The effects of glycosaminoglycan content on the compressive modulus of cartilage engineered in type II collagen scaffolds

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Summary

Objective: The current study determined the unconfined compressive modulus of tissue-engineered constructs with varying sulfated glycosaminoglycan (GAG) density produced by goat articular chondrocytes in type II collagen scaffolds prepared with a range of cross-link densities and various times in culture. The purpose of this work is to establish a basis for future studies employing constructs of selected maturity (e.g., 25%, 50%, or 75% normal GAG content) for cartilage repair in vivo.

Methods: Porous scaffolds (8 mm diameter by 2 mm thick) were fabricated from porcine type II collagen by freeze-drying, followed by dehydrothermal treatment and carbodiimide cross-linking. In a pilot study, passage 3 adult caprine articular chondrocytes isolated from one goat were grown in scaffolds with six cross-link densities for 2, 3, 4, and 6 weeks (n = 3). The goal was to select scaffold cross-link densities and times in culture that would produce constructs with approximately 25%, 50% and 75% the GAG density of native articular cartilage. Based on the results of the pilot study, chondrocytes from three goats were grown in scaffolds with two cross-link densities for three time periods: 3, 5, and 9 weeks (n = 6; one of the cross-link groups was run in quadruplicate). The equilibrium modulus from unconfined compression testing of these samples was correlated with GAG content.

Results: There was a notable increase in GAG density with decreasing cross-link density. Histological analysis verified a chondrogenic phenotype and revealed various amounts of GAG and type II collagen-containing cartilage. The correlation between modulus and GAG density had a linear coefficient of determination of 0.60. One group with a mean GAG density of 22 μg/mm², which was 140% the GAG density of normal caprine articular cartilage, averaged a compressive modulus of 31.5 kPa, which was 10% of caprine articular cartilage tested in this study.

Conclusions: The GAG density and modulus of tissue-engineered constructs can be controlled by the degree of cross-linking of type II collagen scaffolds and time in culture.

Introduction

One of the critical issues related to the implementation of tissue engineering strategies for the treatment of a wide array of problems relates to the degree to which the tissue-engineered construct should be allowed to develop (i.e., mature) in vitro prior to implantation. For example, how mature should a cartilaginous construct, produced in culture, be before it is implanted into a defect in articular cartilage?

In our prior investigations using a canine model, an autologous chondrocyte-seeded type II collagen scaffold cultured for 4 weeks prior to implantation into a defect in the articular surface of the stifle joint resulted in more reparative tissue and a greater percentage of hyaline cartilage after 15 weeks, compared to a chondrocyte-seeded scaffold cultured for only 12 h prior to implantation. While the constructs cultured for 12 h and 4 weeks had different compositions and mechanical behaviors those parameters were not quantitatively evaluated. Therefore, it was not possible to correlate certain features of the make-up of the constructs with the in vivo findings.

Several approaches have been successfully employed for the production of cartilaginous constructs with varying degrees of maturity (e.g., glycosaminoglycan, GAG, content) in vitro. Numerous studies have demonstrated the effects of growth factors individually and in combination on chondrocytes in monolayer and three-dimensional (3-D) cultures. One prior investigation showed a substantial increase in the GAG content of chondrocyte-seeded type II collagen scaffolds grown in serum-free medium supplemented with fibroblast growth factor (FGF)-2, compared to cultures with no FGF-2 supplementation, after only 2 weeks in culture. Another recent investigation of chondrocyte-seeded type II...
collagen scaffolds, however, took an alternative approach to regulate the rate of chondrogenesis in vitro by altering the cross-link density of the scaffolds. The study demonstrated that the cross-link density of the scaffold significantly affected chondrogenesis in vitro. Scaffolds with higher cross-link densities, which underwent cell-mediated contraction, displayed a substantially greater GAG density than scaffolds of higher cross-link density, which did not contract. In order to prepare constructs of a particular diameter for implantation, allowance would have to be made for the amount a construct would contract, in selecting the original diameter of the scaffold to be seeded with cells.

One of the challenging aspects of the investigation of the degree of maturity that a tissue-engineered construct should have prior to implantation relates to the parameters to employ for the evaluation of the maturity of the tissue-engineered construct: morphological/histological, biochemical, and/or mechanical. To begin to address this issue we selected the sulfated GAG density of tissue-engineered cartilaginous constructs as one measure of their degree of maturity, relative to the GAG density of native articular cartilage. The goal is to eventually implant constructs with approximately 25%, 50%, and 75% the GAG density of native cartilage into defects in an animal model. The objective of the current study was to evaluate the GAG density and compressive modulus of chondrocyte-seeded type II collagen scaffolds using various combinations of cross-link density and time in culture; caprine cells were used because a goat model will be used for the in vivo evaluation.

Materials and methods

TYPE II COLLAGEN—GAG SCAFFOLD FABRICATION

Sheets of the type II collagen scaffold, 2.5 mm in thickness, were prepared from a 1% slurry of porcine type II collagen (Geistlich Biomaterials, Wolhusen, Switzerland) in 0.001 N HCl using a freeze-drying protocol found to generate scaffolds with porosity of about 90% and pores with a diameter of approximately 200 μm. Disks, 8 mm in diameter, punched from the sheets were sterilized (and lightly cross-linked) by dehydrothermal treatment and/or chemical treatment. To begin to address this issue we selected the sulfated GAG density of tissue-engineered cartilaginous constructs as one measure of their degree of maturity, relative to the GAG density of native articular cartilage. The goal is to eventually implant constructs with approximately 25%, 50%, and 75% the GAG density of native cartilage into defects in an animal model. The objective of the current study was to evaluate the GAG density and compressive modulus of chondrocyte-seeded type II collagen scaffolds using various combinations of cross-link density and time in culture; caprine cells were used because a goat model will be used for the in vivo evaluation.

CHONDROCYTE CULTURE, SEEDING OF THE SCAFFOLDS, AND VOLUME MEASUREMENTS

Articular cartilage was harvested from the stifle joints of adult Spanish goats immediately postmortem. Chondrocytes were isolated using a previously published protocol, and cultured separately. In a pilot study, passage (P) 2 chondrocytes isolated from one goat were grown in scaffolds with six cross-link densities for 2, 3, 4, and 6 weeks (n = 3). The goal was to select scaffold cross-link densities and times in culture that would produce constructs with approximately 25%, 50% and 75% the GAG density of native articular cartilage. Considering the results of the pilot study, 3 P chondrocyte-seeded scaffolds with two cross-link densities (EDAC1.5 and EDAC2) were cultured for three time periods: 3, 5, and 9 weeks. Based on the available P3 cells (Table II), a sample size of six was achieved using Goat 1 cells to seed three groups, cells from Goat 2 to seed two groups, and cells from Goat 3 to seed the sixth group of scaffolds. For the EDAC1.5 groups, the experiment was conducted in quadruplicate (n = 4), because the pilot data indicated that this treatment might be the most promising to yield the widest range of GAG density.

The scaffolds were seeded with 4 × 10^4 cells and grown in chondrogenic medium as previously described.

The diameters of the cell-seeded matrices and unseeded controls were measured at the end of the culture period using circular templates ranging from 1 mm to 10 mm diameter in 0.5 mm increments. Thickness was measured using a micrometer with a resolution of 0.01 mm. The volume of the constructs was calculated from these dimensions.

MECHANICAL TESTING

Unconfined compression testing was conducted to determine the equilibrium modulus of elasticity, E, of cell-seeded and non-cell-seeded control scaffolds (n = 6) and full-thickness (mean, 0.8 mm) samples of caprine articular cartilage tissue (n = 3 plugs from flat surfaces from one goat knee) of about the same diameter as the scaffolds. A cylindrical polymethyl methacrylate loading plate, 9.5 mm in diameter, was used in the test apparatus. Stress relaxation tests were conducted to steps of 2%, 4%, 6%, 8%, 10%, 15% and 20% engineering strain, with each step applied at a constant rate over 5 s and held for a subsequent 300 s, based on pilot data which showed that values at 300 s were within ±5% of the values at 100 s. The compressive modulus was taken to be the slope of a linear regression line fit to the equilibrium stress and strain calculated at the completion of each step. Tests were accepted if the linear regression coefficient of determination was at least 0.9, as a check for test error such as sample slip or tearing, which would affect the stress–strain relationship (modulus). The multiple measures indicated that the stress–strain relationship was linear (free of strain-stiffening or softening) in most samples, validating the calculation of modulus. Test elimination could usually be traced to a visible tear or unusual slipping of a rounded sample in the test chamber.

BIOCHEMICAL ANALYSIS AND HISTOCHEMISTRY/IMMUNOHISTOCHEMISTRY

At the termination of the cultures and the mechanical tests the constructs were cut into half, for allocation of samples for determination of GAG content using the dimethylmethylene blue dye assay and for Safranin-O histochemical staining and type II immunohistochemistry. Unseeded control scaffolds and samples of goat articular cartilage were also analyzed for GAG content. Cartilage was removed (down to the tidemark) from the two stifle joints of each of the goats (for a total of four samples). Disks (4 mm in diameter) were punched from the samples which ranged in thickness from 0.6 mm to 0.9 mm.

As in prior immunohistochemical studies, the scaffold material did not stain with the antibody, likely due to the breakdown of the epitope in the course of processing the material.

STATISTICS

A sample size of n = 6 was used for most of the analyses using a power calculation based on detecting a significant difference between two groups of 30%, with α = 0.05 and β = 0.05 and a standard deviation (SD) of 15%. P < 0.05 was set as the criterion of significance for statistical tests.

<table>
<thead>
<tr>
<th>Table I</th>
<th>Cross-linking agent molar ratios employing EDAC and NHS. Ratios are shown relative to the carboxyl contained in the collagen scaffold Mean ± S.D.</th>
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<tr>
<td>Cross-link group</td>
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<tr>
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</tr>
<tr>
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<td>1</td>
</tr>
<tr>
<td>EDAC1.5</td>
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</tr>
<tr>
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<th>Table II</th>
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<td>Goat #</td>
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</tr>
<tr>
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<td>10.3</td>
</tr>
<tr>
<td>2</td>
<td>9.5</td>
</tr>
<tr>
<td>3</td>
<td>4.6</td>
</tr>
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</table>
Results

The various cross-link protocols yielded a range of cross-link densities reflected in the SRs (Table I).

As has been observed in previous work\(^{10}\), cell-seeded constructs which were only lightly DHT cross-linked contracted to less than 30% of their original diameter after only 2 weeks. This contraction of the scaffolds was visible after as little as 1 week in culture. The small size of some scaffolds resulted in loss of a few samples in the pilot experiment. Other lightly cross-linked samples were lost owing to their disintegration in culture.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

There was a notable reduction in size of the 3-week constructs related to their cross-link density [Fig. 1(a–d)]. DHT-treated scaffolds contracted to irregular spherical structures only about 2 mm in diameter [Fig. 1(a)]. The DHT- and EDAC1-treated constructs displayed folding of the scaffolds

![Micrographs of chondrocyte-seeded type II collagen scaffolds after 3 weeks in culture: (a) DHT cross-linked; (b) EDAC1.5 group; (c) EDAC2 group; and (d) EDAC3 group. The micrographs in (a)–(d) were stained with Safranin-O and taken at the same magnification. (e) and (f) Micrographs of EDAC1.5 samples after 3 and 9 weeks, respectively, taken at the same magnification. (b)–(d) Planar sections made through the middle part of the disk-shaped scaffold. (e) Safranin-O and (f) type II collagen immunohistochemical stain (brown chromogen).](image)
Chondrogenesis was evident based on the characteristic rounded morphology of cells located in lacunae [Fig. 1(e)], histochemical staining of GAG using Safranin-O stain [Fig. 1(a,c), and (e)], and type II immunohistochemistry [Fig. 1(f)]. Of note was the decrease in chondrogenesis with increasing cross-link density [comparing Fig. 1(a,d)]. For the EDAC1.5, EDAC2, and EDAC3 cross-link groups there was an increase in the amount of tissue containing GAG and type II collagen with time in culture. This was less evident in the DHT group, because of the small size of the constructs and substantial amount of cartilage found by 3 weeks.

Also of note was the inverse relationship between the degree of chondrogenesis and the presence of residual scaffold, which could be distinguished in histological section by its morphology. Little residual scaffold was found in the DHT only samples [Fig. 1(a)] which displayed intense GAG staining and a majority of cells of chondrocytic morphology, whereas much of the initial type II collagen scaffold appeared to remain in the EDAC3 group [Fig. 1(d)] which displayed substantially less cartilage formation. In individual samples in the EDAC1.5 [Fig. 1(b)] and EDAC2 [Fig. 1(c)] groups, regions of cartilage formation with little remaining scaffold could generally be found in the interior portions of the samples with remaining scaffold found in the peripheral regions which displayed no chondrogenesis. While chondrogenesis was clearly evident in the scaffolds in the less cross-linked groups, cells of a non-chondrocytic phenotype were also present in the scaffolds of lower cross-link density. Even though immunohistochemistry for type I collagen was not performed, it was evident that some must be present because the type II antibody did not label all of the newly synthesized matrix. The fibrous appearance of the matrix in cartilaginous regions, including some that stained positive for type II collagen [Fig. 1(f)], indicated the presence of fibrocartilage. The mixed make-up of tissues (hyaline cartilage, fibrocartilage and fibrous tissue) within individual constructs was a common finding. Also of interest was the high cell number density in many of the cartilaginous regions [Fig. 1(e)]. Some cells in these hypercellular areas displayed pyknotic nuclei, and cell debris could also be seen, suggesting the eventual reduction in cell number.

GAG DENSITY

The GAG/volume values for the four goat articular cartilage samples were: 3.3 µg/mm³ and 18.9 µg/mm³ for one goat and 8.6 µg/mm³ and 12.7 µg/mm³ for the samples from the second goat; compared to 27 µg/mm³ for native adult bovine cartilage. Interestingly, there was a strong inverse correlation between GAG density and sample thickness (linear regression analysis; R² = 0.98). Our cartilage samples included the superficial zone which may have been composed predominantly of cell debris and matrix, and also had a decreased GAG content. The inverse correlation might be explained, then, if the GAG-depleted superficial zone increased with increasing thickness, but this will require additional work to resolve. For the purposes of comparative analysis, the GAG content from the pilot experiment was expressed per volume of the constructs and presented relative to the average of the highest GAG density values for the two goats, 15.8 µg/mm³. Thus we chose a conservative approach in estimating the GAG density of the native goat cartilage.

The GAG density of the tissue-engineered constructs generally increased with decreasing cross-link density (Fig. 2). There was no systematic increase in GAG density with time in culture across all cross-link groups (Fig. 2). Of note was the finding that selected constructs achieved GAG densities substantially higher than native cartilage after only 2 weeks in culture.

Based on the goal of producing constructs with approximately 25%, 50%, and 75% the GAG density of native cartilage, it was decided to employ the EDAC1.5 and EDAC2 cross-link protocols for 3, 5, and 9 weeks. For these two cross-link densities, there was a significant five-fold increase in total GAG content with time from about 150 µg at 3 weeks to 750 µg at 9 weeks [Fig. 3(a)]. The total GAG content was comparable for the two cross-link groups at each of the three time periods [Fig. 3(a)]. Three-factor analysis of variance (ANOVA) revealed a significant effect of time on GAG content (P < 0.0001, power = 1), but no effect of cross-link treatment (P = 0.27) or of animal source of cells (P = 0.07, power = 0.51). Fisher’s protected least squares differences (PLSD) post hoc testing showed that the differences in GAG content between time periods were significant. Two-factor ANOVA performed on the EDAC1.5 group demonstrated that there was no effect of quadruplicate set on GAG content, as would be expected. Of note, however, was the effect of animal source of cells on the GAG content for the EDAC1.5 data, with cells from one of the animals resulting in ~30–50% higher values of GAG content after 5 and 9 weeks; two-factor ANOVA (time and animal) revealed a significant effect of animal source of cells on GAG content (P = 0.0003, power = 0.98).

There was a dramatic difference in the volumes of the constructs prepared with these two cross-link protocols [Table III; Fig. 3(b)]. The EDAC1.5 group was about 20% of its original volume at week 3, while the EDAC2 group remained at about 80% of its original volume through the 9-week culture period. Three-factor ANOVA revealed significant effects of cross-link treatment and animal source of cells on volume (both P < 0.0001, power = 1); there was also a significant effect of time on volume, but with a low power (P = 0.04, power = 0.6). In analyzing the data for

![Fig. 2. Pilot study comparing the effect of cross-link group and time in culture on GAG content. GAG content (GAG/volume) is shown as percent of that in native caprine articular cartilage (15.8 µg/mm³).](image-url)
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Non-cell-seeded control scaffolds were found to have a GAG density of 0.14 ± 0.09 µg/mm³ (mean ± SD; n = 4). Owing to the large difference in the volumes of the constructs there was a large difference in the GAG density between the two cross-link groups [Fig. 3(c)], with the EDAC1.5 constructs displaying a three-fold greater GAG density than the EDAC2 group at 3 weeks, two-fold greater at 5 weeks, and 2.5-fold greater at 9 weeks [at 9 weeks, 21.5 ± 2.5, mean ± standard error of the mean (S.E.M.) vs 8.6 ± 1.3 µg/mm³]. For both cross-link groups there was an increase in GAG density with time [Fig. 3(c): the mean value for the 3-week EDAC1.5 group was about 60% of the native value of 15.8 µg/mm³, and the 5-week EDAC1.5 samples exceeded that value.

Three-factor ANOVA revealed significant effects of cross-link treatment (P < 0.0001, power = 1), time (P = 0.0002, power = 0.99), and animal source of cells (P = 0.035, but with a low power = 0.63) on GAG density. Fisher’s PLSD post hoc testing showed significant differences in GAG density between weeks 3 and 5 (P = 0.005) and 3 and 9 (P < 0.0001), but no difference in GAG density between weeks 5 and 9 (P = 0.15). In analyzing the data for the EDAC1.5 group only, two-factor ANOVA (time and quadruplicate set) showed no significant difference in the GAG values in the quadruplicate samples at each of the three time periods.

**COMPRESSIVE MODULUS**

Non-cell-seeded, hydrated scaffolds in the EDAC1.5 cross-linking group had a modulus of 0.88 ± 0.38 kPa (mean ± SD; n = 4) while scaffolds in the EDAC2 group had a modulus of 1.45 ± 0.84 kPa (n = 3). The modulus of native caprine articular cartilage was 314 ± 221 kPa (mean ± SD; n = 3).

The values for compressive moduli for the less cross-linked group (EDAC1.5) were higher than the values for the EDAC2 group at each of the three time periods of culture [Fig. 4(a)]. After 9 weeks the EDAC1.5 modulus was three-fold higher than that of the EDAC2 group (~31.5 ± 3 kPa, mean ± S.E.M., vs 10.7 ± 5 kPa). The moduli of each cross-link group increased with time in culture with the EDAC1.5 group increasing five-fold (6.1 ± 2 kPa to 31.5 ± 5 kPa) from 3 to 9 weeks [Fig. 4(a)]. Even the highest mean value of 31.5 kPa was only 10% of the native modulus, despite the fact that the GAG density for this group was 140% of the native value.

Two-factor ANOVA revealed significant effects of cross-link treatment (P = 0.001, power = 0.94) and time (P = 0.0002, power = 0.991) on modulus. Fisher’s PLSD post hoc testing showed that the differences between individual cross-link groups and among all time points were significant. For the EDAC1.5 group, two-factor ANOVA (time and quadruplicate set) showed a significant effect of time (P < 0.0001, power = 1) but not of quadruplicate samples (P = 0.06, power = 0.69) on modulus. Two-factor ANOVA (time and animal) revealed a significant effect of time on modulus (P < 0.0001, power = 1), but not of animal source of cells (P = 0.35).

The relationship between GAG density (µg/mm³), ρₐ₁GAG, and modulus (kPa), Eₐ₁Comp, from linear regression analysis [Fig. 4(b)], was:

\[
E_{\text{Comp}} = 1 \times \rho_{\text{GAG}}
\]

\[P < 0.0001; R^2 = 0.6\]

where the S.E.M. for the coefficient was ±0.1.
Table III
Dimensions of the constructs cross-linked with the EDAC1.5 and EDAC2 carbodiimide protocols (mm); mean ± s.e.m. (n)

<table>
<thead>
<tr>
<th>Thickness</th>
<th>Diameter</th>
<th>Thickness</th>
<th>Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>1.8 ± 0.2 (4)</td>
<td>8.3 ± 0.2 (4)</td>
<td>2.1 ± 0.2 (3)</td>
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<tr>
<td>3 Weeks</td>
<td>2 ± 0.1 (24)</td>
<td>3.6 ± 0.3 (24)</td>
<td>1.8 ± 0.2 (6)</td>
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<tr>
<td>5 Weeks</td>
<td>2.5 ± 0.1 (20)</td>
<td>4.1 ± 0.3 (20)</td>
<td>1.9 ± 0.1 (6)</td>
</tr>
<tr>
<td>9 Weeks</td>
<td>2.7 ± 0.1 (24)</td>
<td>4.2 ± 0.2 (24)</td>
<td>2.2 ± 0.1 (6)</td>
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</table>

Discussion

This study showed that the cross-link density of the type II collagen scaffolds was a principal determinant of the GAG density of chondrocyte-seeded constructs, thus supporting earlier findings. A notable finding was that the GAG density of some of the constructs substantially exceeded the value for goat articular cartilage after only a few weeks of culture. Recent work growing primary 1-week old calf chondrocytes in scaffold free-culture also found GAG values which exceeded that of native (bovine) cartilage: two-fold at 4 weeks, and then decreasing to two-thirds at 12 weeks. In the pilot study there was no systematic change in the GAG density with time across all groups, while in the follow-up work with the EDAC1.5 and EDAC2 groups there was an increase with time. This might be explained in the groups with lower cross-link density (viz., DHT and EDAC1) by the substantial amount of cartilage that had already formed in the constructs after only 2 weeks.

The increased GAG density in the less cross-linked, more contracted scaffolds was not due to a densification of the GAG that had already formed, because the majority of the contraction occurred before most of the GAG production. Also of importance is that the difference in the GAG density among the constructs was associated with a phenotypic difference in the cells. Cells in the constructs which contracted and had a high GAG density displayed the chondrocytic phenotype of rounded cells in lacunae, whereas cells in constructs with a low GAG density were not chondrocytic in appearance. It has been well-documented that isolated chondrocytes de-differentiate during monolayer culture associated with their adoption of a flattened morphology, particularly in the presence of FGF-2. These cells re-differentiate to chondrocytes in vitro under conditions which allow (or perhaps force) them to assume a spherical shape. The fact that individual constructs can be populated by both re-differentiated and de-differentiated chondrocytes, which may be synthesizing various types of GAG at different rates, complicates investigations of the effects of cross-link density and contracture on the rate of cartilage-specific GAG production.

Contraction of the scaffolds has previously been attributed to α-smooth muscle actin expression. The contracture of the more compliant EDAC1.5 scaffolds resulted in an increase in cell number density in selected regions of the construct. This finding recalls the "condensation" of mesenchymal progenitor cells necessary for the development of cartilage in vivo and in "micro-mass" cultures. Contracture-facilitated aggregation of cells in the scaffolds, increasing the packing density, may have induced chondrocyte expression by increasing cell–cell contacts and/or by favoring a rounded morphology. Once cells re-differentiated in the contracted scaffolds, contracture may have favored chondrogenesis by facilitating the retention of newly synthesized matrix molecules within the collapsed pores of the constructs. The scaffolds of higher cross-link density and stiffness, resisting cell-mediated contraction, may have inhibited/delayed the chondrocytic re-differentiation of the cells by providing an environment permissive of the maintenance of a flattened/elongated morphology of the de-differentiated chondrocytes.

Also of interest was the finding that the greater the degree of chondrogenesis the lesser the amount of residual scaffold. The increased rate of scaffold degradation associated with cartilage formation, i.e., perhaps to be considered a remodeling process, may have been due to enzymes synthesized by the re-differentiated chondrocytes acting on the scaffolds of lower cross-link density.
Cross-link density and culture-time combinations could be found to engineer constructs with mean GAG densities from 20% to 100% of normal canine cartilage density, for constructs ranging in diameter from 3 mm to 7 mm. The results provide a basis for determining the cross-link density and time in culture required to produce constructs of a particular size and GAG density for implantation. While cartilaginous constructs may be formed in scaffold free-cultures, the use of the type II collagen scaffold may provide a wider range of dimensions of the construct and shorter culture times.

The compressive modulus of natural canine articular cartilage reported in this study (314 kPa) compares with values from 300 kPa to 800 kPa for bovine articular cartilage tested under unconfined compression. The compressive modulus of selected constructs reached about 25% of normal. The highest mean modulus was, however, 10% of the normal value, even though the GAG density for the group was about 140% of the native value. Unconfined compression moduli reported for other chondrocyte-seeded scaffolds include: 12 kPa after 28 days in culture for a biodegradable elastomer, poly(1,8-octanediol citrate), construct, and 100 kPa for a hyaluronan scaffold with selected cell-seeding densities and times in culture.

There was only a modest correlation between compressive modulus and GAG density, reflected in the $R^2$ value of 0.60. This is understandable in the light of the known contributions of type II collagen, water content, and other matrix molecules, and microstructure, to the mechanical behavior of articular cartilage. Prior work demonstrated a coefficient of determination of about 0.40 for the correlation between GAG content and equilibrium modulus for tissue-engineered constructs and their contribution to cartilage repair. A self-assembling process in articular cartilage in $\text{vitro}$ is a VA Research Career Scientist Awardee. The type II collagen matrix molecules, and microstructure, to the mechanical behavior of articular cartilage prior work demonstrated a coefficient of determination of about 0.40 for the correlation between GAG content and equilibrium modulus for tissue-engineered constructs and their contribution to cartilage repair.

Acknowledgements

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