Significance: In order to successfully employ a biodegradable magnesium TMJ prosthetic device, the effect of magnesium ion concentration on fibrocartilage must be assessed.

Introduction: Recently, magnesium has been explored as a potential biomaterial for biodegradable orthopedic implants due to its similar mechanical properties to bone[1] and biocompatibility. While magnesium alloys show promise as potential implant materials for bone regeneration, the effect of degrading magnesium on the surrounding soft tissue needs to be determined. In a study done by Fayerebend et al. [2], it was found that high concentrations of magnesium sulfate (MgSO₄) have been shown to support chondrocyte proliferation and dedifferentiation. While these findings demonstrate the effect of magnesium concentrations up to 30 mM on chondrocyte behavior, the degradation products of some magnesium alloys could result in higher concentrations. This generates the need to test for the effects of higher concentrations of magnesium on cell behavior. Additionally, other ions, such as magnesium chloride (MgCl₂), should be tested to ensure that changes in tissue response are not attributed to increased sulfate concentration. Specifically, for TMJ prosthetics made of magnesium alloys, the effect of high concentrations of magnesium on the regeneration of fibrocartilage needs to be investigated.

In tissue engineering approaches for fibrocartilage, goat costal chondrocytes cultured using a scaffoldless approach have proven to be a viable cell source due to their production of high quantities of collagen and GAG. [3, 4] In this study, scaffoldless costal chondrocyte constructs are used as a model to determine the effect of additional magnesium on fibrocartilage extracellular matrix production. The objective of this study was to assess the effect of concentrations of 20, 50, and 100 mM of magnesium chloride (MgCl₂) and magnesium sulfate (MgSO₄) on the mechanical properties of fibrocartilage constructs.

Materials and Methods:
Goat costal cartilage was isolated from the ribs of three young (<1 year) female Boer goats within 24 hours of slaughter. Isolated fibrochondrocytes were passed three times in Dulbecco’s modified Eagle medium (DMEM)/high glucose (Thermo Scientific) 10% fetal bovine serum (Atlantic Biologicals), 1% penicillin-streptomycin (Lonza), 1% non-essential amino acid solution (Thermo Scientific) and 25 µg/ml L-ascorbic acid (Sigma-Aldrich). The passaged cells were seeded in 5 mm² agarose wells at a density of 2 million cells/well using chondrogenic media. [3] Additional concentrations of 20, 50, and 100 mM MgCl₂ (Fisher Scientific) and MgSO₄ (Acros) were sterile filtered into the chondrogenic media. The baseline magnesium ion concentration of the DMEM/high glucose was 0.8 mM MgSO₄. The media was replenished every 48 hours and constructs were cultured in 37°C and 5% CO₂. After two weeks, constructs were transferred to agarose coated plates for an additional 2 weeks.

Unconfined compression testing and analysis was performed according to our published methods. [5] The MTS Insight™ was used to measure changes in force throughout the test. The constructs then underwent 10 cycles of preconditioning at 9% min until 10% strain was reached. [5, 6] Immediately following preconditioning, the samples were compressed until 10% strain was reached, and were allowed to relax for 60 minutes.

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

Results
The results from the compression analysis can be seen in Figure 1. The constructs from the 100 mM MgCl₂ and 100 mM MgSO₄ groups did not have the structural integrity to withstand the 0.05 N preload and were therefore deemed un-testable. The percent decrease in stress relaxation in the 20 mM MgCl₂ group (44.8±7.2%) and no difference between the 50 mM MgCl₂ group compared to the 0.8 mM control (p<0.05). There was also no significant difference between the stress relaxation of the 20 mM MgSO₄ group and the control but there was significant increase in the 50 mM MgSO₄ group (75.7±3.9%) (p<0.05). The tangent modulus of the control constructs was 434.8±46.8 kPa (Figure 3b). There was no statistically significant difference between the 20 mM MgCl₂ group and the 0.8 mM control. The results show a significant decrease in tangent modulus compared to the 0.8 mM MgCl₂ constructs (229.2±14.5) (p<0.05). Again, there was no significant difference in tangent modulus between the 20 mM MgSO₄ constructs and the control. The 50 mM MgSO₄ constructs did have a significantly lower tangent modulus than the control at 126.2±6.3 kPa (p<0.05).

Figure 3: Simple compression analysis of the scaffoldless constructs (n=6) for constructs for 0.8, 20, 50, and 100 mM MgCl₂ and MgSO₄ at 4 weeks. (a) Percent stress relaxation (b) Tangent modulus. The symbol (*) indicates significance (p<0.05) to the 0.8 mM MgCl₂ constructs. Error bars indicate S.E.M.

Discussion
The results of this study demonstrate the mechanical differences between scaffoldless constructs cultured in high concentrations of magnesium. Constructs cultured in concentrations of magnesium of 50 mM or higher exhibited decreased mechanical properties. The tangent modulus for all tested groups (~350 kPa) was in a similar range to that which was obtained for the goat TMJ disc and mandibular condylar cartilage at 10% strain (304±141 kPa and 205±107 kPa, respectively). [5] This demonstrates that the scaffoldless constructs do achieve compressive mechanical integrity which is comparable to that of native tissue. The stress relaxation behavior of the tested constructs was slightly less than that obtained for the goat TMJ disc and mandibular condylar cartilage (85±7% and 85±6%, respectively). [5] The dissimilarities between the native tissue and the engineered constructs in regards to matrix organization would also have an impact on the tensile properties of the tissue, as aligned collagen fibers exhibit high tensile strength axially. It is important to note that this was greater than the compressive instantaneous modulus of scaffoldless constructs reported by Anderson et al. (55.0±14.7 kPa) [3] As the role of magnesium in the regeneration process becomes further understood, the appropriate design criteria for tissue engineered devices can be established. The information from this study provides essential framework for the development of devices that alleviate symptoms in patients suffering from TMDs.

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References: