Dynamic Electrophoretic Assembly of Metal-Phenolic Films: Accelerated Formation and Cytocompatible Detachment

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ABSTRACT: Material-independent coating has emerged as an advanced tool for interface engineering in numerous applications, including drug delivery, single-cell nanoencapsulation, catalysis, and agrotechnology. Despite remarkable progress made in controlled film formation on solid substrates, the high adhesion of coating species, exemplified by metal-phenolic materials, hinders the film detachment and subsequent formation of free-standing films. In particular, there have been no reports on cytocompatible fabrication of bio-friendly free-standing films of metal-phenolic materials and polyphenols, which is highly demanded in biomedical engineering and nanomedicine. Considering the high demand for cytocompatible protocols for cytocompatible free-standing films, in this work, we have developed an electrophoresis-based, biocompatible method, called dynamic electrophoretic assembly (dEPA), for dynamically and locally regulating the cohesion and adhesion processes of metal-phenolic materials under mild conditions. The locally concentrated cohesive process in dEPA increases the film growth rate by two- to three-orders of magnitude, and, importantly, simple current switching weakens only film adhesiveness and yields durable free-standing films under cytocompatible conditions. Cytocompatibility of the materials and processes in dEPA leads to the fabrication of free-standing cell sheets as well as enabling the incorporation of various functional entities, including enzymes, into the metal-phenolic films.

INTRODUCTION

Recent years have witnessed the unforeseen exploitation of the adhesion-cohesion rivalry¹ for material-independent coating and film formation, because the competition between cohesive molecule-molecule and adhesive molecule-substrate interactions determines whether the molecules coalesce or spread out over the surface.^{2–5} In the coating process, adhesion dominates in formation of the first layer, while cohesion dominates in flocculation, precipitation, and gelation.^{6–8} Nonspecific adhesive species, generated *in situ* in the solution phase, are also cohesive, facilitating but simultaneously interfering with the film growth, as exemplified by polydopamine (PD)-⁹ and metal-phenolic network (MPN)-based methods.¹⁰ Their formation near to or at the substrate surface should not be excluded, but the fast termination in film growth suggests predominant or uneven generation in the solution phase. High adhesion, enabling material-independent coating, does not allow for easy film detachment or delamination.^{11,12} Weakened adhesiveness is coupled with weakened cohesiveness, leading to film degradation, not detachment, in the bulk process.^{13–17} In this respect, kinetic and thermodynamic control of adhesive and cohesive processes is highly demanding to dynamically regulate the characteristics of the films being formed and detached. Recent examples include the in-situ oxidation of Fe²⁺ to Fe³⁺, localized in the reaction

space, which directs the localized cohesive interactions—ensuring the continuous film growth—as well as the adhesion-derived first-layer formation.^{18–21} We also reported that relatively stable (i.e., less cohesive) metal-phenolic species led to continuous film growth.^{22–24}

Free-standing thin films and membranes have been fabricated for various applications, such as sensors,^{25,26} catalysis,^{27,28} drug delivery and wound healing,^{29–31} separators,^{32,33} batteries,^{34,35} actuators,^{36,37} electronic devices,^{38,39} and organs-on-a-chips.^{40,41} For example, we have reported the production of cytocompatible free-standing films of nature-derived chitosan/alginate(/sulfated levan) for drug delivery and tissue engineering.^{29,31} Free-standing films and membranes have also been used in cell sheet engineering, as a cell culture platform to enhance the mechanical stability of cell sheets.⁴² In this regard, the MPN films have great potential for cytocompatible free-standing films applicable to bio-related systems, such as cell sheet engineering, because of biocompatibility of the constituents (i.e., Fe³⁺ and tannic acid (TA)) and controllability of film permeability.^{4,43} To date, however, it has proved elusive to detach the MPN films from substrates and fabricate free-standing films under innocuous conditions.^{10,22} In this work, we propose electrophoresis as an approach for dynamically regulating the cohesive and adhesive phenomena in MPN-based, material-independent film formation.

Figure 1. A digital photograph of the dEPA setup, composed of two ITO glasses, connected to an AA battery, in the Fe^{3+} -TA complex.

Specifically, the electrophoretic movement and localization of the metal-phenolic complex lead to a 10-350 fold increase in the rate of continuous film growth, and film detachment is achieved with ease under cytocompatible conditions by simply switching the electrical potential, from positive to negative, at an electrode. Our strategy of dynamic electrophoretic assembly (dEPA) fabricates free-standing MPN films with arbitrary shapes and also with a combination of different metal ions, phenolic molecules, and functional entities.

EXPERIMENTAL SECTION

Materials. TA (Sigma-Aldrich), gallic acid (GA, $\geq 97.5\%$, Sigma-Aldrich), pyrocatechol (PC, $\geq 99\%$, Sigma-Aldrich), pyrogallol (PG, $\geq 99.0\%$, Sigma-Aldrich), iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\geq 98.0\%$, Sigma-Aldrich), gallium(III) nitrate hydrate ($\text{Ga}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$, 99.9%, Sigma-Aldrich), indium(III) nitrate hydrate ($\text{In}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$, 99.9%, Sigma-Aldrich), terbium(III) nitrate hexahydrate ($\text{Tb}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, 99.999%, Sigma-Aldrich), dimethylsulfoxide (DMSO, Sigma-Aldrich), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, $\geq 98.0\%$, Sigma-Aldrich), glucose oxidase from *Aspergillus niger* (Sigma-Aldrich), peroxidase from horseradish (HRP, Sigma-Aldrich), D-(+)-glucose ($\geq 99.5\%$, Sigma-Aldrich), hydrochloric acid (HCl, 35%, OCI Company Ltd.), AA batteries (Energizer), indium tin oxide (ITO)-coated glasses (Fine Chemicals Industry), ITO-coated polyethylene terephthalate (ITO/PET) films (Fine Chemicals Industry), metallic iron nanoparticles ($D = 35\text{--}45\text{ nm}$, Sigma-Aldrich), SiO_2 nanoparticles ($D = 500\text{ nm}$, Sigma-Aldrich), amine-functionalized latex microparticles ($D = 1\text{ }\mu\text{m}$, Sigma-Aldrich), polystyrene (PS) microparticles ($D = 3.20 \pm 0.13\text{ }\mu\text{m}$, Microparticles GmbH), polypropylene (PP) synthetic paper (iLabel), HeLa cells (HeLa, No. 10002, Korean Cell Line Bank), NIH 3T3 fibroblasts (NIH 3T3, No.

21658, Korean Cell Line Bank), Dulbecco's modified Eagle's medium (DMEM, Welgene), fetal bovine serum (FBS, Welgene), penicillin-streptomycin (P/S, 5,000 U/mL of penicillin and 5,000 $\mu\text{g/mL}$ of streptomycin, Welgene), phosphate-buffered saline (PBS, 10 mM, pH 7.4, Welgene), trypsin-ethylenediaminetetraacetic acid (EDTA) solution (0.05% trypsin, 0.53 mM EDTA-4Na in Hank's balanced salt solution, Welgene), CellTracker™ Red CMTPX (Life Technologies), CellTracker™ Green CMFDA (Life Technologies), CellTracker™ Violet. BMQC (Life Technologies), and Live/Dead® Viability/Cytotoxicity Kit for mammalian cells (Life Technologies) were used as received. HaCaT keratinocytes were obtained as a kind gift from Prof. J.-H. Lee at Chungnam National University, Korea. Au substrates were prepared by thermal deposition of Ti (5 nm) and Au (100 nm) onto silicon wafers (Sehyoung Wafertech). De-ionized (DI) water ($18.3\text{ M}\Omega\cdot\text{cm}$) from Milli-Q Direct 8 (Millipore) was used.

Characterizations. Field-emission scanning electron microscopy (FE-SEM) imaging was performed with a FEI Inspect F50 microscope (FEI) with an accelerating voltage of 5 and 10 kV, after sputter-coating with platinum. Energy-dispersive X-ray (EDX) spectroscopy mapping was performed with a Quattro S environmental scanning electron microscope (Thermo Scientific) with an accelerating voltage of 15 kV. X-ray photoelectron spectroscopy (XPS) spectra were acquired with a Sigma Probe (Thermo VG Scientific), and UV-visible absorption spectra were acquired with a UV-2550 spectrophotometer (Shimadzu). The cells were characterized with a confocal laser-scanning microscope (CLSM) (LSM 800, Carl Zeiss). The ellipsometric thickness was measured with an Elli-SE spectroscopic ellipsometer (Ellipso Technology). At least three independent points of each sample were measured, and average values (with at least 9 measurements) were recorded. Inductively coupled plasma optical emission spectrometry (ICP-OES) analysis was performed with an ICP-OES 720 instrument (Agilent). Zeta potential measurements were performed with a Zetasizer Nano ZS90 instrument (Malvern).

Formation and Cytocompatible Detachment of MPN Films by dEPA. (1) *Film formation:* All solutions were freshly prepared for immediate use. Metal-phenolic complex was prepared by mixing a phenolic-molecule solution with an equal volume of the metal ion solution at ambient temperature (final concentrations: $[\text{Fe}^{3+}, \text{Ga}^{3+}, \text{or } \text{Tb}^{3+}] = 18.5\text{ mM}$ and $[\text{TA}, \text{GA}, \text{or } \text{PG}] = 12\text{ mM}$; $[\text{Fe}^{3+}] = 55.5\text{ mM}$ and $[\text{PC}] = 36\text{ mM}$ for Fe^{3+} -PC films; $[\text{Fe}^{3+}] = 9.3\text{ mM}$, $[\text{Ga}^{3+}] = 4.6\text{ mM}$, $[\text{In}^{3+}] = 4.6\text{ mM}$, and $[\text{TA}] = 12\text{ mM}$ for triple-metal $\text{Fe}^{3+}/\text{Ga}^{3+}/\text{In}^{3+}$ -TA films). Two ITO glasses, connected to an AA battery (1.5 V), were immersed in the metal-phenolic complex (10 mL). After pre-determined time of dPEA reaction (Fe^{3+} -TA films: 0 to 12 h; MPN films with different metal ions, phenolic molecules, or multiple-metals: 14 h), the ITO glasses were washed with DI water three times to remove excess metal ions and phenolic molecules. The film thickness was determined based on the cross-sectional SEM images of the MPN films. The stoichiometry of Fe^{3+} , Ga^{3+} , and In^{3+} in the $\text{Fe}^{3+}/\text{Ga}^{3+}/\text{In}^{3+}$ -TA films was quantified by ICP-OES. (2) *Shape control:* A PP mask was placed on an ITO glass, and the dEPA reaction was

Figure 2. Characterizations of the MPN films formed by dEPA. (a) Digital photographs of anodic ITO glasses at different dEPA reaction-times of Fe³⁺-TA complex. (b) Cross-sectional SEM images of Fe³⁺-TA films at different dEPA reaction-times. (c) A graph of Fe³⁺-TA-film thickness versus dEPA reaction-time. (d and e) Cross-sectional SEM images of Tb³⁺-TA and Fe³⁺-PC films after 14 h of dEPA. Scale bar: 1 μ m.

performed for the desired time. (3) *Free-standing film formation*: After the ITO glasses were immersed in PBS, the negative potential was applied to the MPN film-coated ITO glass for 20 min.

Incorporation of Functional Entities into MPN Films.

(1) *Incorporation of functional entities*: The Fe³⁺-TA complex was simply mixed with functional entities (final concentrations: [Fe³⁺] = 18.5 mM and [TA] = 12 mM; [HRP] = 50 units/mL, [iron nanoparticle] = 1 mg/mL, [SiO₂ nanoparticle] = 2.5 mg/mL, [latex microparticle] = 50 μ L/mL, or [PS microparticle] = 1 wt%). Two ITO glasses (or two ITO/PET films), connected to an AA battery (1.5 V), were immersed in the mixture (10 mL). After pre-determined time of dEPA (24 h for HRP; 14 h for iron nanoparticles, SiO₂ nanoparticles, latex microparticles, or PS microparticles), the ITO glasses were washed with DI water three times to remove excess metal ions, phenolic molecules, and

functional entities, and immersed in PBS. (2) *ABTS radical assay of HRP-embedded free-standing Fe³⁺-TA films*: The aqueous indicator solution of glucose oxidase (10 units/mL), ABTS (1 mM), and glucose (40 mM) was prepared. The HRP-embedded Fe³⁺-TA film was incubated in the indicator solution with gentle shaking. After 16 h, 100 μ L of the indicator solution was diluted with 900 μ L of DI water, and the absorbance was measured by UV-visible spectroscopy.

Fabrication of Free-Standing Cell Sheets. (1) *Cell preparation*: HeLa, NIH 3T3, or HaCaT cells were seeded in a T75 cell culture flask with 15 mL of cell culture media (DMEM containing 10% FBS and 1% P/S for HeLa cells and NIH 3T3 fibroblast, or 5% FBS and 1% P/S for HaCaT cells) and incubated at 37 $^{\circ}$ C in a humidified atmosphere containing 5% CO₂. After reaching 80% confluence, the cells were washed with PBS twice and trypsinized in a cell incubator

Figure 3. Characterizations of the multiple-metal $\text{Fe}^{3+}/\text{Ga}^{3+}/\text{In}^{3+}$ -TA film after 14 h of dEPA. (a) SEM image of the film. Inset in the SEM image is a digital photograph of the multiple-metal TA film. (b-f) EDX-mapping images of the film. Scale bars: 30 μm .

for 5 min. Cell culture medium was added after incubation, and the cells were collected by centrifugation and washed with DMEM twice. CellTrackerTM Red CMTPX, CellTrackerTM Green CMFDA, and CellTrackerTM Violet BMQC were used to stain NIH 3T3 fibroblast, HeLa cells, and HaCaT cells, respectively. CellTrackerTM stock solutions (10 mM solution in DMSO) were diluted with DMEM to the 5 μM solutions, and the cells were incubated with the diluted CellTrackerTM solution for 30 min at 37 $^{\circ}\text{C}$. After incubation, the cells were washed twice with DMEM and seeded, at 10^5 cells/mL, on Fe^{3+} -TA films (3 h of dEPA). After 2 d of incubation, the cells on the Fe^{3+} -TA films were characterized by CLSM. (2) *Formation of free-standing cell-sheet*: As-prepared cells were seeded on a Fe^{3+} -TA film and incubated at 37 $^{\circ}\text{C}$ in a humidified atmosphere containing 5% CO_2 for 2 d. The cells on the film were washed with PBS three times and detached under physiological conditions described before. (3) *Cytotoxicity test*: The Live/Dead[®] Viability/Cytotoxicity Kit for mammalian cells was used to determine the viability of the cells on Fe^{3+} -TA films. Calcein AM (5 μL) and ethidium homodimer-1 (20 μL) were dissolved in 10 mL of PBS, and NIH 3T3 fibroblast cell sheets were treated with the solution for 20 min and characterized by CLSM.

RESULTS AND DISCUSSION

The dEPA setup was composed of ITO glasses, connected to an AA battery, in an aqueous solution of the Fe^{3+} -TA complex (Figure 1). In the setup, the Fe^{3+} -TA complex (pH: 1.7) acted as both a self-assembling block for the MPN film and conductive medium with the battery as a power source. In brief, the complex was prepared by mixing a TA solution (40 mg/mL in DI water) with an equal volume of the $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (10 mg/mL in DI water).²² It was apparent to the naked eye, upon the battery connection to the circuit, that only anodic (i.e., positively charged) ITO glass turned dark (bluish black), indicating the charge-

driven formation of Fe^{3+} -TA MPN films (Figure 2a, Figure S1).

We found that our dEPA strategy greatly accelerated the film growth in a continuous fashion (Figure 2b,c), presumably because of the Fe^{3+} -TA complex concentration made high at or near to the anodic electrode by electrophoretic movement and subsequent locally confined cohesive process of the Fe^{3+} -TA complex.^{44,45} The film-growth rate was calculated to be 46.7 nm/h, which was about 7.5-fold higher than that of our previous report without dEPA (6.2 nm/h).²² The Fe^{3+} -TA MPN film with a thickness of 560 nm was formed in 12 h using dEPA, whereas 89 h was required to make the film without electrophoretic control.²² The enhancement in rate of film formation was also observed with other metal ions and ligands, such as Ga^{3+} , Tb^{3+} , GA, PG, and PC (Figure 2d,e, Figure S2): notably, the growth rates for Tb^{3+} -TA and Fe^{3+} -PC films increased by 350 and 100 times, respectively. Quantitatively, the film-growth rate was greatly varied by the identity of cations: for example, it was calculated to be 117 nm/h for Ga^{3+} -TA complex, but it was 24.8 and 46.7 nm/h for Tb^{3+} -TA and Fe^{3+} -TA, respectively (Figure 2, Figure S2). The FE-SEM images also showed that while the Ga^{3+} -TA and Fe^{3+} -TA films were quite rough, composed of submicrometer particulates, the Tb^{3+} -TA film was relatively smooth (Figure S3). With Fe^{3+} as a cation, the identity of polyphenols also affected the film thickness and roughness. These results implied that the chemical composition of the metal-phenolic complex played an important role in the locally confined cohesive process, resulting in the difference in film-growth rate, as reported previously.²³

After confirming the greatly accelerated film formation by the dEPA, we investigated its generalizability and expandability in terms of chemical compositions in the film. Especially, multi-component films have been demanded in various sectors, such as catalysis, where the synergistically

Figure 4. Fabrication and manipulation of the free-standing MPN films. (a) Schematic for the fabrication of free-standing films by dEPA. (b) Digital photograph of a free-standing Fe^{3+} -TA film (letters). (c and f) Folded and unfolded Fe^{3+} -TA films. (d) HRP-embedded, free-standing Fe^{3+} -TA film. (e) Free-standing multiple-metal $\text{Fe}^{3+}/\text{Ga}^{3+}/\text{In}^{3+}$ -TA film. Scale bar: 0.5 cm.

programmed operation of the multi-components is preferred.⁴⁶ A simple case would be multi-metal cascade reactions, and we investigated the possibility of incorporating multiple metals in the film, with the combination of Fe^{3+} , Ga^{3+} , and In^{3+} (molar ratio: 1:0.5:0.5) as a model. The optical and SEM images, after 14 h of dEPA, showed the successful film formation (Figure 3a). The EDX analysis confirmed the presence of the three cations in the film (Figure 3b-f), which was further supported by the ICP-OES analysis. The cation ratio in the film (1:0.8:0.2) was found to be different from that in the coating solution, indicating the different deposition rates of the cations under dEPA conditions. The Fe^{3+} -TA MPN film, as a representative example, was characterized by SEM, UV-visible spectroscopy, and XPS, all confirming successful film formation (Figures S3-S5). Film characterizations indicated that the dEPA process did not alter the chemical characteristics of the MPN films. The UV-visible absorption spectrum showed the ligand-to-metal charge-transfer (LMCT) band of the Fe^{3+} -TA complex at 565 nm (Figure S4). The estimated area ratio of C=O, C-O, and C-C peaks in the C 1s XPS spectrum (33:85:100) was similar to that for the Fe^{3+} -TA film assembled without electrophoretic control (Figure S5).²² The film was also degraded rapidly in the 1 M HCl solution (Figure S6).¹⁰

In addition to the incorporation of multiple cations, which are the self-assembling components with polyphenols in the coating process, to the films, we investigated whether it would be possible to embed or incorporate external entities in the MPN films, because they would endow the MPN film with the functions and properties that were not attainable from the film itself. As a proof-of-demonstration, an enzyme, HRP, was dissolved in the Fe^{3+} -TA complex, and the electrophoretic MPN assembly was performed for

24 h (Figure 4d). We observed that HRP was catalytically active after film formation, confirmed by the ABTS assay, indicating the biocompatibility of the dEPA coating process as well as the presence of HRP in the film (Figure S7). We also incorporated bigger entities, such as iron nanoparticles (FeNPs) (35-45 nm), SiO_2 nanoparticles (500 nm), latex microparticles (1 μm), and polystyrene microparticles (3.2 μm) to the Fe^{3+} -TA films, supported by the FE-SEM analysis (Figure S8). The zeta potentials of the particles became more negative, after 1 min of incubation in the Fe^{3+} -TA solution, indicating the MPN film formation on the particle surfaces (in the case of amine-functionalized latex, the zeta potential was changed to be negative) (Figure S9). The results suggested the potential of the dEPA strategy in incorporation of external entities to the film regardless of sizes, types, and surface charges. Furthermore, the successful introduction of FeNPs to the film was confirmed indirectly by the rotational motion of an ITO-coated PET (ITO/PET) film, which was coated with the FeNP-embedded Fe^{3+} -TA film, at the water-air interface by a magnet (Figure S10 and Movie S1). Taken together, the dEPA-driven, localized cohesive process not only led to accelerated film growth but also can be extended to different components.

The adhesiveness of the MPN films was reduced, without disturbing the cohesion, by simply switching the potential from positive to negative in the dEPA system, leading to film detachment under physiologically relevant conditions (Figure 4a). Film characterizations indicated that the dEPA-based detachment process did not alter the chemical integrity of the MPN films. The UV-visible absorption spectrum showed the LMCT band of the Fe^{3+} -TA complex between 500 and 700 nm (Figure S11). The XPS analysis in

Figure 5. Fabrication of the free-standing cell sheets. (a) Schematic for the fabrication of free-standing cell sheets by dEPA. (b) A digital photograph of NIH 3T3 fibroblast cell sheets on the Fe^{3+} -TA film in the cell culture medium before detachment. (c and d) Confocal laser-scanning microscopy (CLSM) images of (c) fibroblast cells and (d) multiple cell types (NIH 3T3, HeLa, and HaCaT) on the Fe^{3+} -TA films. Red: NIH 3T3 fibroblast; green: HeLa; violet: HaCaT. (e and f) Digital photographs of fabrication of the free-standing cell sheet. (g) A 3D-reconstructed CLSM image of the free-standing fibroblast cell sheet after cylindrical folding. Green: live; red: dead.

indicated that the oxidation state of Fe ions (i.e., Fe^{3+}) and area ratio of C=O, C-O, and C-C bonds (i.e., 35:83:100) were unchanged (Figures S5 and S12).

The free-standing films that are adjustable in shape or pattern would be valuable platforms in a wide range of fields including biomedical and materials science.^{47–49} Shape control was demonstrated by using the dEPA system. We used a PP mask to make certain areas of the electrode exposed to the Fe^{3+} -TA complex, and formed the free-standing films with various shapes including letters and symbols (Figure 4b, Figure S13). The results also indicated that the localized electrostatic repulsion between the MPN film and the cathode played a crucial role in the peeling-off of the MPN films from the electrode. The free-standing film formed was found to be durable: while maintaining the original shape in an aqueous solution (pH 7) for more than six months, the film could be folded and unfolded many times, like paper origami (Figure 4c,f). The film detachment was general, confirmed by the free-standing-film formation of the HRP-embedded Fe^{3+} -TA system and the $\text{Fe}^{3+}/\text{Ga}^{3+}/\text{In}^{3+}$ -TA system (Figure 4d,e), which would enable versatile applications of the functional free-standing films. In the case of the HRP-embedded free-standing film, the ABTS assay clearly showed that HRP was enzymatically

active, indicative of biocompatible characteristics of dEPA-based free-standing-film formation (Figure S7).

It has been a challenging task in the biomaterials field to fabricate cytocompatible free-standing membranes and films with controlled porosity. For example, free-standing membranes could act as a culture platform in microfluidic devices while permitting the penetration of small molecules, such as drug candidates or stimulants, from the medium.^{50–52} The key requirement for free-standing films in this direction is cytocompatibility of the films and, crucially, fabrication processes. For example, in certain cases, it is required to fabricate the free-standing films that have living cells. We envisioned that the dEPA would lead to the formation of free-standing cell sheets without any compromise in cell viability (Figure 5a). Before free-standing-film formation, we verified the cytocompatibility of Fe^{3+} -TA MPN films with various cell types, such as NIH 3T3 fibroblast, HeLa, and HaCaT cells. An efficient adhesion and uniform distribution of fibroblast cells were observed after two days in culture (Figure 5b,c). The co-culture of three cell lines was made on the Fe^{3+} -TA film (Figure 5d, Figure S14), additionally indicating that the MPN film provided the cytocompatible surface environment to the cells. Current switching formed the free-standing cell sheets (Figure

5e), which were bent slightly due to cellular cohesion⁵³ but retained the 2D sheet morphology in solution (Figure 5f). Of importance was the observation that the cell viability remained greater than 90% before and after the film detachment (Figure S15), proving that the electrophoretic regulation was a cytocompatible process. It is to note that there have been few reports that demonstrate the cytocompatible detachment of cytocompatible films. For example, we have previously reported the film detachment and free-standing film formation of nature-derived biocompatible layer-by-layer (LbL) multilayers, but the conditions for film detachment were not compatible with living cells.^{29–31} Therefore, the confirmed cytocompatibility in both materials and detachment conditions in our dEPA system would be beneficial in various bio-related applications, such as tissue engineering, molecular diagnostics, and implantable microdevices, additionally benefiting from innate material-independent coating capability of the MPN. Moreover, it would be feasible to confer bio-operating properties to MPN films by incorporating functional entities. We also believe that the control over film characteristics, such as chemical composition and mechanical stiffness, coupled with permselectivity of the MPN films^{10,21,22,54} would advance cell-sheet engineering and related fields. For example, the “bendability” of the MPN film allowed for the fabrication of cylindrical cellular constructs without any loss of cell viability (Figure 5g): more complex films could be produced by sequentially depositing different components, producing stratified structures with controlled composition.

CONCLUSIONS

In summary, we demonstrated an electrophoretic approach to dynamically and locally regulating the adhesion and cohesion of metal-phenolic species, enabling cytocompatible fabrication of the free-standing films even with living cells. Compared with previously reported methods for fabricating free-standing films,⁵⁵ our dEPA strategy has some advantages and disadvantages. The advantages include: (1) film coating and detachment are rapid and cytocompatible; (2) the fabrication setup is extremely simple and requires only two ITO glasses, connected to an AA battery; (3) it allows incorporation of functional entities, regardless of their sizes, types, and surface charges, into the MPN films of customized architectures, simply by mixing them with the Fe³⁺-TA complex; (4) it is scalable in size and shape, and requires low energy consumption, permitting the industry-level manipulation; and (5) it is not limited to MPN films and can be applied to various free-standing-film formation with tunable thickness. On the other hand, our method is limited to conducting materials, not applicable to insulating substrates. Although it is possible to introduce various properties to the MPN films by incorporation of functional entities, the material scope and corresponding film properties are also limited, especially compared with the LbL strategy that could employ a plethora of building blocks. Nonetheless, we think that the simplicity, efficiency, versatility, and cytocompatibility of the dEPA method would provide an alternative approach to the technological development of biomedical engineering and bioMEMs.

ASSOCIATED CONTENT

This material is available free of charge via the Internet at <http://pubs.acs.org>.

Details of characterization data including digital photographs, SEM images, EDX-mapping images, film-thickness results, UV-visible absorption spectra, XPS spectra, CLSM images, and zeta potential values

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (MSIP2012R1A3A2026403), and the European Research Council grant agreement ERC-2014-ADG-669858 (ATLAS). This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 & UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. Part of this research was conducted and funded by the Australian Research Council (ARC) Centre of Excellence in Convergent Bio-Nano Science and Technology (Project No. CE140100036). F.C. acknowledges the award of an NHMRC Senior Principal Research Fellowship (GNT1135806).

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Dynamic Electrophoretic Assembly (dEPA)

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Title:

Dynamic Electrophoretic Assembly of Metal–Phenolic Films: Accelerated Formation and Cytocompatible Detachment

Date:

2020-09-22

Citation:

Yun, G., Youn, W., Lee, H., Han, S. Y., Oliveira, M. B., Cho, H., Caruso, F., Mano, J. F. & Choi, I. S. (2020). Dynamic Electrophoretic Assembly of Metal–Phenolic Films: Accelerated Formation and Cytocompatible Detachment. *Chemistry of Materials*, 32 (18), pp.7746-7753. <https://doi.org/10.1021/acs.chemmater.0c02171>.

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