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Tailored Functional Surfaces Using Nanoparticle and Protein "Nanobrick" Coatings

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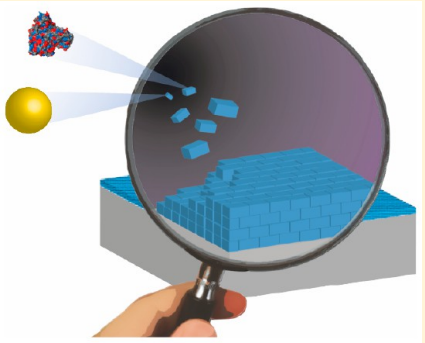
ABSTRACT: Surface properties are an essential feature in a wide range of functional materials. In this article, we summarize strategies developed in our group that employ nanoparticles and proteins as nanobricks to create thin-film coatings on surfaces. These coatings contain tailorable surface functionality based on the properties of the predesigned nanobricks, parlaying both the chemical and structural features of the precursor particles and proteins. This strategy is versatile, providing the rapid generation of both uniform and patterned coatings that provide "plug-and-play" customizable surfaces for materials and biomedical applications.



INTRODUCTION

Engineering the interactions of surfaces with the surrounding environment is crucial to the development of functional materials.[1−3] For example, controlling the interface between materials and biological entities enables the design of smart and responsive biomaterials.[4−6] Surface properties can either result directly from the bulk material surface or can be tailored by applying coatings that impart specific properties and functionalities.[7,8] These surface coatings provide a versatile platform for modulating the interface between the bulk material and the surrounding environment, consequently defining and enhancing the utility of these materials.[9,10]

Small-molecule monolayers have been widely employed for surface modification.[11] Although molecular grafting on surfaces is straightforward, this type of coating can face challenges with coverage efficiency,[12] stability[13] and characterization (particularly for more complex surfaces).[14] Applying thin film coatings on surfaces using polymers,[15,16] proteins,[17,18] and nanoparticles[19,20] as building blocks provides an alternative strategy to tailoring the functionality of surfaces.[21−23] In this feature article, we consider these nanobricks to be pre-engineered or natural nanomaterials that retain their structures and properties when used as coatings. These building blocks (nanobricks) often possess unique chemical and physical properties that can be translated into the surface features required for specific applications.[24,25] By applying these nanobricks to surfaces, a uniform and dense coating of the desired surface functionality can be obtained due to the high surface area of nanomaterials,[26] with stability enhanced through multivalent particle−surface interactions.[27] Additionally, the use of nanoparticles for surface modification automatically provides nanotextured surfaces,[28] in contrast to more traditional topological control employing etching and deposition techniques that increase the processing cost and time.[29]

In our research, we have used the ease of fabrication and characterization of nanoparticles to generate complex surfaces featuring a high degree of control of both physical and surface properties. More recently, we have added proteins (nature's nanoparticles) to our nanobrick tool kit, developing an approach that allows individual proteins to retain their native surface properties, e.g., charge and zwitterionic properties. In this feature article, we summarize our use of nanoparticles and proteins to provide new coating tools (Scheme 1) for a wide range of applications, paving the way for new technologies.

NANOPARTICLE-BASED COATINGS

Strategies to assemble NPs on surfaces can be broadly classified into two categories: supramolecular interactions and covalent bonding (Figure 1). Both approaches feature unique strengths. Supramolecular strategies feature the simplicity and modularity provided by self-assembly and can generate stable coatings through multivalent particle−surface interactions.[30] Covalent surface modification provides even greater stability[31] and can be used for applications requiring harsh conditions such as solvent, high ionic strength, or heat.[32] The chemical functionality of the surfaces can be systematically modulated by pre-engineering the capping ligands on these NPs for which characterization is easy and well-established.[33] Furthermore, the curvature of nanoparticles provides unique topographic properties to the surfaces, which we have used to generate surfaces with decreased fouling behavior.[34]

Immobilization of Nanoparticles on Surfaces Using Dithiocarbamate (DTC) Chemistry. Thiophilic metal and semiconductor NPs can be anchored onto thiol-terminated surfaces via a simple metal−sulfur interaction.[35] However, this approach can generate surfaces that are sensitive to oxidation or to place exchange by other thiols, especially in biological environments.[36,37] Molecules with the carbodithioate ($-CS_2$) moiety of

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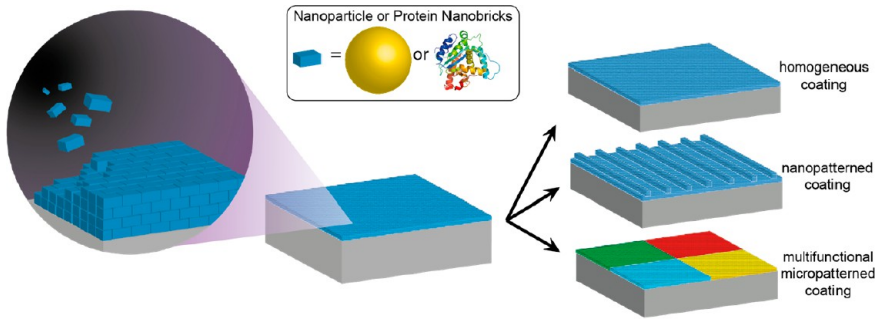
Scheme 1. Surface Coatings Fabricated by Nanoparticle- and Protein-Based Nanobricks



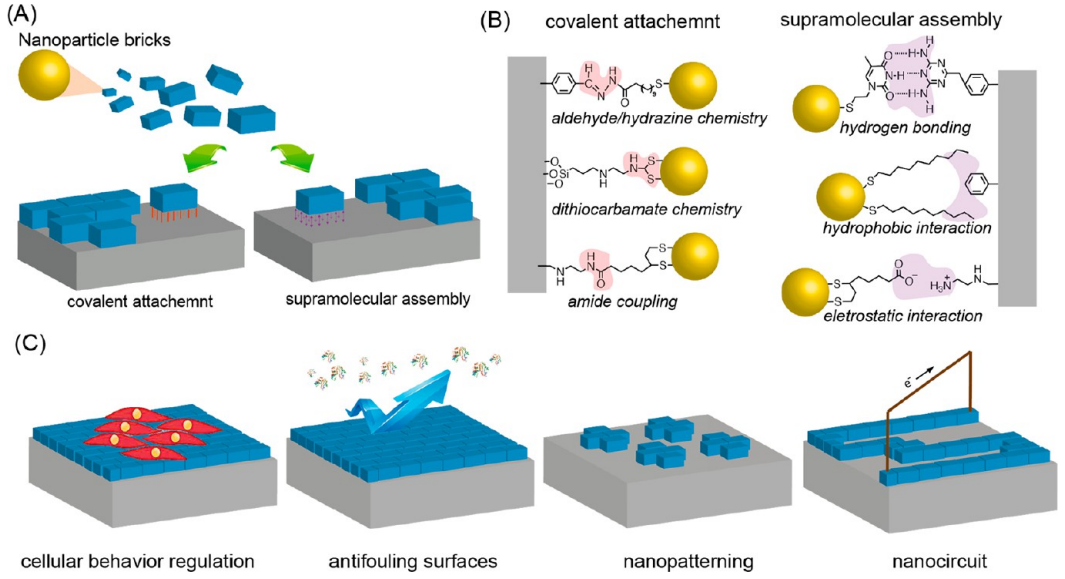


Figure 1. (A) Schematic illustration of NP-based nanobrick coating strategies. (B) Examples of NP coatings using covalent or supramolecular strategies. (C) Schematic illustration of NP-based coating applications.

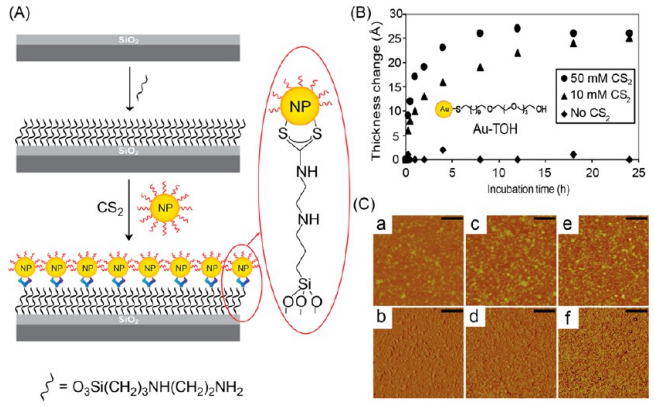


Figure 2. Nanoparticle-based surface modification technique utilizing DTC chemistry. (A) DTC-mediated attachment of NPs to amine-terminated surfaces. (B) Change in thickness as a function of time, as observed through ellipsometry, due to the binding of AuNPs. (C) AFM height and phase images of a surface after incubation times of (a, b) 1/2 h, (c, d) 1 h, and (e, f) 8 h in a solution of 50 mM CS_2 and 100 nM Au-NPs. Scale bars are 250 nm. The *Z* scale is 10 nm. Adapted with permission from ref 39. Copyright 2008, Wiley-VCH Verlag GmbH & Co. KGaA.

dithiocarbamates provide robust linkages to thiophilic surfaces due to their divalent nature and, in the case of gold, the interatomic S−S distance that is ideal for epitaxial adsorption onto Au surfaces.[38] To ensure the robustness of NP immobilization, we developed a dithiocarbamate (DTC)-based approach for the stabilization of thiophilic NPs on amine-terminated surfaces. In this process, a reversible adduct is formed through the reaction of primary or secondary amines on the surface with CS_2. This active group is stabilized when it bonds to the metal/semiconductor NPs, resulting in robust and stable NP-modified surfaces. We demonstrated the utility of the DTC strategy by reacting gold nanoparticles (AuNPs) with an amine-terminated silicon wafer in the presence of CS_2 (Figure 2A).[39] The immobilization of nanobricks on surfaces was monitored by measuring the change in thickness (ellipsometry) and surface topography (atomic force microscopy, AFM). Ellipsometry and AFM measurements showed that a uniform

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AuNP monolayer was formed after 1 day of incubation (Figure 2B,C). A salient feature of this method is that it can be applied to NPs with a variety of surface functionalities, which we demonstrated by generating neutral, negatively charged, and positively charged surfaces using hydroxyl-, carboxylate-, and trimethylammonium-functionalized AuNPs, respectively. In addition, the versatility of this approach was demonstrated using other thiophilic nanoparticles, including FePt NPs and quantum dots (QDs).[39] This DTC chemistry was also used in the reverse fashion to immobilize amine-terminated NPs onto thiophilic surfaces. We attached amine-functionalized silica NPs onto gold substrates, creating a scaffold with dense amine functionality and high surface area that was used for a variety of postfunctionalization technologies.[40] Taken together, these results demonstrate the utility of DTC chemistry for "painting" surfaces with nanoparticles.

Supramolecular Immobilization of NPs. Supramolecular chemistry provides a dynamic and reversible strategy for generating customizable NP coatings.[41] This functionalization strategy takes advantage of the wide range of available noncovalent interactions, including hydrogen bonding,[42] hydrophobic interaction,[43] and electrostatics.[44] In one approach, we used "lock and key" hydrogen bonding to coat surfaces with nanoparticles. Thymine-functionalized QDs (Thy-QD) were immobilized onto diaminotriazine-functionalized polystyrene (PS-Triaz) surfaces via three-point hydrogen-bonding interactions (Figure 3).[45] The three-point interaction provides specificity to this immobilization process, which is beneficial to the fabrication of multifunctional or patterned surfaces. (See the next section.) The specificity of this method was demonstrated by using *N*-methyl thymine-functionalized QDs (MeThy-QD) as a negative control, where no binding to the PS-Triaz surface was observed (Figure 3C,E).

Electrostatic interactions provide another noncovalent tool for surface modification,[46] allowing the use of readily available charged substrates and generating surfaces with direct utility for biomaterial applications. In contrast to small-molecule systems, multivalent NP/surface interactions can provide stable coatings. We developed a versatile technique that uses positively charged polyvinyl *N*-methylpyridine (PVMP) to direct the site-selective deposition of negatively charged citrate-protected AuNPs, producing conductive surfaces (Figure 4A,B).[47] Furthermore, post-functionalization using different thiols enables the fine tuning of surface properties (Figure 4C). Coupling of this simple deposition strategy with the wide range of polymer patterning techniques should allow easy access to a variety of materials, with applications ranging from nanoelectronics to medical devices (Figure 4D–G).

Electrostatic immobilization strategies can also be applied to coating commercially available negatively charged PS surfaces used for cell culture applications. For these uses, we developed a facile strategy for modifying readily available polystyrene cell culture plates with cationic nanoparticles. Angle-resolved XPS showed that a monolayer of AuNPs was formed after incubating the plasma-treated PS plates (Figure 5A,B). We then tested the stability of these AuNP coatings in a cell culture environment. The stability of these monolayers was tested in PBS, cell culture media, and protease. The results indicated that AuNP monolayers on PS remain stable under biological conditions but only when the PS plate was pretreated with plasma either by the manufacturer or in-house (Figure 5C).[48]

Micro- and Nanopatterning of NP-Coated Surfaces. Surface patterning can be readily adapted to NP coating strategies.[49] The key is to create patterned areas that can either facilitate or block reactions and interactions with NP bricks. For example, octadecanethiol (ODT) can be used as a mask to block

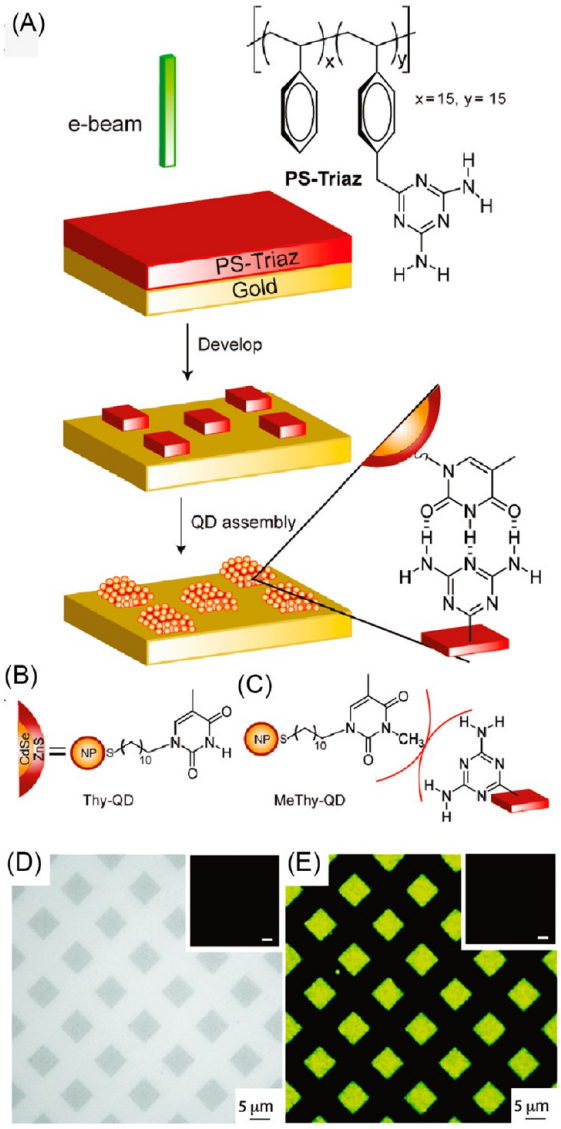

Figure 3. (A) Fabrication of nanopatterned PS-Triaz templates using EBL and postfunctionalization with the complementary Thy-QDs. (B) Structure of Thy-QDs. (C) Schematic representation of the lack of binding between control MeThy-QDs and PS-Triaz templates due to the disruption of hydrogen bonding. (D) Bright-field image of PS-Triaz patterns prior to QD assembly; the inset shows the fluorescent image. (E) Fluorescent image after the assembly of Thy-QDs; the inset shows the patterned surface fluorescent image after incubation in MeThy-QDs. Adapted with permission from ref 45. Copyright 2011, American Chemical Society.

the interaction of Au surfaces by occupying the available bonding areas. We used microcontact printing to generate patterned ODT areas on Au surfaces. After adding amine-functionalized SiO_2 NP and CS_2 to ODT-patterned Au surfaces, NP attachment occurred only on the bare areas.[40] Another strategy for blocking reactions is to physically prevent the coating solution from contacting the surfaces by using nanomolding in capillaries (NAMIC).[50] NAMIC uses a stamp with hard and well-defined features on flat poly(dimethylsiloxane) (PDMS) as the nanomold. The capillaries allowed the reactants (AuNPs and CS_2) to fill in and react at locations that allow large-area patterning of AuNP arrays in a one-step, low-cost process (Figure 6A). The versatility of this patterning method was demonstrated by using both QD (Figure 6B) and FePt NP nanobricks (Figure 6C).

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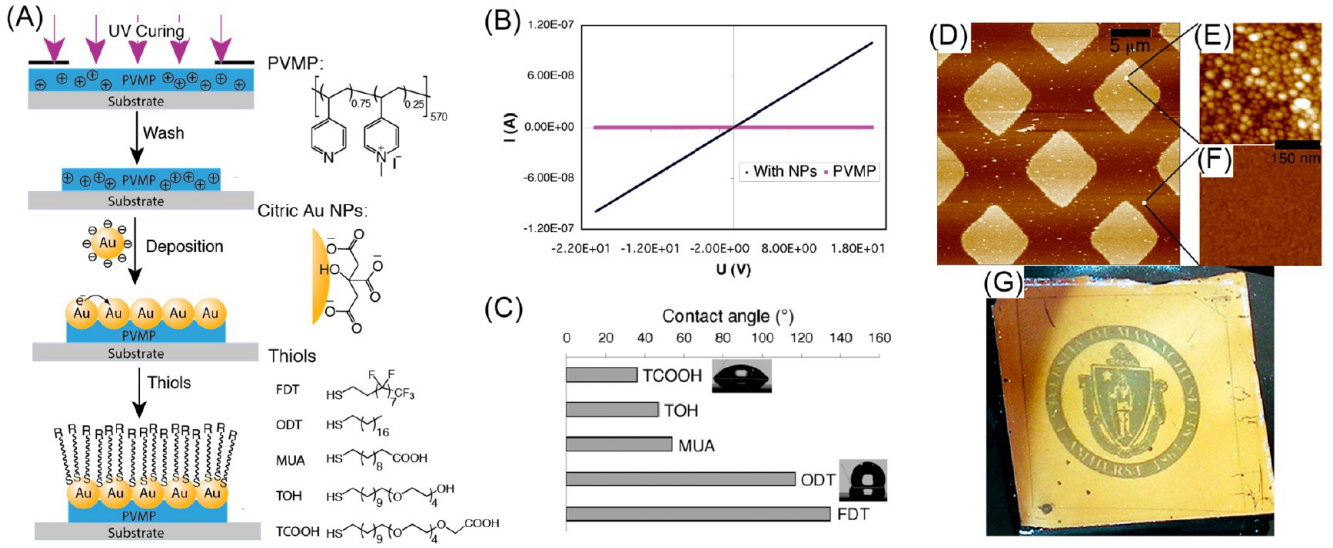
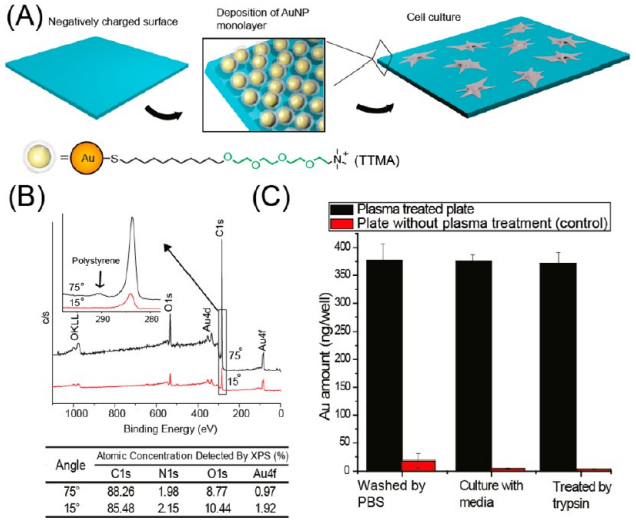


Figure 4. (A) Fabrication of patterned NP surfaces via site-selective adsorption of NPs on photopatterned PVMP films to produce conductive Au films. (B) *I*–*V* curves of the PVMP film before (purple) and after (black) the deposition of NPs. (C) Changes in the water contact angle due to postfunctionalization with different thiols. The insets are representative micrographs of a hydrophilic (top) and hydrophobic surface (bottom). (D) AFM images of patterned NPs films, with close-up images of (E) deposited AuNP and (F) bare silicon areas. (G) Conductive Au film fabricated on a 1 cm × 1 cm glass slide. Adapted with permission from ref 47. Copyright 2007, Wiley-VCH Verlag GmbH & Co. KGaA.



Angle	Atomic Concentration Detected By XPS (%)			
	C1s	N1s	O1s	Au4f
75°	88.26	1.98	8.77	0.97
15°	85.48	2.15	10.44	1.92

Figure 5. (A) Schematic representation of a strategy to generate positively charged AuNP monolayers on plasma-treated polystyrene cell-culture plates. (B) Angle-resolved XPS analysis of the AuNP layer on the polystyrene surface. Relative atomic concentrations of C, N, O, and Au are listed in the table. (C) AuNPs attached to plasma-treated (black) and untreated (red) plates under various cell-culture conditions. Each bar represents the amount of Au remaining in a well of a 96-well plate. Reproduced with permission from ref 48. Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA.

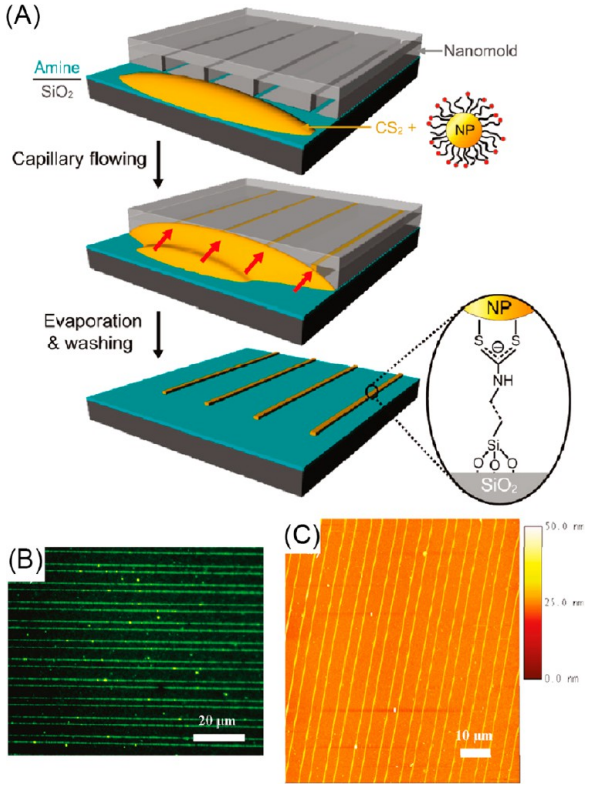


Figure 6. (A) Fabrication of 1D NP arrays using NAMIC coupled with DTC chemistry. (B) Fluorescent image of patterned QD-TOH NPs. (C) AFM topography image of patterned FePt-TOH NPs. Adapted with permission from ref 50. Copyright 2010, American Chemical Society.

In addition to masking, we can use an additive approach that employs reactive areas on the surface to guide NPs to form patterned coatings. This reactive strategy was demonstrated by fabricating poly(ethylenimine) (PEI)-patterned surfaces using nanoimprint lithography (NIL).[51] The amine groups present on the patterned PEI layer were used for multiple strategies of NP immobilization, including amide coupling, DTC, and electrostatic interactions. Moreover, by introducing orthogonal patterns with different interactions, multiple functionalities on the same surface could be generated using a one-step, self-sorting process. For example, negatively charged QDs can be deposited on polyvinyl *N*-methylpyridine (PVMP)-treated areas, and the diaminopyridine-functionalized polystyrene (DAP-PS) can be deposited on thymine-terminated areas (Figure 7).[52]

Controlling Biomaterial Interfaces Using NP Coatings. The creation of functional biocompatible materials for delivery and sensing applications has been a long-term focus of our research program.[53] Nanobrick-based surface modification

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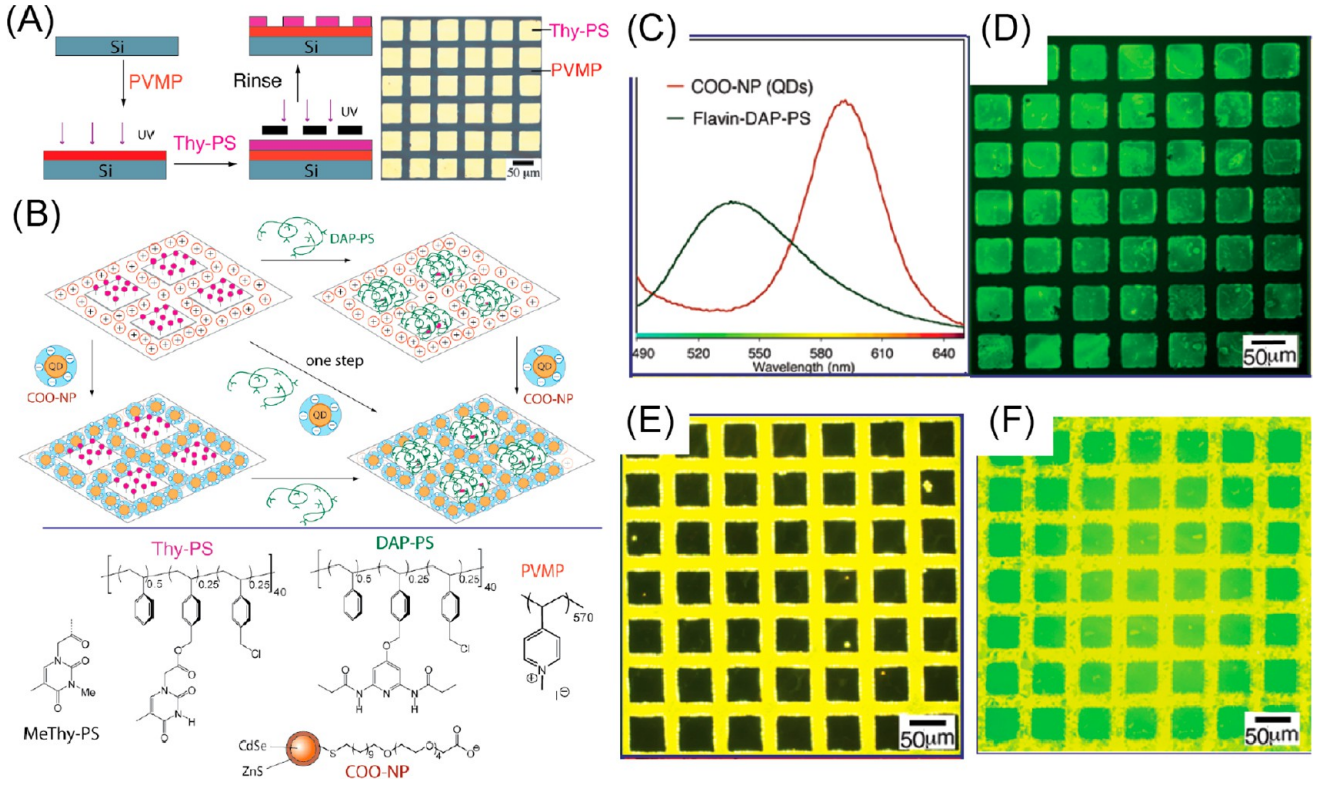

Figure 7. (A) Fabrication of a patterned PVMP/Thy-PS surface and its optical micrograph. (B) One-step sequential orthogonal functionalization process using DAP-PS and COO-NP and the chemical structures of the materials. (C) Fluorescence emission spectra of DAP-PS and COO-NP. Fluorescence microscopy of surfaces modified using (D) DAP-PS, (E) COO-NP, and (F) both components in a one-step process. Adapted with permission from ref 52. Copyright 2006, American Chemical Society.

strategies allow us to parlay the behavior of these engineered nanoparticles into surface properties. As a starting point, the undesired accumulation of proteins on surfaces and devices (i.e., biofouling) has been a challenge when designing biomaterials. In our previous studies, AuNPs were generated with engineered surface ligands that stabilize adsorbed proteins, preventing denaturation.[54] This ability to bind without denaturation was used to generate surfaces using AuNPs that are highly resistant to protein fouling. Surfaces featuring positive, neutral, and negative AuNPs were immobilized onto PEI films via DTC chemistry (Figure 8A). The AuNP-coated surfaces, regardless of the terminal functionalities, significantly reduced the protein fouling as compared to that of bare PEI surfaces (Figure 8B),[55] reducing the propensity of these systems to encounter inflammation and immunogenic responses in patients from protein fouling.[56]

The utility of the AuNP-coated surfaces for cell−surface interfacing was demonstrated by cell adhesion and proliferation studies. All of the surfaces, regardless of the charge, supported cell growth and adhesion, showing excellent biocompatibility (Figure 8C). These surfaces provided templates for cell patterning and alignment, as demonstrated using patterned anionic AuNPs coatings. The resistance of AuNP patterns to protein adsorption provides a high degree of cellular alignment, demonstrating effective communication between the antifouling surface and the cells (Figure 8D−F).[57] Taken together, the use of AuNP nanobricks provides a promising solution for preparing antifouling coatings for biomedical applications.

Surface properties such as morphology, chemistry, and hydrophobicity have strong effects on regulating cellular behavior.[58] Using these antifouling AuNP nanobricks, we developed a powerful platform for studying the role of surface chemical functionality on cell−surface interaction without the complications induced by denatured proteins on surfaces. We used a variety of functional groups to decorate the surfaces by electrostatically depositing cationic AuNP monolayers onto polystyrene substrates. The chemical functionality was controlled by changing the R group on the ligand, as shown in Figure 9A. These AuNPs were immobilized on plasma-treated PS cell culture plates through electrostatic interactions (Figure 5). We used this commercially available and high-throughput platform to study the effect of chemical functionality on cell viability, adhesion, and proliferation (Figure 9B).[48] The cell lines interacted differently with surfaces featuring different functionalities, creating a useful database for the systematic study of the structure−activity relationship of surface functionality and biological responses. Taken together, AuNP nanobrick coatings provide a powerful tool for studying cell−surface interactions and can be used to modulate cellular behaviors.

■ PROTEIN NANOBRICKS

The nanobrick method of generating coatings allows us to translate the surface properties of each nanobrick into the functionality of the bulk surface.[59] Proteins are nature's nanobricks, providing incredible functional and structural diversity.[60] Moreover, proteins are biocompatible and sustainable precursors for generating functional coatings.[61] A vast majority of applications of protein films require stability in aqueous environments.[62,63] However, current methods for stabilizing protein films either (a) employ denaturing conditions that relinquish the surface properties of the protein;[64] (b) use naturally self-assembling proteins that dramatically reduce the variety of proteins that can be used;[65] or (c) employ toxic cross-linkers that adversely affect the

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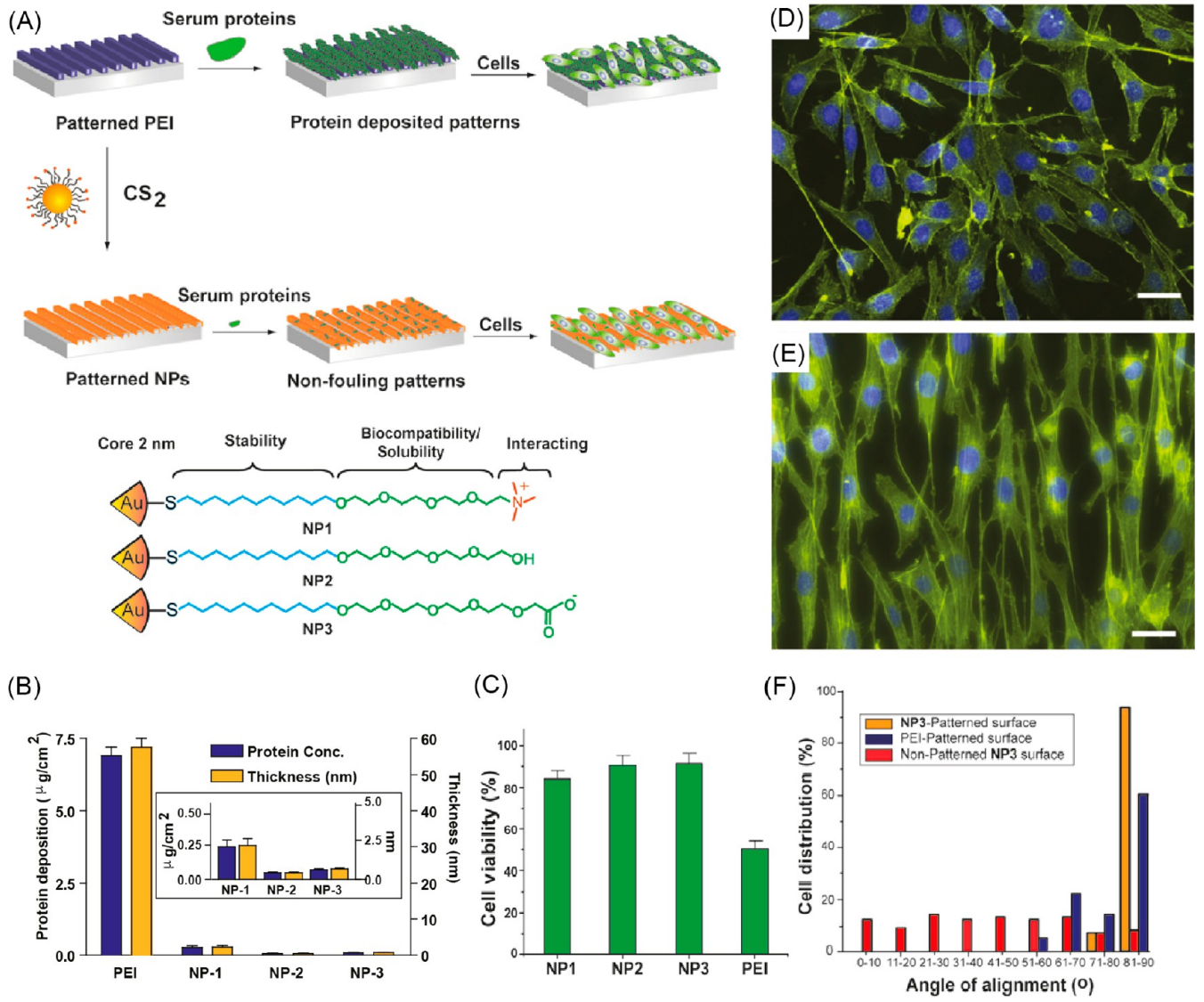

Figure 8. (A) Schematic depiction of the cellular arrangement on pristine PEI and AuNP-coated, PEI-patterned surfaces. (B) Quantity of the protein adsorbed (blue) and thickness of the protein layer (yellow) on PEI- and NP -coated surfaces, as measured by ellipsometry. Inset: deposition on NP-coated surfaces. (C) Relative percentage of cell viabilities of fibroblast cells on the surfaces. Fluorescent micrographs of cells on (D) the nonpatterned NP3 surface showing random cell arrangement and (E) on the patterned NP3 surface showing cell alignment along the pattern. (F) Histogram of the cell alignment angle on surfaces. The scale bar represents 20 μm. Adapted with permission from ref 57. Copyright 2012, Wiley-VCH Verlag GmbH & Co. KGaA.

behavior of protein nanobricks by significantly altering their biocompatibility and/or surface functionality.[66]

Recently, we developed a scalable technology to fabricate protein films that relies on the thermal treatment of proteins in a fluorous environment in order to generate aqueous stable coatings that retain the surface functionality of native proteins, including hydrophilicity, biodegradability, surface charges, and zwitterionic properties. This method can be used with any type of protein (or at least all we have tried) to generate a variety of different surfaces with properties derived from the molecular functionality of the proteins. We employed two strategies to provide this fluorous environment, including heat curing in a fluorous media for 3D substrates[67] and nanoimprint lithography (NIL) for nanopatterned 2D surfaces[78] (Figure 10).

The first key step in this research was establishing the retention of protein structure after heat curing in fluorous media (Figure 11). Heat curing of bovine serum albumin (BSA) films in air results in almost complete denaturation, as determined by circular dichroism.[68] In contrast, heating these films in fluorous media results in only modest changes in protein secondary structure (Figure 11B). The next question is whether the retention of protein structure provided films where the surface properties mirrored those of the native protein, particularly in terms of hydrophilicity and charge. Heating protein films in air creates high water contact angles (Figure 11C) due to the denaturation of the protein and the presentation of hydrophobic residues at the surface to minimize air−film surface energy.[69] In contrast, fluorous-cured films had significantly lower contact angles indicative of a more hydrophilic surface.

One of the key advantages of using proteins as building block is that they come with a wide range of surfaces charges, from cationic to anionic. We explored the translation of surface charge into material surface properties by generating fluorous- and air-cured films from two proteins: anionic BSA (pI 4.8) and cationic lysozyme (Lyso, pI 11). We next performed Kelvin probe force microscopy (KPFM) on the films (Figure 11D).[70] The air-cured films

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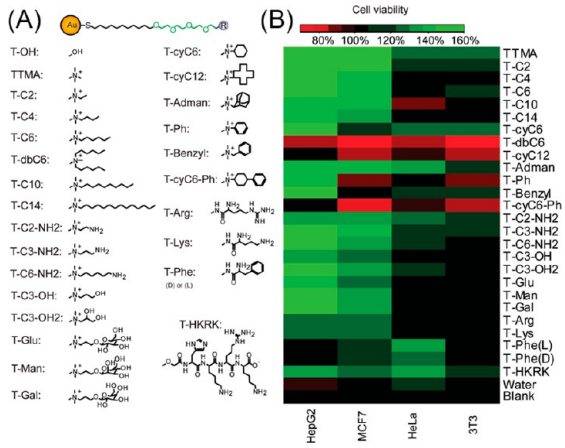

Figure 9. Heat map showing the cell viability of 4 different cell lines on 26 different AuNP coatings. (A) Structures of the ligands on the AuNP nanobricks. (B) Heat map of cell viability influenced by different AuNP coatings. Reproduced with permission from ref 48. Copyright 2014, Wiley-VCH Verlag GmbH & Co. KGaA.

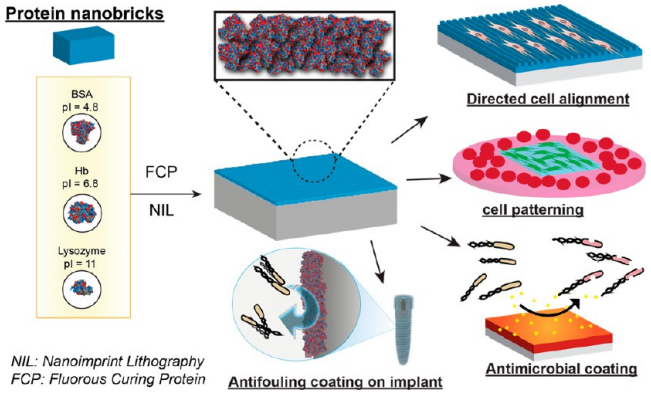

Figure 10. Schematic illustration of protein nanobrick-based coatings and their current applications.

had similar potentials, consistent with burying the protein surface charges in the film. In contrast, the fluorous-cured films featured dramatically different surface charges, with the cationic Lyso film substantially more positive than that derived from the anionic BSA.

Proteins possess unique properties derived from their structural and surface properties.[71] After successfully preserving protein surface charge through fluorous curing, we next wanted to know if this method can be employed to translate protein structure into material surface properties. BSA has been frequently used as a blocker to prevent nonspecific binding in delivery and sensing applications due to its zwitterionic structure and overall negatively charged functionality.[72,73] Therefore, we decided to determine if this property can be translated into resistance to bacterial fouling on BSA-coated substrates, particularly for biomedical applications. Combined with dip-coating deposition, we were able to generate smooth and seamless coatings on complex 3D substrates, i.e., dental implant screws. Complete coverage of protein coating on the screws was observed by using brilliant blue staining and scanning electron microscopy. In addition, the remarkable robustness of BSA coatings was demonstrated by application to a bone-mimicking polyurethane block (Figure 12A,B). We next tested the antifouling property of these BSA coatings by immersing the screws in bacterial solution for 24 h. No adhesion was observed on the BSA-coated screw, while the control surface showed severe bacterial contamination (Figure 12C).

NIL-Based Treatment of Nanopatterned Protein Coatings. In addition to surface charge and hydrophobicity, the morphology and topology of surface coatings are important factors in controlling cell−surface interactions.[74] In particular, nanotopology is a key determinant of cell proliferation and spreading and therefore forms an integral aspect of tissue engineering.[75] NIL has been used for creating nanopatterned surfaces and is suitable for roll-to-roll production.[76] Taking advantage of the fluoruos environment generated by the fluorinated molds commonly used in the NIL process,[77] we empolyed NIL for the fabrication of flat and nanopatterned protein coatings (Figure 13A).[78] In this study, we generated negative, neutral, and positively charged surfaces using BSA (pI 4.8), hemoglobin (Hemo, pI 6.8), and Lyso (pI 11). (Figure 13B). A functional demonstration of the differences in surface properties was obtained through cell adhesion studies. Cationic Lyso coatings provide excellent cytophilicity, while anionic BSA coatings and charge-neutral Hemo coatings are cytophobic and therefore prevent biofouling (Figure 13C). Using a nanopatterned mold, we fabricated Lyso films with a 300 nm grooved pattern to dictate cellular alignment, with strong orientation effects observed (Figure 13D). Together these results demonstrate the simplicity of protein nanobrick-based coatings for generating multifunctional surfaces and adaptability to large-scale production.

Inkjet-Printed Micropatterning of Protein Nanobricks. A major advantage of our fluorous-based strategy is the ability to generate surfaces with different surface properties solely by the choice of protein nanobricks, as demonstrated using cell adhesion studies. This ability to tailor surface cytophilicity is promising for directing cell adhesion to generate patterned cell culture,[79] which is appealing for various biological applications including tissue engineering, sensing, and developing coculture systems.[80] Inkjet printing provides a reproducible method for controlling the precise mixing and deposition of nanomaterials on the surface.[81,82] We fabricated micropatterned BSA and Lyso films with this technique. The parametric control offered by the inkjet printer allows us to modulate the coating properties through combinatorial protein film fabrication (Figure 14A). Results from KPFM studies revealed that, as expected, the surface potential of these mixed protein films increases through increasing the Lyso component (Figure 14B). The use of mixed protein nanobricks to construct coatings with tunable functionalities was further tested by cell adhesion studies. The results obtained using combinatorial and gradient films both demonstrated that the cell−surface interaction on these micropatterned patches was highly dependent on the protein nanobrick components, in which the Lyso nanobrick was responsible for the affinity to the fibroblast cells (Figure 14C,D).[83]

We next generated micropatterned coatings to direct cell attachment on the printed Lyso patches while avoiding the BSA area (Figure 14E). This self-sorted cellular patterning technique is promising for the development of cell arrays as well as coculture platforms.[84] More specifically, side-by-side coculture facilitated research on the cell−cell interaction at the interface,[85] such as phagocytosis-based therapeutics.[86] We demonstrated the potential of a micropatterned protein film for inflammatory studies by coculturing macrophage (RAW264.7) and human embryonic kidney cells (HEK293) side-by-side. Our study revealed that HEK293 attached only to an assigned Lyso patch while RAW264.7 was capable of growing on both BSA and Lyso areas. Using a two-step seeding method, a cell pattern composite

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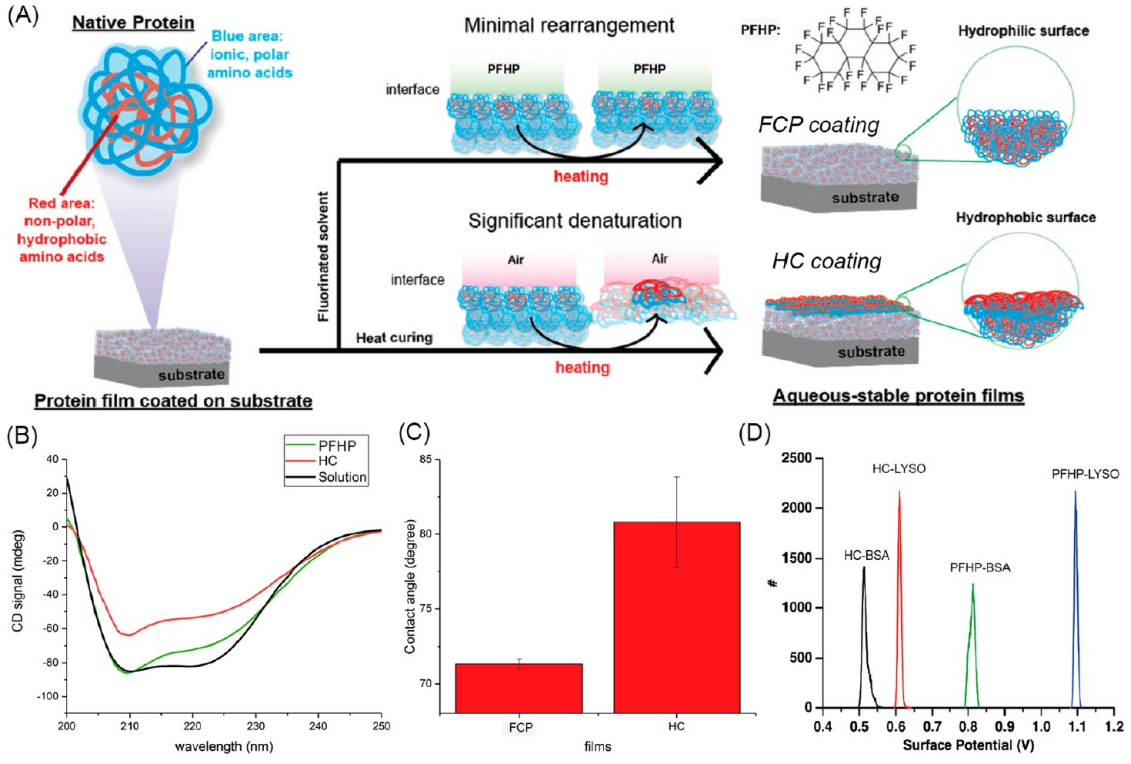

Figure 11. (A) Protein coatings heated in air or fluorous solvent to provide air-stable thin films. Fluorous solvent avoids the denaturation of proteins at the interface, generating hydrophilic films that retain intrinsic properties such as charge and zwitterionic surfaces of the precursor proteins. (B) Circular dichroism spectra of BSA in solution (phosphate buffer) and heat-cured (HC) and fluorous-cured processing (FCP). (C) Contact angle (water) of BSA films stabilized by HC and FCP methods. (D) Surface potential of Lyso and BSA films as quantified by Kelvin probe force microscopy (KPFM). Adapted with permission from ref 67. Copyright 2018, The Royal Society of Chemistry.

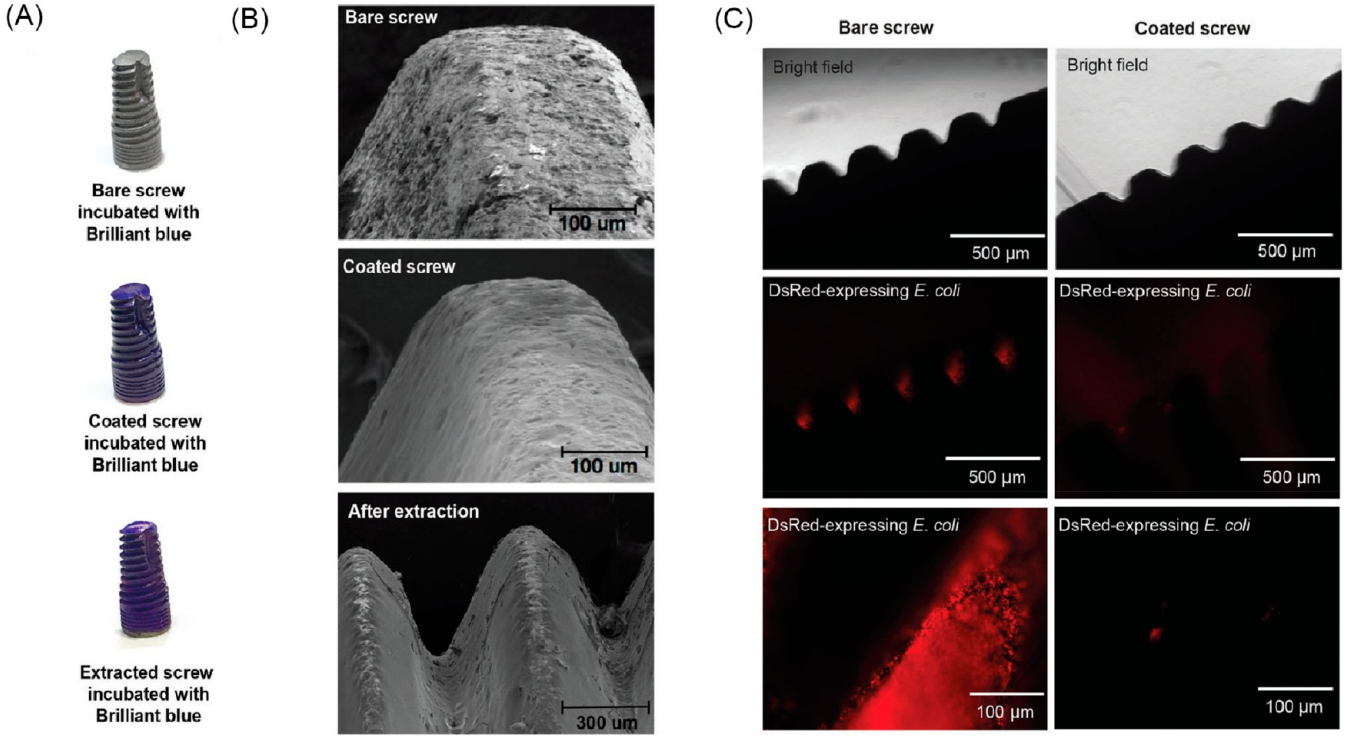

Figure 12. Three-dimensional protein film coating on a dental implant screw. (A) Images of brilliant-blue-stained screws that are bare, BSA-coated, and after extraction from a bone mimic PU block. (B) Scanning electron microscopy images for bare, coated, and extracted screws. (C) Optical and fluorescence microscopy images of DsRed-expressing *E. coli* on bare and coated screws after 24 h of incubation with bacteria. Reproduced with permission from ref 67. Copyright 2018, The Royal Society of Chemistry.

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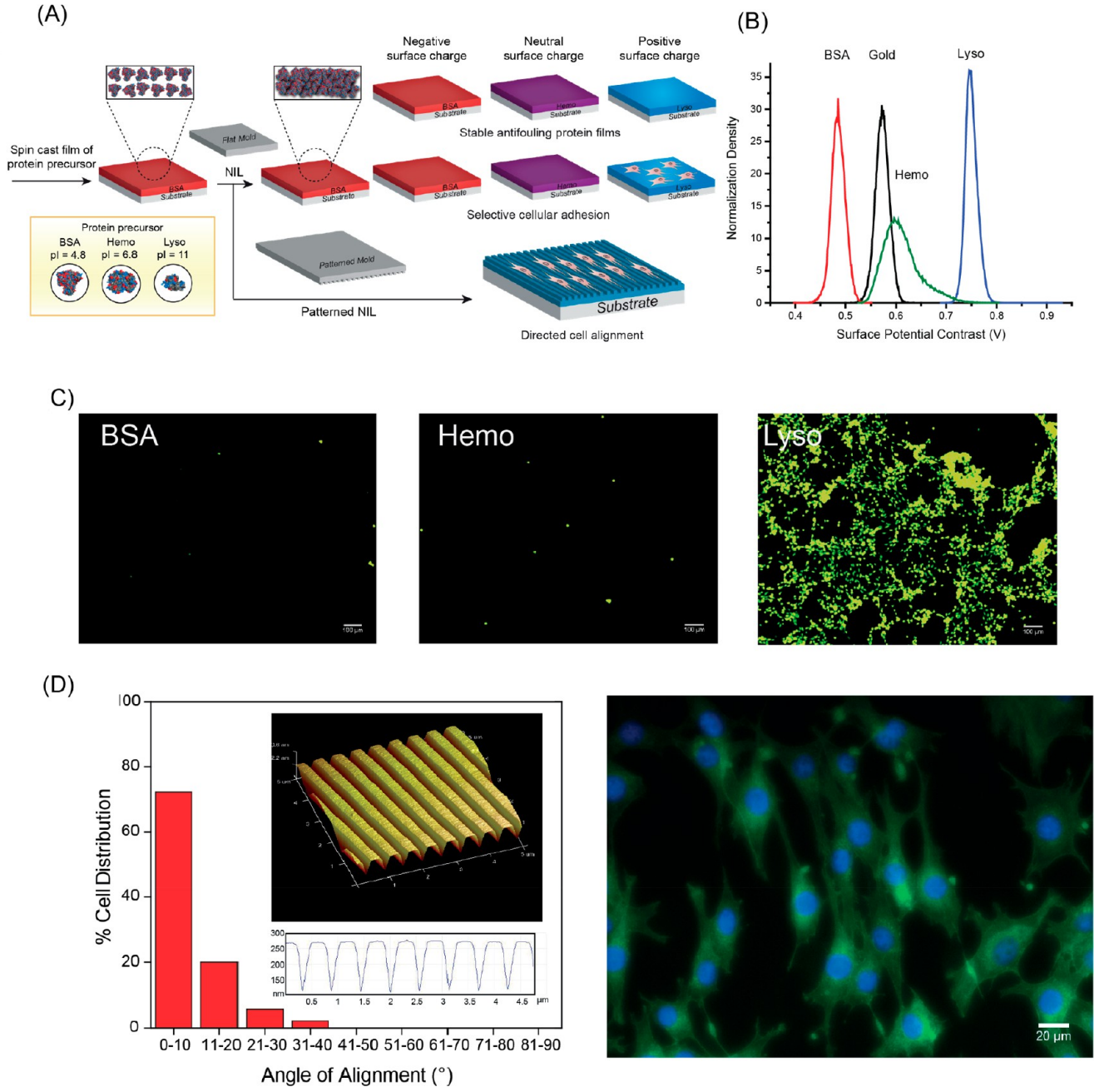

Figure 13. (A) Protein solutions were nanoimprinted using temperature and pressure to generate both planar and patterned surfaces. (B) Surface potential of individual protein films as determined by Kelvin probe force microscopy. (C) Cellular adhesion to protein films. Cells adhered to protein films were stained with calcein AM after 48 h of incubation. Scale bars represent 100 μm. (D) Percentage of cells aligned with the nanoimprinted Lyso pattern and fluorescence micrograph of fibroblast cells. The inset is a 3D AFM micrograph of the patterned Lyso film. Adapted with permission from ref 78. Copyright 2015, Wiley-VCH Verlag GmbH & Co. KGaA.

of multiple cell types was generated (Figure 14F).[87] This side-by-side coculture system has the potential to overcome the limitations of the traditional mixing method, thus providing a better platform for studying intercellular interactions.

Antimicrobial Surfaces Prepared by Protein-Based Coatings. Developing an effective antimicrobial strategy has been one of our main focuses in terms of translational research. Infections caused by the bacterial contamination of medical devices are a serious healthcare concern.[88] Designing a biocompatible coating that possesses antimicrobial activity is, therefore, especially important for medical applications.[89] Several strategies have been proposed for either preventing bacterial adhesion or imparting biocidal capability. For example, zwitterionic polymers and poly(ethylene glycol) generate useful platforms for preventing bacterial adhesion.[90] *N*-Halamine polyurethane films generated by the halogenation of methacrylamide-grafted polymeric films provided antimicrobial dental unit waterline tubing with biocidal activity.[91] However, the biocidal efficacy can be compromised by the accumulation of dead bacterial cells on coatings that cannot prevent biofouling.[92] Combining nonfouling and biocidal properties therefore yields effective anti coatings.[93,94]

Taking advantage of the inherent chemical diversity of the protein surface, we performed postfunctionalization to impart

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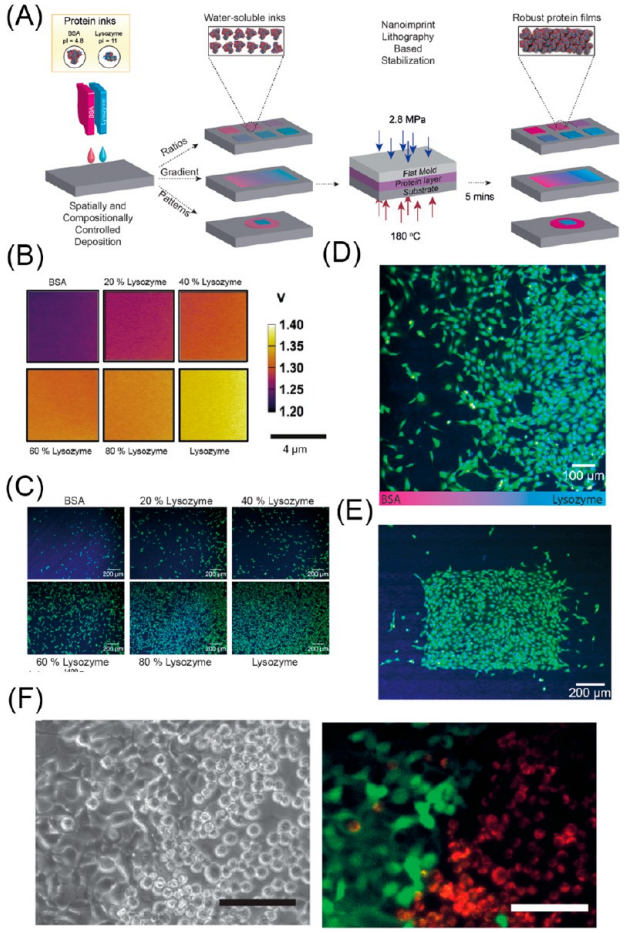

Figure 14. (A) Inkjet printing of protein to control the spatial presentation of the protein "bricks" on the surface. (B) Surface potential determined by KPFM. Protein films were fabricated by varying the BSA/Lyso ratio printed on the surface. (C) Adhesion of mammalian fibroblasts on films with varying ratios of protein components. (D) Adhesion of fibroblasts to protein films using a gradient pattern of BSA to Lyso. (E) Adhesion of fibroblasts to patterned film with discrete Lyso and BSA domains. (F) Optical (left) and fluorescence micrographs (right) of the coculture (green, GFP-expressed HEK293; red, DiD lipophilic tracer labeled RAW264.7). Scale bars represent 100 μm. Adapted with permission from ref 87. Copyright 2016, American Chemical Society.

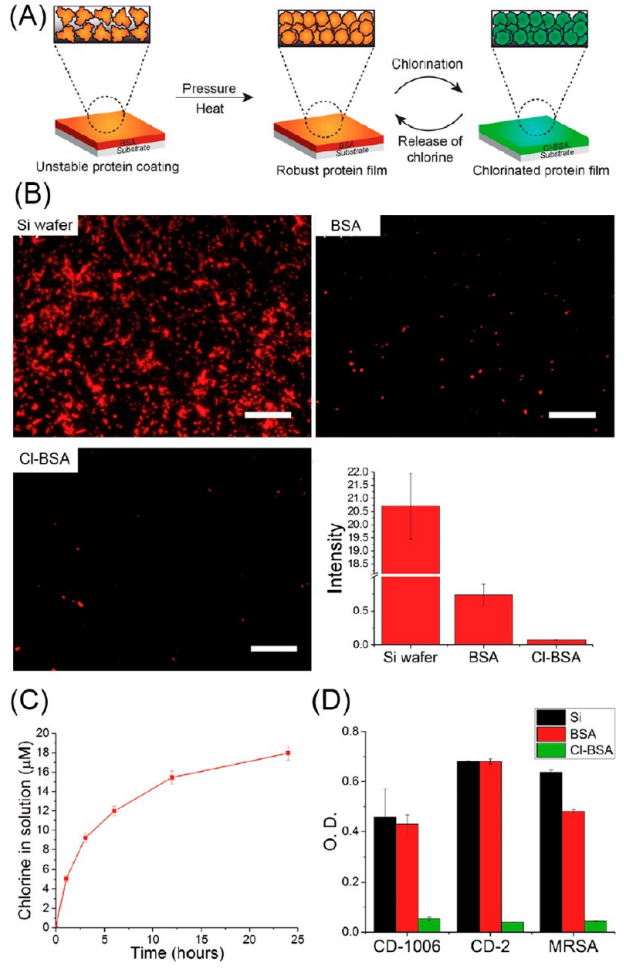

Figure 15. (A) Processing strategy to generate chlorinated protein films. (B) Fluorescent microscopy images and quantitative analysis of bacterial adhesion on Si wafers, BSA, and the Cl-BSA surface after incubation with red fluorescent protein expressing *E. coli* for 24 h. (C) Chlorine content in water after incubating with the Cl-BSA film. (D) Bacterial growth in solution after 24 h of incubation with silica, BSA, and Cl-BSA surfaces for pathogenic strains of Gram-positive (CD-1006 and CD-2) and Gram-negative (MRSA) bacteria. Scale bars are 100 μm. Adapted with permission from ref 96. Copyright 2016, American Chemical Society.

biocidal activity to protein coatings. BSA nanobricks possess cytophobicity and repel bacterial adhesion (Figure 12), thus being ideal candidates for developing antimicrobial coatings. We imparted biocidal activity to BSA coatings by using a commercially available sanitizer, sodium dichloroisocyanurate, to chlorinate protein nanobricks (Figure 15A). These BSA coatings retained their antifouling properties while gaining microbiocidal activity through the slow release of chlorine (Figure 15B). The halogenation reaction took place on cysteine sulfurs on BSA coatings, as verified by the X-ray photoelectron spectra of the chlorinated coatings.[95] After chlorination, chlorine was slowly released from BSA coatings and inhibited bacterial growth, displaying biocidal activity for a wide range of bacterial species (Figure 15C,D).[96]

SUMMARY AND OUTLOOK

Nanobrick-based coating strategies provide a key tool for material science and translational research. In our studies, we have developed immobilization strategies that are versatile and straightforward, generating stable, uniform, and multifunctional coatings with easily tailorable material surface properties. By utilizing these properties, we have developed new coating systems using nanoparticle and/or protein nanobricks that can easily be adapted to several patterning techniques, modulate cellular behavior, and prevent protein/bacterial fouling. Beyond these applications, proteins are emerging as intrinsically sustainable and ecofriendly nanobricks with the capability of modulating material properties based on their structural and chemical diversity. Our nanobrick-based coating strategies open a new avenue for interfacial science, providing a versatile platform for exploring surface properties derived from innumerable choices of nanobricks. While there are several nanobrick precursors still left to be explored, the strategies enlisted are apt to be translated into customizable coatings for applications ranging from nanoelectronics to medical devices, thereby providing new tools for the manufacture and application of functional materials.

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Notes

The authors declare no competing financial interest.

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Li-Sheng Wang received his B.S. degree in applied chemistry in 2007 from National Chi Nan University. He then received his M.S. degree in chemistry with a focus on nanomaterial science from National Tsing Hua University in 2009. He is currently a Ph.D. candidate in Professor Vincent M. Rotello's laboratory studying the use of naturally abundant protein in developing novel biomaterial thin films for antimicrobial applications, including antifouling surfaces and bactericidal coatings.

Sanjana Gopalakrishnan received her B.S. in chemistry from the Indian Institute of Technology, Kanpur in 2016, where she studied the characterization of biomaterials using AFM under Professor T. G. Gopakumar. She is currently pursuing her Ph.D. at the University of Massachusetts at Amherst under the supervision of Professor Vincent M. Rotello. Her current research is focused on developing functional protein-based materials for biological applications.

Vincent M. Rotello is the Charles A. Goessmann Professor of Chemistry and a university distinguished professor at the University of Massachusetts at Amherst. He joined the faculty at the University of Massachusetts in 1993. He has received the Langmuir Lectureship (2010), and in 2016, he received the TREE Award presented by the Research Corporation, the Bioorganic Lectureship of the Royal Society of Chemistry (U.K.), the Australian Nanotechnology Network Traveling Fellowship, and the Chinese Academy of Sciences, President's International Fellowship for Distinguished Researchers. He is a fellow of both the American Association for the Advancement of Science (AAAS) and of the Royal Society of Chemistry (U.K.). He was also recognized in 2014, 2015, and 2018 by Thomson Reuters/Clarivate as a highly cited researcher. He is currently the Editor in Chief of Bioconjugate Chemistry, and his research program focuses on using synthetic organic chemistry to engineer the interface between the synthetic and biological worlds.

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