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Tunable staged release of therapeutics from layer-by-layer coatings with clay interlayer barrier

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ABSTRACT

In developing new generations of coatings for medical devices and tissue engineering scaffolds, there is a need for thin coatings that provide controlled sequential release of multiple therapeutics while providing a tunable approach to time dependence and the potential for sequential or staged release. Herein, we demonstrate the ability to develop a self-assembled, polymer-based conformal coating, built by using a water-based layer-by-layer (LbL) approach, as a *dual-purpose* biomimetic implant surface that provides staggered and/or sustained release of an antibiotic followed by active growth factor for orthopedic implant applications. This multilayered coating consists of two parts: a base osteoinductive component containing bone morphogenetic protein-2 (rhBMP-2) beneath an antibacterial component containing gentamicin (GS). For the fabrication of truly stratified composite films with the customized release behavior, we present a new strategy—implementation of laponite clay barriers—that allows for a physical separation of the two components by controlling interlayer diffusion. The clay barriers in a single-component GS system effectively block diffusion-based release, leading to approximately 50% reduction in bolus doses and 10-fold increase in the release timescale. In a *dual-therapeutic* composite coating, the top GS component itself was found to be an effective physical barrier for the underlying rhBMP-2, leading to an order of magnitude increase in the release timescale compared to the single-component rhBMP-2 system. The introduction of a laponite interlayer barrier further enhanced the temporal separation between release of the two drugs, resulting in a more physiologically appropriate dosing of rhBMP-2. Both therapeutics released from the composite coating retained their efficacy over their established release timeframes. This new platform for multi-drug localized delivery can be easily fabricated, tuned, and translated to a variety of implant applications where control over spatial and temporal release profiles of multiple drugs is desired.

1. Introduction

Recently, the concept of generating multi-component delivery systems that provide localized release of multiple therapeutics over appropriate timescales and with precise doses has been of great interest for many drug delivery and tissue engineering applications [1–3]. In particular, there is a need for a multi-agent delivery thin film platform that can conformally coat complex implant, scaffold and device surfaces and release a range of different kinds of drugs, with independent control of order, timing, and rate of release. Despite the promise of multi-component delivery, the ability to generate a multi-component system with highly tailored release profiles has remained a challenge due to the lack of materials and methods that enable incorporation of a range of sensitive biologic drugs while preserving their activity *and* provide spatial and temporal control over the release of the therapeutics. The layer-by-layer assembly (LbL) technique—a method involving the alternate adsorption of oppositely charged polymers—is one of the most suitable methods for generating multi-component coatings due to its simplicity, ease of application, and water-based assembly [4]. Its conformal nature provides the flexibility to incorporate a broad range of biomaterials, including those with nonplanar complex geometries and large surface area such as microneedles [5] and nanoparticles [6,7]. LbL assembly holds significant promise in the ability to easily tune the loading of materials and control the order and location of multiple layers with nano-scale precision [1,8], and this promise is furthered by recent demonstrations that LbL films provide controlled and tunable release of therapeutics from surfaces [9–11].

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A rapidly expanding area in regenerative medicine and tissue engineering is the development of biomimetic surface coatings on orthopedic implants that can accelerate the bone healing process while preventing infection. Millions of orthopedic implants are performed annually, with bone implant integration being a common clinical issue. However, due to surgical and implant-related complications, approximately 12% of patients have to receive revision replacements within 10 years after surgery [12]. Among the primary reasons for joint failure, implant-related infections create complications for patients and cost close to $2 billion in annual treatment. For this reason, prevention or elimination of infection following a revision operation is key for successful patient recovery. Today's gold standard for treatment of implant-associated infection is two-stage re-implantation, which involves six weeks of antibiotic therapy before introduction of the new implant, and two surgeries. Although relatively effective at eradicating infection, this treatment method has several drawbacks including long periods of hospitalization, morbidity, requirement of a second surgery for removal of the antibiotic beads or spacer, and sometimes increased mortality [13]. Therefore, there is a strong need for a single-stage re-implantation such as a drug–device combination system, which can treat bacterial infection as new bone is generated at the interface of the implant. Recent studies have demonstrated that co-administration of an antibiotic and a growth factor has potential beneficial effects and thus results in more favorable clinical outcomes such as increased bone formation, compared to single administration of the individual antibiotic and growth factor controls [14,15]. A dual-purpose system with customized release behavior can reduce the incidence of implant failure due to post-operative infection and mechanical loosening *in situ* [14,16].

In previous work, we have demonstrated that antibiotics can be released from LbL coated implant surfaces to address infection in a rabbit model [17]; furthermore, we have independently shown the power of single and dual growth factor LbL films to modulate the integration of bone on implant surfaces, and to yield dense and highly vascularized bone in 3D scaffolds in rats [18–21]. Given the advantages of multi-component delivery and the LbL assembly technique, attempting to develop a multi-agent LbL film is a natural next step. Recent efforts have been directed at developing truly stratified LbL films, but unfortunately, many such approaches have been unsuccessful because of interlayer diffusion, a phenomenon that leads to mixing and sometimes exchange of film components during assembly [22,23]. To block interlayer diffusion in the LbL films, we and other groups have investigated a range of methods and materials including polymer barrier layers [24–27] and graphene oxide [9]. Despite the many promising achievements, the aforementioned approaches still present some limitations for certain drug delivery and tissue engineering applications; some covalent chemistries are incompatible with biologic drugs, and newer nanomaterial components such as graphene oxide [28] are still under investigation with regard to their safety as biomaterials.

Laponite clay, a disk-shaped synthetic silicate $Na^{+}_{0.7}[Si_8Mg_{5.5}Li_{0.3}H_4O_{24}]^{-}_{0.7}$ with dimensions of 25 nm in diameter and 0.92 nm in thickness, is readily available, low-cost and is generally regarded as safe (GRAS) by the FDA as a natural clay product; the nanomaterial also exhibits some favorable bioactive properties [29]. Recent studies have demonstrated that laponite can induce osteogenic differentiation of stem cells and develop microenvironments that support tissue regeneration [30,31]. In the area of drug delivery, laponite nanoplatelets have been utilized to modulate release properties because of their intercalation capacity [32–35]. Also, laponite and montmorillonite clays have been used in varying amounts as components of LbL films to enhance their mechanical properties by increasing modulus and durability [36,37]. To this end, laponite clay was considered as a most appropriate two-dimensional barrier material that can physically block interlayer diffusion and sustain release of loaded drugs.

In this study, the primary goal was to develop a multi-agent delivery thin film LbL platform with controlled local release of an antibiotic, gentamicin sulfate, and an osteoinductive growth factor, rhBMP-2, in a manner that is biologically relevant and leads to increased efficacy. Orthopedic implant surfaces modified using this multi-drug LbL coating can fulfill the need for controlled delivery of multiple therapeutic agents for healing bone defects, inducing osteointegration on the implant surface while preventing infection at the implant site. A suitable multi-drug delivery platform would exhibit a rapid release of an antibiotic for the first few days, followed by a sustained release for multiple weeks along with a controlled release of a growth factor. In this article, we fabricated a series combination of an rhBMP-2 film component and a GS component in multilayer films with and without laponite barrier layers with the aim of demonstrating the laponite clay barrier interlayer as an effective means of modulating release. We hypothesize that such an approach can provide a means to achieve this kind of customized delivery behavior, with staggered release of antibiotic followed by active growth factor. To evaluate the bioactivity of the films, the efficacy of both components over their established release timeframes was assessed *in vitro*.

2. Materials and methods

2.1. Materials

Poly(β-amino esters), Poly1 (M_n ~ 10 kDa) and Poly2 (M_n ~ 11 kDa), were synthesized as previously described [38]. Poly(acrylic acid) (PAA, M_w ~ 450 kDa and 1.25 MDa), Chitosan (Chi, M_v ~ 110–150 kDa) poly(diallyldimethylammonium chloride) (PDAC, M_w ~ 200–300 kDa), 3 M sodium acetate buffer (NaOAc, pH 5.2), as well as solvents and common buffers, were purchased from Sigma–Aldrich (St. Louis, MO). Laponite was purchased from Southern Clay Products (Gonzales, TX). Recombinant human BMP-2 (rhBMP-2) was a gift from Pfizer Inc. (Cambridge, MA). Non-radiolabeled gentamicin sulfate (GS) was purchased from Mediatech, Inc. (Herndon, VA), and radiolabeled gentamicin ^{3}H-GS (250 μCi total, 1 mCi/mL in ethanol, 200 μCi/mg) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Silicon wafers were purchased from Silicon Quest International (Santa Clara, CA). All materials and solvents were used as received without further purification.

Staphylococcus aureus UAMS-1 (ATCC 49230) and MC3T3-E1 subclone 4, a mouse preosteoblasts cell, were purchased from ATCC (Manassas, VA). Cation-adjusted Mueller Hinton broth (CaMHB), Bacto agar, and gentamicin standard disks were purchased from BD Biosciences (San Jose, CA). Alpha minimum essential medium (α-MEM), fetal bovine serum (FBS), trypsin-EDTA, and phosphorate buffered saline (PBS) were purchased from Invitrogen (Carlsbad, CA).

2.2. Preparation of polyelectrolyte solutions

For GS component, dipping solutions of poly1 and PAA (M_w ~ 1.25 MDa) were prepared at 2 mg/mL in 100 mM sodium acetate buffer and pH adjusted to 5.0. The dipping solution of GS was at 10 mg/mL in 100 mM sodium acetate buffer. For *in vitro* release studies, a small amount of ^{3}H-GS was added to the 10 mg/mL GS solution to yield the end product of 0.5 μCi/mL; the molar ratio of ^{3}H-GS to regular GS was 1/4000. For the rhBMP-2 component, dipping solutions of poly2 and PAA (M_w ~ 450 kDa) were prepared at 1 mg/mL in 100 mM sodium acetate buffer and pH adjusted to 4.0. The dipping solution of rhBMP-2 was at 40 μg/mL in 100 mM sodium acetate buffer.

2.3. Layer-by-layer film formation

Silicon substrates with dimensions of 0.5 × 2.0 cm^2 were used for all *in vitro* experiments. In all cases, substrates were rinsed with methanol and ultra-pure water, dried under nitrogen, and plasma etched in oxygen at high RF power for 90 s using a Harrick PDC-32G plasma cleaner. The cleaned silicon substrates were immediately immersed in the first cationic solution. First, tetralayer films were fabricated at room temperature using an automated dipping robot (Carl Zeiss HMS Series Programmable Slide Stainer) by alternate dipping in a solution of cationic species for 5 min followed by two consecutive rinse steps in 100 mM sodium acetate baths for 30 and 60 s, and then into anionic species for 5 min followed by the same rinse cycle. The entire cycle was repeated until the desired number of tetralayers was deposited. Following the film deposition, the films were allowed to dry and then stored at 4 °C prior to subsequent analysis.