TISSUE ENGINEERING: Part A
Volume 17, Numbers 1 and 2, 2011
© Mary Ann Liebert, Inc.
DOI: 10.1089/ten.tea.2010.0272

# Maturation State-Dependent Alterations in Meniscus Integration: Implications for Scaffold Design and Tissue Engineering

Lara C. Ionescu, M.S.,[1,2] Gregory C. Lee,[2] Grant H. Garcia, B.S.,[1] Tiffany L. Zachry, M.S.,[1] Roshan P. Shah, M.D.,[1] Brian J. Sennett, M.D.,[1] and Robert L. Mauck, Ph.D.[1,2]

The knee meniscus is a crucial component of the knee that functions to stabilize the joint, distribute load, and maintain congruency. Meniscus tears and degeneration are common, and natural healing is limited. Notably, few children present with meniscus injuries and other related fibrocartilaginous tissues heal regeneratively in immature animals and in the fetus. In this work, we evaluated fetal, juvenile, and adult bovine meniscus properties and repair capacity *in vitro*. Although no changes in cell behavior (migration and proliferation) were noted with age, drastic alterations in the density and distribution of the major components of meniscus tissue (proteoglycan, collagen, and DNA) occurred with development. Coincident with these marked tissue changes, the *in vitro* healing capacity of the tissue decreased with age. Fetal and juvenile meniscus formed a robust repair over 8 weeks on both a histological and mechanical basis, despite a lack of vascular supply. In contrast, adult meniscus did not integrate over this period. However, integration was improved significantly with the addition of the growth factor transforming growth factor-beta 3. Finally, to evaluate engineered scaffold integration in the context of aging, we monitored cellular infiltration from native tissue into engineered nanofibrous constructs. Our findings suggest that maturation processes that enable load bearing in the adult limit endogenous healing potential and identify new metrics for the development of tissue-engineered meniscus implants.

## Introduction

The meniscus is a fibrocartilage found in the knee whose primary function is mechanical, withstanding both compressive and tensile forces that arise with locomotion.[1] The extracellular matrix (ECM) is complex in architecture and composition, and is comprised of circumferentially oriented collagen bundles interspersed with a dense proteoglycan (PG) network.[2] When acute injury or degenerative changes occur, meniscus load-bearing capacity is compromised.[3] Clinically, patients with meniscal tears are treated surgically, with the torn or frayed portion resected or, when possible, reattached.[4–6] When significant damage occurs, meniscus allografts can be utilized as a total replacement.[7] However, despite restoration of pain-free motion with these interventions, the joint compartment remains predisposed to the development of osteoarthritis, as joint mechanics remain altered and nonideal motion and mechanical forces are imparted on the surrounding tissues.[8–10]

The adult meniscus has limited vascularity and healing capacity,[11] and so simple repairs often fail over time. To better study meniscus healing, *in vitro* models have been developed. Early work by Webber and coworkers[12] demonstrated that meniscus sections remain viable in culture media supplemented with bovine serum or in a chemically defined media. Kobayashi and coworkers showed that defects in the outer meniscus heal better than defects in the inner meniscus, at least on a histological basis.[13] More recently, Hennerbichler and coworkers adopted a method commonly employed to assess cartilage-to-cartilage integration[14] to mechanically evaluate meniscus repair *in vitro*.[15] This method involves the formulation of a cylindrical full-thickness concentric defect within a meniscus explant.[15] Integration strength is determined by measuring the force required to extrude the inner core from the outer ring. Using this approach, no measurable differences in mechanical integration were noted comparing the inner and outer regions. This technique has also been used to study the influence and interplay of inflammatory cytokines and matrix metalloproteinases in meniscus healing.[16–20] The organ culture model allows for the careful analysis of healing by region, the influence of soluble factors, and how repair changes over time—factors that are difficult to observe or control in the joint space.

While adult meniscus healing is limited, a number of observations in immature meniscus and related tissues suggest that immature fibrocartilage may possess a greater

[1]McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, Pennsylvania.
[2]Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania.

healing potential. First, younger patients rarely present with acute meniscal tears.[21] The repair capacity may be due to the more complete vascular infiltration of immature meniscus compared to adult meniscus, which is only vascularized in the outer third of the tissue.[22,23] Indeed, repairs to the more vascular outer region of the adult meniscus can have long-term success.[24] Further, *in vitro* studies have shown that juvenile cartilage heals better than adult cartilage even in the absence of a blood supply.[25,26] Similarly, immature rat lateral collateral ligament heals better than mature ligament *in vivo*.[27] In the fetus, complete regenerative healing of tendon and cartilage has been observed.[28–30] Taken together, these findings motivated us to evaluate differences in healing potential between fetal, juvenile, and adult meniscus, as well as to characterize the properties of the meniscus and associated meniscus fibrochondrocytes (MFCs) as a function of age.

Beyond evaluation of natural tissue healing, this *in vitro* meniscus culture system can be used as a test bed for meniscus tissue engineering, similar to approaches used in cartilage tissue engineering.[31–33] Numerous materials have been tested for meniscus tissue engineering, including collagen,[34] small intestinal submucosa,[35,36] estane,[37] polyurethane,[38] hyaluronic acid,[39] agarose gels,[40] and alginate.[41] However, due to the complex loading environment many of these approaches have shown limited success *in vivo*. The only material translated to the clinic in the United States is the Collagen Meniscus Implant (Menaflex), although its efficiency is not yet clearly demonstrated.[34] A polyurethane foam product (Orteq) is also available in Europe, although its use has not been approved by the U.S. Food and Drug Administration. To expand the palette of materials for meniscus tissue engineering, we have developed a nanofibrous electrospun scaffold, with aligned fibers that mimic the native collagen architecture of meniscus. In these scaffolds, MFCs and mesenchymal stem cells infiltrate and deposit ordered matrix, generating an engineered tissue with physiologically relevant mechanical properties.[42–45]

In this work, we evaluated cellular and architectural changes in the meniscus as a function of age. Further, we used an *in vitro* integration model to assess the healing capacity of fetal, juvenile, and adult bovine meniscal defects. To expedite integration, we evaluated the effect of the exogenous addition of the chondrogenic growth factor transforming growth factor-beta (TGF-β)[16,46–48] in explants of different ages. Finally, we established a test bed for the *in vitro* optimization of a tissue-engineered meniscus construct by modifying the meniscus integration model to include an electrospun scaffold.

## Materials and Methods

### *Age-dependent characteristics of bovine MFCs*

Menisci from fetal (mid-gestation), juvenile (0–3 months), and adult (skeletally mature) cows were sterilely dissected with care taken to remove adherent synovium from the meniscal rim. A 5 mm radial slice was taken, minced into 3 $mm^3$ pieces, and plated onto tissue culture plastic (Corning, Sigma-Aldrich, St. Louis, MO) in basal media (Dulbecco's modified Eagle's medium with 10% fetal bovine serum and 1% penicillin/streptomyosin/fungizone). MFCs emerged onto the plate and divided.

After one passage, cells from each age were used to assess proliferation and migration capacity.[49] Briefly, for proliferation, 20,000 cells were expanded in basal media in individual tissue culture-treated six-well plates. Each day, in triplicate, the cells were dislodged using a cell scraper and DNA content analyzed using the PicoGreen assay (Invitrogen, Carlsbad, CA). The experiment was performed with three different donors, with one donor data set shown. To assess migration capacity, a defined wound was created in a confluent monolayer with a 0.1–10 μL pipette. The wells were washed to remove cell debris and filled with basal media. Initial light microscope images were captured of the wound regions and the locations were marked on the plate. Over 15 h, the same regions were photographed. Extent of infiltration was determined in duplicate using ImageJ (Wayne Rasband, NIH) by measuring the change in area of the gap in a given sample over time.

### *Histological and mechanical alterations in bovine meniscus as a function of age*

Axial and radial samples of meniscus (thickness = 5 mm) from each age were fixed in paraformaldahyde, embedded with paraffin, and sectioned. Radial sections were stained with a mixture of Alcian Blue for PGs and Picrosirius Red for collagens. Axial sections were stained with hematoxylin and eosin to identify nuclei.

Unconfined compression testing was performed on native tissue as in Ref.[50]. A 4 mm circular explant was taken from the central region of the meniscus in the vertical axis using a dermal punch. The top and bottom of the explant was removed on a freezing stage sledge microtome to produce cylindrical samples with parallel surfaces (3 mm height, $n = 6$/age). Height was measured using a digital caliper before mechanical testing. Next, a creep test was performed in a phosphate-buffered saline bath with a 0.05 N load applied for 5 min, with displacement noted at equilibrium. Finally, a stress relaxation test was performed by applying three stepwise compressive deformations of 3% of the postcreep thickness (at 0.05%/s) followed by 800 s of relaxation to equilibrium. The compressive equilibrium modulus was calculated from the equilibrium stress and strain values.

### *Biochemical analysis of bovine meniscus as a function of age*

To assess biochemical content of meniscus by age, radial sections from different menisci ($n = 6$/age) were divided into three regions (inner, middle, and outer) and weighed. After lyophilization, samples were reweighed to determine water content and digested in a buffer containing 2% papain at 60°C. The resulting digestate was used to assess DNA content (PicoGreen Assay; Invitrogen), glycosaminoglycan (GAG) content using the 1,9 dimethylmethylene blue (DMMB) Assay,[51] and collagen content using the orthohydroxyproline (OHP) assay with a conversion factor of 7.14.[52] Results are reported normalized to sample dry weight.

### *Integration potential of bovine meniscus as a function of age*

Fetal, juvenile, and adult menisci were isolated as above. Using a sterile 8-mm-diameter dermal punch, circular ex-

plants were excised from the tissue in the vertical axis. The top and bottom portions of the explant were removed with a scalpel to create parallel surfaces, creating a 3-mm-tall cylinder. A 4-mm-diameter dermal punch was used to create an inner core, with care taken to ensure that the cut was complete and that minimal rotation occurred. Explants were cultured for 8 weeks in basal media supplemented with 50 μg/mL ascorbate-2-phosphate. At bi-weekly intervals, two constructs were processed for histology as above. Picrosirius Red-stained sections were viewed under polarized light to assess collagen bundle organization at the injury site. At bi-weekly intervals, 7–12 samples were tested for mechanical integration using a custom testing device (Fig. 4B).[53–55] Briefly, an Instron 5848 was outfitted with a 3.5-mm-diameter indenter in series with a 50 N load cell. This indenter was placed above a plate with a 5-mm-diameter hole. The meniscus sample was placed onto the plate, and the indenter progressed through the defect site at a rate of 0.0833 mm/s.[15] Integration strength was determined by normalizing the maximum force by the contact area between the core and annulus. This study was conducted in duplicate, with one representative data set presented.

### *Integration potential in the presence of TGF-β*

To assess the influence of the proliferative and pro-chondrogenic growth factor TGF-β3 on the long-term culture and integration potential of meniscus, juvenile and adult meniscus explants were constructed as described above and cultured in either basal media supplemented with 50 μg/mL ascorbate-2-phosphate or basal media supplemented with 50 μg/mL ascorbate-2-phosphate and 10 ng/mL TGF-β3 for 8 weeks. Every 4 weeks, 2 samples were fixed for histology and 7–18 samples were tested for integration strength as above. After testing, both portions of the explant were digested for biochemical analysis. Likewise, histological analysis was conducted on fresh samples from each condition and time point.

### *Integration potential of a fibrous scaffold*

To assess the migratory potential of meniscus cells out of native tissue and through a three-dimensional engineered structure, 8-mm-diameter explants of juvenile and adult menisci were generated. As before, a 4-mm-diameter internal core was created, but this time the core was removed and replaced with an electrospun aligned poly(ε-caprolactone) multi-lamellar column. The column was created by spot-welding multiple 4 mm discs of 1-mm-thick scaffold at the center point and press-fitting the column into the meniscus annulus. At 4 and 8 weeks, samples were fixed in paraformaldehyde and frozen in optimal cutting temperature (OCT) sectioning medium. Sections from the top, middle, and bottom of each explant were stained with 4′,6-diamidino-2-phenylindole (DAPI, Prolong Gold; Invitrogen) to identify cell nuclei. A custom MATLAB program based on Ref.[56] was used to quantify cell infiltration by dividing the scaffold into four concentric zones with radii equating 25%, 50%, 75%, or 100% of the total scaffold radius. Images were converted to a binary image (Fig. 8B), and for each zone, the cell density (cells/pixel) was normalized by the native tissue density in three 100×100 pixel squares of native tissue, and multiplied by the average native tissue cell count to allow for comparisons across sections and between juvenile and adult tissue.

### *Statistical analysis*

All statistical analysis was performed using SYSTAT (Chicago, IL) with Bonferonni's *post-hoc* tests and $p < 0.05$. One-way analysis of variance (ANOVA) was used to compare the compressive modulus of meniscus by age. For biochemical analysis, one-way ANOVA for age-related differences and two-way ANOVA for age and region differences were employed. Integration strength was compared with a two-way ANOVA with age and culture duration as independent variables. For the TGF-β integration studies, three-way ANOVA was utilized. Finally, significant

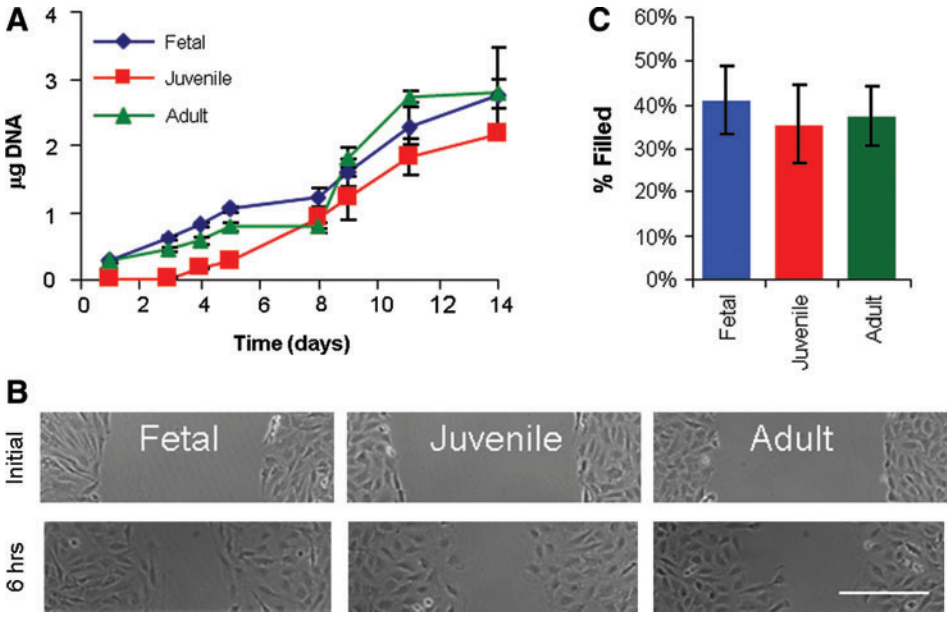


**FIG. 1.** Proliferation and migration of fetal, juvenile, and adult bovine MFCs are comparable. **(A)** MFCs proliferate over 14 days at similar rates ($n = 3$/age). **(B)** Cell migration into a gap was tracked over 15 h. Example images of the initial gap and appearance after 6 h for MFCs from each age (scale bar = 200 μm). **(C)** Image analysis of **(B)** demonstrates similar cell migration rates between MFC ages ($n = 3$/age). MFCs, meniscus fibrochondrocytes. Color images available online at www.liebertonline.com/ten.

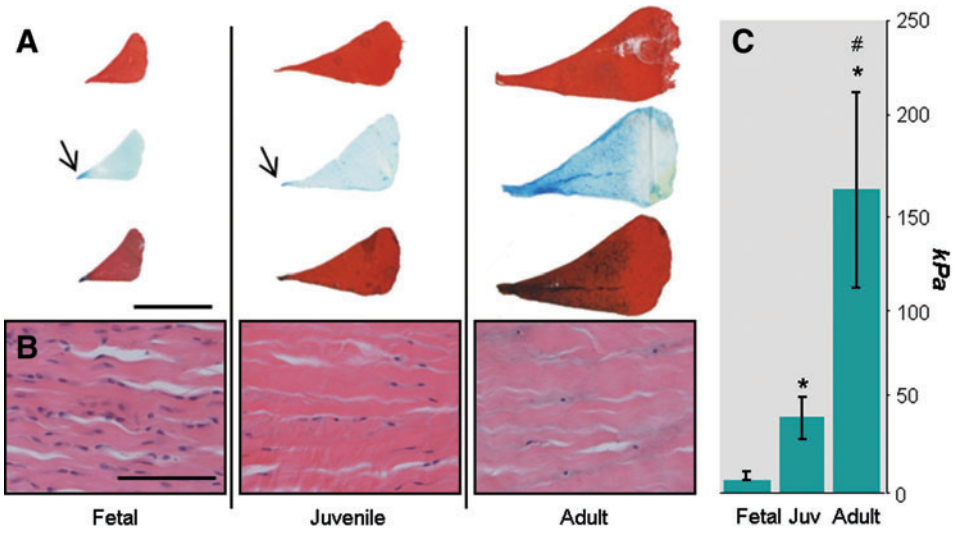


**FIG. 2.** Histological and mechanical properties of bovine meniscus are modulated by age. **(A)** Radial sections from the central region of the meniscus stained with Picrosirius Red (top), Alcian Blue (middle), and a combination of the two (bottom) reveals changes in tissue size and proteoglycan distribution (blue) with age (scale bar = 10 mm). Arrows indicate concentration of Alcian Blue staining at the inner tip. **(B)** Transverse sections of meniscus stained with H&E reveal decreasing cell density with age (scale bar = 100 μm). **(C)** Compressive equilibrium modulus increases significantly with each increase in age ($n = 6$). *Difference from fetal with $p < 0.0001$. #Difference from juvenile with $p < 0.0001$. H&E, hematoxylin and eosin. Color images available online at www.liebertonline.com/ten.

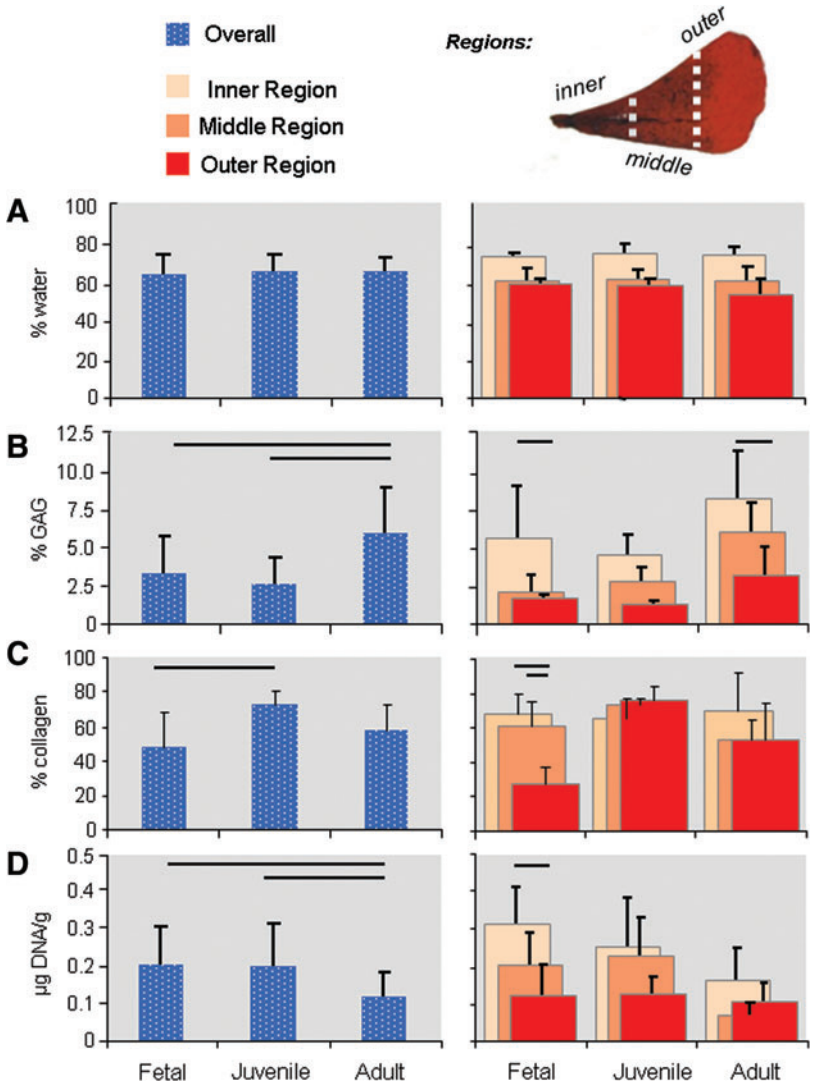


**FIG. 3.** Biochemical content and distribution of the bovine meniscus is modulated by age. **(A)** Water content as a function of tissue wet weight. **(B)** GAG and **(C)** collagen content as a percent of dry weight. **(D)** DNA content per dry weight. $n = 6$/region/age. Bar indicates significant differences between groups with $p < 0.05$. GAG, glycosaminoglycan. Color images available online at www.liebertonline.com/ten.

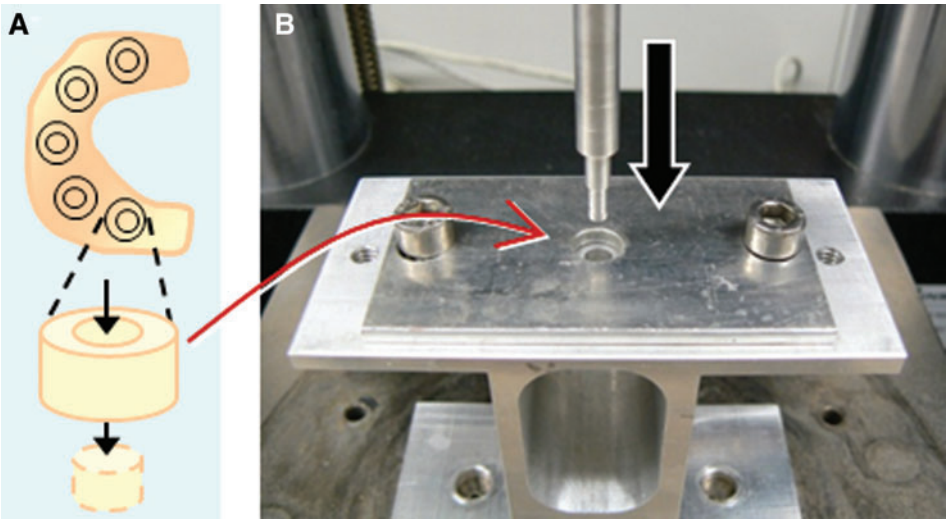


**FIG. 4.** Experimental design for meniscus integration studies. **(A)** Cylindrical explants (8 mm) were removed sterilely from fetal, juvenile, and adult meniscus. After flattening surfaces, central cores were punched with a 4 mm dermal punch, removed, and replaced into the original position to simulate a full-thickness meniscus injury. **(B)** A custom mechanical testing device was used to measure the force required to extrude the inner core from the meniscus explant. Color images available online at www.liebertonline.com/ten.

differences in cellular distribution in the scaffolds were evaluated via two-way ANOVA.

## Results

### *Age-dependent characteristics of bovine MFCs*

The proliferation and migration capacity of MFCs isolated from fetal, juvenile, and adult bovine menisci were compared. Across all ages, proliferation rates were consistent over 14 days (Fig. 1A). Further, no difference in migration into an artificial wound was observed (Fig. 1B, C).

### *Histological and mechanical alterations in bovine meniscus as a function of age*

Histological analysis revealed dramatic structural changes in meniscus through development. The meniscus increased in size with age, particularly in the medial-lateral direction (Fig. 2A). In fetal and juvenile menisci, PG was concentrated in the inner-most tip of the tissue (Fig. 2, arrows) with little staining throughout the rest of the tissue. Adult menisci stained darkly for PG throughout the tissue. Hematoxylin and eosin staining showed a progressive decline of cell density with advancing age (Fig. 2B). Morphologically, the thickness of collagen bundles increased through development (Fig. 2B). The age-dependent changes observed histologically were concurrent with mechanical changes, with ~5-fold improvements in the equilibrium compressive modulus between each age tested.

### *Biochemical analysis of bovine meniscus as a function of age*

Samples from the inner, middle, and outer region of the meniscus were digested to elucidate biochemical changes with development. Overall, water content did not change with age (Fig. 3A). In contrast, fetal and juvenile meniscus contained significantly lower GAG content than adult meniscus (Fig. 3B). Fetal meniscus had significantly more GAG in the inner region than the outer region, although by the juvenile stage, difference by region was less pronounced. Adult meniscus also had significantly more GAG in the inner region compared to outer region. Further, the relative proportion of GAG in the middle region of the meniscus increased in adult specimens. The overall collagen content increased significantly from fetal to juvenile meniscus, but remained the same between juvenile and adult meniscus (Fig. 3C). The outer region of the fetal meniscus had significantly less collagen than the other regions, perhaps due a blending of the synovial rim with the tissue early in development. By juvenile and adult stages, there were no differences in collagen content between regions. Finally, adult meniscus had significantly less DNA content than fetal or juvenile meniscus (Fig. 3D). Cell density in the fetal meniscus showed the largest distribution with location, with significantly more DNA found in the inner region compared to the outer region. With maturation, the distribution of DNA equilibrated.

### *Integration potential of bovine meniscus as a function of age*

For each age, circular meniscus explants with concentric full-thickness defects were formed and cultured for up to 8 weeks (Fig. 4). Histology revealed continual improvements in matrix connectivity at the injury site in fetal and juvenile tissue. In contrast, no integration was seen in adult samples through 8 weeks (Fig. 5A). Viewing the best integrated samples (juvenile, week 8) under polarized light microscopy showed that while new collagen was deposited at the injury site, this new matrix was not aligned or contiguous with the surrounding collagen bundles (Fig. 5A, green arrows).

The mechanical testing results mirrored these histological observations. Fetal explants improved in integration strength over 8 weeks, with significant improvements at 6 and 8 weeks compared to week 0 ($p < 0.05$). Integration strength of juvenile defects increased at a slower pace, with significant improvements by week 8. The integration strength of adult samples did not change (Fig. 5B).

### *Integration potential in the presence of TGF-β*

To further improve repair, juvenile and adult meniscus explants were cultured with and without 10 ng/mL TGF-β3, a concentration previously shown to promote matrix deposition in MFCs.[42,43,57] Addition of TGF-β3 increased matrix

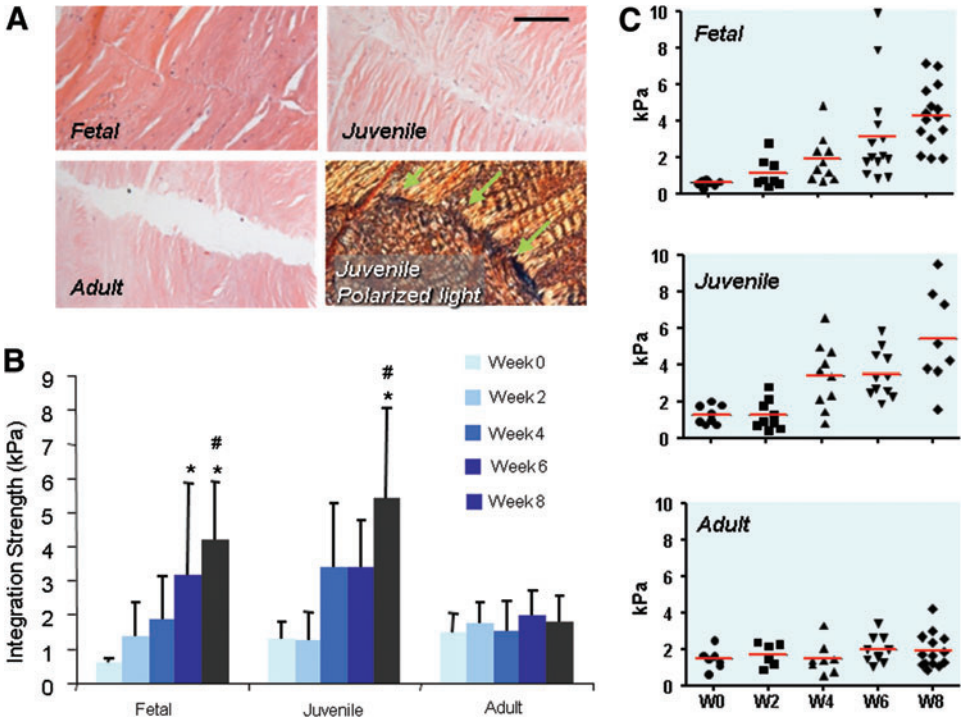


**FIG. 5.** Age-dependent integration of bovine meniscus. **(A)** H&E staining of the injury site at week 8 shows signs of repair in fetal and juvenile tissue, but not in adult tissue (scale bar 200 μm). Lower right: Picrosirius Red staining of 8 week juvenile sample viewed under polarized light reveals lack of collagen continuity at the injury site (arrows). **(B)** Integration strength as a function of meniscus age with time in culture. Integration strength increases significantly for fetal and juvenile samples, but not for adult samples. **(C)** Mechanical testing results demonstrating variability in response between specimens. $n = 7$–12. *Significant difference ($p < 0.05$) from week 0. #Significant difference ($p < 0.05$) compared to adult week 8 (w8). Color images available online at www.liebertonline.com/ten.

deposition at the injury site in both juvenile and adult samples (Fig. 6A, B). Picrosirius Red staining revealed no major changes in collagen levels (Fig. 6C, D). However, Alcian Blue staining of PG was significantly greater in explants cultured in the presence of TGF-β (Fig. 6C, D). Even in the presence of TGF-β, some sections of the injuries appeared to be disconnected. It is unclear if this is an artifact from histology, or if these regions contribute to the variability seen in the mechanical testing results.

The integration strength of both juvenile and adult meniscus explants improved sevenfold by 8 weeks with the addition of TGF-β, reaching 97 and 57 kPa, respectively (Fig. 7A). Continued exposure to TGF-β for 8 weeks resulted in significant improvements from week 4 and compared to basal media alone. Further, juvenile meniscus integration strength was significantly higher than adult meniscus integration strength by 8 weeks. Biochemical analysis of the tissue after mechanical testing showed that GAG content decreased over time when cultured in basal media; GAG content was far below native tissue, particularly for adult tissue (Fig. 7B, dotted line). Conversely, addition of TGF-β maintained native levels of GAG in the explants. Due to the lower initial concentration of GAG in juvenile tissue, the quantity of GAG in the explants cultured in TGF-β did not increase significantly. However, adult explants had almost twofold more GAG by 8 weeks than explants cultured in basal media alone. Further, adult explants contained significantly more GAG than juvenile explants cultured in the same conditions, similar to the trend in native tissue. These quantitative measures of GAG content correlate well with histological staining (Fig. 6C, D). Even in the presence of TGF-β, DNA content in explants fell below native levels (Fig. 7C). Similar to native tissue, adult explants had significantly less DNA than juvenile explants. As shown via histology (Fig. 6C, D) and by quantification (Fig. 7D), collagen content did not change between groups or over time.

### *Integration potential of a fibrous scaffold*

To evaluate integration between engineered materials and native meniscus, poly(epsilon-caprolactone) (PCL) was electrospun in 1-mm-thick aligned sheets and cut into 4-mm-diameter discs. These discs were subsequently spot-welded to one another to form columns (Fig. 8A) that were situated inside juvenile and adult meniscus explants and cultured for 1, 3, and 6 weeks. Despite the significantly higher native tissue density of juvenile tissue, similar numbers of cells populated the scaffolds by 6 weeks (Fig. 8D). The infiltration was progressive and occurred more quickly for juvenile compared to adult explants, with significantly fewer cells in the adult scaffold than in the juvenile scaffold at 3 weeks. No differences were detected in cell density between the bottom, middle, and top of the column, suggesting uniform infiltration from the exterior circular wall of the native tissue. In terms of location (Fig. 8E), most cells were located in the outer-most zone (1) adjacent to the native tissue and the fewest cells reached the center region (4). Although juvenile tissue had almost fivefold higher average cell density, the zonal cell density was similar between juvenile and adult tissue.

## Discussion

While injury to the meniscus is the leading cause for orthopedic surgery,[58] therapeutic options are limited and still often lead to the development of osteoarthritis.[8,9,59,60] In this work, we characterized cell behavior, tissue architecture, and integration potential of the meniscus as a function of age given prior evidence of healing in immature meniscus[21] and in related juvenile[25,27,61] and fetal[28–30] fibrocartilaginous tissues. Further, we established a test bed for the optimization of a tissue-engineered scaffold.

When bovine MFCs were extracted from fetal, juvenile, and adult menisci, age did not alter their behavior in monolayer culture. Consistent with these findings, we have

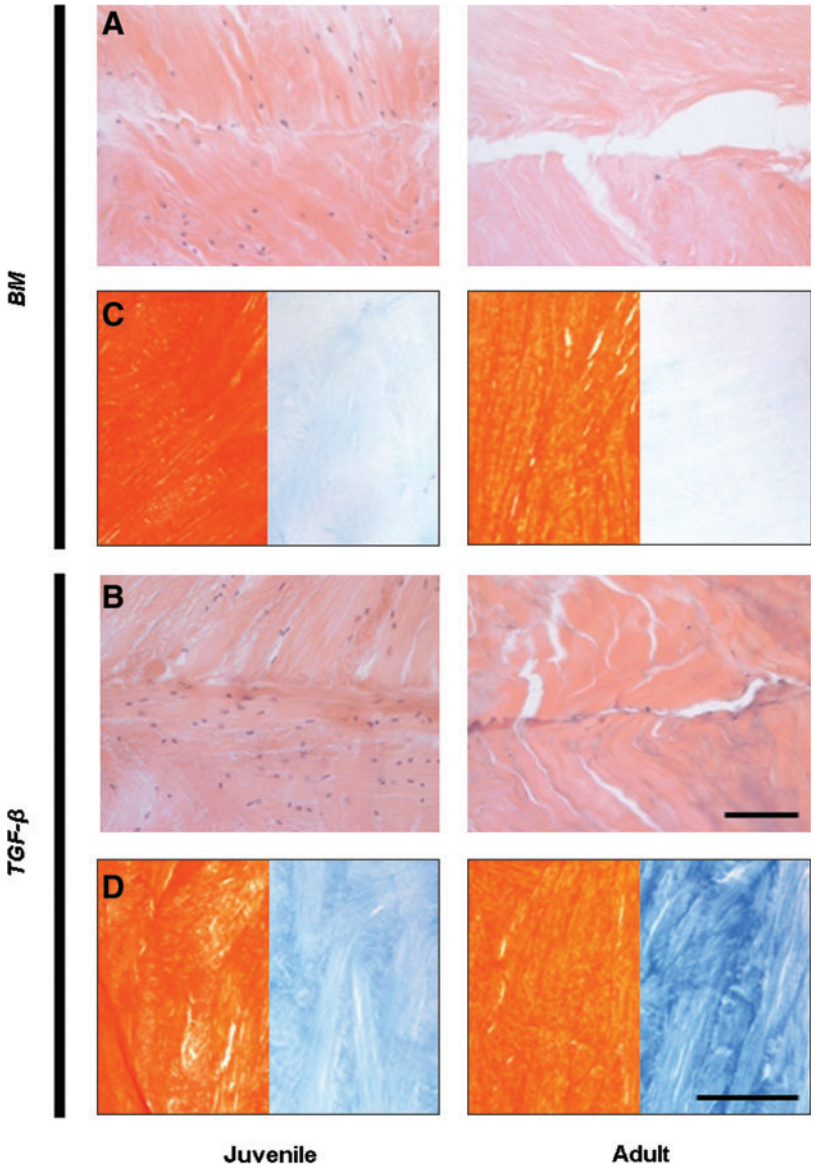


**FIG. 6.** Histological analysis of integration in the presence or absence of TGF-β after 8 weeks of *in vitro* culture. **(A)** H&E staining reveals that while the juvenile sample appears to have healed across the injury site, no obvious integration is visible in the adult sample. **(B)** The addition of TGF-β improved cell and matrix formation at the injury site for both juvenile and adult samples. Scale bar = 100 μm. (**C** and **D**) Picrosirius Red staining (left) is consistent across samples at 8 weeks. However, Alcian Blue staining (right) of proteoglycan reveals significant depletion with culture in basal media compared to TGF-β in both juvenile and adult specimens. Scale bar = 500 μm. TGF-β, transforming growth factor-beta. Color images available online at www.liebertonline.com/ten.

previously shown that in the ovine model, fetal and adult MFCs proliferate and migrate at the same rate.[49] In contrast, related cell types, such as anterior cruciate ligament (ACL) fibroblasts and dermal fibroblasts, lose proliferation and migration capacity with age.[62,63]

At the tissue level, meniscus size and architecture changed with development. In fetal and juvenile meniscus, cell density was higher, collagen bundles were smaller, and PG was concentrated in the inner tip of the tissue. In adult tissue, PG filled the majority of the tissue, DNA content decreased and became more uniform, and collagen fiber bundles increased in thickness. The majority of these changes occurred upon skeletal maturity (i.e., from juvenile to adult), rather than progressively through early development. Our findings are corroborated by other work in the field; similar changes in PG deposition and collagen development were noted by Melrose and coworkers in an ovine model.[64] Chen and coworkers found that PG content increased with age, DNA content decreased, and collagen content increased in the inner region of the meniscus from the fetus to adult in bovine meniscus tissue.[65] Further, Clark and Ogden observed progressive changes in collagen bundle thickness and cell density in human fetal and juvenile meniscus.[23]

These architectural changes corresponded to functional improvements in the compressive moduli concomitant with the increasing load-bearing demands in the joint. The equilibrium compressive modulus (163 kPa) for adult bovine tissue was higher than that found in human tissue (25–37 kPa)[66] and is similar to that reported for porcine meniscus (170–350 kPa).[67] Chen and coworkers found similar compressive properties for fetal tissue, but a significantly lower compressive modulus (30 kPa) in the adult.[65] The differences in adult compressive modulus may be attributed the location from which the samples were obtained; Chen and coworkers tested explants from the inner region perpendicular to the femoral surface of the meniscus, whereas we tested explants taken from the middle region of the meniscus perpendicular to the tibial surface.

The similarities between fetal and juvenile meniscus were also apparent in the explant integration studies (Fig. 5). Both

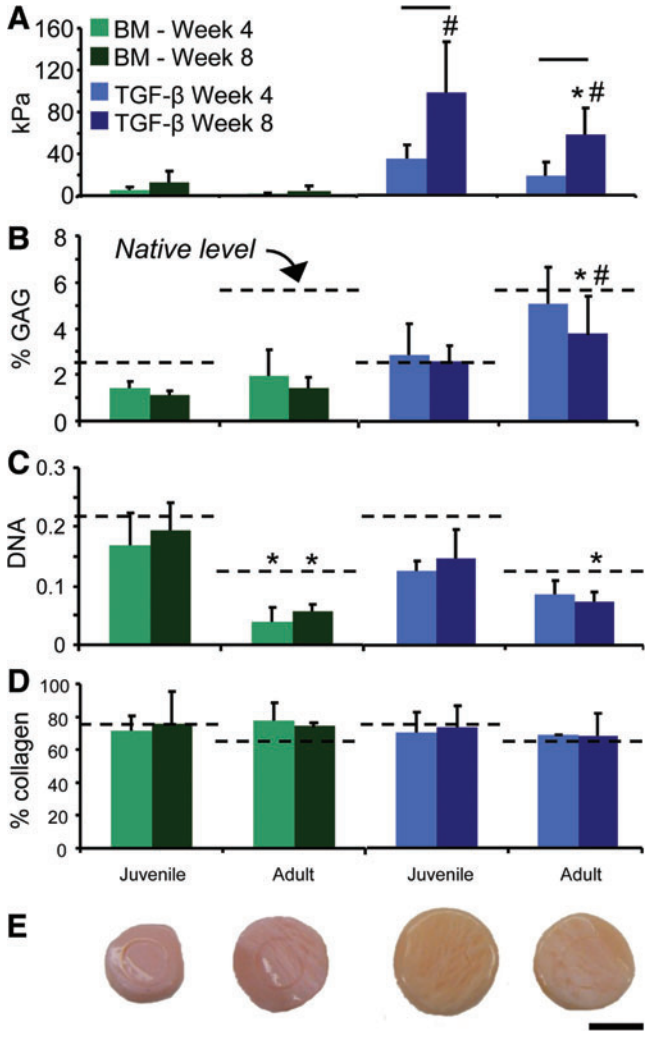


**FIG. 7.** TGF-β improves integration strength and preserves explant GAG content after 8 weeks of *in vitro* culture. **(A)** Integration strength of juvenile and adult defects improved in the presence of TGF-β ($n = 7$–18) compared to basal media (BM). **(B)** GAG content was depleted in the absence and maintained in the presence of TGF-β ($n = 6$) compared to native tissue (dashed line). **(C)** DNA content decreased in all conditions. **(D)** Collagen content remained consistent across all conditions. **(E)** Appearance of explants after 8 weeks in culture. Scale bar = 5 mm. *Significant difference ($p < 0.05$) between ages within a given media condition and time point. #Significant difference ($p < 0.05$) between media conditions at a given time point. Bar indicates significant difference ($p < 0.05$) between time points within a given media condition and age. Color images available online at www.liebertonline.com/ten.

fetal and juvenile defects increased in integration strength by 8 weeks, although fetal explants improved at a faster rate. In contrast, and as is seen clinically in the avascular region, adult meniscus showed very little healing.[24] Interestingly, DiMicco and coworkers demonstrated that fetal cartilage does not heal as well as juvenile cartilage *in vitro*,[25] suggesting that although the meniscus and cartilage are related, the healing mechanisms at young ages may be different between these tissues.

TGF-β is known to promote the deposition of a fibrocartilaginous matrix and the maintenance of phenotype in MFCs.[46,48,57,68] Exposure of meniscus explants to 10 ng/mL of TGF-β3 for 8 weeks improved integration strength significantly, to seven times the level of basal media alone (~100 kPa) (Fig. 7A). Adult meniscus healed significantly better with the addition of TGF-β, though juvenile meniscus improved more than adult meniscus. Previously, McNulty and coworkers[16] utilized a similar *in vitro* model to evaluate the dose responsivity (0.1–10 ng/mL) to TGF-β1 and found a small but significant improvement at the 1 ng/mL dose (12 kPa) after 14 days. TGF-β played a number of roles that may contribute to the observed improvement in healing. First, it maintained the PG content of explants over 8 weeks (Fig. 6D) compared to basal media alone. Imler and coworkers previously showed that the addition of TGF-β to meniscus explants increases sulfate incorporation.[69] Interestingly, GAG content in the explants cultured in basal media were comparable between ages at 8 weeks, despite significantly higher initial GAG content in adult explants. Further, the loss was progressive, with adult meniscus segments failing to maintain native levels of GAG at 8 weeks, even in the presence of TGF-β. While leaching or degradation of PG may an artifact of *in vitro* culture, the power of TGF-β to promote PG deposition to native levels may contribute to the regeneration of meniscus-like ECM at the site of injury. In keeping with these findings, Wilson and coworkers recently showed that PG levels in meniscus explants decreased over time in culture, and that the addition of TGF-β increased PG levels in the tissue.[70] Further, exposure to a broad-spectrum metalloproteinase inhibitor significantly reduced PG loss over time.[71]

While no quantitative improvements in collagen content were found, histology of the injury site suggested that more collagen was deposited with exposure to TGF-β3. Further, we have previously found that immature ovine MFCs deposit more collagen than adult MFCs in the presence of TGF-β when cultured in pellets.[49] DNA levels decreased in all explants to 50%–75% of native levels. Cell death may occur early in culture due to the lack of physiologically relevant stimuli, impaired nutrition, or from the process of defect formation itself. These observations are another artifact of *in vitro* culture, but must be considered when endeavoring to develop *in vitro* protocols for meniscus repair.

Translating this work to tissue engineering approaches, we incorporated electrospun scaffolds into this *in vitro* defect model. These scaffolds support MFC proliferation and matrix deposition over time, and increase in mechanical properties *in vitro* when seeded with both bovine and human cells.[42,43] Despite marked differences in tissue structure and composition, implanting these scaffolds into juvenile and adult meniscus defects yielded surprisingly similar results. While the infiltration of the scaffold was somewhat faster in younger tissue, after 8 weeks the average cell number within the scaffold was comparable, despite significantly more cells adjacent to the scaffold in the juvenile tissue (Fig. 8C). Similar to the behavior in monolayer culture (Fig. 1), migration into and subsequent proliferation in the scaffold were comparable by age, although cells were slightly better distributed in the scaffold containing adult MFCs. The finding suggests that while heightened cell density at the injury site may promote integration, it may not improve colonization and maturation of a tissue-engineered scaffold.

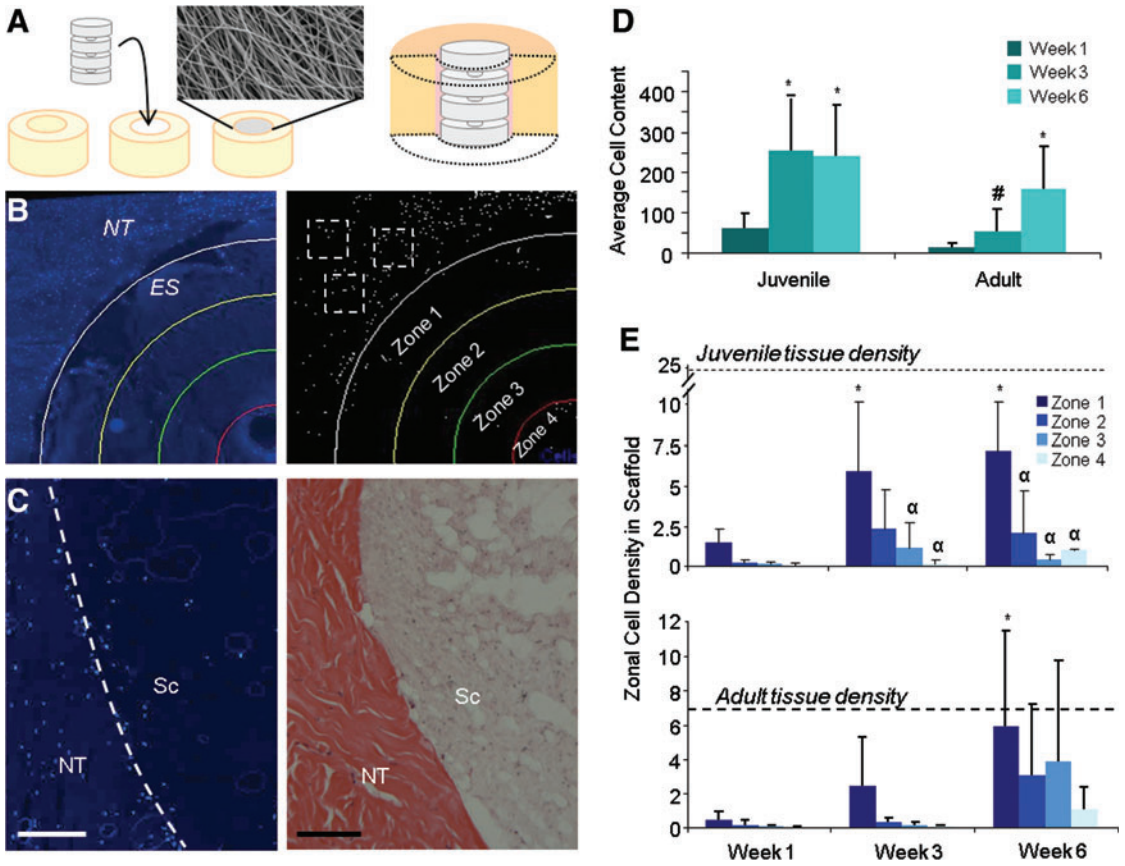


**FIG. 8.** Comparable cellular infiltration in juvenile and adult meniscus defects repaired with nanofibrous scaffolds. **(A)** Cylindrical meniscus explants were formed, and a central core was removed and replaced with a column of electrospun poly(ε-caprolactone) nanofibrous scaffold disks welded together at the central point. **(B)** Cell infiltration into the cylindrical constructs was quantified using a custom MATLAB program. Left: Excerpt of image of the DAPI-stained composite with the electrospun scaffold (ES) region divided to quantify cell infiltration from the surrounding native tissue (NT). Right: Cells were counted in binary images by zone. Native tissue density was determined using three regions from each image (boxes) and averaged across all samples. **(C)** Left: DAPI staining of integration zone between native tissue (NT) and scaffold (Sc). Scale bar = 100 μm. Right: H&E staining of integration zone. **(D)** Significant cell infiltration was seen after 3 and 6 weeks for juvenile and adult meniscus defects, respectively. By week 6, scaffold infiltration was comparable between ages ($n = 9$/age/timepoint). **(E)** Juvenile cells populated the scaffold more rapidly than adult scaffolds, although the overall densities were comparable despite a significantly higher starting density in juvenile native tissue. *Significant difference ($p < 0.05$) from week 1. #Significant difference ($p < 0.05$) between ages. αSignificant difference ($p < 0.05$) from zone 1. Color images available online at www.liebertonline.com/ten.

Identifying distinguishing features of immature, healing meniscus may provide new directions for advancing repair in the adult and tailoring engineered scaffolds to advance regeneration. Specifically, we noted a simultaneous loss of healing capacity with increased GAG concentration, decreased DNA content, and thickening of collagen bundles. Modifying the structure of mature meniscus to more closely resemble immature meniscus might promote healing. While depletion of GAG appears to be an interesting therapeutic option, GAG levels depleted quickly and significantly during *in vitro* culture (Fig. 7B). The addition of TGF-β maintained near-native levels of GAG content and improved integration, suggesting that GAG depletion may in fact hinder meniscus repair rather than promote it. Similarly, the quantity of DNA dropped in all meniscus samples with *in vitro* culture. Although the addition of TGF-β did not change the overall DNA content of the explants, it is possible that small local increases in cell density occurred at the injury sites and bolstered healing. Drug delivery of TGF-β directly to the site of injury at high doses through a system such as the one described in Ref.[72] may support local proliferation at the injury site. While bulk collagen content did not change progressively with tissue maturation, the bundle size increased. No major changes in collagen content were observed during *in vitro* culture, even in the presence of TGF-β. However, it is possible that small increases in collagen content occurred at the injury site. It may be speculated, but remains to be seen, that decreasing collagen bundle size through partial degradation might enhance tissue healing.

Although this work contributes to our knowledge of the meniscus and develops an *in vitro* test bed for the integration of tissue-engineered materials with native tissue, some limitations do exist. First, we only analyzed overall collagen and GAG content, rather than considering individual components that may vary with development. While culturing explants for 8 weeks allowed us to evaluate the reparative capacity of the tissue, this *in vitro* environment is artificial, with no mechanical loading and loss of PG and cellularity.

Also, we have only explored one concentration of TGF-β, whereas other concentrations may have yielded different or improved integration levels. Given that matrix formation, integration, and healing will depend on both anabolism and catabolism, it will be important to explore expression and activity of matrix metalloproteinases and other matrix modulating enzymes in future studies. Further, although cells infiltrated into the electrospun scaffold, minimal matrix was deposited (data not shown) due to a lack of additional matrix stimulating anabolic factors in the media that might also influence migration, proliferation, and tissue formation. Finally, while this *in vitro* test bed provides a facile system for understanding meniscus healing and optimizing scaffold design, it may not translate directly to the *in vivo* articular environment. Future work will include extending these experiments to clinically relevant large animal models, as well as models involving human meniscus tissue to ensure that our *in vitro* findings translate to clinical applicability.

## Conclusions

Overall, this study explored developmental changes in bovine meniscus in terms of tissue architecture, ECM distribution and content, and cell behavior. These changes corresponded to differential integration capacity of the meniscus with aging. Integration was significantly enhanced by the addition of the exogenous growth factor TGF-β, which also served to better maintain overall explant properties. Moreover, we showed that *in vitro* models such as this can be test beds for optimizing engineered meniscus scaffolds before *in vivo* implantation. To glean instructive information regarding healing with age, we noted that while cell behavior did not change with age, the overall balance between cells, GAG, and collagen content in the meniscus changed markedly. While loss of GAG content to immature meniscus levels (a natural consequence of the *in vitro* culture system) did not promote adult meniscus healing, increasing local DNA content or decreasing collagen fiber diameter remain viable targets for enhancing repair. As such, this work identifies new targets for meniscus tissue engineering as well as a method for testing and optimizing potential clinical solutions for meniscus repair.

## Acknowledgments

This work was supported by the National Institutes of Health (R01 AR056624 and T32 AR007132) and the Penn Center for Musculoskeletal Disorders.

## Disclosure Statement

No competing financial interests exist.

## References

1. Sweigart, M.A., and Athanasiou, K.A. Toward tissue engineering of the knee meniscus. Tissue Eng **7,** 111, 2001.
2. Fithian, D.C., Kelly, M.A., and Mow, V.C. Material properties and structure-function relationships in the menisci. Clin Orthop Relat Res **252,** 19, 1990.
3. Herwig, J., Egner, E., and Buddecke, E. Chemical changes of human knee joint menisci in various stages of degeneration. Ann Rheum Dis **43,** 635, 1984.
4. Yoo, J.C., Ahn, J.H., Lee, S.H., Lee, S.H., and Kim, J.H. Suturing complete radial tears of the lateral meniscus. Arthroscopy **23,** 1249, 2007.
5. Farng, E., and Sherman, O. Meniscal repair devices: a clinical and biomechanical literature review. Arthroscopy **20,** 273, 2004.
6. Cicuttini, F.M., Forbes, A., Yuanyuan, W., Rush, G., and Stuckey, S.L. Rate of knee cartilage loss after partial meniscectomy. J Rheumatol **29,** 1954, 2002.
7. Verdonk, R., and Kohn, D. Harvest and conservation of meniscal allografts. Scand J Med Sci Sports **9,** 158, 1999.
8. Shapiro, F., and Glimcher, M.J. Induction of osteoarthritus in the rabbit knee joint: histological changes following meniscectomy and meniscal lesions. Clin Orthop **147,** 287, 1980.
9. Lufti, A.M. Morphological changes in the articular cartilage after meniscectomy. J Bone Joint Surg **57B,** 525, 1975.
10. Cox, J.S., Nye, C.E., Schaefer, W.W., and Woodstein, I.J. The degenerative effects of partial and total resection of the medial meniscus in dogs' knees. Clin Orthop Relat Res **109,** 178, 1975.
11. King, D. The healing of semilunar cartilages. 1936. Clin Orthop Relat Res **252,** 4, 1990.
12. Webber, R.J., York, J.L., Vanderschilden, J.L., and Hough, A.J.J. An organ culture model for assaying wound repair of the fibrocartilaginous knee joint meniscus. Am J Sports Med **17,** 393, 1989.
13. Kobayashi, K., Fujimoto, E., Deie, M., Sumen, Y., Ikuta, Y., and Ochi, M. Regional differences in the healing potential of the meniscus-an organ culture model to eliminate the influence of microvasculature and the synovium. Knee **11,** 271, 2004.
14. van de Breevaart Bravenboer, J., In der Maur, C.D., Bos, P.K., Feenstra, L., Verhaar, J.A., Weinans, H., *et al.* Improved cartilage integration and interfacial strength after enzymatic treatment in a cartilage transplantation model. Arthritis Res Ther **6,** R469, 2004.
15. Hennerbichler, A., Moutos, F.T., Hennerbichler, D., Weinberg, J.B., and Guilak, F. Repair response of the inner and outer regions of the porcine meniscus *in vitro*. Am J Sports Med **35,** 754, 2007.
16. McNulty, A.L., and Guilak, F. Integrative repair of the meniscus: lessons from *in vitro* studies. Biorheology **45,** 487, 2008.
17. McNulty, A.L., Weinberg, J.B., and Guilak, F. Inhibition of matrix metalloproteinases enhances *in vitro* repair of the meniscus. Clin Orthop Relat Res **467,** 1557, 2009.
18. McNulty, A.L., Moutos, F.T., Weinberg, J.B., and Guilak, F. Enhanced integrative repair of the porcine meniscus *in vitro* by inhibition of interleukin-1 or tumor necrosis factor alpha. Arthritis Rheum **6,** 3033, 2007.
19. Wilusz, R.E., Weinberg, J.B., Guilak, F., and McNulty, A.L. Inhibition of integrative repair of the meniscus following acute exposure to interleukin-1 *in vitro*. J Orthop Res **26,** 504, 2008.
20. Hennerbichler, A., Moutos, F.T., Hennerbichler, D., Weinberg, J.B., and Guilak, F. Interleukin-1 and tumor necrosis factor alpha inhibit repair of the porcine meniscus *in vitro*. Osteoarthritis Cartilage **15,** 1053, 2007.
21. Andrish, J.T. Meniscal injuries in children and adolescents: diagnosis and management. J Am Acad Orthop Surg **4,** 231, 1996.
22. Petersen, W., and Tillmann, B. Age-related blood and lymph supply of the knee menisci. A cadaver study. Acta Orthop Scand **66,** 308, 1995.

23. Clark, C.R., and Ogden, J.A. Development of the menisci of the human knee joint. Morphological changes and their potential role in childhood meniscal injury. J Bone Joint Surg Am **65,** 538, 1983.
24. Arnoczky, S.P., and Warren, R.F. The microvasculature of the meniscus and its response to injury. An experimental study in the dog. Am J Sports Med **11,** 131, 1983.
25. DiMicco, M.A., Waters, S.N., Akeson, W.H., and Sah, R.L. Integrative articular cartilage repair: dependence on developmental stage and collagen metabolism. Osteoarthritis Cartilage **10,** 218, 2002.
26. Giurea, A., DiMicco, M.A., Akeson, W.H., and Sah, R.L. Development-associated differences in integrative cartilage repair: roles of biosynthesis and matrix. J Orthop Res **20,** 1274, 2002.
27. Provenzano, P.P., Hayashi, K., Kunz, D.N., Markel, M.D., and Vanderby, R.J. Healing of subfailure ligament injury: comparison between immature and mature ligaments in a rat model. J Orthop Res **20,** 975, 2002.
28. al-Qattan, M.M., Posnick, J.C., Lin, K.Y., and Thorner, P. Fetal tendon healing: development of an experimental model. Plast Reconstr Surg **92,** 1155, 1993.
29. Favata, M., Beredjiklian, P.K., Zgonis, M.H., Beason, D.P., Crombleholme, T.M., Jawad, A.F., *et al.* Regenerative properties of fetal sheep tendon are not adversely affected by transplantation into an adult environment. J Orthop Res **24,** 2124, 2006.
30. Namba, R.S., Meuli, M., Sullivan, K.M., Le, A.X., and Adzick, N.S. Spontaneous repair of superficial defects in articular cartilage in a fetal lamb model. J Bone Joint Surg **80,** 4, 1998.
31. Tognana, E., Chen, F., Padera, R.F., Leddy, H.A., Christensen, S.E., Guilak, F., *et al.* Adjacent tissues (cartilage, bone) affect the functional integration of engineered calf cartilage *in vitro*. Osteoarthritis Cartilage **13,** 129, 2005.
32. Obradovic, B., Martin, I., Padera, R.F., Treppo, S., Freed, L.E., and Vunjak-Novakovic, G. Integration of engineered cartilage. J Orthop Res **19,** 1089, 2001.
33. Maher, S.A., Mauck, R.L., Rackwitz, L., and Tuan, R.S. A nanofibrous cell-seeded hydrogel promotes integration in a cartilage gap model. J Tissue Eng Regen Med **4,** 25, 2010.
34. Rodkey, W.G., DeHaven, K.E., Montgomery, W.H., Baker, C.L.J., Beck, C.L.J., Hormel, S.E., *et al.* Comparison of the collagen meniscus implant with partial meniscectomy. A prospective randomized trial. J Bone Joint Surg Am **90,** 1413, 2008.
35. Welch, J.A., Montgomery, R.D., Lenz, S.D., Plouhar, P., and Shelton, W.R. Evaluation of small-intestinal submucosa implants for repair of meniscal defects in dogs. Am J Vet Res **63,** 427, 2002.
36. Tan, Y., Zhang, Y., and Pei, M. Meniscus reconstruction through coculturing meniscus cells with synovium-derived stem cells on small intestine submucosa—a pilot study to engineer meniscus tissue constructs. Tissue Eng Part A **16,** 67, 2010.
37. Tienen, T.G., Heijkants, R.G., de Groot, J.H., Pennings, A.J., Schouten, A.J., Veth, R.P., *et al.* Replacement of the knee meniscus by a porous polymer implant: a study in dogs. Am J Sports Med **34,** 64, 2006.
38. Ramrattan, N.N., Heijkants, R.G., van Tienen, T.G., Schouten, A.J., Veth, R.P., and Buma, P. Assessment of tissue ingrowth rates in polyurethane scaffolds for tissue engineering. Tissue Eng **11,** 1212, 2005.
39. Kon, E., Chiari, C., Marcacci, M., Delcogliano, M., Salter, D.M., Martin, I., *et al.* Tissue engineering for total meniscal substitution: animal study in sheep model. Tissue Eng Part A **14,** 1067, 2008.
40. Aufderheide, A.C., and Athanasiou, K.A. Assessment of a bovine co-culture, scaffold-free method for growing meniscus-shaped constructs. Tissue Eng **13,** 2195, 2007.
41. Ballyns, J.J., Gleghorn, J.P., Niebrzydowski, V., Rawlinson, J.J., Potter, H.G., Maher, S.A., *et al.* Image-guided tissue engineering of anatomically shaped implants via MRI and micro-CT using injection molding. Tissue Eng Part A **14,** 1195, 2008.
42. Baker, B.M., and Mauck, R.L. The effect of nanofiber alignment on the maturation of engineered meniscus constructs. Biomaterials **28,** 1967, 2007.
43. Baker, B.M., Nathan, A.S., Huffman, G.R., and Mauck, R.L. Tissue engineering with meniscus cells derived from surgical debris. Osteoarthritis Cartilage **17,** 336, 2009.
44. Mauck, R.L., Baker, B.M., Nerurkar, N.L., Burdick, J.A., Li, W.J., Tuan, R.S., *et al.* Engineering on the straight and narrow: the mechanics of nanofibrous assemblies for fiber-reinforced tissue regeneration. Tissue Eng Part B Rev **15,** 171, 2009.
45. Baker, B.M., Handorf, A.M., Ionescu, L.C., Li, W.J., and Mauck, R.L. New directions in nanofibrous scaffolds for soft tissue engineering and regeneration. Expert Rev Med Devices **6,** 515, 2009.
46. Collier, S., and Ghosh, P. Effects of transforming growth factor beta on proteoglycan synthesis by cell and explant cultures derived from the knee joint meniscus. Osteoarthritis Cartilage **3,** 127, 1995.
47. Gruber, H.E., Mauerhan, D., Chow, Y., Ingram, J.A., Norton, H.J., Hanley, E.N., *et al.* Three-dimensional culture of human meniscal cells: extracellular matrix and proteoglycan production. BMC Biotechnol **8,** 54, 2008.
48. Tanaka, T., Fujii, K., and Kumagae, Y. Comparison of biochemical characteristics of cultured fibrochondrocytes isolated from the inner and outer regions of human meniscus. Knee Surg Sports Traumatol Arthrosc **7,** 75, 1999.
49. Ionescu, L.C., Gee, A.O., Sennett, B.J., Liechty, K., and Mauck, R.L. Regenerative Potential of Fetal Meniscus Fibrochondrocytes. The 55th Annual Meeting of the Orthopaedic Research Society, Las Vegas, NV, 2009.
50. Mauck, R.L., Yuan, X., and Tuan, R.S. Chondrogenic differentiation and functional maturation of bovine mesenchymal stem cells in long-term agarose culture. Osteoarthritis Cartilage **14,** 179, 2006.
51. Farndale, R.W., Buttle, D., and Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim Biophys Acta **883,** 173, 1986.
52. Stegemann, H., and Stalder, K. Determination of hydroxyproline. Clin Chim Acta **18,** 267, 1967.
53. Ionescu, L.C., Garcia, G.H., Erickson, I.E., Guevara, J.L., Shah, R.P., Sennett, B.J., *et al.* In Vitro Meniscus Integration is Age Dependent. The 56th Annual Meeting of the Orthopaedic Research Society, New Orleans, LA, 2010, p. 927.
54. Ionescu, L.C., Garcia, G.H., Zachry, T.L., Lee, G.C., Sennett, B.J., and Mauck, R.L. *In Vitro* Meniscus Integration Potential is Inversely Correlated with Tissue Maturation State. American Society of Mechanical Engineers—Bioengineering Division, Naples, FL, 2010.
55. Erickson, I.E., Kestle, S., Burdick, J.A., and Mauck, R.L. In Vitro Cartilage Integration of MSC-Seeded Hyaluronic Acid Constructs. The 56th Annual Meeting of the Orthopaedic Research Society, New Orleans, LA, 2010.

56. Baker, B.M., Gee, A.O., Metter, R.B., Nathan, A.S., Marklein, R.A., Burdick, J.A., *et al.* The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers. Biomaterials **29,** 2348, 2008.
57. Mauck, R.L., Martinez-Diaz, G.J., Yuan, X., and Tuan, R.S. Regional multilineage differentiation potential of meniscal fibrochondrocytes: implications for meniscus repair. Anat Rec **290,** 48, 2007.
58. Garrett, W.E., Swiontkowski, M.F., Weinstein, J.N., Callaghan, J., Rosier, R.N., Berry, D.J., *et al.* American board of orthopaedic surgery practice of the orthopaedic surgeon: part-II, certification examination case mix. J Bone Joint Surg Am **88,** 660, 2006.
59. Englund, M., Guermazi, A., and Lohmander, L.S. The meniscus in knee osteoarthritis. Rheum Dis Clin North Am **35,** 579, 2009.
60. Stärke, C., Kopf, S., Petersen, W., and Becker, R. Meniscal repair. Arthroscopy **25,** 1033, 2009.
61. Mastrangelo, A.N., Haus, B.M., Vavken, P., Palmer, M.P., Machan, J.T., and Murray, M.M. Immature animals have higher cellular density in the healing anterior cruciate ligament than adolescent or adult animals. J Orthop Res **28,** 1100, 2010.
62. Stalling, S.S., and Nicoll, S.B. Fetal ACL fibroblasts exhibit enhanced cellular properties compared with adults. Clin Orthop Relat Res **466,** 3130, 2008.
63. Brink, H.E., Miller, G.J., Beredjiklian, P.K., and Nicoll, S.B. Serum-dependent effects on adult and fetal tendon fibroblast migration and collagen expression. Wound Repair Regen **14,** 179, 2006.
64. Melrose, J., Smith, S., Cake, M., Read, R., and Whitelock, J. Comparative spatial and temporal localisation of perlecan, aggrecan and type I, II and IV collagen in the ovine meniscus: an ageing study. Histochem Cell Biol **124,** 225, 2005.
65. Chen, A.C., Wong, W.M., and Sah, R.L. maturation dependent biomechanical properties of bovine meniscus-0074. Abstract presented at the Orthopedic Research Society Annual Conference.
66. Chia, H.N., and Hull, M.L. Compressive moduli of the human medial meniscus in the axial and radial directions at equilibrium and at a physiological strain rate. J Orthop Res **26,** 951, 2008.
67. Sweigart, M.A., and Athanasiou, K.A. Biomechanical characteristics of the normal medial and lateral porcine knee menisci. Proc Inst Mech Eng [H] **219,** 53, 2005.
68. Verdonk, P.C., Forsyth, R.G., Wang, J., Almqvist, K.F., Verdonk, R., Veys, E.M., *et al.* Characterisation of human knee meniscus cell phenotype. Osteoarthritis Cartilage **13,** 548, 2005.
69. Imler, S.M., Doshi, A.N., and Levenston, M.E. Combined effects of growth factors and static mechanical compression on meniscus explant biosynthesis. Osteoarthritis Cartilage **12,** 736, 2004.
70. Wilson, C.G., Nishimuta, J.F., and Levenston, M.E. Chondrocytes and meniscal fibrochondrocytes differentially process aggrecan during *de novo* extracellular matrix assembly. Tissue Eng Part A **15,** 1513, 2009.
71. Wilson, C.G., Vanderploeg, E.J., Zuo, F., Sandy, J.D., and Levenston, M.E. Aggrecanolysis and *in vitro* matrix degradation in the immature bovine meniscus: mechanisms and functional implications. Arthritis Res Ther **11,** R173, 2009.
72. Ionescu, L.C., Lee, G.C., Sennett, B.J., Burdick, J.A., and Mauck, R.L. An anisotropic nanofiber/microsphere composite with controlled release of biomolecules for fibrous tissue engineering. Biomaterials **31,** 4113, 2010.

Address correspondence to:
*Robert L. Mauck, Ph.D.*
*McKay Orthopaedic Research Laboratory*
*Department of Orthopaedic Surgery*
*University of Pennsylvania*
*36th St. and Hamilton Walk*
*Philadelphia, PA 19104*

*E-mail:* lemauck@mail.med.upenn.edu

*Received: May 7, 2010*
*Accepted: August 12, 2010*
*Online Publication Date: September 27, 2010*