Layer-by-Layer nanoshell build-up onto human Red Blood Cells

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Statement of Purpose: A three dimensional film composed of polyelectrolytes multilayers was built on human red blood cells (RBCs) via the layer-by-layer self assembly technique, which involves the stepwise assembly of cationic and anionic polymers (Decher, G. Science. 1997; 277:1232-37). Chitosan (CH) (Illum, L. 1998; 15:1326–1331) and hyaluronic acid (HA) (Presti D. Cell Biochem Funct. 1994; 12:281-8) were chosen for this study since they are natural, biocompatible and non-immunogenic polymers of opposite charge. The aim of this study is to characterize the coated red blood cells for their application in blood transfusion medicine.

Methods: RBCs were coated via the layer-by-layer self assembly technique which consists in the alternate adsorption of chitosan and hyaluronic acid (Thierry B. Biomacromolecules. 2003; 4: 1564-71). The removal of free unbound polymers was achieved by the membrane filtration technique (Voigt A. Ind. Eng. Chem. Res. 1999; 38: 4037-4043). The coating of each layer was investigated by measuring the zeta potential with the ZetaPlus instrument (Brookhaven Instruments Corporation). Optical microscopy was used to study the RBCs shape and morphology following the coating. Polymer coverage on the coated RBCs surfaces was assessed by confocal microscopy using labeled polymers, rhodamine-CH and fluorescein isothiocyanate-HA. The polyelectrolyte multilayers surface mass on RBCs was monitored by Quartz Crystal Microbalance with energy Dissipation monitoring (QCM-D).

Results/Discussion: Zeta potential studies showed a charge alternation between +30 mV and -50 mV (figure 1). This confirms the successful layers build-up on RBCs. By using the optical microscopy analysis, it was possible to observe the integrity of the RBCs shape and morphology after the coating. The coated RBCs with the labeled polymers reveal a very good coverage on RBCs surface by confocal microscopy and this is demonstrated by a higher intensity on edge RBCs (figure 2). The QCM technique allows the layer by layer build up tracking on the RBCs in real time (figure 3). The mass (µg/cm^2) onto the silica crystal surface without RBCs is negligeable in comparison with the build-up with the RBCs.

Conclusions: Polyelectrolyte multilayers were successfully assembled in nano-organized shells on red blood cells through an electrostatic layer-by-layer self assembly technique. The RBCs's shape and morphology were not affected by the polyelectrolytes multilayers build-up. The second part of this study is to investigate the immunologic reaction of different antibodies with the coated RBCs.

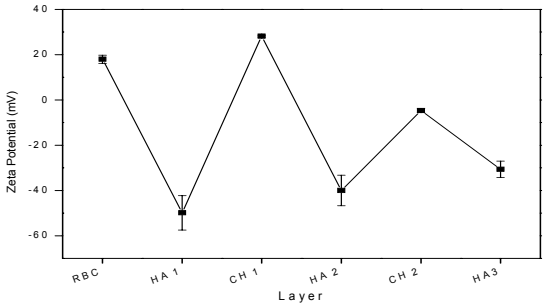

Figure 1: Zeta potential of RBC vs layer deposition of hyaluronic acid and chitosan

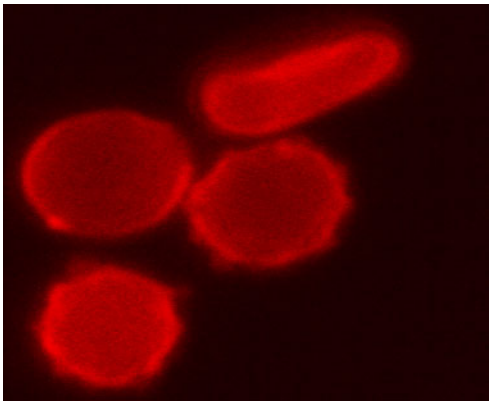

Figure 2: Confocal microscopy of RBCs coated with hyaluronic acid and chitosan. The outer membrane is chitosan-rhodamine.

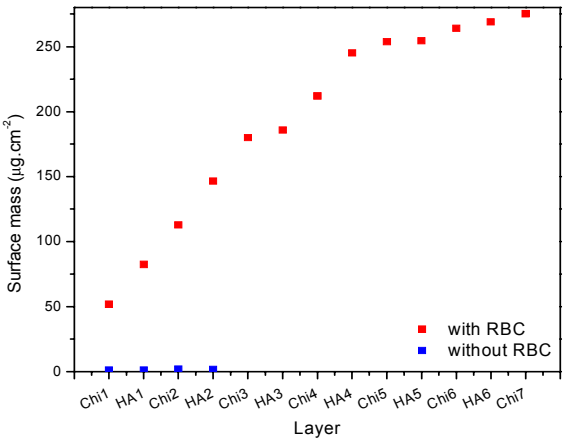

Figure 3: Mass (µg/cm^2) of different chitosan and hyaluronic acid layers onto the crystal surface with and without red blood cells.