Lower back pain is a significant source of morbidity in the United States, with a reported lifetime prevalence as high as 84%, at a total annual cost of as much as $200 billion. The facet joints are synovial joints lined with hyaline articular cartilage that stabilize the vertebral unit during flexion and extension and minimize axial rotation.

During spinal loading, the facet joint is exposed to as much as 25% of the total load under normal conditions, and as much as 47% of the total load in osteoarthritis. This extensive loading results in the application of significant compressive and tensile forces to the articular cartilage surface, which leads to further tissue injury. Additionally, these significant loads may lead to significant pain as a result of the joint’s dense innervations, as reviewed by Kalichman and Hunter.

Although facet joint osteoarthritis has been identified as a significant source of morbidity, current treatments merely strive
to reduce the pain associated with the joint. To the best of our knowledge, no studies have attempted to create a replacement tissue for osteoarthritic facet cartilage. Therefore, a viable replacement tissue may be provided by decellularized xenogenic tissue, in which antigenic intracellular proteins and nucleic acids are eliminated while preserving the functional properties of the tissue’s extracellular matrix. Xenogenic approaches appear very promising because previous studies demonstrated relatively few immune responses in cartilage, as reviewed in detail previously. Additionally, this approach is especially applicable to articular cartilage, in which the tissue’s properties are primarily attributed to its extracellular matrix, which is preserved after decellularization. This process has been used successfully to create an acellular dermal matrix, used clinically as the U.S. Food and Drug Administration–approved human-derived Alloderm product or the porcine-derived Strattice product. Furthermore, decellularization approaches have been used for other musculoskeletal tissues, such as the knee meniscus and the temporomandibular joint disc.

The objective of this study was to determine the effects of 2% sodium dodecyl sulfate (SDS) on cartilage cellularity, biochemical, and biomechanical properties. SDS is an ionic detergent that generally permeabilizes the nuclear and cytoplasmic cell membranes and has been used for decellularizing other musculoskeletal tissues including meniscus and temporomandibular joint, tendon, and ligament. In this study, SDS treatment was examined at 3 application times: 2 hours, 4 hours, and 8 hours. It was hypothesized that cartilage explants could be effectively decellularized while preserving the biomechanical and biochemical, collectively termed functional, properties of the tissue.

**MATERIALS AND METHODS**

**Specimen Preparation**

Cartilage explants were isolated from the distal femur of week-old male calves (Research 87, Boston, Massachusetts) shortly after slaughter. To normalize animal variability, 6 legs were used, and each leg came from a different animal. For each group, 1 explant from each animal was used, for a total of 6 explants per group. Using a scalpel, cartilage was separated from the subchondral bone. Separation of the chondral and bone tissue was verified histologically, and a cryotome was used to ensure uniform tissue thickness before biomechanical testing. After tissue isolation, the chondral explants were wrapped in gauze and soaked in normal saline solution with protease inhibitors (ethylenediamine tetra-acetic acid, benzamidine hydrochloride, N-ethylmaleimide, and phenylmethylsulfonyl fluoride) and frozen until testing. All samples were subjected to 1 freeze-thaw cycle before the assessments.

**Decellularization Treatment**

After thawing, articular cartilage explants (6 per group) were exposed to 2% SDS for 2, 4, or 8 hours. The treatments were based on pilot experiments as well as previous work involving the decellularization of tissue-engineered constructs by our group. All treatments included 0.5 mg/mL DNase type I, 50 μg/mL RNase, 0.02% ethylenediamine tetra-acetic acid, and 1% penicillin/streptomycin/fungizone in phosphate-buffered saline. The treatments were applied in an incubator at 37°C with agitation. After the detergent treatment, the constructs were washed for 2 hours in phosphate-buffered saline at 37°C with agitation. An untreated control group was assessed immediately after thawing, without the treatment or wash steps.

**Histology**

Samples were sectioned at 14 μm after freezing. A hematoxylin and eosin stain was used to examine explants cellularity. GAG distribution was examined with a safranin O/fast green stain, and a picrosirius red stain was used to examine collagen content.

**Quantitative Biochemistry**

Samples were frozen overnight and lyophilized for 48 hours, followed by digestion with 125 μg/mL papain (Sta, St. Louis, Missouri) in 50 mmol/L phosphate buffer (pH 6.5) containing 2 mmol/L N-acetylcysteine (Sigma) and 2 mmol/L ethylenediamine tetra-acetic acid (Sigma) at 65°C overnight. A Picogreen Cell Proliferation Assay Kit (Molecular Probes, Eugene, Oregon) was used for DNA content assessment. GAG content was measured using the Blyscan Glycosaminoglycan Assay kit (Biocolor Ltd., Carrickfergus, United Kingdom), based on 1, 9-dimethylmethylen blue binding. After hydrolysis with 2 N NaOH for 20 minutes at 110°C, total collagen content was quantified using a chloraamine-T hydroxyproline assay.

**Indentation Testing**

Compression testing was performed using creep indentation apparatus, as described previously. For the control explants and the explants treated with SDS for 2 hours, a 2-g (0.02-N) step mass was applied with a 1-mm flat-ended, porous indenter tip, and specimens were allowed to creep until equilibrium, as described elsewhere. For the explants treated with SDS for 4 hours or 8 hours, a 0.7-g (0.007-N) step mass was applied instead to maintain equivalent strains. Strains generally ranged from 5% to 16%. Preliminary estimations of the aggregate modulus of the samples were obtained using the analytical solution for the axisymmetric Boussinesq problem with Papkovich potential functions. The sample biomechanical properties, including aggregate modulus, Poisson’s ratio, and permeability, were then calculated using the linear biphasic theory.

**Tensile Testing**

A uniaxial materials testing system (Model 5565; Instron Corp., Canton, Massachusetts) was used for the determination of tensile properties with a 50-N load cell, as described previously. Samples were cut into a dog-bone shape and clamped outside of the gauge length. The gauge length, thickness, and width were measured with a micrometer. Samples were pulled at a constant strain rate of 1% of the gauge length, and all samples broke within the gauge length. The load-displacement curve and the cross-sectional area of each sample were used to created stress-strain curves, and Young’s modulus was calculated from the linear region of each stress-strain curve.

**Statistical Analysis**

All biomechanical and biochemical assessments were made using N = 6. To compare among treatment groups, a single-factor analysis of variance was used, and a Fisher least significant difference post hoc test was used when warranted. Significance was defined as P < .05.
RESULTS

Gross Appearance and Histology

The cartilage explant thicknesses for the control group and the 2-, 4-, and 8-hour treatment groups were 0.79 ± 0.14, 0.66 ± 0.12, 0.60 ± 0.15, and 0.54 ± 0.15, respectively. The 4- and 8-hour treatment groups had a thickness significantly smaller than the control group.

Figure 1 displays the histological results of the study. Extensive staining for cell nuclei was observed in the hematoxylin and eosin staining of the control group. Treatment with 2% SDS for 2 hours reduced the number of cell nuclei, whereas treatment for either 4 or 8 hours nearly eliminated all nuclei from the explants. Additionally, extensive staining for collagen was observed for all groups. Finally, extensive staining for GAG was observed for the control group and the 2-hour SDS treatment. However, treatment for 4 and 8 hours resulted in no GAG staining.

Quantitative Biochemistry

The DNA/dry weight for the control group and the 2-, 4-, and 8-hour treatment groups was 6405 ± 2272, 6172 ± 785, 4642 ± 547, and 3861 ± 1094 ng/mg, respectively (Figure 2). Only treatment for 8 hours resulted in a significant decrease in DNA content from control. The effects of the decellularization treatments on explant GAG content are found in Figure 3. Treatment for 4 and 8 hours had no effect on GAG content, whereas treatment for 4 or 8 hours resulted in nearly complete elimination of GAG. The GAG/dry weight for the control group and the 2-, 4-, and 8-hour treatment groups was 0.17 ± 0.06, 0.15 ± 0.12, 0.02 ± 0.02, and 0.003 ± 0.007 mg/mg, respectively. The effects of the decellularization treatments on collagen content are shown in Figure 4. There were no significant differences among any of the treatments with collagen/dry weight values of 0.84 ± 0.06, 0.77 ± 0.12, 0.77 ± 0.08, and 0.73 ± 0.09 mg/mg for the control group and 2-, 4-, and 8-hour treatment groups, respectively.

Biomechanical Evaluation

The effects of the various decellularization treatments on explant aggregate modulus are displayed in Figure 5. Treatment with SDS for 2 hours maintained the compressive stiffness, whereas treatment
To the best of our knowledge, this is the first study to decellularize hyaline articular cartilage explants using a detergent-based approach and is the only study to create a replacement tissue directly targeting the facet joint. The objective of this study was to determine the effects of multiple decellularization treatment times on the biomechanical, biochemical, and histological properties of cartilage explants.

The results demonstrated that 2% SDS allows effective tissue decellularization while maintaining tissue functional properties, thus confirming our hypothesis. For example, treatment with 2% SDS for 2 hours maintained the compressive and tensile biomechanical properties as well as the GAG and collagen content while resulting in a decrease in cell nuclei and a 4% decrease in DNA content. Additionally, treatment for 8 hours resulted in complete histological decellularization and a 40% decrease in DNA content while maintaining collagen content and tensile properties. However, a significant decrease in compressive properties and GAG content was observed. Similar results were observed with 4-hour treatment, although the decrease in DNA content was not as great as with 8-hour treatment.

Although 2% SDS treatment for 4 or 8 hours resulted in complete histological decellularization, it is important to note that these treatments did not result in complete elimination of DNA. Based on these results, SDS treatment for 4 and 8 hours was effective for cell and nuclear membrane lysis, as evidenced by complete histological decellularization on the hematoxylin and eosin stain. However, despite the membrane lysis, 60% of the DNA remained in the tissue. Because only 1 concentration of DNAse was used in this study, it is possible that higher concentrations would allow for a more effective elimination of DNA content after membrane lysis. Also, it is possible that adding nucleases...
during the wash step would also allow for a more effective reduction in DNA content because nucleases were only used during detergent treatment in this study. A future study is planned to examine the effects of multiple DNase treatments during both the decellularization treatment and the wash step.

Although the decellularization treatments did not result in complete elimination of DNA in the explants, it is unclear whether DNA elimination is absolutely necessary to prevent an immune response. A recent study by Gilbert et al. demonstrated measurable levels of DNA in several commercially available extracellular matrix scaffold materials used clinically. Additionally, some of the scaffolds also had positive histological staining for nuclear material. However, these scaffold materials have not resulted in significant inflammatory responses with clinical use; therefore, it is possible that the limited amount of DNA present in the explants may not result in a significant host response when used in an in vivo setting. It is also important to consider the relatively immune-privileged nature of the joint space, as reviewed elsewhere. Based on these issues, the exact level of tissue decellularization that must be attained to limit the host immune response is unclear. It is possible that only histological decellularization may be needed to limit the immune response. Future in vivo studies will need to be performed to examine this issue directly and determine what degree of tissue decellularization is required for a facet cartilage replacement tissue. Therefore, it may be possible that the 2-hour treatment would elicit a similar immune response as that of the 8-hour treatment, despite the presence of cells.

Although this study only assessed the effects of decellularization, expression of the α-galactosyl epitope has been described as an important mediator of xenograft immune rejection, as reviewed previously. To circumvent this issue, Stone et al. treated porcine cartilage with α-galactosidase to remove the epitope and found that this treatment significantly reduced the inflammatory response when the porcine cartilage was implanted in cynomolgus monkeys. Therefore, treatment with α-galactosidase may be an important adjunctive therapy to SDS and DNase to completely eliminate the immunogenicity of the tissue, and this issue should be addressed in future studies.

The results of this study were particularly exciting because they serve as an initial step toward the creation of a nonimmunogenic replacement tissue for the facet joint. A previous study demonstrated that lumbar facet cartilage had a thickness of 0.5 mm and compressive and tensile stiffnesses of 500 kPa and 10 MPa, respectively. In this study, decellularization with 8 hours of SDS treatment resulted in a construct with gross morphological and tensile properties, but only had a minimal effect on the DNA content of the tissue. Conversely, treatment for 4 or 8 hours resulted in complete histological decellularization and maintenance of the tensile properties and collagen content; treatment for 8 hours also resulted in a significant 40% reduction in DNA content. Despite the inability to decellularize articular cartilage while maintaining all functional properties, the results of this study are promising and demonstrate the feasibility of creating a decellularized articular cartilage replacement tissue that could be used as a treatment for facet cartilage osteoarthritis.

CONCLUSION

Treatment with 2% SDS for 8 hours seemed to be the most promising method for cartilage decellularization and could be used in future studies. Treatment for 2 hours maintained the biomechanical and biochemical tissue properties, but only had a minimal effect on the DNA content of the tissue. Conversely, treatment for 4 or 8 hours resulted in complete histological decellularization and maintenance of the tensile properties and collagen content; treatment for 8 hours also resulted in a significant 40% reduction in DNA content. Despite the inability to decellularize articular cartilage while maintaining all functional properties, the results of this study are promising and demonstrate the feasibility of creating a decellularized articular cartilage replacement tissue that could be used as a treatment for facet cartilage osteoarthritis.

Disclosure

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**COMMENT**

Elder et al presented basic science information that creatively crafts a foundation for future research. They established a mechanism for articular cartilage decellularization that could lead to clinically effective facet joint cartilage replacement strategies. They studied the process and the biomechanics of such a strategy. This strategy is as promising as any in this area. The authors are to be congratulated for forging forward in this new frontier.

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