

Hybrid polymer-mesoporous silica nanoparticles for simultaneous controlled release of proteins and antibiotics

Sanjib Bhattacharyya, Rowland Han, Paul Ducheyne

Center for Bioactive Materials & Tissue Engineering, Dept. of Bioengineering, University of Pennsylvania, Philadelphia, PA

Statement of Purpose: Newly designed controlled release systems intend to deliver multiple biomolecules for well-defined duration and concentrations to a target site [1]. Before, controlled release concepts were oftentimes limited to the delivery of one drug. Still there are many clinically relevant conditions where a delivery system capable of releasing multiple molecules with different properties such as molecular size and pharmacokinetics would be useful. This applies to bone tissue growth stimulation conditions.

Tissue engineering in general, and bone tissue engineering treatments in specific rely on an optimal combination of scaffolds, growth factors and cells. Therefore, appropriate sustained controlled release of large protein molecules and growth factors is desirable, and the scaffold/carrier material must be of a type that can be localized at the site of bone growth stimulation. As there is always the risk in these bone repair and regeneration conditions that the synthetic bone graft carrier might function as a nidus for infection, infection prevention and treatment is also a paramount consideration. Thus, a controlled release system which is capable of delivering large protein molecules along with antibiotics that can be applied at the site is highly desirable for bone tissue growth stimulation in a variety of clinical conditions.

The objective of this work is to design a composite hybrid drug delivery system which can deliver simultaneously large protein molecules and antibiotics in controlled fashion for extended periods of time. In this study we designed a composite, hybrid drug delivery system which combines an inorganic mesoporous silica nanosphere (MSN) core, with a grafted chitosan surface layer. Trypsin inhibitor (TI), a model protein for bone growth factors and vancomycin, a potent antibiotic commonly used to combat MRSA infection, were incorporated. Herein, we report the simultaneous in-vitro release kinetics over weeks of duration. We also demonstrate that biological activity of both molecules is retained upon release.

Methods: The MSNs were prepared as follows: 1.00 g octadecyltrimethylammonium bromide ($C_{18}TAB$) was dissolved in 480 ml distilled water at 75 °C. The solution was made basic (pH~12) by the addition of 3.5 ml of 2.00M sodium hydroxide (NaOH). After reaching a stable pH=12, 5 ml tetraethylorthosilicate (TEOS) was added dropwise. The reaction temperature was maintained at 75 °C for 2 hrs to give rise to white precipitates. The mixture was filtered and washed several times with deionized water until the supernatant showed a neutral pH (~7). The samples were then dried in vacuum at 80 °C for 2 days. After the synthesis the pores of the MSNs were expanded by hydrothermal treatment. Finally the pore expanded MSNs were calcined at 550 °C for half an hour to remove the surfactant from pores. We synthesized two controlled release systems one of which comprised of TI loaded inside the pores of MSNs and vancomycin within the chitosan surface layer. In the other

system vancomycin was incorporated within the pores of MSNs and TI was loaded in the chitosan surface layer.

Results: Figure 1(a) shows the simultaneous release profile of TI and vancomycin. It is evident that both TI and vancomycin can be released over 5 weeks of time in controlled fashion when the molecules are incorporated within the mesopores of MSNs or in the surface chitosan layer. The biological activity of released TI was determined by using the trypsin inhibitor enzymatic assays (Figure 1(b)), in which the values on the vertical axis correspond to levels of trypsin activity. Since TI reduces the trypsin activity, biologically active TI should reduce the activity of trypsin (Y-axis variable). Thus, all of the curves should slope downward, with steeper slopes corresponding to greater TI potency (increase in TI content leads to a greater decrease in trypsin activity). Figure 1(b) shows the TI activity for 1, 6 and 14 days of release duration. Stock TI dissolved in cold sodium phosphate buffer was used as control. TI released from the nanospheres at different durations demonstrated the normal enzymatic activity comparable to control.

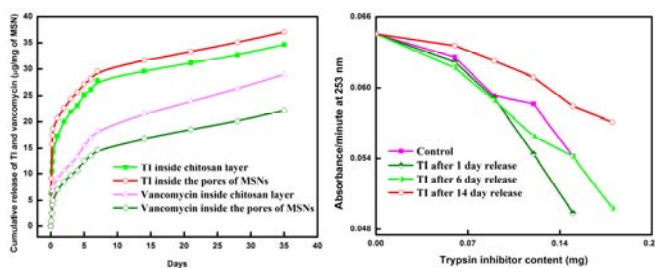


Figure 1(a)

Figure 1(b)

Conclusions: Focusing on the controlled and sustained release of dual molecules of different size simultaneously at the intended site, we synthesized hybrid biopolymer/mesoporous silica nanoparticles controlled release system composed of mesoporous silica nanoparticles as the core with the biopolymer coating on the surface. Prolonged release for at least 5 weeks was observed for both trypsin inhibitor, a protein used to model bone growth factors, and the antibiotic vancomycin. Furthermore, it was demonstrated that both trypsin inhibitor and vancomycin released from the chitosan-MSN materials retain their respective biological activity for the whole duration of release.

References: 1. Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. *Nat. Biotechnol* 2001;19:1029–1034.