Vancomycin Diffusion into Articular Cartilage and its Impact on Compressive Mechanics

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INTRODUCTION: Vancomycin and other antibiotics are commonly used in orthopaedic procedures to prevent and treat infection.[1] In vivo studies have shown that Vancomycin reduces postoperative infection risk after orthopaedic spine surgery and lowers infection rates during periarticular tibia fracture trauma surgery.[2,3] Additionally, soaking grafts in Vancomycin before anterior cruciate ligament reconstruction decreases sepsis and infection rates.[4] Despite these benefits, previous studies have reported decreased cell viability in surrounding tissues like cartilage, when indirectly exposed to Vancomycin, such as after ACL reconstruction.[1] Beyond cell viability, it remains unclear how Vancomycin affects cartilage tissue, including the depth of its penetration into the tissue and its impact on tissue mechanics. Therefore, the objectives of this study were to (1) assess Vancomycin diffusion into articular cartilage and (2) to evaluate the effects of Vancomycin treatment on the mechanical properties of the tissue.

METHODS: Vancomycin powder was reconstituted in sterile DPBS to 2.5 or 5 mg/mL.[4] Diffusion analysis: Osteochondral plugs were harvested from fresh-frozen, male, human cadaver knees. Subchondral bone was removed and cartilage plugs were soaked in Vancomycin (2.5 or 5 mg/mL) for 0, 1, 3, 6, 12 or 24 hr (2 biological + 2 technical replicates). Cartilage was washed for 5 minutes in DPBS, embedded in M1 medium, cryosectioned, and mounted on indium tin oxide slides (Bruker IntelliSlides). Samples were imaged with a timsTOF fleX MALDI-2, and images were processed with custom MATLAB code. Specified ROIs excluded the top and bottom 1 mm to minimize edge effects. Intensity values were averaged along the y-axis using 100 evenly spaced vertical lines and normalized across the sample width (0 = left edge). White pixels were excluded, data from 0.1-0.9 of the normalized width were used to reduce effects of signal loss due to washing, and maximum intensity was defined as the 95th percentile of pixel intensities. Mechanical Testing: Cartilage plugs from never-frozen, neonatal, male, bovine joints were soaked in Vancomycin (2.5 or 5 mg/mL) for 0, 1, or 24 hr, and kept at 4ºC until testing.[1] Samples were rinsed in DPBS and compressive mechanical properties were determined using an Instron 5943 testing system. Stress-relaxation tests were performed with five sequential imposed 5% strain steps.[5] Equilibrium loads were used to calculate stress, and moduli were calculated from the imposed strain and equilibrium stress values.[5] Stress relaxation (%) was calculated as $\text{Stress Relaxation } \% = \frac{\sigma_{Max} - \sigma_{Equilibrium}}{\sigma_{Max}} \times 100$. Statistical Analysis: Diffusion data were analyzed using a linear mixed-effects model. Mechanical data normality was assessed with the Shapiro-Wilk test; normally distributed data were analyzed via one-way ANOVA with Tukey's post-hoc test, while non-normal data were evaluated with the Kruskal-Wallis test and Wilcoxon pairwise comparisons. Proposed histological analysis: While data have not yet been collected, proposed methods for histological analysis are as follows and will be completed prior to the ORS 2026 annual conference. Cartilage plugs harvested from neonatal bovine joints will be soaked in Vancomycin (2.5 or 5 mg/mL) for 0, 1, or 24 hr, fixed in 10% buffered formalin, embedded, and sectioned. Histological slides will be stained with Safranin-o and Picrosirius red, followed by imaging under brightfield (Saf-O) and polarized light (Picro). This data will be used to assess the effects that Vancomycin has directly on the extracellular matrix (ECM) materials vs. indirect via cell death and will bring clarity to the mechanical test outcomes.

RESULTS: MALDI-TOF MS analysis images showed that the depth of Vancomycin diffusion into articular cartilage was dependent on time, as demonstrated by yellow pixels in Fig. 1A, representing the sodium cation adduct of the Vancomycin. When intensity was quantified and mapped over sample thickness, increased Vancomycin treatment time yielded a higher mean intensity across the sample width, Fig. 1B, with all treated samples exhibiting a higher maximum intensity compared to the control. Maximum intensity data, Fig. 1C, showed an increase in intensity with treatment time, with no differences observed between concentrations at each time point (i.e. 1 hr 2.5 mg/mL vs. 1 hr 5 mg/mL). The x-position of the maximum intensity indicated that more maximum intensity events occurred closer to the central half of the sample in specimens that underwent longer treatments (Fig. 1D). There was an increase in max stress and a trend towards an increase in stress relaxation (%) at 25% imposed strain in the control samples compared to the 24 hr 5 mg/mL samples (SR % $p = 0.056$, max stress $p = 0.016$, Fig. 2A&B). There was no statistically significant difference in Young's modulus between treated groups and control (Fig. 2C).

DISCUSSION: This study assessed the diffusion of Vancomycin into articular cartilage over a period of 24 hours and how Vancomycin treatment affected compressive mechanics. The quantification of maximum intensities showed increase of intensity with time of treatment and no statistically significant changes across concentrations at each time point, indicating that time has a greater effect on Vancomycin diffusion into articular cartilage compared to concentration. Additionally, the x-position where the maximum intensity events occurred fell into the center half of the sample more often with the longer soaks, especially for 12 and 24 hr samples at 5 mg/mL. While Young's modulus remained similar in all groups, max stress was increased in control compared to the 24 hr 5 mg/mL samples and the stress relaxation % also trended towards an increase between these groups. This suggests that treated samples had reduced ability to recover after higher imposed strain, possibly reflecting changes to the matrix. Ongoing work is focusing on assessing whether Vancomycin treatment causes alterations in the cartilage matrix via histological analysis and whether any changes to the matrix can be correlated with changes to mechanical properties. Matrix and mechanical alterations will also be assessed after Vancomycin-free culture following the 24 hr soak to determine how the tissue responds to exposure even after direct contact has stopped.

SIGNIFICANCE: These results show that Vancomycin can penetrate into articular cartilage after exposure and longer soak periods affect cartilage's ability to mechanically recover after high imposed strain. Additional histological analysis will be used to assess permanent effects on ECM.

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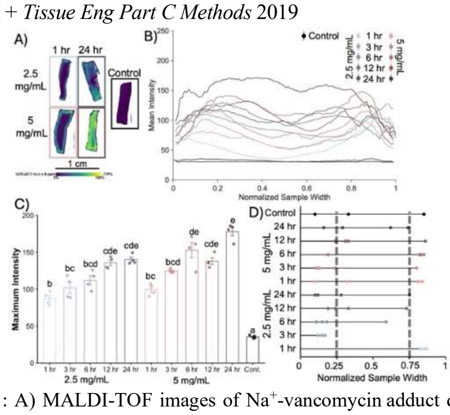

Figure 1: A) MALDI-TOF images of Na$^+$-vancomycin adduct depicting vancomycin diffusion. Yellow pixels indicate increased vancomycin. B) Intensity values of Na$^+$-vancomycin adduct images plotted against normalized sample width. Highest intensity values are assigned to yellow pixels C) Maximum intensities for each group. Different letters indicate significance between groups ($p < 0.05$)

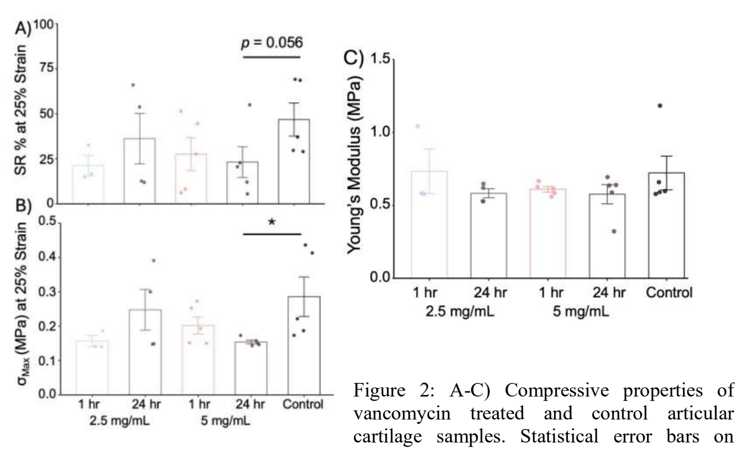

Figure 2: A-C) Compressive properties of vancomycin treated and control articular cartilage samples. Statistical error bars on graphs are ± standard deviation (*=p< 0.05).