Poly (Glycerol Sebacate): A Novel Scaffold Material for Temporomandibular Joint Disc Engineering

Catherine K. Hagandora, BS,^{1,2} Jin Gao, PhD,¹ Yadong Wang, PhD,¹
and Alejandro J. Almarza, PhD^{1,2}

The preponderance of temporomandibular joint (TMJ) disorders involving TMJ disc injury inspires the need to further explore tissue engineering strategies. The objective of this study was to examine the potential of poly (glycerol sebacate) (PGS), a biocompatible, biodegradable elastomer, as a porous scaffold material for the TMJ disc. Goat fibrochondrocytes were seeded on PGS at three seeding densities (25, 50, 100 million cells/mL scaffold), respectively, and cultured for 24 h, 2 weeks, and 4 weeks. The resulting histological, biochemical, and biomechanical properties were determined. Histological staining revealed an abundance of both collagen and glycosaminoglycans (GAG) throughout the high seeding density scaffolds at 4 weeks. There was also a significant increase in the cellular content in all groups over the four-week period, showing that the scaffolds promoted cell attachment and proliferation. The PGS scaffolds supported the deposition of large quantities of extracellular matrix, with differences noted between seeding density groups. At 4 weeks, the medium and high seeding density groups had significantly more collagen per scaffold ($181 \pm 46 \mu\text{g}$ and $218 \pm 24 \mu\text{g}$, respectively) than the low seeding density group ($105 \pm 28 \mu\text{g}$) ($p < 0.001$). At 4 weeks, the medium and high seeding density groups also had a significantly higher GAG content per scaffold ($702 \pm 253 \mu\text{g}$ and $773 \pm 187 \mu\text{g}$, respectively), than the low seeding density group ($324 \pm 73 \mu\text{g}$) ($p < 0.001$). The compression tangent modulus was significantly greater at 4 weeks than 24 h ($123.6 \pm 86 \text{ kPa}$ and $26.2 \pm 5 \text{ kPa}$, respectively) (seeding density groups combined) ($p < 0.001$), with no differences between seeding groups at each time point. After 4 weeks, the tangent modulus of the low seeding density group was in a similar range of the goat TMJ disc ($180 \pm 127 \text{ kPa}$ compared to $304 \pm 141 \text{ kPa}$, respectively). The results show that cell seeding density and culture time do have an effect on both the biochemical and biomechanical properties of PGS scaffolds. These findings demonstrate that PGS has great potential as a scaffold material for TMJ disc engineering.

Introduction

THE TEMPOROMANDIBULAR JOINT (TMJ) is a bilateral joint consisting of the articulation of the condyle of the mandible against the glenoid fossa of the temporal bone. A fibrocartilage disc rests between the condyle and the fossa, acting as a distributor of compressive, tensile, and shear loads during mandibular movements.^{1–4} It is estimated that 10 million Americans suffer from temporomandibular joint disorders,⁵ with up to 70% exhibiting displacement of the TMJ disc,⁶ or an abnormal positional relationship of the disc relative to the mandibular condyle and the articular eminence. When the displaced disc becomes morphologically damaged, surgeons may perform discectomy with or without autograft replacement. While autografts have been demonstrated to prevent the onset of crepitus,⁷ or a grating noise during joint movement, which often occurs in dis-

cectomy alone,^{8–10} they are limited by their propensity to perforate and resorb in addition to increased donor-site morbidity.^{11,12} Since the TMJ disc is relatively avascular and does not spontaneously regenerate or repair itself *in vivo*,¹³ it has become a target for tissue engineering.

The ideal scaffold for engineering the TMJ disc should be biodegradable, sufficiently porous to allow for the diffusion of nutrients to cells,¹⁴ support matrix deposition, and provide adequate mechanical support throughout the regeneration process. Up to this point, TMJ fibrocartilage engineering techniques using alginate,¹⁵ poly glycolic acid (PGA),^{15,16} and poly-L-lactic-acid (PLLA)¹⁷ have been attempted with varying success. The intrinsic challenge of regenerating a suitable TMJ disc replacement is engineering a structure that achieves levels of type I collagen and glycosaminoglycans (GAG) comparable to the native TMJ disc.¹⁸

Departments of ¹Bioengineering and ²Oral Biology, Center for Craniofacial Regeneration, McGowan Institute of Regenerative Medicine, School of Dental Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

Poly (glycerol sebacate) (PGS) is a biocompatible, biodegradable polymer,¹⁹ which has shown great potential as a scaffolding material for soft tissue engineering applications.²⁰ As a first step toward tissue engineering of the TMJ disc, we will determine whether PGS provides an acceptable environment for fibrochondrocyte attachment and matrix production. Furthermore, the optimum cell seeding density of fibrochondrocytes in porous PGS scaffolds must be evaluated.

Unlike TMJ disc cells, which are difficult to obtain and unlikely to provide a viable cell source from diseased tissues, costal cartilage, which is used clinically as an autogenous tissue graft for TMJ reconstruction, can be easily harvested. Additionally, previous studies have shown that costal chondrocytes, which become more like fibrochondrocytes with increased passage number, produce the highest collagen per wet weight to date in scaffoldless tissue culture.^{21,22} The objective of this study was to determine the effect of cell seeding density of goat fibrochondrocytes in PGS scaffolds on the extracellular matrix (ECM) production and biomechanical properties of the tissue-engineered scaffolds. Cells were seeded at concentrations of 25, 50, and 100 million cells/mL of scaffold (low, medium, and high seeding density groups) and were tested histologically and biochemically after 24 h, 2 weeks, and 4 weeks of culture. The mechanical properties of the scaffolds in unconfined compression were assessed at 24 h and 4 weeks. The selected range of low, medium, and high seeding densities: 25–100 million cells/mL scaffold, was based on a study performed by Almarza *et al.*, in which a high seeding density of 120 million cells/mL of PGA scaffold resulted in the highest collagen production by porcine TMJ disc fibrochondrocytes.¹⁶ Saturation effect was noted between medium (30 million cells/mL of scaffold)- and high (120 million cells/mL of scaffold)-density seeding and it was suggested that 75 million cells/mL of scaffold would likely be ideal for porcine TMJ disc cells seeded in PGA scaffolds. Therefore, we decided to test within a similar range of seeding densities for our goat fibrochondrocytes seeded in PGS scaffolds. The 24-h time point was evaluated to determine the cell seeding efficiency as well as a way of measuring the initial compressive strength of the scaffold. Previous studies using goat costal fibrochondrocytes have not shown major ECM deposition beyond 4 weeks, and thus it was chosen as the last time point. The 2-week time point was evaluated to assess the behavior through time. In this study, we evaluated both time points to ensure that, despite degradation of the PGS scaffold, there continued to be an increase in ECM production.

Methods

Cell isolation and culture

Goat costal cartilage was isolated from the ribs of three young (<1 year) female Boer goats within 24 h of slaughter. The fibrocartilage was minced and digested in 2 mg/mL type II collagenase (Worthington) overnight at 37°C and 5% CO₂ with mechanical agitation. Isolated fibrochondrocytes were passaged three times in Dulbecco's modified Eagle's medium (DMEM)/high glucose (Thermo Scientific), 10% fetal bovine serum (Atlantic Biologicals), 1% penicillin–streptomycin (Lonza), 1% nonessential amino acid solution (Thermo Scientific), and 25 µg/mL L-ascorbic acid (Sigma-Aldrich). The cells were frozen after passage one in passage

media with 5% dimethyl sulfoxide and an additional 10% FBS, and then thawed and passaged twice to allow for simultaneous seeding of the scaffolds.

Cell seeding

PGS sheets were prepared as previously described, using salt particles of 75–150 µm, which have been shown to facilitate fibroblast attachment and diffusion.²⁰ Scaffolds were cut from a PGS sheet of approximately 1.3 mm thickness using a 5-mm biopsy punch. Scaffolds were seeded at 25, 50, and 100 million cells/mL of scaffold in 10 µL of chondrogenic media using a 25-gauge needle. Chondrogenic media consisted of DMEM/high glucose (Thermo) supplemented with 1% penicillin–streptomycin (Lonza), 1% nonessential amino acid solution (Thermo Scientific), 1% insulin–transferrin–selenium + premix (BD Biosciences), 0.1 µM dexamethasone (MP Biomedicals), 40 µg/mL L-proline (Acros Organics), and 50 µg/mL ascorbate 2-phosphate (Sigma-Aldrich).²¹ Scaffolds were cultured in 24-well plates coated in 2% agarose. After seeding, the media was replenished every 48 h (1 mL of media per well). Scaffolds were cultured in 37°C and 5% CO₂ on an orbital shaker (approximately 9 orbits per minute) for 24 h, 2 weeks, and 4 weeks.

Histology

Scaffolds ($n=3$ per group) were embedded in the optimal cutting temperature compound (Tissue-Tek) and flash frozen to –80°C. The samples were cryosectioned at 6 µm, fixed in cold acetone, and stained with hematoxylin and eosin to visualize the cellular content and distribution, picosirius red for collagen staining and Safranin-O/Fast green for GAG staining. Immunostaining for collagen types I and II was performed, as previously described,²³ using a Vectastain[®] ABC kit (Vector Laboratories), a monoclonal anti-collagen type I antibody produced in mouse (Sigma), and a anti-human collagen type II produced in mouse (MP Biomedicals).^{23,24} Negative stains were also performed without applying the primary antibody. All images were obtained on a Nikon Eclipse TE2000E inverted light microscope.

Biochemistry

Scaffolds ($n=6$ per group) were dried on the Speed Vac (Thermo) overnight and digested as previously described.²² Briefly, samples were digested at 4°C with constant mechanical agitation in 125 µg/mL papain (Sigma-Aldrich) for 8 days followed by 1 mg/mL elastase (Sigma-Aldrich) digestion for 3 days. All scaffolds were dried and stored in –20°C until digestion was ready to be performed on all samples at once. All assays were performed using this digest. The DNA content was measured using a PicoGreen dsDNA quantitation kit (Molecular Probes, Inc.). The total hydroxyproline content was assessed using the modified protocol of reacting the samples with chloramine T and dimethylaminobenzaldehyde that allows for a colorimetric comparison and compared against collagen standards.²⁵ The total amount of GAG was measured using a dimethylmethylene blue colorimetric assay kit (Biocolor).

Compression testing

Unconfined compression testing and analysis was performed per our published methods ($n=6$ per group).²⁶

Scaffolds were tested at two time points, 24 h and 4 weeks, to compare changes in mechanical integrity as a result of matrix deposition over the 4-week period. The construct diameter was measured before testing using digital calipers. The scaffolds were attached to a compression platen using cyanoacrylate. To estimate construct height, force was applied to the construct until reaching 0.02 N, at which point the crosshead position was noted and the platen was immediately removed. The water bath was then filled with phosphate-buffered saline and the thermocouple was set to 37°C before testing. The MTS Insight was used to measure changes in force throughout the test. The upper platen was lowered within 0.1 mm of the determined construct height and a preload of 0.05 N was applied for 30 min. In the compression testing analysis, the estimated effect of buoyant force is not subtracted from the acquired force reading. However, by determining the construct height using the compression system before filling the water bath, we believe we are minimizing error that may be attributed to buoyant force. By zeroing the load at 0.1 mm above the sample, there is an average travel path of approximately 0.3 mm, which would result in a buoyant force that is well below the accuracy of our load cell. The height at the end of the preload was taken to be the height of the construct and was utilized in subsequent calculations. The scaffolds then underwent 10 cycles of preconditioning at 9% /min until 10% strain was reached.^{26,27} Immediately following preconditioning, the samples were compressed until 10% strain was reached, and were allowed to relax for 60 min.

A tangent modulus was fit to the linear portion of the stress-strain curve using Matlab, defined as the last 2% of 10% strain. The percent relaxation was determined by evaluating the ratio of the stress of the relaxed specimen, with the specimen considered fully relaxed at 60 min, to the peak stress.

Statistical analysis

A two-way analysis of variance was used to assess differences for biochemical and biomechanical values within culture time and seeding density with $p < 0.05$ defined as statistically significant. Tukey's *post hoc* testing was used to examine differences between groups. Outliers were defined as observations with values exceeding 1.5 times the interquartile range and were excluded from the analysis. All statistical analysis was performed using Minitab. All data are reported as average $\pm$ standard deviation of the means.

Results

Gross morphology

The gross morphology of the PGS scaffolds at 24 h and 4 weeks after cell seeding is shown in Figure 1. At all time points and seeding densities, scaffolds were indistinguishable by size, shape, and opacity of the cell-seeded construct.

Histology

The PGS scaffolds stained with hematoxylin and eosin are shown in Figure 2. The images were magnified to show matrix deposition from the edge to the center of the PGS scaffolds. There was an increase in areas of staining with high seeding density and time. The staining reveals prolif-

FIG. 1. Gross morphology of the high seeding density poly (glycerol sebacate) (PGS) scaffolds at 24 h and 4 weeks. Color images available online at www.liebertpub.com/tea

eration of fibrochondrocytes as well as an increase in ECM over time. At 4 weeks, the high seeding density group appeared to have more lacunae than the other groups, corresponding to an increase in the cellular content over time.

The images from the histological assessment of the specific matrix content are shown in Figure 3. The images show matrix deposition from the edge to the center of the PGS scaffolds. No specific staining for GAG or collagen was seen at 24 h, thus images are not shown. All groups stained positively for GAG at weeks 2 and 4. There tended to be an increase in areas of staining with high seeding density and time. There was variability in the way matrix was deposited throughout the scaffolds with some scaffolds showing matrix deposition throughout and others with matrix deposition confined to the edges, not dependent on seeding density.

The picrosirius red stain revealed that all groups also stained positively for collagen at 2 and 4 weeks. (Fig. 3) Corresponding with the biochemistry findings, there was an increase in areas of collagen staining at later time points. Similar to the GAG results, there was a random distribution of collagen throughout the scaffold with some scaffolds exhibiting collagen throughout and others with collagen on one or both edges of the scaffold, not dependent on seeding density.

The scaffolds demonstrated sparse staining for collagen type I that was indistinguishable from the negative control with the primary antibody removed. There was positive staining for collagen type II throughout the scaffolds at weeks 2 and 4. (Fig. 3) A side-by-side comparison of the positive and negative collagen type II stain is shown in Figure 4. At week 4, there were more areas of staining for collagen type II in the center of the scaffolds than in week 2,

FIG. 2. Hematoxylin and Eosin staining of the low, medium, and high seeding density PGS scaffolds at 24 h, 2 weeks, and 4 weeks. Scale bar is 500 μm . Color images available online at www.liebertpub.com/tea

where collagen type II staining was confined to the outer edges of the scaffold.

Biochemical content

The cell content of the scaffolds is shown in Figure 5A. There were no significant differences between seeding density groups within each time point. There was a significant increase in the cellular content at 4 weeks when compared to 2 weeks and 24 h ($6.7 \pm 1.8 \times 10^5$ compared to $4.6 \pm 0.9 \times 10^5$ and $3.7 \pm 1.0 \times 10^5$ cells/construct, respectively (low, medium, and high groups combined)) ($p < 0.001$). Also, the low seeding density group had significantly lower cell content than the medium and high seeding density groups across all time points. ($4.0 \pm 1.3 \times 10^5$ compared to $5.5 \pm 2.1 \times 10^5$ and $5.6 \pm 1.7 \times 10^5$ cells/construct, respectively (24-h, 2-week, and 4-week time points combined)) ($p < 0.001$).

The collagen content of the scaffolds is shown in Figure 5B. There were significant differences in the collagen content of the scaffolds between seeding density groups and time (Table 1) ($p < 0.001$). In the low seeding density group, there was a significant difference in the collagen content between 24 h and 2 weeks ($24 \pm 3 \mu\text{g}$ and $72 \pm 19 \mu\text{g}$ of collagen per construct, respectively). In the medium seeding density group, there was a significant increase in the collagen content at each time point ($38 \pm 6 \mu\text{g}$, $102 \pm 34 \mu\text{g}$, and $181 \pm 46 \mu\text{g}$ of collagen per construct at 24 h, 2 weeks, and 4 weeks, respectively) ($p < 0.001$). Similarly, in the high seeding density group, there was a significant increase in the collagen content at each time point ($36 \pm 7 \mu\text{g}$, $100 \pm 22 \mu\text{g}$, and $218 \pm 24 \mu\text{g}$ of collagen per construct at 24 h, 2 weeks, and 4 weeks, respectively) ($p < 0.001$). There were no significant differences between the low, medium, and high seeding density groups at 24 h and 2 weeks. At 4 weeks, the low seeding density group had significantly lower collagen content per scaffold

FIG. 3. Histological staining of the PGS scaffolds. Row 1: Safranin o/fast green staining for GAG. Row 2: Picrosirius red staining for collagen. Row 3: Collagen type II immunostain. Scale bar is 250 μ m. Color images available online at www.liebertpub.com/tea

($105 \pm 28 \mu\text{g}$) than the medium and high seeding density groups ($181 \pm 46 \mu\text{g}$ and $218 \pm 24 \mu\text{g}$ of collagen per scaffold, respectively) ($p < 0.001$).

The GAG content of the scaffolds is shown in Figure 5C. There were significant differences in the GAG content of the scaffolds between seeding density groups and time (Table 2) ($p < 0.001$). In the low seeding density group, there was a

significant increase in the GAG content between 24 h and 4 weeks ($3 \pm 1 \mu\text{g}$ and $324 \pm 73 \mu\text{g}$ of GAG per scaffold, respectively) ($p < 0.001$). In the medium seeding density group, there was a significant increase in the GAG content at each time point ($9 \pm 2 \mu\text{g}$, $362 \pm 125 \mu\text{g}$, and $702 \pm 253 \mu\text{g}$ of GAG per scaffold at 24 h, 2 weeks, and 4 weeks, respectively) ($p < 0.001$). Similarly, in the high seeding density group,

FIG. 4. Example of collagen type II-positive and -negative stain. Scale bar is 500 μ m. Color images available online at www.liebertpub.com/tea

FIG. 5. Biochemical content of PGS scaffolds at 24 h, 2 weeks, and 4 weeks: **(A)** cell content per construct, **(B)** collagen content per construct, and **(C)** glycosaminoglycan (GAG) content per construct. Error bars indicate average $\pm$ standard deviation. The symbol (*) indicates statistical difference between groups at each time point $p < 0.001$.

there was also an increase in the GAG content at each time point ($10 \pm 3 \mu\text{g}$, $338 \pm 57 \mu\text{g}$, and $773 \pm 187 \mu\text{g}$ of GAG per scaffold at 24 h, 2 weeks, and 4 weeks, respectively) ($p < 0.001$). There were no significant differences between the low, medium, and high seeding density groups at 24 h and 2 weeks. At 4 weeks, the low seeding density group had significantly lower GAG content per scaffold ($324 \pm 73 \mu\text{g}$) than the medium and high seeding density groups ($702 \pm 253 \mu\text{g}$ and $773 \pm 187 \mu\text{g}$ of GAG per scaffold, respectively) ($p < 0.001$).

Compression

The results from the compression analysis are shown in Figure 6. There was a significant increase in peak stress of the cell-seeded PGS scaffolds at 4 weeks ($4.1 \pm 0.7 \text{ kPa}$ at 24 h and $10.3 \pm 6.1 \text{ kPa}$ at 4 weeks) ($p < 0.001$). (Fig. 6A) There were no significant differences in peak stress between seeding density groups at each time point. Similarly, there was a significant increase in equilibrium stress over the 4-week period ($3.6 \pm 0.7 \text{ kPa}$ at 24 h and $5.4 \pm 1.4 \text{ kPa}$ at 4 weeks) ($p < 0.001$) with no differences between the seeding density group at each time point. (Fig. 6B) The percent stress relaxation of the 4-week scaffolds was significantly greater than the 24-h scaffolds ($38.3 \pm 11.5\%$ and $14.5 \pm 4.2\%$, respectively) ($p < 0.001$). (Fig. 6C) There were no significant differences in

stress relaxation between the seeding density group at each time point. The tangent modulus was significantly greater at 4 weeks than 24 h ($123.6 \pm 86.0 \text{ kPa}$ and $26.2 \pm 5.0 \text{ kPa}$, respectively) ($p < 0.001$). (Fig. 6D) Again, there were no significant differences between the seeding density groups at each time point.

Discussion

The results show that PGS shows great potential as a substrate for fibrocartilage regeneration. Cell seeding density and culture time did have an effect on both the biochemical and biomechanical properties of the scaffolds. The histological results confirm the biochemistry findings, indicating an increase in matrix deposition both with time and cell seeding density. Histology also indicated that cells did migrate and deposit matrix throughout the scaffold. This demonstrates that the PGS scaffolds are of a sufficient porosity to support both the migration of cells and diffusion of nutrients. The immunostaining revealed a sparse distribution of type I collagen throughout the scaffold and positive staining for type II collagen. This is consistent with what has been reported when using the same cell type and media formulation.^{21,22} Increasing the collagen type I/II ratio remains a critical challenge in fibrocartilage tissue engineering. It is possible that cyclic compression or the *in vivo* mechanical

TABLE 1. DIFFERENCES IN μg OF COLLAGEN IN RESPONSE TO TIME (24 H, 2 WEEKS, AND 4 WEEKS) AND SEEDING DENSITY (LOW, MEDIUM, AND HIGH)

Collagen (μg)	Low	Medium	High
24 h	24 $\pm$ 3 D	38 $\pm$ 6 CD	36 $\pm$ 7 CD
2 weeks	72 $\pm$ 19 BC	102 $\pm$ 34 B	100 $\pm$ 22 B
4 weeks	105 $\pm$ 28 B	181 $\pm$ 46 A	218 $\pm$ 24 A

Groups that do not share a letter are statistically significant ($p < 0.001$).

TABLE 2. DIFFERENCES IN μg OF GLYCOSAMINOGLYCANS IN RESPONSE TO TIME (24 H, 2 WEEKS, AND 4 WEEKS) AND SEEDING DENSITY (LOW, MEDIUM, AND HIGH)

GAG (μg)	Low	Medium	High
24 h	3 $\pm$ 1 C	9 $\pm$ 2 C	10 $\pm$ 3 C
2 weeks	199 $\pm$ 27 BC	362 $\pm$ 125 B	338 $\pm$ 57 B
4 weeks	324 $\pm$ 73 B	702 $\pm$ 253 A	773 $\pm$ 187 A

Groups that do not share a letter are statistically significant ($p < 0.001$).

FIG. 6. Biomechanical properties of PGS scaffolds at 24 h and 4 weeks. **(A)** peak stress, **(B)** equilibrium stress, **(C)** percent stress relaxation, and **(D)** tangent modulus. Error bars indicate average $\pm$ standard deviation. The symbol (*) indicates $p < 0.001$ for all groups between time points.

environment is necessary to achieve the proper collagen type I/II ratio.

There was an increase in the cellular, GAG, and collagen content over the 4-week period and an increase in ECM deposition at higher seeding densities. The average amount of collagen produced by goat costal chondrocytes in PGS at 4 weeks in the high cell seeding density group ($218 \pm 24 \mu\text{g}$ collagen per construct) exceeded that which has been reported previously using porcine TMJ disc cells and PGA scaffolds for TMJ disc engineering.¹⁶ In the high seeding density group, there was $3.5 \pm 0.5\%$ collagen and $12.1 \pm 2.3\%$ GAG per dry weight at 4 weeks which is comparable to scaffoldless constructs, which have shown to achieve $6.9 \pm 0.5\%$ collagen and $16.5 \pm 1.3\%$ GAG per dry weight at 4 weeks.²³ In contrast, the native goat TMJ disc is $45.7 \pm 19.6\%$ collagen and $2.1 \pm 1.2\%$ GAG per dry weight.²⁶ Additionally, there was an increase in cell density over time, indicating that, even at high seeding densities, the scaffolds did not reach saturation, and there was sufficient diffusion of nutrients into the scaffold to support cell proliferation.

The tangent modulus of the low seeding density group at 4 weeks ($180 \pm 127 \text{ kPa}$) was in the range of what has been reported for the goat TMJ disc ($304 \pm 141 \text{ kPa}$).²⁶ There was a wide range of maximum stress obtained in the low seeding density group at 4 weeks ranging from 5–14 kPa, which, based on histological evidence, was likely due to variations

in the way matrix was deposited throughout the scaffold (edges vs. middle). Similarly, there was a significant increase in percent stress relaxation between 24 h and 4 weeks, which ranged from $33 \pm 11\%$ to $45 \pm 13\%$ in all three seeding density groups, compared to $85 \pm 7\%$, which was reported in the goat TMJ disc.²⁶ The increase in the tangent modulus of the PGS scaffolds over the 4-week period provides evidence that the degradation rate of the material is slow enough to allow for the regeneration of ECM and restoration of mechanical integrity. The change in stress relaxation behavior is a positive indicator that the tissue-engineered constructs are becoming more viscoelastic, and better emulating the mechanical behavior of the native TMJ disc.

In this study, the amount of collagen and GAG per scaffold exceeded that which has been reported using TMJ disc cells on PLLA scaffolds with exogenous growth factors.¹⁷ A noteworthy advantage to using the PGS scaffold material is avoidance of scaffold contraction observed in PGA nonwoven meshes, which have shown to reduce in volume by over 50% in 4–6 weeks.²⁸ In this study, the dimensions of the unseeded PGS scaffolds were indistinguishable from the 4-week cell-seeded scaffolds. In contrast, scaffoldless constructs using the same cell type and media formulation exhibit varying morphology dependent on the culture time.²² Furthermore, scaffoldless constructs fail to support cell viability and matrix production in the center of the construct,

likely due to limitations in nutrient diffusion.²³ However, the fibrochondrocytes utilized in the study were able to successfully deposit matrix throughout the PGS scaffold, indicating that using scaffolding materials with sufficient porosity is key to supporting cell growth and ECM production. A limitation in this study was the non-homogenous cell and matrix distribution within the PGS scaffolds, which could be ameliorated by using smaller scaffolds, multiple injections of cells during seeding, cyclic compression, and/or spinner flasks.¹⁵ Additionally, to make the engineered tissue more fibrocartilagenous, steps must be taken to adjust the collagen type I and GAG content closer to the native tissue. To increase the collagen type I/II ratio, the incorporation of combinations of growth factors, such as insulin-like growth factor-I, transforming growth factor- β 1, or basic fibroblast growth factor, could be employed.²⁹ Chondroitinase could also be utilized to deplete the excess GAG production, allowing more space for the production of additional collagen. It has also been shown that the application of mechanical stimuli during culture could potentially enhance type I collagen production and organization of fibrochondrocytes.³⁰ In future studies, the effect of mechanical stimulation on collagen production will be assessed by analyzing gene expression. Finally, the use of coculture with costal chondrocytes and TMJ disc cells could also be used to increase collagen type I production.³¹

This study demonstrated the potential of PGS as a scaffold material for fibrocartilage engineering, with the highest seeding density and longest time point producing the greatest amount of ECM. It has been shown that fibrochondrocytes respond to the application of mechanical forces by producing elevated amounts of ECM.³⁰ It is hypothesized that the elastomeric properties of PGS will allow for the transduction of force to cells seeded on the scaffold, enhancing both the production and organization of collagen. Therefore, a future aim is to test the effect of the application of both static and cyclic compression on the matrix production of fibrochondrocytes seeded on PGS scaffolds. Additionally, the ability of autologous cell-conditioned scaffolds to promote the regeneration of TMJ fibrocartilage *in vivo* will also be assessed.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Alejandro J. Almarza, PhD

Department of Bioengineering

Center for Craniofacial Regeneration

McGowan Institute of Regenerative Medicine

School of Dental Medicine

University of Pittsburgh

566 Salk Hall, 3501 Terrace Street

Pittsburgh, PA 15261

E-mail: aja19@pitt.edu

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