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Translation of Protein Charge and Hydrophilicity to Materials Surface Properties using Thermal Treatment in Fluorous Media

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Protein-based materials provide an inherently biocompatible and sustainable platform for the generation of functional materials. Translating protein properties into protein films resistant to aqueous degradation is crucial for most applications such as tissue engineering and controlled drug delivery. Current methods to stabilize protein films use three main strategies: employing the relatively limited variety of naturally self-assembling proteins, using added cross-linkers or heat curing. While the cross-linking strategy generates functionally diverse structures, unreacted additives retained in cross-linked protein films can adversely affect their final behavior. Traditional heat curing results in hydrophobic surface and loss of protein inherent properties. We demonstrate here a scalable, additive-free, fluorous media assisted thermal treatment for the fabrication of stable, hydrophilic protein films. This approach is general in terms of protein building block, retaining much of their native structure and surface properties upon heating. We demonstrate the versatility of this strategy through fabrication of antifouling coatings on complex three-dimensional surfaces. The utility of these films as biomaterials is highlighted through the generation of highly biocompatible non-fouling surfaces and regulation of cellular adhesion through choice of protein precursor.

Conceptual Insight

Proteins have intrinsic charge properties that would be highly useful for materials applications. Translating these molecular properties to surface behaviour is challenging due to loss of protein structure upon film stabilization. Thermal treatment in fluorous media retains protein charge, secondary structure, biodegradability and hydrophilicity in protein coatings. This fluorous-based approach is additive-free and versatile in terms of protein precursors and the coating of substrates with 3D geometry, providing a new strategy for the generation of designer biomaterials.

Introduction

Protein-based materials provide a uniquely sustainable and biocompatible platform for biological applications.[1,2,3] The inherent structural and surface diversity of proteins makes them versatile building blocks for functional materials for use in medical implants,[4] tissue engineering,[5,6,7] drug delivery,[8,9,10] and bioelectronics.[11, 12,13] Furthermore, the aqueous processability and biodegradability of proteins produces minimal environmental impact, making them ideal building blocks for eco-friendly materials.[14]

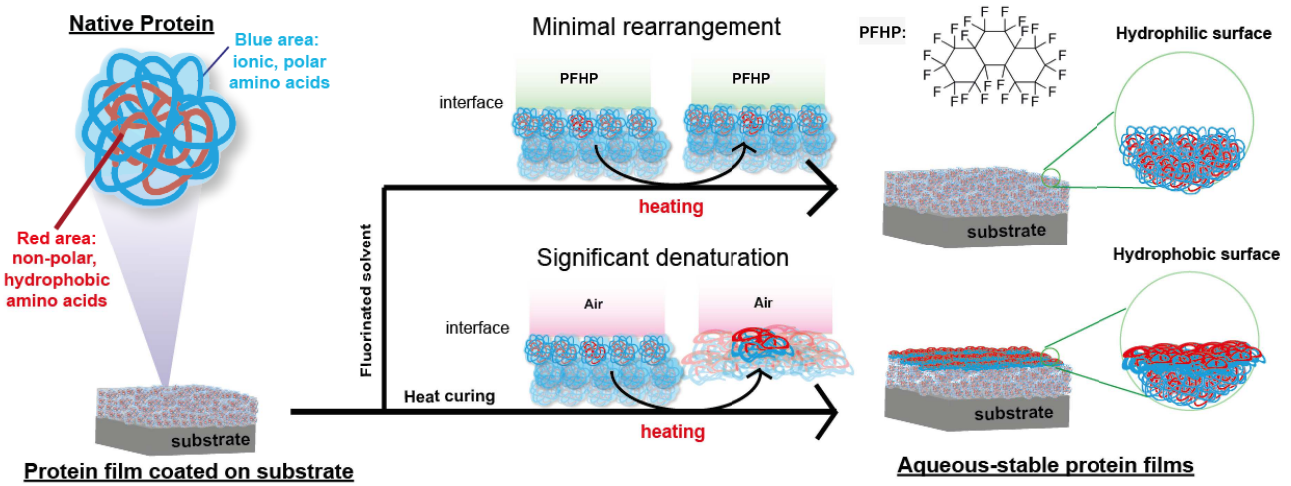

Figure 1: Methods for protein film fabrication. Proteins were spin-cast and then heated in either fluorous solvent (perfluoroperhydrophenanthrene, PFHP) or air to generate stable thin films. Fluorous solvent provides an environment that prevents protein denaturation at the interface, resulting in hydrophilic films that retain intrinsic properties of the precursor proteins. In contrast, heat curing in air results in protein denaturation to minimize surface energy, resulting in the generation of hydrophobic films.

The vast majority of applications of protein films require stability in aqueous environments[15,16] Current strategies to produce aqueous-stable protein films include: (i) Using a relatively limited range of naturally self-assembling proteins, such as silk fibroin, to produce stable protein films.[17] However, post-functionalization techniques[18] or protein engineering[19] are required to generate films with diverse surface properties while using this strategy. (ii) Employing cross-linkers to create polymeric structures by covalent bonding of proteins.[20] However, the unreacted additives retained by the resulting cross-linked protein film can adversely alter film properties.[21,22] Moreover, many commercially available cross-linkers are toxic and therefore hinder the applicability of such materials in biological systems. [23] (iii) Heat-curing provides a universal and readily employed platform for fabricating films from any protein precursor.[24] Traditional heat curing, however, results in denaturation of the protein precursors. This loss of structure leads to hydrophobic films that do not retain the surface properties of the native protein, in particular their overall charge and inherent zwitterionic nature. As a result, these hydrophobic surfaces can induce severe protein/bacterial fouling,[25] and trigger immune responses.[26]

Beyond their eco- and biocompatible composition, the use of proteins as building blocks for materials applications has the potential to leverage structural components of proteins, such as charge and hydrophobicity, to provide control over film surface properties.[27] We previously developed an additive-free, thermal nanoimprint lithography (NIL)-based methodology for developing protein-based functional biomaterials on two-dimensional substrates.[28] These NIL-stabilized films retained substantial native protein structure, concomitantly providing inherently hydrophilic, zwitterionic and biodegradable films. Through choice of protein, the surface charge of these films could be readily controlled. Due to the nature of NIL, however, this method could only be used for flat (2D) surfaces.

The NIL-based strategy for generating stable protein films employed a combination of heat and pressure applied using a fluorosilane-modified stamp. We hypothesized that the retention of surface properties of protein films upon heating in the NIL process was potentially due to the fluorous environment provided by the fluorinated stamp,[29] as opposed to the effect of the compression pressure. Fluorous media, also known as perfluorocarbon fluids are inert, stable and immiscible with water or hydrocarbons. These unusal immiscibility and stability properties have led to the use of fluorous media as an alternative to water for performing polymerization.[30] We hypothesized that these immiscible and non-reactive properties prevent the dissolution of protein films in the heating media, as well as inhibit protein denaturation of the surfaces upon heating, resulting in the formation of water stable and hydrophilic protein films. This hypothesis was tested by the comparison of surface properties of protein films heat cured in a fluorous solvent versus those heated in air (Figure 1). The protein films cured in the fluorous environment retained a much higher degree of native protein structure and were substantially more hydrophilic than those heated in air. We report here the creation and characterization of stable protein films through heat treatment in fluorous media, and demonstrate the versatility of this strategy through fabrication of antifouling coatings on complex three-dimensional surfaces.

Results and Discussion

Our initial protein stabilization and denaturation studies focused on bovine serum albumin (BSA, an anionic protein). Protein films of thickness ~200 nm were generated by spin-casting 10% w/w BSA solution onto plasma-cleaned substrates (Figure S1). These water-soluble films were next stabilized by heating at 180°C in perfluoroperhydrophenanthrene (PFHP), air, or using NIL. The secondary structure of protein building blocks in each film was characterized using circular dichroism spectroscopy. Consistent with

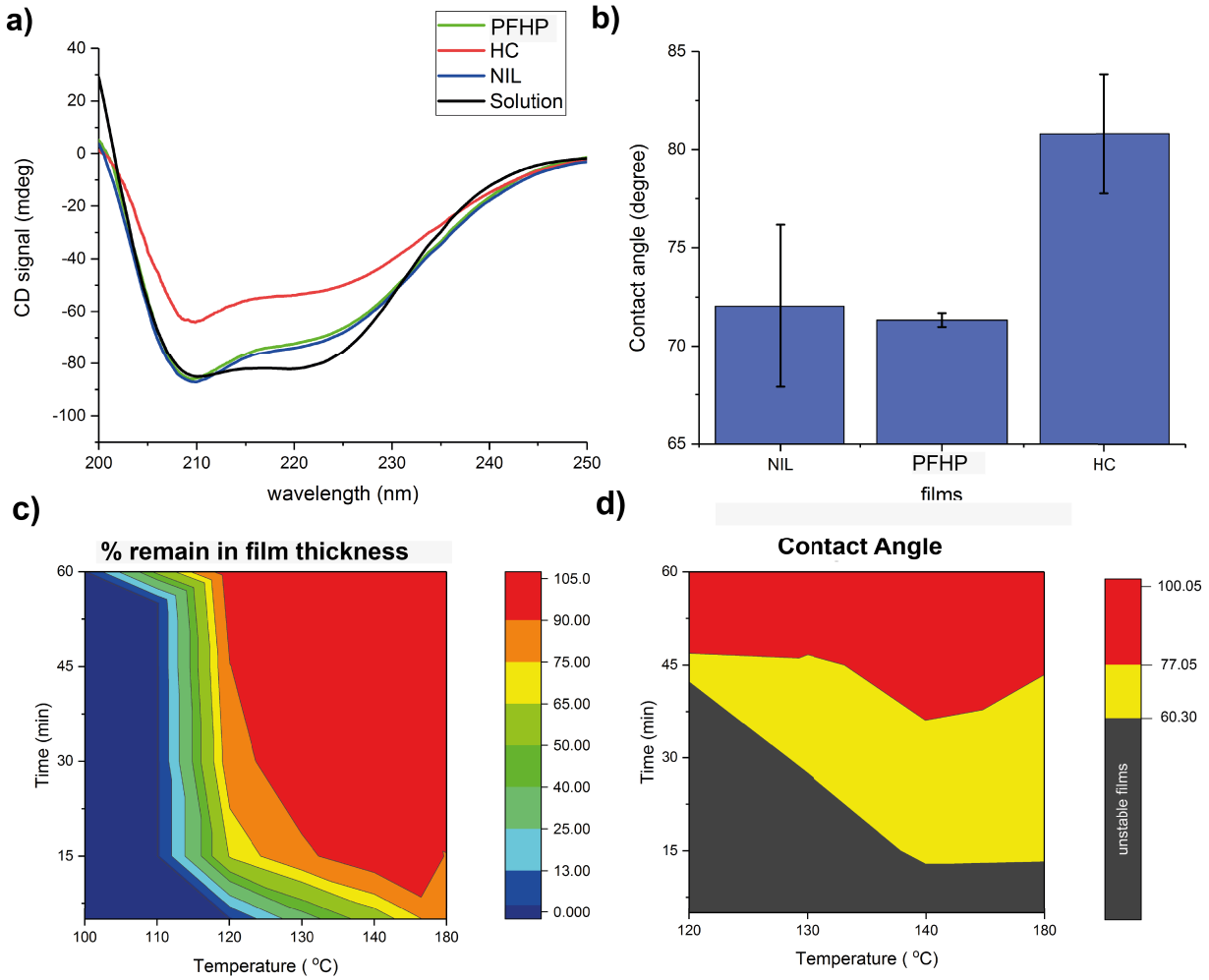

Figure 2: Structural and surface characterization of protein films. a) Circular dichroism spectra of BSA in phosphate buffer (solution), and BSA films prepared by nanoimprinting (NIL), heat-curing (HC) and stabilizing in PFHP (PFHP). b) Water contact angle on BSA films stabilized by NIL, PFHP and heat-curing methods. c) Heat map showing the effect of time and temperature on film stability in water. Films were washed for 1 min with water and the thickness measured by ellipsometry after drying. d) Heat map showing the effect of time and temperature on film hydrophilicity. Water contact angle was measured by static sessile drop method using 2 µL of water.

our prior studies, a substantial amount of the secondary structure was retained in films stabilized by PFHP and NIL. In contrast, protein films stabilized by traditional heat-curing (HC) resulted in essentially complete loss of native structure (Figure 2a). To verify that the deposition method does not affect the protein structure, we also measured the CD spectra for dip-coated films before and after stabilization. The results showed a similar trend as spin-coated films (Figure S3). Protein denaturation induces surface hydrophobicity due to the migration of hydrophobic residues to the film surface to minimize interfacial energy. The correlation between structure retention and surface hydrophobicity of protein films was quantified through contact angle measurement. NIL and PFHP stabilization methods both provide hydrophilic surface (Figure 2b). In contrast, heat-curing in air generates hydrophobic surface (Figure 2b). Taken together, these results indicate that fluorous environment prevents proteins from significant denaturation while heating, thus enabling the fabrication of hydrophilic protein films.

The processing temperature and time in PFHP method were varied to determine the conditions at which aqueous stability was achieved and hydrophilicity of protein films was maintained (Figure 2c-d). The results demonstrate that stable films were generated at temperatures > 140°C in 15 min when heating in PFHP. These films were stable in PBS without degradation or dissolution for more than 10 days (Figure S4). In addition, no residue of fluorine in protein films was observed even at the highest operation temperature (Figure S2), indicating that no chemical reaction occurred between fluorous solvent and protein films, nor was any solvent entrained in the film. Stability can also be achieved at lower temperatures by prolonging the heating time. However, such films tend to be slightly more

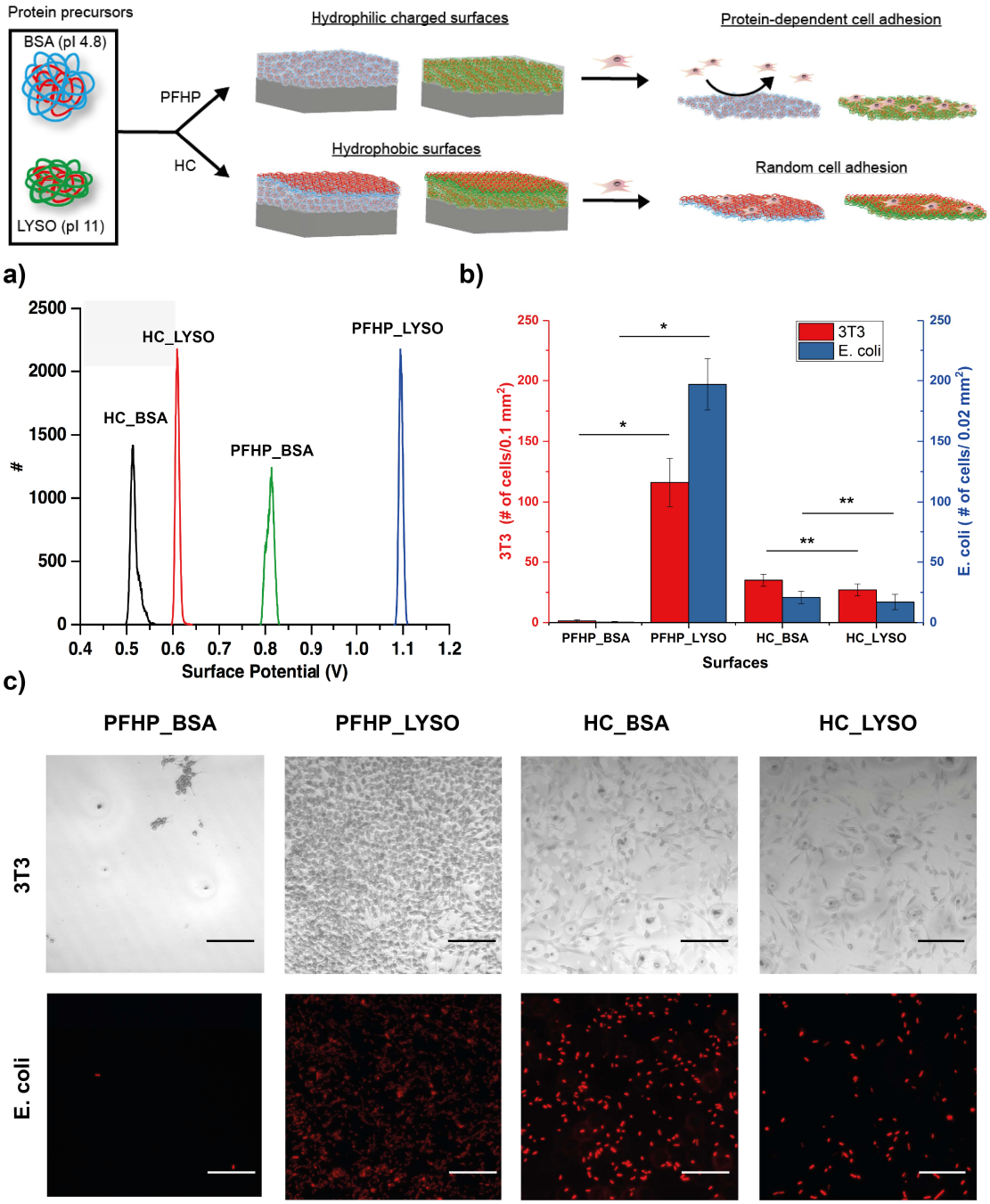

Figure 3: Cellular adhesion to protein films. a) Surface potential of BSA and LYSO films fabricated by HC or PFHP method as determined by Kelvin probe force microscopy (KPFM). b) Number of mammalian cells (3T3) and bacterial cells (E. coli) adhered on protein films. c) Optical and fluorescent microscopy images for mammalian and bacterial cells adhered on protein films. Scale bars are 200 μm and 60 μm for 3T3 and E. coli respectively. *p<0.0005, **p>0.05 (n=5).

hydrophobic owing to longer exposure to elevated temperatures (Figure 2d). Although the protein films stabilized in the fluorous environment were stable in aqueous solution, they are digested by proteases, e.g. trypsin (Figure S5), demonstrating their biodegradeability.

The retention of protein structure and surface hydrophilicity of films heated in fluorous solvent implies that proteins' molecular properties, such as degradability and surface charge, can be translated into macroscopic films for different biomaterial applications. The translation of surface charge into protein films was demonstrated using cationic lysozyme (LYSO, pI 11) and anionic BSA (pI 4.8) as protein precursors. The surface potential of resulting films was quantified using Kelvin Probe Force Microscopy (KPFM). As expected, the PFHP-LYSO surface exhibits a more positive surface potential as compared to PFHP-BSA (Figure 3a). The

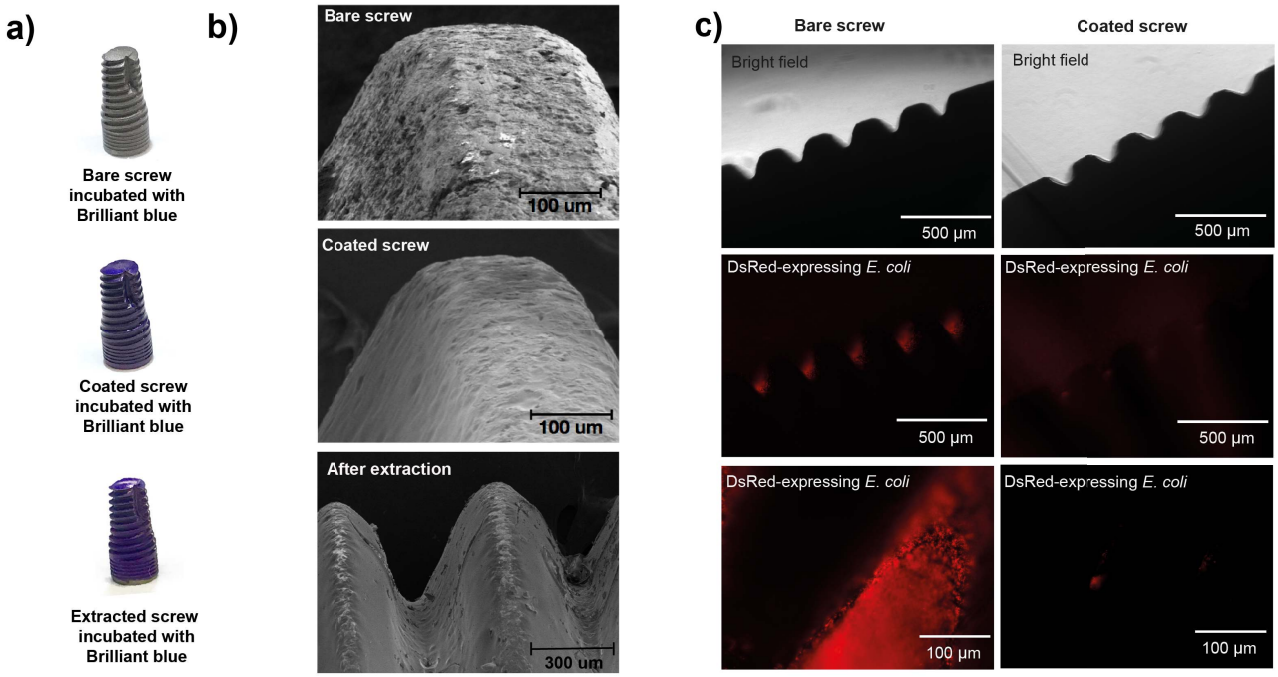

Figure 4: Three-dimensional protein film coating on dental implant screw. a) Images of brilliant blue stained screws that are bare, BSA coated, and extracted from a bone mimic PU block. b) Scanning electron microscopic images for bare, coated, and extracted screws. c) Optical and fluorescent microscopy images of DsRed-expressing *E. coli* on bare and coated screws after 24 hours incubation.

potential difference between PFHP-LYSO and PFHP-BSA is 0.28V, which remains consistent with our previous research with NIL films. In contrast, hydrophobic heat-cured films present a lower surface potential for both BSA and LYSO surfaces, and the difference between the surface potential is significantly lesser. The loss of surface property of protein precursors in traditional heat cured films was presumably due to the oxidation reactions that occur upon heating.[31]

The surface properties of protein films can be tailored to the biomaterial application. For example, positively charged surfaces promote cellular attachment, which can be employed for tissue engineering. Conversely, zwitterionic or negatively charged surfaces are suitable for bio-inert coatings, especially for medical implants.[32] Based on our capability of controlling the surface potential of protein films, a functional demonstration of charged protein films was performed by cellular adhesion studies. 3T3 fibroblast cells were seeded onto the protein films for 24 hours and examined by microscopy after washing with PBS (Figure 3b). PFHP-LYSO provided excellent adhesion for 3T3 cells, while PFHP-BSA showed essentially complete anti-fouling. These results are in agreement with our previous observations with the NIL films. In contrast, heat-cured films show no control of cellular adhesion, indicating the surface properties of protein precursors were lost during the stabilization process. Similar behavior of protein films was observed with bacterial adhesion. PFHP-BSA showed complete resistance to bacterial fouling, while PFHP-LYSO triggered a strong interaction with bacteria. These results, along with the contact angle studies, indicate the importance of the use of the fluorous environment in the retention the surface hydrophilicity and consequently the ability to control protein films properties.

The stabilization of protein films using heating in fluorous media provides a technology for generating seamless protein coatings on three-dimensional (3D) substrates. Medical devices with complex geometry, e.g. dental and orthopedic implants, are often susceptible to bacterial contamination.[33] Protein films, being biocompatible, are potentially adavantageous candidates for antifouling coatings on such implants. Based on the cellular adhesion studies, BSA was chosen to generate antifouling coating on dental implant screws as a functional demonstration. The BSA coatings showed comparable antifouling properties with conventional polyethylene glycol coated surfaces. Moreover, the BSA coatings continued to prevent bacterial fouling over prolonged exposure for 3 days. In addition, similar antifouling property was observed regardless of the deposition methods (Figure S6, S7). An oxygen plasma-cleaned screw was dip-coated with 20% w/w BSA solution, and the coating was stabilized using heating in PFHP. To verify that the coating was uniform and seamless, coated and uncoated screws were stained by incubating in a Brilliant Blue staining solution for 10 min. The protein film is prone to strong electrostatic interaction with Brilliant Blue resulting in a blue-colored screw after washing. In contrast, the bare screw showed no retention of Brilliant Blue after washing (Figure 4a). Another evidence of uniform coating was observed by scanning electron microscopy. The topography of the coated is smooth as compared to that of the bare screw, which is explained by the attachment of a uniformly-coated thin protein film. The retention of functionality of the BSA film was demonstrated by incubating both bare and coated screws in DsRed-expressing *E.* coli for 24 hours. Fluorescence microscopy images show that the BSA-coated screw prevents bacteria adhesion uniformly throughout the screw while

substantial amounts of *E.* coli were observed on the bare screw, especially between the threads. To test the mechanical stability of BSA coatings, the BSA-coated screws were screwed into a synthetic bone mimic PU block (10 PCF polyurethane foam, Sawbones) then extracted via unscrewing.[34] Although there were some small cracks observed in SEM image (Figure 4b), the overall coating remained attached after extraction (Figure 4a). These results demonstrate that the PFHP-stabilized protein films can be employed to generate anti-fouling BSA coatings on medical implants such as dental screws.

Conclusions

In summary, we have demonstrated that the fluorous environment provided by PFHP preserves protein structure upon heating, preventing protein denaturation and hydrophobic rearrangement at the interface, while generating stable films. Thus, aqueous-stable protein films were fabricated without the use of additives. The protein film retained intrinsic physical properties from the precursor proteins that allowed for the fabrication of biocompatible and versatile functional protein films by altering the choice of protein precursors. The versatility of this coating procedure was demonstrated through generation of antifouling coatings on medically relevant 3D substrates. Taken together, our study provides a scalable and generalizable route for the creation of surface derived from the vast variety of naturally-occurring as well as engineered proteins. Moreover, it widens the scope of functional protein coatings to medically relevant 3D devices such as antifouling implants and drug eluting stents.

Methods

Materials: BSA and Lyso were purchased from Fisher Scientific and used without further purification. Perfluoperhydrophenanthrene and tetradecafluorohexane were purchased from Sigma-Aldrich. Silica wafers were purchased from WRS Materials. Quartz microscopy slides were purchased from Electron Microscopy Sciences. MilliQ water was purified by using a Millipore water purification system. Titanium dental implant screw was purchased from Alpha Bio Tec.

Film preparation: 10% w/w solutions of protein in MilliQ water were spin-coated at 300 rpm for 25 seconds onto an oxygen plasma cleaned silicon substrate or quartz slides, yielding a thin film of protein.

Protein film stabilized by fluorous solvent: As prepared protein films were incubated in preheated perfluoperhydrophenanthrene solvent at 180 °C for 15 mins, following by washing with tetradecafluorohexane.

Protein film stabilized by Nanoimprint Lithography (NIL): Nanoimprinting of protein films was performed by using a Nanonex NX-2000 nanoimprinter with silicon molds. Imprinting was performed at various temperatures and pressures for 5 min. All molds were treated with heptadecafluoro-1,1,2,2-(tetrahydrodecyl) dimethylchlorosilane at 90 °C for 2 days in a vacuum chamber.

Kelvin probe force microscopy (KPFM): KPFM measurements were performed on a commercial AFM (Asylum Research MFP-3D; Santa Barbara, CA) using a Ti/Ir coated silicon tip (f ~ 70 kHz; k ~ 2 N/m (ASYELEC-01)) to probe the surface potential. During the measurement, the silicon substrate was kept at ground and the tip sequentially scanned along the top of each sample surface to collect the surface potential. All KPFM images were acquired at a scan rate of 0.6 Hz, a 3 V_{AC} applied tip bias, and a 10 nm fixed separation between the tip and sample surface during the second pass.

3D coating: Dental implant screw was cleaned by oxygen plasma before dip coating with 20% w/w BSA solution. The screw was dried in a flame hood for 3 hours before heating in fluorous solvent. After washing with tetradecafluorohexane, the screw was dried by nitrogen gas.

Cell Culture: Mouse fibroblast cells 3T3 (ATCC CRL-1658) were cultured in Dulbecco's modified Eagle's medium (DMEM; ATCC 30-2002) supplemented with 10% bovine calf serum (ATCC 30-2030) and 1 % antibiotics in T75 flasks. Cells were maintained at 37 °C in a humidified atmosphere of 5 % CO_2 and were sub-cultured once in 4 days.

Cell Adhesion: 3T3 cells grown in T75 flasks were washed with phosphate buffered saline (PBS), trypsinized with 1X trypsin and collected in DMEM media. Cells were centrifuged and were re-suspended in fresh DMEM media and counted by using a hemocytometer. Protein film coated surfaces were placed in a six-well plate where 3T3 cells were added to each well (100000 cells/well) and incubated for 48 h at 37 °C in a humidified atmosphere of 5 % CO_2.

Bacteria adhesion: DsRed-expressing E. coli bacteria were inoculated in 3 mL LB broth and grown to stationary phase at 37 °C. The cultures were then diluted to O.D 0.1 in an M-9 media supplemented with 1 mM IPTG (isopropyl β -D-1-thiogalactopyranoside).[35] 2 mL of the dilution was poured onto the surfaces kept in 12 well culture plates. The surfaces were kept at 25 °C and the bacteria were allowed to grow for 24 hours. In general, the surfaces with bacteria were rinsed with PBS three times before analysis under the microscope.

To test the extent of anti-fouling over prolonged exposure, the surfaces were challenged with DsRed-expressing E. coli bacteria for 3 days. The cultures were as described above on Day 1. The bacteria solution was replaced with fresh OD 0.1 bacteria solution each day for two additional days. The surfaces were washed and characterized as described above on Day 4, after a total exposure of 3 days.

Characterization: Bright field images and fluorescence were detected by using an Olympus IX51 microscope with excitation wavelengths of 470 nm and 535 nm. AFM imaging of the surfaces was done on a Dimensions 3000 (Veeco) in tapping mode using a RTESP7 tip (Veeco). The film thickness of the protein films was measured by a Rudolph Research Auto EL ellipsometer. Far-UV circular dichroism (CD) spectra were measured on a JASCO J-720 spectropolarimeter with a quartz cuvette of 1 mm path length at 25 °C. The spectra were recorded from 200 to 260 nm as an average of three scans at a rate of 20 nm/min. X-ray photoelectron spectroscopic (XPS) analysis was performed on a Physical Electronics Quantum 2000 spectrometer using a monochromatic Al Kα excitation at a spot size of 10 mm with pass energy of 46.95. Chemically

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distinct species were resolved using a Gaussian Lorentzian function with nonlinear least-squares fitting procedure.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

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† Electronic Supplementary Information (ESI) available: SEM image and XPS spectrum of BSA films, AFM image of BSA film for protease degradation. See DOI: 10.1039/c000000x/

[1] F. G. Omenetto, D. L. Kaplan, *Science,* 2010, **329**, 528-531.

[2] L.-S. Wang, A. Gupta, B. Duncan, R. Ramanathan, M. Yazdani, V. M. Rotello, *ACS Biomater. Sci. Eng.,* 2016, **2**, 1862-1866.

[3] Y.-C. Chen, H.-C. Yu, C.-Y. Huang, W.-L. Chung, S.-L. Wu, Y.-K. Su, *Sci. Rep.,* 2015, **5**, 10022.

[4] L. Uebersax, T. Apfel, K. M. R. Nuss, R. Vogt, H. Y. Kim, L. Meinel, D. L. Kaplan, J. A. Auer, H. P. Merkle, B. von Rechenberg, *Eur. J. Pharm. Biopharm.,* 2013, **85**, 107-118.

[5] R. Zhu, Y.-X. Chen, Q.-F. Ke, Y.-S. Gao, Y.-P. Guo, *J. Mater. Chem. B,* 2017, **5**, 5009-5018.

[6] C. Dhand, S. T. Ong, N. Dwivedi, S. M. Diaz, J. R. Venugopal, B. Navaneethan, M. H. Fazil, S. Liu, V. Seitz, E. Wintermantel, R. W. Beuerman, *Biomaterials*, 2016, **104**, 323-338.

[7] H. Knopf-Marques, M. Pravda, L. Wolfova, V. Velebny, P. Schaaf, N. E. Vrana, P. Lavalle, *Adv. Healthc. Mater.,* 2016, **5**, 2841-2855.

[8] M. Rezvanian, N. Ahmad, M. C. I. Mohd Amin, S.-F. Ng, *Int. J. Biol. Macromol.,* 2017, **97**, 131-140.

[9] M. A. Brenckle, B. Partlow, H. Tao, M. B. Applegate, A. Reeves, M. Paquette, B. Marelli, D. L. Kaplan, F. G. Omenetto, *Adv. Funct. Mater.*, 2016, **26**, 44-50.

[10] C. S. B. Gil, V. S. B. Gil, S. M. Carvalho, G. R. Silva, J. T. Magalhaes, R. L. Orefice, A. Mansur, H. S. Mansur, P. S. O. Patricio, L. C. A. Oliveira, *New J. Chem.*, 2016, **40**, 8502-8510.

[11] Q. Wang, M. Jian, C. Wang, Y. Zhang, *Adv. Funct. Mater.*, 2017, **27**, 1605657.

[12] Y. Shon, H. Kim, H. S. Hwang, E. S. Bae, T. Eom, E. J. Park, W.-S. Ahn, J. J. Wie, B. S. Shim, *Sensors Actuators B Chem.,* 2017, **244**, 1-10.

[13] M. Oliviero, R. Rizvi, L. Verdolotti, S. Iannace, H. E. Naguib, E. Di Maio, H. C. Neitzert, G. Landi, *Adv. Funct. Mater.*, 2017, **27**, 1605142.

[14] J. Gu, Y. Su, P. Liu, P. Li, P. Yang, *ACS Appl. Mater. Interfaces* 2017, **9**, 198-210.

[15] D. Wang, Y. Ha, J. Gu, Q. Li, L. Zhang, P. Yang, *Adv. Mater.,* 2016, **28**, 7414-7423.

[16] Y. Zhao, Q. Jiang, H. Xu, N. Reddy, L. Xu, Y. Yang, *J. Biomed. Mater. Res. Part B Appl. Biomater.,* 2014, **102**, 729-736.

[17] G. H. Altman, F. Diaz, C. Jakuba, T. Calabro, R. L. Horan, J. Chen, H. Lu, J. Richmond, D. L. Kaplan, *Biomaterials*, 2003, **24**, 401-416.

[18] D. K. Kim, J. I. Kim, T. I. Hwang, B. R. Sim, G. Khang, *ACS Appl. Mater. Interfaces*, 2017, **9**, 1384-1394.

[19] Y. Kambe, K. Kojima, Y. Tamada, N. Tomita, T. Kameda, *J. Biomed. Mater. Res. Part A*, 2016, **104**, 82-93.

[20] P. J. Nowatzki, D. A. Tirrell, *Biomaterials*, 2004, **25**, 1261-1267.

[21] J. Sun, L. Yang, M. Jiang, Y. shi, B. Xu, H. Ma, *J. Chromatogr. B*, 2017, 1054, 57-63.

[22] R. Güzel, A. Ersöz, İ. Dolak, R. Say, *Mater. Sci. Eng. C*, 2017, **79**, 336-342.

[23] D. R. Smith, R.-S. Wang, *Environ. Health Prev. Med.*, 2006, **11**, 3-10.

[24] N. Rangavajhyala, V. Ghorpade, M. Hanna, *J. Agric. Food Chem.*, 1997, **45**, 4204-4208.

[25] S. Chen, L. Li, C. Zhao, J. Zheng, *Polymer.,* 2010, **51**, 5283-5293.

[26] D. F. Moyano, Y. Liu, D. Peer, V. M. Rotello, *Small*, 2016, **12**, 76-82.

[27] L.-S. Wang, B. Duncan, R. Tang, Y.-W. Lee, B. Creran, S. G. Elci, J. Zhu, G. Yesilbag Tonga, J. Doble, M. Fessenden, M. Bayat, S. Nonnenmann, R. W. Vachet, V. M. Rotello, *ACS Appl. Mater. Interfaces*, 2017, **9**, 42-46.

[28] E. Jeoung, B. Duncan, L.-S. Wang, K. Saha, C. Subramani, P. Wang, Y.-C. Yeh, T. Kushida, Y. Engel, M. D. Barnes, V. M. Rotello, *Adv. Mater.*, 2015, **27**, 6251-6255.
[29] M. Sarkari, I. Darrat, B. L. Knutson, *Biotechnol. Prog.*, 2003, **19**, 448-454.
[30] D.-W. Zhu, *Macromolecules*, 1996, **29**, 2813-2817.
[31] K. R. Millington, H. Ishii, G. Maurdev, *Amino Acids*, 2010, **38**, 1395-1405.
[32] C. Subramani, K. Saha, B. Creran, A. Bajaj, D. F. Moyano, H. Wang, V. M. Rotello, *Small*, 2012, **8**, 1209-1213.
[33] E. H. Abdulkareem, K. Memarzadeh, R. P. Allaker, J. Huang, J. Pratten, D. Spratt, *J. Dent.*, 2015, **43**, 1462-1469.
[34] J. Raphel, J. Karlsson, S. Galli, A. Wennerberg, C. Lindsay, M. G. Haugh, J. Pajarinen, S. B. Goodman, R. Jimbo, M. Andersson, *Biomaterials,* 2016, **83**, 269-282.
[35] X. Li, Y.-C. Yeh, K. Giri, R. Mout, R. F. Landis, Y. S. Prakash, V. M. Rotello, *Chem. Commun.*, 2015, **51**, 282-285.

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