

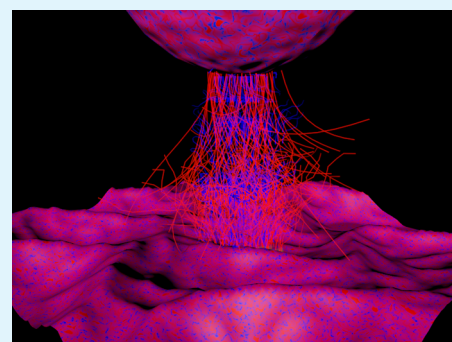
Nanometer-Thick Hyaluronic Acid Self-Assemblies with Strong Adhesive Properties

Andrew Marais,^{*,†,‡} Samuel Pendergraph,^{†,‡} and Lars Wågberg^{†,§}

[†]Division of Fibre Technology, School of Chemical Science and Engineering, and [§]The Wallenberg Wood Science Centre, School of Chemical Science and Engineering, KTH Royal Institute of Technology, Stockholm, SE-100 44 Stockholm, Sweden

S Supporting Information

ABSTRACT: The adhesive characteristics of poly(allylamine hydrochloride) (PAH)/hyaluronic acid (HA) self-assemblies were investigated using contact adhesion testing. Poly(dimethylsiloxane) spheres and silicon wafers were coated with layer-by-layer (LbL) assemblies of PAH/HA. No increase in adhesion was observed when surfaces covered with dried LbL films were placed in contact. However, bringing the coated surfaces in contact while wet and separating them after drying resulted in an increase by a factor of 100 in the work of adhesion (from one to three bilayers). Herein we discuss the adhesion in PAH/HA and PAH/poly(acrylic acid) assemblies. PAH/HA assemblies have potential application as strong biomedical adhesives.



KEYWORDS: hyaluronic acid, layer-by-layer, self-assembly, adhesion, poly(dimethylsiloxane)

The layer-by-layer (LbL) deposition technique^{1,2} has been extensively used over the past few years as a means to produce controlled functionalized surfaces in applications such as biomedical devices and electronics.³ In this study, LbL assemblies of high-molecular-weight (1.6 MDa) hyaluronic acid (HA) and low-molecular-weight (15 kDa) poly(allylamine hydrochloride) (PAH) were used to tailor the adhesive properties of model surfaces. HA is a linear and high-molecular-mass anionic biopolymer consisting of repeating units of $\beta(1-4)$ -linked *N*-acetyl-D-glucosamine and D-glucuronic acid linking other units with $\beta(1-3)$ bonds.⁴ It is naturally present in the human body (e.g., synovial liquid) and is commonly used in biomedicine for ophthalmology, drug delivery, and surgery.⁵⁻⁷ Combined with poly(L-lysine), HA has been used in LbL films for drug delivery, cell adhesion, and tissue engineering.⁸⁻¹¹ More recently, HA has been investigated in LbL structures associated with PAH, and its ability to form nanostructured films¹² was demonstrated. In the same study, the wet adhesive properties of PAH/HA were investigated at the nanoscale using the atomic force microscopy (AFM) colloidal probe technique, and the results showed the influence of the contact time between the films before pull-off experiments as well as the number of layers deposited on the surface and which type of polymer that was in the most external layer. Very strong wet adhesion was achieved between two PAH/HA films when the films were assembled in 10 mM NaCl.

The aim of the present investigation was to investigate the adhesive properties of the PAH/HA system (assembled in 10 mM NaCl) at the macroscopic level. This biocompatible system could potentially be used in biomedical applications such as tissue engineering as a robust adhesive. In order to

provide information on the adhesive features of a given system at the macroscopic scale, the Johnson–Kendall–Roberts (JKR) theory is commonly used.¹³ On the basis of this theory, Chaudhury and Whitesides developed an experimental protocol to measure adhesion using poly(dimethylsiloxane) (PDMS) caps.¹⁴ Following a similar approach, PAH/HA films were assembled on PDMS spheres with a diameter of ca. 3 mm (Figure S1 in the Supporting Information, SI) as well as on silicon wafers. Previous studies have already shown the exponential growth of the PAH/HA film thickness with the number of layers deposited on silicon wafers under specific salt conditions (10 mM NaCl) by quartz crystal microbalance with dissipation (QCM-d) and dual polarization interferometry (DPI) techniques.^{12,15} QCM-d was used here to demonstrate the similar growth behavior for PAH/HA deposited onto PDMS substrates, showing that both PDMS spheres and silicon wafers were coated similarly (Figure S2 in the SI).

In the first adhesion test, the two dried PAH/HA-coated model surfaces were brought in contact and pulled apart in the dry state, and the force required to separate the two surfaces was recorded. The force/displacement results (Figure S3 in the SI) showed that the LbL deposition of a PAH/HA film onto the model surfaces did not increase the adhesion in the dry state compared to an uncoated PDMS sphere. Dry thin LbL films did not form a strong contact under such conditions because of their surface roughness and high modulus, as

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previously shown for LbL assemblies of PAH and poly(acrylic acid) (PAA).¹⁶

In order to promote stronger contact between the two PAH/HA films and highlight the importance of intermixing between the LbL films in contact, the two LbL-coated model surfaces were placed in contact in the wet state (using neutral pH Milli-Q water) and dried overnight under controlled conditions of temperature (23 °C) and relative humidity (30%) prior to testing, as schematically depicted in Figure 1.

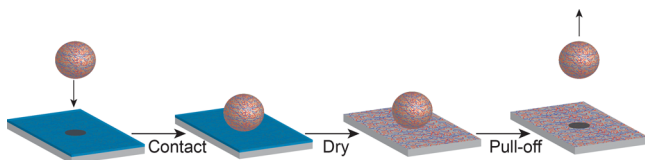


Figure 1. Schematic of the wet adhesion measurement procedure, where the LbL-coated PDMS spheres were brought in contact with the LbL-coated SiO₂ surface in the wet state. The surfaces were then dried in contact before being pulled off.

The force/displacement curves were plotted in Figure 2A for systems coated with different numbers of bilayers of PAH/HA.

The increasing amount of bilayers adsorbed onto the surfaces had a substantial effect on the adhesive strength, with critical pull-off forces increasing by a factor of 10 between one and three bilayers adsorbed onto the surfaces. To observe the macroscopic effect of the nanometer-thick LbL coating, photographs of the silicon wafers (at the vicinity of the contact region) were taken after the pull-off experiments (Figure 2B). The micrographs of these areas revealed that the deposition of PAH/HA films as thin as two bilayers (i.e., 5 nm thick according to DPI measurements¹²) produced adhesive strengths strong enough to enter a regime where both adhesive failure of the interface and cohesive failure of the PDMS probe coexist, as shown schematically in Figure 2C. The presence of small fragments of PDMS remaining on the silicon wafer after the pull-off test indicated cohesive failure of the probe. This cohesive failure of the PDMS probe was more pronounced when three PAH/HA bilayers (ca. 12 nm thick¹²) were adsorbed onto the surfaces.

In order to elucidate the improved adhesive properties of the different systems brought in contact in the wet state, the contact radii as well as the stiffness values were recorded and plotted as a function of the number of bilayers adsorbed onto the model surfaces. Both the contact radius and mechanical

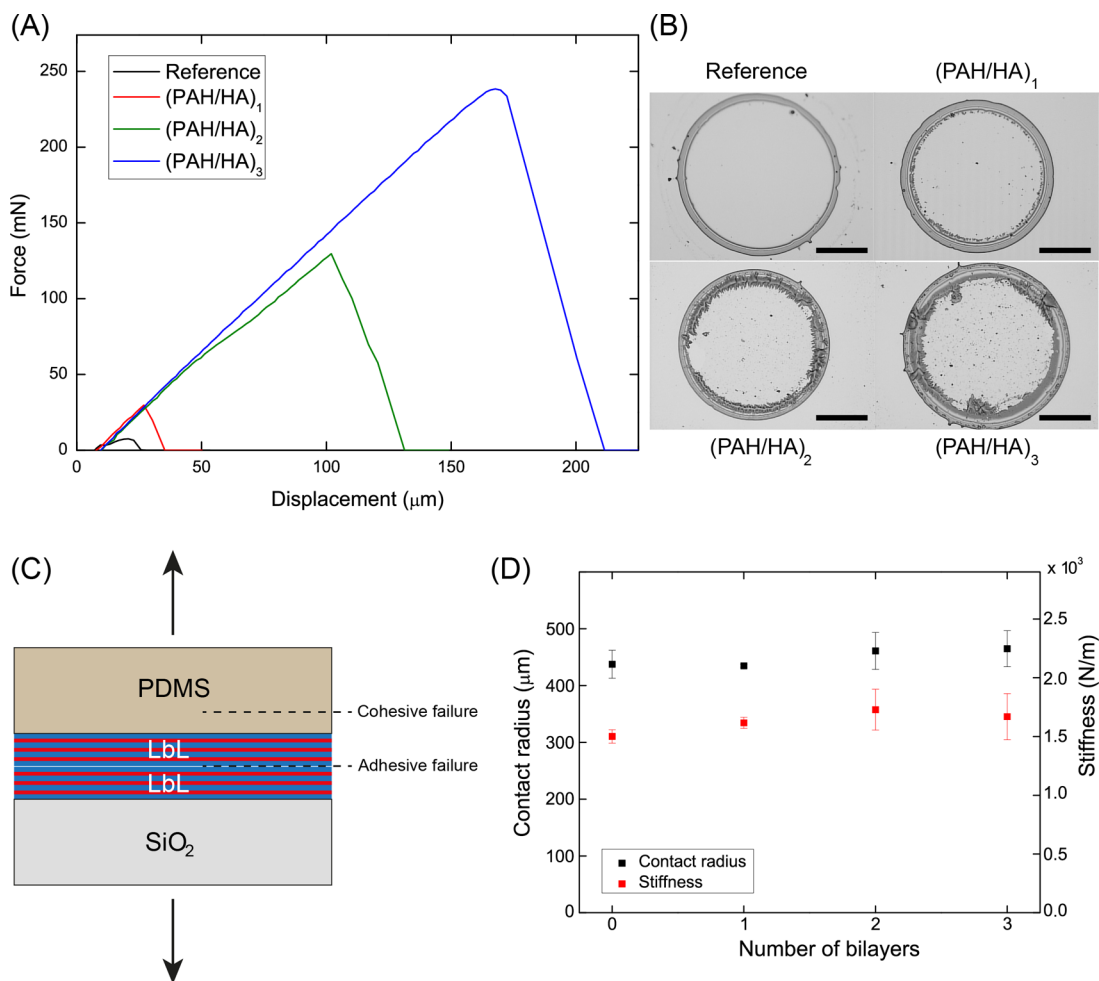


Figure 2. (A) Force/displacement curves for systems coated with zero, one, two, and three bilayers of PAH/HA, brought in contact in the wet state and dried overnight. (B) Photographs of the contact zones of the corresponding surfaces after testing (the scale bars are 250 μm). (C) Schematic of the interface between the two LbL-coated model surfaces. (D) Plot of contact radius (left) and mechanical stiffness (right) versus the number of bilayers for PAH/HA systems.

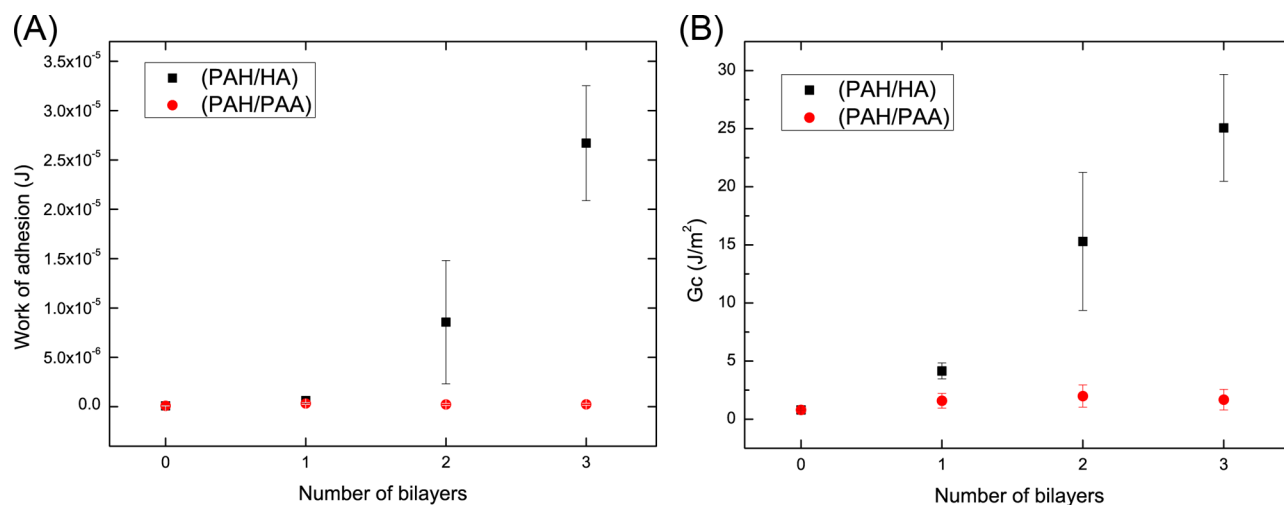


Figure 3. (A) Work of adhesion (calculated from the area under the force/displacement curves) and (B) critical energy release rate, as a function of the number of bilayers adsorbed onto the surfaces, for the PAH/HA and PAH/PAA systems.

stiffness, taken from the force versus displacement plots, were constant as a function of the thickness (Figure 2D) and varied within experimental error regardless of the bilayer number (ca. 10–15%). The normal adhesive force has been previously shown to scale to the square root of both the area and stiffness.¹⁷ Having both initial contact area and stiffness relatively constant regardless of the amount of polymer adsorbed, the adhesive forces were dictated primarily by the interfacial interactions. The improved adhesive properties were attributed to the interactions between the LbL films.

The work of adhesion values for the different configurations were calculated by integration of the force/displacement curves, and the results are shown in Figure 3A. The substantial increase observed in the force/displacement curves translated into similar trends in terms of the work of adhesion with an increase from 6.38×10^{-7} to 2.67×10^{-5} J for systems coated with one to three bilayers. This gain in adhesion corresponded to approximately 1 order of magnitude for each bilayer added, which was a significant increase considering the thickness of the formed layers.

In order to give context to the PAH/HA system, similar experiments (using the same polymer and salt concentrations as well as the same protocol to coat the surfaces and test them) were performed with another LbL polymeric system combining PAH and PAA. This LbL system was chosen because it was previously shown to behave similarly to the PAH/HA system, with exponential film growth upon the addition of salt and strong adhesive properties at the nanoscale evaluated with AFM measurements.^{18,19} The work of adhesion for the PAH/PAA system (Figure 3A), displayed a minimal increase of adhesion with increasing numbers of bilayers adsorbed onto the model surfaces. As previously shown with AFM colloidal probe measurements,^{12,19} the adhesive properties at the nanoscale of PAH/HA were stronger than those of PAH/PAA at the nanoscale. At the macroscopic scale, the difference between the adhesive properties of the two systems was even more pronounced. Assemblies of PAH/PAA from one to three bilayers were not strong enough to translate into differences in adhesion at the macroscopic scale, whereas assemblies of PAH/HA show a significant increase in adhesion for each bilayer added.

The critical energy release rate (G_c) was also determined for all of the samples at a constant velocity of 100 $\mu\text{m}/\text{min}$ using eq 1, where F_c is the critical pull-off force and R the radius of curvature for the sphere.

$$F_c = \frac{3}{2}\pi G_c R \quad (1)$$

The values of G_c for different numbers of bilayers adsorbed on the surfaces are shown in Figure 3B for both the PAH/HA and PAH/PAA systems. Similar to the work of adhesion, there was a significant increase in G_c when the number of bilayers of PAH/HA adsorbed onto the surfaces increased. However, when PAH/PAA was adsorbed onto the model surfaces, G_c remained significantly lower regardless of the number of bilayers adsorbed.

When two LbL-coated surfaces were joined in the wet state and in the presence of salt, the polymer chain mobility was promoted and the LbL films could form better molecular contact. As demonstrated by Kocavevic et al.,²⁰ LbL films showed a “glassy”, immobile behavior when assembled without salt, whereas the films showed a liquid behavior when assembled in the presence of salt. The interdiffusion process was also favored by an increased contact time between the two surfaces, similarly to what was previously shown for PAH/HA at the nanoscale using AFM colloidal probe measurements in the wet state.¹² We believe that the improved contact and chain mobility gave rise to intermixing within the PAH/HA LbL systems brought in contact. This intermixing provided a substantial increase in adhesion with the number of bilayers adsorbed onto the surfaces.

The necessity of water for the establishment of a strong contact at the interface between two PAH/HA LbL treated surfaces had already been observed in a previous study in which cellulosic fibers were used as a substrate. Fibers were LbL-coated and brought in contact in the wet state using a conventional papermaking approach in order to form a fibrous network, which was then dried. The fibrous networks formed with the treated fibers displayed a very high strainability.²¹ In the present study, a similar approach was adopted because the LbL-coated model surfaces were brought in contact in the wet state and dried in contact before performing the pull-off tests,

and comparable trends were observed in the results, with a dramatic gain in the work of adhesion.

Furthermore, the requirement of a compliant interface (promoting the contact at the interface between the two coated model surfaces) to achieve such adhesive properties was expected. Using the JKR equation for adhesion (eq 2), the scaling relationship for the contact radius (a) with the bulk modulus of the system (K) is $\sim 1/K^{1/3}$:

$$a^3 = \frac{R}{K} [F + 3\pi GR + \sqrt{6\pi GRF + (3\pi GR)^2}] \quad (2)$$

where F is the load, R is the probe radius, and G is the energy release rate. Because there was a 5 orders of magnitude difference in the modulus between the glass and PDMS spheres, we expected the contact area to be substantially greater with a PDMS probe simply because of the discrepancy in the bulk modulus, promoting therefore adhesion of the system.

The exact molecular mechanisms governing the very high increase in adhesion upon deposition of PAH/HA onto a surface are not clear yet, but it can be suggested that the presence of both hydrogen-bond acceptors (carboxylic groups) and hydrogen-bond donors (amine groups) in the PAH/HA system promotes the secondary interactions acting like cross-links within the LbL film, as pointed out by Cordier et al.²²

In conclusion, the adhesive properties of LbL assemblies of PAH/HA were investigated using contact adhesion testing. When the LbL surfaces were joined in the dry state, no adhesion increase compared to uncoated surfaces could be detected. However, when small volumes of water were added to the coatings brought in contact, the polymer chain mobility was enabled, and strong adhesive contacts were formed. The improvement of the adhesion was shown to be dependent on the mechanical stiffness of the system, where softer substrates promoted higher contact areas at lower loads. PAH/HA displayed substantially higher adhesion compared to another commonly studied LbL assembly that forms strong electrostatic contacts. The complex molecular behavior of HA suggests that the adhesion is not simply due to the electrostatic assembly of the film but also to other molecular interactions. These promising results from PAH/HA show the potential of a robust adhesion in biomedical applications with minimal amounts of material (nanometer-sized coatings).

■ ASSOCIATED CONTENT

■ Supporting Information

QCM-d data for PAH/HA and PAH/PAA systems assembled on PDMS model surfaces, force versus displacement curves for noncoated and (PAH/HA)₃-coated systems tested in dry conditions, and a plot of the contact radius and stiffness as a function of the number of deposited bilayers for the PAH/PAA system. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b03760.

■ AUTHOR INFORMATION

■ Corresponding Author

*E-mail: marais@kth.se.

■ Author Contributions

‡These authors contributed equally.

■ Notes

The authors declare no competing financial interest.

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