Development of a Multi-functional Red Blood Cell Analog Using Polyelectrolyte Complex Microparticles

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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. This study is focused on the synthesis and characterization of polyelectrolyte complex (PEC) chitosan-alginate microparticles. Physico-chemical and rheology properties of the PEC microparticles crosslinked with copper ions were studied to determine the set of parameters that would yield microspheres with deformable properties for applications in biomimesis of erythrocytes and drug delivery.

Methods: Crosslinked alginate microspheres were prepared by a modified water-in-oil (W/O) emulsion/gelation technique. The polymer used was alginate from Keltone (LV). Copper nitrate (Sigma) was used as the crosslinking agent. Sodium alginate was dissolved in dH_2O at a 3% (w/v) concentration and emulsified in an oil phase containing cyclohexane (Sigma) and Pluronic® L61 (kindly donated by BASF). A second aqueous solution containing 0.5M of the crosslinking agent was added to the emulsion under continuous stirring. After separation of the two phases, particles were collected by filtration through a 45-µm mesh.

Cu-alginate microparticles were coated using the layer-by-layer (LbL) absorption technique. The coating always started with the positive polymer and ended with the negative polymer. Microcapsules with zero, two, six, and ten alternating layers were fabricated. The cationic polymer used was a high-deacetylated, low-molecular-weight chitosan oligosaccharide lactate (Mw < 5000, 90% deacetylation) from Aldrich. Alginate was the anionic polymer used. After coating the particles, crosslinking ions were removed.

Surface morphology of the microparticles was examined with SEM after gold-palladium coating, and size distribution was determined with the Coulter LS13320 (from Beckman). To test particles' deformability, the micropipette aspiration technique was used [4]. The viscosity and stability of the particles in simulating plasma medium was measured using the Wells-Brookfiled Cone/Plate Digital Viscometer System (Brookfield, Stoughton, MA) with a CP-52 conical spindle. The MTT cell proliferation assay was used to determine cell survival and recovery.

Results / Discussion: Particles presented a narrowed-range size distribution; diameter of uncoated particles ranged from 10-20 µm. Particle size decreased when increasing the number of coatings. PEC microparticles presented a very rough surface morphology with some aggregations which is attributed to the PEC coatings (Figure 1). After removing cross-linkers of coated microspheres, alginate capsules were deformable and remained stable using the micropipette technique under physiological pressures (Figure 2). Viscosity values of uncoated microparticle suspensions were significantly higher compared to the viscosity of blood. However, the average viscosity for solutions of bilayer PEC particles was 5.90 mPa•s, which is not significantly different from the viscosity values of human blood (Figure 3). The highest level of toxicity was shown by uncoated Cu-crosslinked particles at 1000 µg/ml. The rest of the samples seemed not to affect cells at the conditions studied.

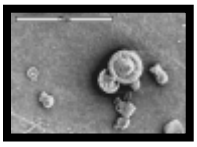

Figure 1. SEM of PEC chitosan-alginate microspheres. Original magnification = x1000; bar denotes 50 µm.

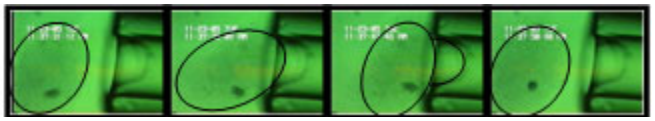

Figure 2. Optical micrograph of hexa-layer PEC capsule during micropipette aspiration. Original magnification = x400.

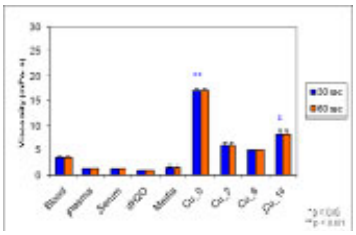

Figure 3. Viscosity values of PEC microparticles. Viscosity values for blood, plasma, and serum were obtained from Rosenson [5].

Conclusions: After removal of copper ions, multiple-layer microspheres presented deformability properties under micropipette aspiration. Particles suspended in simulated plasma presented viscosity values similar to blood's values. Stability of particles after ion removal can be enhanced by multiple coatings. In addition, multiple coatings decreased toxicity of heavy-metal crosslinked particles.

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). [5] (Rosenson. Clinical Chemistry 42, 1996, No. 8, 1189-1195).