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Falk Bernsmann, Ludovic Richert, Bernard Senger, Philippe Laval, Jean-Claude Voegel, et al.. Use of dopamine polymerisation to produce free-standing membranes from (PLL-HA)_n exponentially growing multilayer films. *Soft Matter*, 2008, 4 (8), pp.1621-1624. <10.1039/B806649C>. <hal-00667266>

HAL Id: hal-00667266

<https://hal.science/hal-00667266v1>

Submitted on 13 Apr 2023

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Use of dopamine polymerisation to produce free-standing membranes from (PLL-HA)_n exponentially growing multilayer films

Falk Bernsmann,^{ab} Ludovic Richert,^{ab} Bernard Senger,^{ab} Philippe Lavalle,^{ab} Jean-Claude Voegel,^{ab} Pierre Schaaf^c and Vincent Ball^{*ab}

In this communication, we demonstrate that dopamine is able to undergo a polymerisation process in (PLL-HA)_n polyelectrolyte multilayer films, and that this polymerisation is of the same nature as in solution at pH 8.5. This polymerisation changes the chemical composition and decreases the mobility of the PLL chains in the film, and ultimately allows the easy detachment of the film as free-standing membranes with 0.1 M HCl solutions.

Catechols play an important role as antioxidant molecules, and catecholamines like adrenaline, noradrenaline and dopamine are well known neuromediators. The electrochemical behaviour of these molecules has been known for a long time.^{1,2} It has also been found that 3,4-dihydroxy-L-phenylalanine (DOPA), a hydroxylated form of the natural amino acid L-phenylalanine, is an important constituent of the proteins found near the plaque–substrate interface of mussels,³ which are able to adhere to virtually any kind of substrate and to withstand strong shear stresses. The molecular mechanism allowing for such a strong adhesion has been elucidated recently by means of atomic force microscopy (AFM) measurements,⁴ and the observation that catechols like dopamine establish strong interactions with metal, metal oxide or polymer surfaces has allowed the introduction of a new paradigm in the field of surface modification.⁵ In this surface functionalisation method, a dopamine solution in a 50 mM Tris buffer at pH 8.50 is simply put in contact with the material of interest, yielding a film about 50 nm in thickness after a reaction time of 20–25 hours.⁵ The reaction occurs not only on the surface of the investigated material but also in solution and offers the advantage of a one-step surface functionalisation, which is efficient on virtually any kind of material. As a first application of this concept, it has been demonstrated that block copolymers made from dopamine and L-lysine (which plays also an important role in the adhesion behaviour of the mussel foot proteins) with *N*-(methoxyethyl)glycine allow the production of very stable antifouling surfaces resistant to both protein adsorption from serum and to adhesion of 3T3 fibroblasts on the surface of the functionalised material.⁶ In addition, it appears that

proteins like the bone morphogenic protein 2 (BMP2) can be modified at their N-terminus with poly(dihydroxyphenylalanine). This allows the production of new affinity fusion tags that can be used to functionalise the surfaces of biomaterials with molecules, which in turn allow the control of their interactions with biological fluids.⁷ As another application of the strong interaction of dopamine with metal oxide surfaces, it has been demonstrated that the adsorbed catechol can be used to initiate atom transfer radical polymerisation of poly(methyl methacrylate) from the surface of titanium dioxide nanoparticles.⁸ From the same perspective of producing reservoirs of active molecules, able to elicit a favourable response of cells entering into contact with the functionalised biomaterial, such as cell adhesion and differentiation, the use of polyelectrolyte multilayer films (PEMs)^{9–12} has become a very popular field of research. These PEMs can also be loaded with catalytic sites¹³ and used as templates to confine chemical reactions.¹⁴ The ability of PEMs to be a matrix in which chemical reactions take place depends on the permeability of the film with respect to the reactants which have to diffuse in the film. The use of dopamine, which is expected to diffuse into highly hydrated polyelectrolyte multilayer films such as those made from HA and PLL,¹⁵ may offer some opportunities not only from the point of view of performing polymerization in the film, but also with some important consequences for the film properties, such as its permeability and mechanical properties. Indeed, the incorporation of inorganic nanoparticles has been shown to increase the stiffness and the ultimate strength of PEMs.^{16–18} It is the aim of this communication to demonstrate that dopamine is able to diffuse into the (PLL-HA)_n PEM bulk, to undergo polymerisation and to reduce the mobility of the PLL in the film. The notation (PLL-HA)_n is used to denote a film constructed by the alternate deposition of *n* layers of poly-L-lysine (PLL) and *n* layers of hyaluronic (HA). The effective production of a polymer derived from dopamine is shown by the easy detachment of (PLL-HA)₂₃ films as free-standing membranes provided the contact time between the PEM and the dopamine solution is greater than a critical time, which is around 4 hours. In addition, the release of the films as thin (less than 4 μm) free-standing membranes is performed under *mild conditions*, namely in the presence of 0.1 M hydrochloric acid. Most of the detachment experiments of PEMs, but not all,^{19,20} have been performed in the presence of either hydrofluoric acid^{16,17} or by dissolving a polymeric film (such as cellulose acetate) serving as a substrate for the deposition of the PEM.²¹

As an initial trial we wanted to check if dopamine, under conditions at which no polymerisation occurs (namely at pH 5.9 in presence of 0.15 M NaCl), is able to diffuse into a (PLL-HA)_n film and to be retained there after the film is rinsed with dopamine-free solutions. The idea behind this experiment was to load the multilayer film with dopamine and *to trigger its polymerisation by means of an*

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external pH change. This would allow us to separate dopamine polymerisation in the film from events occurring in solution, which is not possible if polymerisation occurs simultaneously in solution and possibly in the (PLL-HA)_n multilayer film. Unfortunately, even though dopamine is able to diffuse into a (PLL-HA)₁₂ film, we found that it is rapidly desorbed from it upon contact with a dopamine-free NaCl solution, as shown by UV-vis spectroscopy (Fig. SI 1†). Hence we had to investigate if polymerisation occurs simultaneously in the solution as well as in the interior of the film and if the polymer remains in the film upon buffer rinse.

Consequently we investigated the initial events leading to dopamine polymerisation in solution in order to find the optimal polymerisation conditions. The initial increase in absorbance at a wavelength of 500 nm as a function of the dopamine concentration in the presence of Tris buffer at pH 8.5 and at temperatures ranging from 20 to 23 °C was investigated by UV-vis spectroscopy (Fig. SI 2). In fact, the solutions develop a strong absorbance in the whole visible spectrum, resulting in a brown colour. We chose the absorbance at 500 nm as a representative example. It appears that after a linear increase of the initial rate of absorbance increase per second, v_0 , for dopamine concentrations below 1 g L⁻¹, a plateau is reached (Fig. 1).

The linear regime is consistent with a reaction of apparent first order with respect to dopamine. To explain the plateau we noted that electrochemical investigations have identified the oxidation of dopamine into a quinone as the first step leading to polymerisation.² The only possible oxidant to induce the oxidation of dopamine in water in the absence of a working electrode is the dissolved oxygen. This allowed us to assume that the reaction is limited by the depletion of oxygen at high dopamine concentrations, an assumption that is supported by a dramatic decrease of the initial rate of absorbance increase after intensive argon bubbling of the solution (Fig. 1).

If the initial step of the reaction is oxidation of dopamine, following eqn (1) and eqn (2), this initial step should be reversed by a decrease in pH according to Le Châtelier's principle.

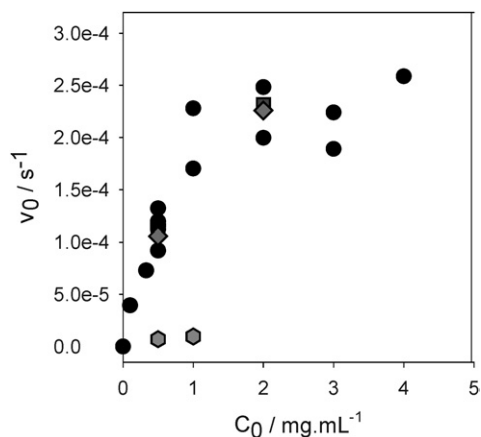
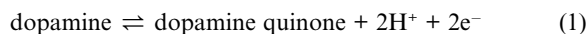
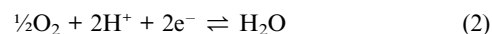


Fig. 1 Evolution of the initial rate of dopamine oxidation v_0 measured at $\lambda = 500$ nm as a function of the initial dopamine concentration C_0 (circles). The same experiments were performed in the same Tris buffer at pH = 8.5 but in the presence of HA at 1 g L⁻¹ (squares), PLL at 1 g L⁻¹ (diamonds) and after intensive degassing of the buffer with Ar bubbling (hexagons).



This is indeed the case, as shown in Fig. SI 3: when the dopamine solution is acidified to pH = 3.45 after 5 min of reaction, the brownish colour of the solution totally disappears and the absorbance at 500 nm decreases to zero. However, when the reaction is allowed to proceed for a longer time, the relative decrease in absorbance after addition of HCl is lower (Fig. SI 3). This is also true when the pH is decreased to 1.0 (data not shown) showing that after a longer time the initially formed dopamine quinone has been consumed to yield stable species, most probably 5,6-dihydroxy-indole, which can undergo polymerisation.² It has also to be noted that the initial rate of absorbance increase is not dependent on the presence of HA or PLL in the Tris buffer (Fig. 1). This does not necessarily mean that after longer reaction times the amino groups of PLL cannot compete with the amino groups of the dopamine quinone in the formation of leucodopaminechrome by internal cyclisation.^{1,2} This aspect will be investigated in forthcoming studies.

We then investigated if this polymerisation mechanism could also proceed in PEMs made from PLL as a polycation and HA as a polyanion. UV-vis spectroscopy shows that after 14 h of contact between vertically held (PLL-HA)_n films deposited on quartz slides and a dopamine solution at 2 g L⁻¹ in 50 mM Tris at pH 8.5, the absorbance of the films at 500 nm scales approximately in proportion to the film thickness (Fig. 2).

This strongly suggests that within this time, dopamine is able to diffuse homogeneously in the volume of the (PLL-HA)_n films and to undergo polymerisation there, simultaneously with the polymerisation in solution. When the reaction time was only 4 hours, a downward deviation occurred in the absorbance *versus* thickness curve in the case of thicker films (Fig. 2). This demonstrates that the thicker the film is, the more time is needed to obtain an homogeneous filling with the polymer, which is most probably poly(5,6-indolequinone).²

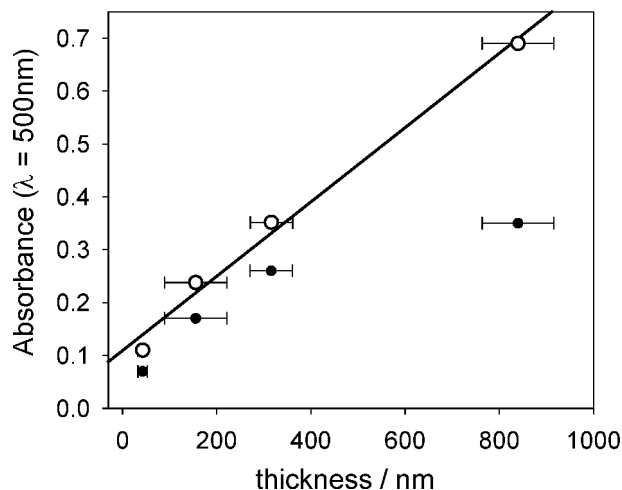


Fig. 2 Relationship between the film thickness (measured by ellipsometry in the dry state and before contact with the dopamine solution) and the absorbance at 500 nm in the case where the contact with dopamine (2 g L⁻¹ in 50 mM Tris buffer at pH 8.5) extended over 4 h (●) and 14 h (○). The horizontal error bars correspond to one standard deviation over the thickness determination. The full line corresponds to a linear regression to the experimental data in the case where polymerization was allowed to take place for 14 h.

The films were also characterised after contact with a dopamine solution by infrared spectroscopy in the attenuated total reflection mode (ATR-FTIR), atomic force microscopy (AFM) and confocal laser scanning microscopy (CLSM). ATR-FTIR spectroscopy shows that the absorption bands due to the polymer (each measurement was done after buffer rinse to remove non-polymerised species from the film) increase up to 25 h of contact with a film made from 12 PLL-HA layer pairs. In addition, the bands are very close to those obtained when the dopamine solution is put in contact with the ZnSe substrate not covered with a PEM. Since the (PLL-HA)₁₂ film is thicker than the penetration depth of the evanescent wave used to probe the film, as demonstrated by the absence of absorbance increase when three additional layer pairs are deposited on a (PLL-HA)₉ film, the observation of bands due to the polymer grown from dopamine shows that the reaction proceeds not only in the part of the film in direct contact with the solution but also in the lowest part of the film (Fig. SI 5). After long incubation of the (PLL-HA)₁₂ film with a dopamine solution at pH 8.5, one observes not only an absorbance increase of the bands attributed to the polymer formed from dopamine (see Fig. SI 5 parts B and C) but also an increase of the bands attributed to the (PLL-HA)₁₂ film (these bands are labelled in Fig. SI 5, parts A and C). The intensity increase of the bands attributed to PLL and HA in the film upon prolonged incubation with a dopamine solution can only be due to a *shrinking* of the film. This film shrinking during dopamine polymerisation leads to an increase in the PLL and HA concentration in the film and hence an increase in absorbance.

AFM topography measurements show that the film roughness increases progressively as the contact time with the dopamine solution increases (Fig. SI 6 and Table SI 1). This might be due to the formation of polymer aggregates at the surface of the film or to the possible deposition of polymer aggregates from solution. Nevertheless, the root-mean-square roughness after 9 h of contact with the dopamine solution only reaches close to 55 nm, which is negligible with respect to the average film thickness of about 4 μm (as estimated by CLSM). The CLSM virtual z-sections (Fig. 3) confirm the observation from AFM that the films remain flat. Furthermore they show that the fluorescence intensity, originating from PLL labelled with fluorescein isothiocyanate (PLL-FITC), progressively decreases. This originates from an inner filter effect: upon polymerisation the absorbance increases over the whole visible spectrum, leading to an accessibility decrease of the incoming photons to PLL-FITC in the films as well as to a re-absorption of the photons emitted by PLL-FITC. Due to this inner filter effect, we could not image the PLL-FITC-loaded films after long contact with a dopamine solution due to the fact that the film lost its transparency.

After having shown that polymerisation occurs in the (PLL-HA)_n films and that it affects their composition and roughness, we aimed to

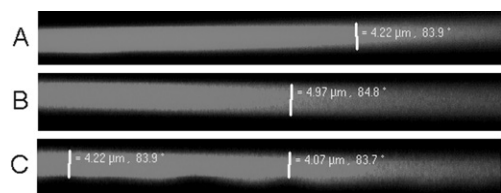


Fig. 3 (A) CLSM z-sections of (PLL-HA)₃₀-PLL-FITC films and after 1 h of contact with dopamine solutions at (B) 0.25 g L⁻¹ and (C) 1 g L⁻¹. The length of the sections in the sample plane is 230 μm.

investigate if the polymer grown in the PEM films affects the mobility of PLL chains in the film. To this end, we examined the fluorescence recovery after photobleaching of (PLL-HA)₃₀ films loaded with a final layer of fluorescein isothiocyanate conjugated PLL (PLL-FITC). Experiments were performed on native films as well as on films put in contact with dopamine at 1 g L⁻¹ for one hour. It clearly appears (Table 1) that the fraction of mobile PLL-FITC chains as well as their diffusion coefficient is significantly reduced upon contact with polymerizing dopamine (even if the PEM film may not be homogeneously filled with polymer; see Fig. 1). The mechanism of the origin of the reduction in PLL mobility can only be speculated on at this time: it can either be due to chemical crosslinking of PLL with the polymerizing dopamine *via* its amino group (which is able to compete with the amino group of dopamine upon its Michael-type addition to the quinone ring)² or to a steric effect. In this latter case, some polymer islands derived from dopamine would grow in the (PLL-HA)₃₀ film, increasing the tortuosity of the film. However, the fraction of mobile chains would not be significantly affected, which is in contradiction with our experiments. To assess the question of the interaction between the polymer derived from dopamine and the constituents of the PEM film, we plan to carry out some nuclear magnetic resonance experiments in the future.

As a final experiment, we tried to detach the (PLL-HA)_n films put in contact with polymerizing dopamine from their substrates. Native (PLL-HA)_n films behave as liquids or very soft gels²² and disintegrate

Table 1 Diffusion coefficient D and fraction p of mobile PLL-FITC molecules in a (PLL-HA)₃₀-PLL-FITC PEM film exposed for one hour to a dopamine solution of concentration C_0 in Tris 50 mM buffer at pH 8.5 and at ambient temperature. The results were obtained by FRAP and are mean values over three measurements \pm one standard deviation

$C_0/g\ L^{-1}$	$D/\mu m^2\ s^{-1}$	p
0	0.058 ± 0.014	0.84 ± 0.04
1	0.032 ± 0.006	0.50 ± 0.11

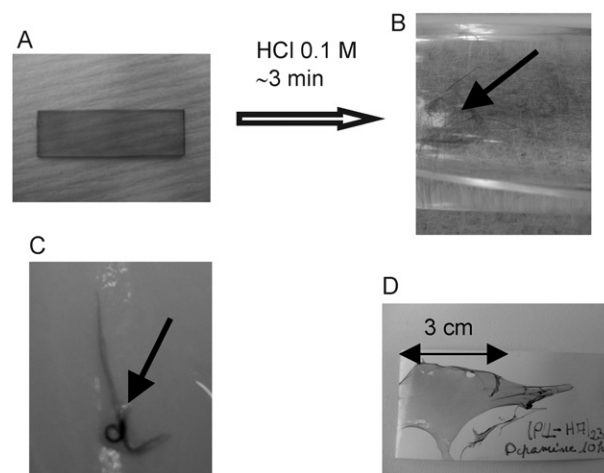


Fig. 4 A free-standing membrane of a (PLL-HA)₂₃ film (A). After rinsing with distilled water, drying under a stream of nitrogen, cutting of the edge of the glass slide and immersing it in a 0.1 M HCl solution for 3 min (B). Depending on the contact time with the dopamine solution (2 mg mL⁻¹ in 50 mM Tris buffer at pH = 8.5) one obtains pieces of a membrane (B and C, displayed with an arrow, after 4 h) or a membrane that can be easily deposited on glossy paper (D, after 10 h).

instantaneously when brought into contact with pure water. This is in contrast with (PLL-HA)₂₃ films put in contact with dopamine (in Tris buffer at pH 8.5) for at least 4 hours (Fig. 4A): the film remains unchanged and adherent to the support. When the edge of the film is cut with a razor blade and put in hydrochloric acid solution at pH 1, the film detaches from the substrate either as small pieces when the contact time with dopamine is 4 hours (Fig. 4B) or as a replica of the initial glass or quartz support for polymerisation times of 10 h. After one hour the level of brown colour is so low that one cannot distinguish if pieces of the film are detached.

The obtained membranes withstand shear stress in solution and can be easily deposited on glossy paper (Fig. 4D). This is a remarkable property because the membrane does not carry inorganic loads and is thinner than 4 µm (a (PLL/HA)₃₀ film loaded with dopamine has a thickness of about 4 µm, as seen in Fig. 3). Since no film detachment is obtained when a glass slide covered with a film is put in 10⁻² M and 10⁻³ M HCl solutions, detachment might well occur through positive charging of the silanol groups of the glass slide, whose point of zero charge lies at around pH 2. The (PLL-HA)₂₃ film is anchored to the glass slide with the positively charged PLL. This may explain the membrane detachment *via* electrostatic repulsion when the pH of the solution is decreased to 1.

It has to be noted that after 10 h of contact with the dopamine solution (at pH 8.5), the (PLL-HA)₂₃ film might not be homogeneously filled with polymer, as anticipated from Fig. 2. Nevertheless a full membrane can be detached from such a film (Fig. 4D): This implies that the filling of the film does not need to be homogeneous but that a critical level of film modification needs to be reached in order to obtain its detachment as a free-standing membrane. The exact nature of the film modification still needs to be determined.

In the future we aim to detach the (PLL-HA)_n films loaded with polymerised dopamine under more biologically friendly conditions by depositing them on biodegradable polyester films before loading with dopamine.

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