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Long-lasting Antifouling Coating from Multi-Armed Polymer

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Abstract

We describe a new antifouling surface coating, based on aggregation of a short amphiphilic four-armed PEG-dopamine polymer into particles, and on surface binding by catechol chemistry. An unbroken and smooth polymeric coating layer with an average thickness of approximately 4 microns was formed on top of titanium oxide surfaces by a single step reaction. Coatings conferred excellent resistance to protein adhesion. Cell attachment was completely prevented for at least eight weeks, although the membranes themselves did not appear to be intrinsically cytotoxic. When linear PEG or four-armed PEG of higher molecular weight were used, the resulting coatings were inferior in thickness and in preventing protein adhesion. This coating method has potential applicability for biomedical devices susceptible to fouling after implantation.

1. Introduction

Long-term implant performance is highly dependent on host-material interactions occurring at the interface with the surrounding biological milieu.[1, 2] For example, protein accumulation onto biosensor surfaces (fouling) may reduce device sensitivity and efficacy,[3] while cell proliferation on coronary stents can lead to the formation of new blockages within an artery.[4] Several approaches have been utilized to minimize these unwanted interactions at the material surface to optimize device outcome. These approaches and applications include: self-cleaning surfaces[5], photo-activated materials[6], peptide based materials,[7] biocide releasing coatings[8] and microtopography.[9] Of these methods, the deposition of thin polymer films with appropriate antifouling properties has proven to be among the most promising.[10]

Immobilization of poly(ethylene glycol) (PEG) on surfaces in order to prevent fouling has been a very common strategy due to that molecule's excellent antifouling properties and demonstrated safety.[11] However, existing immobilization strategies are limited by low polymer densities and relatively thin coatings.[12, 13] Consequently, coatings are often susceptible to thermal and oxidative degradation,[14] which lowers their long-term stability

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Supporting Information Available: ^{1}H NMR spectra, macroscopic images, QCM-D, Static water contact angles, profilometery, cytotoxicity and X-ray photoelectron spectroscopy (XPS). This material is available free of charge via the Internet at http://pubs.acs.org

and decreases their performance in preventing nonspecific adsorption.[15] For example, cell proliferation was seen after 1 week on titanium surfaces coated with single linear monomethoxy-terminated PEGs that were conjugated to single DOPA residues and applied by simple adsorption. [14]Cells cultured on PEG-based films approximately 100 nm in thickness on titanium substrates developed 100% confluence after 10 weeks (similar to growth on uncoated surfaces).[16]

Amphiphilic polymers have been used for in-situ gel formation[17] and surface modification.[18]The amphiphilicity of these polymers allows them to aggregate into particles such as micelles and vesicles;[19] gelation of those particles into surface coatings occurs through a process of particle aggregation and breakdown.[20] While coating surfaces by aggregation of amphiphilic polymers is relatively simple, its main drawback is the weak adherence between the coating and the subjacent surface, which may decrease its longevity compared to covalently bonded coatings.[21]

Here, we present a one-step method to form a thick and stable surface coating (Figure 1), based on the aggregation of a short amphiphilic four-armed PEG-dopamine polymer (PEG_4-dopamine) into particles and subsequent surface binding by catechol chemistry. Our strategy does not require surface modification or harsh conditions, and it can be applied, in principle, to any metal surface susceptible to bio fouling. We have designed and synthesized a short amphiphilic four-armed poly(ethylene glycol) polymer (500 Da/arm) functionalized with terminal dopamine (153 Da) groups (PEG_4-dopamine). A short four-armed molecule was selected as a core structure because star-shaped polymers are capable of forming more stabilized particles and denser surface coverage compared to linear polymers of similar molecular weight.[22-24]

The dopamine group provided the amphiphilicity to facilitate aggregation of PEG_4-dopamine into particles; it has been demonstrated that polymers containing high dopamine content are likely to aggregate into stable particles due to the amphiphilic nature of the catechol group.[25] The dopamine group also provided anchoring to the metallic substrate. The covalent bonding of catechols to metal oxides has been studied extensively[26-27] and has been attributed to the formation of a stable bidentate modes of H-bonding and metal coordination.[28] It has been demonstrated that catechol molecules such as dopamine and 3,4-dihydroxy-L -phenylalanine (DOPA) can form strong bonds with a wide range of inorganic and organic materials, including noble metals, oxides, polymers, semiconductors, and ceramics.[29] This has led, for example, to the development of useful coating techniques for hydrophobic membranes,[30] heparin immobilization[31] and for reducing the toxicity of biomaterials in vivo.[32]

We hypothesized that low molecular weight, amphiphilic PEG_4-dopamine will aggregate into particles in aqueous media, suitable for coating surfaces by mechanisms described above, and that the ability of dopamine to bind metal surfaces covalently will increase the longevity of the coatings. Together, these attributes could produce a thick and long-lasting polymeric coating.

2. Experimental Section

Materials

All chemicals and reagents were obtained from commercial sources. Dopamine hydrochloride, K_2SO_4, N-morpholinopropanesulfonic acid (MOPS), sodium acetate, phosphate buffered saline (PBS), and ethanol were purchased from Sigma-Aldrich, Inc (St Louis, MO). Four-armed succinimidyl glutarate-terminated pentaerythritol core polyethylene glycol (2000 Da) was purchased from Nanocs, Inc. (NY, NY). Spectra/Por®

dialysis membranes (MWCO: 1000 Da) were purchased from Spectrum Labs (Rancho Dominguez, CA). Deuterium oxide was purchased from Cambridge Isotope Laboratories, Inc. (Cambridge, MA). Water used in polymerization and dialysis (DDW) was distilled and purified using a Nanopure® water purification system (Thermo Scientific, Bremen, Germany). The UV/Vis spectra were recorded with a Varian Cary 50 Bio UV-Visible Spectrophotometer (Agilent, Santa Clara, CA). Titanium discs (10 mm dia.) were obtained from J.G. Machine Co., Inc. (Wilmington, MA). Round glass coverslips (12mm dia.) were purchased from Electron Microscopy Sciences, Fort Washington, PA. Quartz crystal microbalance (QCM) sensors with a 150 nm TiO_2 coating were purchased from (Q-Sense, Sweden). Cell culture reagents and media were purchased from Gibco Life Technologies (Grand Island, NY).

Synthesis and characterization—1.0 g of four-armed PEG-succinimidyl glutarate and 2.5 g dopamine hydrochloride (1:6.5 PEG termini: dopamine mole ratio) were separately dissolved in 20 and 30 mL DDW, respectively. The polymeric solution was added drop-wise to the dopamine solution and the pH was then adjusted to 5.9 ± 0.1 by 0.1 M NaOH and left to stir for 1 hour at 4 °C. Then, the pH was retained at 5.9 ± 0.1 and the reaction was allowed to mix overnight at 4 °C. The solution was then dialyzed six times (1000 MWCO dialysis membrane, Spectra/Por®, Rancho Dominguez, CA) against 3L of DDW containing a few drops of 0.1 M HCl (pH 3.5–4.0) followed by lyophilization. PEG_4-dopamine with a molecular weight of 10,000 Da (2500 Da/arm) was synthesized in a similar fashion by reacting 1.0 g succinimidyl glutarate-terminated poly(ethylene oxide), 4-arm, (Sigma-Aldrich, Inc (St Louis, MO) and 0.5 g dopamine hydrochloride (1:6.5 PEG termini:dopamine molar ratio, conversion rate >90%, confirmed by NMR). Linear Methoxy-PEG dopamine was synthesized in a similar fashion by reacting 1.0 g methoxy-PEG succinimidyl glutarate ester, 2000 Da (Creative PEG works, Winston Salem, NC), and 0.62 g dopamine hydrochloride (1:6.5 molar ratio, conversion rate >90, confirmed by NMR). The chemical structures of the molecule as well as the efficiency of the modification were determined by ^1H NMR using a Varian Mercury (Palo Alto, CA) 400 MHz spectrometer in DMSO-d_6 (Figure S1). The dopamine content was determined by comparing the integral value of pentaerythritol methylene protons at $\delta = 3.5$ to the phenolic protons of the dopamine at $\delta = 6.6$. MALDI–TOF MS analyses were performed using a Microflex (Bruker Daltonics, Billerica, MA, USA) equipped with a pulsed nitrogen laser operating at 337 nm. Small oligomer positive ion spectra were acquired in linear mode over an m/z range from 1000 to 5000 using a 20-kV accelerating voltage and a 150-ns delay extraction time. The spectrum for each spot was obtained by averaging the results of 200 laser shots. The analysis was performed by spotting on the target plate 1.0 μL of the sample mixed with an equal volume of the matrix solution, 20 mg/ml DHB (Sigma Aldrich), in CH3CN/H2O (50:50, v/v) containing 0.1% (v/v) trifluoroacetic acid (Sigma Aldrich, St Louis, MO), and allowing it to air dry. A Zetasizer Nano ZS (Malvern Instruments, Worcestershire, United Kingdom) was used to determine the size and size distribution of particles in the buffer solution. EVOS fluorescent microscope (AMG, Bothell, WA) was used at 40× magnification to image the particles.

Surface preparation—Titanium discs and glass coverslips were cleaned by sonication in detergent and methanol for 5 minutes each, with multiple washes in DDW in between. Substrates were then dried in a stream of filtered N_2, before exposure to O_2 plasma (Harrick Scientific, Pleasantville, NY) at ≤ 150 torr and 100 W for five minutes to obtain clean surfaces. Glass substrates were subsequently coated with 50 nm of commercially pure titanium using electron-beam evaporation (Sharon Vacuum, Brockton, MA) to obtain optically transparent titanium surfaces.

Surface modification—Immediately before modification, clean titanium substrates were exposed to O_2 plasma at ≤ 150 torr and 100 W for five minutes to obtain a TiO_2 surface layer. A solution composed of a 4:1 mixture of 0.6M K_2SO_4 and 0.1M MOPS was prepared. PEG_4-dopamine polymer was dissolved in the K_2SO_4/MOPS solution to the appropriate concentration, titrated with 15% sodium acetate solution to achieve pH 6.0, then sterile filtered with a 0.22 μm filter) to remove contaminants. Surface modification with PEG_4-dopamine was performed by immersing substrates in coating solution (0.5 or 2 mg/mL) with constant shaking at 25 RPM. Titanium surfaces were held horizontally throughout the procedure. Coated substrates were exhaustively rinsed with DDW and dried in a stream of filtered N_2 before further use.

Contact angle measurements—Modified and unmodified titanium substrates were loaded onto the stage of a Kruss DSA 100 (Hamburg, Germany) contact angle goniometer. Drops of DDW were placed on the surfaces, and the resulting image was captured and analyzed using the proprietary drop shape analysis software. Reported static angles were calculated by using a tangential fitting method and by averaging the angles from both the left and right sides of the droplet. At least 9 static angle measurements were obtained per experimental condition, and all analyses were performed at the same temperature and humidity.

Scanning electron microscopy—Surface coating morphology was observed under a JEOL-5600LV scanning electron microscope (SEM) (Peabody, MA), using an accelerating voltage of 5kV in backscattered mode. PEG_4-dopamine-coated titanium discs and coverslips were mounted on aluminum stubs using double-sided adhesive tape and sputter coated with gold/palladium (80:20) using a Hummer 6.2 Sputter Coating System (Anatech; Battle Creek, MI) before imaging.

Elemental analysis—Modified and unmodified samples were mounted on standard sample studs using double-sided adhesive tape. Survey and high-resolution spectra were collected on a FEI DualBeam™, model Nova Nanolab 200 (XT Nova Nanolab, Hillsboro, OR). All binding energies were calibrated using the Ti 2p peak (458.7 eV). Samples were first analyzed using a broad survey scan (160.0 eV pass energy), then a medium resolution scan (40.0 eV pass energy) at a 20-degree glancing angle. The higher resolution data was then used to calculate the surface atomic compositions for each sample (Table S1). Curve fitting of the C1s peaks was used to determine the relative compositions of the different types of carbon bonds (Figure S7).

Profilometry—Coatings were scratched using a razor blade and the step height and surface profile of the coating layer examined using a KLA-Tencor P-16+ surface profilometer (Milpitas, CA) with a 2 μm radius diamond-tipped stylus. Four samples were examined in each group, with four height measurements taken along the scratch, for a total of 16 data points per group. Higher resolution scans were then performed to obtain detailed 2D and 3D surface profiles (Figure S8). Apex Profiler 7.21 software was used for data analysis and reporting. The roughness of the surface was determined by measuring roughness root-mean-square (Rrms) average of the height (z) taken from the mean data plane,[33] expressed as

$$R_{rms} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(Z_n - \overline{Z}\right)^2}$$

where Z is the is the vertical height of the surface, $\bar{Z}$ is the center plane, and N is the number of points in the sample area.

QCM-D measurements

Immediately prior to use, TiO_2-coated QCM sensors were cleaned by sonication in 2% SDS solution, rinsed with ultrapure water, then exposed for 20 min in a UV/ozone chamber. All measurements were performed utilizing temperature control at a working temperature of 37 °C to avoid drifts in frequency (*f*) and dissipation (*D*). Up to 4 separate measurements were performed in parallel at a flow rate of 0.1 ml/min. For each measurement, K2SO4/MOPS buffer was first loaded into the measurement chambers until stable baselines were obtained. PEG4-dopamine solutions (0.5 and 2.0 mg/mL) and a control PEG4 solution were then introduced into the liquid cell to replace the K2SO4/MOPS buffer. For the protein resistance measurements, TiO_2-coated QCM sensors were first modified with PEG4-dopamine as described above before insertion into the measurement chamber of a Q-sense E4 system. The system was equilibrated in PBS buffer for 10 minutes to attain a stable baseline. For antifouling experiments up to 4 separate measurements were performed in parallel. For each measurement, PBS buffer was first loaded into the liquid cell until stable baselines were obtained. Fetal bovine serum solution (100% Gibco, Grand Island, NY) was subsequently introduced into the liquid cell allowing interaction with surfaces. After 20 minutes, PBS buffer was introduced into the cell until the frequency signal came to equilibrium to allow the removal of weakly bound protein. Changes in *f* and *D* were monitored and recorded in real-time using proprietary Q-Sense software (QSoft). All raw data was subsequently analyzed using the QTools software package. Frequency and dissipation curves were fitted to a Voigt viscoelastic model to yield relevant mass, thickness and kinetic information. The adsorbed mass (Δm) can be obtained from the frequency change (Δf) according to the Sauerbrey equation:

$$\Delta m = -\frac{C}{n}\Delta f$$

where *C* is the mass-sensitivity constant *C* (=17.7 ng cm2 Hz-1 at *f=5* MHz) and *n* is the overtone number.

Protein attachment—Ti surfaces were used as substrates for protein attachment assays. Surfaces were immersed in bovine serum albumin (BSA) conjugated with Alexa Fluor 647 (purple color, 100 μg/mL in PBS) for 12 h at 37 °C. Protein solution covered the surface throughout the experiment. For long-term studies, new substrates were incubated in PBS for two weeks prior to immersion in BSA solution. The fluorescence intensities of microscopy images (AMG $EVOS_{fl}$, Bothell, WA; λex=647 nm) were quantified by ImageJ (National Institutes of Health, Bethesda, MD). The mean and standard deviation of the measurements were reported (n=4).

Cell attachment—Glass coverslips coated with 50 nm c.p. Ti were used as model substrates for the cell attachment assays. The thinness of the titanium film allowed for substantial light transmission and enabled the tracking of cell attachment via phase contrast microscopy. NIH3T3 mouse fibroblasts were purchased from ATCC (Manassas, VA) and maintained at 37 °C and 10% CO_2 in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) containing 10% fetal bovine serum and 100 μg/mL of penicillin and 100 U/mL of streptomycin. Cells of passage 3-8 were harvested using 0.25% trypsin-EDTA, resuspended in fresh media, and counted using a hemocytometer. Cells were seeded onto modified and unmodified titanium substrates in 24-well plates at 1×10^4 cells/well and

maintained at 37 °C and 10% CO_2. For long-term studies, substrates were reseeded with 1 × 10^4 cells/well twice a week. At periodic intervals, equal volumes of calcein AM and ethidium homodimer-1 (Live/Dead kit, Invitrogen, Grand Island, NY) were diluted 1:1000 in DPBS and added to each cover slip. After 15 minutes of incubation, cover slips were gently washed with DPBS and then imaged using an AMG $EVOS_{fl}$ (Bothell, WA) fluorescence microscope fitted with GFP and RFP filter cubes at 10× magnification. Quantitative cell attachment data was obtained by acquiring images of five random fields of view for each substrate at each time point. Live/dead cell density was determined using ImageJ. The mean and standard deviation of the measurements were reported (n=4).

In vitro cytotoxicity—Coated titanium coverslips were placed into a 24-well plate and sterilized in 70% ethanol for 1 hour, and then dried in stream of filtered N_2. 1 mL of complete DMEM was added to each coverslip, and incubated for 48 hours at 37 °C. Subsequently, 100 μL aliquots of the supernatant were added to NIH3T3 cells pre-seeded in a 96-well tissue culture plate at a density of 5,000 cells per well. At 24 hours, cell viability was determined using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (MTS) kit (Promega; Madison, WI) following manufacturer instructions. Absorbances were measured at 490 nm using the Biotek SynergyMx microplate reader (Winooski, VT) following a two hour incubation. Cells were also monitored visually to confirm the results of the MTS assay. Cell viability was normalized to that of control unexposed cells. Measurements were performed in quadruplicate.

Statistical analysis—Results are presented as means ± standard deviations. Statistical comparisons were performed with Instat 3.10 software (Prism 5, GraphPad, San Diego, CA). One-way analysis of variance (ANOVA) was used to test the significance of the differences between the treated groups. Tukey's test was used for post comparison of specific groups. $P < 0.05$ was considered statistically significant. N was equal to or greater than 4 in all cases.

3. Results and Discussion

Four-armed PEG (2000 Da) with N-hydroxysuccinimide (NHS) functional groups was reacted with dopamine hydrochloride solution to yield a viscous, golden-brown PEG_4-dopamine polymer (Figure S2A). 77% of the PEG end-groups were functionalized with dopamine as confirmed by ^1H NMR spectrum (Figure S1), comparable to reported efficiencies achieved with functionalization of various linear and branched PEG polymers.[34, 35] The presence of multiple small peaks between 6.3-7.1 ppm indicate the formation of oxidized dopamine derivates[36]; integration ratios calculated for each proton suggested an oxidation rate of about 10% of the dopamine moieties. Coatings were prepared on clean titanium discs by immersion into dilute aqueous PEG_4-dopamine solutions (0.5 and 2.0 mg/mL, Rrms=2.02 ± 0.5 and 1.34 ± 0.3, respectively. Titanium was selected as a model substrate for coating due to its widespread use in biomedical implants and biosensors.[37] Elevated temperature and ionic strength conditions were used to achieve higher densities of surface bound polymer by reducing chain repulsion during the grafting reaction.[38] The dissolution of PEG_4-dopamine (2 mg/mL) in buffer solution resulted in the formation of spherical particles (Figure 2A), 3.7 μm by DLS (Figure 2B); polydispersity index = 0.16. Diameter measurements by DLS were in agreement with data acquired by microscopy. The aggregation mechanism of amphiphilic, multi armed polymers in water involves two aspects:[39] a) the immediate, interaction, attributed to the amphiphilic nature of PEG_4-dopamine and to the formation of hydrogen bonds and, b) the molecular packing which plays an important role in the hydrodynamic volume of the particle.[40] The micron scale diameter can be explained by the high content of hydrophilic PEG (~77 w/%) which swelled and prevented closer packing in an aqueous environment.[41] Other multiarmed amphiphilic polymers with PEG contents higher than 60% have been found to form particles

with diameters of up to 100 μm.[42] Dopamine moieties probably constitute the core due to their slightly hydrophobic nature. Dopamine is able to interact with the metallic surface due to the instability of PEG-based particles, which cannot maintain their structure during the process of surface fixation.[43] The fact that our coatings were homogenous and uniform is consistent with this view.

Surfaces were treated for 18 h in buffer (0.6 M K_2SO4 with 0.1 M MOPS buffered to pH 6.0) at 60°C. After extensive rinsing in double distilled water (DDW) to remove unbound PEG_4-dopamine and particles, a distinctive golden-brown film was observed on modified surfaces (Figure S2B). Coated surfaces (2 mg/mL) were immersed in methylene chloride for 2 hours under mechanical agitation. A small portion of the coating was recovered and the PEG_4 – dopamine, as well as the original PEG_4–dopamine were scanned by UV-vis spectroscopy (Figure S3). Both spectra revealed a narrow, dominant peak at 280 nm, characteristic of non-oxidized dopamine groups.[23] A slight broadening of the peak, in the range of 310-370 nm was observed in the recovered PEG_4–dopamine, confirming the oxidation process revealed by ^1H NMR spectroscopy.

Coating mass versus time curves (Figure S4) were determined with a quartz crystal microbalance with dissipation monitoring (QCM-D). This method, based on the high sensitivity of an oscillating piezoelectric crystal to mass changes, has been used to investigate the real-time kinetics of small molecule and protein adsorption onto surfaces.[43, 44] High particle adsorption rates were observed with both 0.5 and 2.0 mg/mL polymer solutions within 2 and 4 hours of incubation, respectively (Figure S4 and S5). The apparent leveling off of data is due to the QCM-D sensing limit, which is typically less than several hundred nanometers[45]. A control composed of four-armed PEG without dopamine end-groups (PEG_4) showed no particle formation nor adsorption onto surfaces, demonstrating the importance of dopamine to the coating procedure.

Static water contact angle measurements (Figure S6) demonstrated the successful functionalization of titanium surfaces with the hydrophilic PEG_4-dopamine coating. Contact angles decreased from 51° for unmodified titanium to 34° and 15° for the 0.5 and 2 mg/mL PEG_4-dopamine modified surfaces, respectively. The greater surface wettability observed with the 2 mg/mL PEG_4-dopamine can be explained by a higher PEG density of the coating layer.[46] Coating morphology was characterized by scanning electron microscopy (SEM; Figure 2 C and D) along and on either side of a full-thickness scratch created by a laboratory razor blade. Modified surfaces showed distinct differences in topography dependent on the PEG_4-dopamine coating concentration. 0.5 mg/mL PEG_4-dopamine coatings formed discontinuous globular polymer structures approximately 10 microns across and spaced 1-5 microns apart (Figure 2C). In contrast, the 2 mg/mL PEG_4-dopamine coatings were continuous, homogeneous and uniform films which partially concealed the ridged, grooved microstructure of the titanium discs (Figure 2D).

Complete coverage of the titanium surface by the 2 mg/mL coating was further verified by X-ray photoelectron spectroscopy (XPS). Qualitative analysis survey scans were taken to determine the atomic compositions of unmodified and modified surfaces (Table S1). Unmodified surfaces exhibited strong peaks from oxygen and titanium in a ratio (O/Ti) of 2.2, which is close to the theoretical value of 2.0 indicative of the thin oxide passivation layer that naturally forms on titanium.[47] There was also a moderate carbon signal, likely due to hydrocarbon contamination[48] from chemicals contacted during the manufacturing process and storage in air.[49,50] High-resolution spectra (Figure S7) of the unmodified surfaces showed predominantly C-C/C-H bonds (284.6 eV), also consistent with surface hydrocarbon contamination.[51] After coating of the titanium, a significant increase in carbon and nitrogen was observed, as well as a significant decrease in oxygen; titanium was undetectable. The

disappearance of the titanium signal suggested that the PEG_4-dopamine coating was thicker than the maximal penetration of the photoelectron beam (~10 nm) and/or dense enough to obscure the underlying titanium completely (Figure 2E). The large increase in the carbon signal (68% vs. 24%) can be attributed to the modification of the surface with the coating layer. High-resolution spectra (Figure S7) of modified surfaces exhibited a predominant C-O peak (286.0 eV) attributed to the ether backbone of the PEG coating layer.

The thickness of the 2 mg/mL PEG_4-dopamine coating, determined by SEM (Figure 2F) and by profilometry (Figure S8), was in the range of 3.2-4.0 μm (n=8), which is also the range of the particles. The tendency of particles to form a coating layer with thicknesses very close to the particles' diameters in solution has been reported.[52]

Surfaces used in subsequent protein and cell fouling studies were modified using the 2 mg/mL coating solution, due to the complete surface coverage achieved at this concentration. Protein adsorption on PEG_4-dopamine modified surfaces was examined using QCM-D. A significant increase in adsorbed mass was observed upon exposure of both modified and unmodified surfaces to whole bovine serum (Figure 3) for twenty minutes. After rinsing with buffer to remove any unbound serum proteins, the final adsorbed mass of protein on unmodified titanium surfaces was approximately 125 ng/cm^2, comparable to values previously reported in the literature.[53] In contrast, the final amount of protein adsorption on coated surfaces was close to the 2 ng/cm^2 sensitivity limit of QCM-D, demonstrating the excellent protein resistance conferred by the PEG_4-dopamine coating.

To determine the long-term performance of PEG_4-dopamine coatings, Ti surfaces with or without PEG_4-dopamine coating were exposed to bovine serum albumin (BSA) conjugated with Alexa Fluor 647 (100 μg/mL in PBS) for 12 hours at 37 °C. The surfaces were then imaged and the fluorescent intensities quantitated with ImageJ software. All PEG_4-dopamine surfaces demonstrated excellent protein resistance, with fluorescent intensities equal to those of Ti surfaces that had not been exposed to protein (Figure 4). That resistance was also seen after a second 12-hour challenge with labeled BSA following 2 weeks in PBS. Titanium surfaces coated with a linear mPEG-dopamine (2 mg/mL) with a molecular weight similar to the sum of those in PEG_4-dopamine (2000 Da) also showed excellent protein resistance initially, but that property was greatly reduced after 2 weeks in PBS. The PEG_4-dopamine coatings remained stable for at least 10 weeks in cell culture media, as seen in the maintenance of their thickness (~4 μm) and attachment to the Ti surface (Figure 5). To explore the ability of PEG_4-dopamine modified surfaces to resist cell attachment, NIH3T3 fibroblasts were seeded twice weekly (days 0, 3, 7, 10, 14, etc.) onto titanium-coated glass slides, and cell attachment quantified by fluorescence microscopy at regular intervals. Fibroblasts were chosen as a model for mammalian cells due to their strong tendency to adhere on most substrates and their important role during wound healing and integration of biomaterials.[54] Cells readily attached to unmodified titanium surfaces, forming a confluent monolayer within a week. In contrast, PEG_4-dopamine modified surfaces were entirely resistant to cell attachment and remained cell-free for at least eight weeks (Figure 6). To explore the possibility that the cellular resistance of PEG_4-dopamine was due to a soluble toxic component of the coating, cells were incubated overnight in culture media that had been exposed to PEG_4-dopamine-coated titanium surfaces for 48 hours. Cell viability was similar to that of cells not exposed to the experimental surfaces (Figure S9).

Previously reported coatings utilizing high molecular PEGs functionalized with catecholamine moieties did not report coatings as thick as we have demonstrated here[47, 55] which suggests a possible influence of the molecular weight on the coating process. To assess the relationship between PEG molecular weight and the thickness of the resulting layer, we functionalized a higher molecular weight four-armed PEG with dopamine end

groups (PEG_4-dopamine, 2500 Da/arm), and used a concentration of 2 mg/mL with the same conditions as described above for surface modification. This larger PEG_4-dopamine did not form particles (as was evident by light microscopy) and created significantly thinner coatings that were no longer visible by eye. Surface profilometry showed an average coating thickness of 107 nm, suggesting that the low molecular weight of the PEG_4-dopamine played a major role in particle formation and in the achievement of a thick polymer network. The effect of PEG MW on coating thickness may be related to the fact that the 10,000 Da PEG_4-dopamine contains less than 6 w% dopamine (one dopamine per 2500 Da arm), while the 2000 Da PEG_4-dopamine contains 26 w% dopamine (one dopamine per 500 Da arm). Polymers with high dopamine content are more likely to aggregate into particles.[25] Particle size can bear a close relationship to the thickness of the resulting coatings.[56]

4. Conclusions

We have developed a new coating based on aggregation of a short arm amphiphilic polymer to form 3D microparticles, and cathechol chemistry that demonstrated binding to a solid substrate. The coating was produced by a single mild reaction, making it amenable to large-scale production. The versatile catechol group will allow modification of various metal oxide surfaces to provide excellent resistance against proteins and cells for extended durations. This coating approach could be extended to other amphiphilic multi-armed polymer systems for coating biomedical devices and diagnostic tools and for drug delivery from surfaces.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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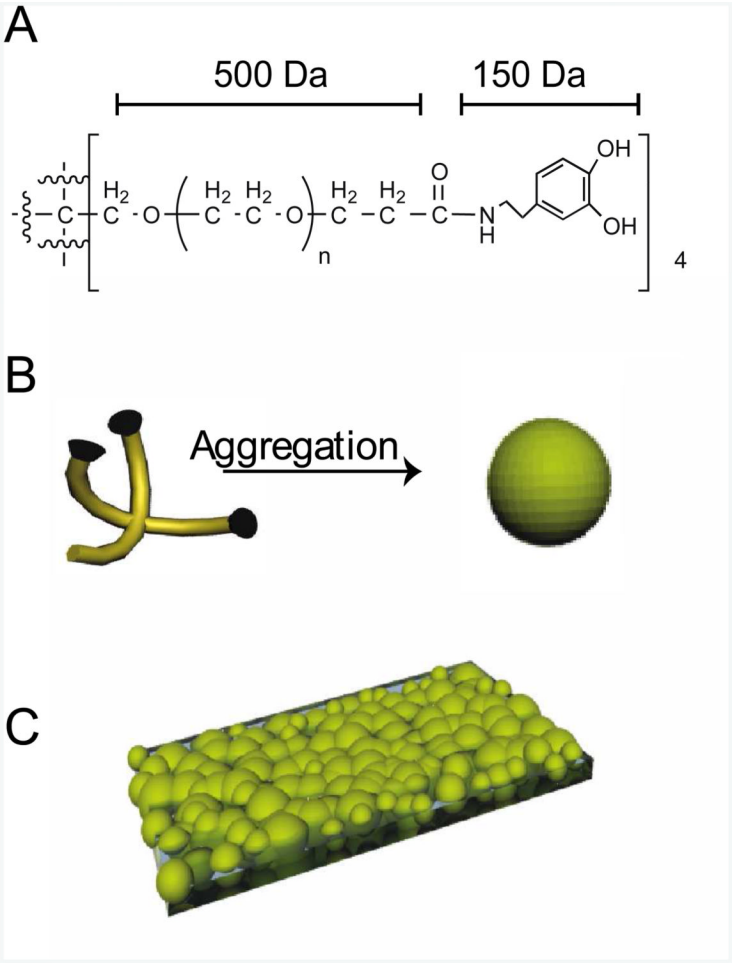

Figure 1.

Schematic illustration of methodology to coat metal surfaces by aggregation of amphiphilic polymers. (A) Chemical structure of PEG_4-dopamine, a four-armed monomer composed of a poly(ethylene glycol) (PEG) core (Mw = 2000 Da) and dopamine end groups. (B) Aggregation of PEG_4-dopamine in aqueous solution into particles. (C) A dense network of PEG_4-dopamine particles is attached to the surface by dopamine groups.

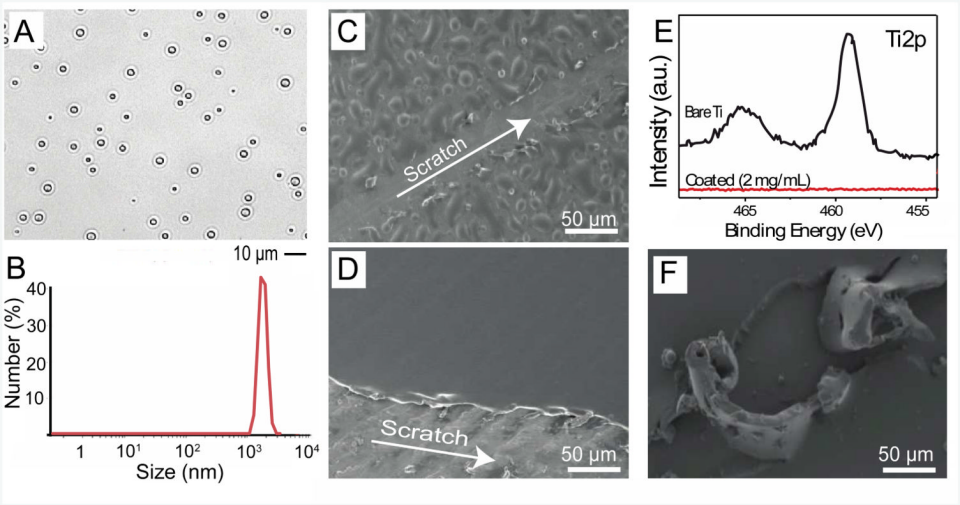

Figure 2.

Particle formation and surface analysis of modified titanium surfaces. A) Optical micrographs of particles from PEG_4-dopamine in aqueous buffer (pH 6.0). B) Size distribution of particles in the same buffer. C and D) SEM images of surfaces coated by 0.5 (C) or 2 (D) mg/mL PEG_4-dopamine solutions. The arrows indicate a deep scratch made on the coatings. (E) XPS Ti2p spectra of titanium surfaces before (black) and after (red) coating with 2 mg/mL PEG_4-dopamine solution. (F) SEM image of a peeled coating layer indicating micron-scale thickness.

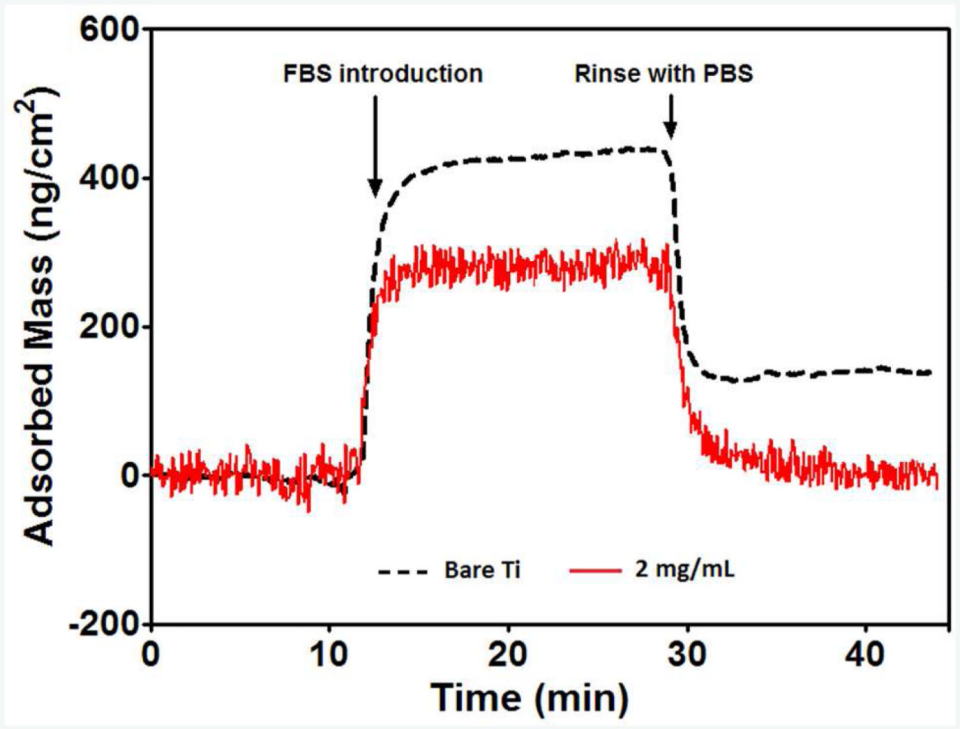

Figure 3.
Real time QCM-D measurements of serum protein adsorption on coated (red) and uncoated (black) titanium sensors.

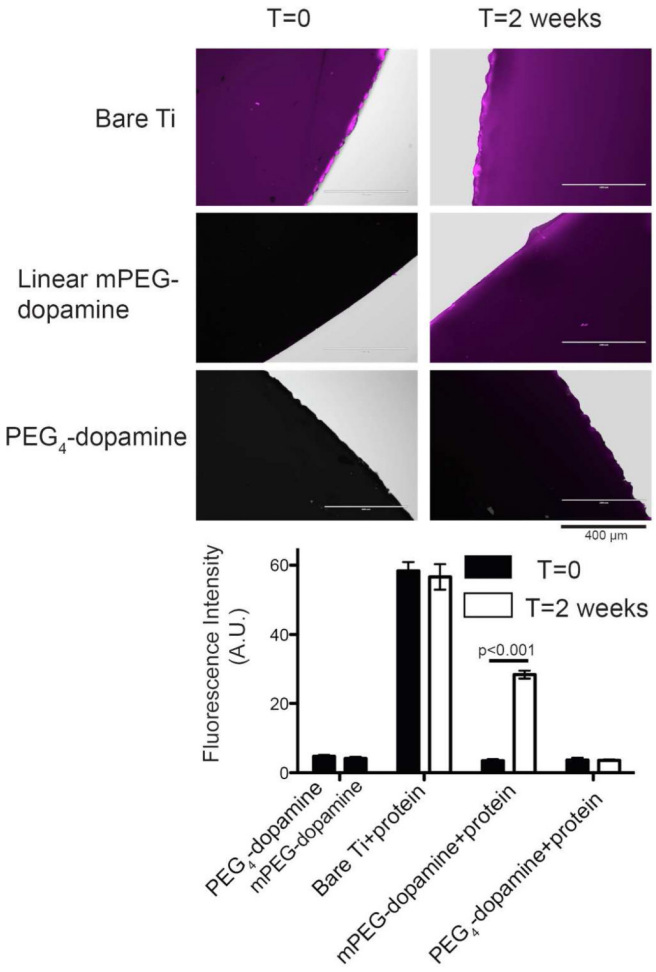

Figure 4.

Adsorption of Alexa Fluor 647-labeled bovine serum albumin (purple color) to Ti surfaces (black) with or without PEG_4-dopamine and linear methoxy polyethylene plycol (mPEG)-dopamine coatings, after a 12-hour incubation. Adsorption was tested before and after incubation in PBS for 2 weeks. The fluorescence intensities were quantified using Image J analysis of images obtained by fluorescence microscopy (λ_{ex}=647 nm). “Protein” = Alexa Fluor 647-labeled bovine serum albumin.

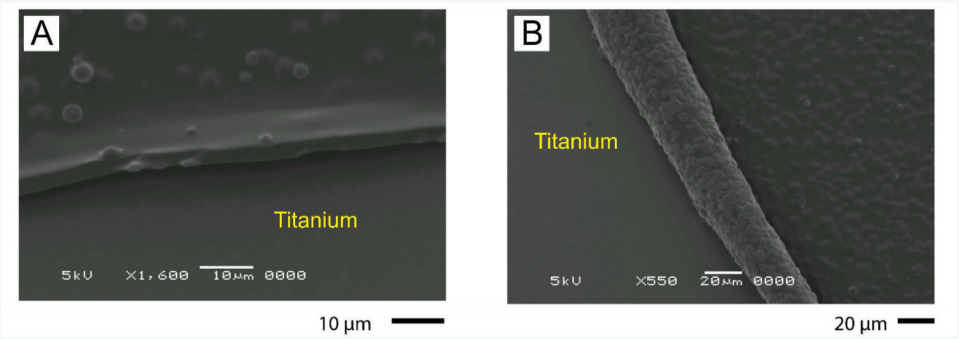

Figure 5.
SEM of coated titanium surfaces (2 mg/mL PEG_4-dopamine) after 10 weeks of incubation in cell culture media at 37 °C. The coating layer has been scratched and peeled away from the substrate surface, exposing the titanium below. (A) View of the scratched edge of the coating, revealing micron-scale thickness. (B) Lower power view of the same coating. Note the presence of a particulate component to the coating.

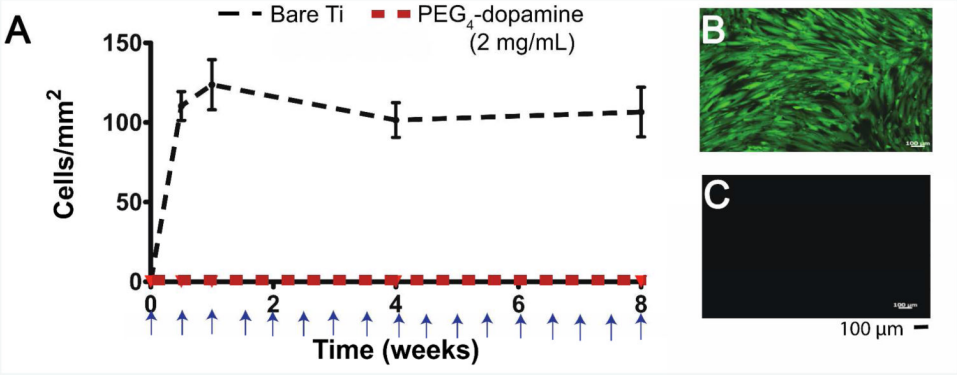

Figure 6.

Cell attachment studies. (A) PEG_4-dopamine-modified titanium demonstrates long-term resistance to NIH3T3 fibroblast attachment. Blue arrows indicate challenge with fresh cells. Data are means ± standard deviations (n=4). (B, C) Fluorescence microscopy images of fibroblast attachment at 1 week on (B) bare titanium and (C) the lack of attachment on PEG_4-dopamine-modified titanium.