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Dynamic Mechanical Loading Enhances Functional Properties of Tissue-Engineered Cartilage Using Mature Canine Chondrocytes

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Objective: The concept of cartilage functional tissue engineering (FTE) has promoted the use of physiologic loading bioreactor systems to cultivate engineered tissues with load-bearing properties. Prior studies have demonstrated that culturing agarose constructs seeded with primary bovine chondrocytes from immature joints, and subjected to dynamic deformation, produced equilibrium compressive properties and proteoglycan content matching the native tissue. In the process of translating these results to an adult canine animal model, it was found that protocols previously successful with immature bovine primary chondrocytes did not produce the same successful outcome when using adult canine primary chondrocytes. The objective of this study was to assess the efficacy of a modified FTE protocol using adult canine chondrocytes seeded in agarose hydrogel and subjected to dynamic loading.
Method: Two modes of dynamic loading were applied to constructs using custom bioreactors: unconfined axial compressive deformational loading (DL; 1 Hz, 10% deformation) or sliding contact loading (Slide; 0.5 Hz, 10% deformation). Loading for 3 h daily was initiated on day 0, 14, or 28 (DL0, DL14, DL28, and Slide14).
Results: Constructs with applied loading (both DL and Slide) exhibited significant increases in Young's modulus compared with free-swelling control as early as day 28 in culture ($p < 0.05$). However, glycosaminoglycan, collagen, and DNA content were not statistically different among the various groups. The modulus values attained for engineered constructs compare favorably with (and exceed in some cases) those of native canine knee (patella groove and condyle) cartilage.
Conclusion: Our findings successfully demonstrate an FTE strategy incorporating clinically relevant, adult chondrocytes and gel scaffold for engineering cartilage replacement tissue. These results, using continuous growth factor supplementation, are in contrast to our previously reported studies with immature chondrocytes where the sequential application of dynamic loading after transient transforming growth factor-β3 application was found to be a superior culture protocol. Sliding, which simulates aspects of joint articulation, has shown promise in promoting engineered tissue development and provides an alternative option for FTE of cartilage constructs to be further explored.

Introduction

Currently there are two prevailing approaches regarding implantation of tissue-engineered cartilage *in vivo*. One approach advocates implanting the cell–scaffold immediately into the defect site and relies on the *in situ* biological and loading environment to foster construct development.[1–6] The other approach is to first cultivate constructs *in vitro* to allow some elaboration of extracellular matrix and promote functional material properties with the goal of minimizing the risk of cracking following implantation, and improving long-term outcome. The concept of functional tissue engineering (FTE) has promoted the use of physiologic loading bioreactor systems to cultivate engineered cartilage tissues with functional load-bearing properties of articular cartilage.[7]

Previous studies have established that mechanical loading regulates the normal maintenance of articular cartilage *in vivo*[8,9] and *in vitro*.[10–17] Investigators using dynamic or cyclic loading conditions have shown that the biosynthetic response is strongly dependent on magnitude and frequency of the applied load,[13,14,17–20] with slow frequency loading

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generally resulting in suppression of proteoglycan (PG) synthesis, whereas more rapid loading frequencies result in stimulated synthesis.[21]

Using agarose as the scaffold material, we have demonstrated that in culture, applied dynamic compressive loading can modulate extracellular matrix composition and/or structural organization of engineered constructs, which promotes development of engineered cartilage tissues with a Young's modulus that matches native values.[22,23] Agarose has now been used as a component of a next-generation autologous chondrocyte implantation therapy (Cartipatch) for repair of cartilage defects in humans.[24,25] As our previous successes with applied dynamic compressive loading was primarily based on primary juvenile bovine chondrocytes, the efficacy of mechanical loading needs to be tested on passaged mature chondrocytes, which are more clinically relevant but have been shown to exhibit diminished capacity to produce a mechanically functional cartilage extracellular matrix.[26]

Under *in vivo* conditions, articular cartilage is subjected to compressive loading and sliding, which may promote shear forces.[27] Recent studies have shown that application of tissue shear enhanced matrix synthesis, especially the production of collagen, in cartilage explants.[28–31] Thus, the other objective of this study was to investigate whether sliding contact loading with a curved loading platen, which applies compressive deformational loading (DL) coupled with sliding-induced frictional shear forces, would increase extracellular matrix accumulation and improve the mechanical performance of the tissue-engineered cartilage.

Motivated by the above, we hypothesize that dynamic mechanical loading (compressive loading and sliding contact loading) will enhance the functional properties of the developing tissue-engineered cartilage.

Materials and Methods

Sample preparation and tissue culture

Chondrocyte-seeded agarose hydrogel disks were prepared as previously described.[23] Briefly, primary canine chondrocytes were harvested from the femoral condyles of 2–4-year-old dogs via digestion in 0.05% (w/v) collagenase (Sigma Chemicals, St. Louis, MO) for 11 h. Primary canine cells were expanded in expansion medium (Dulbecco's modified Eagle's medium, 5% fetal bovine serum, 1 ng/mL transforming growth factor-β3 [TGF-β3], 5 ng/mL fibroblast growth factor-2, and 10 ng/mL platelet-derived growth factor-ββ) for two passages before being encapsulated in 2% (w/v) low-melt agarose (Type VII; Sigma Chemicals) in phosphate-buffered saline (PBS) at 30×10^6 cells/mL. Disks (ø 4.00 mm) were cored from the slabs and cultured in defined serum-free chondrogenic medium (Dulbecco's modified Eagle's medium, 1% insulin transferrin selenium [ITS] + Premix, 50 μg/mL L-proline, 0.1 μM dexamethasone, 0.9 mM sodium pyruvate, antibiotics), supplemented with ascorbate (50 μg/mL). TGF-β3 (10 ng/mL; R&D Systems, Minneapolis, MN) was administered throughout the entire duration of the culture. Media were changed three times a week.

Mechanical loading

The prescribed compressive loading was applied as described previously.[23,32] Briefly, the loading protocol consisted of a nominal ± 5% compressive sinusoidal strain at 1 Hz frequency, superposed above a 10% tare strain, in unconfined compression with impermeable loading platens, for 3 h/day, 5 days/week. Dynamic compressive loading was carried out at 37°C and 5% CO_2 in a humidified incubator. Free-swelling (FS) controls were positioned adjacent to the loading device. The dynamic sliding contact load was applied with a custom-designed sliding contact bioreactor (Fig. 1A). The protocol produced 10% peak compressive loading coupled with oscillatory sliding contact, induced by curved loading platens sliding over the top surface of the constructs. The effective compressive loading frequency was 0.5 Hz, achieved by holding the four loading platens stationary while rotating the lower dish containing the constructs back and forth for 180° in each direction at the angular velocity of 1.25 revolutions per second (Fig. 1A). Dynamic compressive loading was applied starting either from day 0, 14, or 28 to 56, resulting in three experimental groups, which were DL0 (56 days of loading), DL14 (42 days of loading), and DL28 (28 days of loading) and the sliding contact loading was applied starting from day 14 to 56 (Slide14, 42 days of sliding) (Fig. 1B).

Mechanical testing

The spatially averaged mechanical properties of construct disks were evaluated at selected time points using a custom table-top testing device.[23] The equilibrium Young's modulus (E_Y) was determined under unconfined compression at 10% strain, followed by tests for dynamic moduli at 0.1, 0.5, and 1 Hz and 1% strain amplitude.

Frictional testing

Friction measurements were performed at room temperature in a physiological buffered saline (PBS) bath in a previously described custom-designed testing apparatus.[33] The friction apparatus consists of a sliding stage (Nutec, Deer Park, NY) with a motion control module (ACS Tech 80, Maple Grove, MN) to provide reciprocal translational motion and a stepper micrometer to provide normal load application (Oriel Instruments, Stratford, CT), connected to a linear variable differential transformer to measure specimen deformation (HR100; Shaevitz Sensors, Fair-field, NJ). Normal and frictional loads were measured with a multiaxial load cell (JR3, Woodland, CA). Tissue-engineered cartilage specimens were placed within a small recess at the center of the test chamber. The friction measurements (cartilage-on-glass) were performed in unconfined compression creep, under reciprocal sliding motion consisting of 100 cycles over a range of ± 4.5 mm, at 1 mm/s. Under this testing configuration, the friction coefficient rises monotonically over time, from a minimum value denoted by μ_{min} to a steady-state equilibrium value denoted by μ_{eq}, as shown in previous studies[33,34] (Fig. 2A). The pausing interval between two consecutive sliding cycles was gradually increased as the friction coefficient increased. This resulted in fewer sliding cycles during the later stage of the test, when the friction was high, helping to minimize tissue wear. A creep load of 0.445 N (corresponding to a normal stress of 0.035 MPa) was ramped up in 5 s and held constant for the duration of the friction test (3600 s). The friction force and normal force were averaged over the

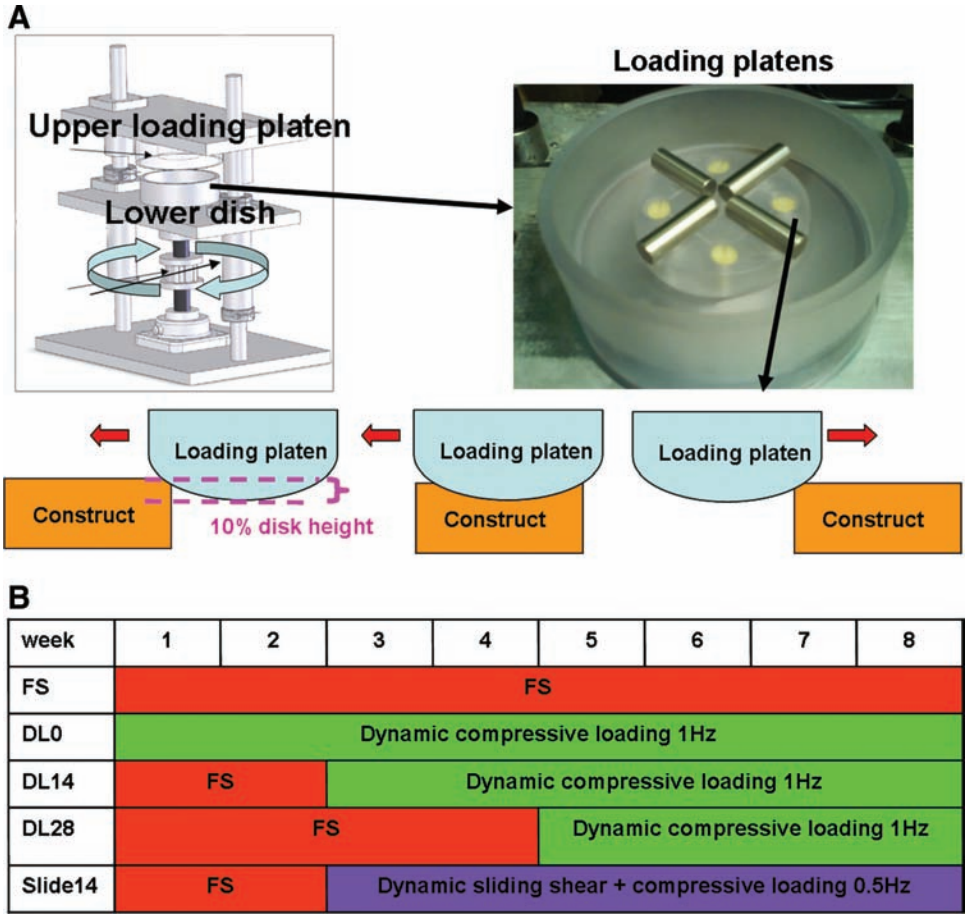

FIG. 1. (**A**) Design of the sliding bioreactor. (**B**) Temporal application of mechanical loading in different groups. FS, free-swelling; DL, deformational loading; Slide, sliding contact loading. Arrows indicate the direction of platen motion. Color images available online at www.liebertonline.com/ten.

back and forth portions of each cycle of reciprocal motion. This averaging procedure eliminates the contribution of the tangential force that may arise when the top and bottom faces of the cylindrical sample are not perfectly parallel. The time-dependent friction coefficient was determined from the ratio of the average friction force and normal force within each cycle; μ_{min} and μ_{eq} were used in the statistical analyses.

Biochemical analysis

One-half of each construct was weighed wet, lyophilized, reweighed dry, and digested in 0.5 mg/mL Proteinase-K (Fisher Scientific, Pittsburgh, PA) at 56°C for 16 h. The PicoGreen assay (Invitrogen, Carlsbad, CA/Molecular Probes, Eugene, OR) was used to quantify the DNA content of the constructs, with Lambda phage DNA (0–1 mg/mL) as a standard.[35] The glycosaminoglycan (GAG) content was measured using dimethylmethylene blue (Sigma Chemicals) dye-binding assay, with shark chondroitin sulfate (0–50 mg/mL) as a standard.[36] The overall collagen content was assessed by measuring the orthohydroxyproline content via dimethylaminobenzaldehyde and chloramine T assay. Collagen content was calculated by assuming a 1:7.5 orthohydroxyproline-to-collagen mass ratio.[37] The collagen and GAG contents were normalized to the disk wet weight and DNA content.

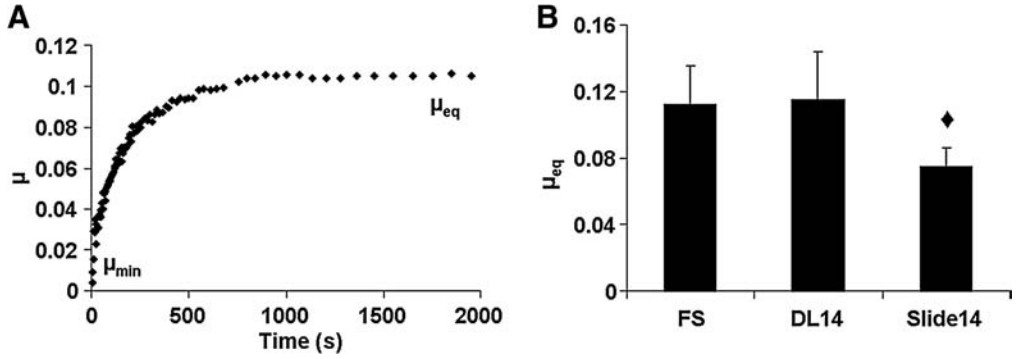

FIG. 2. (**A**) Representative plot of the friction coefficient with respect to time. (**B**) Equilibrium modulus of the constructs from the FS, DL14, and Slide14 groups on day 56; $^{\blacklozenge}p < 0.05$ versus FS and DL14; $n = 5$.

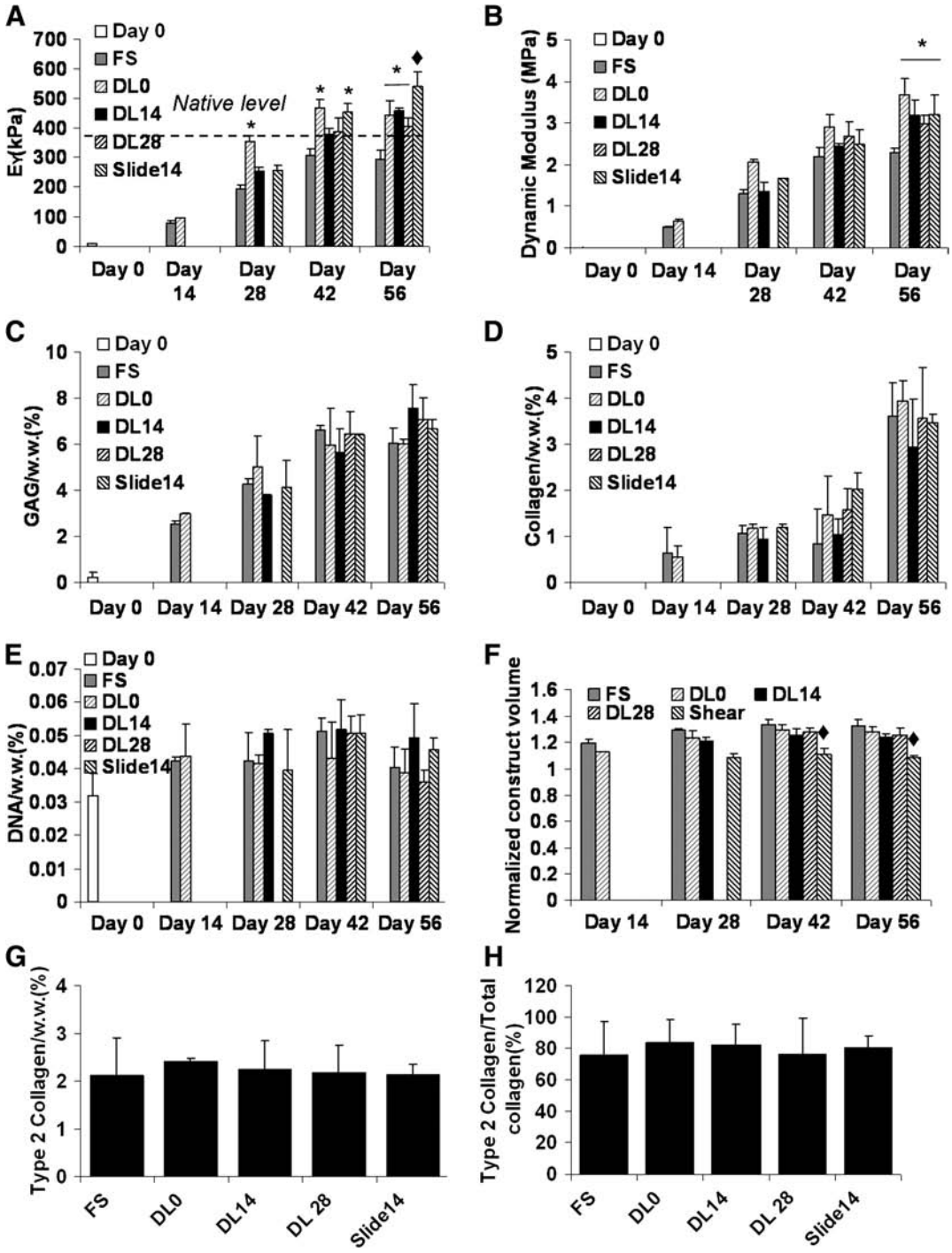

FIG. 3. (**A**) Equilibrium modulus and (**B**) dynamic modulus of the tissue-engineered constructs. (**C**) Glycosaminoglycan (GAG) content, (**D**) total collagen content, and (**E**) DNA content per construct (normalized to wet weight of the constructs). (**F**) Volume of the tissue-engineered constructs (normalized to the day 0 value). Type II collagen content of the constructs normalized to the wet weight (**G**) and total collagen (**H**) on day 56. *$p < 0.005$ versus FS; ♦$p < 0.05$ versus all other groups; $n = 4$.

Sandwich enzyme-linked immunosorbent assay (ELISA) against type II collagen was performed using a commercially available detection kit (Chondrex, Redmond, WA).

Histological analysis

The other halves of the constructs were fixed in a fixative solution (5% acetic acid, 3.7% formaldehyde, 70% ethanol) for 24 h and stored in 70% ethanol solution. After serial dehydration in ethanol, the constructs were embedded in paraffin (Fisher Scientific), sectioned to 8 μm, and mounted onto microscope slides. The samples were then dewaxed, rehydrated, and stained with Safranin-O (Sigma Chemicals) and Picrosirius Red (Sigma Chemicals) dyes to determine the distribution of GAG and collagen, respectively. To view the distribution of superficial zone protein (SZP) in the con-

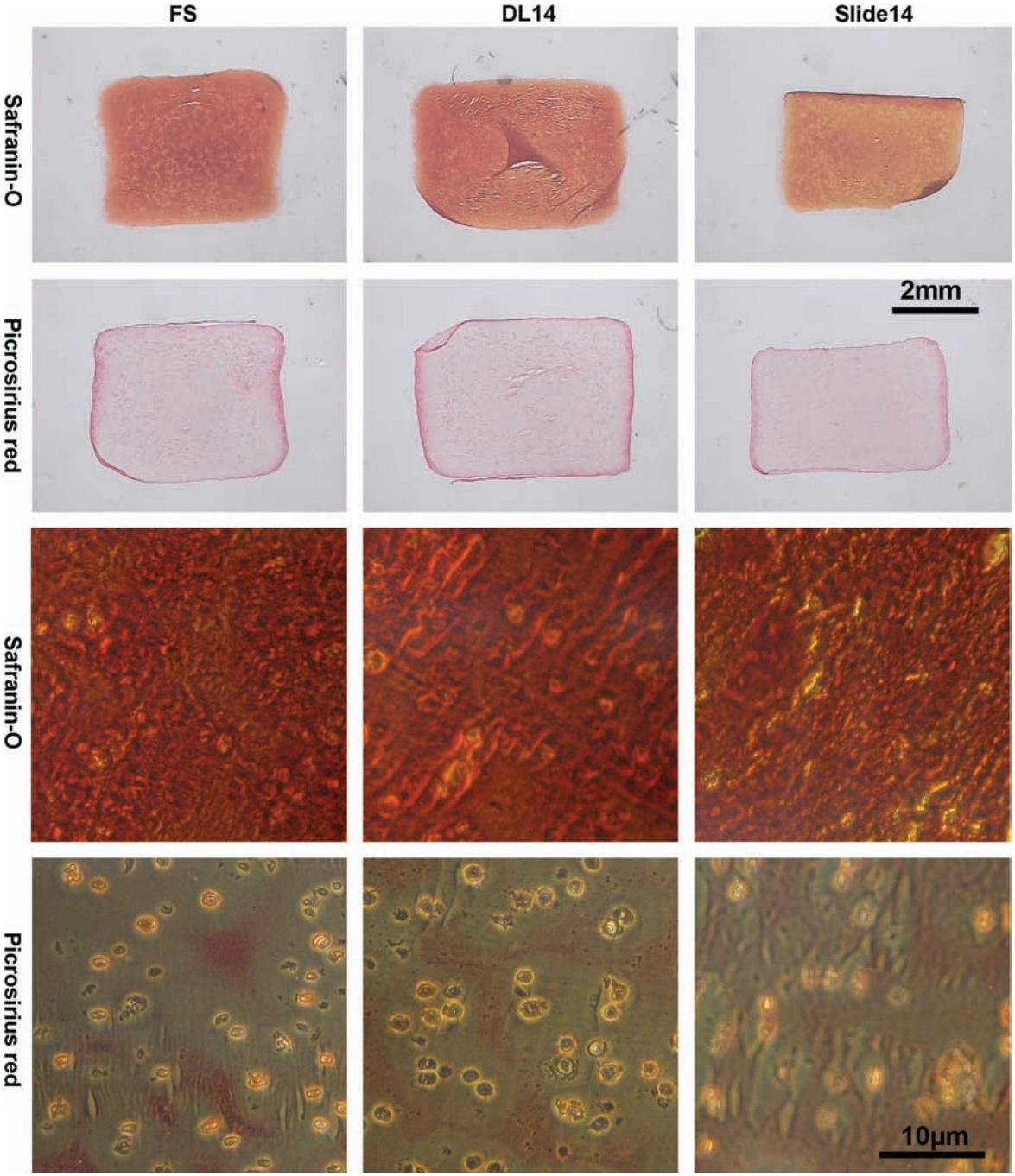

FIG. 4. Safranin-O and Picrosirius Red staining of the histological sections of the FS, DL14, and Slide14 groups on day 56 (taken at two magnifications). Color images available online at www.liebertonline.com/ten.

structs, sections were stained as described previously by Krishnan *et al*.[38] Briefly, sections were dewaxed, rehydrated, washed three times in PBS for 2 min each, and then blocked with 10% normal goat serum (NGS, in PBS) for 10 min at room temperature. This was followed by incubation with primary, rabbit-derived antibody (06A10; kindly provided by Dr. Carl Flannery, Wyeth Research Division, Cambridge, MA) at a concentration of 24 mg/mL in 10% NGS for 12 h at 4°C. Sections were then washed with PBS and incubated with Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (Molecular Probes) at 10 mg/mL in 10% NGS for 1 h at room temperature. After washing with dH_2O, samples were treated with propidium iodide nucleic acid stain (Molecular Probes) at 10 mg/mL for 5 min to view nuclei, washed three times with dH_2O, and coverslipped with Gel = Mount (Biomedia, Foster City, CA). On each slide, one section was maintained as a nonimmune control, following the procedure described earlier but with 10% NGS substituted for primary antibody. Immunohistochemistry staining against type IX collagen was performed similarly as described earlier, with primary antibody obtained from a commercial source (Abcam, Cambridge, MA).

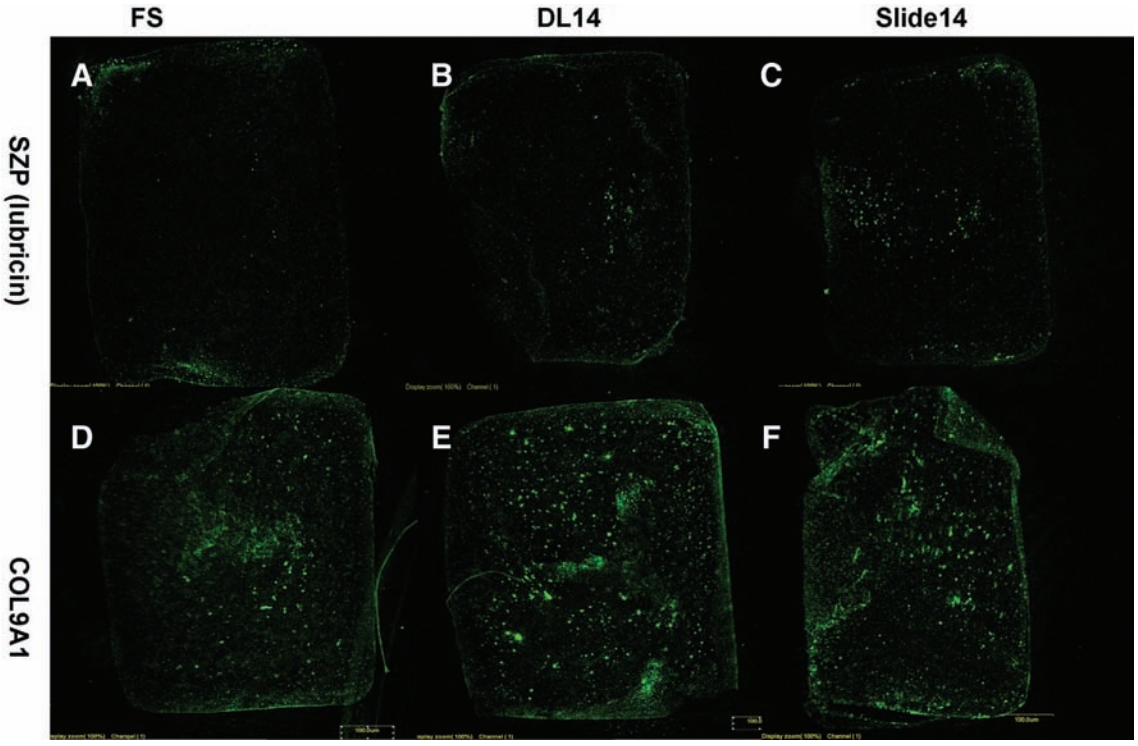

FIG. 5. (**A, D**) Immunohistochemistry staining against SZP (superficial zone protein, or lubricin) and COL9A1 on histological sections of the FS, (**B, E**) DL14, and (**C, F**) Slide14 groups on day 56. Color images available online at www.liebertonline.com/ten.

Statistical analysis

Statistica (Statsoft, Tulsa, OK) was used to perform statistical analyses using two-way analysis of variance and the Tukey honest significance difference (HSD) *post hoc* test of the means ($n = 4$–6 samples per group), with culture duration and experimental groups as independent factors.

Results

The group subjected to dynamic compressive loading from day 0 (DL0) developed a significantly higher Young's modulus on day 28 than all other groups (DL0: 352 ± 22 kPa vs. FS: 193 ± 14 kPa, DL14: 253 ± 14 kPa, and Slide14: 258 ± 16 kPa; Fig. 3A). On day 42, the Young's modulus of both DL0 and Slide14 groups became significantly higher than that of the FS group. On day 56 the group subjected to sliding contact (Slide14) became significantly stiffer than all other groups (DL0, DL14, DL28, and FS) in terms of Young's modulus (Slide14: 541 ± 48 kPa vs. DL0: 445 ± 46 kPa, DL14: 456 ± 8 kPa, DL28: 404 ± 29 kPa, and FS: 294 ± 31 kPa; Fig. 3A). Further, on day 56, all the groups subjected to dynamic loading (DL0, DL14, and DL28) exhibited significantly higher Young's modulus than the FS group. It was also noted that all the mechanically loaded groups (DL0/14/28 and Slide14) reached or even surpassed the Young's modulus of native canine knee cartilage (native E_Y: 380 ± 105 kPa, obtained from canine condyle cartilage explants using the same testing protocol) starting from day 42, whereas the FS group remained below the native level (Fig. 3A). Similarly, on day 56, all the groups subjected to loading (DL0, DL14, DL28, and Slide 14) showed significantly higher dynamic modulus than the FS group (Slide14: 3.20 ± 0.45 MPa, DL0: 3.67 ± 0.41 MPa, DL14: 3.18 ± 0.38 MPa, and DL28: 2.98 ± 0.19 MPa vs. FS: 2.30 ± 0.10 MPa; Fig. 3B). However, the GAG and collagen contents of all groups were not significantly different from each other at all time points (Fig. 3C, D). There was no significant difference in the DNA contents among all the groups either (Fig. 3E). The volume of the constructs increased with culture time and the FS group showed the largest swelling (Fig. 3F). There was no significant difference among groups until days 42 and 56, when the construct volume of the Slide14 group exhibited less swelling than all the other groups (Fig. 3F). The results of ELISA indicated no significant difference in type II collagen content between the FS and loaded groups or between different loaded groups on day 56 (Fig. 3G). The type II collagen made up around 70–80% of the total collagen content (Fig. 3H).

Safranin-O and Picrosirius Red staining of the histological sections from different groups were examined at various magnifications and showed no significant difference in terms of staining intensity and spatial distribution of the staining (Fig. 4). Immunohistochemistry staining showed that lubricin (SZP) was primarily deposited in the peripheral area of the constructs. There appeared to be no significant difference in the intensity of staining against lubricin in the Slide14 samples when compared with the FS samples (Fig. 5). Samples from the FS, DL14, and Slide14 groups all showed intense staining against COL9A1 (Fig. 5).

The equilibrium friction coefficient of the constructs subjected to sliding contact loading (Slide14) was significantly lower than that of constructs from the FS and DL14 groups (Fig. 2B). There was no significant difference in the minimum friction coefficient among different groups (data not shown). Sliding was also applied continuously for 48 h on acellular

4% agarose disks, which possessed higher mechanical stiffness needed for friction testing. The results showed that sliding did not significantly alter the equilibrium friction coefficient of acellular agarose disks when compared with that of the FS acellular agarose constructs (data not shown).

Discussion

It has been established that mature chondrocytes exhibit diminished capacity to produce a mechanically functional cartilage extracellular matrix.[26] Studies have shown that the mitotic and synthetic activities of human articular cartilage chondrocytes decline with age,[39,40] and an age-related decline in response to anabolic factors such as insulin-like growth factor-I is known to occur.[41] However, our results demonstrated that dynamic mechanical loading is able to enhance the functional properties of tissue-engineered cartilage using mature chondrocytes to native levels (Fig. 2A). Specifically, this study shows that both dynamic compressive loading and reciprocal sliding contact loading can promote mechanical stiffness of the tissue-engineered cartilage *in vitro*.

Our previous study indicated that a delayed loading regimen (dynamic compressive loading applied after termination of TGF-β3 supplementation on day 14) had beneficial effects on the maturation of the tissue-engineered constructs seeded with immature bovine chondrocytes, whereas a continuous loading regimen starting from day 0 of the culture appeared to be detrimental.[22] In contrast, in this study, this differential response of different loading regimens was not observed in constructs seeded with mature canine chondrocytes. Dynamic compressive loading applied on days 0, 14, and 28 all enhanced the mechanical stiffness of the constructs by a similar extent after 56 days of culture. Loading initiated on day 0 (DL0) showed no negative effect on tissue stiffness after 14 days and resulted in higher stiffness on day 28 than for constructs loaded from day 14 (DL14). This outcome suggests that the earlier loading period, from day 0 to 14, may have primed the cells from the DL0 group to be more responsive to subsequent loading applied from day 14 to 28 than the cells from DL14 group. The beneficial effect of earlier loading gradually diminished and eventually the groups receiving delayed loading (DL14, DL28) caught up with the DL0 group in terms of mechanical stiffness. This finding implies that applying mechanical loading earlier will accelerate the maturation of the tissue-engineered cartilage and, therefore, shorten the *in vitro* culture period, which is costly, prior to implantation. The length of the *in vitro* culture is critical to the eventual clinical application of tissue-engineered cartilage. On one hand, the Young's modulus of the DL0 group was already close to the native level of canine cartilage on day 28. On the other hand, the length of the *in vitro* culture will ultimately depend on how stiff the engineered cartilage needs to be to achieve the optimal *in vivo* repair. If only a subphysiological mechanical property (for instance, 70% of the native level) is needed for successful repair, then the *in vitro* culture period can be further shortened.

By transitioning from an immature bovine model to a more clinical relevant mature canine model, a number of parameters such as species, age, and cell passaging were introduced. A previous study showed that similar to mature canine chondrocytes, mature bovine chondrocytes also need continuous supplementation of TGF-β3 to elaborate ECM.[42] This suggests that the age of the cells plays an important role. On the other hand, it was found that unpassaged mature canine chondrocytes were inferior to passaged mature canine chondrocytes in matrix elaboration after three-dimensional encapsulation.[43] Therefore, passaging with priming growth factors may have an effect on the mature canine chondrocytes. Lastly, a separate study in our laboratory has shown that using the same protocol as used in this study (two-dimensional expansion, three-dimensional encapsulation, and growth factor supplementation), engineered cartilage seeded with human osteoarthritic chondrocytes (from adult donors) developed a respectable Young's modulus of around 180 kPa.[44] Hence, it is reasonable to speculate that *in vitro* culture protocols optimized from this canine model can be instrumental to developing strategies for human cartilage repair in future.

There was no significant difference in biochemical composition found among all groups. Mechanical stiffness of the tissue-engineered constructs generally correlates with the content of major matrix components, that is, GAG and collagen.[45,46] However, mechanical stiffness is not solely determined by GAG and collagen content. As shown by Mauck *et al.*, dynamic compressive loading can increase the mechanical stiffness of bovine chondrocyte-seeded constructs without elevating their bulk GAG and collagen content.[47] Considering the lesser swelling observed in the mechanically loaded versus FS constructs (Fig. 2F), the higher stiffness observed in the dynamically loaded constructs may be attributed to an improved organization of the collagen network.[48,49] Minor matrix components, such as cartilage oligomeric matrix protein (COMP),[50,51] type IX collagen,[52] and link protein,[53,54] can also modulate the mechanical stiffness of the constructs and may potentially account for the difference in mechanical properties between the loaded groups and the FS group. However, in this study, we did not observe noticeable differences in type IX collagen deposition among different groups, as assessed qualitatively from immunohistochemistry staining. Quantitative assays such as ELISA would be needed in future studies to ascertain any possible difference in the production of these crosslinking matrix molecules.

Polarized light microscopy was performed on the histological sections stained with Picrosirius Red at various magnifications and no significant difference in the pattern of birefringence was found between different groups (data not shown). Together with the finding that the collagen II content of the constructs was similar in all experimental groups, this suggests that neither the organization nor the bulk content of collagen fibrils is the primary reason for the elevated mechanical properties in the mechanically loaded constructs.

Dynamic loading in unconfined compression subjects the constructs to axial compression and radial and circumferential tension. In addition, the faces of the construct are occluded by the loading platens during the loading segment of the daily duty cycle, leaving only the lateral cylindrical surface of the construct exposed to nutrient diffusion.[55,56] In contrast, the sliding contact bioreactor produces a migrating contact area as the platens reciprocate in a periodic fashion across the sample surface. Thus, over each cycle of loading, the top

surface of the construct becomes periodically exposed to nutrient diffusion, complementing nutrient supply from the lateral surface. This loading mode reflects that found with physiologic joint loading and promotes sustained interstitial fluid pressurization in the constructs during *in vitro* culture with loading.[57]

No significant differences were observed in the minimum friction coefficient, μ_{min}, among the various groups. This outcome is not unexpected, because the value of μ_{min} is regulated mostly by interstitial fluid pressurization upon initial loading[33] and is not expected to differ among the groups. Over time, as the interstitial fluid pressure subsides, the friction coefficient rises toward its equilibrium value, which is significantly regulated by boundary friction mechanisms that may depend on the composition of the extracellular matrix and interactions among different components of the matrix.[58] The constructs subjected to sliding contact loading (Slide14 group) exhibited a lower equilibrium friction coefficient than other groups, which may be attributed to a higher content of boundary lubricants such as SZP/lubricin. Previous studies have shown that dynamic shear can increase the production of SZP/lubricin.[29,31] However, in this study, immunohistochemistry staining against SZP only provided a qualitative assay. Therefore, future work is needed to analyze the synthesis of lubricin quantitatively using ELISA or quantitative polymerase chain reaction assays.

Future work remains to be completed to better explain the underlying causes responsible for the differences in mechanical properties observed between loaded and FS constructs. Additionally, *in vivo* evaluation is planned, which will ultimately test whether enhanced mechanical properties (with loading) translates to a functional tissue repair. These animal studies will also permit study of the repair of cartilage defects with engineered constructs of similar mechanical properties obtained with loading or FS conditions in culture, providing a means to identify the intrinsic beneficial effects of mechanical preconditioning of chondrocytes (i.e., preparing chondrocytes for seeing joint loading) that may exist.

Conclusion

Our findings successfully demonstrate an FTE strategy incorporating clinically relevant, adult chondrocytes and hydrogel scaffold[25] for engineering cartilage replacement tissue. These results using continuous growth factor supplementation are in contrast to our previously reported studies with immature chondrocytes where the sequential application of dynamic loading after transient TGF-β3 application was found to be a superior culture protocol.[32] Applied DL provides a physical stimulus to chondrocytes in addition to enhanced nutrient transport.[23,59] Sliding contact, which simulates aspects of joint articulation, has shown promise in promoting engineered tissue development[60,61] and provides an alternative option for FTE of cartilage constructs to be further explored. The ability to cultivate engineered cartilage that can achieve native mechanical properties using adult chondrocytes is clinically significant. To survive joint loading, engineered cartilage constructs should possess substantially similar mechanical properties to the surrounding host tissue before implantation, especially if they have to support most of the articular contact stresses as a result of the local congruence of the articular surfaces. Further, we believe that constructs that have been mechanically loaded prior to implantation in the joint loading environment will perform better than unloaded constructs, reflecting loading-induced differences in tissue structure and composition as well as cell preconditioning to physical stimuli.

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Disclosure Statement

No competing financial interests exist.

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