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# A new biodegradable polyester elastomer for cartilage tissue engineering

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Received 13 May 2005; revised 3 August 2005; accepted 25 August 2005

Published online 10 January 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30607

**Abstract:** The objective of this study is to assess whether a new biodegradable elastomer, poly(1,8-octanediol citrate) (POC), would be a suitable material to engineer elastomeric scaffolds for cartilage tissue engineering. Porous POC scaffolds were prepared via the salt-leaching method and initially assessed for their ability to rapidly recover from compressive deformation (% recovery ratio). Controls consisted of scaffolds made from other materials commonly used in cartilage tissue engineering, including 2% agarose, 4% alginate, non woven poly(glycolic acid) (PGA) meshes, and non woven poly(L-lactide-co-glycolide) (PLGA) meshes. Articular chondrocytes from bovine knee were isolated and seeded onto porous disk-shaped POC scaffolds, which were subsequently cultured *in vitro* for up to 28 days. POC scaffolds completely recover from compressive deformation, and the stress-strain curve is typical of an elastomer (recovery ratio > 98%). Agarose gel (2%) scaffolds broke during the compression test. The recovery ratio of 4% alginate gel scaffolds,

PLLA, and PGA were 72, 85, and 88%, respectively. The Young's modulus of POC-chondrocyte constructs and cell-free POC scaffolds cultured for 28 days were  $12.02 \pm 2.26$  kPa and  $3.27 \pm 0.72$  kPa, respectively. After 28 days of culture, the recovery ratio of POC-chondrocyte constructs and cell-free POC scaffolds were 93% and 99%, respectively. The glycosaminoglycan (GAG) and collagen content at day 28 was 36% and 26% of that found in bovine knee cartilage explants. Histology/immunohistochemistry evaluations confirm that chondrocytes were able to attach to the pore walls within the scaffold, maintain cell phenotype, and form a cartilaginous tissue during the 28 days of culture. © 2006 Wiley Periodicals, Inc. *J Biomed Mater Res* 77A: 331–339, 2006

**Key words:** cartilage; tissue engineering; elastomer; citric acid; biodegradable polymer

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## INTRODUCTION

Osteoarthritis is a joint disease, which is characterized by articular cartilage degeneration, erosion, and eventually loss of cartilage tissue on the joint surface. This disease affects more than 20 million people in the United States, and by 2030, about 70 million Americans will be at risk for osteoarthritis.<sup>1</sup> It is one of the most frequent causes of physical disability among adults, particularly those aged 65 or older and professional athletes.<sup>1,2</sup> The treatment of cartilage injuries and osteoarthritis has been a challenge to the orthopaedic research and clinical communities because of the very limited capacity

for cartilage regeneration and the limited success of the few surgical techniques that are available to repair damaged cartilage (e.g. mosaicplasty and autologous chondrocyte implantation).<sup>3–5</sup> Recent efforts at cartilage repair have focused on tissue-engineering methods to create cartilaginous tissue *in vitro* to enable cartilage transplantation.<sup>6–13</sup> Tissue engineering requires the use of a three-dimensional scaffold for cells to grow on and differentiate properly. The ideal scaffold should be biocompatible, biodegradable, allow for adequate cell loading, facilitate cell proliferation and differentiation, and possess mechanical and physical properties that are suitable for the target application.<sup>12,14–17</sup> In an attempt to increase the quality of engineered cartilage tissue, scaffolds loaded with cells are often subjected to external mechanical stimuli. The implanted cartilage constructs are also constantly exposed to compression and shear forces that are produced by joint movement. These forces are very important for maintaining the homeostasis of cartilage metabo-

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Contract grant sponsor: National Science Foundation

lism. Therefore, the mechanical properties of the scaffolds used must be conducive to mechanical conditioning regimens.<sup>13,18</sup>

Several investigators have demonstrated the importance of cyclic compressive and shear forces during cartilage development *in vitro* and *in vivo*.<sup>19–28</sup> The results are encouraging, although variable and far from satisfying.<sup>12</sup> Scaffold materials that have been used in cartilage tissue engineering studies have included agarose, alginate, polyglycolic acid (PGA), poly-L-lactic acid (PLLA), or poly(L-lactide-co-glycolide) (PLGA).<sup>19–21,29–33</sup> These scaffolds have limited elasticity and may be prone to elastic deformation when challenged with compressive strain. These undesirable scaffold mechanical properties may partially explain the conflicting results regarding the effects of cyclic mechanical strain on GAG and collagen formation.<sup>12</sup> To address the issue of how scaffold mechanical properties may influence the development of engineered tissue, we have been developing a novel class of biodegradable polyester elastomers referred to as poly(diols citrates). These novel elastomers have the following advantages: nontoxic monomers, relatively simple synthesis that can be carried out under mild conditions without addition of toxic catalysts or crosslinking reagents, controllable mechanical and biodegradation properties, easy processing, and inherent surface affinity for various cell types.<sup>17,34</sup> Depending on the synthesis conditions, total degradation may take 6 months to 2 years. When subjected to tensile tests, these elastomers can be stretched to three times their original length with 100% recovery.<sup>17</sup> Based on the elastomeric and biodegradation properties of poly(diols citrates), we hypothesized that they could be potentially better suited for use as a biodegradable scaffold to improve the quality of engineered cartilage when applying long-term cyclic compression and shear strain on the cell-scaffold construct *in vitro*. Also, *in vivo*, an elastomeric scaffold may better respond to compression and shear forces produced by mobilization of the joint, thereby improving the quality of the regenerated tissue. However, as a first step towards testing the hypothesis, it is necessary to characterize the ability of these materials to support chondrocyte attachment, proliferation, matrix synthesis, and cell differentiation. Therefore, the objective of this study is to assess whether a newly developed poly(diols citrate), poly(1,8-octanediol citrate) (POC) could be used as a scaffold for cartilage tissue engineering.

## MATERIALS AND METHODS

### Materials

All chemicals, cell culture medium and supplements were purchased from Sigma–Aldrich (St. Louis, MO), except

where indicated. Immunohistochemistry (IHC) staining kits were purchased from Vector Laboratories (Burlingame, CA). Collagenase type II was purchased from ICN Biomedical (Aurora, OH). Papain was purchased from Worthington Biomedical Corporation (Lakewood, NJ). Type II collagen antibody was purchased from Neomarkers Corporation (Freemont, CA). PGA (65.13 mg/cm<sup>3</sup>, 97% porosity) and PLLA (69.9 mg/cm<sup>3</sup>, 97% porosity) scaffolds were purchased from Albany International Research (Mansfield, MA). The complete synthesis and chemical characterization of poly(1,8-octanediol-co-citrate) are described elsewhere.<sup>17</sup>

### Fabrication, characterization, and mechanical testing of POC scaffolds

#### Scaffold fabrication and characterization

The details of the synthesis and characterization of poly(diols citrates) have been published elsewhere.<sup>17,34</sup> Briefly, POC prepolymer was synthesized by melting a 1:1 mol ratio of citric acid and 1,8-octanediol under nitrogen at 160–165°C, followed by stirring at 140°C for ~1 h. The prepolymer was dissolved in 25% (w/v) 1,4-dioxane solution, followed by addition of sieved salt (90–120 μm) that served as a porogen. The resulting slurry was cast into poly(tetrafluoroethylene) (PTFE) molds and postpolymerized (cross-linked) at 80°C for 4 days. Salt in the resulting composite was leached out by successive incubations in water (produced by Milli-Q water purification system, Billerica, Mass, USA) every 12 h for 96 h. The resulting sponge-like porous scaffold was freeze-dried and then stored in a desiccator under vacuum before use. Disk-shaped scaffolds were punched out for the experiments. Scaffold cross-sections were observed by scanning electron microscopy (Hitachi S-3500, Hitachi High Technologies America, Pleasanton, CA), and the pore size data were obtained using image analysis software (Image-Pro® Plus V.4.0, Silver Spring, MD). Over 50 measurements were taken and the data were expressed as mean ± standard deviation. The porosity of the scaffold was measured using a method based on Archimedes' principle, as described elsewhere.<sup>35</sup>

#### Compression testing and recovery from deformation of POC scaffolds

The compressive Young's modulus of disk-shaped porous scaffolds ( $N = 6$ , 2.4 mm in thickness and 6 mm in diameter) was determined from the initial slope of the stress–strain data obtained from an Instron 5544 mechanical tester (Instron Canton, MA) operated in compression mode at a cross-head speed of 2 mm/min and a force of 10 N. The initial thickness of each scaffold was measured with an electronic caliper before application of the compressive force. The thickness of each scaffold was recorded 1 min after the removal of the compressive force for calculation of the compressive recovery ratio. The compressive recovery ratio was used to assess the capability of the scaffolds to recover from deformation and was calculated by using Eq. (1),<sup>36</sup> where  $L_1$

and  $L_0$  are the final and initial thickness of the samples after and before compression, respectively.

$$\text{Recovery (\%)} = \frac{L_1}{L_0} \times 100 \quad (1)$$

For comparison purposes, the compressive recovery ratio was measured for other similarly-sized scaffolds that are commonly used for cartilage tissue engineering. These included alginate (4% w/v), agarose (2% w/v), poly(glycolic acid) (PGA) non woven meshes (6 mm in diameter, 1.7 mm thick), and PLLA non woven meshes (6 mm in diameter, 2.4 mm in thickness).

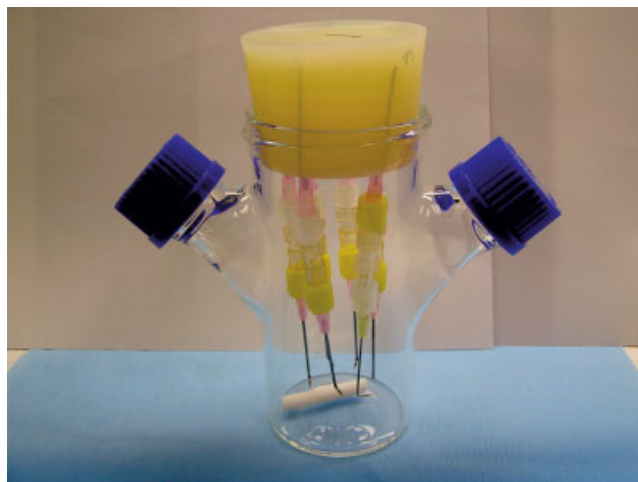
### Chondrocyte isolation, cell seeding, and *in vitro* culture of the POC-chondrocyte constructs

#### Isolation of primary chondrocytes

Full-thickness cartilage was surgically removed from femoral condyles and tibial platform of 4 bovine (17–19 months old) knee joints obtained from a local slaughter house (Swissland Packing, Chicago IL). Cartilage explants from all of the joints were sliced into small pieces ( $3 \times 3 \times 3 \text{ mm}^3$ ) and pooled together. Cartilage slices were digested with 0.15% (w/v) collagenase type II overnight at 37°C on an orbital shaker set at 40 rpm, as described previously.<sup>6</sup> The resulting cell suspension was filtered with a 150- $\mu\text{m}$  pore nylon mesh and washed four times with PBS via centrifugation. Chondrocytes were counted with a hemocytometer using trypan blue staining to take into account the presence of dead cells.

#### Construct culture

A stirred spinner flask was used for the culture of the constructs (Fig. 1).<sup>11,37</sup> Twenty-four POC scaffolds (6.0 mm in diameter and 2.4 mm in thickness) were gas-sterilized and threaded onto 21-gauge needles, which were attached to a silicon stopper. POC scaffolds were prewetted with fully supplemented medium (FSM), containing DMEM, 10% FBS, 100 unit/mL penicillin, 100  $\mu\text{g}/\text{mL}$  streptomycin, 0.05 mg/mL vitamin C, 0.1 mM nonessential amino acid, 10 mM HEPES, and 0.4 mM proline. The silicon stopper with the attached POC scaffolds was assembled onto the opening of the 200 mL spinner flask, which contained a 100 mL cell suspension at a concentration of  $4.8 \times 10^5$  cells/mL. The spinner flask was put on a stir plate and the cell suspension was stirred at a speed of 50 rpm in an incubator at 37°C with 5%  $\text{CO}_2$ . The cell culture medium was changed every other day. Cell-POC constructs ( $N = 8$  per time point) were collected after 7, 14, and 28 days of culture and evaluated via SEM, histology and IHC (collagen type II) and biochemical assays (DNA, glycosaminoglycan (GAG), hydroxyproline (total collagen)). Day 28 samples were subjected to mechanical testing before they were processed for biochemical analysis.



**Figure 1.** Digital picture of the spinner flask set up used to culture the cell-scaffold constructs. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

### Compression testing (unconfined) of POC-chondrocyte constructs

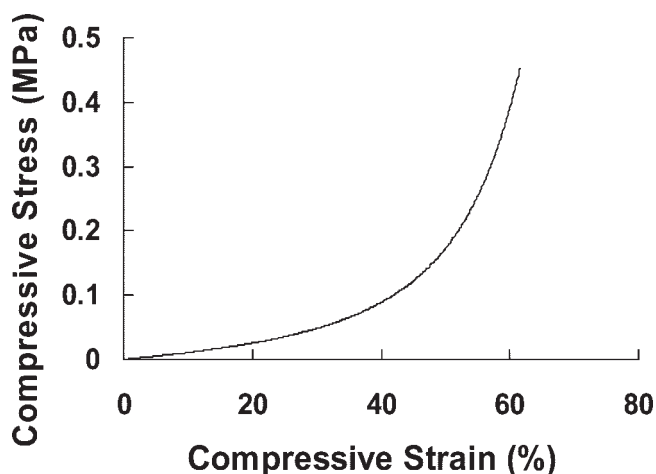
POC-chondrocyte constructs ( $N = 6$ ) that were cultured in FSM for 28 days were collected and washed in PBS three times, 5 min each time. The compressive Young's modulus at 10 N was determined using an Instron 5544 mechanical tester (10 N load cell and operated at a crosshead speed of 2 mm/min), as described earlier. The recovery ratio was also calculated. As a control, the Young's modulus and recovery ratio were also measured for cell-free POC scaffolds incubated in FSM for 28 days.

### Biochemical analysis of POC-chondrocyte constructs

Constructs were digested with 1 mL 0.0125% papain (w/v) at 60°C overnight and assayed for DNA, collagen, and GAG, as described later. Six samples in each experimental group at each time point were used for biochemical analysis. Each sample was measured in duplicate. The results are presented as means  $\pm$  standard deviation. Student's *t*-test was used to evaluate the significance of difference of cell proliferation, GAG, and collagen content in PGA and POC constructs at individual time point. Values were considered to be significantly different if  $p < 0.05$ .

#### DNA assay

Briefly, a 10  $\mu\text{L}$  sample of each digested construct was mixed with 90  $\mu\text{L}$  of tris-sodium-EDTA buffer and 2 mL of 0.1  $\mu\text{g}/\text{mL}$  Hoechst 33258 dye. The mixtures were read at 365 nm excitation and 458 nm emission with a fluorometer (Turner Designs, Sunnyvale, CA).<sup>38</sup> Cell numbers were calculated from DNA content (0.0077 pg DNA/cell).



**Figure 2.** Typical compressive stress–strain curve of POC (porosity and pore size are  $(90 \pm 2)\%$  and  $(106 \pm 26) \mu\text{m}$ , respectively).

### Collagen assay

Briefly, a 100  $\mu\text{L}$  sample of each digested construct was hydrolyzed in 900  $\mu\text{L}$  of 6N HCl at 115°C overnight. The pH value of each sample was adjusted to neutral. Samples were mixed with 0.5 mL of 1.57% (w/v) chloramine-T and 0.5 mL of 17.44% (w/v) *p*-dimethylaminobenzaldehyde solutions. The OD<sub>595</sub> value was then read with a spectrophotometer (Gary 50 Bio, Varian Australia PTY LTD, Australia).<sup>39</sup> This assay measures hydroxyproline content, which was converted to collagen content (collagen content ( $\mu\text{g}$ ) = hydroxyproline content ( $\mu\text{g}$ )/0.11).

### GAG assay

Briefly, a 100  $\mu\text{L}$  sample of each digested construct was mixed with 2.5 mL of 0.0016% (w/v) dimethylmethylene blue dye. The OD<sub>525</sub> value was then read with a spectrophotometer (Gary 50 Bio, Varian Australia PTY LTD, Australia).<sup>40,41</sup>

### Histological and immunohistochemical evaluation

Constructs were fixed in 10% buffered formalin overnight, dehydrated with a series of graded ethanol, and embedded in paraffin. Tissue sections were stained with the following: hematoxylin and eosin (HE), to assess cell distribution and morphology, Safranin-O, to assess GAG distribution, and Masson's Trichrome, to assess total collagen distribution. For IHC, slides with tissue samples were deparaffinized and incubated with mouse anti type II collagen antibody for 1 h, biotinylated anti mouse IgG for another hour, and ABC-alkaline phosphatase reagent for 30 min. The staining signal was developed by incubating the slide with the enzyme's substrate for 30 min at room temperature.

### Scanning electron microscopy (SEM) evaluation

Constructs collected at day 2, 14, and 28 were washed with PBS, fixed with 2.5% glutaraldehyde overnight, dehydrated with serial ethanol, and lyophilized overnight (FreeZone 6, Labconco, Kansas City, MO). Samples were coated with gold by using sputter coater (108auto, Cressington Scientific Instruments, Cranberry Twp., PA) and observed under SEM.

## RESULTS

### Mechanical testing

Compression tests on POC scaffolds produced a stress–strain curve characteristic of an elastomeric scaffold (Fig. 2). The compressive Young's modulus is 10.40 kPa. POC scaffolds (dry) completely recovered from deformation (98.7% recovery) (Table I). The 2% agarose gel samples broke up into small pieces during the compression test. The recovery ratio of 4% alginate gel after compression was 72.33% ( $p < 0.05$ ) and that of PGA and PLLA was 88.85 and 85.34%, respectively ( $p < 0.05$ ) (Table I).

Compression testing of POC-chondrocyte constructs cultured in FSM for 28 days revealed that Young's modulus was  $12.02 \pm 2.26$  kPa, which is significantly higher than that of POC control scaffolds ( $3.27 \pm 0.72$  kPa) that were cultured for 28 days (Table II). The compressive recovery ratio of POC-chondrocyte constructs, 93.27%, was not significantly different from that of control POC scaffolds, 99.44% ( $p > 0.05$ ) (Table II).

### Biochemical analysis

As shown in Figure 3(B), chondrocytes seeded in the POC scaffold proliferated during the culture period of 28 days. The cell number in the constructs was more than doubled at day 28 compared with that of day 7. GAG [Fig. 3(C)] and collagen [Fig.

**TABLE I**  
**Recovery from Compressive Deformation (within 1 min)**

	Scaffold Thickness (mm)		Recovery Ratio (%)	<i>p</i> Value <sup>a</sup>
	<i>L</i> <sub>0</sub>	<i>L</i> <sub>1</sub>		
Alginate	$1.51 \pm 0.07$	$1.09 \pm 0.11$	$72.33 \pm 4.32$	<0.05
PLLA	$2.35 \pm 0.05$	$2.01 \pm 0.06$	$85.34 \pm 4.01$	<0.05
PGA	$1.68 \pm 0.03$	$1.48 \pm 0.11$	$88.08 \pm 3.65$	<0.05
POC	$2.34 \pm 0.19$	$2.31 \pm 0.08$	$98.72 \pm 3.64$	–

<sup>a</sup> *p* Value is derived by comparing scaffold recovery ratios to that of POC ( $N = 6$ ).

**TABLE II**  
**Compression Testing of POC and POC-Chondrocyte**  
**Constructs Cultured *in vitro* for 28 days**

	Compressive modulus (kPa)	Compressive Recovery (%)
POC	3.27 ± 0.72	99.44 ± 0.48
POC with chondrocytes	12.02 ± 2.26	93.27 ± 6.22
p value <sup>a</sup>	<0.05	>0.05

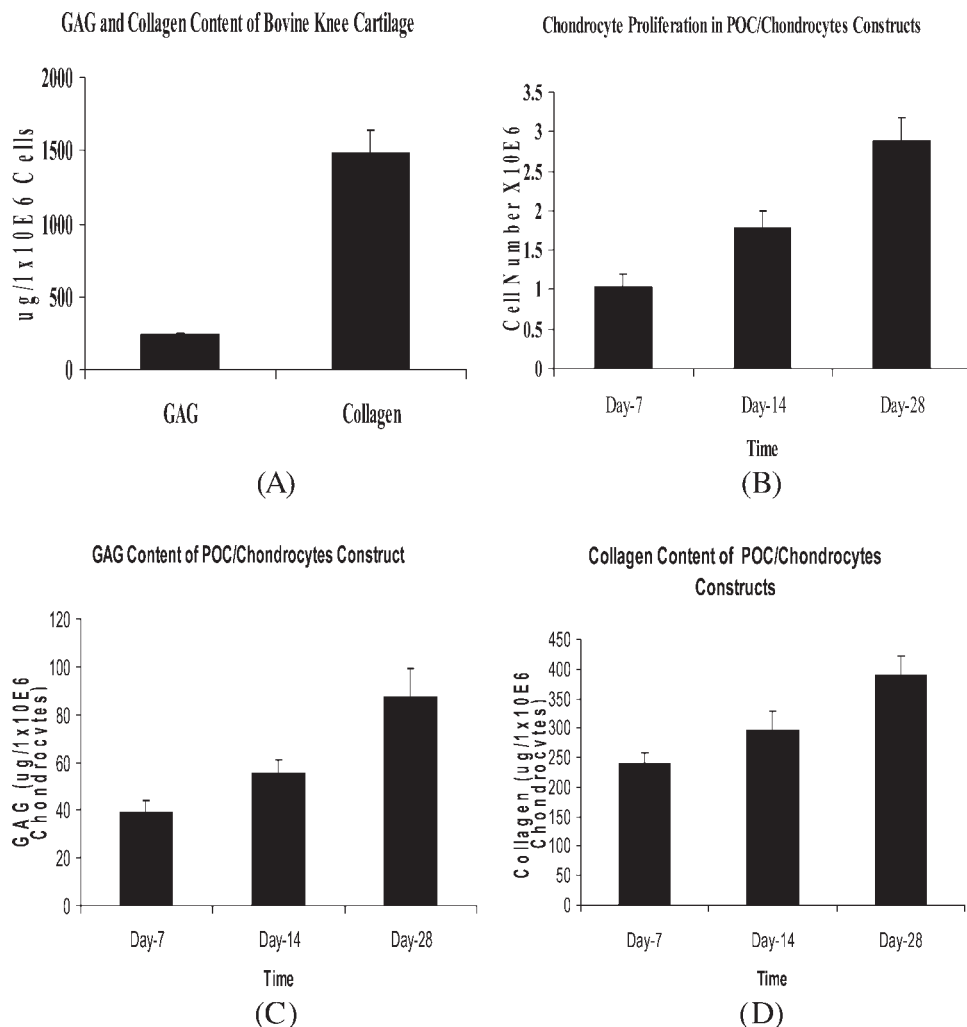
<sup>a</sup> p Value is obtained by comparing POC with POC-chondrocytes cultured for 28 days (N = 4–6 samples).

3(D)] content in POC constructs also increased throughout the duration of the study (on a per million cell basis). The cell number, GAG, and collagen content significantly increased at each time point ( $p < 0.05$ ). After 28 days in culture, the GAG and collagen content in POC constructs was 36%

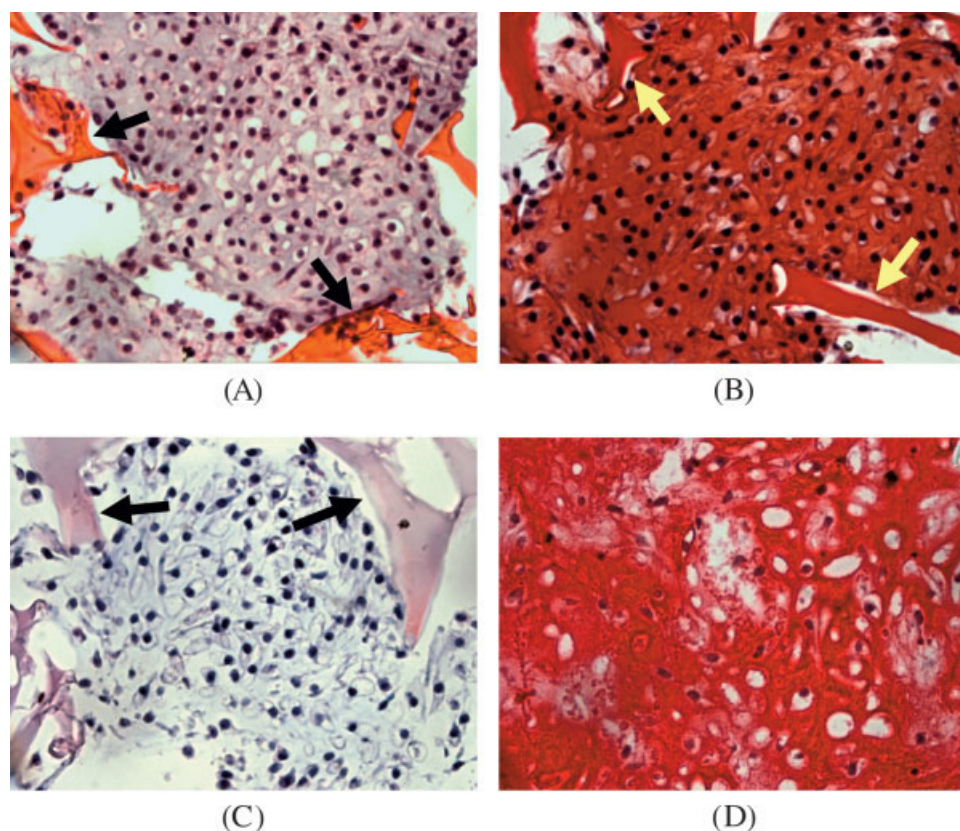
and 26% of that found in bovine knee cartilage, respectively [Fig. 3(A,C,D)].

**Histology, IHC, and SEM evaluation**

Cartilaginous tissue was formed within POC scaffolds (Fig. 4). Chondrocytes in lacuna were evenly distributed within the tissue. Safranin-O staining revealed GAG synthesis and deposition in the extracellular matrix of the neo-cartilage [Fig. 4(B)]. Collagen network formation in the interterritorial space was confirmed by Trichrome staining [Fig. 4(C)]. Type II collagen was expressed in the constructs [Fig. 4(D)]. The mean pore size of the POC scaffold was  $106 \pm 26 \mu\text{m}$  with a porosity of  $(90 \pm 2)\%$  [Fig. 5(A)]. Chondrocytes attached to the pore walls within the POC scaffold, and maintained their spherical shape [Fig. 5(B)]. The extracellular matrix around chon-



**Figure 3.** A: GAG and collagen content of bovine knee cartilage. B: Cell proliferation in POC-chondrocyte constructs. C: The observed difference between mean GAG content in constructs at different time points is significant ( $p < 0.05$ ). D: The observed difference between mean collagen content in constructs at different time points is significant ( $p < 0.05$ ).



**Figure 4.** Cell distribution and extracellular matrix production within 28-day POC-chondrocyte constructs ( $\times 400$ ). Arrows indicate remnants of the POC scaffold (A) hematoxylin and eosin, (B) safranin-O (GAG), (C) trichrome (collagen), and (D) Type II collagen. [Color figure can be viewed in the online issue, which is available at [www.interscience.wiley.com](http://www.interscience.wiley.com).]

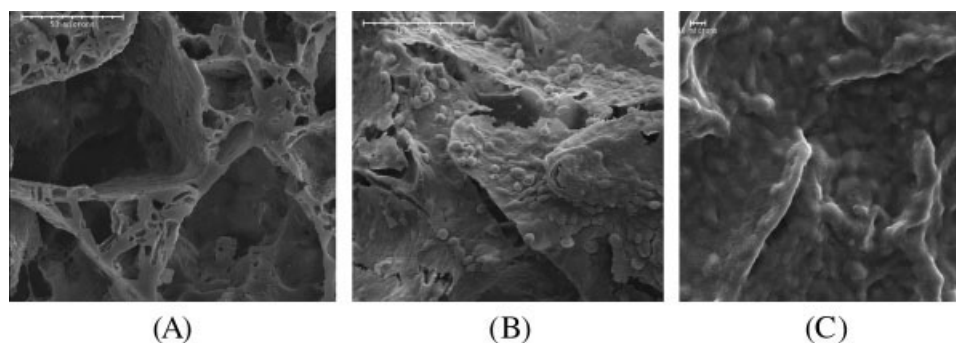
drocytes and in the interterritorial space was observed in the POC constructs by day 28 [Fig. 5(C)].

## DISCUSSION

Poly(diols citrates) are a new class of biodegradable polyester elastomers that can potentially be used in tissue engineering applications, where repeated mechanical stimulation of the cell-scaffold construct is important.<sup>12</sup> As several investigators have shown that cyclic mechanical compression can influence cartilage development *in vitro* as well as *in vivo*, poly(diols citrates) could potentially play a major role in scaffold design for cartilage tissue engineering.<sup>19–28</sup> This work describes the evaluation of POC as a scaffold for cartilage tissue engineering. 1,8-Octanediol was chosen as the monomer because it is the largest aliphatic diol that is water soluble, a property that should be advantageous to the degradation process as the formation of insoluble complexes due to the diol released into the surrounding medium is unlikely. It is also the first poly(diols citrate) synthesized in our lab and described in the literature.<sup>17</sup> As shown in Figure 2, the stress-strain curve of POC is similar to that of a typical

elastomer. The recovery ratio of 98.7% for the dry POC scaffolds reported in this study is consistent with POC porous scaffold recovery ratios that were reported by us in a previous study.<sup>17</sup> In the study of Yang et al., the recovery ratio of porous POC scaffolds, calculated before and after subjecting the scaffold to 500 cyclic compressions, remained constant at 99%. The results support further investigation into the use of POC as a candidate material to design improved scaffolds that are amenable to mechanical stimulation. Its degradation and mechanical properties can be modulated by changing the conditions used to crosslink the prepolymer, i.e., temperature and time.<sup>17</sup> Optimal degradation characteristics of poly(diols citrates) for cartilage tissue engineering are currently under investigation.

The capability to resist permanent deformation and exhibit instant recovery is very important for improving the quality of engineered cartilage constructs *in vitro* and maintaining the homeostasis of cartilage metabolism *in vivo*. Agarose gel was too fragile to withstand a compression force of 10 N. Alginate gel could withstand the compression force of 10 N, but it was significantly deformed and its time for close to 100% recovery was far beyond the 1 min criteria defined in the study (Table I). Non woven PGA and



**Figure 5.** Scanning electron micrographs of (A) POC scaffold before cell seeding, scale bar = 50  $\mu\text{m}$ , (B) POC construct at day 14, scale bar = 100  $\mu\text{m}$ , and (C) POC construct at day 28, scale bar = 10  $\mu\text{m}$ .

PLLA mesh scaffolds maintained their shape, but they could not recover their original thickness after the compression force (Table I). Therefore, our data suggest that POC has the best capability among the tested scaffold materials to recover from deformation in a rapid manner.

The Young's modulus of POC-chondrocyte constructs cultured for 28 days was significantly higher than that of dry POC scaffolds and cell-free POC scaffolds cultured for 28 days in FSM. The compression recovery ratio was greater than 90% for all groups. The biocompatibility of POC *in vivo*, as assessed by subcutaneous implantations, has previously been described.<sup>17</sup> In the study described herein, we show that POC can support chondrocyte attachment, proliferation, and differentiation *in vitro*. These findings are a first step towards the assessment of POC as a scaffold suitable for mechanical conditioning for cartilage tissue engineering. According to the DNA assay results, the number of chondrocytes present within porous POC scaffolds increased to almost 3-fold throughout the 28 days of culture. Total GAG and collagen content also increased steadily, approximating 36% and 26%, respectively, of the concentrations found in bovine cartilage explants (on a per million cell basis) [Fig. 3(A,C,D)]. Histology of the constructs after 28 days of culture show uniform cell, GAG, and collagen distribution (Fig. 4). The cell proliferation rate, GAG content, and collagen content in POC-chondrocyte constructs are comparable with those obtained with chondrocytes seeded on poly(glycolic acid) (PGA) mesh scaffolds that were cultured under conditions similar to the POC scaffolds (data not shown). Therefore, POC is a biodegradable polyester that is permissive to *in vitro* chondrogenesis in a manner that is consistent with a commonly used fibrous polymer scaffold (PGA) but with the added advantages of controllable elasticity, ease of synthesis, and reduced cost.<sup>17,34</sup>

The ability of a POC scaffold to support chondrocyte differentiation was assessed via IHC and SEM because chondrocyte de-differentiation to a fibroblast-like phenotype is often a concern when culturing these cells on synthetic material surfaces. POC scaffolds are

hydrophilic (water-in-air contact angle of POC films is 38°) and possess interconnected pore walls [Fig. 5(A)]. The presence of type II collagen and the maintenance of spherical cell morphology were used as primary markers of cell differentiation, as described by others.<sup>41,42</sup> Type II collagen was present in the newly formed extracellular matrix [Fig. 4(D)], and cells maintained a spherical shape after attachment and matrix synthesis on POC scaffolds [Fig. 5(B,C)]. These findings are consistent with those of Papadaki et al. In that study, the authors cultured bovine articular chondrocytes on poly(ether-ester) block copolymers and determined that the optimum degree of surface hydrophilicity for the retention of chondrocyte differentiation had a water-in-air contact angle of 37°. The degradation products of POC, primarily citric acid, 1,8-octanediol, and low molecular weight oligomers thereof, did not appear to inhibit new cartilage formation as over time, cells and cartilaginous matrix filled most of the pore volume within the scaffold [Fig. 5(C)]. Citric acid is a natural metabolite of the body (Krebs cycle). 1,8-Octanediol, at concentrations of up to 0.5 wt %, did not inhibit human aortic smooth muscle cell viability and proliferation on tissue culture polystyrene, as assessed by MTT assay (data not shown). Given the degradation rate of POC, the concentrations of citric acid and 1,8-octanediol that are released are expected to be within physiologically tolerable limits, and this expectation is supported by the *in vitro* data presented herein as well as *in vivo* data presented elsewhere.<sup>17</sup> Both monomers are also small enough to be cleared by the kidneys *in vivo*. These findings demonstrate that bovine articular chondrocytes can attach, proliferate, and synthesize extracellular matrix that includes type II collagen when grown on a hydrophilic poly(diols) such as POC.

## CONCLUSIONS

In summary, this study evaluated the key mechanical characteristics of a scaffold prepared from a newly

developed elastomer, POC, and its capacity to support chondrocyte attachment, proliferation, and differentiation *in vitro*. POC's elastomeric characteristics are expected to be conducive to its use as a biodegradable scaffold when applying long-term cyclic and shear strain on the cell-scaffold construct *in vitro* to increase the GAG and collagen content of engineered cartilage. Furthermore, when implanted in the body, the elastomeric properties of a POC-chondrocyte construct may be better suited to undergo and adequately transfer local compression and shearing forces produced by joint mobilization, thereby enhancing *in vivo* cartilage regeneration.

The authors thank Swissland Packing Co. for donating the bovine knees for the study.

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