

Regional Effects of Enzymatic Digestion on Knee Meniscus Cell Yield and Phenotype for Tissue Engineering

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An abundant cell source is the cornerstone of most tissue engineering strategies, but extracting cells from the knee meniscus is hindered by its dense fibrocartilaginous matrix. Identifying a method to efficiently isolate meniscus cells is important, as it can reduce the cost and effort required to perform meniscus engineering research. In this study, six enzymatic digestion regimens used for cartilaginous cell isolation were used to isolate cells from the outer, middle, and inner regions of the bovine knee meniscus. Each regimen in each region was assessed in terms of cell yield, impact on cell phenotype, and cytotoxicity. All digestion regimens caused an overall upregulation of cartilage-specific genes *Sox9*, collagen type I (*Col 1*), collagen type II (*Col 2*), cartilage oligomeric matrix protein, and aggrecan (*AGC*) in cells from all meniscus regions, but was highest for cells isolated using 1075 U/mL of collagenase for 3 h (high collagenase). In response to isolation, outer meniscus cells showed highest upregulation of *Sox9* and *Col 1* genes, whereas greatest upregulation for middle meniscus cells was seen in *Col 1* expression, and *Col 2* expression for inner cells. Cell yield was highest in all regions when subjected to 45 min of 61 U/mL pronase followed by 3 h of 1075 U/mL collagenase (pronase/collagenase [P/C]) digestion regimen (outer: 6.57 ± 0.37 , middle: 12.77 ± 1.41 , inner: $22.17 \pm 1.47 \times 10^6$ cells/g tissue). The second highest cell yield was achieved using the low collagenase (LC) digestion regimen that applied 433 U/mL of collagenase for 18 h (outer: 1.95 ± 0.54 , middle: 3.3 ± 4.4 , inner: $6.06 \pm 2.44 \times 10^6$ cells/g tissue). Cytotoxicity analysis showed higher cell death in the LC group compared with the P/C group. Self-assembled constructs formed from LC-isolated cells were less dense than constructs formed from P/C-isolated cells, and P/C constructs showed higher glycosaminoglycan content and compressive moduli than LC constructs. All isolation methods tested resulted in similar phenotypic changes in meniscus cells from each region. These results indicate that, compared with other common isolation protocols, the P/C isolation method is able to more efficiently isolate meniscus cells from all regions that can produce tissue engineered constructs.

Introduction

SUCCESSFUL ENGINEERING of meniscus tissue is a valuable goal, as the knee meniscus has a limited capacity for self-repair following injury or disease, which compromises the tissue's ability to perform basic, protective functions such as load transmission and distribution. Engineered replacement tissue may restore these functions before further degeneration of the joint ensues. However, meniscus tissue engineering strategies often require a large number of cells to create tissues with appropriate biochemical and biomechanical properties. Primary meniscus cells are often used for this purpose, and have shown great promise in creating tissue engineered constructs with properties similar to native cartilage.¹⁻⁸ To obtain meniscus cells for tissue engineering

efforts, enzymatic digestion is needed, but little is known about the effects of isolation on the phenotype of meniscus cells, or which digestion technique is most effective for extracting cells from the meniscus.

Isolating cells from fibrocartilaginous tissues such as the meniscus is difficult due to the abundance of fibrous extracellular matrix (ECM). Various techniques have been employed to extract cells from cartilaginous tissues including collagenase type II, trypsin, pronase, and hyaluronidase but their efficiency has not been compared or reported for meniscus cell isolation. These enzymes differ in substrate specificity, and therefore meniscus cell isolation techniques often use sequential digestion protocols to break down the tissue.⁹⁻¹² Collagenase type II is comprised of a variety of enzymes produced by the bacterium *Clostridium histolyticum*,

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and cleaves various sites along the collagen triple helix.^{13–15} Trypsin is a serine peptidase derived from the digestive system that breaks down polypeptide chains into shorter fragments.^{16–18} Similar to trypsin, pronase is made up of a variety of serine proteases produced by the bacterium *Streptomyces griseus*.^{19–24} Pronase has very broad substrate specificity and is able to break down proteins into their constituent amino acids, in contrast to other enzymes whose end-products are often poly- or oligopeptides.^{13,25,26} Hyaluronidase is an enzyme present in bovine testes and produced by certain bacteria, which digests the glycosaminoglycan (GAG) hyaluronan.^{27,28}

While a formal comparison of enzymes has not been performed for meniscus cell isolation, a similar analysis has been carried out for articular chondrocytes. A study on the effects of enzymatic digestion of hyaline cartilage on chondrocytes revealed that digesting the tissue with a high concentration of collagenase for a short period of time resulted in the least phenotypic changes in the cells.¹⁰ However, other digestion techniques involving a low concentration of collagenase for a long period of time, pronase followed by collagenase, or trypsin followed by collagenase, allowed for a greater cell yield than the high collagenase (HC) treatment, but also caused more phenotypic changes in the cells overall.¹⁰

Because of the regional differences in meniscus biochemistry and cell phenotype, it is possible that different isolation techniques may be necessary for optimal cell yield from each region. The inner portion of the meniscus is similar to hyaline cartilage in that it contains the majority of sulfated GAGs and collagen type II (*Col 2*), whereas the middle and outer meniscus regions contain a higher proportion of collagen type I (*Col 1*).^{29–33} The morphology of meniscus cells also becomes progressively more fibroblast-like peripherally in the meniscus, with the inner region cells more rounded and chondrocyte like and the outer cells containing more cellular processes.³⁴ In addition to morphology, regional cell phenotypic differences are observed; cells in the outer meniscus show high gene expression for *Col 1*, while cells from the inner region display high gene expression for *Col 2* and aggrecan (*AGC*).^{35,36} These differences in biochemical content regionally within the meniscus, as well as cell morphology and phenotype may dictate the type of digestion technique optimal for regional cell yield. The different cell populations residing in each meniscus region may also be affected differently in terms of phenotype to different digestion enzymes and protocols.

Therefore, the objectives of this study were (i) to compare enzymatic digestion techniques for each of the three meniscus regions in terms of overall cell yield and viability, and (ii) to determine the effect of these isolation techniques on cell phenotype. The cells from the most promising digestion techniques were also used in a tissue engineering modality as a first step to determine the most promising isolated cell population for cartilage tissue engineering purposes. The self-assembly method was used as a model tissue engineering strategy, as it has been used previously with meniscus cells yielding constructs with promising biochemical and biomechanical properties.^{2–4} It was hypothesized that different digestion regimens would cause varying phenotypic changes in different regions of the meniscus, and that the digestion regimen yielding the highest number of cells could be used in the self-assembling process. The identification of a meniscus digestion method with high cell yield and minimal

detrimental phenotypic changes is of great value to the tissue engineering field, allowing for a more efficient and cost-effective source of cells for further research.

Materials and Methods

Tissue dissection

In phase 1, five medial menisci were harvested from one-week-old bovine knee joints. The anterior and posterior horns of the menisci and connective tissue from the outer portion were carefully removed. Each meniscus was then separated into inner, middle, and outer radial regions. Each region was portioned into eight sets of tissue, and the wet weight of each set was measured and recorded. One of the sets was placed in RNA-later and analyzed with real-time polymerase chain reaction (RT-PCR), and another was digested in papain and assayed for total DNA content using a PicoGreen[®] Assay Kit (Invitrogen).

Cell isolation

The other six sets of tissue were minced to $\sim 1 \text{ mm}^3$, and subjected to one of six tissue digestion regimens: 430 U/mL collagenase type II (Worthington) for 18 h (low collagenase [LC]), 1075 U/mL collagenase type II for 7 h (HC), 61 U/mL pronase (Sigma-Aldrich) for 1.5 h followed by 1075 U/mL collagenase type II for 3 h (pronase/collagenase [P/C]), 2.5 mg/mL (0.25%) Trypsin (Invitrogen) for 45 min followed by 1075 U/mL collagenase for 3 h (trypsin/collagenase [T/C]), 433 U/mL hyaluronidase (Sigma) for 45 min followed by 1075 U/mL collagenase for 3 h (hyaluronidase/collagenase [H/C]), and 433 U/mL hyaluronidase for 30 min followed by 2.5 mg/mL Trypsin for 30 min followed by 1075 U/mL collagenase for 3.5 h (hyaluronidase/trypsin/collagenase [H/T/C]). Tissues were digested at a concentration 43.73 mg/mL of digestion solution, and all digestion steps were carried out at 37°C with gentle shaking. All enzymes were reconstituted in DMEM containing 1% P/S/F and 1% nonessential amino acids, and collagenase solutions contained an additional 10% FBS. After each digestion regimen, cell solutions were filtered through a 70 μm cell strainer, and centrifuged at 700 $\times g$ for 5 min. Cells were then washed once with PBS and resuspended in PBS before being counted with a Z2 Coulter Counter (Beckman-Coulter). Cells were counted three times and the average and standard deviation of cell counts was calculated. Cell count data were analyzed using a two-way analysis of variance (ANOVA), with a significance level of $p < 0.05$.

Quantitative RT-PCR

Cells from each digestion regimen and regional tissue controls were also analyzed using quantitative RT-PCR for mRNA abundance of cartilage-specific genes *Sox9*, *Col 1*, *Col 2*, cartilage oligomeric matrix protein, and *AGC*. Total RNA was extracted from native tissue using an RNAqueous Kit, and from cells using an RNAqueous-Micro Kit (Ambion). Total RNA was reverse transcribed using the SuperScript[™] III First-Strand Synthesis System (Invitrogen), and then PCR was performed on the resulting cDNA for cartilage-specific genes using SYBR[®] Green PCR Mastermix, 80–100 ng of sample cDNA, and 900 nM of each primer. PCR analysis was performed using a RotorGene 3000, and each run used the following protocol: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C

for 15 s and 60°C for 60 s. Bovine *18s rRNA* was used as a housekeeping gene for each of the digestion techniques and tissue control. All fold-change calculations were determined by normalizing data to native tissue controls from each region and were calculated using the formula $y = 2^{\Delta\Delta C_t}$, where $\Delta\Delta C_t$ represents the difference in takeoff cycle between experimental and control groups. Specific primers for each gene were identified from the literature^{37–40} and are listed in Table 1. Gene expression data were analyzed using a two-way analysis of variance (ANOVA), with a significance level of $p < 0.05$.

Fluorescent staining of necrosis

Cells from the highest yielding digestion regimens were also fluorescently stained to visualize cell nuclei and necrotic cells. Hoechst 33342 was used to stain cell nuclei, and ethidium homodimer III was used to stain cells in a necrotic state (Biotium).

Tissue engineering

In phase 2, the two digestion techniques with highest cell yield were used to extract cells from 3–4 g of total medial menisci. The cells were counted and total cell yield per gram of tissue was determined. An aliquot of cells from each digestion technique was stained for live and dead cells using a Live/Dead Viability/Cytotoxicity Kit (Invitrogen). The remaining cells were then seeded into 5 mm agarose molds at 5.5×10^6 cells per well in 0.5 mL of chondrogenic medium containing DMEM with 1% penicillin/streptomycin/fungizone, 1% nonessential amino acids, 10^{-7} M dexamethasone, 5 mM L-ascorbic acid 2-phosphate, 0.4 mM L-proline, and 10 mM sodium pyruvate. Constructs were cultured for 4 weeks in an incubator at 37°C with 5% CO₂ and 0.5 mL of media was changed every day. At the end of the culture period, each construct's diameter and thickness was measured. Constructs were then either analyzed for total GAG content using a dimethyl-methylene blue assay kit (Biocolor), or total collagen content using a hydroxyproline assay, or were mechanically tested using an unconfined compression stress-relaxation test at 10% strain. Compressive testing was initiated with 15 cycles of preconditioning at 0–5% strain, and data were fit with a Kelvin solid viscoelastic model to determine the modulus of relaxation (E_r) and instantaneous modulus (E_i) as previously described.⁴¹

Results

Cell isolation

Tissue specimens from the inner, middle, and outer meniscus were portioned from five medial menisci and minced

in preparation for digestion. Qualitatively, it was observed that the inner meniscus was the easiest to mince, while the outer meniscus was more tough and fibrous, necessitating much more time and effort to achieve the desired $\sim 1 \text{ mm}^3$ fragments. In phase 1, six different isolation methods were used to isolate cells from the inner, middle, and outer regions of the bovine meniscus. Qualitative differences in the viscosity of resultant cell solution were observed among the digestion regimens, with the P/C protocol producing the least viscous solution compared with the HC and LC protocols, which seemed the most viscous. The number of cells per gram of tissue was determined for each isolation regimen (Fig. 1). Statistical analysis showed that the overall cell yield was highest for the inner meniscus compared with the outer meniscus. The middle meniscus cell yield trended higher than the outer region but was not statistically different from either the inner or outer regions. All isolation regimens were able to extract cells from all regions, and the P/C treatment resulted in the highest overall cell yield from all regions (outer: 6.57 ± 0.37 , middle: 12.77 ± 1.41 , inner: $22.17 \pm 1.47 \times 10^6$ cells/g tissue). Additionally, the P/C regimen, when applied to the inner meniscus region, produced a cell yield closely matching that of native tissue (outer: 33.88 ± 0.07 , middle: 23.55 ± 1.17 , inner: $19 \pm 0.16 \times 10^6$ cells/g tissue). Overall cell yields from the other isolation regimens were not statistically different from each other, but the LC treatment trended higher than the rest (outer: 1.95 ± 0.54 , middle: 3.3 ± 4.4 , inner: $6.06 \pm 2.44 \times 10^6$ cells/g tissue).

Fluorescent staining of necrosis

Fluorescent staining of cell nuclei and necrotic cells was carried out for the highest-yielding digestion regimens, P/C and LC. Visual comparison of necrotic cells to total cells showed more necrotic cells present in LC-digested specimens from all regions than in the P/C-digested specimens (data not shown).

Gene expression

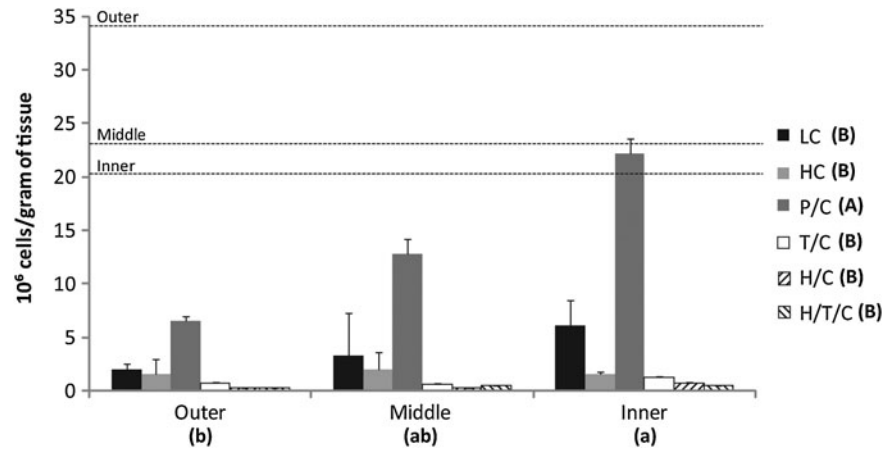
Gene expression of digested cells from each meniscus region using six different isolation protocols was compared with native gene expression from each region (Fig. 2). In general, isolated cells from all regions showed an increase in gene expression for all genes studied compared with native tissue controls. In the outer region, *Sox9* and *Col 1* gene expression displayed the most upregulation, whereas *Col 2* gene expression was least affected by isolation. Among the digestion regimens, the outer region cells showed the highest gene upregulation when isolated using the HC protocol, while the gene expression of cells resulting from the LC

TABLE 1. QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION FORWARD AND REVERSE PRIMERS

Gene	Forward primer (5'–3')	Reverse primer (5'–3')	Target (bp)	Accession No.	Reference
<i>18S rRNA</i>	CAAATTACCCACTCCCGACCC	AATGGATCCTCGCGGAAGG	114	DQ066896.1	37
<i>SOX9</i>	ACGCCGAGCTCAGCAAGA	CACGAACGGCCGCTTCT	71	AF278708	38
<i>Col 1</i>	CATTAGGGGTCACAATGGTC	TGGAGTTCCATTTTCACCAG	97	NM_174520	39
<i>Col 2</i>	AACGGTGGCTTCCACTTC	GCAGGAAGGTCATCTGGA	69	X02420	39
<i>COMP</i>	TCAGAAGAGCAACGCAGAC	TCTTGGTCGCTGTCAAA	72	X74326	40
<i>AGC</i>	GCTACCCTGACCCTTCATC	AAGCTTTCTGGGATGTCCAC	76	U76615	39

Col 1, collagen type I; *Col 2*, collagen type II; *COMP*, cartilage oligomeric matrix protein; *AGC*, aggrecan.

FIG. 1. Cell yield from different meniscus regions using six isolation methods. The number of cells per gram of tissue was determined for each isolation regimen in each radial region of the meniscus. For comparison, native tissue average cellularity is depicted for each meniscus region (dashed lines). Overall, cell yield from the inner region was highest, followed by the middle and outer regions, respectively. Among the isolation regimens, the pronase/collagenase (P/C) treatment resulted in the highest overall cell yield from all regions. Results were analyzed using a two-way analysis of variance (ANOVA), with significance set at $p < 0.05$. Groups not connected by the same letter are statistically different. LC, low collagenase; HC, high collagenase; T/C, trypsin/collagenase; H/C, hyaluronidase/collagenase; H/T/C, hyaluronidase/trypsin/collagenase.



isolation regimen was the only group not statistically different from native tissue. In response to isolation, middle meniscus cells showed the most upregulation in *Col 1*, and the least upregulation in *Col 2*. The digestion regimen resulting in the highest overall gene expression levels was the HC protocol, and the gene expression of cells from the H/T/C protocol was not statistically different from native tissue. Inner meniscus cells showed the highest overall upregulation in *Col 2*, and *AGC* gene expression was least affected by isolation. The HC protocol produced the highest overall gene expression upregulation, whereas the gene expression of cells from the LC and P/C protocols was not statistically different from native tissue.

Tissue engineering

In phase 2, constructs were formed from cells isolated using the LC and P/C protocols. Cell yield was similar to results from phase 1, with P/C method yielding ~50% more cells than the LC method (Fig. 3A). Live-dead staining of the resultant isolated cells showed more dead cells relative to live cells in the LC-isolated population relative to the P/C-isolated cells (Fig. 3B). After 4 weeks in culture, constructs from each group were compared in terms of gross morphology, GAG content, collagen content, and compressive mechanics (Fig. 4). Constructs formed from LC-isolated cells displayed statistically higher thickness compared with P/C constructs, though construct diameters were not different between groups. P/C constructs showed higher GAG content, but no difference was observed in collagen content between groups. Stress-relaxation unconfined compression testing of the two groups showed higher modulus of relaxation and instantaneous modulus for P/C constructs relative to LC constructs.

Discussion

This study sought to compare the regional effects of enzymatic digestion techniques for meniscus tissue engineering. Six different digestion protocols were characterized based on regional meniscus cell yield, viability, and phenotype, and the protocols yielding the highest number of cells

were tested in a tissue engineering modality. In agreement with the proposed hypothesis, meniscus cells from different regions showed varying phenotypic changes following isolation, and the protocol yielding the highest number of cells (the P/C isolation method) was successfully implemented in the self-assembling process.

In phase 1, isolation of cells from the different regions of the meniscus showed wide variation. Specifically, the cell yield from the inner meniscus, regardless of digestion regimen, was higher than the yield from the outer region. Although the outer meniscus contained the highest concentration of cells ($\sim 34 \times 10^6$ cells/g tissue), isolation using the P/C protocol was able to extract less than 20% of these cells. In contrast cell yield from the inner meniscus using the P/C protocol was similar to the expected native tissue cellularity. The reason for this difference needs further investigation, but could be due to higher GAG content in the inner meniscus facilitating digestion solution uptake and enzymatic action, or insufficient release of cells from the outer region matrix increasing the viscosity of the isolation solution and impeding cell pelleting during centrifugation. To our knowledge, this disparity in cell yield from these two regions has not been reported previously. As the meniscus is wedge shaped, however, the relatively low cell yield from the outer region maybe somewhat mediated by the abundance and higher cellularity of outer tissue compared with inner meniscus tissue. The resulting distribution of cells from enzymatic isolation has implications for tissue engineering as meniscus cells from different regions show varying synthetic profiles, which affect the biochemical and mechanical properties of the engineered constructs they form.

Interestingly, cell yield was found to be statistically higher using the P/C isolation regimen in all regions of the meniscus. This protocol involved 45 min of pronase treatment followed by 3 h of HC treatment. Since the P/C treatment resulted in higher cell yield than the HC treatment, it follows that the introduction of pronase was necessary to achieve such a high cell yield. Although both trypsin and pronase are serine peptidases, they differ in substrate specificity, with pronase having a wider range of proteolytic activity than

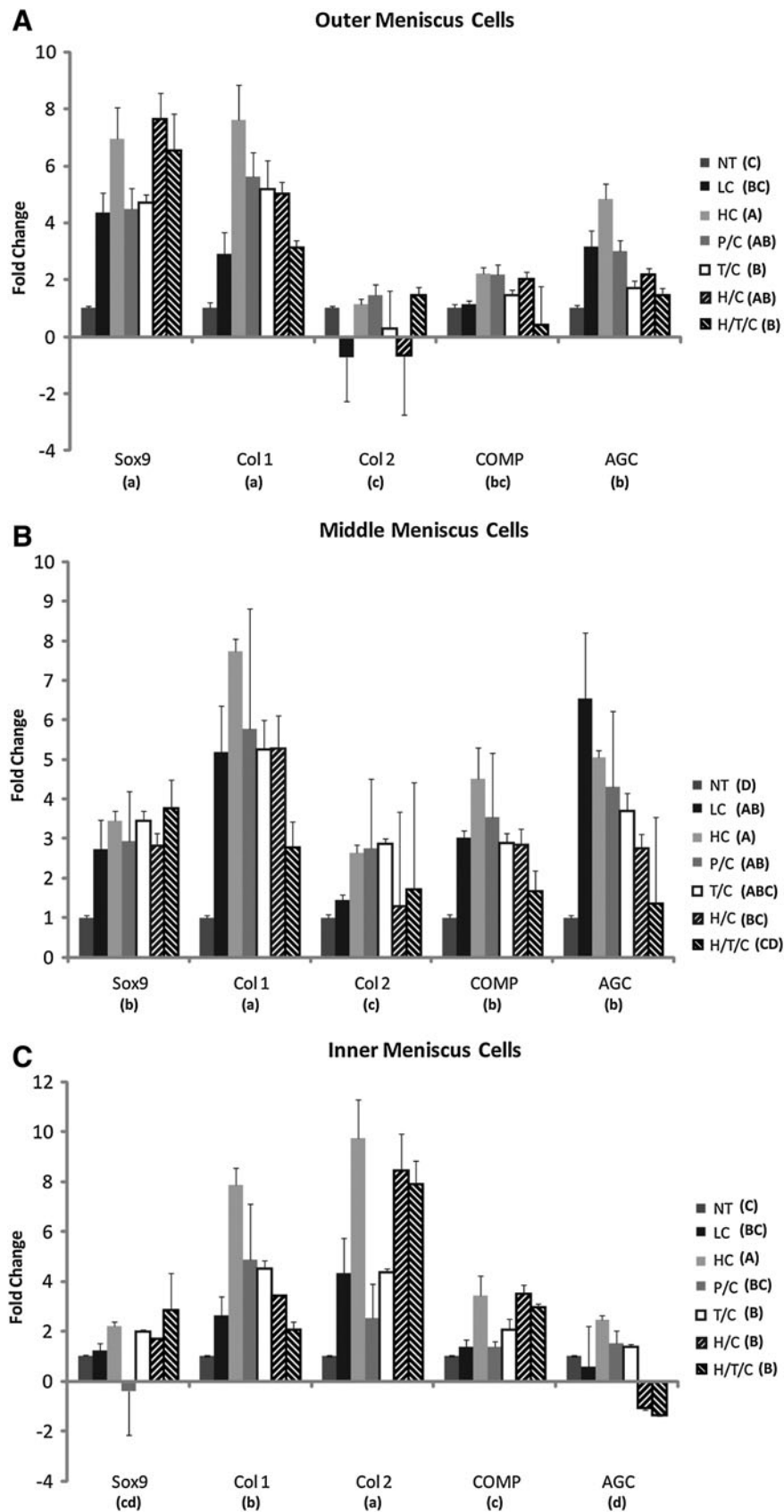


FIG. 2. Gene expression of meniscus cells in response to isolation. Outer (A), middle (B), and inner (C) meniscus cells were isolated from NT, or subjected to one of six isolation regimens: LC, HC, P/C, T/C, H/C, or H/T/C. Gene expression levels were normalized to native tissue values for *Sox9*, *Col 1*, *Col 2*, *COMP*, and *AGC*. Results for each cell type were analyzed using a two-way ANOVA, $p < 0.05$. Groups not connected by the same letter are statistically different. NT, native tissue; *Col1*, collagen type 1; *Col2*, collagen type 2; *COMP*, cartilage oligomeric matrix protein; *AGC*, aggrecan.

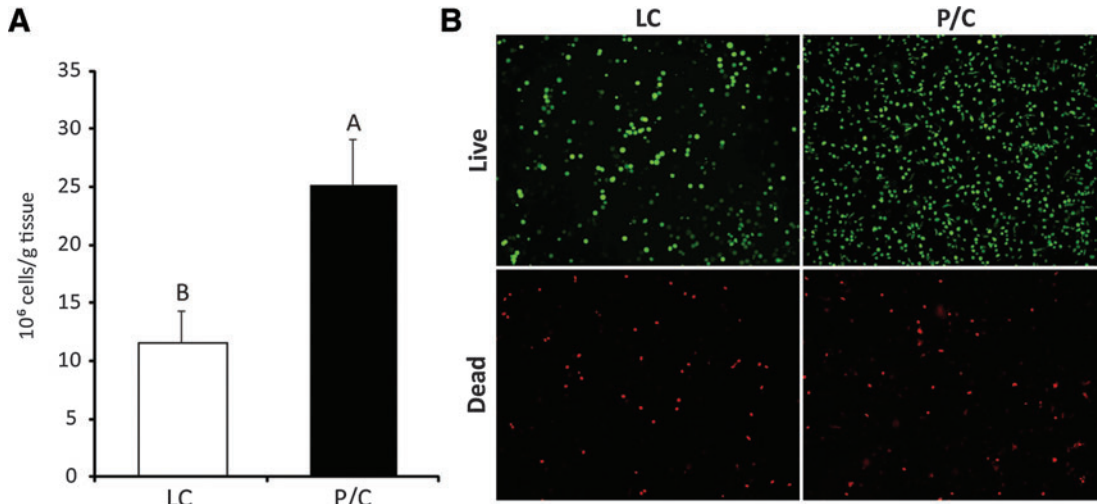


FIG. 3. Phase 2 cell yield and live-dead analysis. **(A)** Cells from whole medial menisci were isolated using the LC and P/C protocols from phase 1. Cell yield was higher for the P/C method. **(B)** Live-dead staining of the resultant isolated cells showed more dead cells relative to live cells in the LC-isolated population compared with the P/C-isolated cells. Quantitative data were analyzed using a Student's *t*-test, with significance set at $p < 0.05$. Groups not connected by the same letter are statistically different. Color images available online at www.liebertonline.com/tec

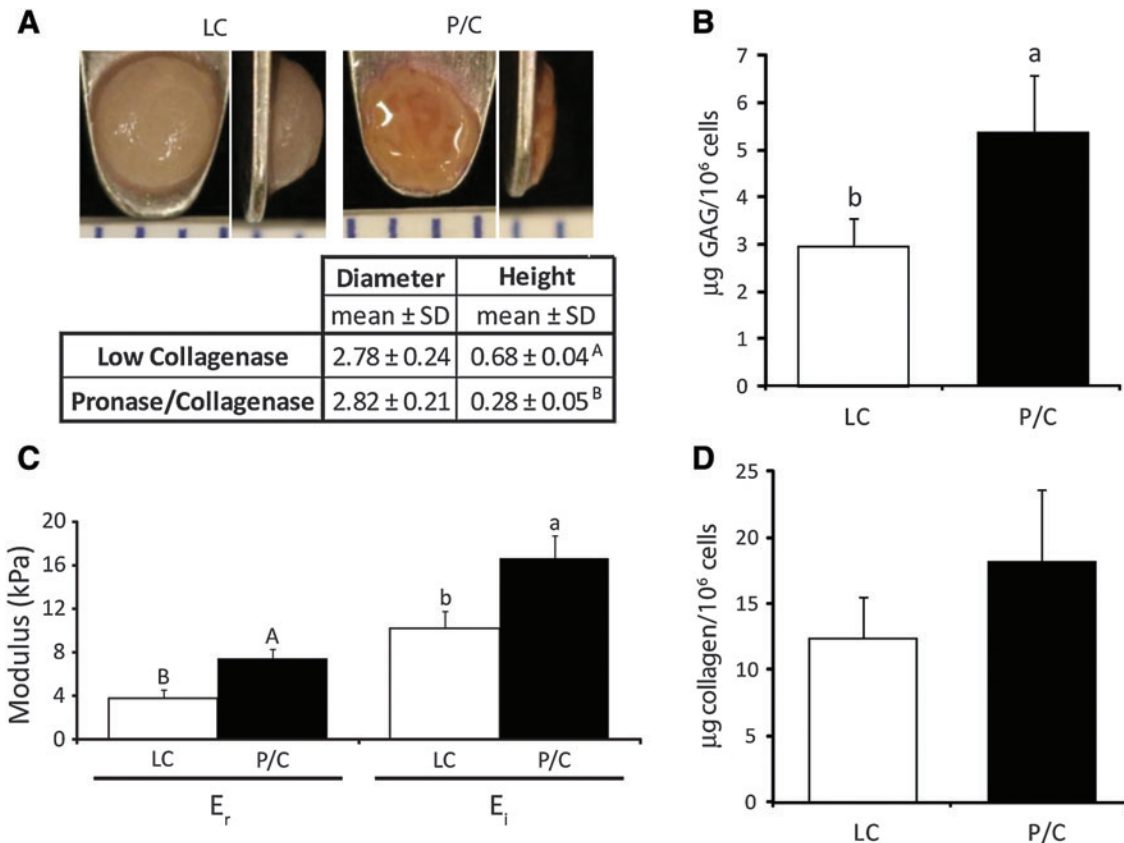


FIG. 4. Phase 2 gross morphology, biochemistry, and compressive mechanics. Self-assembled constructs were formed using cells isolated by the LC or P/C method **(A)**. LC constructs were significantly thicker than P/C constructs; however, construct diameter was not different between groups. Biochemical analysis of GAG and collagen content **(B and D, respectively)** showed increased GAG content per cell in the P/C constructs, but no difference in collagen content between groups. P/C constructs showed higher modulus of relaxation (E_r) and instantaneous modulus (E_i) compared with LC constructs when subjected to unconfined compression stress-relaxation at 20% strain **(C)**. Student's *t*-tests were performed on each data set, with significance set at $p < 0.05$. Groups not connected by the same letter are statistically different. GAG, glycosaminoglycan. Color images available online at www.liebertonline.com/tec

trypsin. In agreement with the present findings, previous reports comparing the efficacy of trypsin and pronase have reported more favorable tissue digestion with pronase, with digestion occurring more rapidly and the resulting cell solution containing fewer cell clumps.^{42,43} As pronase is known to act on a wide variety of substrates, breaking proteins down into their individual amino acids, it is likely that pretreatment of meniscus tissue with this enzyme increases tissue permeability and allows the following collagenase enzyme better access to collagen molecules throughout the tissue. The high cell yield resulting from the P/C isolation protocol represents a vast improvement over other common digestion methods, and maybe applicable to other fibrocartilaginous or fibrous tissues such as the temporomandibular joint disc or tendons. More studies are needed, however, to optimize the concentrations and application times of pronase and collagenase to minimize cell death and maximize efficiency of cell isolation.

Additionally, no other combinatorial treatment with HC (T/C, H/C, or H/T/C) resulted in the same increase in cell yield, indicating that the present pronase application acts differently on meniscus tissue than either hyaluronidase or trypsin. Compared with trypsin and pronase, hyaluronidase is known to have higher substrate specificity, concentrating primarily on the hyaluronan molecule.^{44,45} It has also been reported that specificity of this enzyme to hyaluronan is dependent on molecular weight, with more cleavage occurring on high-molecular-weight molecules.⁴⁵ While this enzyme may be effective for digestion of GAG-rich tissue such as articular cartilage, the meniscus contains relatively little GAG, limiting the efficacy of hyaluronan for meniscus cell isolation.^{9,33}

As hypothesized, phenotypic changes in isolated meniscus cells were observed in all regions. Although a general upregulation of cartilage-specific genes was noted in all isolated cells compared with native tissue, gene expression was found to be altered differently in isolated outer, middle, and inner meniscus cells. This variation in phenotypic response based on meniscus regions corresponds to the known differences in native meniscus cell morphology and phenotype.^{34,35} Specifically, outer meniscus cells showed the highest upregulation in *Sox9* and *Col 1* expression, middle cells in *Col 1* expression, and inner cells in *Col 2* expression. Outer and middle meniscus cells, therefore, show similar phenotypic changes in response to tissue digestion, and the gene most affected (*Col 1*) also corresponds to the most abundant ECM protein, *Col 1*, in these regions. Similarly, increased inner meniscus cell expression of *Col 2* also corresponds to the most abundant collagen protein found in the inner region.³¹⁻³³ These results suggest that upon liberation from their resident ECM, meniscus cells respond by upregulating genes commensurate with restoring their native environment. This behavior is different than that observed in freshly isolated articular chondrocytes, in which gene expression of *Col 2* and *AGC* was unaffected or decreased following tissue digestion, providing more evidence of the distinction between articular chondrocytes and meniscus cells.³¹ The general upregulation of cartilage-specific genes in meniscus cells isolated from native tissue may indicate that isolation of these cells stimulates them to produce cartilage matrix components during *in vitro* culture or tissue engineering.

The P/C protocol proved to be an efficient mode of extracting meniscus that had higher viability than LC-isolated cells. Cytotoxicity analyses on cells isolated using LC and P/C protocols revealed fewer dead cells in the P/C-isolated population in both phase 1 and phase 2. As cell viability following isolation of meniscus cells is rarely reported in the literature, it is difficult to compare these results to published data. While the mechanism by which P/C isolation preserved cell viability is unknown, it maybe related to the relatively short digestion regimen of the P/C protocol relative to the LC protocol. Regardless of the mechanism, however, the increased cell viability observed in P/C-isolated cells is an attractive property for use of these cells in tissue engineering.

In addition to increased viability, isolated P/C cells also showed more promising biochemical and mechanical properties when used in a tissue engineering modality. In phase 2, tissue engineering showed increased GAG content and compressive properties in constructs formed from P/C-isolated cells compared with constructs formed from LC cells. However, LC constructs were also significantly thicker than their P/C counterparts. This difference in thickness maybe attributed to the characteristics of the cell solution seeded. The LC protocol resulted in a much more viscous cell solution than the P/C protocol, perhaps due to the presence of higher-molecular-weight digestion fragments in the LC isolate. In contrast, the P/C-isolated cells settled to the bottom of the agarose well readily, which may have aided cell-cell contacts and subsequent protein synthesis, as has been theorized to be important in self-assembled articular chondrocyte constructs.⁴⁶ Further, the higher compressive properties of P/C constructs relative to LC constructs maybe related to the increased GAG content of P/C constructs compared with LC constructs as GAGs are known to be important for maintaining compressive properties in tissue such as articular cartilage.^{47,48} The P/C cells, therefore, appeared to be more active in the self-assembling process than LC cells. These results indicate that P/C cells are a promising population for tissue engineering purposes, and a more comprehensive investigation of their potential to form engineered cartilaginous tissue is warranted in future work.

In summary, the P/C isolation regimen resulted in (i) higher cell yield from all meniscus regions than other protocols tested, (ii) higher cell viability than cells isolated using the LC protocol, and (iii) cells that produced tissue-engineered constructs with higher GAG content and compressive properties than the LC regimen. Therefore, the P/C meniscus cell isolation method is an efficient way to harvest meniscus cells, regardless of region, with relatively low cytotoxicity. This method can be widely applicable to tissue engineering strategies using a variety of fibrocartilaginous or fibrous tissues. However, more studies are warranted to identify the optimal concentrations and durations of P/C tissue digestion, and the conditions under which isolated cells are best used for tissue engineering.

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Disclosure Statement

No competing financial interests exist.

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