Published in final edited form as:

Biomaterials. 2013 December ; 34(38): 10287–10295. doi:10.1016/j.biomaterials.2013.09.028.

Mutant MCP-1 Protein Delivery from Layer-by-Layer Coatings on Orthopaedic Implants to Modulate Inflammatory Response

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Abstract

Total joint replacement (TJR) is a common and effective surgical procedure for hip or knee joint reconstruction. However, the production of wear particles is inevitable for all TJRs, which activates macrophages and initiates an inflammatory cascade often resulting in bone loss, prosthetic loosening and eventual TJR failure. Macrophage Chemoattractant Protein-1 (MCP-1) is one of the most potent cytokines responsible for macrophage cell recruitment, and previous studies suggest that mutant MCP-1 proteins such as 7ND may be used as a decoy drug to block the receptor and reduce inflammatory cell recruitment. Here we report the development of a biodegradable, layer-by-layer (LBL) coating platform that allows efficient loading and controlled release of 7ND proteins from the surface of orthopaedic implants using as few as 14 layers. Scanning electron microscopy and fluorescence imaging confirmed effective coating using the LBL procedure on titanium rods. 7ND protein loading concentration and release kinetics can be modulated by varying the polyelectrolytes of choice, the polymer chemistry, the pH of the polyelectrolyte solution, and the degradation rate of the LBL assembly. The released 7ND from LBL coating retained its bioactivity and effectively reduced macrophage migration towards MCP-1. Finally, the LBL coating remained intact following a femoral rod implantation procedure as determined by immunostaining of the 7ND coating. The LBL platform reported herein may be applied for *in situ* controlled release of 7ND protein from orthopaedic implants, to reduce wear particle-induced inflammatory responses in an effort to prolong the lifetime of implants.

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1. Introduction

Total joint replacement (TJR) is a very common and successful procedure performed over 1 million times annually in the US on hips and knees alone. Despite the success of current TJR procedures, 38,000 revision total hip replacements (THRs) and 39,000 revision total knee replacements (TKRs) were performed in 2006, leading to costs in excess of 1 billion dollars for the procedures alone.[1–2] Revision procedures are more costly and have a less predictable outcome. The revision procedure often results in increased bone loss, the requirement for bone grafts and more expensive implants. By the year 2030, the percentage of Americans over 65 years is expected to increase to 37%, from 19% in 1985, and result in further increasing needs for THRs and TKRs.[3–4] Furthermore, TJRs are now been performed in younger patients with more active lifestyles, which places increased demands on their TJR. As a result, the major long-term problem associated with TJRs is in the generation of wear particle debris.

One major factor that contributes to the failure of TJR is the production of wear particles, which is inevitable for all TJRs. Wear begins during the initial “bedding in” phase of newly implanted TJRs and continues with use. The majority of TJRs incorporate a polyethylene bearing surface, and polyethylene wear particles are phagocytized by macrophages, which initiates activation of the inflammatory cascade. Pro-inflammatory cytokines, chemokines, prostanooids, reactive oxygen intermediates (ROIs) and other factors are released locally into the synovial fluid and interfacial tissues.[5–6] These inflammatory mediators activate local osteoclasts and interfere with mesenchymal cell proliferation, differentiation and function, which delays initial implant osseointegration leading to peri-prosthetic bone resorption. Cells of the monocyte/macrophage lineage (macrophages, foreign body giant cells and osteoclasts) are among the key cells that perpetuate the inflammatory reaction to wear particles of orthopaedic implants. The process begins when monocytes/macrophages are recruited to the site of wear particle production. Once monocytes/macrophages are recruited to the joint space, the presence of wear particles stimulates over-expression of inflammatory cytokines. Such inflammation in the joint space would also delay bone implant integration, and can result in implant failure. Therefore, there is a strong need to develop methods to modulate the inflammatory responses at the implantation site of orthopaedic implants to inhibit undesirable macrophage trafficking.

Macrophage chemoattractant protein-1 (MCP-1) is one of the major cytokines responsible for monocyte/macrophage cell recruitment. The wild type MCP-1 protein is 76 amino acids long and signals through the C-C chemokine receptor type 2 (CCR2) and CCR4 receptors on the surface of monocytes. The N-terminal residues of MCP-1 are crucial in activating the MCP-1 signaling pathway. Previous studies have created mutant MCP-1 by removing the first 8 residues (QPDAINAP), and the resulting protein MCP-1 (9-76) can bind to CCR2 and CCR4 without activating the pathway.[7–8] It has also been shown that removal of residues 2–8 (MCP-1 (1+9-76), also known as 7ND, produced a similarly inhibitory effect on monocyte recruitment.[9–11] *In vivo* release of 7ND from gene eluting stents was shown to attenuate stenosis in rabbits and monkeys by effectively reducing inflammatory cell recruitment.[12] These results suggest that mutant MCP-1 proteins such as 7ND may be

used as a promising decoy drug to block the receptor, thereby inhibiting the undesirable monocyte/macrophage recruitment.

To modulate inflammatory responses at the implant site and minimize peri-prosthetic bone loss, it would be highly desirable to deliver 7ND locally to the peri-prosthetic space. A bolus delivery of 7ND at the time of implantation may inhibit the early stage recruitment of monocytes/macrophages, however, controlled release of 7ND would be preferred to achieve a prolonged anti-inflammatory response. To achieve local delivery of 7ND, one promising strategy is to coat the protein onto the surface of the implant. Layer-by-layer (LBL) deposition provides an effective and facile method for loading biological molecules onto irregularly shaped material surfaces, and allows higher amounts of drug loading and more controlled release than surface adsorption. The LBL process involves sequential deposition of positively and negatively charged polyelectrolytes on the implant surface. Charged biomolecules can be substituted in place of a polyelectrolyte layer at any time throughout the layering process. The process can be repeated multiple times until the desired amounts of biomolecules have been loaded. However, most previously reported LBL platforms require lengthy coating process and the use of a large number layers to avoid burst release. For example, a 48-hr LBL coating process was required to achieve sufficient BMP-2 loading on the surface of PCL scaffolds for prolonged release and enhanced bone formation *in vivo*. [13] To decrease the initial burst release from a titanium surface, methods have been developed to add a large number of layers followed by chemical crosslinking of free amines within the LBL assembly [14]. However, this multi-step process is lengthy and requires incubation at low pH (pH 5.5) for up to 12 hours, which may result in the loss of biological activity of the drugs.

To overcome the above limitations, we have recently reported a LBL platform that allows controlled protein release from polymeric scaffolds over a few weeks using a small number of layers that is an order of magnitude less than conventional methods. [15] We also found that protein loading and release kinetics can be optimized by varying chemical structure of the positively charged polyelectrolytes. [15] Based on these observations, we hypothesize that our recently reported LBL platform may be applied to achieve multilayered coating on orthopaedic implants, for local delivery of 7ND proteins to decrease monocyte migration towards MCP1, thereby decreasing wear-particle initiated inflammatory responses (Figure 1). We further hypothesize that the release kinetics of 7ND can be modulated by varying the structures of polyelectrolytes used in LBL assembly. To test our hypotheses, we synthesized a small library of cationic polyelectrolytes with diverse chemical structures for constructing LBL assemblies. We then examined the effects of varying polymer structure and order of deposition on protein loading and release. Scanning electron microscopy was used to visualize LBL coating on the surface of titanium rods over time. Using an optimized layering strategy, we then coated 7ND onto the surface of titanium rods and evaluated the effects of cationic polyelectrolyte structure on 7ND release kinetics. The bioactivity of released 7ND from LBL coating was examined by quantifying the migration of THP1, a human monocytic cell line, using a transwell assay. Finally, we evaluated the LBL stability during a femoral implantation procedure by press fitting the coated titanium rods into the

distal femur, and compared the surface structures pre- and post-implantation using SEM and fluorescence microscopy.

2. Materials and Methods

2.1. Poly(β -amino)ester Synthesis

End-modified poly(β -amino ester)s (PBAEs) were synthesized as previously described.[16] Briefly, a diacrylate monomer (C or D) (Sigma Aldrich, St. Louis, MO) was reacted with 5-amino-pentanol (32) (Sigma Aldrich) through a Michael addition reaction at 90 °C (Figure 2A). Both acrylate-terminated PBAEs were subsequently modified with four different amine end groups (Figure 2B) using molecules as shown in Figure 2C. Following an overnight reaction at room temperature, amine-terminated PBAEs were extracted in anhydrous diethyl ether (Fisher Scientific, San Jose, CA), dissolved in anhydrous DMSO (Fisher Scientific) and stored at -20 °C until use.

2.2. Preparation of polyelectrolyte solutions

The positively charged polyelectrolyte, PBAE, was dissolved in phosphate buffered saline (PBS) (Life Technologies, Grand Island, NY) or sodium acetate (25 mM) (Fisher Scientific) to reach a concentration of 10 mg/ml. Polystyrene sulfonate (PSS) (Sigma Aldrich) or chondroitin sulfate (CS) (Sigma Aldrich) were selected as the negatively charged polyelectrolytes and dissolved in H₂O or sodium acetate (25 mM) to reach 3 mg/ml. 7ND protein was dissolved in H₂O containing bovine serum albumin (1 mg/ml) (Fisher Scientific) or sodium acetate (25 mM) to a final concentration of 30 μ g/ml. BSA acts as a carrier protein for 7ND during the deposition process.

2.3. Combinatorial screening for the choice of polyelectrolytes on minimizing burst release from LBL coating

To examine the effects of varying negatively charged polyelectrolytes, both chondroitin sulfate (CS) (Groups 1–4) and polystyrene sulfonate (PSS) (Groups 5–8) were used as the negatively charged polyelectrolytes throughout the layering process for binding 7ND. Within each subset of groups, 4 PBAEs with different functional end-group chemistry were used as the positively charged polyelectrolytes including C32-130, C32-135, C32-140, C32-117. Alternatively, 7ND was bound directly to the positively charged PBAEs with various end-group chemistry (Groups 9–12 and 13–16), and PSS was used as the negatively charged layer for constructing the LBL assembly. Previous studies have demonstrated efficient deposition and controlled release using low pH due to an increase in polyelectrolyte charge density. To further examine the effects of varying pH, LBL coating was performed at pH5 (Groups 9–12) by dissolving polyelectrolytes and 7ND in 25 mM sodium acetate or neutral pH solution (Groups 13–16). For groups 13–16, 7ND was dissolved in BSA solution of 0.1% (w/v) (Groups 9–16). Detailed procedure for LBL assembly is described below.

2.4. Layer-by-layer coating of 7ND on titanium rods

Hollow A-40 titanium rods (6 mm, 21 G) were purchased from New England Small Tube, Litchfield, NH. 7ND recombinant protein is a mutant MCP-1 protein which lacks the N-terminal amino acids 2 through 8, and has been shown to function as a dominant negative

inhibitor of MCP-1.[9, 17] The 7ND in the current study was kindly provided by Dr. Egashira at Kyushu University, Japan. Titanium rods were surface etched first in acid solution to aid layer-by-layer (LBL) attachment. Specifically, titanium rods were placed in concentrated hydrochloric acid solution (37.3% w/v, Fisher Scientific) for 2 hours. The rods were then washed extensively with H₂O and rinsed 3 times in 70% ethanol (Fisher Scientific) for a total of 15 mins. The rods were air dried before LBL deposition as illustrated in Figure 3A, with precursor layers on the bottom and biological layers containing 7ND on the top. Each layer was deposited for 5 mins, with the exception of 7ND layers, which were deposited for 10 mins. The rods were washed twice in H₂O between every layer with 30 secs per wash. To measure the protein release from the LBL coating, coated rods were immediately placed in 150 µl of PBS containing 0.05% (w/v) BSA and supernatant was collected periodically, and replaced with fresh PBS/BSA. BSA was included in the collection solution to prevent 7ND adhesion to the tissue culture plastic. Collected supernatant from various time points was stored at -80 °C until time of analysis. Given the similarities in structure between 7ND and MCP-1, released 7ND was quantified using Enzyme Linked Immunosorbent Assay (ELISA) for MCP-1 (Peprotech, Rocky Hill, New Jersey). The accumulative release of 7ND was monitored up to 1 week.

2.5. Characterizing the effects of LBL coating on implant surface morphology

To examine the morphology changes on implant surface during the coating process, titanium rods were characterized before and after the LBL coating using scanning electron microscopy (SEM) and fluorescence microscopy. Rods were surface coated with 50 Angstroms of palladium before imaging. Micrographs were obtained using a SEM (Sigma, Zeiss, Oberkochen, Germany). Immunofluorescent imaging was also performed to allow direct visualization of 7ND deposition on the titanium rod. An MCP-1 protein detection kit was used as MCP-1 antibodies are cross-reactive with 7ND. Rods were incubated in 150 µl of a biotinylated rabbit anti-hMCP-1 (Peprotech) diluted 1:120 in diluent (PBS + 0.05% Tween-20 + 0.1% BSA) for two hours. Detection antibody solution was then removed from the wells, and rods were incubated in 150 µl of Avidin-FITC conjugate (Life Technologies) diluted 1:200 in diluent for 45 minutes. After incubation, rods were washed in PBS containing 0.05% (w/v) Tween-20, allowed to dry, and then imaged under fluorescence microscopy (Axio Observed 3.1, Zeiss).

2.6. Cell Culture

THP-1 cells (Human acute monocytic leukemia cell line) (Cat #: TIB-202, ATCC, Manassas, VA) were cultured in Dulbecco's Modified Eagles Media supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin (all from Life Technologies) and 0.05 mM β-mercaptoethanol (Fisher Scientific). Cells were maintained at 37 °C in a humidified atmosphere of 5% carbon dioxide.

2.7. Chemotaxis Assay

To determine if released supernatant remained bioactive, a cell chemotaxis assay was performed. Released supernatant from each rod at day 1 (55 µl/rod) was lyophilized overnight, and then resuspended in 55 µl of fully supplemented THP-1 cell culture medium containing 6.0×10^4 cells and incubated for 1 hour. Chemotaxis assays were performed using

a ChemoTx® Disposable Chemotaxis System (NeuroProbe, Gaithersburg, MD) containing 8 μm pores. The inflammatory cytokine (10 ng/mL MCP-1) was placed in the bottom chamber while 55 μL of cells/supernatant were placed in the upper chamber. A linear dilution of THP-1 cells was also placed in the bottom chamber which served as a means to quantify the number of migrated cells. Following a 90-min incubation period, cells in the upper chamber were removed by aspiration and the porous membrane was removed. Cells in the bottom chamber were transferred to a 96 well plate. Each well of the bottom chamber was washed with 30 μL H_2O which was transferred to the respective wells in the 96-well plate. The plate was transferred to a -80°C freezer for 2 hours and returned to room temperature until fully thawed. The freeze-thaw cycle was repeated 2 more times. To quantify the number of migrated cells, 100 μL of PicoGreen (Invitrogen) dye was added to each well and fluorescence was read at 480/520 nm using a plate reader (Spectramax M2e, Molecular Devices, Sunnyvale, CA). DNA content was converted to cell number using the linear dilution of THP-1 cells.

2.8. Evaluating the stability of LBL coating during a femoral implantation procedure

Fresh cadavers of six 7–8 weeks old C57Bl/6 male/female mice used for colony maintenance were obtained from Stanford animal facility with institutions approval. The patellar tendon was exposed through a 5 mm skin incision and the intercondylar notch of the distal femur was accessed through a medial parapatellar arthrotomy. A series of needles (25–21 gauge) was then used to manually drill through the intercondylar notch to gain access to the medullary cavity. LBL coated titanium rods were fluorescently labeled as described above prior to implantation. A coated and fluorescence labeled titanium rod connected from one end to vinyl tubing, was then press fit into the medullary canal of the distal femur through the drill hole. To minimize the artificial damage to the coating, the femur with the rod still in place was dissected free of any soft tissues, detached from the hip and knee joints and split open by superficial, longitudinal incision. The rod was then lifted off from the remaining bone bed by holding it by the vinyl tubing. Titanium rods were imaged pre- and post- implantation via SEM and fluorescent microscopy as described above. Analysis of fluorescence was accomplished using NIH ImageJ software by quantifying density of brightness for each rod. The background brightness density was subtracted from the rod brightness density to determine the corrected total fluorescence (CTF).

2.9. Statistical analysis

Minitab™ (Minitab Inc., USA) software was used for statistical analysis. One-way analysis of variance (ANOVA) with a Tukey's post-hoc analysis was used to determine statistical significance between groups while a paired T-test was used to directly compare two groups. A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Constructing 7ND-containing LBL assembly using PBAE

PBAEs were synthesized by a 2-step Michael addition reaction, in which various amine end capping groups were conjugated to hydrolytically degradable backbone polymers (Figure 2). The resulting PBAE polymers were positively charged and used as the cationic layers for

LBL assembly. PBAEs have been used for LBL assembly for the controlled release of DNA, [18] in which DNA is negatively charged and PBAE serves as the cationic layers during LBL assembly. Proteins can be either positively or negatively charged depending on the pKa of the proteins of interest, therefore protein has been used both as the anionic or cationic layer during LBL assembly.[19] MCP-1 has a pKa of 9.81, therefore it is expected to be positively-charged at neutral pH, and 7ND is expected have a similar pKa given its structural similarity to MCP-1.

3.2. The choice of polyelectrolytes modulates the initial burst release of 7ND from LBL coating

LBL deposition and release can be influenced by a number of factors including polyelectrolyte structure, layering order and pH. One of greatest challenges in applying LBL coating for drug release is the initial burst release of biomolecules, and prior LBL design often required the use of a few hundred layers to prevent the initial burst release.[13, 20–21] To identify a LBL coating design using a few layers while minimizing the initial burst release from LBL coating, we performed a combinatorial screening study and constructed 16 groups of LBL coatings by varying polyelectrolytes of choice for binding 7ND protein, the order of deposition, and pH (Figure 3A). We found that polystyrene sulfonate (PSS) resulted in much higher loading efficiency of 7ND than chondroitin sulfate, as total amount of 7ND released from PSS-based LBL coatings were two orders of magnitude higher than the total 7ND released from CS-based LBL coatings (Figure S1). When 7ND was bound to the anionic layers using CS or PSS, all groups (groups 1–8) resulted a rapid initial burst release within 1 hour. (Figure 3B and Figure S1) This may in part explain the high number of layers required when using the anionic layer as the protein binding layers in previous studies.[13, 20–21] When 7ND was bound to cationic layers using PBAE polymers (Groups 9–16), loading efficiency was further increased as shown by the total amount of released 7ND protein (Figure S1). LBL coatings constructed at pH5 generally resulted in a burst release (groups 9–12), whereas LBL coating constructed at neutral pH using BSA-containing 7ND proteins overcame the rapid burst and extended release beyond 48 hours. The presence of BSA resulted in an overall negative charge in the 7ND protein solution, which we speculate to enhance the binding between the 7ND protein layer and the underlying positively charged PBAE layer. The presence of BSA will also preserve the concentration and structure of 7ND during the loading process by preventing adhesion to the surrounding well. Using C32-130 and C32-135 as the cationic polyelectrolytes resulted in a gradual release from LBL coatings within the first 70 hours. C32-130 also led to higher amount of total 7ND release and was chosen for further experiments.

3.3. Microscopy imaging confirmed effective 7ND loading using LBL coating

Successful 7ND loading via LBL deposition was confirmed by SEM and fluorescent microscopy. Un-coated rods have an un-even surface with pits resulting from etching in hydrochloric acid, which was applied to decontaminate the implant surface and increase surface area for polyelectrolyte binding (Figure 4A and 4C). Following the LBL coating, the etched pits were largely filled with the protein coating, creating a smooth appearance on the surface of the implants (Figure 4B and 4D). As the protein coating mainly resides within the pits, it may also help minimize delamination of the coating during the press-fit implantation

procedure. The presence of 7ND within the coating was also confirmed through immunolabeling, which showed uniform coating of 7ND across the titanium rod (Figure 4F), and minimal background signal can be observed prior to the coating (Figure 4E).

3.4. Gradual 7ND release can be controlled by varying PBAE chemistry

To further modulate prolonged release of 7ND over time, we synthesized two PBAE polymers terminated with the optimal end group (130) with different degradation rate (C and D). Polymer C contains 4 consecutive carbons in the backbone while polymer D contains 6 consecutive carbons, hence polymer D has increased hydrophobicity and is expected to degrade more slowly. We expect slower degradation would lead to more stable LBL assembly, thereby prolonged protein release over time. This was confirmed by the accumulated 7ND protein release data (Figure 5), in which C32-130 released protein over a 2 day period while D32-130 continued to release protein up to day 7. D32-130 also led to 6-fold higher amount of total released 7ND protein compared to C32-130 (Figure 5). Previous LBL studies have demonstrated controlled protein release over a similar time frame, however over 100 layers were required to achieve the delayed release [20]. In the current study, we achieved controlled release of proteins using only 14 layers in total, with 4 layers containing 7ND. The rate of protein diffusion may be delayed by increasing the number of layers applied to the implant surface which will also result in a higher amount of 7ND loading. Likewise, the concentration of 7ND released from the implant surface may be increased by using a higher concentration of 7ND during the layering process.[15] In the current study, we have used a concentration of 30 µg/ml 7ND, while previous studies have used growth factor concentrations up to 75 µg/ml for.[15] Interestingly, D32-130 released significantly more 7ND than C32-130. The results suggest that D32-130 has a greater affinity for 7ND or perhaps D32-130 enhances growth rate of the LBL assembly. As each layer is influenced by the charge and homogeneity of the underlying layer, it is possible that D32-130 encourages deposition of the proceeding layer, hence increasing the final concentration of 7ND on the coated implant surface.

3.5. Degradation of LBL coating on implant surface

Previous LBL studies have suggested that biomolecule release from LBL assembly is influenced by both diffusion and polymer degradation.[22] To monitor the degradation of LBL coating over time, SEM analysis was performed on titanium rods coated using D32-130 for up to 21 days. (Figure 6) Uncoated rods showed etched pits with clearly defined edges, which was covered by a smoother coating after the LBL assembly. SEM showed minimal degradation of the coating on the implant surface over the first 7 days, which suggest that diffusion is likely to be the primary mechanism by which 7ND gets released from the coating. The coating remains visible until day 15, and titanium substrate became visible at day 21. Therefore protein release over 1 week is likely to be modulated by both diffusion and layer degradation. As the goal of the present study is to develop an anti-inflammatory coating, the prolonged presence of non-degradable polymers would be less desirable. The PBAE polymers used in our study are hydrolytically degradable, which facilitates the clinical translation of the platform. It should be noted that polymer concentration in the peri-implant space would be minimal as the LBL coatings are generally nanoscale.[23–24]

3.6. Released 7ND retained its bioactivity to inhibit THP-1 cell migration

In our study, LBL deposition was performed in aqueous conditions and neutral pH to facilitate the retention of protein bioactivity. We have previously shown that fibroblast growth factor retains its activity when released from a similar LBL assembly.[15] To confirm that released 7ND protein retained its biological activity, we examined the ability of released 7ND to inhibit THP-1 cell migration towards MCP-1 using a trans-well migration assay. When no MCP-1 is applied and no 7ND is present, minimal cell migration was observed. MCP-1 induced substantial THP-1 cell migration, and supernatant from LBL coatings that do not contain 7ND (PBS/BSA) did not reduce cell migration. In contrast, supernatant from titanium rods coated with 7ND using polymers C32-130 or D32-130 substantially reduced THP-1 cell migration towards MCP-1 to a level that is comparable to fresh 7ND (Figure 7). These results confirm that 7ND released from the current LBL platform retained its biological activity to modulate inflammatory cell migration.

3.7. The LBL coating remained stable after femoral implantation procedure

Most orthopaedic implants are designed as press fit implants, therefore one prerequisite for an effective biological coating is to remain stable and overcome de-lamination through such forceful procedure. Delamination is common with techniques such as plasma spraying as the coating can be easily detached during the abrasive implantation procedure, although recent advances have improved bonding strength between implant and coating.[25] To examine the stability of our LBL coating, we implanted titanium rods coated with fluorescently-labeled 7ND into the medullary canal of a mouse femur. Fluorescent microscopy and SEM were employed to characterize the implant surface before and after implantation (Figure 8). Fluorescence imaging showed strong fluorescence on the rod surface both before and after the implantation procedure, with no noticeable difference in fluorescence intensity. SEM analysis also confirmed the presence of LBL coating on the titanium rods, with comparable morphology observed before and after implantation. Quantitative analysis of the fluorescent intensity on the coating also confirmed the implantation procedure did not result in a statistically different level of 7ND protein on the rod surface, suggesting that stability of the LBL platform reported here can meet the demands of orthopaedic implantation procedure and withstand the abrasive forces during the press-fit procedure. It should be noted however that these results are based on the forces required to press fit the implant into a mouse femur. Scale up studies are required to determine if the coating will withstand the forces during TJR in humans.

4. Conclusion

In summary, here we report the development of a LBL-based, biodegradable platform for introducing biological coatings to orthopaedic implants to modulate inflammatory responses to wear particles. Our results showed that protein loading and release can be modulated by varying the polyelectrolytes of choice, the polymer chemistry, order of deposition, and pH. Our lead condition resulted in efficient 7ND loading and prolonged release of 7ND for at least 1 week, and released 7ND retained its biological activity to inhibit macrophage migration towards MCP-1. Finally, the LBL coating demonstrated great stability and remained comparable before and after the femoral implantation procedure. Together, our

results suggest the LBL platform reported herein as a promising strategy for introducing biological coatings to various orthopaedic implants, and prolonged 7ND delivery from such coatings may be used to modulate anti-inflammatory responses to prolong the lifetime of current orthopaedic implants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the Stanford Bio-X interdisciplinary initiative award and the National Institute of Health (2R01AR055650-05). We would also like to acknowledge the Ellenburg Chair in Surgery at Stanford University for funding.

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Figure 1.

Schematic demonstrating the design concept of the study, with controlled release of 7ND protein from layer-by-layer (LBL) coating on orthopaedic implants to modulate inflammation. The released 7ND is designed to bind the MCP-1 receptor located on macrophages without activating the pathway, hence 7ND acts as an antagonist for MCP-1 to inhibit inflammation.

Figure 2.

Synthesis schemes of poly(β -amino esters) (PBAE)s with different backbone or end-group chemistry via a 2-step Michael addition reaction. (A) Acrylated monomers (C or D) were reacted with amine-terminated monomer (32) to first produce acrylate-terminated PBAE (X-32-Ac) (X represents either C or D). (B) The acrylated terminated PBAEs were further modified to add amine end groups. (C). Structures of four different amine molecules used for end-capping of PBAEs.

Figure 3.

A combinatorial study to examine the effects of varying polyelectrolyte structure and deposition order on preventing burst release of 7ND from the LBL coating. (A) Schematic design of a total of 16 groups were screened with varying polycation structures and order of polyelectrolyte deposition. The precursor layers were shown on the bottom, and biological layers containing 7ND on the top. All LBL deposition were performed at pH7, with the exception of groups 9–12, in which polycations were dissolved in sodium acetate and performed at pH5. 7ND was dissolved in H₂O for all groups, with the exception of groups 13–16 where 7ND was suspended in 0.1% (w/v) BSA solution. (B) Percentage of 7ND released within the first 24 hrs showed burst release in most groups. Groups 1 and 2 (using C32-130 or C32-135 throughout layers at pH7) were identified as optimal combinations with no burst release. CS: chondroitin sulfate, PSS: Polystyrene sulfonate.

Figure 4.

Characterization of surface morphology of titanium rods before and after LBL coating using scanning electron microscopy and fluorescence imaging. (A, B) SEM images demonstrating the surface of the etched titanium rod before and after LBL deposition. (C, D) Higher magnification of SEM images showed LBL coating resulted in a smooth finish on the surface. (E, F) Fluorescence imaging Immunostaining was performed to confirm the presence of 7ND. Fluorescent images of un-coated (E) and coated (F) rods are shown.

Figure 5.

Release of 7ND as determined by ELISA. LBL was performed using 4 different cationic polymers (C32-130, C32-135, D32-130, D32-135) and release was performed in PBS containing 0.05% BSA at 37°C. Data is presented as mean $\pm$ standard deviation. * indicates statistical difference between groups ($p < 0.05$).

Figure 6.
SEM micrographs of LBL coating degradation. Titanium rods coated with a LBL process using D32-135, were imaged at various time points over a 3 week incubation in PBS containing 0.05% BSA at 37°C. Scale bar = 3 μm

Figure 7.

Effects of released 7ND from LBL coatings (using C32-132 or D32-130) on inhibiting migration of THP-1 cells towards MCP-1 (10 ng/ml). PBS/BSA or coatings contain no 7ND were included showed no inhibitory effects on THP-1 cell migration. Supernatant from 7ND-containing LBL coatings demonstrated comparable level of inhibitory effects on THP-1 cell migration as freshly prepared 7ND solution (+ ctrl). Solution containing no 7ND and no MCP-1 induced only baseline level of THP-1 migration (– ctrl). Data is presented as mean $\pm$ standard deviation. Bars with similar letters are not statistically different from each other ($p < 0.05$).

Figure 8.

Effects of implantation procedure on the stability of LBL coating, shown by fluorescence imaging and SEM. Fluorescently-labeled 7ND Immunolabeling was performed prior to implantation (A) and re-imaged post implantation (B). SEM images were also obtained to confirm results obtained from immunolabeling. (C and D) Average fluorescent signal across the surface of 6 rods pre and post implantation was quantified to confirm coating stability (E). Data is presented as mean $\pm$ standard deviation.