Encapsulated chondrocyte response in a pulsatile flow bioreactor ☆

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Abstract

We have developed a bioreactor-based millifluidic technique that allows for dynamic culture conditions and measurement of the fluid flow impinging upon a three-dimensional tissue engineering scaffold. Chondrocytes in scaffolds have been shown to require mechanical stimulation to produce an extracellular matrix that resembles native cartilage. This study investigates the effect of pulsatile flow on chondrocyte response in a model poly(ethylene glycol) dimethacrylate hydrogel. Bovine chondrocytes were encapsulated in the hydrogel and cultured for 7, 14 and 21 days at pulsatile flow frequencies of 0.5 Hz (15 ml/min) and 1.5 Hz (17 ml/min). The scaffolds cultured under dynamic conditions were compared to those cultured under static (non-flow) conditions. Quantitative real-time reverse transcription polymerase chain reaction was used to quantify collagen type I, collagen type II and aggrecan gene copy numbers as markers for chondrocyte phenotypic expression. Histological sections stained with hematoxylin & eosin, and Alcian blue confirmed chondrocyte morphology and matrix formation. Interestingly, regulation of the collagen type II gene was particularly sensitive to the flow conditions. The understanding of the cell response to encapsulation and flow could be used to identify the appropriate culture conditions necessary to design and develop hydrogel carriers to promote the formation of extracellular matrix as well as to further our knowledge of chondrocyte mechanobiology.
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Keywords: Cartilage; Tissue engineering; Poly(ethylene glycol) hydrogels; Three-dimensional scaffold; Biomaterials

1. Introduction

Regeneration of cartilage using tissue engineering technologies has been an active area of biomedical research for the repair of degenerative and injured cartilage conditions. Various research groups have shown that mechanical stimulation of chondrocytes in three-dimensional (3-D) matrices help develop and maintain the proper cell phenotype [1–7]. For connective tissues such as cartilage, the proper phenotype was generally defined by the type and amount of extracellular matrix (ECM) produced. Articular cartilage is largely composed of collagen type II and proteoglycans such as aggrecan. Collagen type II comprises approximately 90% of all collagen in human articular cartilage, and aggrecan comprises approximately 80–90% of all proteoglycans in human articular cartilage matrix [8]. Tissue-engineered cartilage containing similar amounts of these matrix components may have the necessary mechanical properties to serve as a replacement for damaged cartilage.

One challenge for engineering new cartilage is determining the appropriate culture conditions that promote the

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formation of functionally equivalent tissues [9–11]. Two popular approaches to provide the necessary mechanical signals for promoting chondrocyte phenotype are direct mechanical stimulation and stimulation by fluid stress. There is a large body of work on direct mechanical stimulation of cartilage explants and tissue-engineered constructs, many of which use the amplitude and frequency of compressive or shear loading as tissue culture variables. It has been observed that static compression up to 60% strain reduced the ECM produced by chondrocytes in alginate gels or cartilage explants, but cyclic strains increased the [^{35}S]-sulfate (proteoglycan content) and [^{3}H]-proline (collagen content) incorporation [12]. The same study also found significant frequency dependence in the chondrocyte response, where the ECM production monotonically increased as the rate of compression increased from 0.01 to 1 Hz. Other studies also observed a strong dependence in the chondrocyte response on the magnitude of the static strain over which the oscillatory compression was superimposed [13].

In addition to direct mechanical stimulation, many bioreactors employ fluid stresses to promote the development of proper phenotype [14–18]. Comparing scaffolds cultured under static conditions with those cultured in mixed-flask and rotating-vessel bioreactors, the tissues developed in the rotating vessel bioreactor are superior [19]. Both the glycosaminoglycan (GAG) and collagen production in constructs cultured in the rotating vessel were significantly higher than those from the mixed flasks. The difference in bioreactor results has been attributed to the flow patterns; mixed flasks subject the cell–scaffold constructs to a turbulent flow while rotating vessels subject the samples to a laminar flow. Tissues from rotating vessels have concomitantly higher equilibrium modulus and dynamic stiffness, even approaching that of native cartilage [20].

The challenge with designing rotating vessel bioreactors is that samples must be dynamically suspended in a laminar rotational field that balances centrifugal and buoyant forces [21]. Chondrocytes respond sensitively to the hydrodynamic conditions, but the vessel rotation rate must be set to keep the construct in a constant state of freefall. Thus, the culture conditions are dictated in part by engineering constraints instead of entirely by what would be optimal for promoting tissue development. While it is possible to vary the vessel design to match the chondrocyte requirements, having a model bioreactor system to test the effects of flow and soluble factors on cell–scaffold constructs could be quite valuable.

In this paper, we use millifluidic techniques to build a perfusion bioreactor capable of applying fluid flows to chondrocytes embedded in photopolymerized hydrogels. Millifluidic techniques are well suited to bioreactor design because the fabrication is straightforward. We hypothesize that mechanotransduction of chondrocytes is a key variable that can be used to regulate the gene expression and ECM production by applying fluid stress to chondrocytes encapsulated in poly(ethylene glycol) dimethacrylate (PEGDM) gels.

2. Materials and methods[2]

2.1. Polymer

Poly(ethylene glycol) (PEG; 4000 g/mol), methacrylic anhydride (MA), ethyl ether and triethylamine (TEA) were purchased from Sigma–Aldrich and used as received. Dichloromethane was purchased from Sigma–Aldrich and dried over activated molecular sieves (4 Å) prior to use. The photoinitiator Irgacure 2959 (2-hydroxy-l-[4-(hydroxyethoxy)phenyl]-2-methyl-l-propanone, I2959) was obtained from Ciba Specialty Chemicals and used as received. PEGDM was prepared following the method of Lin-Gibson et al. [22].

2.2. Millifluidic bioreactor fabrication

A schematic representation of the millifluidic bioreactor chamber is shown in Fig. 1. The millifluidic device consisted of a 6.3 mm thick polydimethylsiloxane block (PDMS; Sylgard 184, Dow Corning Corp., Midland, MI) sandwiched between two glass slides. Prior to assembling the device, the bioreactor chamber was created by punching a hole (6.5 mm diameter) through the center of the PDMS block. A Teflon disk (6.5 mm diameter, 2.0 mm thick) was inserted into the chamber to position the hydrogel scaffolds directly in the path of the inlet and outlet piping. The inlet and outlet piping (outer diameter = 1.5 mm, inner diameter = 670 μm) were inserted through the PDMS and attached to silicone tubing (Masterflex, ColeParmer, Vernon Hills, IL) that was gas permeable to carbon dioxide and oxygen. The silicone tubing thus permitted gas exchange for oxygen uptake and maintenance of culture medium pH. The fluid circuit (Fig. 2) consisted of the pulsatile displacement pump (FiberCell, Bellco Glass, Vineland, NJ) drawing from a two-sided arm cap and bottle (Bellco Glass, Vineland, NJ) to deliver consistent flow rates to the scaffolds cultured within the bioreactor chamber. The pump (FiberCell, Bellco Glass, Vineland, NJ) was calibrated to give accurate and consistent flow rates of 2–40 ml/min depending on tubing size, thickness and length at frequencies of 0.5 and 1.5 Hz. The bioreactor chambers were sterilized with ethanol and ultraviolet (UV) light prior to use. The tubing, reservoir and all other parts were sterilized in an autoclave prior to use.

2.3. Chamber flow measurement

The bioreactor chamber was calibrated for fluid flow using a liquid flow sensor (Omega Engineering, Inc., Stamford, CT) and for fluid pressure using a pressure transducer
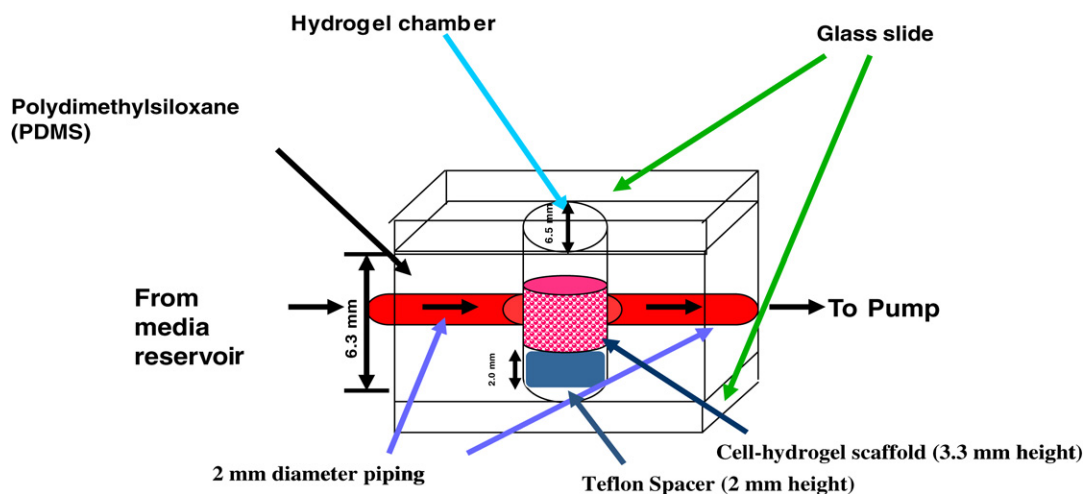
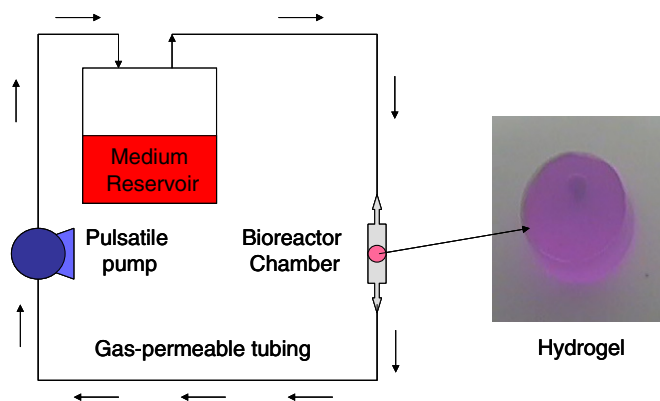Fig. 1. Schematic representation of the hydrogel bioreactor chamber.

Fig. 2. Schematic diagram of the perfusion bioreactor system with the media flow loop indicated.

(Entran Devices, Inc., Fairfield, NJ) calibrated for absolute pressure between 0 and 10 psi with 10 V excitation and sensitivity of 25.44 mV (converted to kPa). The location of the flow sensor in the fluid circuit was behind the bioreactor chamber and the placement of the pressure transducer was over the opening of the hydrogel chamber. Using these devices we were able to measure the flow and fluid pressure in the chamber with and without the hydrogel.

2.4. Cell culture

Bovine chondrocytes were isolated as described and obtained from NIH [23]. Cells were cultured in 150 cm² tissue culture flasks (Corning Glass Works, Corning, NY) using chondrocyte growth medium and maintained at 37 °C in a humidified incubator at 5% CO_2 by volume in air. The chondrocyte growth medium was composed of Dulbecco's modified Eagle's medium (BioWhitaker, Walkersville, MD), 10% by volume fetal bovine serum (FBS) (Gibco-Invitrogen, Grand Island, NY), 1% by volume minimum essential medium vitamin solution (Gibco, Carlsbad, CA), 50 µg/ml L-ascorbic acid 2-phosphate (Sigma, St.

Louis, MO) and 1% by volume antibiotics (penicillin/streptomycin) (Cellgro, Herndon, VA). Cell culture medium was replaced every 2 days. Cells in a dedifferentiated state obtained at passages 8–9 were used in this study to confirm the diminished chondrocyte phenotype before encapsulation within the hydrogel. Chondrocytes were harvested using 0.25% trypsin plus 1 mmol/l EDTA (Gibco-Invitrogen). Viable cells were counted using a flow cytometer (Guava Technologies Inc., Hayward, CA) following the standard protocol for the Guava Viacount Flex kit (Guava Technologies Inc.).

2.5. Cell-seeded hydrogel preparation

Photopolymerized hydrogels were prepared as previously described [24]. PEGDM ($M_n \approx 4000$ g/mol) was used to form hydrogels for the current study. The structure of the hydrogel (10% by mass preparation) consisted of dense, crosslinked methacrylate clusters embedded in a soft solution-like matrix. This particular hydrogel exhibited a weak correlation length of approximately 150 Å as measured by small angle neutron scattering and was mechanically robust. The shear modulus was 53 ± 5 MPa [25]. Briefly, the gel solution was prepared by sterilizing solid macromer (PEGDM) for 20 min under UV irradiation and then dissolving to a concentration of 10% by mass in sterile chondrocyte growth medium containing 20% FBS (Premium Select, Atlanta Biological, Atlanta, GA). Chondrocytes were added for a seeding density of 200,000 cells per 100 µl of gel solution. The photoinitiator I2959 was dissolved in water at high concentrations, sterilized using a 0.22 mm syringe filter, and added to the cell–macromer gel solution for a final concentration of 0.05% by mass. A Teflon mold was assembled, and 100 µl of the cell–macromer–initiator gel solution was placed into each cylindrical opening of the mold to create cylindrical hydrogel samples (6.1 mm diameter, 2.0 mm height) by photopolymerization using a long-wave (365 nm) UV lamp (Spectroline

ENF-240 C, Westbury, NY) for 10 min. These hydrogel scaffolds were then placed into chondrocyte growth medium in tissue culture plates and allowed to swell and equilibrate before loading into the millifluidic bioreactors. The cells encapsulated within the gel were labeled using calcein-AM (Molecular Probes Inc., Eugene, OR) and observed with an inverted epifluorescent microscope (Nikon Eclipse TE 300, Melville, NY, 20× objective). The diameter of the hydrogel scaffold (6.3 mm) was smaller than the bioreactor chamber diameter (6.5 mm), resulting in slight leakage around the perimeter of the scaffolds. For dynamic culturing studies, the hydrogels were placed into the bioreactor chamber cultured at 0.5 Hz (15 ml/min) and 1.5 Hz (17 ml/min) under pulsatile flow conditions and the whole system was placed in a 5% CO_2 incubator. This experiment was conducted two times with a sample number of 3 for each time point (7 d, 14 d and 21 d). Replacement of cell culture medium was not necessary over the duration of the experiments since a large excess of media was used in the reservoir. Under static conditions the cells were encapsulated in hydrogels and cultured in dishes without flow. The Day 0 control chondrocytes were cells cultured on tissue culture polystyrene (TCPS) before encapsulation within the hydrogel.

2.6. Histology

At 7, 14 and 21 days, hydrogel scaffolds from static (no flow) and dynamic culture were removed, rinsed with phosphate-buffered saline (PBS) and fixed in PBS containing 4% paraformaldehyde for 30 min. The 4% paraformaldehyde was removed and the scaffolds were washed twice with PBS, embedded in paraffin, sectioned and stained with Alcian blue for sulfated proteoglycan matrix production and hematoxylin & eosin (H&E) for cell morphology using standard histological protocols. Cross sections of the hydrogel scaffold were 10 μm in thickness.

2.7. Quantitative RT-PCR

Gene expression profiles of bovine chondrocytes within the hydrogels were analyzed using quantitative real-time reverse transcription polymerase chain reaction (RT-PCR). Cells were harvested from TCPS at day 0 (TCPS static) and from cells cultured in hydrogel scaffolds for 7, 14 and 21 days under static and flow conditions. Cell lysates were harvested from three separate samples for each time point. Lysates were stored in liquid nitrogen until the time for RNA extraction. The RNA extraction was carried out using the materials and protocol provided in the RNeasy Kit from Qiagen (Qiagen, Valencia, CA). The protocol was followed according to the manufacturer's specification using the QIAshredder (Qiagen). The RNA was immediately treated with RNA Secure after elution from the membrane (Ambion, Austin, TX) and stored at −20 °C.

The primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), collagen type I (collagen I), collagen type II (collagen II) and aggrecan were adapted from Ronziere et al. [26] and Hunter et al. [27]. The primers were as follows: sense 5′-ATCACTGCCACCCAGAAGAC-3′, anti-sense 5′-ATGAGGTCCACCACCCTGTT-3′, GAPDH [26]; sense 5′-GCCGAGACTTGAGACTCAGCCACCCA-3′, anti-sense 5′-GATAGGCAGGCGAGATGGCTTGTT-TG-3′, collagen I [27]; 5′-AACGGTGGCTTCCACTT-CAG-3′, anti-sense sense 5′-TGCCCAGTTCAGGTCT-CTTAGAG-3′, collagen II [27]; and sense 5′-ACCGA-TACGAGATCAATG-3′, anti-sense 5′-CTGTAGTCT-GCGCTTGTA-3′, aggrecan [27] (MWG Biotech, Inc., High Point, NC).

RT-PCR was carried out using the QuantiTect SYBR Green RT-PCR Kit and protocol (Qiagen) on an iCycler instrument (Bio-Rad, Hercules, CA) [28]. The protocol utilizes the following thermal parameters: reverse transcription, 30 min at 50 °C; activation step, 15 min at 95 °C; three-step cycling, denaturation for 30 s at 95 °C; annealing for 2 min at 57 °C, extension for 2 min at 72 °C for 35 cycles. A melt curve between the temperatures of 50 and 95 °C was subsequently performed to analyze the products generated in increments of 1 °C. In order to ensure RT-PCR product specificity, samples were run on a 2% by mass agarose gel and stained with 10 μg/ml ethidium bromide (Sigma). After isolation from the gel, the resultant cartilage phenotypic marker products were sequenced using the Big Dye Terminator Kit (ABI, Foster City, CA) on a 310 DNA Genetic Analyzer (ABI). The detection limits for the assay were in the range of 10^3 for all samples. The standard curves were adjusted accordingly to ensure that the sample fell within the linear portion of the standard curves for statistical purposes. Further, triplicate samples of the standard curve were run for every assay and the average was utilized for sample analysis.

3. Results

Light microscopy showed deformation of the hydrogel within the chamber in response to the frequency of pulsed fluid flow. These observations correspond to the flow rate measurements shown in Fig. 3, which plot the flow rate and fluid pressure as a function of time for pulsed frequencies of 0.5 and 1.5 Hz with (thin red lines) and without (thick black lines) a hydrogel. For an empty bioreactor chamber pulsed at 0.5 Hz (Fig. 3, top left), the flow rate appeared to be sinusoidal and peaked at ~20 ml/min and declined to ~5 ml/min. Although the flow rate never decayed to zero during the cycle, the corresponding fluid pressure peaked at 4 kPa and declined to ~0 kPa just before the next pulse (Fig. 3, bottom left). The phenomena of the pressure drop to near zero but the flow rate still being measurable is probably due to an artifact of the flow sensor device, which measures the rotation of a turbine wheel that generates the measured voltage used to determine the flow rate. Any residual rotation of the wheel between pulses would lead to slightly increased flow rate. Therefore, the peak heights of the flow graphs are taken
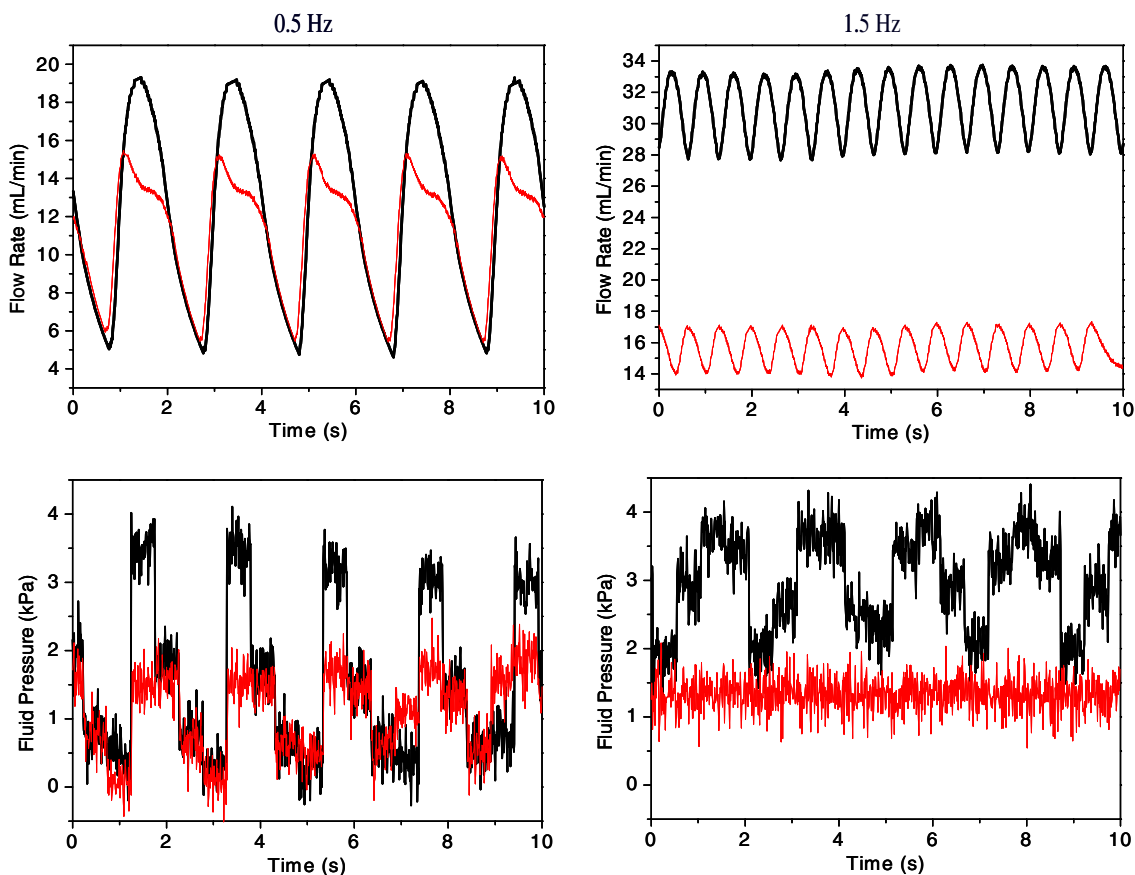
Fig. 3. Flow rate measurements are shown for 0.5 and 1.5 Hz at 10 s intervals. Flow rate (0.5 Hz (top left), 1.5 Hz (top right)) and fluid pressure (0.5 Hz (bottom left), 1.5 Hz (bottom right)) for the bioreactor chamber without a hydrogel scaffold (thick black line) and the bioreactor chamber containing a hydrogel scaffold (thin red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

as a measure of total flow rate (i.e., 15 ml/min for 0.5 Hz and 17 ml/min for 1.5 Hz frequency). When a hydrogel was added to the chamber for the 0.5 Hz flow, (Fig. 3, top left), the flow rate curve shifted from ~17 ml/min to 15 ml/min and included a plateau that persisted for approximately 0.5 s at ~14 ml/min. The corresponding fluid pressure oscillated between 2 and 0 kPa. At the higher pulsatile frequency of 1.5 Hz, the flow through the empty chamber (Fig. 3, top right) peaked at ~33 ml/min and the fluid pressure oscillated between 4 and 2 kPa. Adding the hydrogel to the chamber (Fig. 3, thin red line) reduced the peak flow rate to ~17 ml/min. In addition, the fluid pressure changed from its cyclical nature to a steady pressure of approximately 1.25 kPa. The deformation of these highly viscoelastic hydrogels under oscillating strain are complex and beyond the scope of this paper. However, we note that under the current experimental conditions, gels cultured at 0.5 Hz fully relax to their resting position with frequency oscillation, whereas gels cultured at 1.5 Hz were not able to relax to its rest position.

3.1. Histology

The cells cultured within the hydrogels were completely dispersed and showed round or oval morphology indicative of the normal 3-D conformation experienced by chondrocytes in vivo. Cell viability within the cell–hydrogel scaffolds after 14 days of static culture has already been established [22]. The histological sections stained the same regardless of whether the section was taken from the edge or center of the hydrogel sample. In addition, slices of the hydrogel cross section showed no difference in staining due to depth. After staining of the histological sections with H&E, we observed a pronounced maturation of the cells encapsulated in hydrogels cultured for 14 and 21 days, which was indicative of a redifferentiation response (Fig. 4). The variations in the intensity of Alcian blue staining also indicated a greater production of sulfated proteoglycan matrix in the scaffolds cultured under pulsatile flow conditions as compared to those cultured under static conditions (Fig. 4). This was taken as a marker for redifferentiation of the chondrocytes within the hydrogel in the millifluidic bioreactor. Moreover, proteoglycan aggregates around the cells became more defined over time with dynamic culturing also demonstrated by the Alcian blue staining. The chondrocytes within the statically cultured gels stained faintly for matrix production after 14 and 21 days. It was concluded that over time there was increased cellular function towards redifferentiation under pulsatile flow conditions.
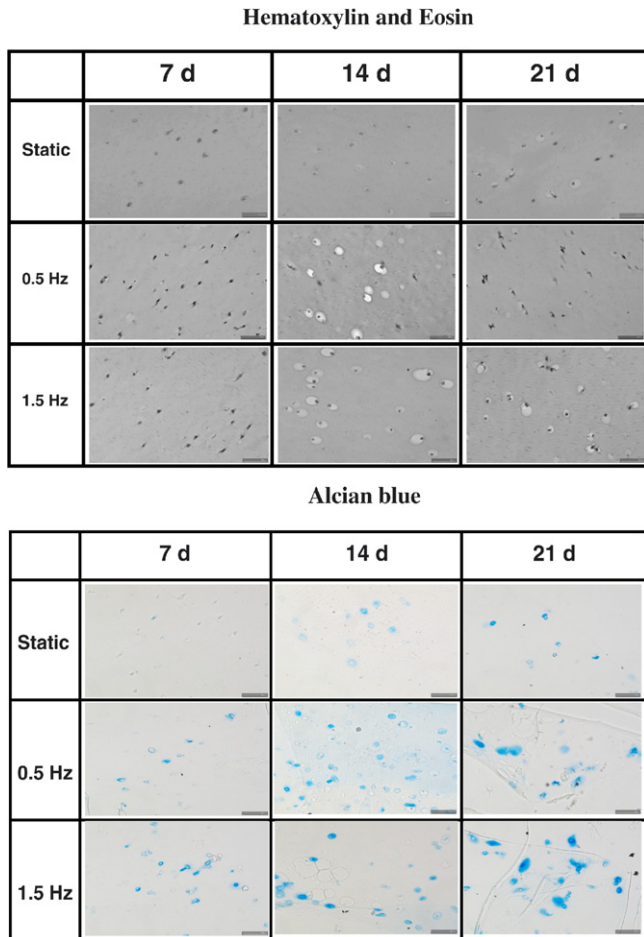
Hematoxylin and Eosin

Alcian blue

Fig. 4. Histological characterization of chondrocyte response within PEGDM hydrogels with mechanical stimulus (0.5 and 1.5 Hz) and without mechanical stimulus (static). Bar = 50 μm.

3.2. RT-PCR

Quantitative RT-PCR was conducted to determine the gene expression levels of aggrecan and collagen types I and II. These genes were identified as the major phenotypic markers for chondrocytes [29–32]. The GAPDH gene did not modulate by flow or culture with the PEGDM hydrogels. The bar graphs denoting the gene expression of aggrecan and collagen types I and II were normalized to GAPDH levels and are shown for chondrocytes at day 0 taken from TCPS before encapsulation (TCPS static) and after 7, 14 and 21 days of static and dynamic culturing within 3-D hydrogels (Fig. 5). The RT-PCR analysis demonstrated significant gene expression profile differences between the dynamic and static (no flow) conditions. The gene expression of aggrecan was expressed by chondrocytes encapsulated in hydrogels for both dynamic and static conditions. However, collagen type II was only expressed in hydrogels under dynamic conditions and collagen type I was only expressed in chondrocytes at day 0 before encapsulation within the hydrogel. Markers were considered not expressed when they fell below the detection limits of the RT-PCR. The upregulation also appears to be strongly dependent on time and frequency. After 7 days, there was only a slight upregulation of the genes of collagen type II and aggrecan at a flow rate of 15 ml/min and 0.5 Hz, whereas an overall increase in the upregulation of the genes at a flow rate of 17 ml/min and 1.5 Hz was detected with collagen type II as the dominant gene expressed. At day 14, the trends reverse and the 0.5 Hz pulse condition gives an overall increase in gene regulation with collagen type II as the dominant gene expressed. The cells that were pulsed for 14 days at 1.5 Hz showed aggrecan as the dominant gene expressed. At day 21, the dominant gene expressed was aggrecan, regardless of pulsed frequency. This may mean that by day 14 the cells have become more acclimated to their mechanical environments. It should also be noted that after 14 and 21 days, the static cultured gels were expressing only the aggrecan gene. These findings indicate that chondrocytes need mechanical stimulus to properly express the chondrocyte differentiation markers (i.e., collagen type II) needed for the development of a tissue-engineered cartilage.

4. Discussion

In this study, the effect of mechanical stimulation by pulsatile flow on phenotype expressed by chondrocytes encapsulated in PEGDM hydrogels was investigated for 7, 14 and 21 days of culture. The consistent and reproducible pulse-dependent mechanical stimulation of the encapsulated chondrocytes showed quantitatively greater collagen type II and aggrecan gene expression with pulsatile flow frequencies of 0.5 Hz at 15 ml/min and 1.5 Hz at 17 ml/min as compared to static controls. Collagen type II gene expression was only observed when the chondrocytes received pulsed mechanical stimulation, whereas aggrecan was present at day 14 for all chondrocytes encapsulated within the hydrogels. The function of collagen type II is to provide tensile strength and the function of aggrecan is to provide compressive stiffness to native cartilage tissue [33]. As a result, these studies suggest that mechanical stimulation was necessary for producing a phenotypic gene expression profile that had the appropriate mechanical properties to allow the chondrocytes to adjust to their environment. In addition, the presence of collagen type II and aggrecan without collagen type I expression indicated that the cells have redifferentiated toward their phenotype within the mechanically stimulated 3-D hydrogel system.

These results are consistent with previous findings based on applying an osmotic pressure under static conditions and hydrodynamic/osmotic pressure under flow conditions to the cells [34–36]. Mow et al., in a study on articular cartilage explants, described how fluid flow from the high-pressure upstream side to the low-pressure downstream side had its energy consumed by compaction of the ECM [36]. This effect was called flow-induced compaction of the ECM and caused pressure gradients through the depth of the cartilage tissue. The significance of the flow-induced compaction on the ECM was that frictional drag effect is
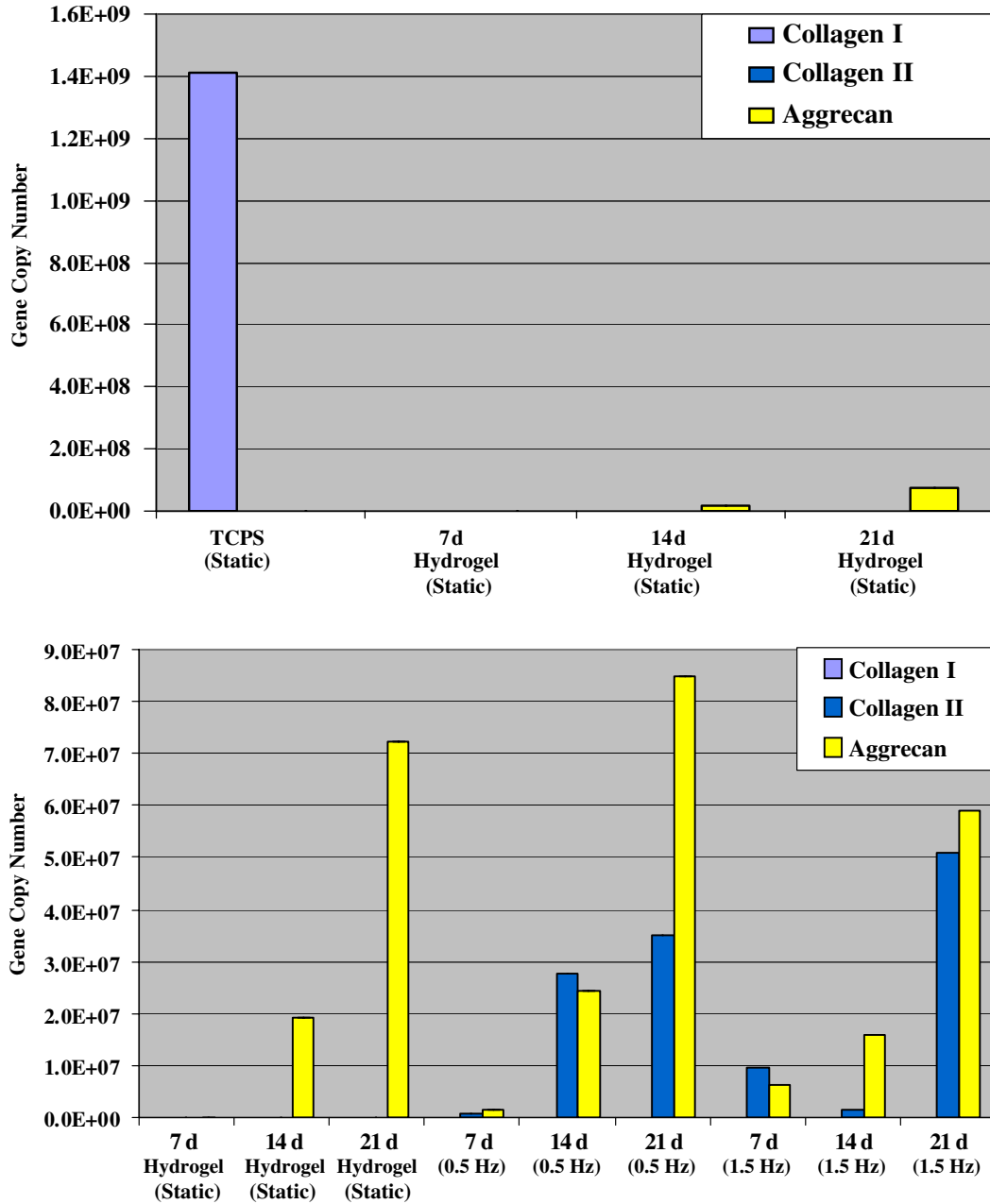
Fig. 5. Gene copy number of collagen type I, collagen type II and aggrecan was quantified from chondrocyte hydrogel samples. Collagen I was not detected in the 3-D hydrogels. Error bars are representative of one standard deviation from the mean of triplicate samples harvested from two separate populations of chondrocyte cells, and are an estimate of the standard uncertainty. The error bars are smaller than the line thickness shown ($p < 0.001$). Results show the upregulation of aggrecan due to encapsulation within the hydrogel under static conditions. In addition, the upregulation of collagen type II gene expression is the result of continuous pulsatile stimulus.

likely to be one of the major effects of fluid flow through the porous-permeable ECM. The description of this effect was very similar to that observed during pulsed flow within our bioreactor chamber, as the hydrogel scaffolds also experienced a flow-induced compaction. In addition, the flow induced compaction could explain the drop in fluid pressure from 4 kPa to <2 kPa observed when the hydrogel was placed in the bioreactor chamber. But interpretation of the flow-induced compaction phenomena was difficult due to the perpendicular flow impingement on the hydrogel

within the bioreactor system and minor fluid loss around the perimeter of the scaffold.

The quantitative RT-PCR analysis confirmed the histological section observations and showed the effect of pulsatile fluid flow conditions on the upregulation of collagen type II and aggrecan production. Interestingly, our data suggested that collagen type II was not always co-expressed with aggrecan. Under dynamic conditions, production of both matrix markers increased while only aggrecan was expressed under static conditions. No collagen type I was

detected for the chondrocytes encapsulated in the hydrogels under static or dynamic conditions, suggesting that these bovine chondrocytes had redifferentiated.

5. Conclusions

We have developed a novel pulsatile millifluidic bioreactor to study the cellular behavior of chondrocytes when exposed to fluid flow. The chondrocytes were encapsulated in model 3-D hydrogel systems which mimic the 3-D conformational environment of natural cartilage tissue. The present study indicates that dedifferentiated bovine chondrocytes cultured in PEGDM gels showed variations in proteoglycan synthesis and matrix deposition as a result of different mechanical stimulus environments. Further investigations are needed to define the flow rates and pressures that can be used to regulate the proteoglycans and collagen type II synthesized by chondrocytes under differing mechanical culturing conditions. The deformational complexities of this experiment have led us to believe that analysis of the flow rates and the pressures being applied to the cell–hydrogel scaffold would provide greater insight into the cellular mechanotransduction events which mediate the response of chondrocytes and the culture conditions needed to develop tissue-engineered cartilage.

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