Published in final edited form as:

Adv Mater Interfaces. 2014 September ; 1(6): . doi:10.1002/admi.201400145.

High ionic strength formation of DOPA-melanin coating for loading and release of cationic antimicrobial compounds

Jinghao Kuang, Jason L. Guo, and Phillip B. Messersmith*

Biomedical Engineering Department Northwestern University 2145 Sheridan Road, Evanston, IL 60208 (USA)

Keywords

DOPA melanin; coating; antimicrobial; gentamicin; *Staphylococcus aureus*

Melanins are widespread in nature, providing pigmentation, photoprotection, anti-oxidant, metal binding and other biological properties.^[1] An important subclass of melanins is eumelanin, which forms from tyrosine through a pathway involving 3,4-dihydroxyphenylalanine (DOPA) oxidation, intramolecular cyclization, oligomerization, and aggregation to form an insoluble and heterogeneous solid.^[2,3] Research on eumelanins is in part motivated by an interest in understanding structure-property relationships in the context of biological function,^[4] but also due to a growing interest in the interesting optical, electrical properties and technological applications of melanin thin films.^[5–7]

Synthetic and natural melanins are insoluble in many solvents and this property represents a challenge for processing into useful forms such as thin films. In the past, the deposition of melanin-like thin films on substrates has been accomplished by solution casting,^[8,9] (electro)spraying,^[10,11] spin coating,^[12–14] electrochemical deposition,^[7,9,15] and pulsed laser deposition.^[16] In addition to generally requiring the solubilization of melanin in organic solvent or aqueous NaOH or ammonia, most reported methods are either line-of-sight, require sophisticated equipment, employ complex multi-step protocols, or can only be performed on conducting substrates. Due to these limitations, there remains a significant need for simple and versatile approaches to melanin thin film deposition. Methods for forming melanin thin films that avoid the need for significant infrastructure and that can accommodate a variety of substrate compositions, shapes and configurations, will accelerate the development of practical applications for this interesting class of bioinspired materials.

A simple and versatile method to modify surfaces with melanin-like coatings was recently reported, involving dip-coating of substrates into an aqueous solution of catecholamine mimics of DOPA-rich mussel adhesive proteins.^[17,18] The prototypical catecholamine that has dominated the literature in this field is dopamine, which undergoes auto-oxidation in mildly basic and aerated aqueous solution to form an adherent polydopamine (PDA) film on virtually any substrate.^[17] PDA, also referred to as dopamine-melanin because of its

*philm@northwestern.edu.

Supporting Information is available online from Wiley InterScience or from the author.

chemical similarity to eumelanin, can then act as a primer for further modification leading to numerous applications such as biomolecule immobilization, surface energy modification, biomineralization and biosensing.^[19,20] Molecules of interest can also be co-deposited simultaneously with dopamine in a one-step reaction to create surfaces with desired properties.^[21–23]

Several derivatives of dopamine and other catecholamines polymerize in a similar fashion onto a wide variety of substrates. For example, norepinephrine was shown to polymerize into adherent films with the added ability of initiating ring-opening polymerization of ϵ -caprolactone due to the presence of a hydroxyl group not found on dopamine.^[24] Other molecules containing both catechol and amine functionalities have been used to coat various surfaces to enable DNA immobilization^[25] and surface polymerization of antifouling brushes.^[26]

It would be of interest to spontaneously polymerize DOPA-melanin (DM) films on a variety of substrates in a similar fashion as PDA, as this would further accelerate technological applications of eumelanin-like thin films. In comparison to PDA, DM films would be expected to exhibit a higher concentration of free carboxylic acids that can be exploited for a variety of applications. However, historical reports of the alkaline auto-oxidation of DOPA in water or low ionic strength buffer resulted in a melanin-like product which is soluble^[27–29] or a solution-stable supramolecular nanoaggregate.^[30] Consistent with this behavior, we as well as others have experienced difficulty forming adherent films from DOPA using the standard conditions developed for spontaneous PDA film formation (buffered aerated H₂O, pH 8–9), especially on negatively charged substrates such as TiO₂ and SiO₂.^[31] Greco et al. reported the spontaneous formation of core-shell melanin particles by autooxidation of DOPA in the presence of cysteinyl-DOPA melanin (CDM).^[32] The CDM particle appeared to play a crucial role in DOPA oxidation and epitaxial deposition of the DM shell, and it is therefore unclear if the method could provide a general approach to DM thin film formation on other substrates. A few reports showed the in-situ formation of thin DOPA-melanin (DM) coatings on polymeric membranes to improve wettability^[33,34] and to act as a primer for covalent immobilization of molecules,^[35–37] however in-situ grown DM films have only been shown to achieve thicknesses of 10 nm or less under conditions that typically yield much thicker films using dopamine. Thus, compared to PDA, the literature demonstrates that DM films are considerably more difficult to grow on substrates by spontaneous autooxidation of DOPA.

In this communication, we report the unexpected finding that spontaneous in-situ autooxidative deposition of DM films proceeds in a facile manner at high ionic strength. Thick adherent DM films formed on a wide variety of substrates, including metal oxide, noble metal and polymer. DM films possess many of the desirable characteristics of PDA films but with some advantages, namely higher hydrophilicity and the ability to electrostatically bind and release cations. The latter property was exploited for fabricating antibacterial coatings by loading and release of a cationic aminoglycoside from DM films.

The ionic strength dependence of DM film formation was revealed by exposing representative examples of noble metal (Au), oxide (TiO₂), and polymer (polycarbonate,

PC) to aqueous solutions of DOPA (5 mM) in bicine buffer (10 mM, pH 8.5) at NaCl concentrations from 0 – 1M and measuring the thicknesses of DM films that formed after 16 h using spectroscopic ellipsometry (**Fig. 1A**). On PC and TiO₂ substrates, DM films were not detected in the absence of NaCl but increased in thickness with increasing ionic strength, plateauing at about 80 nm and 40 nm, respectively, at NaCl concentrations of 250 mM and above. This behavior was in stark contrast to PDA coatings, whose thickness was found to be independent of the NaCl concentration (**Fig. S1**). DM formation on Au was unusual in that a thin 20 nm film formed without NaCl, rising to 140 nm at 250 mM NaCl before decreasing to about 80 nm upon further increases in NaCl concentration. Although the origin of this anomalous behavior is not known at this time, the observation was reproducible and we hypothesize the phenomenon could be related to electrostatic induction of the Au substrate by the negatively charged DM film. Nevertheless, at 500 mM of NaCl and above, the DM thickness on Au was similar to that on PC (~80 nm).

We also investigated the role of ionic strength on DM film formation by using other salts in place of NaCl in the bicine buffer. The Debye length of these buffers were determined using the equation $k^{-1} (nm) = \frac{0.304}{\sqrt{I(M)}}$, where k^{-1} is Debye length in nm and $I(M)$ is the total molar ionic strength of the salt, DOPA and bicine in the solution.[38] The results revealed that when either NaCl or Na₂SO₄ was used, the DM film thickness on PC rose dramatically only when the the Debye length was reduced to less than 0.7 nm (Fig. 1C). This supports the hypothesis that high ionic strength enabled the formation of DM films by electrical screening, reducing the Debye length and hence the repulsion between the negatively charged DOPA molecules and their reaction products. When CaCl₂ was used, an even lower concentration compared to Na₂SO₄ was required to form 50 nm DM films on PC (Fig. S2). However, the interpretations of the effects of divalent salts may be more complicated because of the ability of divalent cations to electrostatically bridge two DOPA molecules.

On the basis of these preliminary results, Buffer A (10 mM bicine, pH 8.5, 250 mM NaCl) was chosen as the optimal buffer for DM formation and was used in a kinetic study to observe the growth of DM films on substrates exposed to 5 mM DOPA. Film growth exhibited a sigmoidal shape, with film thickness growth occurring most rapidly between 8 – 24 h (**Fig. 1B**). The DM coatings were conformal and adherent to all substrates tested, although DM grown on TiO₂ for longer than 36 h delaminated in patches when rinsed with H₂O, perhaps due to electrostatic repulsion between DM and the negatively charged TiO₂ surface (the same films did not delaminate when rinsed with 150 mM NaCl). In contrast, PDA formed in Buffer A did not show a sigmoidal growth curve after the first time point of 1 h (**Fig. S3**). Like PDA, DM films appear as dark coatings, depending on the deposition time and thickness (**Fig. S4**), due to broad-band absorption (**Fig. S5**) similar to that of eumelanin.[39]

Fourier transform infrared spectroscopy in attenuated total reflection mode (ATR-FTIR) was used to compare the differences in functional groups between DM and PDA films deposited on Au. A major distinction between DM and PDA was the presence of peaks at 1600 cm⁻¹ and a shoulder at 1700 – 1730 cm⁻¹, corresponding to COO⁻ (asymmetric stretching) and

COOH (stretching), respectively (**Fig. S6**).^[40] These data suggest that carboxylic acid groups are present in DM and exist in both the protonated and carboxylate form.

X-ray photoelectron spectroscopy (XPS) was used to further characterize the DM coating formed after 16 h. Compared to the pristine substrates, DM modified substrates exhibited increased N content, reaching a N/C ratio similar to the 0.11 theoretical N/C ratio of DOPA (**Table S1**). O/C ratios were found to be less than or equal to the 0.44 theoretical O/C ratio of DOPA. Importantly, substrate-specific signals such as Au and Ti were either eliminated or drastically reduced, suggesting the formation of an adventitious DM film thicker than the typical XPS analysis depth (~ 10 nm). We also noted the presence of about 3% Na in all the DM films, likely due to the presence of Nacarboxylate salts as suggested by the FTIR data.

Scanning electron microscopy revealed that the DM film obscured the underlying substrate and possessed a rough or porous surface on all substrates (**Fig. 2A & S7**). All three DM modified substrates were almost completely wetting ($< 10^\circ$), which we attribute to the presence of a high density of ionized carboxylate groups. In contrast, the advancing contact angles of PDA-modified substrates were about 35° (**Fig. 2B-C**), which is consistent with the literature.^[17] The improved wetting of DM films may be advantageous for applications in water treatment and purification, where typically hydrophobic polymer membranes are now being treated with PDA coatings to facilitate wetting.^[41,42] The significantly lower contact angle for DM films compared to PDA may enable additional performance improvements for these applications.

Like PDA, the ability of DM films to adhere to different classes of substrates is likely related to the presence of the catechol functional group, as it is capable of participating in a wide variety of interactions, such as metal coordination, hydrogen bonding and π stacking.^[43] The structure of PDA has been the subject of several recent reports.^[44–46] One hypothesis is that PDA has a structure related to eumelanin, which is commonly believed to consist of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole carboxylic acids (DHICA) as subunits.^[39] Recent work by Vecchia et al. suggests that dopamine first oxidizes at high pH into its quinone, which then reacts via multiple pathways resulting in a complex product containing subunits of uncyclized dopamine, DHI cyclized from dopamine, and pyrrolicarboxylic acid moieties.^[45] As DOPA is a precursor of natural eumelanin, the formation of DM is expected to more closely mimic biological melanogenesis (**Scheme S1**) which involves enzyme mediated DOPA oxidation into dopaquinone, cyclization into cyclodopa, conversion into dopachrome, rearrangement by *dopachrome tautomerase* (DCT) into DHICA and DHI, which then undergo oxidative polymerization into eumelanin.^[47] In the absence of DCT, the majority of dopachrome undergoes decarboxylation to form DHI instead of DHICA.^[48] In contrast, our FTIR and XPS data suggests that a significant presence of carboxylic acid is preserved in DM films formed in high salt buffer. It is possible that charge screening of the deprotonated carboxylates of DOPA and its derivatives at high ionic strength leads to an increase in aggregation and surface binding. The exact composition of DM films, presence of uncyclized DOPA subunits, linkages between subunits, and their oxidation states are still unknown and will require further investigation.

To illustrate a functional advantage afforded by residual carboxylic acids in DM compared to PDA, DM films were exploited for binding and release of the cationic aminoglycoside gentamicin (GM). GM is effective against a wide spectrum of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA)^[49] which is one of the largest causes of nosocomial infections leading to high morbidity and mortality.^[50] Substrates were coated with DM or PDA and then immersed in either H₂O or a GM solution (5 mg/mL in H₂O) for 16 h. Thickness measurements revealed that both DM and PDA films on all substrates decreased in thickness by about 1 – 5 % in H₂O, which we surmise may be due to loss of loosely bound DM and PDA (**Fig. 3A**). In GM solution, PDA films decreased in thickness by a similar amount (1 – 3%) whereas DM films on all the three substrates swelled by about 10 – 15%. XPS revealed that loading of GM into DM-coated substrates resulted in an increase in N/C ratio and a decrease in the O/C ratio, which is consistent with the incorporation of GM into the DM films (**Table S1**). Additionally, the virtual loss of Na signal suggests that GM had been incorporated via cationic substitution for Na⁺ ions which were initially associated with the carboxylates in the DM film. These results suggest that GM loaded into DM but not significantly into PDA.

The release of GM from DM was investigated by immersing coated PC substrates (PC/DM/GM) into Dulbecco's Modified Eagle Medium (DMEM) and measuring GM release using an enzyme-linked immunosorbent assay (ELISA). As shown in **Fig. 3B**, a 56 nm thick DM film released 0.95 µg/cm² of GM over 4 h. The total amount of GM loaded and released from DM films could be tuned easily by varying the DM thickness (**Fig. 3C**), which in turn was controlled by coating time or by multiple coating cycles with rinsing and drying in between steps, a method which had previously been shown to form thicker PDA films.^[51] The composition of inorganic salts in the release medium was found to influence GM release (**Fig. S8**), suggesting that Na⁺, Mg²⁺ and Ca²⁺ found in DMEM play a role in GM release.

A Kirby-Bauer disk diffusion assay was performed to evaluate the ability of GM-loaded DM films to inhibit *S. aureus* growth. Coated and uncoated PC substrates were placed onto agar plates that were inoculated with *S. aureus* and incubated for 18 h. As shown in **Fig. 3D**, incubation with DM/GM resulted in a zone of inhibition of 16.7 mm, indicating that GM was released from the coating to inhibit bacterial growth away from the substrate. In contrast, bare PC, PC treated with GM (PC/GM), PDA, PDA/GM, and DM did not show any zones of inhibition (**Table S2**). To show that GM-loaded DM was not only bacteriostatic but also bactericidal, we performed a death assay in which planktonic *S. aureus* were incubated with substrates for 4 h followed by enumeration of surviving bacteria. Bacteria exposed to GM-loaded DM exhibited substantial bacterial killing, whereas all other coatings had statistically similar survival rates as bare PC (**Fig. 3E**). Together, these experiments demonstrated that only DM films were able to load and release sufficient GM to inhibit and kill *S. aureus*, illustrating a significant advantage of DM over PDA that is likely correlated to the presence of carboxylate groups in DM.

In conclusion, we have developed a facile substrate-versatile surface modification technique that exploits high ionic strength to polymerize DOPA into thick adherent DM films under aqueous, mildly alkaline conditions. Spontaneous deposition of DM films by immersion

coating represents a noteworthy simplification compared to previously employed DM coating methods, affording an expansion in the range and configuration of substrates capable of supporting DM films. Compared to PDA films that have been more extensively studied, DM films exhibit enhanced wettability and can be loaded with cationic guest molecules. This was exemplified by demonstrating loading and release of GM from DM films to kill *S. aureus* and will foreseeably work with a wide variety of other cationic aminoglycosides. The ease of formation and reversible cation-binding properties of DM films may lead to new applications of catecholamine coatings for preventing bacterial colonization of surfaces.

Experimental

DOPA and dopamine polymerization

L-DOPA (10 mM) was first dissolved in H₂O, then mixed in equal volumes with 2X Buffer A (10 mM bicine, pH 8.5, 250 mM NaCl). Dopamine.HCl (5 mM) was directly dissolved in Buffer A. Substrates were placed into a 24-well plate and immersed in the DOPA or dopamine solutions. PC samples were allowed to float via surface tension, face down. Gaps in the lid of the 24-well plate provided the solutions access to oxygen in the air. After coating for desired times, the substrates were thoroughly rinsed with H₂O and dried with N₂.

GM loading and release

Substrates coated with PDA or DM were exposed to a 5 mg/mL GM solution in H₂O overnight (16 h) before rinsing with H₂O and blow-drying with N₂. GM loaded substrates were placed into 24-well plates containing 1 mL of DMEM. GM concentration in the release solution was measured using ELISA (Bioo Scientific, TX).

Characterization

The thicknesses of all films were measured using an ESM-300 spectroscopic ellipsometer (J. A. Woollam, Lincoln, NE) at multiple angles of incidence using wavelengths from 400 to 1000 nm. PDA and DM layers were fit to the refractive index and thickness of a Cauchy model with initially fixed coefficients ($A_n=1.45$, $B_n=0.01$). SEM was performed on 5 nm Os coated samples at 10 kV using a Hitachi SU8030.

Bacteria inhibition and death assays

PC substrates were coated for 20 h twice with DM (~90 nm) or PDA (~80 nm) and then loaded with GM as described above. *S. aureus* (ATCC 29213) was expanded overnight in tryptic soy broth (30g/L) and centrifuged twice at 4000 rcf for 5 min with saline rinses. For the Kirby-Bauer assay, bacteria were resuspended to 10⁸ CFU/mL in 150 mM saline, of which 100 μ L was spread onto 4 mm thick cation-adjusted Müller-Hinton (CAMH) agar. The test samples (12 mm diameter round PC) were placed onto the agar for incubation (18 h, 35 °C) before measuring zones of inhibition. GM disks (10 μ g GM, 6 mm diameter, BD, NJ) were used for positive control. For the death assay, the bacteria were resuspended at 10⁷ CFU/mL in DMEM (ATCC) with 10% calf bovine serum (ATCC), of which 100 μ L was exposed to each substrate in 24-well plates. The positive control was the same bacterial solution with 50 μ g/mL of GM. After 4 h incubation (37 °C, 5% CO₂), the well plates were sonicated for 2 min to release any adhered bacteria, the bacterial solution was serially

diluted and plated on CAMH agar. Enumeration was performed after 24 h incubation (37 °C, 5% CO₂).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This research was supported by grants R01EB005772 and R37 DE014193 from the National Institutes of Health (NIH). J.K. was supported by a National Science Scholarship from the A *STAR Graduate Academy of Singapore.

References

1. Nicolaus RA. Hermann Paris. 1968
2. d' Ischia M, Wakamatsu K, Napolitano A, Briganti S, Garcia-Borron J-C, Kovacs D, Meredith P, Pezzella A, Picardo M, Sarna T, Simon JD, Ito S. Pigment Cell Melanoma Res. 2013; 26:616. [PubMed: 23710556]
3. Lerner AB, Fitzpatrick TB. Physiol. Rev. 1950; 30:91. [PubMed: 15403662]
4. Meredith P, Powell BJ, Riesz J, Nighswander-Rempel SP, Pederson MR, Moore EG. Soft Matter. 2006; 2:37.
5. d' Ischia M, Napolitano A, Pezzella A, Meredith P, Sarna T. Angew. Chem. Int. Ed. 2009; 48:3914.
6. Mostert AB, Powell BJ, Pratt FL, Hanson GR, Sarna T, Gentle IR, Meredith P. Proc. Natl. Acad. Sci. 2012; 109:8943. [PubMed: 22615355]
7. Kim YJ, Wu W, Chun S-E, Whitacre JF, Bettinger CJ. Proc. Natl. Acad. Sci. 2013; 110:20912. [PubMed: 24324163]
8. Lorite GS, Coluci VR, da Silva MIN, Dezidério SN, Graeff CFO, Galvão DS, Cotta MA. J. Appl. Phys. 2006; 99:113511.
9. Borghetti P, Goldoni A, Castellarin-Cudia C, Casalis L, Herberg F, Floreano L, Cossaro A, Verdini A, Gebauer R, Ghosh P, Sangaletti L. Langmuir. 2010; 26:19007. [PubMed: 21077619]
10. Abbas M, Ali M, Shah SK, D'Amico F, Postorino P, Mangialardo S, Guidi MC, Cricenti A, Gunnella R. J. Phys. Chem. B. 2011; 115:11199. [PubMed: 21853992]
11. Abbas M, D'Amico F, Morresi L, Pinto N, Ficcadenti M, Natali R, Ottaviano L, Passacantando M, Cuccioloni M, Angeletti M, Gunnella R. Eur. Phys. J. E. 2009; 28:7.
12. Ambrico M, Ambrico PF, Cardone A, Ligonzo T, Cicco SR, Mundo RD, Augelli V, Farinola GM. Adv. Mater. 2011; 23:3332. [PubMed: 21671448]
13. Bothma JP, de Boor J, Divakar U, Schwenn PE, Meredith P. Adv. Mater. 2008; 20:3539.
14. Wünsche J, Cicoira F, Graeff CFO, Santato C, Mater J. Chem. B. 2013; 1:3836.
15. Díaz P, Gimeno Y, Carro P, González S, Schilardi PL, Benítez G, Salvarezza RC, Creus AH. Langmuir. 2005; 21:5924. [PubMed: 15952843]
16. Bloisi F, Pezzella A, Barra M, Chiarella F, Cassinese A, Vicari L. J. Appl. Phys. 2011; 110:026105.
17. Lee H, Dellatore SM, Miller WM, Messersmith PB. Science. 2007; 318:426. [PubMed: 17947576]
18. Lee H, Rho J, Messersmith PB. Adv. Mater. 2009; 21:431. [PubMed: 19802352]
19. Lynge ME, van der Westen R, Postma A, Städler B. Nanoscale. 2011; 3:4916. [PubMed: 22024699]
20. Ball V, Del Frari D, Michel M, Buehler M, Toniazio V, Singh M, Gracio J, Ruch D. BioNanoScience. 2012; 2:16.
21. Kang SM, Hwang NS, Yeom J, Park SY, Messersmith PB, Choi IS, Langer R, Anderson DG, Lee H. Adv. Funct. Mater. 2012; 22:2949. [PubMed: 23580891]
22. Chen X, Yan Y, Müllner M, van Koeven MP, Noi KF, Zhu W, Caruso F. Langmuir. 2014; 30:2921. [PubMed: 24597595]

23. Zhang Y, Thingholm B, Goldie KN, Ogaki R, Städler B. *Langmuir*. 2012; 28:17585. [PubMed: 23205620]
24. Kang SM, Rho J, Choi IS, Messersmith PB, Lee H. *J Am Chem Soc*. 2009; 131:13224. [PubMed: 19715340]
25. Ham HO, Liu Z, Lau KHA, Lee H, Messersmith PB. *Angew. Chem. Int. Ed*. 2011; 50:732.
26. Kuang J, Messersmith PB. *Langmuir*. 2012; 28:7258. [PubMed: 22506651]
27. Binns F, King J. a. G. Mishra SN, Percival A, Robson NC, Swan GA, Waggott A. J. *Chem. Soc. C Org*. 1970:2063.
28. Felix CC, Hyde JS, Sarna T, Sealy RC. *J Am Chem Soc*. 1978; 100:3922.
29. Chio S-S, Hyde JS, Sealy RC. *Arch. Biochem. Biophys*. 1982; 215:100. [PubMed: 6284038]
30. Watt AAR, Bothma JP, Meredith P. *Soft Matter*. 2009; 5:3754.
31. Bernsmann F, Ball V, Addiego F, Ponche A, Michel M, Gracio J. J. de A. Toniazzo V, Ruch D. *Langmuir*. 2011; 27:2819. [PubMed: 21332218]
32. Greco G, Panzella L, Gentile G, Errico ME, Carfagna C, Napolitano A, d' Ischia M. *Chem. Commun*. 2011; 47:10308.
33. Azari S, Zou L. *J. Membr. Sci*. 2012; 401–402:68.
34. Ni M, Teo JCM, bin Ibrahim MS, Zhang K, Tasnim F, Chow P-Y, Zink D, Ying JY. *Biomaterials*. 2011; 32:1465. [PubMed: 21145586]
35. Sui Y, Gao X, Wang Z, Gao C. *J. Membr. Sci*. 2012; 394–395:107.
36. Zhu L-P, Yu J-Z, Xu Y-Y, Xi Z-Y, Zhu B-K. *Colloids Surf. B Biointerfaces*. 2009; 69:152. [PubMed: 19117736]
37. Rai A, Perry CC. *J Mater Chem*. 2012; 22:4790.
38. Israelachvili, JN. *Intermolecular Surf. Forces Third Ed.*. Israelachvili, JN., editor. Academic Press; San Diego: 2011. p. 291-340.
39. Meredith P, Sarna T. *Pigment Cell Res*. 2006; 19:572. [PubMed: 17083485]
40. Silverstein, RM.; Webster, FX.; Kiemle, DJ. *Spectrometric Identification of Organic Compounds*. John Wiley & Sons; 2005.
41. McCloskey BD, Park HB, Ju H, Rowe BW, Miller DJ, Chun BJ, Kin K, Freeman BD. *Polymer*. 2010; 51:3472.
42. Dreyer DR, Miller DJ, Freeman BD, Paul DR, Bielawski CW. *Chem. Sci*. 2013; 4:3796.
43. Waite JH. *Int. J. Adhes. Adhes*. 1987; 7:9.
44. Hong S, Na YS, Choi S, Song IT, Kim WY, Lee H. *Adv. Funct. Mater*. 2012; 22:4711.
45. Della Vecchia NF, Avolio R, Alfè M, Errico ME, Napolitano A, d' Ischia M. *Adv. Funct. Mater*. 2013; 23:1331.
46. Liebscher J, Mrówczy ski R, Scheidt HA, Filip C, H dade ND, Turcu R, Bende A, Beck S. *Langmuir*. 2013 DOI 10.1021/la4020288.
47. Simon JD, Peles D, Wakamatsu K, Ito S. *Pigment Cell Melanoma Res*. 2009; 22:563. [PubMed: 19627559]
48. Palumbo A, d' Ischia M, Misuraca G, Prota G. *Biochim. Biophys. Acta BBA - Gen. Subj*. 1987; 925:203.
49. Kotlus BS, Wymbs RA, Vellozzi EM, Udell IJ. *Am. J. Ophthalmol*. 2006; 142:726. [PubMed: 17056356]
50. Hadler JL, Petit S, Mandour M, Cartter ML. *Emerg. Infect. Dis*. 2012; 18:917. [PubMed: 22607942]
51. Bernsmann F, Ponche A, Ringwald C, Hemmerlé J, Raya J, Bechinger B, Voegel J-C, Schaaf P, Ball V. *J. Phys. Chem. C*. 2009; 113:8234.

Figure 1. DM film formation at pH 8.5. (A) Effect of NaCl concentration on DM film thickness after 16 h. (B) Growth of DM film (250 mM NaCl) as a function of time. (C) Effect of debye length of buffers containing either NaCl or Na₂SO₄ on DM film thickness on PC after 16 h.

A

B

C

Figure 2.

Surface morphology and wetting properties of DM films. (A) SEM image of 23 nm thick DM on TiO₂ formed after 16 h. (B) Advancing water contact angles of DM and PDA formed after 16 h on various substrates. * indicates angles less than 10 °. (C) Images of advancing water droplet on pristine PC, PC coated with PDA, and PC coated with DM, respectively.

D

E

Figure 3.

Binding and release of a cationic antibacterial compound from DM films. (A) Percentage thickness change of DM or PDA after 16h exposure to H₂O or GM solution. (B) GM release from PC/DM/GM over 4h. (C) 4h GM release from PC/DM/GM as a function of DM film thicknesses. (D) Zones of inhibition of *S. aureus* after 18 h incubation of PC coated with DM/GM (bottom) or PDA/GM (top). (E) Death assay showing survival of planktonic *S. aureus* after 4 h exposure to PC coated with PDA and DM.