Effects of Polymer End-Group Chemistry and Order of Deposition on Controlled Protein Delivery from Layer-by-Layer Assembly

Michael Keeney, † Melina Mathur, ‡ Emily Cheng, § Xinming Tong, † and Fan Yang*,†,‡

Departments of Orthopaedic Surgery, Bioengineering, and Computer Science, Stanford University, Stanford, California 94305, United States

Supporting Information

ABSTRACT: Layer-by-layer (LBL) assembly is an attractive platform for controlled release of biologics given its mild fabrication process and versatility in coating substrates of any shape. Proteins can be incorporated into LBL coatings by sequentially depositing oppositely charged polyelectrolytes, which self-assemble into nanoscale films on medical devices or tissue engineering scaffolds. However, previously reported LBL platforms often require the use of a few hundred layers to avoid burst release, which hinders their broad translation due to the lengthy fabrication process, cost, and batch-to-batch variability. Here we report a biodegradable LBL platform composed of only 10 layers with tunable protein release kinetics, which is an order of magnitude less than previously reported LBL platforms. We performed a combinatorial study to examine the effects of polymer chemistry and order of deposition of poly(β-amino) esters on protein release kinetics under 81 LBL assembly conditions. Using the optimal “polyelectrolyte couples” for constructing the LBL film, basic fibroblast growth factor (bFGF) was released gradually over 14 days with retained biological activity to stimulate cell proliferation. The method reported herein is applicable for coating various substrates including metals, polymers, and ceramics and may be used for a broad range of biomedical and tissue engineering applications.

1. INTRODUCTION

Proteins are widely used for drug delivery and tissue engineering applications to promote desired cellular processes or tissue responses. Most proteins are unstable under physiological conditions and are subject to quick denaturation and degradation by various enzymes in circulation. Given the short half-lives of proteins in vivo, platforms that enable prolonged and tunable protein release while maintaining their biological activity would be highly desirable. Layer-by-layer (LBL) assembly involves sequential deposition of positively and negatively charged polyelectrolytes on material surfaces and can be utilized to construct drug delivery depots by incorporating drugs of interest into self-assembled nanoscale thin films. To incorporate biomolecules into LBL coatings, charged biomolecules can be used as one type of polyelectrolyte layer, and assembly can be repeated to achieve the desired concentration of biomolecules on the surface. One advantage of applying LBL assembly for controlled release of biomolecules is its aqueous-based assembly process, which avoids the use of organic solvents that are needed in many other drug loading platforms. Given the fragile nature of most biomolecules, the mild fabrication conditions of LBL assembly make it an attractive candidate for controlled drug release and provides a more physiological environment for preserving protein bioactivity.

When the LBL assembly process is used, proteins, DNA plasmids, and peptides have been deposited onto the surface of tissue engineering scaffolds or medical implants to promote desired tissue formation. Poly(β-amino) ester based hydrolytically degradable multilayer films have been used to encapsulate bone morphogenetic protein-2, which led to up to two weeks of release in vitro and enhanced ectopic bone formation in vivo. Dual growth factor release (bone morphogenetic protein-2 and vascular endothelial growth factor) has also been demonstrated from the surface of polycaprolactone/β-tricalcium phosphate scaffolds. Vascular endothelial growth factor was released over the first 8 days, while bone morphogenetic protein-2 was released over 2 weeks. Such a multiphasic release profile was designed to trigger blood vessel formation followed by bone formation, hence, mimicking the natural events of bone development. When implanted intra-muscularly in a rat model, the group receiving dual growth factor delivery showed greater bone formation compared to the group receiving single growth factor alone, suggesting potential synergistic benefit of releasing multiple types of growth factors in a coordinated manner. Similarly, biphasic release of DNA plasmids from LBL assemblies has been shown to direct sequential transfection when complexed with a nonviral transfection agent in vitro. Growth factor-eluting multilayers may also be coupled with osteoconductive base layers to create osteophilic multilayer coatings for promoting osteogenic differentiation of human mesenchymal stromal cells.
The base layers containing hydroxyapatite nanoparticles complexed with chitosan created an osteoconductive surface for MSCs, while the upper poly(β-amino) ester layers facilitated gradual release of bone morphogenetic proteins over several days.

Despite the promise of applying LBL assembly process for controlled release of proteins, translation of LBL technology for clinical applications has been slow. This lack of progress is, at least in part, due to one key limitation associated with the current LBL platforms: large number of layers is needed to achieve gradual release. Previous reports have shown that a minimum of 80 layers were required for the LBL coating to avoid burst release, and increasing the number of layers up to 170 (approximately a 28 h process) led to a more linear release over a 10 day period.4,20 This platform achieved release kinetics and concentrations that are clinically relevant, which represents a significant progress in the field. However, scaling up such platforms for broad applications remains challenging due to the lengthy fabrication process, which requires significant amounts of materials and time, and is subject to batch-to-batch variability.

To overcome the aforementioned limitations, novel LBL platforms that allow gradual protein release with reduced processing time would be highly desirable. Polyelectrolyte hydrophobicity has been shown to affect the release of DNA from LBL films, which suggests that chemical structure of the polyelectrolyte21 may play an important role in regulating protein binding and release from the LBL assembly. Furthermore, the chemical structure of poly(β-amino) ester, such as the end group chemistry, significantly affects their ability to complex DNA into nanoparticles for gene delivery.22 Based on these findings, here we hypothesize that protein loading efficiency and release kinetics from LBL films may be controlled by tuning the chemical structures of the polyelectrolytes used for LBL assembly. Polyelectrolyte location has also been shown to have a significant effect on release of biomolecules. Barrier layers have been introduced in an effort to delay biomolecule release or to develop sequential release platforms.23,24 We hypothesize that protein release may be controlled by the order of polyelectrolyte deposition, that is, choosing polyelectrolytes with different chemical structures for the base layers versus the upper protein loading layers. To test our hypotheses, we synthesized a set of poly(β-amino) esters with 10 different end-group chemistries for LBL assembly and examined the corresponding effects on protein loading efficiency and release kinetics under 81 combinatorial conditions. It should be noted that the number of layers used in our LBL platform is only 10 layers, which is an order of magnitude lower compared to the previously reported platforms. This new platform, if successful, would substantially reduce the amount of time and materials required for the LBL assembly. To facilitate rapid quantification of protein release from the combinatorial LBL assembly groups, we developed a high-throughput screening assay using bovine serum albumin as a model protein, and protein release was monitored for 14 days.

To examine the biological activity of protein released from LBL coatings, basic fibroblast growth factor (bFGF) was coated onto PLGA scaffolds using the lead poly(β-amino) ester and order of deposition. The release kinetics of bFGF was measured using ELISA and the biological activity of the released bFGF over 14 days was examined using a cell proliferation assay.

2. MATERIALS AND METHODS

2.1. Materials. All materials were purchased from Sigma-Aldrich unless otherwise stated.

2.2. Polymer Synthesis. Acrylate terminated poly(β-amino) ester (POLY) was synthesized as described previously.20 Briefly, 1,533 g S-aminophenyl-1-pentanol (Alfa Aesar, Ward Hill, MA) was combined with 3,255 g butanediol diacrylate and stirred overnight at 90 °C. To introduce different end-group chemistries, 10 different types of amine monomers (A–J) were chosen. To examine the effects of polymer structure chemistry on protein binding and release from LBL assembly, we have chosen the above end-group chemistry with structure diversity, including linear, branched, ring structures, and varying degree of hydrophobicity and amine presentation. Amine monomers (10 mmol) were mixed with 5 g acrylate-terminated POLY in the presence of tetrahydrofuran (Fisher Scientific, Houston, TX) and stirred overnight. The products were then precipitated with diethyl ether and dissolved in anhydrous DMSO (100 mg/mL). All materials were stored at −20 °C until use. The resulting 10 end-modified polymers share the same POLY backbone and are termed POLY-X, with X referring to the specific end-group chemistry, as shown in Figure 1.

![Figure 1. Chemical structures of the polymer backbone (POLY-) and various end groups. Acrylate terminated poly(β-amino) ester (POLY-) was end-capped with 10 different chemical groups (A–J) to serve as the positively charged polyelectrolyte for LBL assembly.](image)

2.3. Preparation of Polyelectrolyte Solutions. POLY-X was diluted to 30 mg/mL in phosphate-buffered saline (PBS; Invitrogen, Carlsbad, CA) and buffered to pH 7 with 0.01 M sodium hydroxide. Chondroitin sulfate (CS) and bovine serum albumin (BSA) were prepared in water at 0.1 and 1 mg/mL, respectively. Basic BFGF (PeproTech, Rocky Hill, NJ) was prepared by dissolving the growth factor at a concentration of 75 µg/mL in 1 mg/mL BSA containing 6 mM Tris.

2.4. Construction of LBL Films Encapsulating Bovine Serum Albumin. The film was constructed following the LBL coating process as depicted in Figure 2. Unless otherwise stated, layers were deposited on the surface of poly(lactic-co-glycolic acid) (PLGA; 50/50% wt/wt) scaffolds. PLGA scaffolds were prepared by first dissolving PLGA in chloroform (100 mg/mL), followed by aliquoting 35 µL of the polymer solution into each well within a 96-well plate. The polymer solution was left to air-dry overnight and the resulting PLGA scaffolds were used for all LBL film assemblies. Two base layers of (CS/POLY-X) were deposited on the surface of the PLGA scaffold followed by two trilayers of (BSA/CS/POLY-X). Each layer was deposited for 5 min, followed by two washes for 30 s each in sterile deionized water to remove any unbound polyelectrolyte. The concentration of BSA solution before and after deposition was...
measured using the BCA Protein Assay (Pierce, Rockford, IL). The wash solution was also collected and quantified to account for unbound protein losses. Total amount of BSA deposition was determined by calculating BSA decrease in the BSA solution during the coating process.

2.5. Protein Deposition on Different Material Substrates. To demonstrate the versatility of the LBL coating technique, fluorescein isothiocyanate (FITC) labeled BSA was deposited on PLGA (50:50% wt/wt), titanium alloy (grade 9), and calcium phosphate. Sintered calcium phosphate scaffolds were a generous gift from Prof. John Jansen (Radboud University, Nijmegen). LBL deposition was performed as described in section 2.4; however, FITC-labeled BSA replaced the nonlabeled BSA.

2.6. Characterization of BSA Release. Protein release from the film coated on PLGA scaffolds was conducted in PBS at 37 °C, in which hydrolytic degradation of POLY-X allowed the release of protein. At a series of different time points, the supernatant was collected and replaced with fresh PBS. All collected samples were frozen at −20 °C until analyzed. The amount of released BSA was determined using a BCA Protein Assay Kit (Pierce) following the manufacturer’s protocol. Briefly, 25 μL of collected sample was added to 200 μL of BCA assay reagent in a 96-well plate and incubated at 37 °C for 30 min. The color change was detected using a plate reader (Spectramax M2e, Molecular Devices, Sunnyvale, CA) measuring absorbance at 562 nm. The absorbance readings were converted into protein content using a BSA standard curve. The absorbance readings were converted into protein content using a BSA standard curve.

2.7. Coupling Polymers with Different Chemistry for Constructing the Base and Upper Layers. To determine the effect of polyelectrolyte location on protein release, LBL films were prepared using polyelectrolyte couples. A polyelectrolyte couple is formed when two different polyelectrolytes are used to fabricate the base and upper layers of the film. The coating procedure was performed as described in section 2.4. A total of 81 polyelectrolyte couples were formed and protein release studies were performed, as described in section 2.6. A nomenclature was assigned to identify the polymers used to form a polyelectrolyte couple, for example, a polyelectrolyte couple comprising of POLY-C in the base layer and POLY-A in the upper layer is assigned the code CA.

2.8. Construction of LBL Films Encapsulating Basic Fibroblast Growth Factor. bFGF (PeproTech, Rocky Hill, NJ) is a known potent stimulating factor for the proliferation of human adipose derived stem cells (hADSCs). bFGF was coated onto the surface of PLGA scaffolds using POLY-1 following the same procedure described for encapsulating BSA. Following deposition of four trilayers containing bFGF, the films were washed 2 times for 30 min each with PBS to remove any unbound growth factor. Scaffolds were immersed in PBS containing 40 mg/mL BSA, and supernatant was collected daily and replaced with fresh PBS/BSA. All collected samples were stored at −20 °C until analyzed. The amount of bFGF in the supernatant was quantified using an enzyme-linked immunosorbent assay (ELISA) specific for bFGF (900-M08, PeproTech, Rocky Hill, NJ) following the manufacturer’s protocol.

2.9. Cell Culture. Human adipose-derived stromal cells (hADSCs) were isolated from excised human adipose tissue from informed and consenting patients following procedures as previously described.25 hADSCs were subcultured upon 90% confluence until passage 5 before use in growth medium consisting of Dulbecco’s Minimal Essential Medium (DMEM, Invitrogen, Carlsbad, CA), 100 units/mL penicillin, 100 μg/mL streptomycin, 10% (v/v) fetal bovine serum (FBS), and 10 ng/mL bFGF. hADSCs were plated at a density of 12000 cells per well in 96-well plates and left to attach overnight.

2.10. Determine the Biological Activity of the Released bFGF. To determine the biological activity of the released bFGF from the film, cell culture medium was replaced with the supernatant released from the scaffolds. Briefly, 12.5 μL of samples collected from each time point were mixed with 187.5 μL of cell culture medium and added to hADSCs. Cells were cultured for 48 h and followed by quantification of cell number using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) following manufacturer’s protocol. hADSCs cultured in the presence of freshly supplemented bFGF (10 ng/mL) were included as a positive control, and hADSCs cultured in cell culture medium only without bFGF were included as a negative control. All samples were incubated at 37 °C for 2 h and absorbance was read with a plate reader at 490 nm.

2.11. Statistical Analyses. Minitab (Minitab Inc., U.S.A.) software was used for statistical analysis. One-way analysis of variance (ANOVA) with a Tukey’s or Dunnett’s posthoc analysis was used to determine statistical significance between groups. A Tukey’s analysis was used for all pairwise comparisons while a Dunnett’s analysis was used for multiple comparisons to a control group.

3. RESULTS AND DISCUSSION

LBL coating is a promising technology for depositing biological signals onto the surface of medical implants such as cardiovascular stents or orthopedic implants, as well as tissue engineering scaffolds. Here we report a combinatorial study that examines the effects of polyelectrolyte chemical structure and their order of deposition on tuning protein release from LBL assembly. Using the lead “polyelectrolyte couples”, we demonstrated highly efficient coating and gradual protein release from LBL assembly using as few as 10 layers in total. Our platform allows efficient coating of proteins onto the surface of a broad range of materials commonly used for biomedical applications including titanium, PLGA, and calcium phosphate (Figure 2). The fabrication process was performed using aqueous conditions at physiological pH, thereby facilitating the preservation of protein bioactivity. Our results also demonstrated that protein deposition and release can be controlled through modification of the small molecule end-groups on the terminus of POLY.

3.1. Effects of Varying POLY Chemical Structures on Protein Deposition. LBL protein deposition is governed by charge and pH of both protein and polyelectrolytes used for the self-assembly.26 Under optimized conditions, LBL can be employed to deposit large quantities of protein on a scaffold surface, but a large number of layers are often required to avoid burst release.1,4,11,20 LBL assembly has been used to deliver growth factors in vivo for ectopic bone formation.1 In previously reported platforms, in order to get increased protein deposition and avoid burst release, increasing the number of
layers was often required. To overcome such limitations, here we aim to achieve efficient loading and controlled release from LBL assembly by tailoring the polyelectrolyte chemistry used for the assembly, while minimizing the number layers needed for the LBL assembly.

We first examined the effect of the chemical structure of POLY, the cationic polyelectrolyte used for LBL assembly, on the efficiency of protein deposition. The end-group chemistry of POLY structure was shown to dramatically impact the loading efficiency of proteins (Figure 3). In the two extreme cases, POLY-J totally blocked protein deposition, while POLY-E led to over 40 μg of BSA deposition using a single trilayer of protein/CS/POLY. The remaining POLYs led to different degrees of protein deposition in between. Among all the POLY structures tested, POLY-I and POLY-G are the only two that do not contain two primary amine groups and were associated with much lower protein deposition than most other POLYs. POLY-E, on the other hand, contains the highest number of primary amine groups and showed the highest protein loading efficiency. These results suggest that increasing number of primary amine groups may increase protein loading efficiency due to increased charged density. POLY-J is the only polymer that contains an aromatic ring in its end group structure and led to complete blocking of protein deposition, despite the presence of two primary amine groups. It is likely that the conjugation effect of the aromatic ring decentralizes the lone pair electron of nitrogen, hence, decreasing the alkalinity. The positive charge density of a polymer with aromatic amine groups may increase protein loading efficiency due to decreased alkalinity. The protein deposition efficiency may also be influenced by varying the concentration of protein solution or the duration of the deposition step (see Supporting Information).

3.2. Effects of Varying POLY Chemical Structures on Protein Release. One major limitation of the current LBL platforms is the need for using 100−200 layers to avoid burst release.1 By varying the end-group chemistry of POLY, here we showed gradual release of proteins over two weeks using as few as 10 layers in total, which includes the four precursor layers and two trilayers of protein/POLY/CS complex (Figure 4). This allows markedly reduced time, cost, and materials needed for constructing LBL assemblies for controlled release. Out of the 10 POLYs examined, four of them led to an instant burst release, while the rest led to a varying degree of gradual release over two weeks. Interestingly, the four POLYs that led to the highest protein deposition (POLY-B−E) are structurally similar, and all led to a burst release. The key to achieve controlled release is to find a balance between protein immobilization and release. A previous study has investigated the influence of polymer structure on the degradation profiles of multilayered polyelectrolyte assemblies fabricated from sodium poly(styrene sulfonate) and three structurally related POLYs with varying charge density and hydrophobicity. While changes in POLY structures did not influence film thickness, increasing POLY hydrophobicity prolonged the degradation profile of the resulting LBL films from 50 h to 15 days.21 These results suggest the possibility of tuning protein release from LBL assembly by changing the structure of the degradable polymamines used to fabricate the film. One of our lead polyelectrolytes, POLY-I, was found to be most effective in avoiding burst release (Figure 4) and led to an almost linear release curve over 2 weeks. The end group of POLY-I contains piperidine group, which is also present in the lead polymer structure from a previous study.21 In our study, degradability of POLY was also shown to be important for controlling the release of proteins. POLY-B and POLY-F share similar structures, except POLY-B contains ester bonds, which allows faster degradation in aqueous conditions. LBL assembly using POLY-B led to a typical burst release profile, which is likely due to the rapid degradation of the capping molecules. In contrast, LBL assembly based on POLY-F led to gradual protein release for up to two weeks. Overall, protein release from LBL assembly is a complex process that may be influenced by multiple parameters such as polyelectrolyte degradation rate, strength of the protein−polyelectrolyte binding, and diffusivity of the polyelectrolyte layers.

3.3. Effects of POLY Deposition Order on Protein Release Kinetics. In our design, the LBL assembly is involves initial deposition of base precursor layers followed by the upper biological layers that contain the proteins of interest. The precursors layers are composed of two repeated bilayers (POLY-X/CS) and serves to prime the surface of the substrate (e.g., metals, polymers, or ceramics) to facilitate efficient deposition of the following biological layers. The biological layers are composed of two repeated trilayers (protein/CS/POLY-X) and their main function is to tune the release kinetics of encapsulated proteins (Figure 5A). This leads to a total of 10
layers for LBL assembly, which is an order of magnitude less than the previously reported LBL platforms.

Previous studies have commonly used PEI as the polyamine when constructing the base layer and switching to POLY for the upper biological layers. In our LBL assembly design, we used POLY for both base priming layers and the upper biological layers and deposited only 10 layers in total. Given the different functions of the priming layers and biological layers, we hypothesized that different POLY structures may be preferable for each component to achieve the balance of immobilization while still allowing gradual release. To test this hypothesis, we designed a combinatorial study composed of 81 "polyelectrolyte couples", using POLY-X with 9 varying end groups as base layers or upper biological layers. POLY-J was not included due to its complete blocking of protein deposition.

Figure 5B summarizes the percentage of released proteins at 24 h. By varying the "polyelectrolyte couples", a broad range of protein release was observed ranging from 10 to 100% (Figure 5B). Both POLY chemistry and order of deposition were shown to impact the protein release profile. POLY-B–E led to burst release when used as both base and upper layers, however, such burst release may be reduced to some degree by coupling these polyelectrolytes with different POLYs as upper polyelectrolytes such as POLY-A, G, I, and F.

We have extracted a full data set for POLY-I to further examine the respective influence of polymer structure used for upper and base layers on protein release up to 14 days. When the upper layer POLY was fixed (POLY-I) while changing the base layer POLY, a broad range of protein release kinetics was obtained from a burst release (e.g., C–I) to a linear gradual release over two weeks (G–I; Figure 5C). In contrast, fixing base layer POLY while changing upper layer POLY has a more mild effect on protein release over time (Figure 5D). Together, our results suggest that the structure of polycations for the base layers plays a dominant role in determining the protein release, while the choice of polycations for the upper layers allows further fine-tuning of protein release, but to a lesser degree.

The advantage of such "polyelectrolyte coupling" is further highlighted in Figure 6, whereby a rapidly releasing polyelectrolyte (POLY-B) was combined with a slow releasing polyelectrolyte (POLY-F) to produce an initial burst followed by a period of sustained release (B–F). Such ability to tune protein release kinetics can provide useful tools to guide desirable cellular behavior. For example, a previous study has shown that a burst release of VEGF followed by sustained VEGF delivery promoted endothelial cell proliferation and sprouting compared to a constant delivery of VEGF.27

3.4. Protein Released from LBL Film Retained Its Bioactivity. By varying the polycation structure and order of deposition, we have demonstrated efficient protein loading and tunable protein release kinetics using as few as 10 layers. To confirm that such platforms would be clinically translatable, it is
important to verify that released proteins retained their biological activity. As described previously, one advantage of our LBL platform is its mild fabrication conditions, and all reagents were prepared at neutral pH and in aqueous solution. LBL assembly is commonly performed under slightly acidic conditions (pH 5–6) to increase charge density of the polyelectrolytes.\(^{11,18,28−31}\) The coating conditions however can have a negative effect on growth factor stability, for example, exposure to organic solvents can lead to loss of the native tertiary structure while exposure to low pH can accelerate chemical cleavage of peptide bonds.\(^{32−34}\) To evaluate the biological activity of proteins release from our LBL platform, basic fibroblast growth factor (bFGF) was used as a model protein and coated on the surface of PLGA films. bFGF was prepared at a concentration of 75 \(\mu\)g/mL in 0.1% BSA (wt/ vol) and POLY-I was used as the positively charged polyelectrolyte. BSA is used as a carrier protein to minimize loss during fabrication and also to provide a net negative charge during deposition. A gradual release of bFGF was observed over the course of 15 days, with a total amount of 177 ng bFGF released from a surface 31 mm\(^2\) (Figure 7). bFGF is a known potent factor to stimulate the proliferation of human adipose derived stem cells (hADSCs). The supernatant containing bFGF released from each day was collected and its ability to stimulate hADSC proliferation was measured. Cells receiving bFGF-eluting supernatant from all the time points led to enhanced cell proliferation relative to the negative control (cells receiving media without the addition of FGF). Proliferation decreases using supernatant from later time points due to slower release of bFGF at later time points (Figure 8). These results confirm that proteins released from the LBL platform reported herein can effectively retain their biological activity over at least two weeks.

In our study, we have chosen bovine serum albumin and basic fibroblastic factor as two model proteins to demonstrate the efficacy of our platform for efficient loading and controlled release of proteins with retained biological functions. It is also important to note that the results reported here could be generalized for releasing other types of proteins with varying physiochemical properties as well. This is due to the fact that bovine serum albumin was intentionally chosen to be a carrier protein during the LBL assembly, thereby the charge will be dominated by the albumin rather than the growth factors themselves. In previously reported platforms, growth factors were directly used for LBL assembly. As such, the charge will be determined by the growth factors and optimal conditions will depend on the isoelectric point of corresponding growth factor.\(^{26}\) Compared to previous methods, our platform is more versatile and facilitates generalization of the optimized platform for delivering a broad range of proteins with varying physiochemical properties.

4. CONCLUSION

Here we report a combinatorial study to examine the effects of polymer chemistry on regulating protein loading and release from LBL films. By tailoring polymer end-group chemistry and order of deposition (base vs upper layers), protein release can be tuned across a broad range. This provides a powerful tool for coating various medical implants or tissue engineering scaffolds for local release of biologics with desirable release kinetics. Using the optimal “polyelectrolyte couples” for constructing the base and upper layers of the LBL film, gradual protein release for up to 2 weeks can be achieved using as few as 10 layers, which is an order of magnitude less than the previously reported LBL platform. This is especially significant for industrial translation, as this LBL technology platform reduces...
the amount of required material, hence, cost and time for production. Furthermore, the film construction process retains biological activity and released bFGF continued to stimulate cell proliferation for up to 2 weeks. The method reported herein is applicable for coating various substrates, including metals, polymers, and ceramics, and may be used for a broad range of biomedical and tissue engineering applications.

**REFERENCES**


**AUTHOR INFORMATION**

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors would like to thank the Donald E. and Delia B. Baxter Foundation, the McCormick Faculty Award, the Stanford Bio-X Interdisciplinary Initiative grant and the 3M Nontenured Faculty Grant for funding.

**ASSOCIATED CONTENT**

Supporting Information

Polymer characterization via NMR, along with data on the protein deposition as a function of time and protein concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

**REFERENCES**