across different samples. During image capture from the fluorescent microscope, exposure time was standardized to 1/4 second to prevent varying signal intensity from exposure time rather than gene expression. Images were converted to 8-bit grayscale format to simplify processing. Because there was mainly one channel of color (green), there was not significant data loss during this conversion. A histogram was then constructed to analyze the distribution of pixel values. This distribution list was then imported into an Excel (Microsoft) spreadsheet, and the pixel value (0-256) was multiplied by the number of pixels in the image that contained that value. The Total Image Value (TIV) was defined as the sum of all the pixel values

multiplied by the pixel counts and was directly proportional to the amount of fluorescence. Furthermore, the size of the samples (in pixels) was kept constant to avoid different TIV due to increased pixel count rather than fluorescence. This allowed for comparison between images. Due to the 3-D conformation of the carriers, at any given focal length, some cells were in focus while others were out of focus. As such, it was not possible to perform an analysis based on average fluorescence per cell or to correct for the background fluorescence since no threshold could be defined that would distinguish between background and an out-of-focus chondrocytes.

Luciferase assay

At the end points of cell seeding/implantation, the cell-containing carriers were grounded in 1x Luciferase Cell Lysis buffer (Promega, Madison, WI), and the luciferase activity was determined by using the Luciferase Assay kit (Promega) as previously described^{19,20}. Each condition was done in duplicate.

Statistical analysis

The Analysis of Variance (ANOVA) was used to analyze the means of the different treatment groups as defined by different carriers. This was done in place of the standard t-test to avoid an inflation of the type I error rate inherent with multiple comparisons. The independent variable was defined to be the treatment group, in this case the various carriers. The dependent variable was the transgene expression (e.g., the luciferase activity). All analysis was performed with SAS (SAS, Cary, NC).

Alcian Blue staining and hematoxylin-eosin (H & E) staining

At the endpoint of each assay condition, cell-containing carriers were fixed in 1% glutaraldehyde solution overnight, and then subjected to snap freezing with Tissue-Tek OCT (Sakura Finetek, Torrance, CA) in liquid nitrogen. Five-micron sections were prepared and stained with Alcian Blue (pH 1.0 to stain for strongly sulfated mucosubstances, such as chondroitin, dermatan, or keratin sulfates) or H & E staining. The stained samples were imaged under a bright field microscope.

RESULTS

Isolation and Culture of Articular Chondrocytes

Articular cartilage was harvested and cultured as described in Methods. Several trials were conducted to optimize the recovery of viable primary chondrocytes. Previous studies have used collagenase at 1mg/ml with an overnight incubation¹⁶. We used varying concentrations of collagenase, including 0.1, 1.0, 2.5 and 5 mg/ml, and varying incubation times ranging from 2.75-20 hours. Representative results are displayed in Fig. 1A. Our results indicate that an efficient isolation of chondrocytes was achieved using a slightly higher concentration of collagenase for a shorter period (vs. overnight) of digestion, while more dead cells were also observed at higher concentrations of collagenase (Fig. 1A). In general, the isolated chondrocytes grew rather slowly during the first three to four days, but then grew faster upon reaching higher densities. Morphology of the cultured cells was examined under a bright field microscope at 2, 5, 7, and 10 days after plating. As shown in Fig. 1B, the

cultured articular chondrocytes adopted a mostly round to short spindle-like cell phenotype, similar to the expected morphology of chondrocytes repopulated under *in vitro* monolayer culture condition.

Recombinant Adenoviral Vector-mediated Gene Transfer in Primary Articular Chondrocytes

As shown in Fig. 2A, robust GFP expression was evident at 24 hours after infection, and continued to increase at 72 hours after infection. The GFP expression was observed at least four weeks after infection. Fig. 2B shows a histogram visually depicting the increasing expression of GFP using the TIV to compare the fluorescence at different time points. These results show that primary articular chondrocytes can survive adenoviral infection and effectively express the reporter gene GFP.

Survival and Gene Expression of Primary Articular Chondrocytes Implanted in Four Scaffold Carriers

Chondrocytes were infected and seeded onto the respective carriers and examined at days 2, 5, and 10. Representative results from three independent experiments are shown in Fig. 3A. At all three time points, GFP expression was observed in all four types of scaffold carriers. However, the intensity of GFP signal was significantly higher in the cells seeded in the OPLA three-dimensional culture (Fig. 3A, bottom row) at each time point than those in the other three carriers. This is demonstrated by the histograms comparing the TIV of the different carriers at each time point (Fig. 3B). It should be pointed out that GFP was moderately expressed in the cells seeded in type I collagen sponge (Fig. 3A, top row), while the hyaluronan carrier (MW = 220 Kd) was the least favorable for chondrocyte growth and gene expression (Fig. 3A, second row). The GFP signal in these three-dimensional cultures was observed at least four weeks after infection. These findings were reproducible in three independent batches of experiments. It is noteworthy that due to 3-dimensional configuration of the chondrocytes in the different carriers, any photograph at a given focal plane contained some cells out of focus.

Quantitative Analysis of the Survival and Gene Expression of Primary Articular Chondrocytes Implanted in Four Different Scaffold Carriers

In order to determine gene expression of the chondrocytes implanted in the scaffold carriers in a quantitative manner, we sought to use a recombinant adenoviral vector constitutively expressing the luciferase gene (AdGL3-Luc), one of the most commonly used reporters for quantitative gene expression. Chondrocytes were infected AdGL3-Luc and seeded into the respective carriers. Luciferase activity was determined at 24 and 48 hours after seeding. As shown in Fig. 4, the highest luciferase activity among the four types of carriers was observed in the cells seeded in the OPLA carrier. Specifically, the luciferase activity in cells seeded in type I collagen, hyaluronan, and fibrin glue scaffold carriers was 20% to 30% of that in OPLA carrier at both time points. These findings were reproducible in two independent experiments. However, it should be pointed out that luciferase activity in the cells seeded in OPLA carrier was approximately 50% lower than that in the cells seeded without any carriers, suggesting that the survival and gene expression of primary articular cartilage cells

may be indeed affected by 3-dimensional culture conditions. The ANOVA analysis indicates that the difference in luciferase activity among the four different carriers was statistically significant ($p < 0.001$). These results show that the different carriers can significantly affect transgene expression. Taken together, our quantitative data are consistent with the GFP signal intensity shown in Fig. 3. Collectively, our results demonstrate that OLPA may be a more cell-friendly scaffold carrier for primary articular chondrocytes.

Matrix Production by Articular Chondrocytes Seeded in the OPLA Scaffold Carrier

We next sought to determine whether articular chondrocytes in the OPLA 3D culture produced cartilage matrix. Briefly, primary articular chondrocytes were seeded into OPLA plugs and fixed at four weeks. The sections were stained with Alcian blue (Fig. 5 B and D), while the plain sections are shown in Fig. 5A and C. Alternatively, the fixed OPLA carriers were first subjected to Alcian blue staining, and then frozen sectioning (Fig. 5E). The fixed OPLA carriers were also stained with hematoxylin-eosin (Fig. 5F). Consistent with the properties described in the manufacturer's instructions, the OPLA carrier was a highly porous scaffold. Cultured articular chondrocytes in the OPLA carrier indeed produced cartilage matrix-like materials as demonstrated by Alcian blue staining (Fig. 5 D and E), which contained clusters of cellular components in the carrier (Fig. 5F), although a fair amount of the OPLA carrier and its cartilage matrix was lost during fixation. Taken together, these results indicate that the primary articular chondrocytes can survive and produce cartilage matrix in the OPLA 3-D culture.

DISCUSSION

In this study, we characterized the cell viability and gene expression of primary articular chondrocytes seeded in four types of 3D cultures, including type I collagen sponges, hyaluronan, fibrin glue, and polylactic acid. To the best of our knowledge, no comparison study has been carried out to determine how different scaffolds support cell growth, gene expression, and matrix production. Here, we show differences in chondrocyte viability and transgene expression in four types of cell carriers.

In this study, we chose to use a recombinant adenoviral vector to transduce articular chondrocytes for the following reasons: 1) recombinant adenoviruses remain one of the most efficient means of gene transfer to a broad range of cell types, including chondrocytes;²¹ 2) recombinant adenoviruses have already been successfully used to infect articular chondrocytes *ex vivo*, resulting in sustained, but not indefinite, expression;^{22,23} 3) we anticipate use of an adenoviral vector in future studies for delivery of therapeutic genes. The major limitation of adenoviruses comes from their propensity to induce inflammation. However, this may be limited by controlling the dosage and by using the adenovirus in a relatively avascular space such as articular cartilage. Since adenoviral vectors can effectively infect cells in a short period of time (e.g., <30 minutes), they can potentially be used intraoperatively to infect implanted cells for a brief period after they have been isolated. This approach has been used with considerable success in animal models of spinal fusion²⁴. Such an approach would potentially allow surgeons to treat articular cartilage lesions with transduced cells immediately, as opposed to performing a staged procedure with

chondrocytes expanded and infected *ex vivo*. Nonetheless, this procedure requires further investigation to determine whether this approach is applicable in the context of articular cartilage injuries.

One of the prominent features of naturally healed cartilage lesions is the preponderance of fibrocartilagenous repair tissue. The cells which populate this fibrous repair tissue are primarily spindle-shaped, and synthesize type I collagen⁶. This is in contrast to normal articular chondrocytes. The resulting repair tissue is, therefore, histologically and mechanically distinct from surrounding normal hyaline cartilage⁷. In a long term follow-up of 101 patients after ACI procedures, Peterson et al.¹⁵ found that most graft failures of the medial femoral condyle occurred in lesions that were fibrous in appearance, and concluded that type II collagen was highly correlated with good to excellent long-term outcomes. With good clinical results correlated to the presence of type II collagen, it follows that techniques to promote and sustain synthesis of type II collagen might be of further benefit.

Currently, ACI for full-thickness cartilage defects utilize chondrocytes grown on a monolayer culture^{16,25}. However, previous reports suggest that chondrocytes in monolayer culture tend to adopt a fibroblastic dedifferentiated morphology and synthesize predominantly type I collagen³⁻⁵. This may predispose these cells to form fibrocartilagenous repair tissue rather than hyaline cartilage. The use of a three-dimensional scaffold is one method of maintaining the chondrocyte phenotype. Thus far, several types of tissue scaffolds have been investigated, including fibrin glue, collagen gels and sponges, hyaluronan esters, polylactic and polyglycolic acid, as well as alginate and composite matrices¹⁰⁻¹³. These different scaffolds share many of the prerequisites for successful cell implantation and growth.

Fibrin glue as a chondrocyte scaffold for articular defects held much promise initially. Previously reported uses of fibrin glue include arthroscopic meniscal repair^{26,27} and as a scaffold for chondrocytes to reconstruct the external ear¹⁷. These uses suggest that fibrin glue may be both chondrocyte friendly and stable within a joint. Our results confirm that chondrocytes were viable when embedded within a fibrin clot (see Fig.s 3 and 4). However, we found fibrin glue to be sub-optimal as a surgical scaffold. First, its tackiness and difficulty in handling prevented confident placement. Further, van Susante reported that fibrin glue in an intra-articular environment (e.g., goat knee) was gradually replaced by fibrous tissue after three weeks²⁸. In such duration, fibrin glue did not offer enough biomechanical support for use as a chondrocyte scaffold in weight bearing portions of the joint. Finally, Brittberg found that fibrin within articular defects may actually impede healing by impairing chondrocyte migration¹³. Nonetheless, because it does not adversely affect cell viability, fibrin glue may continue to play a role as an adjunct in ACI to seal the carrier and chondrocytes into the cartilage defect. This is especially relevant to certain surgical techniques such as ACI because it may avoid the harvesting and suturing of a periosteal patch.

Type I collagen sponges are commercially available, with a significant body of knowledge regarding their uses and modifications. It has been used successfully in intra-articular defects to deliver recombinant proteins¹⁴. Gruber et al showed that chondrocytes from

vertebral discs maintained round morphology and expressed type II collagen when implanted on type I collagen sponge²⁹. However, Nehrer et al compared chondrocyte morphology and matrix production on type I and type II collagen sponges and found that at two weeks, the type II sponges showed higher amounts of DNA and proteoglycan after biochemical analysis³⁰. Type II collagen sponges would be theoretically more appealing and have many of the same material properties as the type I sponges, with the added benefit of increasing cartilage matrix. However, we are unaware of any commercially available type II collagen sponges. There have been some reports of homemade type II collagen scaffolds, but these have been neither standardized nor reproduced^{10,31}.

Hyaluronan represents an interesting biologic carrier. Various studies have suggested a clinical benefit from injection of intra-articular hyaluronan³². Potential mechanisms of hyaluronan may include anti-inflammatory, lubrication, stabilization of the cartilage and matrix, or alterations in normal wound healing and granulation tissue formation³³. Multiple formulations of hyaluronan have received FDA approval as injectables. However, an injectable would be too liquid to be used as a cell scaffold. Recently, hyaluronan has been modified to form a solid sponge or mesh through different chemical processes, such as esterification (hyaff, hyalofil, ACP). Initial results suggest this may be a good cell scaffold. In order to examine the effects of hyaluronan, we directly suspended the chondrocytes in viscous hyaluronan, and then plated the mixture to form a gel-like carrier. Our results demonstrate that the cells indeed remained viable and continued to express the transgene although the transgene expression was not as high as with the OPLA.

Poly(lactic acid) (PLA) and its derivative polyglycolic acid (PGA) are synthetic materials and widely used in Orthopaedic surgery. Both PLA and are biocompatible^{34,35}. The first commercial product of a copolymer of PLA and PGA was Vicryl suture (J&J), composed of 8% PLA and 92% PGA. Currently used PLA and PGA implants include arthroscopic tacks, screws, and suture anchors³⁶. OPLA is a special formulation of PLA and is synthesized from D,D-L,L poly(lactic acid), which is manufactured as a non-compressible sponge with a pore size of 100-200µm. As a dental barrier membrane, the scaffold was found to be structurally functional at 13 weeks and completely resorbed at 12 months. Previous work examined its material properties and found it suitable as a chondrocyte carrier^{37,38}. Our results demonstrate that primary articular chondrocytes are viable and effectively express the marker gene GFP and reporter gene luciferase in the OPLA carrier. Of the four carriers tested, OPLA had the highest transgene expression, suggesting that OPLA may be the best carrier in terms of supporting transgene expression.

While the scaffolds may improve the quality of the regenerated articular cartilage by encouraging cell adhesion, cell growth, gene expression, and matrix formation, the use of scaffolds has the potential to significantly alter the surgical practices needed for chondrocyte transplant. It is conceivable that cells may be preloaded onto a scaffold, which can then be press-fit into an articular cartilage defect. This would obviate the need to harvest and suture a periosteal patch and decrease surgical time, while simultaneously improving outcomes. It may also avoid morbidities associated with the current practice of allograft transplants. It would be ideal to combine the mechanical benefits of allograft transplantation with the

improved biologics in chondrocyte transplantation. Similar techniques may also be applied to the use of mesenchymal stem cells in place of harvested chondrocytes.

CONCLUSIONS

We characterize the viability and transgene expression of primary articular chondrocytes cultured in the three-dimensional structures provided by four types of scaffold carriers, including type I collagen sponges, hyaluronan, fibrin glue, and OPLA. While all four carriers support the survival of primary articular chondrocytes, our findings strongly suggest that OPLA may represent one of the most cell-friendly scaffolds among the four carriers tested, as judged quantitatively by the transgene expression in these carriers. Furthermore, OPLA supports the generation of cartilage matrix. Future efforts should be directed towards using these carriers (especially OPLA) for cell-based gene therapy in articular cartilage repairs in animal models.

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FIGURE 1.

(A) Optimization of rabbit articular chondrocyte harvest. Articular cartilage tissues were retrieved from skeletally mature New Zealand White Rabbits and subjected to collagenase I digestion using various concentrations (0.1, 1.0, 2.5, 5.0 mg/ml) for 5 hours. Recovered articular cartilage cells were cultured in complete DMEM/F12 medium supplemented with 20% fetal calf serum. Cultured chondrocytes under different digestion conditions were shown at three days after isolation. (B) Isolation and culture of rabbit articular chondrocytes. After optimization, articular chondrocytes were isolated using 1.0 mg/ml of collagenase for 5 hours. Morphology of the cultured cells was examined under a bright field microscope at 2, 5, 7, and 10 days after plating. Magnification, 200x.

FIGURE 2.

(A) Recombinant adenovirus-mediated efficient gene transfer into primary articular chondrocytes. Cultured articular chondrocytes were seeded in 24-well cell culture plates and infected with an adenoviral vector expressing green fluorescent protein (i.e., AdGFP) at a pre-optimized titer (multiplicity of infection, or MOI = 50). Expression of GFP was examined at 24, 48, and 72 hours after infection using fluorescence microscopy. (B) Histogram displaying the Total Image Value (TIV) of fluorescence. The total amount of fluorescence was calculated with NIH ImageJ software to quantify the amount of GFP expression. See text for more details.

FIGURE 3.

(A) Survival and gene expression of primary articular chondrocytes seeded in four different scaffold carriers. Subconfluent articular chondrocytes were infected with AdGFP at the MOI = 50. At 24 hours after infection, cells were collected by trypsinization and suspended in complete DMEM/F12 medium. Approximately 50 μ l cells (approx. 2×10^6 cells per seeding) were mixed with hyaluronic acid (HA) or fibrin glue, or added to type I collagen sponge or OPLA carriers. The cell-containing carriers were placed in 48-well cell culture plates, which were kept in a well-moistened CO₂ incubator. Expression of GFP was examined at 2, 5, and 10 days after cell seeding with fluorescence microscopy. Representative results from three independent experiments are shown. (B) Histogram comparing Total Image Value (TIV) of the different carriers at different time points. The TIV was calculated with the NIH ImageJ software for each time point for each carrier and the results are displayed in the histogram.

FIGURE 4.

Quantitative assessment of transgene expression of primary articular chondrocytes seeded in four different scaffold carriers. Subconfluent rabbit articular chondrocytes were infected with AdGL3-Luc at the MOI = 50. At 24 hours after infection, cells were collected by trypsinization and suspended in complete DMEM/F12 medium. Approximately 50 μ l cells (approx. 2×10^6 cells per seeding) were mixed with hyaluronic acid (HA) or fibrin glue, or added to type I collagen sponge or OPLA carriers. The cell-containing carriers were placed in 48-well cell culture plates, which were kept in a well-moistened CO₂ incubator. Luciferase activity was assayed at 24 and 48 hours after cell seeding. Each assay conditions were done in duplicate. ANOVA analysis was conducted on the quantitative data (see text for detail). Representative results from two independent batches of experiments are shown.

FIGURE 5.

Matrix production of articular chondrocytes seeded in the OPLA scaffold carrier. Primary articular chondrocytes were seeded into OPLA plugs (approximately 2×10^6 cells per seeding). Controls (OPLA Only) were done with medium only. At four weeks after plating, OPLA carriers were fixed with 1.0% glutaraldehyde. The fixed OPLA carriers were embedded in OCT medium and frozen sectioned (*a* to *d*), with some sections stained with Alcian blue (*b* and *d*). Alternatively, the fixed OPLA carriers were first subjected to Alcian blue staining, and then frozen sectioning (*e*). The fixed OPLA carriers were also stained with hematoxylin-eosin (*f*). Each condition was performed in triplicate. Representative results from two independent experiments are shown.