One-Step Multipurpose Surface Functionalization by Adhesive Catecholamine

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Surface modification is one of the most important techniques in modern science and engineering. The facile introduction of a wide variety of desired properties onto virtually any material surface is an ultimate goal in surface chemistry. To achieve this goal, the incorporation of structurally diverse molecules onto any material surface is an essential capability for ideal surface modification. Here, a general strategy for surface modification is presented in which many diverse surfaces can be functionalized by immobilizing a wide variety of molecules. This strategy functionalizes surfaces by a one-step immersion of substrates in a one-pot mixture of a molecule and a catecholamine surface modification agent. This one-step procedure for surface modification represents a standard protocol to control interfacial properties.

1. Introduction

Control of surface properties is important in materials science, chemistry, and biotechnology. For example, the shapes and dimensions of nanomaterials are controlled by surface-bound molecules,[1,2] the development of bio-sensors and DNA/protein/peptide microarrays relies largely on the surface chemistry,[3,4] and the surface properties of drug delivery vehicles and medical devices are important factors in their biocompatibility and therapeutic performance.[5,6] For these applications, various materials such as synthetic organics, inorganics, ceramics, and their composites are currently used, onto which a number of surface properties introduced by chemical conjugation, polymerization, mineralization, and physical adsorption have been used. In general, surface modification approaches are developed case-by-case, and depend on the relationship between material properties and tethering molecules. For example, to modify gold and/or silver nanoparticle surfaces, mostly thiol-containing molecules have been used, and for synthetic non-degradable polymers, hydrolysis,[7] aminolysis,[8] and plasma treatment[9] has been utilized. These methods often involve time-consuming synthesis to incorporate appropriate chemical groups for surface tethering. Also, functionalization of polymeric surfaces is widely considered to be a challenging task, often requiring multi-step procedures.

Previously, a facile surface coating method based on the polydopamine (pD) was demonstrated.[10] pD coating methods are inspired by the amino acid composition of mussel adhesive proteins.[11,12] The pD coating has been demonstrated to functionalize a wide array of material surfaces, including superhydrophobic surfaces,[13] and allows for the formation of multifunctional, organic/inorganic ad-layer of proteins,[14] oligonucleotides,[15] polysaccharides,[10] metal ions,[16] hydroxyapatites,[17] or gold nanoparticles.[18] Although pD coating has been widely implemented, it has been limited to molecules presenting either amine or thiol groups.

Here we develop a pD-mediated, one-pot surface immobilization strategy that is applicable not only to versatile materials but also chemical functionalities. In this study, molecules co-dissolved with dopamine are immobilized onto surfaces during the pD formation (i.e. alkaline pH-induced polymerization of dopamine-hydrochloride). This approach is shown to be effective in modifying surfaces of a wide variety of oxides, noble metals, synthetic polymers, and ceramics. Moreover, molecules with a wide range in sizes (10^2 to 10^6 Da) and with various chemistries containing carboxyl, amine, thiol, quaternary ammonium, and/

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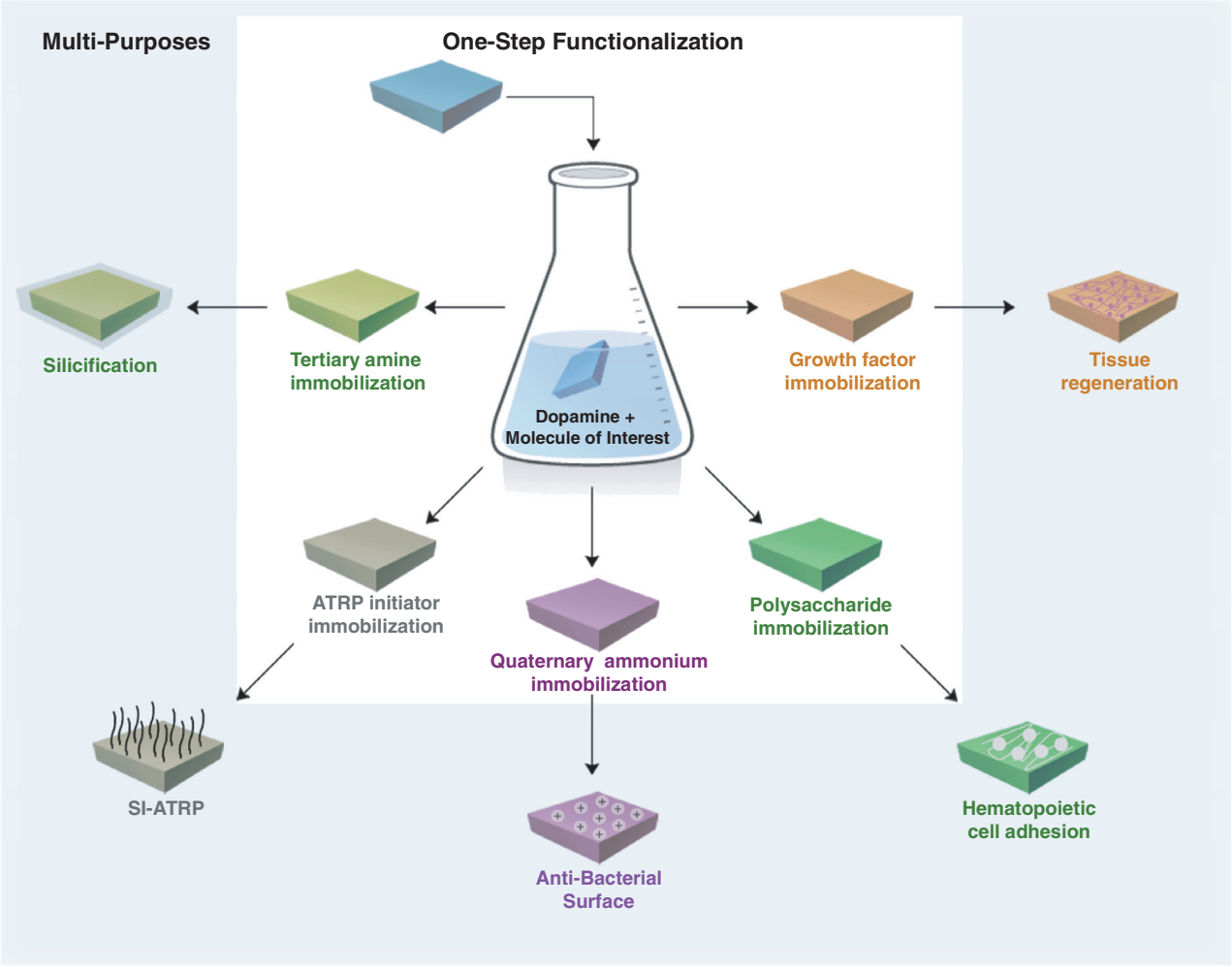

Figure 1. Schematics of the one-step modification of solid substrates. Numerous types of functionalized solid substrates were created for a variety of applications, including surface-initiated polymerization, biomineralization, antibacterial and cell-adhesive surfaces, and tissue regeneration.

or catechol groups are immobilized onto surfaces by a single step. Depending on chemical and biological functions of immobilized molecules, applications such as tissue engineering by growth factor immobilizations, hematopoietic cell adhesion by polysaccharide immobilizations, and anti-bacterial surface preparations were demonstrated. Furthermore, when initiators were immobilized, secondary modifications such as surface-initiated polymerization and bio-mineralization could be performed (**Figure 1**).

2. Results and Discussion

The one-pot pD surface modification consists of three components: a molecule to be immobilized, dopamine, and a substrate. The molecules of interest are co-dissolved with dopamine-hydrochloride in a weak alkaline solution (pH of 8.0 to 8.5) in which the substrates are immersed. After a certain amount of time, typically several hours, the molecule of interest is immobilized onto the immersed substrates regardless of material properties.

The first experimental model system is surface-initiated atom transfer radical polymerization (SI-ATRP). SI-ATRP has been widely used as a 'grafting-from' method to introduce various properties on surfaces.[19] The advantage of ATRP over other polymerization techniques is that nearly monodisperse polymer chains can be grown on the surfaces. However, the initiators used in SI-ATRP are commonly alkanethiols or organosilane derivatives. These initiators allow one to perform SI-ATRP on noble metals and oxide surfaces.[20,21] In contrast to the existing approaches, we demonstrated that the SI-ATRP can be performed on various material surfaces, including indium tin oxide (ITO), glass, silicon oxide (SiO_2), aluminum oxide (Al_2O_3), gold (Au), platinum (Pt), titanium oxide (TiO_2), poly(methyl methacrylate) (PMMA), polycarbonate (PC), poly(vinyl chloride) (PVC), polystyrene (PS), cyclic olefin copolymer (COC), and polytetrafluoroethylene (PTFE) (**Figure 2**). A mixture solution containing the initiator, 2-bromo-*N*-[2-(3,4-dihydroxy-phenyl)-ethyl]-2-methyl-propionamide, and dopamine effectively primed the various substrates by immobilizing the initiator onto the surfaces (Figure 2a). Among the substrates, the Au surface was chosen for analysis because it is compatible to many surface

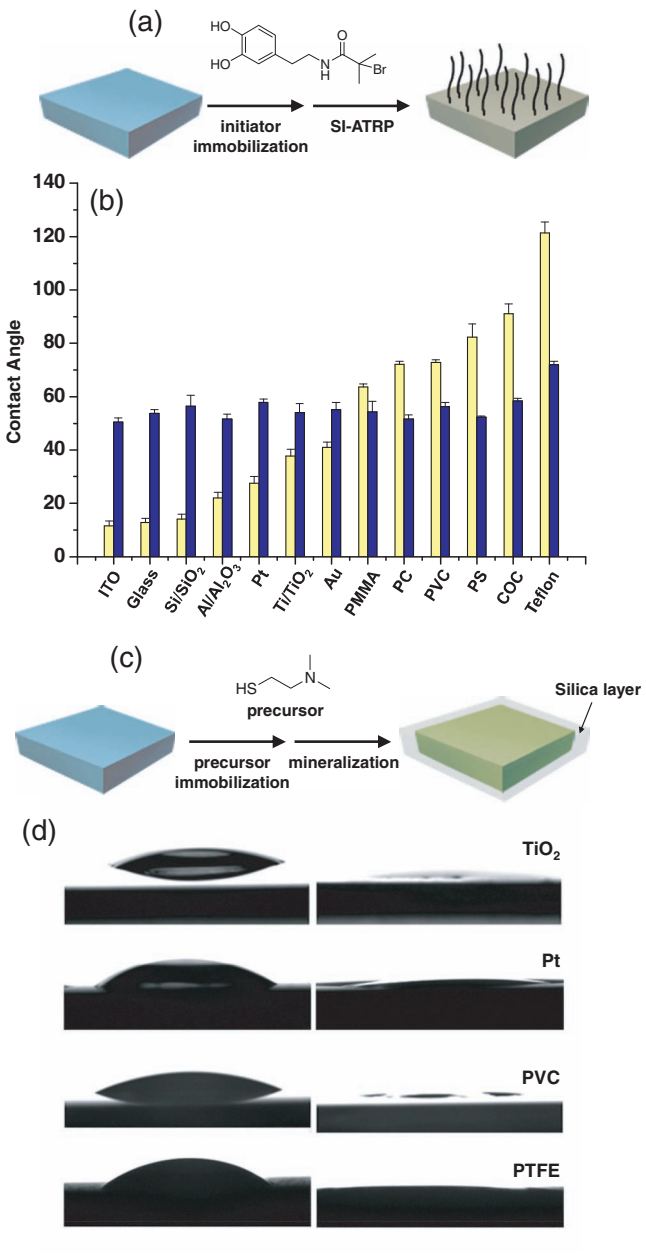

Figure 2. a) The chemical structure of the ATRP initiator, 2-bromo-*N*-[2-(3,4-dihydroxyphenyl)-ethyl]-2-methyl-propionamide. The immersion of the substrates into a solution of the initiator and dopamine enabled surface immobilization of the ATRP initiator, which subsequently allowed SI-ATRP to proceed. b) Water contact angles of a variety of unmodified (yellow) and polymerized substrates (blue). c) Structure of a precursor molecule, 2-dimethylaminoethanethiol, for biomineralization. The immersion of various substrates into a solution of the mineralization template molecule and dopamine enabled facile mineralization on surfaces. d) Water contact angles on precursor-immobilized (left) and mineralized (right) substrates.

analysis tools such as X-ray photoelectron spectroscopy (XPS) and infrared (IR) spectroscopy. The initiator-unique Br 3d peak (69.8 eV; Figure S1a, Supporting Information) was detected by XPS, and the amide signal (1523 and 1624 cm^{-1}; Figure S1c, Supporting Information) was observed by IR spectroscopy. These results demonstrated that the ATRP initiator was successfully immobilized on the surface. Subsequently, ATRP experiments were performed. XPS analysis of the Au substrate that underwent a 4-h polymerization using the monomer of 2-(dimethylamino)ethyl methacrylate (DMAEMA) showed the expected atomic compositions of poly(DMAEMA) without signals from the underlying substrate (Au) or the initiator. Carbon (C1s), nitrogen (N1s), and oxygen (O1s) from poly(DMAEMA) were detected (Figure S1b, Supporting Information), and the IR spectrum showed strong C-H (2770–2972 cm^{-1}) and C = O (1731 cm^{-1}) peaks from the poly(DMAEMA) (Figure S1d, Supporting Information). The goniometric measurement of the poly(DMAEMA)-immobilized Au substrate was 55°, which is consistent with all other polymerized substrates, despite the difference in wettability of the unmodified substrates (10°–120°) (Figure 2b).

Replacement of the ATRP initiator with other biologically relevant molecules that are co-dissolved with dopamine allow for bio-mineralization (Figure 2c,d), antibacterial coating (**Figure 3** a–f), and polysaccharide conjugations for hematopoietic cell culture (Figure 3g–i).

Biological silicification, found in the skeletal frames of diatoms[22] and sponges,[23] involves the formation of inorganic silica under mild and aqueous conditions. Characterization of biosilicificated materials revealed the presence of the unusual tertiary-amine-containing amino acid, methylated lysine, in silaffins. This tertiary amine in silaffins plays a critical role in biomineralization by sequestering precursor ions.[24] This discovery has triggered a number of biomimetic approaches utilizing tertiary amine functionalities to form silica on surfaces,[25] but biomineralization on a number of surfaces remains a challenge. As a proof of concept, we examined the silicification on diverse material surfaces by one-pot modification method. A tertiary-amine-containing precursor, 2-dimethylaminoethane thiol, was immobilized onto the surfaces with pD, and the resulting surfaces were immersed in a monosilicic acid solution to achieve material-independent biomineralization, specifically biosilicification (Figure 2c). Silicification of a wide range of material surfaces, including Pt, TiO_2, PVC, and PTFE, was demonstrated (Figure 2d). The contact angles of the surfaces with the immobilized precursor were approximately 32° (Figure 2d, left); these surfaces became super-hydrophilic, with contact angles of less than 10° after silicification (Figure 2d, right). XPS analysis showed new Si 2s and 2p peaks, indicating successful silicification (Figure S2a,b, Supporting Information). Scanning electron microscopy (SEM) demonstrated nanometer-scale formations of silica on the surfaces (TiO_2; Figure S2c, Supporting Information), which is similar to previous reports of the changes in surface morphology after such reactions.[25] When the biomineralization precursor was replaced with cetyltrimethylammonium bromide (CTAB), a quaternary ammonium compound, the substrate exhibited strong antibacterial properties.[26] The addition of silica microbeads (50 to 100 μm in diameter) to the CTAB and dopamine solution and gentle stirring overnight resulted in CTAB-incorporated beads (Figure 3a). When the CTAB-incorporated silica beads were incubated in bacterial cell media, they exhibited strong anti-bacterial properties to both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Red and green

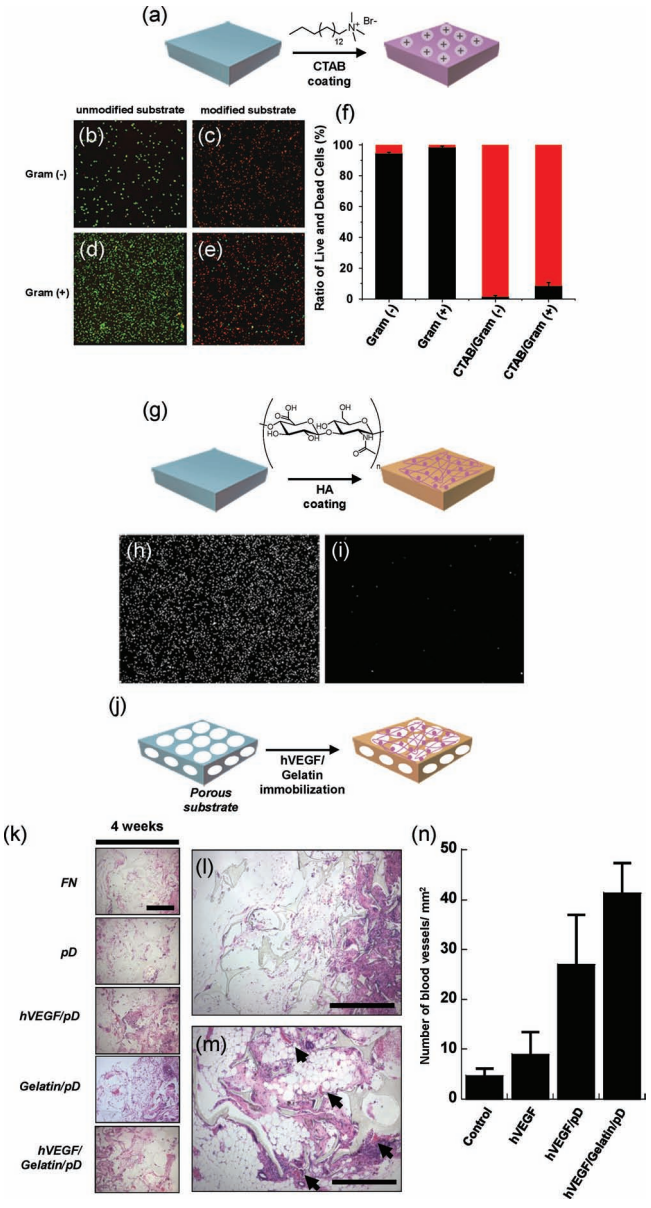

Figure 3. One-step preparation of surfaces for biological applications, such as the creation of antibacterial (a–f), hematopoietic cell-adhesive (g–i), and tissue regenerative surfaces (j–n). Surfaces that were functionalized by CTAB by the one-step co-dissolution of CTAB and dopamine exhibited antibacterial properties against Gram-negative *E. coli* (c) and Gram-positive *S. aureus* (e). f) Quantification of live (black) and dead (red) cells. M07e cells adhered to HA-immobilized Si Surfaces [5286 ± 746 cells/cm^2 (h)], but not to pD-coated surfaces [23 ± 7 cells/cm^2 (i)]. j) One-step immobilization of hVEGF on PLLA/PLCL scaffold surfaces for blood vessel recruitment and host cell migration. k) hVEGF and/or gelatin that were immobilized with pD resulted in significantly higher cellularity and blood vessel formation within the scaffold when compared to fibronectin, gelatin, and pD coatings alone. l) hVEGF immobilization on PLLA/PLCL did not influence the blood vessel recruitment. m) hVEGF immobilization with pD showed blood vessels in proximity to the bioactive scaffolds. Bar = 200 μm n) Quantification of blood vessel quantity within the scaffolds. hVEGF that was physically coated on PLLA/PLCL did not influence the blood vessel recruitment.

fluorescence indicated dead and live bacterial cells, respectively (Figure 3c,e). As a control, silica beads that were modified only with pD (no CTAB) had no anti-bacterial activity (Figure 3b,d). Quantitatively, 98% of the *E. coli* and 91% of the *S. aureus* were killed by CTAB-immobilized microbeads (Figure 3f, 3rd and 4th bars), but most cells (more than 95%) remained alive when the pD-modified microbeads were used, instead of CTAB-immobilized microbeads (Figure 3f, 1st and 2nd bars). The simple incorporation of CTAB onto surfaces could potentially be useful to fabricate wet-resistant anti-bacterial surfaces.

In addition to small molecules, macromolecules such as hyaluronic acid (HA, average molecular weight of 130 kDa) can be attached to substrates using this approach. HA triggers intracellular signals that can, in part, explain the mechanisms of lymphocyte activation,[27] leukocyte migration across the vascular endothelium,[28] and the self-renewal of hematopoietic stem cells.[29] We found that chemically unmodified HA was easily immobilized onto silicon surfaces during oxidative polymerization of dopamine. In contrast, existing methods either chemically modify HA or physically adsorb unmodified HA.[30] The immobilized HA molecules interact with CD44 receptors that are expressed on the membrane of human megakaryocytic M07e cells, resulting in stable cellular adhesions on the silicon surfaces (Figure 3h). This conjugation enabled a dramatic conversion of non-adhesive surfaces into adhesive ones for anchorage-independent M07e cells (Figure 3i).

The utility of pD-mediated one-pot surface functionalization was also evaluated for tissue engineering. In general, the recruitment of blood vessels into engineered tissues is an important step for long-term engraftment and survival of the engineered tissue. One approach to this end involves the surface grafting of a growth factor, vascular endothelial growth factor (VEGF), which stimulates the new blood vessel formation. We hypothesized that the grafting of VEGF onto the surface of porous biomaterials would efficiently recruit the host blood vessels toward the scaffolds.

A one-step immersion of poly(L-lactic acid)/poly(lactide-co-ε-caprolactone) (PLLA/PLCL) scaffolds into a mixture of human VEGF (hVEGF), gelatin, and dopamine resulted in the surface immobilization of hVEGF (Figure 3j). Gelatin was supplemented into the solution to enable the formation of a gelatin-hVEGF-dopamine layer on the scaffolds for the efficient presentation of hVEGF to incoming cells. The scaffolds were subcutaneously implanted in mice to determine the success of blood vessel recruitment into the scaffolds. After 4 weeks, the scaffolds exhibited successful in-growth of blood vessels and recruitment of cells, compared to the control groups, scaffolds coated with fibronectin (FN), pD, hVEGF/pD, or gelatin/pD (Figure 3k). Close examination of the blood vessels in the hVEGF-immobilized scaffolds indicated that they were in close proximity or in contact with the scaffold surfaces, suggesting that the immobilized hVEGF promoted blood vessel recruitment (Figure 3m). Furthermore, the quantification of blood vessel in-growth showed that the hVEGF/gelatin/pD scaffolds supported a large number of blood vessels per area (mm^2) (Figure 3n).

The anti-bacterial, HA-immobilized, and hVEGF-tethered surfaces are examples of on-demand preparations of functionalized surfaces for various purposes. In addition to taking advantage of the versatile surface modification, another advantage is a substantial number of molecules that are available from commercial sources. Molecules with carboxyl, amine, quaternary ammonium, enediol, or thiol groups, and with a large range of

their molecular weight, can be immobilized (Figure 2,3). Thus, one can bypass time-consuming chemical synthesis.

During the course of one-pot modification, several factors may influence the effective surface functionalization as well as coating thickness. We hypothesize that the co-existence of catechol and amine is crucial. Tyramine, a compound that is structurally similar to dopamine without the hydroxyl group at the third carbon of the phenyl ring, resulted in poor surface immobilization with a coating thickness of approximately 0.8 nm (Figure S3, Supporting Information, the 1st bar). Catechol alone also did not exhibit such a robust surface coating capability (Figure S3, Supporting Information, the 2nd bar). However, dopamine showed a significantly different result, with a coating thickness of approximately 20 nm after 6 h of coating (Figure S3, Supporting Information, the last bar). The stability of the immobilized molecules in the pD layer might strongly depends on the stability of pD layer. Previous reports showed that peptide nanowires with pD layer were stable even after ultrasonication, whereas the nanowires without the pD layer were completely removed upon the same treatment.[31] In addition to the mechanical stability, chemical stability of pD layers was also reported in which the pD layer was shown to be highly resistant to strong acid.[10,17] Therefore, the immobilized molecules whitin the pD layer migh exhibit similar chemical and mechanical stabilities.

3. Conclusions

In summary, we have presented a simple strategy of surface modification that can be applied to the surface of many diverse materials. Utilizing this technique, biomineralization and SI-ATRP were performed on various surfaces, and CTAB, hVEGF, and HA were immobilized for anti-bacterial substrates, tissue regeneration, and the adhesion of hematopoietic cells, respectively. The studies demonstrated here indicate that various functional compounds can be effectively immobilized onto diverse surfaces without the requirement of further time-consuming chemical syntheses. Although the coating method developed here is limited by polar organic molecules, we believe that this approach could have broad utility for the rapid development of modified surfaces for a variety of applications.

4. Experimental Section

4.1. Materials

Dopamine hydrochloride (98%, Aldrich), 2-dimethylaminoethanethiol hydrochloride (95%, Aldrich), tetramethyl orthosilicate (TMOS, 99+%, Aldrich), sodium phosphate dibasic (99%, Sigma), sodium phosphate monobasic (99%, Sigma), Trizma base (99.9%, Sigma), Trizma hydrochloride (99%, Sigma), copper(I) bromide (99.999%, Aldrich), 2,2′-dipyridyl (99+%, Aldrich), phosphate buffered saline (PBS, Sigma), sodium hydroxide (NaOH, 96%, Junsei), hydrochloric acid (HCl, Junsei), cetyltrimethylammonium bromide (CTAB, 99+%, Acros), Sodium hyaluronate (HA, 130 kDa, Lifecore), acetone (99%, Daejung Chemicals & Metals), and methanol (HPLC grade, Merck) were used as received. 2-(Dimethylamino)ethyl methacrylate (DMAEMA) was purchased from Aldrich and used after removing the inhibitor by column chromatography with basic alumina. ATRP initiator was prepared according to the previous results.[32] Ultrapure water (18.3 MΩ cm) from the Human Ultrapure System (Human Corp., Korea) was used.

4.2. Polymerization-Initiator Coating and Surface-Initiated Polymerization

4.2.1. Polymerization-Initiator Coating on Solid Substrates

Dopamine (2 mg) and polymerization initiator (12 mg) were dissolved in a mixture solution of methanol and Tris buffer (pH 8.5) (5 mL, MeOH:buffer = 3:2, v:v), and the solid substrate was immersed in the solution. After overnight coating at room temperature, the substrate was taken, rinsed with deionized water and dried under a stream of argon.

4.2.2. Atom Transfer Radical Polymerization on Polymerization Initiator-Coated Substrates

The initiator-coated substrate was placed in a Schlenk flask, and then the flask was degassed under vacuum and purged with argon. Into a separately prepared Schlenk flask, CuBr (0.014 g) and 2,2′-dipyridyl (0.031 g) were added, and the mixture was degassed under vacuum and purged with argon. To the Schlenk flask containing the catalysts, were added degassed water (8.36 mL) and purified DMAEMA (1.64 mL). The solution was transferred to the Schlenk flask containing the initiator-coated substrate by using a syringe. The resulting mixture was stirred for 4 h at room temperature, and the poly(DMAEMA)-coated substrate was taken, rinsed with methanol, and dried under a stream of argon.

4.3. 2-Dimethylaminoethanethiol Coating and Biomineralization

4.3.1. 2-Dimethylaminoethanethiol Coating on Solid Substrates

Solid substrate was immersed in a Tris buffer solution of the dopamine hydrochloride (10 mM, 10 mL, pH 8.5) for 45 min at room temperature. After that, Tris buffer solution of 2-dimethylaminoethanethiol hydrochloride (10 mM, 10 mL) was added and surface functionalization was further performed for another 5 h at room temperature. The substrate was rinsed with deionized water and dried under a stream of argon.

4.3.2. Biomimetic Silica Formation on 2-Dimethylaminoethanethiol-Coated Substrates

Monosilicic acid was formed by stirring a 1 mM HCl solution of TMOS (100 mM) for 15 min at room temperature, and the solution of monosilicic acid (5 mL) was added to a 100 mM aqueous phosphate buffer (pH 6.0, 5 mL) containing the 2-dimethylaminoethanethiol-coated substrate. After 1 h-incubation, the substrate was taken, washed with deionized water and dried under a stream of argon.

4.4. CTAB Coating and Anti-Bacterial Activity Test

4.4.1. CTAB Coating on Solid Substrates

50 mg of silica beads were dispersed in an aqueous dopamine solution (10 mM, 0.8 ml). After that, CTAB (10 mM, 0.2 ml) and NaOH solution (0.5 M, 2 μL) were added, sequentially. The resulting silica beads were washed with PBS twice after overnight coating.

4.4.2. Anti-Bacterial Activity Test of CTAB-Coated Substrate

50 mg of CTAB-coated silica beads were dispersed in 500 mL of PBS solution. 2 mL of bacterial cell suspension (~10^9 bacteria/mL) was added into the solution, and the mixture was incubated at 37 °C for 20 min. Viability of the bacterial cells was investigated by a live/dead staining method (LIVE/DEAD BacLight Bacterial Viability Kit, Invitrogen) after separation of silica beads from the mixture solution.

4.5. HA Coating and Adhesion of M07e Cells

Dopamine (5 mg) and HA (50 mg) were dissolved in a Tris buffer solution (pH 8.5, 5 mL), and the silicon substrate was immersed in the solution. After overnight coating at room temperature, the substrate was taken, rinsed with deionized water and dried under a stream of argon. M07e cells (DMSZ, Germany) were adapted to grow in IMDM (Sigma) supplemeted with 2.5% FBS (Hyclone), 10 ng/mL GM-CSF (Berlex Laboratories), and 1 mg/mL gentamicin sulfate (Sigma). Cells were maintained in exponential growth phase between 5×10^5 and 1×10^6 cells/mL. Normal-force cell adhesion assays were performed. Briefly, M07e cells were stained by 5 μg/mL of calcein AM in PBS and incubated in normal growth media on surfaces for 2 h prior to removal of non-adherent cells by inverted centrifugation at 30 rcf in sealed bags filled with PBS. Image analysis of pre- and post-spin images was performed to calculate the percent cell adhesion on surfaces. The cells were stained directly in the wells via addition of 40 μL of calcein AM (diluted to 5 μg/mL PBS) 30 min prior to pre-centrifugation imaging.

4.6. hVEGF Coating and Tissue Engineering

4.6.1. hVEGF Coating on the Solid Substrate

Three dimensional porous PLLA/PLCL scaffolds were fabricated as previously described with slight modifications.[33] Briefly, PLLA/PLCL 1:1 were dissolved in chloroform to yield a solution of 5% (w/v) polymer. Polymer solution (0.25 mL) was loaded into molds packed with 0.4 g of sodium chloride particles. The solvent was allowed to evaporate overnight and the sponges were subsequently immersed for 12 h in distilled water (changed every 2 h) to leach the salt and create pore structures. The sponges were soaked in 75% (v/v) ethyl alcohol overnight, washed three times with PBS, and coated with hVEGF (10 μg/mL). For hVEGF coating, PLLA/PLCL scaffold was immersed in a Tris buffer solution of the dopamine hydrochloride (10 mM, 10 mL, pH 8.5 with or without 0.1% gelatin) and hVEGF (10 μg/ml) for overnight at room temperature. The scaffolds were rinsed with deionized water prior to transplantation.

4.6.2. Subcutaneous Transplantation into Athymic Nude Mouse

Cell free scaffolds were subcutaneously transplanted into the dorsal region of 6 to 8-weeks-old athymic mice for duration of 4 weeks.

4.6.3. Histology

For histological analysis, constructs were fixed in 4% paraformaldehyde, dehydrated in serial ethanol dilutions, and paraffin embedded. Constructs were cut into 5 μm sections and stained with H&E.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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