Bioengineering of red blood cells by the Layer-by-Layer self-assembly technique

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Statement of Purpose: Shortages in the blood supply have potential life-threatening consequences for patients requiring rapid and urgent blood transfusion. We report here a new means of inducing the immunocamouflage of red blood cells (RBCs) using the Layer-by-Layer (LbL) deposition of Chitosan-graft-phosphoryl choline (CH-PC), Poly-L-lysine-graft-polyethylene glycol and Alginate (AL) onto functional RBCs. The aim, therefore, was to coat fresh and functional red blood cells (RBCs) with a multilayered polymeric shell. We then assessed their interaction with anti-blood group antibodies for their potential application in blood transfusion medicine.

Methods: RBCs were coated via the layer-by-layer self-assembly technique with the alternate adsorption of CH-PC, PLL-PEG and AL.[1] The shell build-up was monitored by quartz crystal microbalance with dissipation factor (QCM-D) upon the deposition of each polyelectrolyte layer. A transmission electron microscopy (TEM) was used to reveal the formed multilayers on the RBC surface. The integrity of the coated RBCs was assessed by means of optical microscopy, and by measuring the percentage of lysis spectrophotometrically at 540 nm using a Drabkin's reagent. Furthermore, by using fluorescence microscopy, antibodies interactions with the coated and non-coated RBC were investigated.

Results: The pH of the solution was kept at 6.2 in order to ensure sufficient protonation of the amine groups of CH-PC.[2] A set of 4 bilayers [(AL/CH-PC)$_4$] was deposited onto RBCs suspended in 10 mM PBS pH 6.2, followed by a set of 5 layers [(AL/PLL-PEG)$_2$/AL)].[3] The frequency and dissipation shift after the addition of the polymeric layers confirmed the successful and stable layers build-up on the RBCs (Figure 1). The thickness of the shell was estimated to be ~ 300 nm, based on the observation of transmission electron micrographs of coated RBCs. Optical micrographs of bare and coated RBCs confirmed that the morphology of the cells was preserved upon LbL construction. The percentage of hemolysis is 3.4% after each coated layer which is similar to the non-coated cells. Fluorescence microscopy demonstrated that the successful build-up of the polymeric system of CH-PC, PLL-PEG and AL on the RBCs prevented the interaction of the anti-A and CD44 antibodies with the antigens at the RBC's surface (Figure 2).

Conclusion: Polyelectrolyte multilayers were successfully assembled in nano-organized shell on RBCs through an electrostatic layer-by-layer self-assembly technique. The RBCs' shape, morphology were not affected by the polyelectrolytes multilayers build-up. The multilayered nanoshell composed of CH-PC, PLL-PEG and AL built on non-fixed RBCs succeeded in preventing antibodies interactions with the specific antigens on the RBC surfaces. This approach is low cost and effective with a potential application in the production of universal red blood cells.

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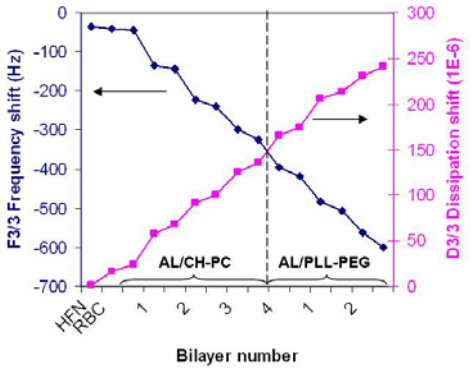

Figure 1. Evolution of frequency and dissipation shifts during the LbL build-up of AL and CH-PC for bilayer 1 to bilayer 4 followed by the alternation between AL and PLL-PEG from bilayer 1 to 2.5 deposited on RBCs' surface.

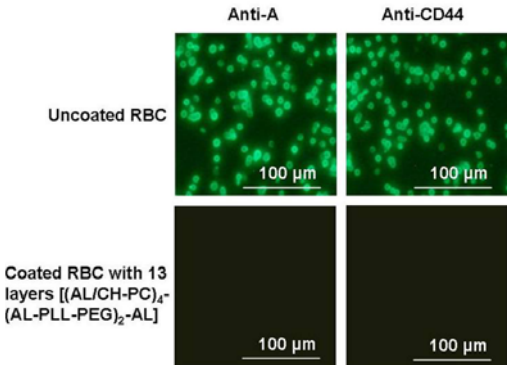

Figure 2. Fluorescence micrographs of uncoated and coated RBCs with 13 layers [(AL/CH-PC)4-(AL/PLL-PEG)2/AL)]. The binding of anti-A (at 1000x dilution) and anti-CD44 (at 200x dilution) to uncoated RBCs.

Reference:

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