**Development of a Multi-functional Red Blood Cell Analog**

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**Introduction:** Since 1990s numerous groups have been trying to develop a red blood cell analog due to the HIV epidemic of the time [1,2]. Even though some of these substitutes are now in phase III of clinical trials, their use is very limited due to side effects and short half life time within the human body [3]. As a result, there is still a need for an effective erythrocyte analog with minimum immunogenic and side effects, so that it can be used for multiple applications. Besides the imperative need of a blood substitute for in vivo use, there is also a need of it for in-vitro testing of medical devices and products. In this study we investigated the use of synthetic polymers and natural biodegradable polymers as possible materials for the development of biodegradable, biocompatible, and multi-functional particles with rheological characteristics similar to erythrocytes for in vitro testing of medical devices and products, and as a drug delivery system.

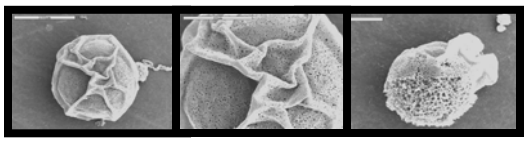
**Methods:** Polyester microspheres were prepared by the water-oil-water (W/O/W) emulsion technique. The polymer used was D,L-poly lactic acid (PLA) (Mw 350 kDa) from Birmingham Polymers. In addition to PLA, pluronics® with different HLB (kindly donated by BASF) were used as surfactants. Pluronic® was dissolved at different concentrations in phosphate buffer saline (PBS), pH 7.4, and emulsified in methylene chloride containing 2% (w/w) PLA. This emulsion was added to 1.5% polyvinyl alcohol (PVA) solution at 4°C under continuous stirring at 1500 rpm for 30 min.

Natural polymeric microspheres were prepared by an air-sprayed crosslinking technique. The polymer used was alginate from Keltone (LV). Calcium chloride and copper nitrate at different concentrations were used as crosslinking solutions. Sodium alginate was dissolved in $dH_2O$ at different concentrations. The alginate solution was extruded through the inner lumen of a double device into the stirred crosslinking solution.

Ca-alginate particles and Cu-alginate particles were coated with high molecular weight chitosan and chitosan oligosaccharide lactate (Mw < 5000) from Aldrich. A chitosan solution containing $CaCl_2$, at different concentrations, was added to crosslinked-alginate particles dispersed in $dH_2O$. Different batches were made differing in the concentrations of chitosan solutions and number of coatings. Particles were coated one, five, and nine times. For multiple-layer particles, chitosan and alginate coatings were used in an alternating way. After coating the particles, crosslinking ions were removed by using EDTA from Sigma and albumin from Cellgro.

To characterize the particles, three tests were performed: SEM analysis, size distribution, and micropipette aspiration technique. Surface morphology of the microspheres was examined with SEM after gold-palladium coating, and size distribution was determined with a light microscope and Axiovision imaging software (from Zeiss). To test particles' deformability, the micropipette aspiration technique was used [4].

**Results / Discussion:** Microspheres made with PLA and pluronics® were not deformable when using the micropipette technique, except for particles made with PLA and P105. In addition to elasticity properties, surface morphology was different between particles made with PLA and all other pluronics® and PLA-P105 particles. The latter presented a core-shell structure (Figure 1). The PLA core was very porous as well as the P105 shell. During micropipette experiments, it was seen that the shell was deformable although the core was not.



**A** **B** **C**

**Figure 1.** SEM of PLA-P105 Particles. (A) 400X, (B) 1000X, and (C) 800X.

Crosslinked-alginate particles coated with high molecular weight chitosan did not yield good results; therefore, only chitosan oligosaccharide was used for all further coatings. Coated Ca-alginate particles were not deformable when using the micropipette technique. Furthermore, mono-layer and multiple-layer coated particles were not stable after removing the calcium ions. Coated Cu-alginate particles were not deformable when using the micropipette as well. However, when copper ions were removed, particles presented elastic properties. Particles deformed and were completely aspirated into the micropipette tip. Best results were obtained with Cu-alginate particles coated five times (Figure 2).

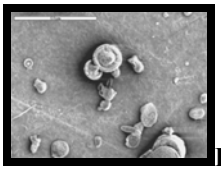


**Figure 2.** SEM of Chitosan-coated Cu-alginate particles (1000X).

**Conclusions:** After removal of copper ions, multiple-layer coated copper-alginate particles presented deformability properties similar to erythrocytes' under micropipette aspiration. Particle stability after ion removal can be enhanced by multiple coatings with low molecular weight (high percent deacetylation) chitosan oligosaccharide. Further studies need to be conducted to compare other rheological properties of red blood cells and our particles. In addition, cell studies are needed to assess the particles' immunogenic effects.

**References:** [1] (Chang, TMS. Artificial Organs. 2004;28 (3):265-270). [2] (Spahn, D. Adv Drug Deliv Rev. 2000;40:143-151). [3] (Winslow, RM. Adv Drug Deliv Rev. 2000;40:131-142). [4] (Thomas, SJ. Transfusion. 2003;43:502-508).