Localized, On-Demand Antibiotic Delivery Using Ultrasound-Responsive Alginate with Chitosan Coating for Infection Prevention in Spinal Fusion

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INTRODUCTION: Postoperative infections in spinal fusion surgery pose significant risks, highlighting the need for localized antibiotic delivery. Hydrogels offer tunable mechanics and controlled release, and emulsion loading can reduce early drug loss. Ultrasound-responsive systems enable on-demand drug release, while chitosan coatings enhance mechanical stability. This study investigates alginate-based hydrogels with perfluorohexane emulsions and chitosan coatings, evaluating ultrasound-triggered vancomycin release and antibacterial efficacy against *Staphylococcus aureus*.

METHODS: Hydrogel constructs (1 mL) were fabricated from 10 mg/mL low-viscosity alginate (Sigma) crosslinked with a $CaCO_3$/GDL system at 20/40 mM (Sigma) and loaded with 0.25 mg vancomycin (Athenex). In Sample A, the drug was encapsulated within 15 µL perfluorohexane emulsions, whereas Sample B incorporated the same emulsion but was subsequently exposed to ultrasound stimulation. Sample C served as a control with vancomycin directly mixed into the alginate matrix without emulsions. Following gelation, each formulation was immersed in a 1.5% (w/v) chitosan solution for 1.5 h to produce coated variants (CHA, CHB, CHC). Ultrasound treatment was applied using an SU-101 HIFU transducer (2.5 MHz, 5.5 MPa) in rastering mode for approximately one hour. Rheological properties were assessed at 10 Hz with a Texas Instruments rheometer. Vancomycin release was monitored for seven days using a Tecan spectrophotometer, and antibacterial activity was determined by the Kirby-Bauer disk diffusion assay against methicillin-sensitive *Staphylococcus aureus* (MSSA, ATCC).

RESULTS SECTION: Sample A exhibited the greatest stiffness among the uncoated groups, with a storage modulus of 945 ± 225 Pa, while Sample B showed a slightly reduced value of 901 ± 150 Pa, and Sample C demonstrated the lowest stiffness at 670 ± 162 Pa. The addition of chitosan coatings significantly increased mechanical strength across all formulations ($p < 0.05$), with the most pronounced effect observed in CHC, which reached 6738 ± 830 Pa. Relative to the uncoated samples, the coatings enhanced stiffness by approximately fourfold in A, threefold in B, and tenfold in C. Drug release on day 1 followed the order C (~65%) > CHC (~57%) > B (~30%) > CHB (~24%) > A (~20%) > CHA (~28%). Ultrasound stimulation notably accelerated release from B, producing a 1.7-fold increase compared to A. By day 7, cumulative release rose to ~40% for A, ~49% for CHA, ~73% for B, ~53% for CHB, while both C and CHC approached full release. Antibacterial activity, measured by the Kirby–Bauer assay, revealed the largest inhibition zone for C (11.8 mm, ~0.25 mg/mL on day 1), followed by CHC (11.3 mm, ~0.19 mg/mL). Ultrasound-treated B maintained activity over time, reaching 8.2 mm (~0.03 mg/mL) at day 7, while CHB produced a smaller zone of 7.4 mm (~0.02 mg/mL) on day 1. Both A and CHA remained below 7 mm (~0.016 mg/mL), with no significant difference between them ($p > 0.99$). These results were consistent with the spectrophotometric release data. Comparisons further showed that B retained stronger activity than CHB at day 7 ($p = 0.014$). Initially, C outperformed CHC (day 0, $p = 0.028$), though this difference diminished by day 1 ($p = 0.29$) and reversed by day 7 ($p = 0.13$). Two-way ANOVA demonstrated a significant interaction between treatment group and time ($p < 0.05$), confirming the contribution of chitosan coatings to prolonging antimicrobial efficacy.

DISCUSSION: Emulsion-loaded hydrogels effectively reduced early drug loss and allowed for externally controlled, on-demand antibiotic delivery, while chitosan coatings enhanced mechanical stability without compromising antibacterial activity. Drug release patterns closely matched antimicrobial performance, showing that ultrasound stimulation prolonged efficacy and aligned with the clinical need to prevent bacterial colonization and biofilm formation beyond the early postoperative phase. Although spectrophotometric measurements occasionally overestimated release due to chitosan interference, Kirby-Bauer assays confirmed consistent antibacterial activity across formulations. Overall, the integration of emulsion retention, ultrasound responsiveness, and chitosan reinforcement positions this system as a promising strategy for localized infection control in spinal fusion surgery, warranting further *in vivo* validation.

SIGNIFICANCE/CLINICAL RELEVANCE: These adaptable hydrogels offer an ultrasound-responsive platform for localized antibiotic delivery, reducing systemic exposure and enhancing infection prevention, particularly in spinal fusion surgery.