Layer-by-Layer Assembly of Biocompatible Polyelectrolytes on pH and Temperature Responsive Microgels

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Statement of Purpose: Layer-by-layer (LbL) sequential adsorption of both synthetic and natural-derived polyelectrolyte pairs, known to mediate biological processes such as cell and protein adhesion, were successfully assembled on temperature- and pH-sensitive poly (*N*-isopropylacrylamide)-co-(methacrylic acid), PNIPAAm-PMAA, microgels: the polyssacharides chitosan, CHI, with dextran sulfate, DS; the polypeptides poly (L-lysine), PLL, with poly (glutamic acid), PGA; and the synthetic polymers poly (allylamine hydrochloride), PAH, with poly (acrylic acid), PAA. The microgels were initially prepared by polymerization in supercritical carbon dioxide ($scCO_2$), rendering pure, monodisperse microparticles. The impact of the polyelectrolytes assembly conditions on the stimuli-responsive behavior of the coated microgels was assessed. PNIPAAm-PMAA microgels exhibit reversible swelling responses to both temperature and pH stimuli. These systems have great potential for applications in which environmentally triggered changes in size, water content or net charge may be explored, namely for biosensing or controlled drug delivery (1).

LbL assembly has attracted extensive attention as a versatile method to control surface properties of materials and incorporate high-loadings of biomolecules (2). It has been shown that LbL sequential adsorption of polyssacharides and polypeptides onto PNIPAAm-PMAA microgels changes the swelling degree and native thermoresponsive behavior depending on the assembly conditions (3). Herein, these polymers as well as biocompatible synthetic polyelectrolytes were explored for coating the PNIPAAm-PMAA microgels to assess the potential of LbL as a method for further functionalization of these systems.

Methods: Synthesis of cross-linked microgels containing 90 mol % of NIPAAm and 10 mol % of MAA was performed in $scCO_2$. More details on the polymerization were subject of a separate study.

The LbL assembly was performed by adding a microgels aqueous dispersion (pH~5) to a polyelectrolyte solution to final microgel concentration of 0.5 mg/mL and stirring for 15 min. Since the microgels were negatively charged the first layer was always a polycation (CHI, PLL or PAH). The solutions pH was adjusted to a value at which the polyelectrolytes were mostly ionized: pH 4 for CHI and 6 for DS; pH 7.4 for both PLL and PGA; and pH 6 for PAH and 9 for PAA. The excess of polyelectrolyte was removed by three centrifugation cycles. Zeta potential measurements were performed at assembly pH in a ZetaPALS analyzer (Brookhaven Instruments Corp). Microgel size at 24 ^{o}C and 37 ^{o}C was determined using a Zeiss Axioskop 2 optical microscope with temperature control (THMS 600 hot stage, Linkam Scientific Instruments). The Fourier-transform infrared spectra (FT-IR) of the microgels and lyophilized coated microgels were obtained in a Thermo Nicolet 6700 FT-IR.

Results: The synthesized PNIPAAm-PMAA microgels present thermoresponsive behavior according to the dispersion pH: while at pH 4, below the acrylic acid pK_a (~ 5), the microgels diameter decreases upon heating, from 17.2 ± 3.0 μm at 24 ^{o}C to 5.6 ± 0.4 μm at 37 ^{o}C, no significant differences in microgel swelling are observed at pH 7. Upon adsorption of the polycations, microgel diameter decreased, especially for PLL at pH 7.4 and PAH at pH 6, and volume phase transition is observed. Probably, the interaction between the polycations and the MAA groups allowed the thermodynamically-driven coil-to-globule transition of the NIPAAm segments of the microgels. The subsequent adsorption of the polyanions lead to an increase in microgel diameter to slightly above the uncoated value. Furthermore, thermoresponsive behavior at physiological conditions was not observed, as the polycation below is now interacting with both the MAA groups and polyanion. The successful LbL assembly of the polyelectrolytes was also shown by charge reversal upon sequential adsorption of polycation and polyanion as assessed from zeta potential measurements (Fig. 1).

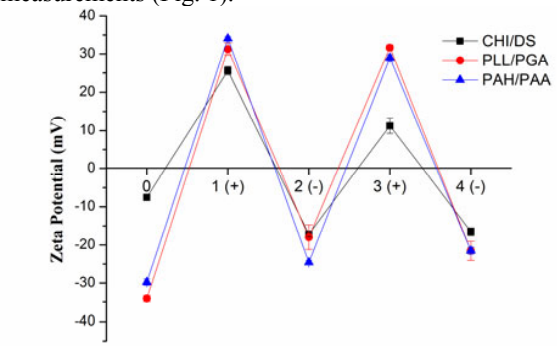

Figure 1. Zeta Potential measurements as a function of the layers assembled on PNIPAAm-PMAA microgels.

In addition, there was an increase in the relative proportion of the polyelectrolytes characteristic bands on the FT-IR spectra of the assembled microgels.

Conclusions: The successful LbL assembly of biocompatible synthetic and natural-derived polymers on PNIPAAm-PMAA microgels was shown by size and zeta potential measurements, as well as FT-IR spectral analysis. This method may be used to further tune PNIPAAm-PMAA responsive microgels and/or incorporate bioactive species. Future studies include assessing the impact of LbL assembly on the microgels mechanical properties and on their interaction with cells.

References: (1) Hoare, T., Langmuir. 2008; 24: 1005-1012.

(2) Picart, C. Curr med chem. 2008. 15: 685-697.

(3)Díez-Pascual, A.M. J Colloid Interface Sci. 2010. 347: 79-89.