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Citation	Macdonald, Mara L., Natalia M. Rodriguez, Nisarg J. Shah, and Paula T. Hammond. Characterization of Tunable FGF-2 Releasing Polyelectrolyte Multilayers. <i>Biomacromolecules</i> 11, no. 8 (August 9, 2010): 2053-2059.
As Published	http://dx.doi.org/10.1021/bm100413w
Publisher	American Chemical Society
Version	Author's final manuscript
Accessed	Sun Jan 24 03:20:03 EST 2016
Citable Link	http://hdl.handle.net/1721.1/79042
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Detailed Terms	

Published in final edited form as:

Biomacromolecules. 2010 August 9; 11(8): 2053–2059. doi:10.1021/bm100413w.

Characterization of tunable FGF-2 releasing polyelectrolyte multilayers

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Abstract

Fibroblast growth factor 2 (FGF-2) is a potent mediator of stem cell differentiation and proliferation. Although FGF-2 has a well-established role in promoting bone tissue formation, flaws in its delivery have limited its clinical utility. Polyelectrolyte multilayer films represent a novel system for FGF-2 delivery that has promise for local, precisely controlled and sustained release of FGF-2 from surfaces of interest including medical implants and tissue engineering scaffolds. In this work, the loading and release of FGF-2 from synthetic hydrolytically degradable multilayer thin films of various architectures is explored; drug loading was tunable using at least three parameters (number of nanolayers, counterpolyanion, and type of degradable polycation) and yielded values of 7-45 ng/cm² of FGF-2. Release time varied between 24 hours and approximately five days. FGF-2 released from these films retained *in vitro* activity, promoting the proliferation of MC3T3 pre-osteoblast cells. The use of biologically derived counterpolyanions heparin sulfate and chondroitin sulfate in the multilayer structures enhanced FGF-2 activity. The control over drug loading and release kinetics inform future *in vivo* bone and tissue regeneration models for the exploration of clinical relevance of LbL growth factor delivery films.

Keywords

drug delivery; Fibroblast growth factor 2 (FGF-2); polyelectrolyte multilayer; heparin; poly(β -aminoester)

1. Introduction

Fibroblast Growth Factor-2 (FGF-2) is a potent biologic capable of helping to direct the stem cell differentiation and proliferation of cells as diverse as hepatocytes^{1, 2}, neurons^{3, 4}, cartilage⁵, and bone. It therefore represents a tremendous potential opportunity in affecting clinical outcomes across a number of different medical conditions in which exogenous cell transplantation, or the recruitment of native stem cells, is a possible strategy. In particular, there is a clear need for enhancing bone creation and regeneration. A bone defect that is too large to bridge can occur in response to trauma, during treatment of bone non-union, or when resecting bone tumors, leading to a lesion which cannot heal⁶. In reconstructive surgery, frequently the need for allograft bone tissue outstrips the supply available⁷, particularly in elderly and pediatric populations, limiting therapeutic intervention. In both of

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Supporting Information Available Graph showing proliferative response of MC3T3 preosteoblast cells to varying concentrations of FGF-2 added to 1% serum concurrently with film sample exposure to the cells. Dose response can be seen to varying concentrations of FGF-2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

these circumstances, it has been found that the introduction of FGF-2 can greatly enhance the rate of healing and the ability of natural bone to bridge defects⁸⁻¹¹ due to its ability to promote proliferation both in pre-osteoblast cell populations to increase bone production¹², and also in endothelial cell populations which enhance blood supply to the growing tissue¹³. FGF-2 has further bone forming roles in increasing inorganic phosphate transport¹⁴ and in stimulating vascular endothelial growth factor (VEGF) secretion¹⁵. However, bolus injections of growth factor are rapidly cleared, leaving little time to exhibit therapeutic effects^{16, 17}. Furthermore, supraphysiologic doses of growth factor are needed for injections due to growth factor dilution in a larger body volume (opening the possibility of off-target side effects), loss of growth factor activity on exposure to blood, and clearance from the blood stream. There is therefore a need for a biomaterial that will locally release sustained low levels of active growth factors for long periods of time.

One means of accomplishing this is to create polymer coatings that can safely contain and release even sensitive biologic drugs in an active form within nanolayered film architectures for controlled release directly from implanted surfaces. Such an implant coating could be beneficial for an orthopedic hip implant, a stent, an implantable tissue engineering scaffold, or a range of surgical fixtures such as screws or sutures. Such release from implant surfaces calls for new, surface based methodologies that allow coating of a current mechanical device rather than traditional bulk polymer encapsulation methods. This can be achieved using Layer-by-Layer (LbL) assembly¹⁸; in this technique, positively and negatively charged polymers are adsorbed sequentially onto a charged surface to build a film. LbL assembly is advantageous in that the films are created through a gentle, aqueous process that preserves fragile drug activity^{7, 19, 20}, while the resulting films can be made to be biodegradable, thin, and completely conformal to the device of interest, with easily tunable drug incorporation and film architecture²⁰⁻²². Furthermore, LbL opens the possibility of sequestering multiple drugs in different layers of the film and creating the opportunity to sequentially release several growth factors or other therapeutic agents such as antibiotics or anti-inflammatory agents^{23, 24} through surface erosion of the film. Finally, because conformal LbL deposition is possible on a wide variety of biomedically relevant materials, including titanium, ceramic, polymer, and glass, there are many potential medical applications of LbL.

Early drug release experiments with LbL films using hydrogen bonded films showed that these films fall apart rapidly at near-neutral pH, allowing a rather instantaneous method of drug release²⁵. However, such release is impossible to tune, and on a shorter time span than most controlled drug delivery applications require. Another approach is to pre-construct LbL films out of inert polymers and then load drug into the permeable network for diffusive or pH induced release²⁶⁻²⁸. While such porous LbL systems work well for the delivery of small molecules, the delivery of substantially larger protein therapeutics is difficult, as the therapeutic gets trapped in the polymer mesh and cannot diffuse out. Therapeutic drugs can either be encapsulated in the core, or in the LbL film surrounding the core itself²⁹. Drugs in the core can either be crystalline drug which therefore is ready for solubilization and release after LbL coating^{30, 31}, encased in a polymer which is subsequently leached out^{32, 33}, or absorbed into the capsule once the template core is dissolved³⁴. However, as non-degradable polymers are typically used, release is diffusive in nature and thus not easily tuned, and such microcapsules do not take advantage of the conformal nature of LbL in the implant setting, in addition to typically employing high ionic strength or high pH as a means of release (conditions rarely encountered in drug delivery applications *in vivo*). These approaches are thus not suitable for proteins.

Although proteins, and in particular growth factors, have been incorporated in LbL films^{19, 22, 35}, it is not typical for these LbL films to incorporate synthetic polymers that are designed to allow biodegradability and growth factor release, which can sometimes hamper

efforts to tune drug release due to the constraints of existing biopolymers³⁶. The approach taken in this work is to incorporate a cationic polymer from the class of poly(β -aminoester)s (PBAEs)³⁷. These polymers are created through the simple Michael addition of two commercially available classes of starting materials. Work in our group and others has shown that PBAEs readily incorporate in LbL films³⁸, and that the drug delivery characteristics of PBAEs from LbL can be varied through varying the polymer architecture^{36, 39}, allowing strong control over release through polymer design. It was found that increasing the hydrophobicity in LbL films led to extended release by decreasing water cleavage of the ester bond up until a critical point, at which the films would quickly fall apart and release all of the film contents due to film instability³⁹. Taken together, the ready incorporation of PBAEs in LbL films combined with our deep understanding of the tunability of these polymers represent an excellent opportunity to tune drug release profiles from LbL films via polymer design³⁸. The poly(β -aminoester)s used in this paper, denoted Poly1 and Poly2, have the same structural characteristics (Figure 2), differing by the addition of two methylene units in the backbone of Poly2, increasing the hydrophobicity in the region next to the ester bond which is hydrolytically cleaved and therefore decreasing degradation.

A negatively charged polyanion is also required for film construction. Here, heparin sulfate and chondroitin sulfate were chosen to exploit advantageous interactions each of these molecules has with FGF-2. Heparin is well known to highly increase the mitogenic potential of FGF-2 by assisting its receptor binding^{40, 41} while preserving FGF-2 from heat, pH changes, and proteolysis⁴². Specific binding sequences⁴³ have been discovered which allow FGF-2 to bind and be sequestered in heparin⁴⁴, leading to a developing body of work on the use of heparin for controlled delivery⁴⁵⁻⁴⁸. However, because it is difficult to modify and optimize biopolymers such as heparin as a release material, there are challenges in addressing their inherent materials limitations, such as undesirable release profiles or unintended side effects. Chondroitin, a natural extracellular matrix biopolymer found in cartilage with anti-inflammatory properties has also been shown to enhance FGF-2 mitogenic activity⁴⁹. In addition, in a previous study the ability to tune the release profile³⁶ from LbL films has been demonstrated, and chondroitin has potential synergism in creating bone and cartilage⁵⁰⁻⁵².

The aim of the present work is to investigate the characteristics of this novel FGF-2 carrier film that combines the advantages of biopolymer enhancement of FGF-2 mitogenic activity with the fine control over release afforded by synthetic polymers, with a focus on creating a conformal thin film that can be coated on implanted medical devices to enhance tissue growth once implanted *in vivo*.

2. Materials and Methods

2.1 Materials

Linear poly(ethylenimine) (LPEI, Mn = 25000) was bought from Polysciences, Inc (Warrington, PA). Poly (sodium 4-styrenesulfonate) (PSS, Mn = 1000000) and chondroitin were purchased from Sigma-Aldrich (St. Louis, MO). Heparin sodium salt was obtained from Celsus Laboratories (Cincinnati, OH). Poly(β -amino ester)s (PBAEs) Poly 1 and Poly 2 (Figure 2) are cleavable through the ester bond, and differ only in an additional 2 methylene units in the backbone. These PBAEs were synthesized as previously described³⁷. Fibroblast Growth Factor-2 (17.2 kDa, pI = 9.6) and FGF-2 ELISA kits were obtained from Peprotech (Rocky Hill, NJ). Glass slides (for substrates) were obtained from VWR Scientific (Edison NJ).

2.2 Preparation of polyelectrolyte solutions

LPEI and PSS dipping solutions contained 10mM polymer with respect to repeat unit, adjusted to pH 4.25 and 4.75 respectively. Dipping solutions were prepared in sodium acetate buffer (pH 5.1, 100mM) in the following concentrations: heparin, chondroitin, Poly 1 and Poly2 prepared at 2mg mL⁻¹, and FGF-2 prepared at 1.65 g mL⁻¹.

2.3 Film construction and characterization

Glass substrates (1" × 1/4") were plasma etched with room air using a Harrick PDC-32G plasma cleaner on high RF power for 5 minutes. Ten base layers of (LPEI/PSS) were deposited upon plasma etched substrates to create a surface area 3/4" × 1/4" with a Carl Zeiss HSM series programmable slide stainer with 5 minutes of soaking per polymer followed by 3 rinses in deionized water. Slides with ten base layers were sequentially dipped in baths of polymers and growth factors to create the following tetralayer architecture: (PolyX/heparin/FGF-2/heparin)_n where the PolyX is either Poly1 or Poly2 and n refers to the number of tetralayers deposited on the substrate. A typical dipping protocol is 10 minutes in Poly 1 solution, 3 washes, 7.5 minutes in heparin solution, 3 washes, 10 minutes in FGF-2 solution, 2 washes and 7.5 minutes in heparin solution with 3 washes (all washes were performed with non-pH adjusted deionized water). The thickness of films was measured by scoring the samples with a razor blade and measuring the step height using a P10 Surface Profiler with a 2 μm tip radius stylus.

2.4 Release characterization

FGF-2 films were released into 1 mL of 1% serum medium, consisting of alpha Minimum Essential Medium (αMEM) supplemented with 1% fetal bovine serum and 1% penicillin streptomycin (Invitrogen, Carlsbad CA) at 37°C. At a series of different time points, 0.5 mL of medium and eluted material was removed and 0.5 mL of fresh release medium was replaced. Samples were analyzed using Enzyme Linked ImmunoSorbent Assay (ELISA) development kits and cell proliferation assays (see below) according to manufacturer instructions. ELISA analysis of the release medium (1% serum in αMEM) yielded no FGF-2 above the lower detection limit of the test (62.5 pg/mL). At the conclusion of the experiment, residual film was scraped from the surface with a razor blade and analyzed with ELISA to determine the total FGF-2 load and fractional release.

2.5 Cell culture

MC3T3 E1S4 (ATCC, Manassas, VA) were maintained in growth medium consisting of α-MEM supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were split when subconfluent and used until passage 15.

2.6 FGF-2 activity assay

750 MC3T3 cells were seeded in 96 well plates in 100 uL of growth medium and allowed to attach overnight. The medium was changed to 1% serum and after 24 hours 10 uL of eluted material from each sample or control was mixed with 90 uL of 1% serum and applied in triplicate to the wells. After 72 hours, bromodeoxyuridine (BRDU) was placed in the release medium (BRDU kit, Roche Applied Science, Indianapolis, IN) for 4 hours. Similar to [³H] thymidine assays, BRDU is incorporated into the DNA of proliferating cells, which is later analyzed using ELISA. Plates were analyzed according to manufacturer instructions.

3. Results and Discussion

3.1 Film assembly characterization

Multilayer films with a tetralayer architecture were created by alternately dipping FGF-2, which exhibits a net positive charge under physiological conditions (isoelectric point = 9.6), with a non-cytotoxic polyanion and a hydrolytically degradable polycation from a series of poly(β -amino-esters). Figure 1 depicts a schematic of the LbL dipping process and resulting tetralayer architecture of the films, which is denoted through this paper as [PolyX/ Polyanion/FGF-2/Polyanion]_n, where the term in brackets represents one repeat unit (tetralayer) of film and n represents the number of repeat units deposited on the substrate. Two poly(β -amino-esters), Poly1 and Poly2, were utilized in this work; they are identical structurally save for two additional methylene units in the backbone of Poly2. Figure 2 shows the structure of both polymers. Previous work^{36, 39} has shown that in drug delivery applications, use of Poly2 typically results in longer release periods than Poly1. This has been attributed to increased hydrophobicity from the methylene units adjacent to the hydrolytically cleavable ester bond, which decreases water access to the bond and results in longer degradation times than Poly1^{36, 39}.

LbL films were successfully constructed varying both the polyanion and polycation, resulting in the three following architectures: [P2/chondroitin/FGF-2/chondroitin], [P2/heparin/FGF-2/heparin], and [P1/heparin/FGF-2/heparin]. The correlation between thickness of the films and number of tetralayers deposited was tracked with profilometry for the three film formulations in Figure 3. All film architectures tested exhibited a two-regime buildup behavior with a brief period for which thin films with nanometer scale thicknesses are formed, followed by a linear building regime. This characteristic growth pattern has been observed in many LbL systems reported in the literature^{36, 53, 54}. When the film is composed of a few tetralayers (10-20 tetralayers or less), an exponential or superlinear growth behavior is observed in which each additional tetralayer incorporates more material than the previous layer due to interdiffusion of the polymers into the film during deposition. This regime gives way to the second, diffusion-limited linear growth regime, which grows by approximately 200 nm per tetralayer; it is thought that this linear thickness increase is the result of diffusion limitations over the timeframe of the adsorption/absorption step from interdiffusion behavior.

This film growth behavior is typically observed in LbL systems for which one or more of the film components can diffuse into the film during the dipping process, and has been attributed to film rearrangement due to this diffusive process during dipping^{36, 53-56}. This is typically true of biological systems in which lower polymer backbone charge density prevails, or in cases when small, rapidly diffused molecules are entrapped in a film⁵⁷. Such exponential buildup can be advantageous for drug delivery applications, due to the rapid buildup and incorporation of drug compared to linearly building LbL films. The thickness per tetralayer of film, 100 to 200 nm, is an order of magnitude larger than typical values for electrostatic multilayer systems.

3.2 FGF-2 loading and release

After ensuring that FGF-2 containing films would assemble properly, the release characteristics and drug loading of the films were tested. Figure 4 shows the cumulative release of FGF-2 release samples incubated at 37°C in 1% serum cell medium from 50 tetralayer films of each film formulation of interest. [Poly1/heparin/FGF-2/heparin] films exhibit a high level of release over the first 24 hour period with low-level release thereafter, while Poly2 films show a sustained 3- or 5-day release. These results are interesting from several perspectives.

The release curve has a classic power law shape. Previous experiments with LbL PBAE films show that surface erosion contributes a dominant mechanism of release in these LbL films^{36, 58}, which would be anticipated to yield a linear release profile. In this release mechanism, it is hypothesized that degradation of the polymer backbone yields shorter polymer fragments which can more easily break the ionic crosslinks with the film surface than can the full length polymer, allowing the polymer to elute off of the surface and therefore release drug entrapped below it. The power law curve suggests that in addition to surface erosion, a second release mechanism, likely diffusion, is also at work, accounting for the additional curvature to the release profile. ..

The drug loading is affected by both the polycation and polyanion used, with maximum loading observed with P2/H (42 ng/cm²), and minimum loading with P1/H (8 ng/cm²). Comparing first the effect of the polycation, it is seen that P2/H contains five times as much FGF-2 as did P1/H. This may be due in part to the increased hydrophobicity along the P2 backbone, combined with a slightly lower charge density, resulting in a lower effective ionic crosslink density within the film, and hydrophobic pockets within the film that may increase secondary interactions with FGF-2. It has been posited that a lower ionic crosslink density can lead to looser, loopier polymer chain conformations within the film that may also more readily accommodate protein sequestration within the multilayer, and, indeed, model protein Poly2 films are thicker and load more protein than their Poly1 equivalents³⁶. As hypothesized, Poly2 films do release for longer periods of time than Poly1 films. To compare release profiles, the released amounts are plotted as percent of total release in Figure 5; here it is seen that [P2/heparin/FGF-2/heparin] films release over a span of 5 days with 85% of release occurring around day 4 while similar films made with Poly1 ([P1/heparin/FGF-2/heparin] release 85% of their contents in 1 day with slow release of the rest over an additional 2 days. . This supports the notion that, because Poly2 is more hydrophobic and thus less degradable by ester hydrolysis, it takes longer for the film to erode and release its contents.

The polyanion also affects film loading and release. Comparing P2/H and P2/C films, total drug loading capacity of the films is affected, with P2/C films loading approximately 30% of the FGF-2 loaded into the P2/H film. One contributing factor is likely the specific interaction between FGF-2 and heparin, which allows more FGF-2 to be incorporated. However, studies with the model protein lysozyme, which has similar isoelectric point and molecular weight to FGF-2, also showed that P1/H films loaded more lysozyme than P1/C films, indicating that specific binding interactions are not the only contributing factor. In that work, it was posited that specific interactions allow better film packing and increased drug density³⁶. The release of [Poly2/chondroitin/FGF-2/chondroitin] films takes place over about three days while the release of [Poly2/heparin/FGF-2/heparin] films takes place over 5 days; these results are consistent with the work done with lysozyme in which heparin films release over a longer period of time.

In Figure 6, [P2/heparin/FGF-2/heparin] films ranging from 10 to 50 tetralayers thick were released to generate a family of curves, showing that the delivered dose and the time span of release of FGF-2 from these LbL films can be easily tuned simply by changing the number of tetralayers used. In comparing the release times of each number of tetralayers, it is interesting to note that the release curves overlap during the early phase of release, until the film is exhausted (so, for example, the forty and fifty tetralayer release curves overlap until the forty tetralayer film releases its entire load). This is consistent with a surface erosion mechanism of release, in which each layer releases its contents until the entire film thickness is eroded, yielding release profiles that release for the same time span and release rate within the family of curves.

Finally, it was of interest to pursue the fractional release from the film. After 10, 20, 30, 40, and 50 tetralayers were done releasing as detected by ELISA, any remaining visible film was scraped from the surface with a razor blade and vortexed in collection medium. The resulting, non released fraction was run on ELISA to detect unreleased FGF-2 in the polymer matrix. In all cases, the fractional release was 95% or higher of the total drug load (Table 1).

3.3 In vitro assessment of activity

FGF-2 is known to be a potent mitogen for the pre-osteoblastic MC3T3 cell line^{59, 60}, as well as for human osteoblasts⁵⁹, which increases the population of pre-osteoblast cells available to become bone cells *in vivo*. The FGF-2 released from the film was tested in a BRDU proliferation assay (Figure 7). In this assay, only proliferating cells are labeled with BRDU which is later detected by ELISA. FGF-2 released from all three film formulations of interest (P2/C, P2/H, P1/H, Fig 7A, B, C, respectively) shows robust (three or more fold) increases in proliferation over control cells which received no FGF-2. This released FGF-2 maintains proliferative activity far past the period of release from the film, as proliferation rates are elevated for up to 12 days in culture, indicating that FGF-2 retains high levels of activity while in the film. Concentrations of free FGF-2 (100, 50, 5, 0.5 and 0 ng/cm²) introduced into the medium show a dose dependence on FGF-2 (Supplemental figure S1). Interestingly, FGF-2 released from LbL films demonstrate increased ability of up to eight fold negative control values to enhance proliferation compared to the free FGF-2 (about 2 fold), which may be due to the co-release of heparin or chondroitin sulfate. Both heparin and chondroitin are known to increase the mitogenic activity of FGF-2⁴⁹, and thus represent a synergistic addition to the film.

To rule out the possibility that heparin, or another film component released from the film, could increase proliferation alone by helping native FGF-2 present in the cell medium serum bind to MC3T3, films were created without FGF-2 (only inactive components) and were released and tested on cells. All other film components (Poly1, Poly2, chondroitin, and heparin) alone or in combination, showed no proliferative effect on the MC3T3 cells (Fig 3D). Thus, the mitogenic activity of FGF-2 encapsulated within these LbL films is preserved and enhanced by co-delivery with heparin or chondroitin.

4. Conclusions

Although growth factors have an acknowledged ability to aid in the formation of new bone tissue, which is still critically needed in many clinical applications, its delivery for clinical applications thus far has been flawed or limited due to loss of activity and inability to control release in a sustained fashion. FGF-2 is one of a family of growth factors that, if released in combination or sequence with other growth factors, could lead to an enhanced bone formation response that would be clinically relevant. LbL is an ideal candidate drug delivery system for such co- and sequential release schemes due to the possibility of sequestering different growth factors in different layers of a constructed film²⁴ that can be used to coat a bone implant or an engineered scaffold for wound healing. In this work, we demonstrate the first successful incorporation and sustained controlled release of FGF-2 from a synthetic, biodegradable polymer LbL drug delivery system intended to work locally at the site of new bone formation. FGF-2 release is tunable through the polycation, polyanion, and number of tetralayers used to construct the film; FGF-2 released from the film exhibits enhanced ability to promote proliferation in pre-osteoblast cells compared with FGF-2 medium supplementation due to co-release of heparin or chondroitin sulfate. We show the ability to release 45 ng/cm² of growth factor with retained bioactivity over 12 days. While other LbL methods that rely on post-manufacture surface adsorption of growth factor onto LbL films^{28, 61} can deliver similar amounts of growth factor, because the growth

factor is surface-bound rather than sequestered with a degradable synthetic polymer, no control can be exerted over its release profile, nor is sequential release accessible. In other cases ^{7, 62}, the amounts released are modest, rendering autologous *in vivo* cell response difficult to achieve. Thus here, we show a unique system which retains LbL's true potential in biomedical surface modification by combining both a high degree of loading of growth factor in multilayer films while preserving the possibility of sequential release of multiple growth factors or other therapeutic agents. These results significantly enhance our understanding of growth factor delivery from LbL films in a way that will enable *in vivo* bone regeneration models in future work, and represent a necessary step in understanding how LbL can be used to treat clinically relevant problems in growth factor delivery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. David Lynn for helpful and synergistic discussions, and Dr. Robert Langer for his support. Financial support for this work is gratefully acknowledged from Deshpande Center grant 009216-1 and the National Institutes of Health grant 1-R01-AG029601-01. M. Macdonald is thankful for a National Science Foundation Graduate Research Fellowship. This work made use of MRSEC Shared Facilities supported by the National Science Foundation under Award Number DMR-0213282.

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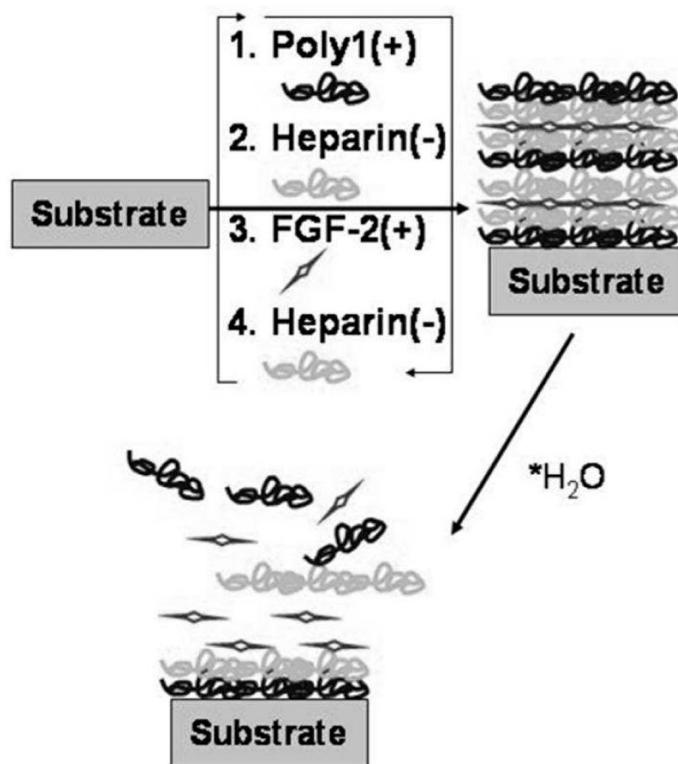
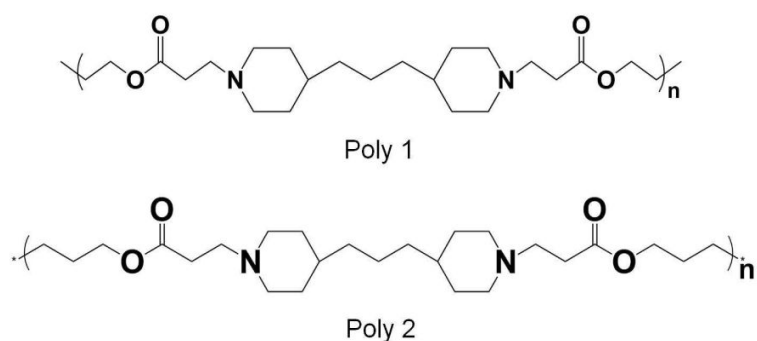


Figure 1. Schematic of the Layer-by-Layer tetralayer architecture employed in this paper and mechanism of release, in which surface erosion through polymer hydrolysis allows for sustained, local delivery of growth factor.

**Figure 2.**

The poly(beta-aminoester)s used in this paper, denoted Poly1 and Poly2, have the same structural characteristics, save for the addition of two methylene units in the backbone of Poly2, increasing the hydrophobicity in the region next to the ester bond which is hydrolytically cleaved and therefore decreasing degradation.

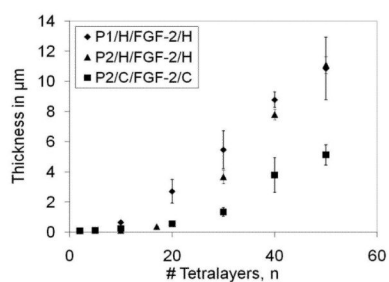


Figure 3.

Film growth is monitored by profilometry. A typical two regime building process is observed in all film formulations, indicating superlinear building and therefore good drug loading potential. In the figure above P1 and P2 = Poly 1 and 2 respectively, H = heparin, and C = chondroitin.

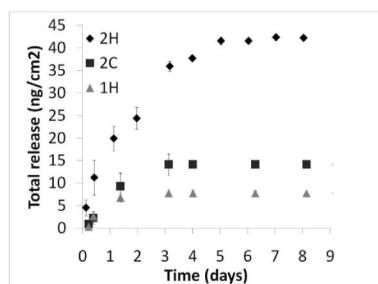


Figure 4.
Release profiles for different 50 tetralayer LbL film architectures

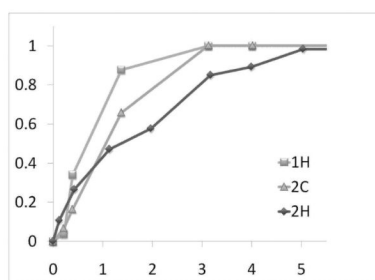


Figure 5. Release from different 50 tetralayer LbL architecture films plotted as a fraction of total release show release profiles for P2 films which have a slower release rate than the release from a P1 film.

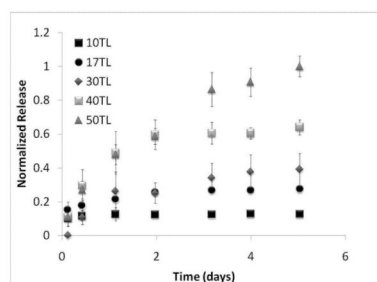


Figure 6.
Release is tunable with number of tetralayers, using [P2/Heparin/FGF-2/Heparin] film as an illustration

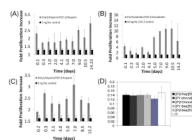


Figure 7.

Released FGF-2 from LbL films retains proliferative effect on MC3T3 cells. Compared with control cells which were not exposed to FGF-2 from the films (“zero” in (A), (B), (C)), cells which were exposed to FGF-2 released from films of different noted architectures showed a marked increase in proliferation rate as measured by BRDU incorporation. (D) All film components tested other than FGF-2 show no positive or negative effect on proliferation compared with control cells not exposed to LbL films (y axis variables are arbitrary units).

Table1

Fractional release of [P2/heparin/FGF-2/heparin] films of varying numbers of tetralayers. Greater than or equal to about 95% release is seen at all numbers of tetralayers, suggesting that all FGF-2 incorporated is released.

# Tetralayers	Percent released
50	98.8
40	100.0
30	94.8
17	95.7
10	99.5