Injectable Antibacterial Dressings for the Treatment of Chronic Rhinosinusitis (CRS)

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Introduction

- Chronic rhinosinusitis (CRS) is a chronic health condition that affects the nasal and paranasal sinus cavities.
- CRS affects approximately 11.5% of the U.S. adult population.
- CRS is associated with opportunistic bacterial invasion, recurrent infections, and biofilm formation worsening outcomes.
- Treatment options for patients with CRS include systemic and/or topical administration of antibiotics and anti-inflammatory agents.
- Systemic antibiotics can be effective against planktonic bacteria but are suboptimal in eradicating bacterial biofilms.
- Topical application of corticosteroids and antibiotics have demonstrated limited efficacy in patients due to the currently available delivery systems.
- Moreover, the predefined physical shape of the stent limits its application to patients with a permissive anatomical form of the sinus.

Objective

- Develop a tunable drug delivery system that conforms to the sinonasal cavity and provides: 1) controlled release of silver nanoparticles (AgNp) and hyaluronic acid (HA) while 2) increasing the residence time in the sinus cavity using a silk-elastinlike protein polymer (SELP).

Materials and Methods

- *In vitro* biofilm prevention assay (crystal violet (CV) assay): *P. aeruginosa* and *S. aureus* were inoculated in M63 (minimal media) in sterile 96 well plate at a concentration of 10^6 CFU/mL. Three different concentrations of AgNp along with positive and negative controls were added to individual wells and incubated at 37 ^{0}C for 24 hours at static condition. After incubation, planktonic bacteria were removed by washing the plate several times. The biofilm was stained with 0.1% CV and the plates were washed and air dried. CV attached to cells were solubilized using 30% acetic acid solution. Biofilm formed were quantified using optical density measurement at 550 nm.
- Anti-inflammatory property of HA: THP-1 cells were used to optimize the HA concentration. Briefly, THP-1 cells were polarized to macrophages with the help of PMA. The macrophages were then treated with lipopolysaccharide (LPS) (1µg/ml) and different concentrations of HA (0.5, 1.0 and 2.0 mg/mL). The anti-inflammatory property was assessed by quantifying the release of pro-inflammatory cytokines (IL-6 and IL-8) using ELISA.
- Radial diffusion assay: Plates were prepared with molten agar containing overnight cultures of *P. aeruginosa* and *S. aureus* at a concentration of 1x 10^6 CFU/mL. Plates were punctured and formulation was added to the well. Plates were incubated in a humid chamber, overnight at 37 ^{0}C in static condition. Zone of inhibition (ZOI) was measured using ImageJ.
- Rheology: Rheological measurements of different formulations were performed on Kinexus ultra+ rheometer using 2^o cone spindle. Oscillatory test was used to measure viscosity over the temperature range of 1 to 37 ^{0}C. Viscosity measurement was followed by oscillatory sweep at 37 ^{0}C with fixed frequency (6.283 rad/sec) to measure the storage (G') and loss (G'') moduli.

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Results and Discussion

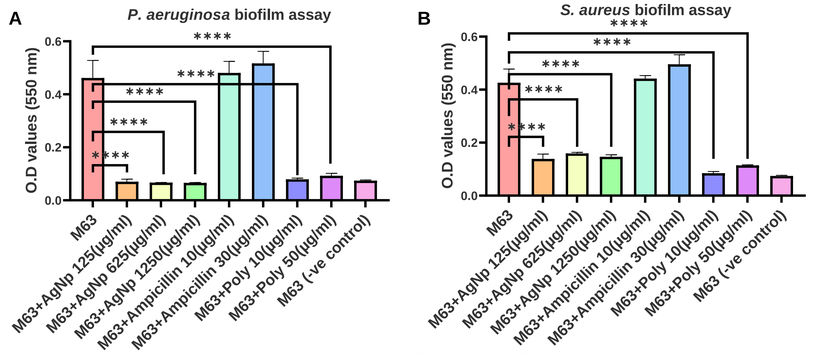

Figure 1: Biofilm prevention property of AgNp - CV assay. The concentration of AgNp tested prevented biofilm formation when tested with A) *P. aeruginosa* and B) *S. aureus* and was comparable to antibiotics.

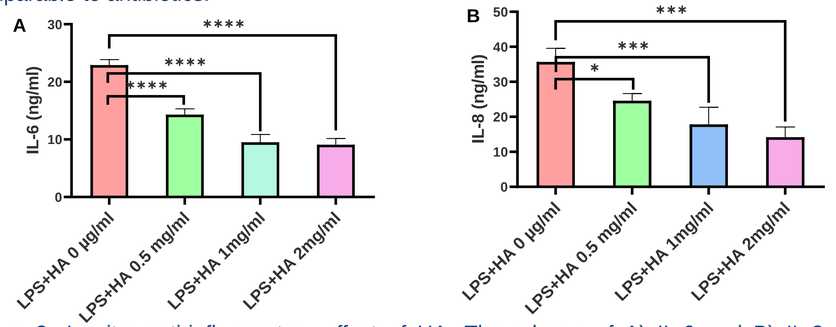

Figure 2: *In vitro* anti-inflammatory effect of HA. The release of A) IL-6 and B) IL-8 from macrophages were determined after exposing to LPS (1 µg/mL) and treating with different concentrations of HA. The presence of HA reduced the cytokine release compared to LPS exposed cells.

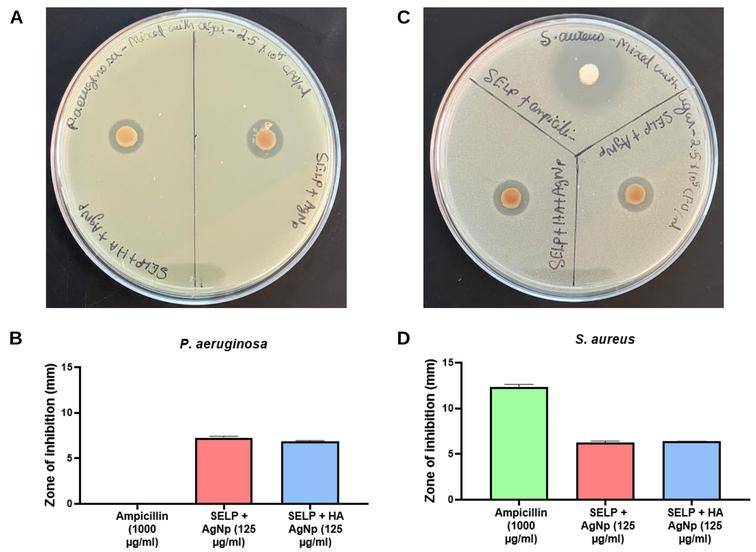

Figure 3: *In vitro* anti-bacterial effect of entire formulation – Radial diffusion assay. A) & B) ZOI and quantification of ZOI obtained for *P. aeruginosa,* respectively. C) & D) ZOI and quantification of ZOI obtained for *S. aureus,* respectively. The ZOI was similar with/without HA in the formulation. The AgNp formulation showed ZOI for both the strains tested.

Results and Discussion

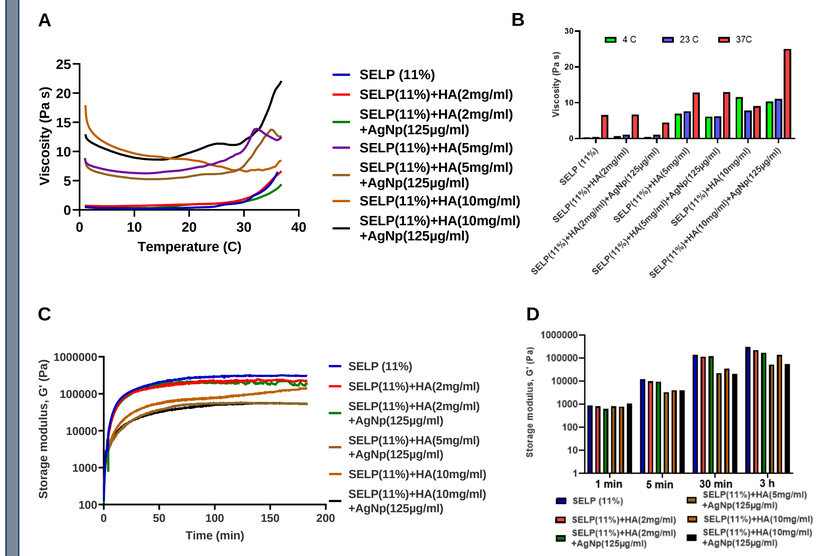

Figure 4: Rheological properties of different formulations. A) Viscosity trend as the temperature increases. B) Viscosity values at different temperature. C) Storage modulus trend. D) Storage modulus at different temperature. The rheological measurements show that incorporation of 2 mg/mL HA did not alter the thermo responsive property of SELP. The viscosity of the formulation showed that they can be easily administered through a catheter.

Conclusions

- The selected concentration of AgNp and HA show *in vitro* anti-bacterial and anti-inflammatory effect.
- The formulation containing SELP, HA and AgNp showed antibacterial effect against both the strains tested.
- The incorporation of HA and AgNp did not alter the thermoresponsive property of SELP.

Future Directions

- The log reduction of bacterial growth should be determined using viable count assay.
- Cytocompatibility of the formulation should be tested with macrophages and human nasal epithelial cells.
- *In vitro* biofilm prevention property of the formulation should be assessed using scanning electron microscopy.

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