Layer-by-Layer Assembly on Hydrogel Surfaces and Control of Human Whole Blood Coagulation

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Poly(vinyl alcohol) hydrogels were coated with thin polymer films based on layer-by-layer assembly of dextran sulfate and chitosan. Fabrication of thin multilayer films on the hydrogels was verified in several methods. Anti- versus pro-coagulation of human whole blood was observed on the hydrogels with the outermost surfaces of dextran sulfate and chitosan, respectively.

Hydrogels have potential technological and biomedical applications, because of their unique technological properties due to the three-dimensional network structures.[1] Coatings of hydrogel surfaces are of great importance in the case that the hydrogels contact with biological atmosphere. There is, however, no general methodology for the coating of hydrogel surfaces so far. It is difficult to react other molecules with surface components of hydrogels and to coat hydrogels with other molecules by means of conventional casting and spin coating methods, because hydrogels swell with water molecules, and because the functional groups do not present in some cases on surfaces.

Layer-by-layer (LbL) assembly produces ultrathin polymer films on solid substrates, and can be generated by alternate immersion of substrates into solutions of oppositely charged polymers.[2] The outermost layer of LbL assembly potentially demonstrates the properties of the corresponding polymer. Although LbL assembly can be applied to various types of solid substrates, applications to hydrogel surfaces for functionalization of the hydrogels have not been reported. In this work, poly(vinyl alcohol) (PVA) hydrogels conventionally prepared were coated with anionic dextran sulfate (Dex) and cationic chitosan in the LbL way. And, the anti- versus pro-coagulant activities of human whole blood on hydrogels coated with respective outermost surfaces were demonstrated.

PVA (Nacalai) hydrogel was prepared by using 5 mol% of a cross-linker, glutaraldehyde against the total units of PVA, and were cut into disk shapes (diameter, 10 mm; thickness, 1 mm).[3] Dex (Wako) (Mw 5.0×10^5) and chitosan (Wako) (Mw 1.4×10^6) were selected as cationic and anionic polymers for LbL assembly, respectively. PVA hydrogels were immersed into aqueous chitosan solution (1 mg ml^{-1}) for 10 min at ambient temperature, and then washed with ultra pure water for 30 s. The hydrogels were immersed again into aqueous Dex solution (1 mg ml^{-1}) for 10 min, and the same procedure was repeated. NaCl was added to both solutions at 1 M to obtain solutions with greater ionic strength. A single step in the assembly process involves immersion of the hydrogels into one polymer solution. Although the assembly was started with chitosan, the starting polymer did not affect the assembly process, because the initial deposition process is based on entanglement between PVA and the corresponding polymer.

LbL assembly normally produces ultrathin polymer films with a nanometer-ordered thickness. It is, therefore, difficult to quantitatively analyze these films, especially on hydrogel surfaces. Accordingly, the films on hydrogels were stained with a cationic dye, methylene blue (MB), via 10-min immersion in the aqueous dye solution at a concentration of 0.002 wt%. In our preliminary experiments, the amount of MB adsorbed on the films was related to total amount of polymers assembled. PVA hydrogel, which had been stained with MB, was made clear by subsequent immersion in water for 3 days (Figure 1a), indicating that MB that had diffused into the hydrogel was readily desorbed. On the other hand, PVA hydrogel coated with 20-step LbL assembly still retained blue color after immersion for 3 days in water (Figure 1b). The blue color also persisted near the surface of the hydrogel formed with larger assembly steps. This retention of MB could be seen with naked eyes, when hydrogel was cut into two pieces. In fact, the blue color of the assembly prepared in the presence of 1 M NaCl was deeper than that prepared without NaCl, indicating that the former film was thicker than the latter one. These results strongly suggest that the hydrogel was coated with thin films.

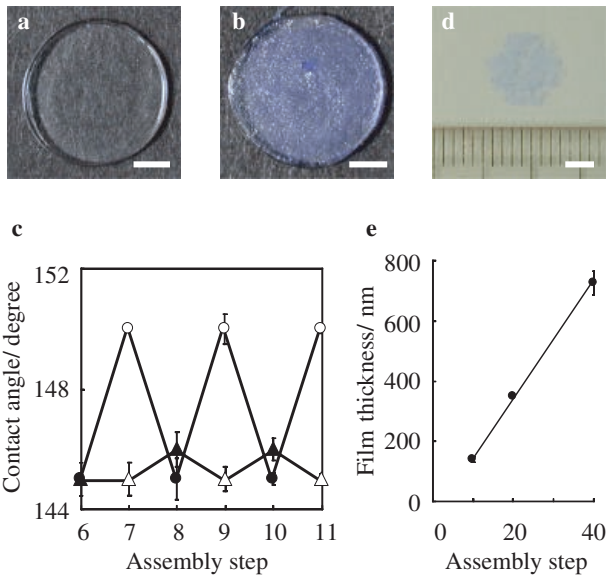

Figure 1. (a) PVA hydrogel stained with MB and subsequently immersed in water for 3 days, (b) hydrogel coated with 20 step LbL assembly, similarly stained and immersed in water, (c) static contact angles of assemblies prepared in the absence (triangle) and presence (circle) of 1 M NaCl using air bubbles in water against assembly step (open and closed symbols indicate assembly steps of chitosan and Dex, respectively), (d) thin film peeled off the coated hydrogel (Figure 1b), and (e) plot of the thickness of an assembly prepared in the presence of 1 M NaCl against assembly step. The scale bars in Figures 1(a), (b), and (d) indicate 3 mm.

To analyze the LbL assemblies, the static contact angles of the 6- to 11-step assemblies prepared in the absence and presence of 1 M NaCl were measured, as shown in Figure 1c. Zigzag behavior was not clearly observed for the film prepared without NaCl, but was observed for the films prepared in the presence of 1 M NaCl. The clear zigzag behavior of the latter case indicates construction of the film in an LbL manner. Increment of thickness of the Dex-chitosan assembly in the presence of NaCl has already been demonstrated using solid substrates.[4] The coiled polymers seems to adsorb in the presence of NaCl, possibly due to a relaxation of the electrostatic repulsion in the polymers dissolved in an aqueous phase. A process similar to polymer deposition appears to occur on the hydrogel surfaces. X-ray photoelectron spectroscopy of the hydrogel surface coated with a Dex-chitosan assembly prepared in the presence of 1 M NaCl also supported LbL deposition. Peak ratios between $S_{2p3/2}$ and N_{1s} ($S_{2p3/2}/N_{1s}$), representing Dex and chitosan, respectively, were estimated to be 0.61 and 3.34 for 19- (chitosan surface) and 20-step (Dex surface) assemblies, respectively, indicating that the outermost surface was predominantly composed of a single component of the assembled polymer.

The thickness of the films prepared on the hydrogel surface was then determined. The thicker films could be peeled off from the hydrogel in a similar size of the hydrogel when the films were put on a glass substrate (Figure 1d). This is due to the lateral mechanical strength of multilayers. The films that did not contact with dried substrates were stable. The thickness of the films without staining could be measured using the scratching mode of an atomic force microscope after transferring onto a glass substrate. For the film prepared in the presence of 1 M NaCl, the thicknesses of 10-, 20-, and 40-step assemblies were 140, 349 and 727 nm, respectively. Note that when the films were peeled off the PVA hydrogels, there was no visible absorption of MB for the PVA hydrogels. The thickness obtained by this method potentially included the thickness of the PVA hydrogel since assembled polymers should be incorporated into the hydrogel surfaces in the initial assembly steps. Accordingly, the mean thickness of the assembly at each Dex-chitosan cycle was estimated from the slope of a plot of the thickness against assembly step between steps, 10–40, of which the coefficient of variation was 0.999, as shown in Figure 1e. The mean thickness was estimated to be 39 nm. The mean thickness of one cycle of the Dex-chitosan assembly prepared on a silver substrate in the presence of 1 M NaCl has already been demonstrated to be approximately 40 nm, although this value was estimated from steps 5–10.[4a] These results indicate that the deposition of chitosan and Dex was similar on the hydrogel surfaces as that on solid substrates.

Alternate bioactivity due to the surface components is an attractive property for potential biomedical applications of hydrogels coated with LbL assembly. Anti- versus pro-coagulation of whole human blood was analyzed by immersion of the hydrogels into fresh human whole blood (supplied by H. Sakaguchi, 22 years old). After incubation for the indicated times, the hydrogels were gently rinsed with phosphate buffered saline and photographed, as shown in Figure 2. Blood coagulated on the surface of a PVA hydrogel after 30 min. Blood coagulated on the surface of hydrogels coated in the absence of NaCl after 15 min. These observations indicate that the hydrogels were coated with the polymers, and that anti- versus pro-coagulation

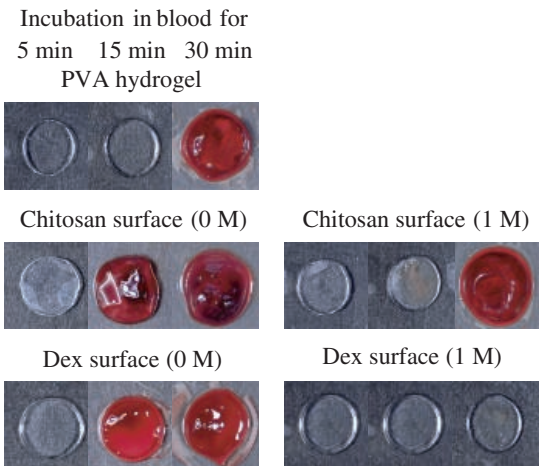

Figure 2. Coagulation of whole human blood on PVA hydrogels non-coated and coated with Dex-chitosan LbL assemblies prepared in the absence and presence of 1 M NaCl.

was not observed due to incomplete LbL assembly, as confirmed by static contact angle measurements. Although the coagulation was faster than that on the hydrogel alone even on the Dex surface, deposition of chitosan as an inner layer seems to accelerate coagulation, possibly due to presence of the chitosan near the hydrogel surface. On the other hand, anti- versus pro-coagulation was observed when the outermost surfaces of Dex and chitosan were assembled in the presence of 1 M NaCl. The anti-coagulation property of the films was maintained even after 3 h. Anti-coagulation on the chitosan surface was also maintained for 15 min, which was longer than that observed for the chitosan surface prepared in the absence of NaCl. This property seems to be similarly derived from the anti-coagulant activity of the Dex present in the inner layers. In fact, the amount of chitosan deposited on the hydrogel surface seems to be smaller than that of Dex.[4a,b] The present anti- versus pro-coagulation of human blood is the first demonstration of alternating bioactivity of hydrogels dependent on the chemical components of the surface layer of the deposited thin films.

Hydrogels were coated with thin Dex-chitosan films using LbL assembly. Anti- versus pro-coagulation of human whole blood on assemblies with outermost surfaces composed of Dex and chitosan was demonstrated. Further studies involving coating of hydrogels with other bioactive polymer combinations as well as controlled release of drugs from the hydrogels is now in progress.

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