Influence of Surface Modification on Anti-bacterial Activity of Chitosan Membranes.

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Introduction: Chitosan, a biodegradable polysaccharide with anti-bacterial properties has shown promise for use in various biomedical applications including wound dressing and tissue engineering. Although it is known to support the activity of a wide range of cells, the cell-material interactions of chitosan are not completely understood. Blending chitosan with Poly(ε-caprolactone) (PCL) to overcome limitations on its mechanical properties resulted in improved support to cellular viability compared to chitosan (Sarasam A. Biomaterials. 2005;26:5500-5508.). Mechanical properties are also tunable to some extent. The exact mode of its anti-bacterial activity has not been established although it is believed that electrostatic attraction between positively charged chitosan and negatively charged bacterial cell walls binds and breaks the cell wall resulting in cell death. The focus of this study was to investigate the surface-dependent anti-bacterial properties of chitosan to two representative strains of Gram-positive and Gram-negative bacteria.

Materials and methods: 3mL of 2% chitosan (85% deacetylated, molecular weight >310kD) dissolved in 3% acetic acid was mixed with 10mL of different concentrations of PCL (MW 80kD) dissolved in glacial acetic acid to obtain blends of various mass ratios of chitosan and PCL. Blend solutions were dried at 55°C to obtain uniform membranes. Surface analysis of membranes was done by atomic force microscopy at ambient conditions in tapping mode at a scan rate of 1Hz. Surface roughness factors were calculated by associated software. *Streptococcus mutans* (ATCC 25175) and *Actinobacillus actinomycetemcomitans* (ATCC 42719) bacteria were grown to their early growth phase in Brain Heart Infusion broth at aerobic conditions and 37°C. Membranes were cut into 2cm×2cm strips, neutralized by immersion in 90% ethanol, washed thoroughly in sterile PBS and suspended in bacterial cultures. To test the effect of neutralization, some membranes were neutralized with 1N NaOH. Transient changes in optical density of the broth were monitored. After 24hr, membranes were retrieved, fixed in 3.7% paraformaldehyde and observed under Scanning Electron Microscope.

Results and Discussion: Neutralization of chitosan with 1N NaOH resulted in increased bacterial adhesion but did not affect proliferation (Figure 1), whereas ethanol neutralization inhibited adhesion although it did not lower

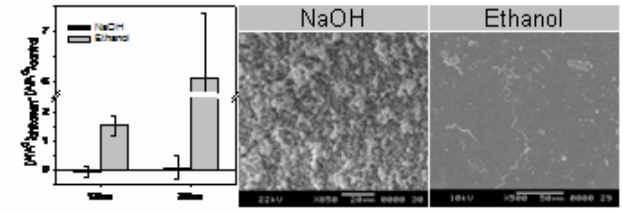

Figure 1. Effect of neutralization of chitosan membranes on proliferation and adherence of *Streptococcus mutans*.

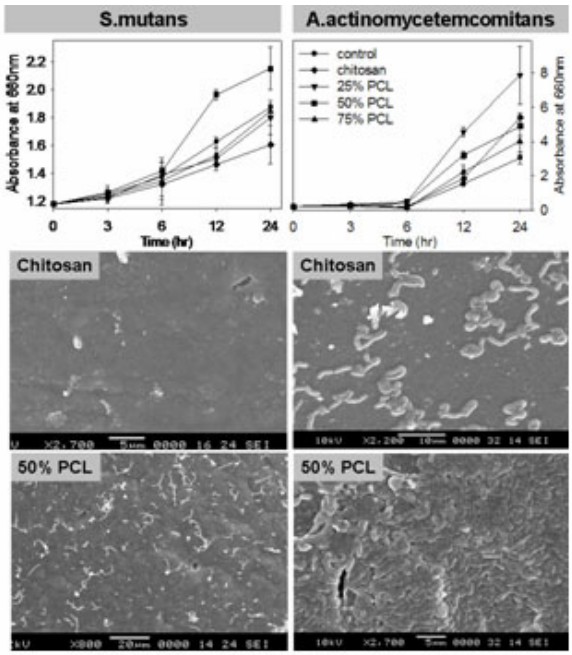

Figure 2. Anti-bacterial activity of chitosan-PCL blend membranes: Transient changes in optical density of bacterial broths and SEM images of bacterial adherence.

proliferation. This suggests that anti-bacterial activity of chitosan is contact dependent. Further, the anti-adherence property of chitosan was lost upon blending with PCL which could be attributed to non-antibacterial nature of PCL (Figure 2). Although it was not bactericidal, chitosan supported least growth and was more anti-bacterial to Gram positive *S.mutans* than to Gram-negative *A.actinomycetemcomitans*. AFM analysis indicated that roughness of chitosan increased to a maximum in 50% PCL (Figure 3). Topographic images also showed preferential orientation of fibers on the blends. Increased roughness might be more conducive for growth of cells and bacteria. Blending with hydrophobic PCL might also have altered the hydrophilicity of chitosan which in turn influences protein adsorption and adherence.

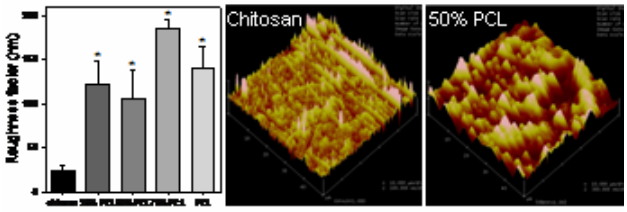

Figure 3. Surface analysis by AFM: Roughness factors and height images of membranes.

Conclusions: Anti-bacterial activity of chitosan was highly contact-dependent. Surface modifications by different neutralization methods and blending with PCL resulted in altered bacterial activity which was attributed to changes in charge distribution and roughness of chitosan membranes.