Building biomedical materials layer-by-layer

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In this materials perspective, the promise of water based layer-by-layer (LbL) assembly as a means of generating drug-releasing surfaces for biomedical applications, from small molecule therapeutics to biologic drugs and nucleic acids, is examined. Specific advantages of the use of LbL assembly versus traditional polymeric blend encapsulation are discussed. Examples are provided to present potential new directions. Translational opportunities are discussed to examine the impact and potential for true biomedical translation using rapid assembly methods, and applications are discussed with high need and medical return.

Paula T. Hammond

David H. Koch Chair Professor in Engineering, Department of Chemical Engineering, Koch Institute of Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

E-mail: hammond@mit.edu

One of the most rapidly growing means of generating thin films in the field of materials science has been the alternating adsorption based layer-by-layer (LbL) assembly method. This method is based on the simple alternating adsorption of complementary multivalent species on a substrate via electrostatic interactions, hydrogen bonding, or other secondary interactions[1-3]. LbL assembly has particular potential in the area of biomaterials[4-9] because it enables nanometer level control of the composition of a thin film, and the generation of highly complex, tailor-made coating compositions[10,11]; however, unlike other molecular level deposition techniques, thin films are constructed in water at room temperature, preserving the activity of sensitive proteins, nucleic acids, and other functional biomacromolecules. It has been demonstrated that LbL multilayers can beautifully and conformally coat structures as small as 10 nm diameter gold nanoparticles[12,13], and surfaces with nanoscale complexity, and yet can also be used to coat large scale macroscopic three-dimensional objects. Finally, multilayer thin films can be designed to exhibit high levels of biocompatibility[14-18] both *in vitro* and *in vivo*, and can be used to incorporate both large and small molecules, as well as a number of organic and inorganic nanomaterials. A particularly unique aspect of this approach is the ability to build drugs in separate sets of layers, with the potential for sequential delivery[19,20]. These properties enable us to explore LbL as a means of achieving controlled drug release from thin films and coatings with high drug density (Fig. 1).

Several common paradigms of traditional polymer encapsulation are shifted when we examine the enabling approach of alternating assembly. It becomes possible to consider the incorporation of very different types of drug molecules at high loadings without the same issues of stability that are problematic to polymer blends. The potential for co-release of two or more drugs with different release profiles, or even time-dependent staged delivery becomes plausible through the use of top-down compartmentalized regions of LbL thin films[19-21]. Finally, several different modes of release can be introduced using this versatile

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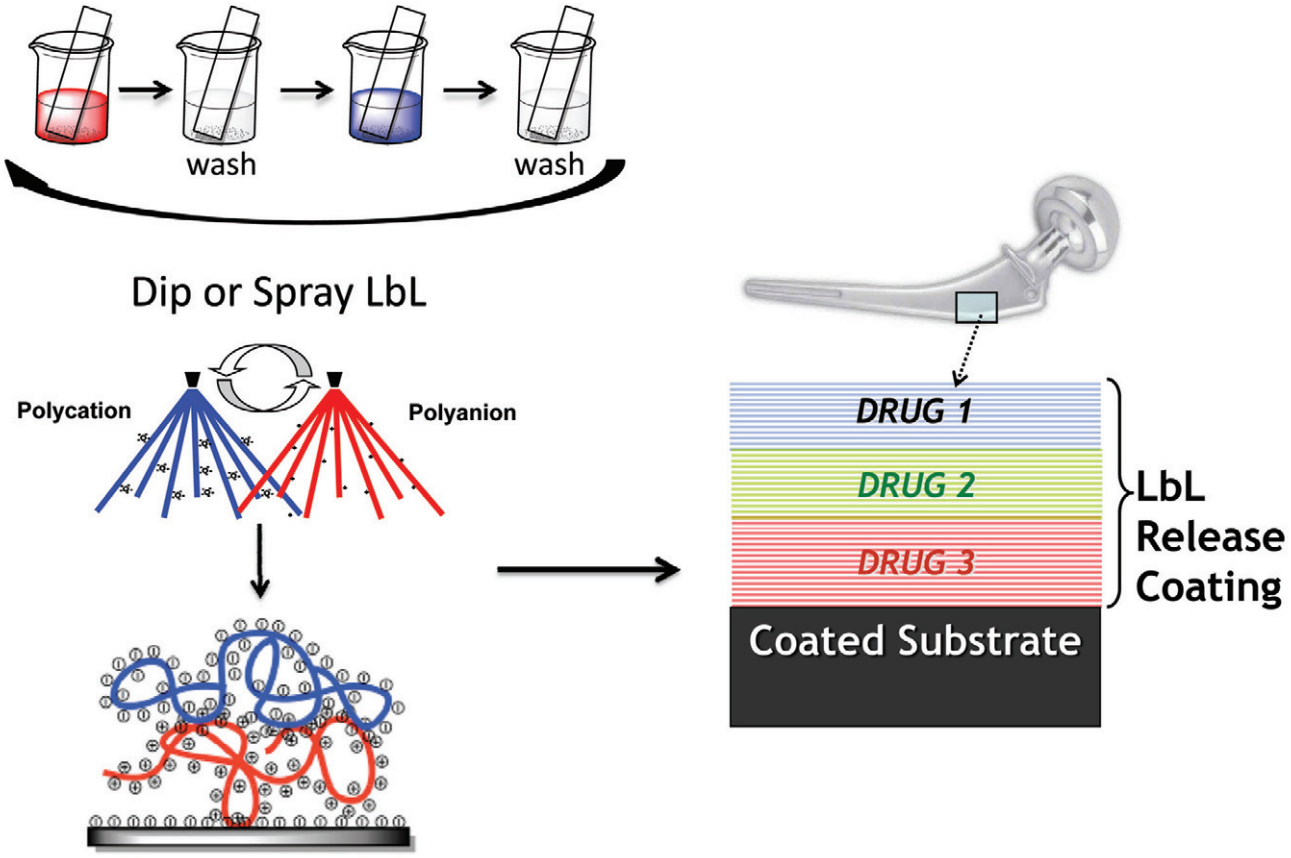


Fig. 1 Water based layer-by-layer (LbL) assembly methods enable the incorporation of therapeutics directly into a thin film conformal coating. The LbL process involves alternate adsorption of charged or other complementary species onto the intended substrate from aqueous solutions. Traditional approaches involve dipping the substrate in dilute solutions; more rapid assembly approaches include automated Spray-LbL, which increases the range of substrate types that can be coated. Multiple drugs can be incorporated into nanometer scale coatings with precise control of dose and controlled release from biomedical implant and device surfaces.

technique through various means of passive and active film disassembly, destabilization, or degradation. In the next decade, it will be important to move LbL approaches beyond laboratory successes and toward clinical translation. Such translation relies on the ability to show the power of multilayer systems in meaningful biomedical applications, as well as the potential for commercial manufacturability of the LbL process. The field has been growing rapidly, and many researchers have contributed to the development of new LbL drug delivery systems[7,9,22]; in this review perspective, a few selected examples from our research team will be provided in three general areas. Among the systems discussed, emphasis is placed on those that have been demonstrated with *in vitro* and *in vivo* studies, and on demonstrations of the use of rapid assembly and versatile LbL application methods that can help to move LbL biomaterials systems from lab to clinic with true translation to pharmaceutical and biomedical applications.

Controlling release of therapeutics from surfaces

A unique advantage to the use of LbL assembly is its ability to incorporate drugs in high concentrations within a multilayer thin film. Traditional polymers used in blends with small molecules for release matrices can generally only incorporate 2 to 5 wt% drugs while maintaining reasonable stability; larger quantities tend to lead to phase separation, destabilization of the blend, and bolus release behavior. In an alternating LbL assembly that consists of two to four components, for example, the direct adsorption of a pharmaceutical actually utilizes the drug as one of the layers within the film, stabilized by electrostatic, hydrogen bonding, or a combination of these interactions. The loading of drugs at high densities – often ranging from 10 to 40 wt% – with nanofilms that can conformally coat a broad range of surfaces creates new opportunities for the release of therapeutic molecules from surfaces for localized drug release.

Gentamicin is a simple, water soluble aminoglycoside that kills bacterial cells by inhibiting their protein synthesis; it is commonly used in hospital settings to address *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus* (Staph) and other common bacterial species for skin, bone, and soft tissue infections[23]. When introduced orally, gentamicin only resides in the body for a few hours before being eliminated, thus significantly lowering the impact of the antibiotic on a local infected region. On the other hand, gentamicin has been introduced into polymer

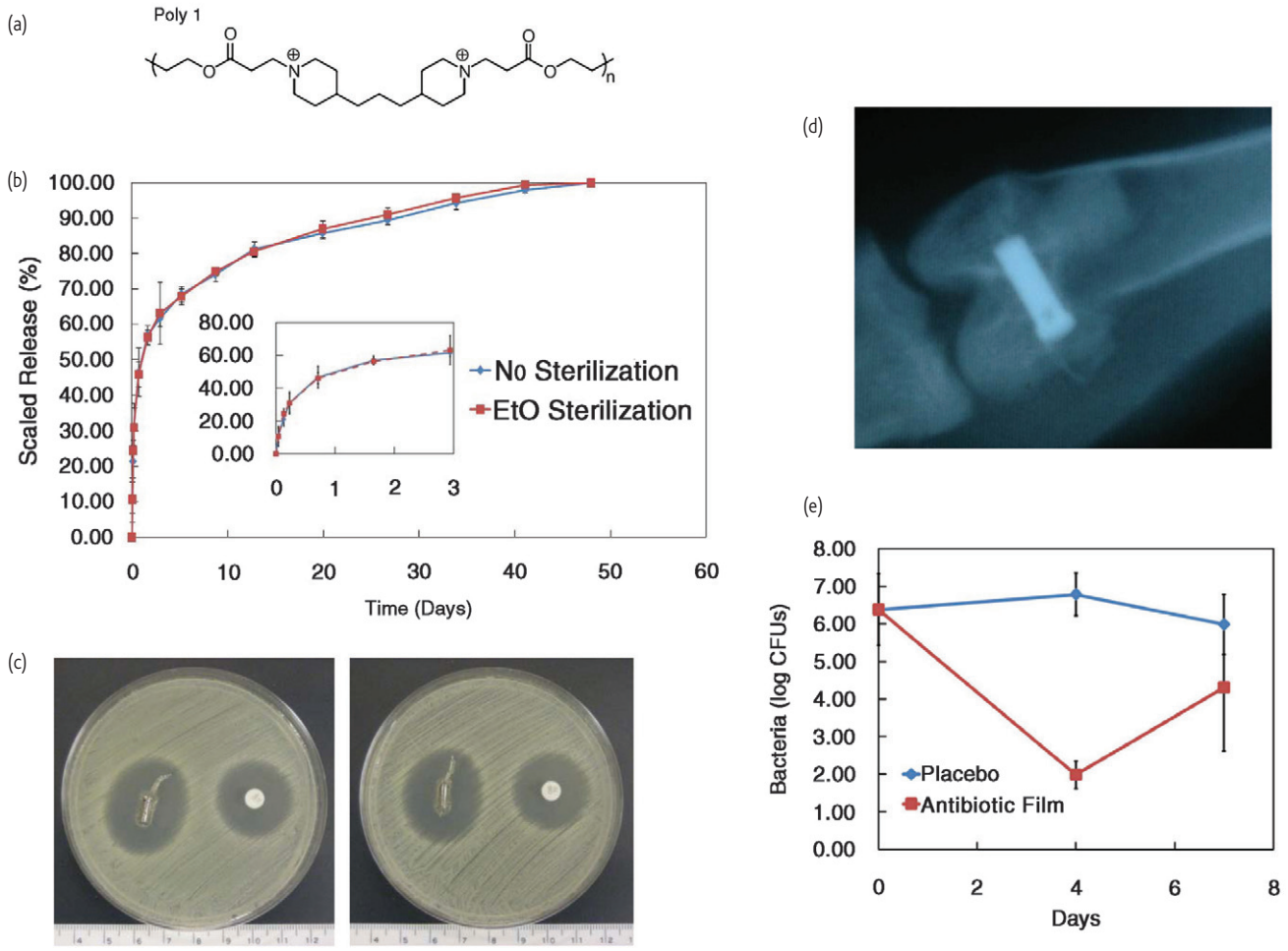


Fig. 2 Gentamicin sulfate (GS) antibiotic containing LbL films are generated via direct alternation with positively charged gentamicin sulfate in tetralayers with poly(acrylic acid) (PAA) and Poly 1 (shown in (a)). (b) Cumulative amount of gentamicin released from[Poly1/PAA/GS/Anion] films. (c) Kirby-Baur assay comparing sterilized and unsterilized LbL GS coatings on a titanium rod implant. (d) micrograph of rabbit femor with titanium rod placed in drilled defect (e) Results from first infection model indicates significant impact on bacteria count within a highly infected wound site by day four. Reproduced from[27] with permission from Elsevier.

composites such as methyl methacrylate based bone cement used for securing whole joint orthopedic implants to prevent infections[24]. The limitation of such systems is that gentamicin can only be incorporated into the cement at low concentrations without sacrificing the integrity of the polymer cement – thus it cannot be used to eliminate an existing infection[25]. By directly layering the positively charged gentamicin sulfate (GS) drug molecule with negatively charged polyelectrolytes in an alternating LbL tetralayer with a degradable polycation and a biologically derived polyanion, it is possible to generate gentamicin release thin films that are on the micron to submicron scale in terms of thickness, and provide uniform coatings on biomedical implants such as orthopedic implants, sutures, cardiovascular, and opthalmalogical implants[26,27]. The degradable component utilized in this example was a poly(β-aminoester) (PBAE), shown as Poly1 in Fig. 2a, which is layered with GS and polyacrylic acid (PAA)[27]. When LbL multilayers are built from the repeat {Poly1/PAA/ GS/PAA}, the final thin film exhibits a large rapid release of gentamicin from the surface, followed by sustained release over multiple weeks; this release behavior is due to freely absorbed GS molecules incorporated into the film in the final step GS absorption step, yielding a combination release mechanism of rapid drug out-diffusion plus slow hydrolytic degradation of GS containing layers. In the example shown in Fig. 2b, 200 tetralayer films release a total of 550 μg/cm^2 of gentamicin sulfate from a titanium implant surface, with an initial rate over the first few days of 11 μg/cm^2/day, followed by a constant linear release over the following four week period of 4 μg/cm^2/day. The gentamicin released from the films retains all of its activity, maintaining the same potency as the free drug (Fig. 2c). More important with regard to the translation of this kind of approach for biomedical implants is the demonstration that the released antibiotic can lower infection *in vivo*. Using a rabbit bone infection model, titanium inserts coated with the LbL gentamicin film were shown to alleviate even very severe established bone infection in animals[27] (Fig. 2d, e).

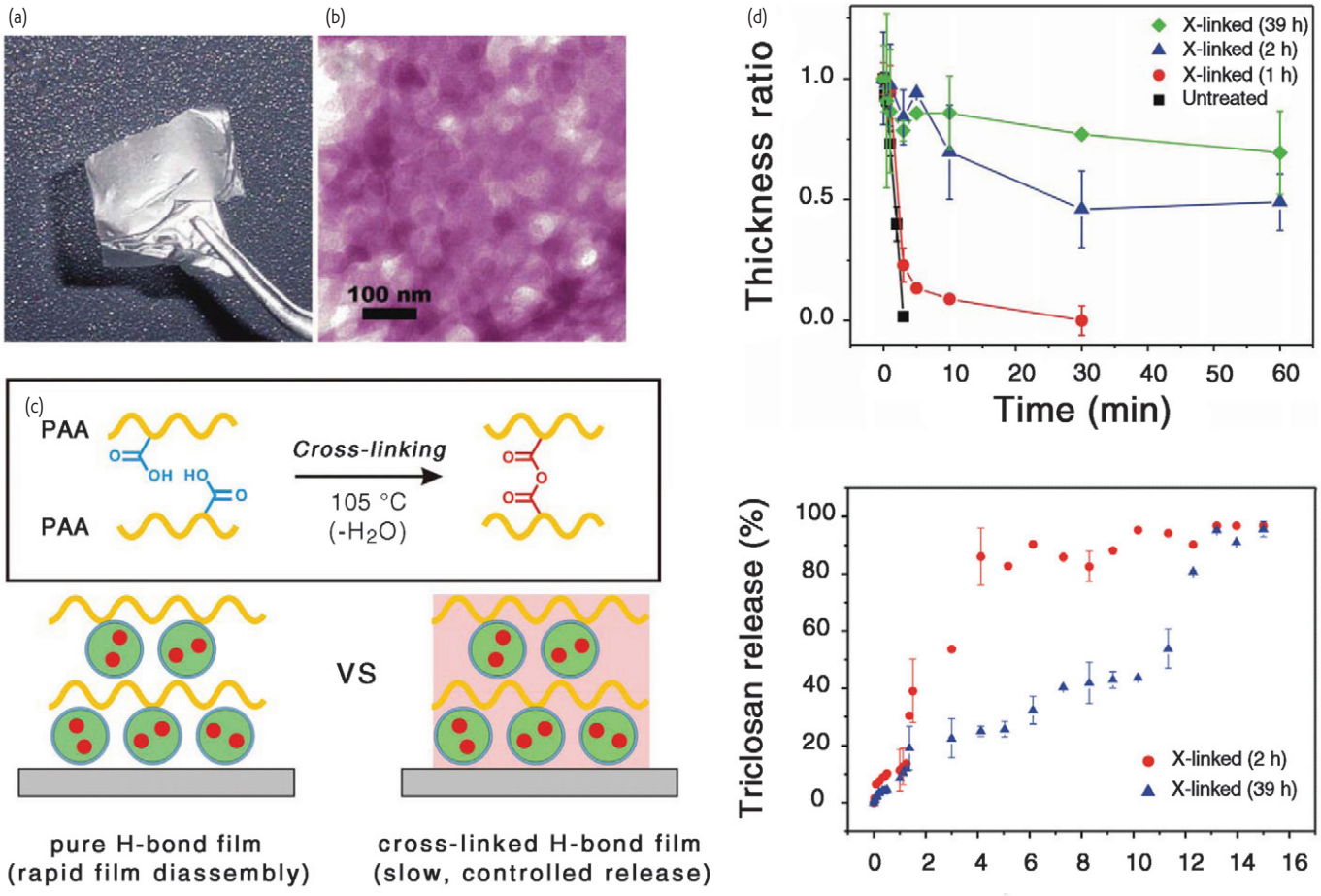


Fig. 3 (a) Photograph image and (b) TEM micrograph of a cross-section of free-standing (PEO-b- PCL/PAA)60 film. (c) Once films are constructed, thermal cross-linking significantly enhances the stability of the film and slows the drug release profile. (d) Change of film thickness as measured by profilometry. The thickness of the as-prepared film rapidly decreases upon introduction to PBS buffer, whereas the cross-linked films undergo much slower film deconstruction. (e) cross-linked film releases drugs over an extended period of time, (six days for two ourh cross-link, thirteen days for 39 hour cross-linked film). Reproduced from[35] by permission of The Royal Society of Chemistry.

There are numerous important therapeutics that are not charged, and do not necessarily have good water solubility. In these cases, it is possible to adapt LbL to a range of different carrier species that can readily be introduced into multilayer systems. For example, polycyclodextrins (PolyCDs) with charged groups can be used to sequester hydrophobic drugs such as anti-inflammatory agents, and subsequently can be layered into multilayer thin films to create stable thin films[28]. A series of non-steroidal anti-inflammatory drugs (NSAIDs)[29] were examined for the assembly of a negatively charged PolyCD-NSAID complex with PBAE's that exhibit different rates of hydrolytic degradation[28]. By varying the composition of degradable polycation chosen, the rate of degradation could be modulated in these systems. On the other hand, for any given degradable polyion, the normalized rates of drug release were statistically identical regardless of which NSAID was incorporated, yielding a controlled and constant release over multiple days and demonstrating a platform technology that enables the incorporation of many different molecules while achieving predictable release characteristics. The NSAIDS examined, including commonly used diclofenac and flurbiprofen, exhibited cyclo-oxygenase (Cox-2) inhibition when released *in vitro* to cells stimulated to yield an inflammatory response. Release in these films is linear with no initial bolus. Therapeutic levels of drug are eluted from film coatings just a few hundred nanometers in thickness, with release over several days to over four weeks. Hydrophobic antibiotics, including Ciproflaxin, which is a broad spectrum antibiotic known for its high efficacy, can also be delivered in its active form using PolyCD complexes[28]. The films themselves are clear, thin, highly conformal, and exhibit excellent optical properties; ongoing work is now targeted toward development of these films for opthalmological applications such as intraocular lenses. It is also possible to combine the technique of direct drug incorporation with the use of a carrier such as a PolyCD; this kind of approach has been successfully demonstrated with the generation of LbL delivery thin

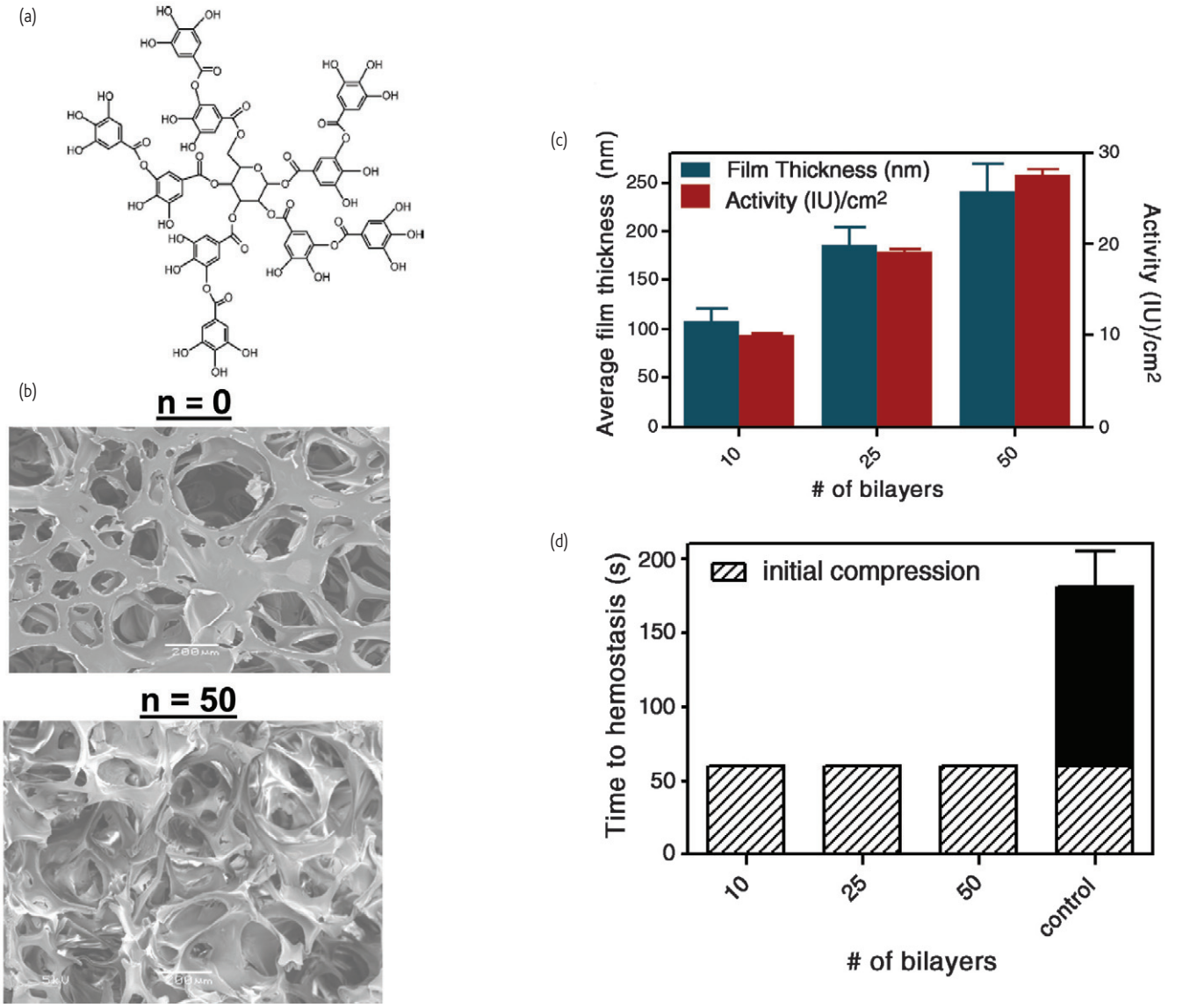


Fig. 4 Hemostat sponge created for battlefield medicine applications contain LbL films with blood clotting protein, thrombin (shown in a). (b) Spray-LbL enables the uniform coating of hydrogen bonded LbL thin films on a highly porous absorbent sponge. (c) In vitro hemostatic activity of film coated gelatin sponge. (d) Time to hemostasis following sample applicaon (controls were sponges with a monolayer coating of BPEI). Reproduced from[42] with permission from Wiley.

films that simultaneously release the broad spectrum charged antibiotic vancomycin and an anti-inflammatory drug[30].

Micelles, liposomes or other colloidal carriers and nanomaterials such as nanoparticles may also be used as drug carriers that can be directly introduced into multilayer thin films[31-34]. Of particular interest is the use of simple FDA approved components to generate thin films with a broad range of release behaviors. It is possible to generate alternating multilayers of the water soluble and biologically safe poly(ethylene oxide) (PEO) with poly(acrylic acid) (PAA) at low pH through hydrogen bonding between the PEO ether oxygens and the PAA acid groups. We have used this knowledge as the basis for the design of LbL thin films consisting of alternating drug loaded micelles from block copolymers of polycaprolactone (PCL)-PEO with moderate to high molecular weight PAA[35]. The films that are formed at low pH are micron-scale in thickness, and generate coatings or cohesive free-standing films, as shown in Fig. 3[35]. When these thin films are exposed to a plasma pH of 7.4, the acid groups become highly charged, the hydrogen bonds are disrupted, and electrostatic repulsion causes the film to rapidly disassemble, releasing its contents and dissolving entirely in a matter of seconds. Such a rapid release approach may be of interest for the rapid delivery of agents to a wound site or the release of oral therapeutics on the tongue or cheek, for example. On the other hand, this same LbL assembly can be treated using heat, or other methods to dehydrate the film and generate anhydride bonds between carboxylic acid groups in

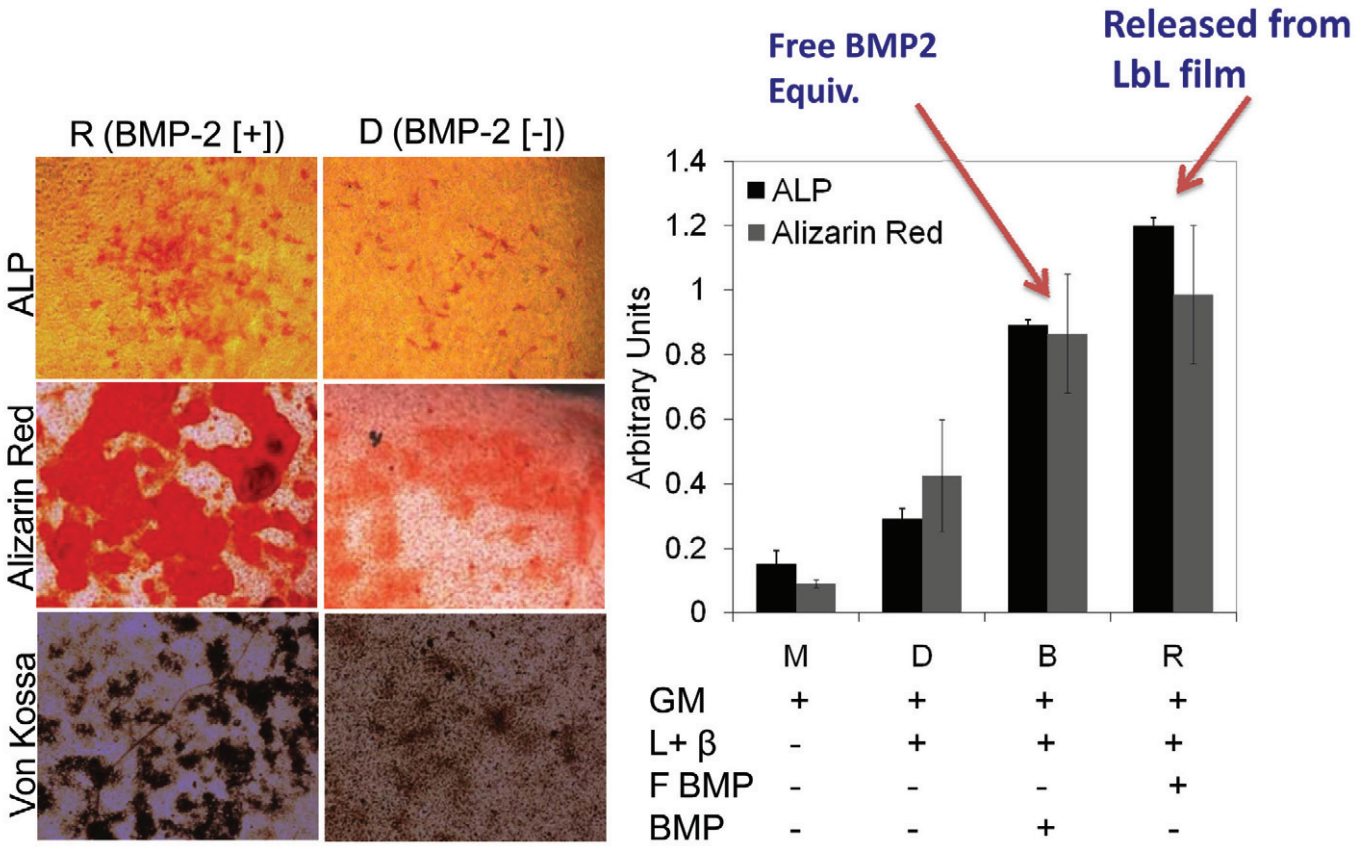


Fig. 5 In vitro studies of bone differentiation with MC3T3 Pre-Osteoblasts. (Left) Bioactive BMP-2 released from LbL films (sample R) induces differentiation of MC3T3 pre-osteoblasts to an osteoblast phenotype compared with differentiation medium alone (sample D). Alkaline Phosphatase (ALP) staining (red) shows early (day six) activation of the bone differentiation cascade; At 28 days of culture Alizarin Red staining for calcium deposition (red), and Von Kossa staining for mineralization of the calcium matrix (black) confirm the differentiation process to osteoblasts. (Right) Quantification of the staining signal (three readings on four independent experiments; error bar is standard deviation) shows that the increase in activity of BMP-2 released from LbL films (R) over BMP-2 positive control (B) (matched 90 ng/mL for both samples) is statistically significant. A single factor ANOVA test allowed rejection of the null hypothesis for both assays; a Tukey test showed that B and R are statistically different with $p < 0.01$ for ALP assay. Both B and R outperform differentiation medium alone (D) or growth medium alone(M). GM = growth medium; L+β = L-ascorbic acid and β-glycerol phosphate (differentiation factors); F BMP = LbL Film-released BMP-2; BMP = BMP-2 added directly to the medium. Reproduced from[58] with permission from Elsevier.

the film. Such films erode away at a much slower rate, dependent on the degree of anhydride crosslinking, leading to a near-linear release of the same cargo over two weeks. The micellar nanocarriers, when released, can penetrate the surrounding tissue of an implant, wound bed, or surgical incision and deliver cargo to these localized regions following the hydrolytic breakdown of the PCL core, which is prone to take place over longer time periods than the anhydride. Use of a different hydrogen bonding component, such as a polyphenol like tannic acid[36], yields a different set of release conditions and systems that can be incorporated into the film. The hydrogen bonding of PEO based micelles with tannic acid, for example, generates films that are stable above pH 7.4, thus allowing the integration of a broad range of components at biologic pH or lower[35].

For direct translation of these systems toward realistic applications, we can introduce a means of rapidly assembling LbL coatings on a variety of surfaces. One approach that has proven to be particularly versatile is the use of Spray-LbL[37-41], in which each alternating component is introduced via a short spray exposure of aqueous polyelectrolyte or drug solution, followed by a spray rinse. Because each cycle for adsorption is reduced to a few seconds, this approach is orders of magnitude faster than traditional dip-LbL methods; however, another important advantage in the biomedical arena is that it allows us to coat a wider variety of surfaces. Flat and uniform, dimensionally complex, and highly porous substrates all become available for LbL application using this approach. Particularly challenging surfaces include those that are both porous and water absorbent, such as gelatin sponges that were designed to absorb many times their weight in blood plasma to address bleeding and help induce hemostasis. Thrombin (Fig. 4a), a native protein that induces rapid hemostasis in a wound by triggering a part of the blood clotting cascade, was introduced in a layer-by-layer thin film through the simple alternation of thrombin with tannic acid[42] based on hydrogen bonding interactions between the protein and the

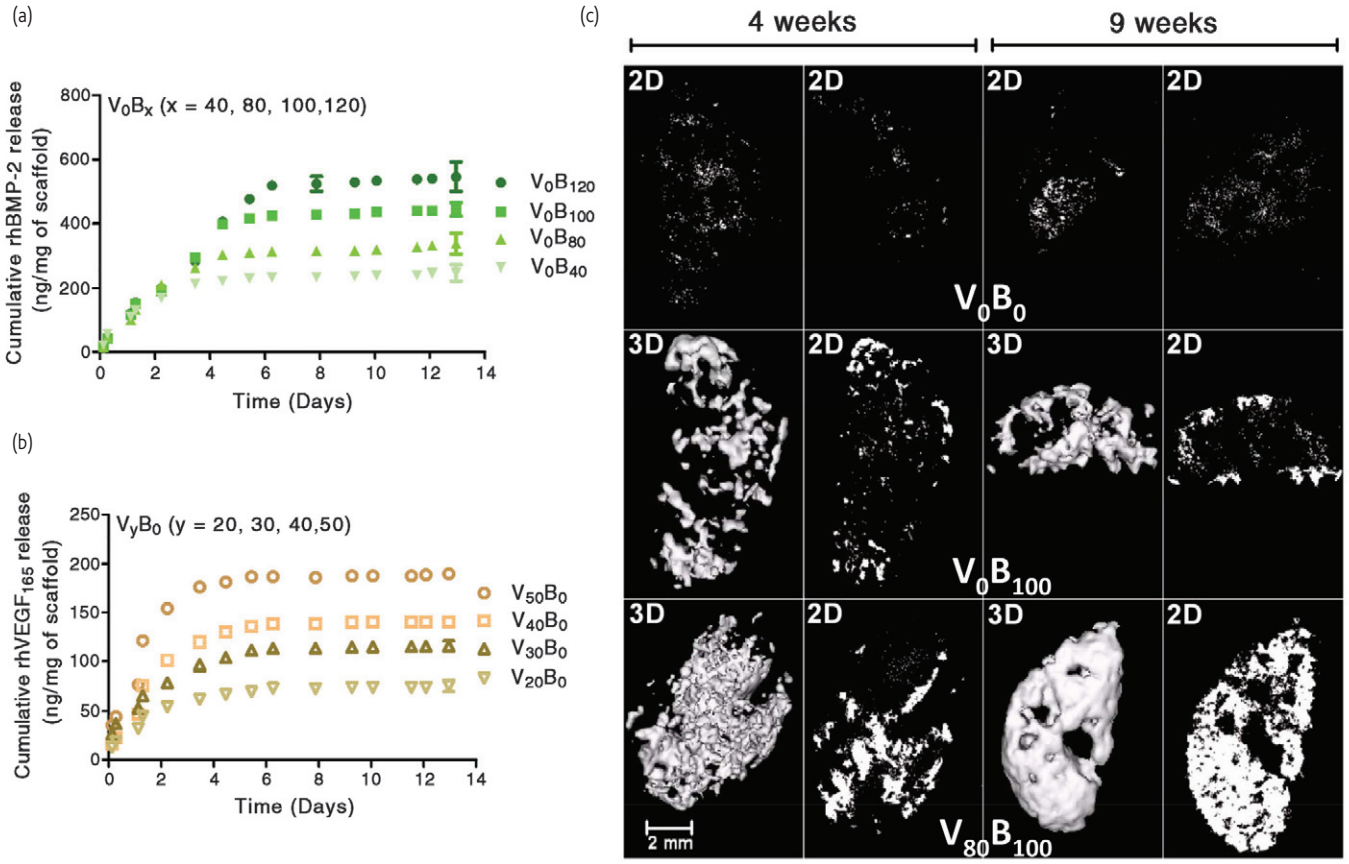


Fig. 6 Combination BMP-2/VEGF growth factors yield higher bone density within implants. Growth factor loading was controlled by varying the number of layers dipped on the scaffold. rhBMP-2 released over a period of about one to two weeks, whereas rhVEGF eluted completely within a shorter time period of four to six days depending on number of layers used. (a) Release profiles for BMP containing films. (b) Release profiles of VEGF containing films. (c) (Top row) Micro CT results of bone tissue formation. Control scaffolds without growth factors produce no detectable bone over the duration of the study (Middle row) In single growth factor rhBMP-2 films lacking rhVEGF165, bone formation is restricted to the periphery of the scaffold at four weeks and nine weeks. (Bottom row) As a result of increased vascularity, scaffolds releasing rhVEGF165 demonstrate a smooth, continuous profile in the ectopically formed bone which matures from four weeks to nine weeks to fill the entire scaffold. Reproduced from[60] with permission from Elsevier.

small molecule, which is a key component found in teas and wine tannins, and a natural anti-oxidant[43]. As shown in Fig. 4b and c, the original porous morphology of the sponge is preserved using vacuum-assisted Spray-LbL; the film conformally coats the surfaces of the pores without significantly filling the pores, leaving them free and accessible for water uptake. The thrombin contained within the films was found to be fully active when compared to the free protein, and large therapeutic amounts of active thrombin can be incorporated into the sponge due to its large surface area. When tested *in vivo* with a pig spleen bleeding model, it was found that upon compression on a heavily bleeding wound, even a 10-bilayer treated LbL thrombin gelatin sponge can stop bleeding at least three times faster, than the uncoated gelatin sponge. Broad spectrum antibiotics, such as vancomycin, can also be coated on these sponges to yield systems that remain functional, and can even improve the sponge absorbency[44]. The ability to coat micro and even nanoporous materials leads to numerous possibilities in the areas of wound care, control of adhesions for surgical implants, and ready storage of localized medical treatments in general for emergency care.

New LbL routes to tissue regeneration

An exciting area in which multilayer assembly presents true potential is regenerative medicine and tissue engineering[4,7,45,46]. A number of highly sensitive growth factors regulate the differentiation, proliferation, and signaling of stem cells that determine the formation of new tissues and key processes such as wound healing. These growth factors are typically administered directly to tissues of interest with bolus injections, or encapsulated in depots that lead to bolus release in the body; such mass release of growth factor proteins generally leads to a large amount of clearance of the active protein, with only a small amount of the expensive biologic therapeutic remaining to exact

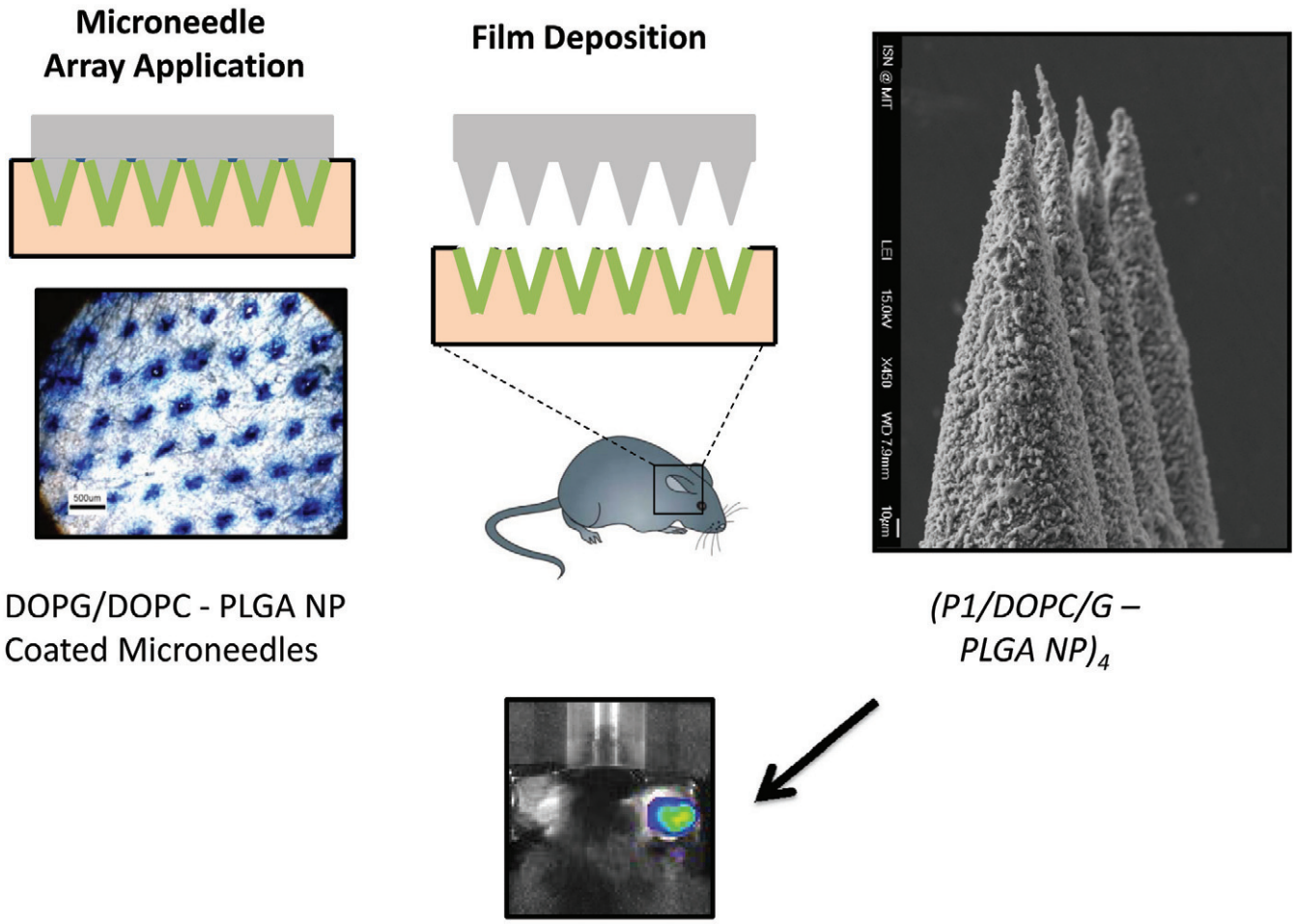
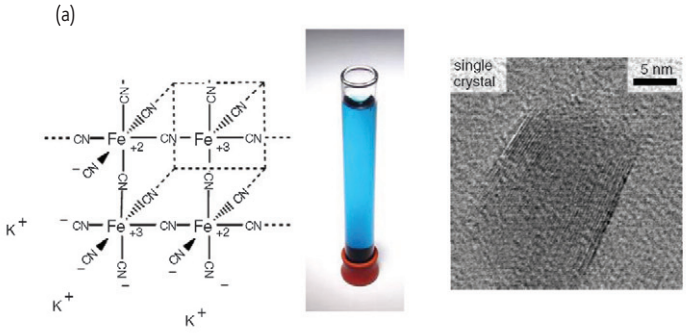


Fig. 7 Schematic and data illustrate the coating of microneedles with hydrolytically degradable Poly 1 and lipid-coated PLGA nanoparticles that encapsulate DNA. The microneedles are able to penetrate into the mouse ear, from which the thin film elutes over time based on the choice of degradable polycation. DNA that contains the luciferase gene is expressed in the mouse ear over a range of timeframes, depending on the number of bilayers and time allowed for release. Reproduced from[62] with permission from Wiley.

its influence on tissue development[47-49]. A more extended release of smaller, controlled quantities of growth factor would generally replicate the natural process of tissue generation, while yielding a controlled and localized process with high pharmacological yield; however, most polymeric carriers can only contain limited amounts of protein in a stable blend formulation due to thermodynamic and other practical constraints. Furthermore, key to the maintenance of protein activity is the avoidance of solvent, higher temperatures, or pH conditions sufficient to denature proteins, conditions that are commonly needed to achieve solubilization in traditional materials systems. A unique aspect of LbL assembly is the fact that it is an all-aqueous, room temperature process that can be readily adapted to the incorporation of a broad range of proteins at high loadings[50]. When incorporated from aqueous solutions at appropriate pH and ionic strength, it has been shown that proteins in LbL films retain their higher order structure and activity[51-53]. In early experiments, LbL films of lysozyme with negatively charged polysaccharides and PBAE polymers led to loadings of fully active protein in the milligram range (over 1000 µg/cm^2) in 10 to 15µm thick thin films[54]; the film release characteristics can be tuned depending on the size and overall charge density of the protein and the choice of complimentary polyions from a few days to as many as 30 days. The controlled nature of localized release from surfaces enables much lower doses of high cost growth factors to be effective for specific applications.

Bone morphogenetic growth factor 2 (BMP2) is one of the key growth factors involved in the differentiation of adult mesenchymal stem cells to osteoblasts, or bone cells, and is considered a potential treatment for the *in vivo* regrowth of bone for orthopedic applications[47,48,55-57]. By generating thin films that alternate BMP2 with chondroitin sulfate and a PBAE degradable polycation, delivery of

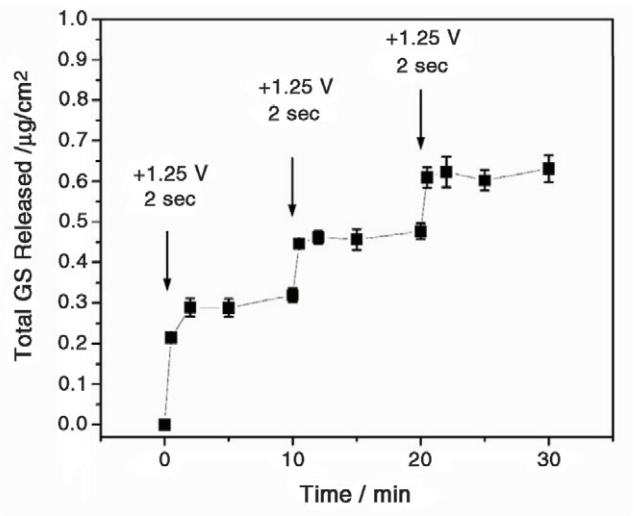
(a)



(b)

Pulse length at +1.25 V	Normalized Fraction of GS Released in 30 min
0 sec	0.18 ± 0.02
2 sec	0.50 ± 0.04
5 sec	0.58 ± 0.11
30 sec	0.78 ± 0.03

(c)



(d)

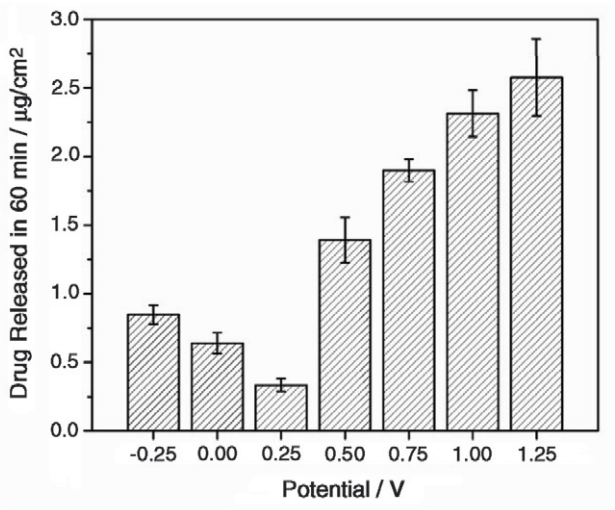


Fig. 8 (a) Prussian Blue nanocrystals are redox-active materials that lose their negative charge upon electrochemical oxidation at low chemical potential. (b) Total amount of gentamicin released from a Chi(PB/Chi)5-(PB/GS)50 film over 30 minutes at different applied potentials. (c) Drug release profile from a Chi(PB/Chi)5(PB/GS)75 film with two second pulses of 1.25 V to turn drug release "on", followed by 30 second pulses at 0.25 V to turn drug release "off". The films are sufficiently stable to allow for on/off, or pulsatile, drug release controlled by the applied potential. (d) Total amount of gentamicin released from a Chi(PB/Chi)5(PB/GS)50 film in one hour at different applied potentials. Reprinted with permission from[69]. © 2010 American Chemical Society.

the protein has been achieved in a controlled fashion over a period of 4 to 7 days or more, depending on the number of layers incorporated in the thin films[58]. The released BMP2 exhibits all of the activity of the original BMP2 protein, as shown in Fig. 5; interestingly, *in vitro* assays actually indicate a slight increase in BMP2 activity, likely due to the use of polysaccharides that have binding sites on BMP2 and ligands for binding target cell receptors, thus increasing the bioavailability and uptake of the protein by cells. Conformal coating of osteoconductive tricalcium phosphate polycaprolactone composite scaffolds with BMP2 containing LbL films can generate a tissue engineering matrix. When these scaffolds are implanted in the intramuscular regions of rats, well-formed and oriented bone is formed around the scaffold region. In this process, BMP2 protein eluted from the scaffold recruits MSCs from the bone marrow to the site of implantation, induces their differentiation into osteoprogenitor cells, and their proliferation in the tissue to form substantial bone.

For tissues to integrate effectively into a scaffold or around an implant, typically a vascular system that is well-developed is needed to provide blood, oxygen and nutrients via blood flow, and a pathway for cell precursors to populate the developing tissue[56,59]. We investigated the construction of separate sets of LbL films containing the vascular endothelial growth factor (VEGF), which recruits endothelial precursors and enables angiogenesis, and BMP2[60]. By generating a series of LbL nanolayers that release VEGF, atop a series containing BMP2, it is possible to observe the simultaneous but independent release of the two factors, with VEGF releasing over a period of two to three days and BMP2 releasing over a more extended time period of five to seven days (Fig. 6a). The amount of protein released in each case

is directly dependent on the number of layers; the modular nature of the protein release enables the design of complex release profiles that can be independently tuned for each protein type – a capability very difficult to achieve with traditional polymer encapsulation methods. Also shown in Fig. 6b, the *in vivo* results of implants comparing BMP2-only versus BMP2/VEGF releasing LbL scaffolds illustrate the substantial impact of introducing synergistic growth factors. Bone in-growth occurs throughout the entire scaffold region, in contrast to just the shell around the implant achieved with just BMP2, yielding a solid bone structure throughout the scaffold matrix at nine weeks for the dual growth factor system. The bone formed with dual growth factors is also higher in bone mineral density, and appears to be highly vascularized. These results indicate the promise of the LbL approach for tissue engineering applications, including the generation of bone tissue around orthopedic implants, and bone in large fractures and bone defects. By extending the work to additional growth factors that stimulate different stem cell and tissue progenitors, great potential exists for the *in vivo* regeneration of cartilage, the repair of large open wounds, blood vessel and skin regeneration from a broad range of scaffolds and substrates.

Controlled micro-release thin film platforms

Although, as demonstrated in the preceding examples, LbL is making avenues in the delivery of molecules from large macroscopic areas for implants and tissue engineering, the method is also particularly well-suited to the delivery of small but controlled amounts of drug from micron scale structures or small thin film surfaces and devices. One area that offers rich opportunities for layer-by-layer technology are transdermal delivery - applications in which nano- to microgram quantities of a drug are delivered across the skin for therapeutic delivery or vaccines. Vaccines are a particularly attractive area of potential growth for transdermal release because the skin is heavily populated with immunological cells, and the typical use of needles presents opportunity for infection and greatly lowers patient compliance. A simple transdermal delivery system was constructed with LbL films of a model antigen protein, ovalbumin, in bilayers with PBAE polycation[61]. Rapid release occurs with the films due to the low charge density and relatively small size of ovalbumin; when the films were placed on tape-stripped mouse ears, ovalbumin was taken up by the Langerhans cells – dendritic cells that reside in the skin – and an immune response was observed through the uptake of ovalbumin to the lymph nodes and the release of specific cytokines. Synergistic effects can be obtained with the addition of oligonucleotide agents within the multilayer film that stimulate T cell response; significantly increased amounts of cytokine production and an even more robust immune response were observed, demonstrating the power of dual delivery of synergistic compounds[61].

This early success led to the investigation of more translational modes of delivery. The use of tape-stripping in the animal experiment was needed to remove the dense *stratum corneum*, which is the thick top layer of skin that is difficult for hydrophilic biomacromolecules to penetrate. By coating arrays of microneedles using the Spray-LbL technique, it is possible to use a physical means of penetrating the *stratum corneum*, while still providing a solid thin film means of delivering proteins and/or other biomolecules directly to the lower layers of the skin – approximately 20 to 30 microns and lower – where the Langerhans and other immunological cells reside[62]. We have demonstrated the delivery of DNA encapsulated within degradable lipid-modified poly(L-lactic-co-glycolic acid) (PLGA) nanoparticles; the negatively charged DNA nanoparticles are alternated with degradable PBAE polycation to achieve nanolayered coatings on PLGA microneedle arrays as shown in Fig. 7. In this case, it was possible to show gene transfection of cells in the areas of penetration with dose dependent response. Transfection was observed even with a low number of bilayers, and longer release times or larger numbers of layers led to enhanced gene response. The ease of assembly of coated microneedle arrays, combined with the rapid process times from Spray-LbL and the high efficacy observed in these systems offers great promise for true translational applications. Furthermore, applications in the delivery of proteins, hormones, DNA, or siRNA for other medical applications may also be of interest in the future.

Other areas of interest in multilayer assembly include the release of drugs based on electrochemical potential. Here, the concept of manipulating charge within the components of LbL thin films to cause film destabilization and disassembly can lead to unique means of controlling release from patterned drug containing thin films on the remote application of small localized fields. Furthermore, LbL thin films can be printed directly onto surfaces[63-66] for the generation of LbL thin film micro-patches that can release different drugs passively at different rates from a number of surfaces, and lift-off methods can be used to generate free-standing micron-scale elements, which may be of interest for microparticle delivery systems. An earlier approach to electrochemically stimulated release was first observed when redox-active Prussian Blue (iron hexacyanoferrate) nanoparticles, capable of undergoing changes in charge from −2 to neutral, were introduced into films in the charged state; when small potentials were switched on in the film, the negative charge was reversibly removed, causing the film to destabilize due to charge imbalance[67]. In more systematic studies, it was possible to show the release of large macromolecules[68], and finally of a small molecule drug such as gentamicin with films of Prussian Blue[69], which is a safe, FDA approved compound often used for the treatment of heavy metals, as shown in Fig. 8; the films can be systematically pulsed multiple times to release controlled nano- to microgram quantities of drugs. More recently, it has also been possible to generate electrically controlled release systems based on the localized disruption of hydrogen bonding in LbL thin films composed of biocompatible components[70]. In general, numerous approaches to microfabrication and LbL thin film manipulation can lead to unique micro-delivery methods that

can be accessed using a broad range of triggers – from temperature to remote control field-assisted release.

Conclusions

In conclusion, a number of new possibilities have been opened up through the promise of LbL techniques. The conformal nature of this thin film process enables the coating of a broad range of biomedical devices, including implant surfaces, porous and nonporous scaffolds for tissue engineering, and microscale release devices for localized delivery within the body or through the skin. The ability of LbL films to incorporate a broad range of drugs, including small molecules and sensitive biologic drugs, while retaining drug activity is particularly enabling, and may open the door to new therapeutic approaches in modified implants, wound healing and remediation, cardiovascular stents, and passive or actively triggered microscale release to localized regions. Remaining challenges in the field include the enhancement of mechanical properties, optimization of film assembly conditions to enable long term storage of multilayer systems, and the exploration of new triggered and responsive release mechanisms. Ultimately, the ability to generate materials capable of synergistic and sustained multi-drug release in the form of an ultrathin conformal coating using new rapid assembly methods and approaches that access three-dimensional and complex geometries on a commercial scale will provide new avenues for translation in the biomaterials field. mt

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