Effects of heavy metals in the development of multi-functional microparticles with deformable properties

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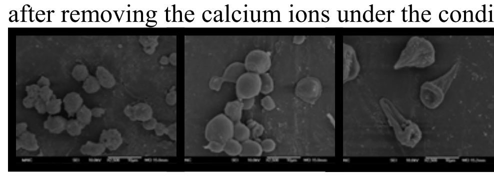
Introduction: Since 1990s numerous groups have been trying to develop a red blood cell analog due to the HIV epidemic of the time [1,2]. Even though some of these substitutes are now in phase III of clinical trials, their use is very limited due to side effects and short half life time within the human 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 different crosslinkers for the development of polyelectrolyte complex 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). Calcium chloride, zinc chloride, and copper nitrate (Sigma) were used as crosslinking solutions. 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 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.

Ca-, Zn-, and Cu-alginate particles 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 by using DMEM cell culture medium supplemented with bovine serum albumin (BSA).

To characterize the particles, four tests were performed: SEM analysis, size distribution, micropipette aspiration, and cytotoxicity studies. Surface morphology of the microspheres 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 MTT cell proliferation assay was used to determine cell survival and recovery. Two time points were studied: a short-term test to demonstrate particles' toxic effects on cells and a long-term test to demonstrate survival, the retention of cell regenerative capacity.

Results / Discussion: Particles made with W/O emulsion/gelation technique using cyclohexane as the organic phase presented a narrowed-range size distribution; diameter of uncoated particles ranged from 5-15 µm. Particle size decreased when increasing the number of coatings. There was approximately a 50% size reduction after the tenth coating. Particles crosslinked with copper presented a tear-drop shape as oppose to the spherical shape of particles crosslinked with zinc and calcium (Figure1). After removing cross-linkers of coated microspheres, zinc- and copper-alginate capsules were deformable and remained stable using the micropipette technique under physiological pressures. Coated calcium-alginate particles were not stable after removing the calcium ions under the conditions tested.



A B C

Figure 1. SEM of (A) calcium-, (B) zinc-, and (C) copper-crosslinked alginate microspheres. Original magnification = x2500; bar denotes 10 µm.

Results from the MTT assay are presented in Figure 2. The highest level of toxicity was shown by uncoated copper- and zinc-crosslinked particles at 1000 µg/ml. The rest of the samples seemed not to affect cells at the conditions studied.

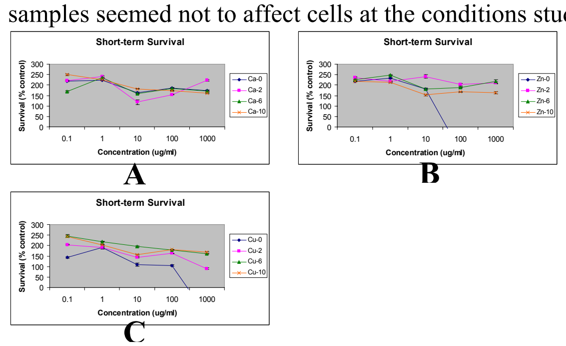


Figure 2. Survival of human fibroblast cells exposed to (A) calcium-, (B) zinc-, and (C) copper-crosslinked alginate microspheres at different concentrations.

Conclusions: After removal of copper ions, multiple-layer coated zinc- and copper-alginate microspheres presented deformability properties under micropipette aspiration. Particle stability after ion removal can be enhanced by multiple coatings with low molecular weight (high percent deacetylation) chitosan oligosaccharide. In addition, multiple coatings decreased toxicity of heavy-metal crosslinked particles. Further studies need to be conducted to compare other rheological properties of red blood cells and our 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).