Biofilm-Degrading Enzyme Delivery from Chitosan Nanoparticles

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Statement of Purpose: Catheter-related blood stream infections (CRBSI) are the leading cause of nosocomial blood stream infections and are associated with significant morbidity and mortality in critically ill patients. Treatment for CRBSI is often difficult due to the microorganism's development of resistance to the drug being used. Development of the resistance is directly correlated with the formation of biofilms, caused when bacteria adhere to the surface of the catheters in community-like complexes[1]. In order, to overcome antibiotic resistance a drug delivery vehicle composed of chitosan crosslinked with tripolyphosphate (TPP)[2] and genipin was loaded with a biofilm degrading enzyme, β-N-Acetylglucosaminidase (NAG). Nanoparticle processing parameters were then studied. We hypothesize that sustained release of NAG for biofilm degradation can reduce the formation of biofilms and increase the effectiveness of antibiotics to aid in reducing CRBSIs.

Methods: Low molecular weight chitosan (>75% deacetylation, 23 cps) was dissolved in dilute .875 % acetic acid. TPP was dissolved in D.I. water at a .5 % concentration and the pH adjusted to 4.5. The two solutions were then mixed at a 2.5:1 (CS/TPP) ratio while being sonicated (Microson XL 2000, Misonix) at room temperature. Lastly, a 1 mM genipin solution (dissolved in D.I. Water) was added to the nanoparticles at the same ratio as TPP. Nanoparticles were separated through high speed centrifugation (Sorvall RC-6) at 40,000 x g for 30 minutes at 10°C. The nanoparticles were imaged at 1 kV by SEM (Carl Ziess EVO 40) after being air dried on a copper TEM grid and sputter coated with gold palladium. Swelling properties of nanoparticles at pH 7 (phosphate buffered saline) and pH 5 (sodium citrate buffer) were characterized by size changes measured by digital light scattering (DLS). A model protein, Bovine Serum Albumin (BSA) was loaded into the nanoparticles by mixing the enzyme with chitosan (5 mg/mL) before particle formation. After centrifugation, the supernatant was measured using a spectrophotometer and then compared against a standard curve. The encapsulation efficiency was determined by following (Total Amt. BSA – Free Amt. BSA)/(Total Amt. BSA). The release of BSA from the particles was measured in pH 7 and pH 5 buffers at 0, 1, 2, 4 and 8 hours.

Results:
Images of chitosan nanoparticles prepared via ion interactions with TPP and genipin were taken. Figure 1 shows spherical particles with the average size of 328.5 nm.

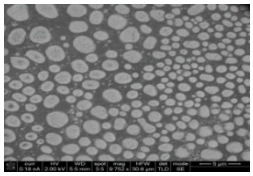

Figure 1: SEM CS Nanoparticles

In the swelling study it was seen that the nanoparticles resuspended in pH 5 buffer swelled to about 1 µm greater than those in the pH 7 buffer.

BSA was first used to determine encapsulation efficiency. The highest efficiency was 55.5 % for the 1 mg/mL BSA. After 4 hours, it was observed that 0.4 mg/mL BSA was released from the particles at pH 5 buffer at hour 4 and continued to increase. Little release was seen when the particles were in pH 7 buffer (Figure 3).

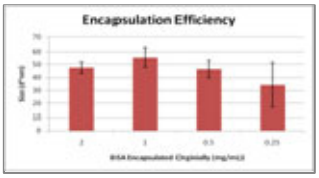

Figure 2: Encapsulation Efficiency

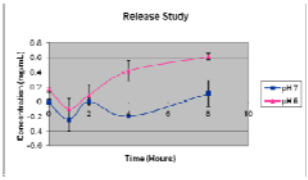

Figure 3: Release Study

Conclusions: From the image studies it can be seen that the nanoparticles are spherical. This is desired; in order, to have controlled uniform release. The average size of the nanoparticles was 328.5 nm, which correlates to the same diameters found in other studies that measured size by DLS.

It was also shown that the nanoparticles exhibit pH dependent swelling properties. This is necessary; in order, for the NAG to diffuse outwards in the pH 5 microenvironments of biofilms. It is also important because NAG is active at pH 5. The encapsulation efficiency is approximately 55.5 % but a higher efficiency is desired, meaning the nanoparticles must be optimized by varying the molecular weight of the CS and pH of the TPP solution. This also needs to be done in regards to the release of the enzyme since the data does not show a burst release; instead, release starts at approximately hour 4. The release study also does not follow the swelling trend. It is hypothesized that this is due to the noise in the measuring method. Future studies will also include encapsulating the enzyme after the genipin crosslinker is used to reduce the potential of the enzyme crosslinking.

In conclusion more studies need to be completed to better optimize the nanoparticles for this application. Additionally, a more accurate method for measuring enzyme release needs to be explored before NAG is encapsulated. However, it is hypothesized that the nanoparticles have the potential to degrade at pH 5; thereby, releasing the active NAG to degrade the biofilms.

References:

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2. P. Calvo, C. Remuñān-López, J.L. Vila-Jato, and M.J. Alonso. "Novel Hydrophilic Chitosan-Polyethylene Oxide Nanoparticle as Protein Carriers." Journal of Applied Polymer Science (1997) 63:125-132