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RESEARCH ARTICLE

Multilayer thin-film coatings capable of extended programmable drug release: application to human mesenchymal stem cell differentiation

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Abstract The promise of cellular therapy lies in healing damaged tissues and organs in vivo as well as generating tissue constructs in vitro for subsequent transplantation. Postnatal stem cells are ideally suited for cellular therapies due to their pluripotency and the ease with which they can be cultured on functionalized substrates. Creating environments to control and successfully drive their differentiation toward a lineage of choice is one of the most important challenges of current cell-based engineering strategies. In recent years, a variety of biomaterials platforms have been prepared for stem cell cultures, primarily to provide efficient delivery of growth or survival factors to cells and a conductive microenvironment for their growth. Here, we demonstrate that repeating tetralayer structures composed of biocompatible poly(methacrylic acid), poly(acrylamide), and poly(ethylene oxide)-*block*-poly(ε-caprolactone) micelles arrayed in layer-by-layer films can serve as a payload region for dexamethasone delivery to human mesenchymal stem cells (MSCs). This architecture can induce MSC differentiation into osteoblasts in a dose-dependent manner. The amount of Dex loaded in the films is controlled by varying the deposition conditions and the film thickness. Release of Dex is tuned by changing the amount of covalent cross-linking of multilayers via thermal treatments. The multilayer architecture including payload and cell-

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adhesion region introduced here are well suited for extended cell culture thus affording the important and protective effect of both Dex release and immobilization. These films may find applications in the local delivery of immobilized therapeutics for biomedical applications, as they can be deposited on a wide range of substrates with different shapes, sizes, and composition.

Keywords Layer-by-layer · Multilayer · Controlled release · Human mesenchymal stem cells · Differentiation · Dexamethasone

Introduction

Surface coatings incorporating therapeutic molecules have recently begun to play an important role in the design of biomedical devices, implantable biomaterials, industrial bioprocesses, and engineered biological interfaces [1–7]. A promising strategy to develop such a biomedical platform is by creating multifunctional polymer coatings that can safely encapsulate and release sensitive biological molecules in an active form within multilayer architectures for controlled release directly from film surfaces [8–14].

Layer-by-layer (LbL) deposition has been widely used to fabricate multilayer architectures by complementary interactions between components, such as positively and negatively charged materials [15, 16] or materials that have hydrogen bond donor and acceptor groups [17, 18], where each layer is adsorbed sequentially onto a surface to achieve a film. Due to a growing interest in applying multilayer architectures to biomedical applications, erodible multilayers that degrade in a controlled manner via disassembly are being explored as a potential platform for controlled release drug delivery over extended time periods [8, 19, 20]. Automated LbL deposition methods are advantageous in that the films are prepared under mild aqueous conditions that preserve drug bioactivity. Furthermore, incorporation of various therapeutics into multilayers for systematic release has been studied from small-molecule steroids and antibiotics to macromolecules such as protein therapeutics, active proteins, enzymes, nucleic acids, and plasmid DNA [16, 21–28]. Multilayers can be conformally coated onto substrates with simple or complex geometries to load therapeutics and control their release over a defined time period. More recently, multilayer thin films have been developed to permit the release of drug dosages with desired periods of time ranging from minutes to days. Thus, there is tremendous potential to use these polyelectrolyte films as cell differentiation platforms. Multilayer thin films are well suited for this application due to the flexibility these structures afford in terms of biocompatibility, material properties, cost of application, and the ability to finely tune surface characteristics as desired. The ability to achieve precise and extended-term drug delivery will enable a broader set of clinical applications where precision dosing must occur over longer time spans [29–33].

Human mesenchymal stem cells (MSCs), also known as multipotent stromal cells are adult progenitors that maintain the potential to differentiate into numerous cell types found within adult connective tissues. A wide range of functionally, morphologically, and transcriptionally distinct phenotypes can be derived from a common MSC precursor. This intrinsic multipotent property, coupled with the ability to isolate large quantities of these cells from the bone marrow, highlights the potential of MSCs as a cell source for regenerative medical applications [34–37]. MSCs are influenced by their physical microenvironment (stiffness and geometry), as well as by biochemical cues from small molecules, and exogenous, paracrine, and autocrine signaling. The utility of thin-film multilayer surfaces derives from the ability to influence all aspects of the MSC niche. Tuning of surface features and delivery of biochemical signals in the form of small molecules are both possible using this approach.

In this work, we present a simple strategy to prepare functional surfaces with tunable and durable biological activity by taking advantage of LbL-assembled multilayer thin films with embedded small bioactive molecules to guide MSC differentiation. To safely encapsulate drugs, polymeric micelles are introduced into LbL films. Specifically, we have constructed LbL films of repeating tetralayer structures composed of biocompatible poly(acrylamide) (PAAm), poly (methacrylic acid) (PMAA), and poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) micelles. In this $(PMAA/PAAm/PMAA/PEO\text{-}b\text{-}PCL)_n$ (n=number of tetralayers) architecture, hydrogen bonding for both PMAA/PAAm, and PMAA/PEO-*b*-PCL were employed to build multilayers. We demonstrate that the resulting films degrade in a repeatable and predictable manner to release drug-loaded micelles into the surrounding cell culture medium under physiological conditions. Furthermore, we are able to tailor the rate of film degradation and drug release by varying the degree of covalent anhydride cross-linking between carboxylic acid groups in PMAA, thus slowing degradation to the desired rate. Since micelle incorporation into multilayer thin films and encapsulation of drugs into micelles do not require specific chemical modifications during the fabrication process, and introducing further cross-linking permits control over the release profile of drugs, this approach is particularly well suited for incorporating active hydrophobic therapeutics for various biological and biomedical coatings.

Experimental section

Materials PMAA (Mw 15,000 30 % aqueous solution) and PAAm (Mw 5,000,000 1 % aqueous solution) were obtained from Polysciences Inc. (Warrington, PA). Amphiphilic

block copolymer, PEO-*b*-PCL (Mn (PEO), 5,000; Mn (PCL), 6,500; and PDI, 1.3) was purchased from Polymer Source (Montreal, Canada). Poly(diallyldimethyl ammonium chloride) (PDAC; Mw=200,000–350,000 g/mol) and poly (sodium 4-styrenesulfonate) (PSS; Mw=70,000) were purchased from Aldrich. PDAC (Mw=200,000–350,000 g/mol) and PSS (Mw=70,000) were purchased from Aldrich. Sodium hyaluronate (or hyaluronic acid (HA), Mn=1.76 MDa) was purchased from Lifecore Biomedical, Inc. (Chaska, MN). Collagen I from rat-tail, collagen I from calf skin, collagen IV from human placenta, and dexamethasone (Dex) were obtained from Sigma. All other reagents and solvents were purchased from Aldrich and used as received. Quartz slide, silicon wafer, Teflon, and polypropylene were used as substrates for the LbL assembly and cleaned extensively prior to the deposition.

Block copolymer micelle formation Block copolymer micelles of PEO-*b*-PCL were prepared according to a modification of a previously published method. Briefly, a stock solution of PEO-*b*-PCL was freshly prepared in tetrahydrofuran (THF) at a concentration of 10 mg/mL. Then, 200 mL of stock solution was placed in a vial with gentle stirring. To this solution was gradually added 5.0 mL of Millipore water (18 Mcm) with vigorous stirring. After an additional 1 h of stirring, the resulting suspension was subjected to dialysis against Millipore water for over 24 h (Spectra/Por 4 regenerated cellulose membrane; MWCO, 12,000–14,000) to remove any residual solvent. The resulting PEO-*b*-PCL micelle was filtered prior to use. The pH of the resulting micelle suspension was adjusted with 0.10 M HCl solution right before LbL film formation. For Dex loading, we followed a modification of previously published protocol. Briefly, Dex solution in THF (concentration, 1.0 mg/mL) was dropwise added to 5.0 mL of the micelle suspension prepared above (0.40 mg/mL). The emulsion was vigorously stirred overnight with a loose cap to evaporate the organic solvent. The solution obtained was centrifuged (4,500 rpm at 10 min) and filtered through a 0.45-μm syringe filter. The drug content in a micelle was evaluated by measuring the characteristic absorbance of Dex in a solvent mixture of MeOH and H_2O (9:1, *v*/*v*), employing a calibration curve with a known concentration of Dex using an Agilent 8,453 ultraviolet–visible (UV–vis) spectrometer.

LbL film preparation All LbL films were assembled with a modified programmable Carl Zeiss HMS slide stainer. Typically, films were constructed on various substrates with approximate size of 1~2 $in.^2$. The O_2-plasma-treated substrate was first dipped into PMAA aqueous solution (10 mM at pH 3) for 10 min and rinsed three times with water (pH 3) for 1 min each. Subsequently, the substrate was introduced into aqueous solution of PAAm (10 mM at pH 3), PMAA aqueous solution (10 mM at pH 3), and PEO-*b*-PCL micelle solution (0.40 mg/mL at pH 3) for 10 min and washed again three times with water (pH 3) for 1 min each. This cycle provides one tetralayer of PMAA, PAAm, PMAA, and PEO-*b*-PCL micelle, denoted "PMAA/PAAm/PMAA/PEO-*b*-PCL." The dipping process was repeated until the desired number of bilayers was obtained. Cell adhesion region was prepared by spin-LbL deposition (2,500 rpm at 30 s). Prepared multilayer films are annealed in oven at 150 °C for predetermeaned time in vacuum. All cell adhesive solutions were filtered through a 0.22-μm sterile filter prior to spinning. Sterility of the underlying layers was achieved as a result of the annealing heat treatment at 150 °C for several hours. All films were stored dry in individual tissue culture containers under sterile conditions at room temperature prior to use and were used within 24 h of fabrication. Films were submersed in enough cell culture medium to cover their surfaces and placed in a tissue culture incubator for 20 min prior to cell seeding in order to equilibrate film temperature at 37 °C.

Surface morphology The surface morphology of BCM multilayer films was investigated by field emission scanning electron microscopy (XL30FEG, Philips)

Measurement of film thickness Following deposition, films were immediately removed from the final rinsing bath and nitrogen dried. Film thickness was determined either by ellipsometry at ten different predetermined locations on the film surface or by profilometry at three different scratch sites.

UV–vis spectroscopy The UV–vis spectra were obtained using a PerkinElmer Lambda UV–vis spectrometer. Dex showed the absorbance peak centered at 238 nm.

Cell culture All MSCs used in this work were passage three human bone marrow derived mesenchymal stromal cells. Passage two human MSCs were obtained from the Texas A&M Health Sciences Center and expanded one additional passage before each experiment. All experiments were conducted using cells derived from the same patient lot. Cells were expanded in Dulbecco's modified Eagle's medium supplemented with 16.5 % fetal calf serum from Atlanta Biologicals using a specific lot recommended by Texas A&M HSC for MSC, 4.5 g/L glucose, L-glutamine, sodium pyruvate, nonessential amino acids, and penicillin/streptomycin at 37 °C and 5 % CO_2. Media was changed every 72 h. All films were seeded at initial densities of 5,000 cells/cm^2.

Alkaline phosphatase activity assay Alkaline phosphatase activity was measured using the colorimetric substrate para-nitrophenylphosphate, which develops a yellow end-product readable at 405 nm (pNPP ALP Kit, Sigma-Aldrich). Samples were incubated at room temperature for 60 min then stopped with 2 N NaOH. Standard curves were generated using dilutions of *p*-nitrophenol. Activity was

normalized to total protein content as measured by BCA assay (Pierce). Identically treated blank control films (no cells) were used to assess the amount of protein contributed by the cell adhesion region in order to correct for background protein in the normalization.

Quantitative real-time PCR RNA extracts were prepared using the Qiagen RNeasy kit. Samples were normalized to 500 ng total RNA content prior to quantitative real-time PCR (qRT-PCR) cycling. All biological replicates were aliquoted into three technical replicates. Primers and OneStep qRT-PCR kits were from Qiagen. Realtime optical readings were performed using the Chromas 4 system. Abundance levels for each gene (A) of interest were calculated from the take-off cycle (Ct) and efficiency (E) for each gene using: $A=1/(1+E)$Ct. All sample abundances were normalized to GAPDH abundance for each experimental condition.

Results and discussion

To develop a polyelectrolyte multilayer film with tunable drug loading and controlled release properties, we created three functional regions in a composite multilayer thin film (Fig. 1a). First, a nondegradable base layer was prepared directly onto the substrate to mitigate substrate effects and build up sufficient surface charge for subsequent multilayer adsorption by precoating a planar silicon wafer or glass slide with ten and a half bilayers of PDAC and PSS (ca. 67 nm thick). Next, a payload region composed of a drug-loaded micellar nanocontainer was fabricated. Dex is a synthetic glucocorticoid that can induce early commitment along an osteogenic lineage when applied to MSCs [38–40]. Dex was encapsulated within the hydrophobic compartments of PEO-*b*-PCL micelles and assembled into multilayer thin films. The Dex containing PEO-*b*-PCL (PEO-*b*-PCL^{dex}) micelles had a hydrodynamic diameter of 98.8±13.4 nm in solution, measured by dynamic light scattering. The payload region consisted of 20 tetralayers of electrostatically and hydrogen-bonded PMAA/PAAm/PMAA/PEO-*b*-PCL^{Dex} and was terminated with 3.5 (PMAA/PAAm) bilayers. The advantages of the tetralayer architecture are: (1) even distribution of Dex in the payload region, (2) ability to generate anhydride bonds between PMAA groups with thermal treatment to control the film cross-linking density of the tetralayer, and (3) the ability to easily control the amount of Dex incorporated. The topmost region of the film was cell adhesive prepared by collagen, polysaccharide and disaccharide to promote cell adhesion at top of multilayer film (Fig. 1a). It is noted that the Dex molecule is stable at temperatures up to

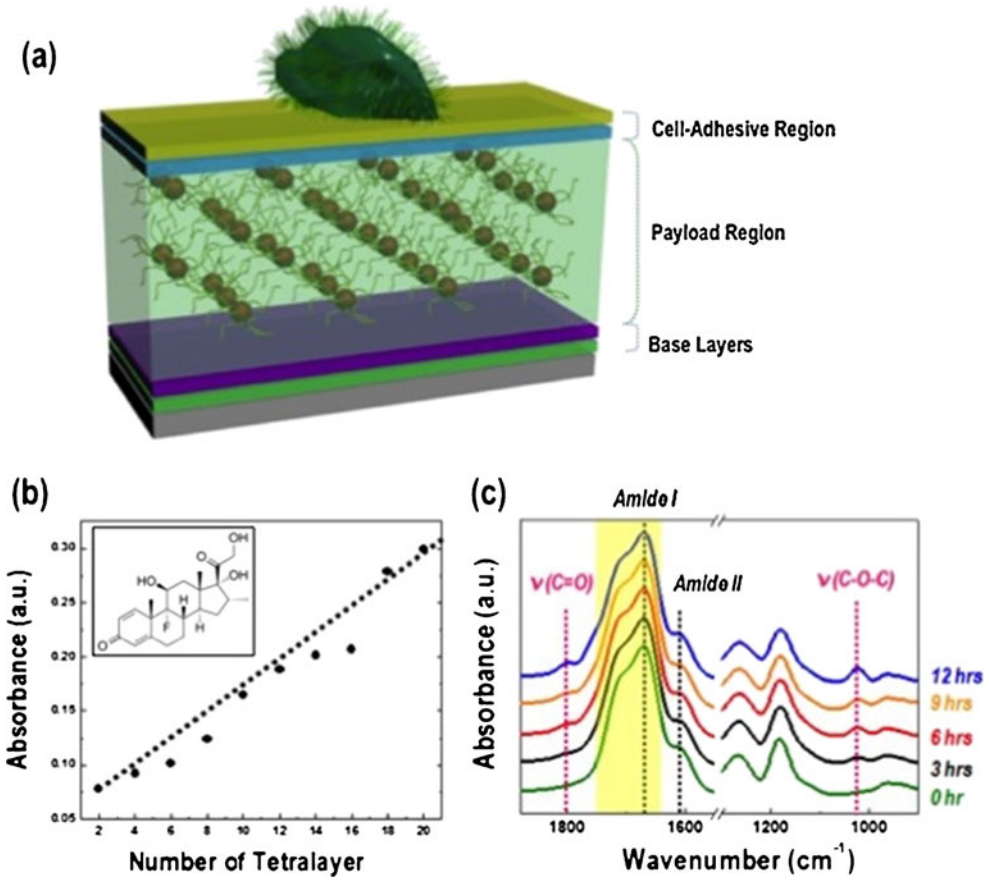

Fig. 1 **a** The schematic illustration of the thin-film multilayer platform. **b** UV–vis absorption of (PMAA/PAAm/PMAA/PEO-*b*-PCL^{dex})$_{20}$ multilayer films as a function of tetralayer number. The absorption peak at 238 nm, which originates from Dex in PEO-*b*-PCL micelles, was used for absorbance measurements here (structure of Dex (*inset*)). **c** FT-IR absorption spectra of multilayer films with different heat treatment times

above 200 °C, as was demonstrated by both differential scanning calorimetry and thermogravimetric analysis in previously reported literature, [41] and that there is no indication of loss of function for this small molecule drug when exposed to temperatures below 200 °C. The thermal annealing temperatures used in this study were performed at 150 °C, well below the degradation temperature for the drug.

The Dex absorbance peak at 238 nm was monitored as the (PMAA/PAAm/PMAA/PEO-*b*-PCL^{dex})$_n$ films were assembled. The absorbance at 238 nm grows linearly with increasing tetralayer numbers of (PMAA/PAAm/PMAA/PEO-*b*-PCL^{dex}) (Fig. 1b) demonstrating linear multilayer film growth. The average thickness per tetralayer was 67.1± 6.8 nm. Covalent cross-linking in the LbL films was achieved through heat treatment at 150 °C under vacuum for up to 12 h. Figure 1c is the FT-IR spectrum of the heat-treated (PMAA/PAAm/PMAA/PEO-*b*-PCL^{dex})$_{20}$(PMAA/PAAm)$_{3.5}$ payload region with respect to time. New peaks at 1,042 and 1,804 cm^{-1} appeared after heat treatment that are attributed to anhydride bond formation by PMAA-PMAA (acid anhydride) through a condensation process. However, the peaks for amide and anhydride overlap as indicated by the asymmetric and symmetric signatures of C=O in PEO-*b*-PCL (1,780^{-1} to 1,660 m^{-1}). Therefore, covalent-bonded cross-linking derived from heat treatment was indirectly confirmed through the formation of anhydride peaks (i.e., 1,042 and 1,804 cm^{-1}). The average film thickness of the payload region before heat treatment was 1,432±87 nm while after treatment it decreased to 1,227±15 nm, a reduction of about 7 %; polymer shrinkage to this extent is typically also observed in bulk polymer systems following imide or anhydride cross-linking. Heating the multilayer film at 150 °C for 12 h introduces chemical cross-links that stabilize the multilayer assembly even at pH 7.4 in phosphate-buffered saline (PBS) solution. The heat-treated multilayer film experiences an 8.5 % film thickness reduction after 60 h of immersion in PBS solution. Films that are not cross-linked by heat treatment dissolve immediately when immersed in PBS due to the immediate ionization of the PMAA groups, and disassembly of the hydrogen-bonded multilayers due to electrostatic repulsion; cross-linking the film for shorter time periods such as 3 h, on the other hand, does not enhance the film resistance to the PBS buffer to a large extent due to a low degree of anhydride linkage formation. It is noted that no catalyst or activation group was used to introduce cross-links in this study; however, such approaches such as the use of NHS and DCC coupling agents could be introduced here, as well as accelerating agents for anhydride formation to achieve much more rapid reaction times.

A cell adhesive region was introduced onto the payload region to control MSC adhesion to the substrate; various native biological macromolecules that contain specific binding motifs for cell adhesion receptors were examined. Positively charged collagen I (most abundant form of collagen) from rat tail, collagen III from calf skin, and collagen IV (most interstitial tissues) from human placenta, as well as negative-charged HA, heparin, and the synthetic polyanions, PMAA, and poly(acrylic acid) (PAA). Triple helix-rich fragments of collagen are well-known adhesion substrates to cells, and HA is a highly attractive natural biomaterial due to its role in the extracellular matrix [42] and its influence on cell behavior [43]. Heparin, a highly sulfated glycosaminoglycan, is widely used as an injectable anticoagulant and has the highest negative charge density of any known biological molecule [44, 45]. PMAA and PAA are also well-known FDA-approved biocompatible polyelectrolytes [46, 47]. Alternating adsorption of the different collagens with each of the polyanionic species was used to fabricate a series of 4.5 bilayer LbL film surfaces for cell adhesion, which were screened for cell attachment and cytocompatibility (see Electronic supplementary material). We examined the effect of the various surfaces on initial cell adhesion. Phase-contrast micrographs were taken 24 h after MSC seeding in MSC growth medium at densities optimal for differentiation. Among the combinations, multilayers prepared with PMAA and PAA spontaneously peeled off in PBS buffer, which is not suitable for cell adhesion. On the other hand, among the other multilayer systems examined (i.e., collagens I and III multilayered with HA and heparin) exhibited better cell adhesion, higher cell density, and better stability in solution. A 4.5 bilayer of (collagen I/HA), with average thickness of 112±19 nm, was selected as the cell adhesive surface region to be used for further experiments.

The surface morphology of both the payload and cell-adhesion regions were observed using SEM (Fig. 2). Dex-encapsulated PEO-*b*-PCL micelles remain distinguishable on the surface as aggregates, as shown in Fig. 2a. These films exhibit high roughness on the scale of a few microns, which is presumably the result of aggregation of micelles that occurs during the LbL assembly process, as shown in Fig. 2a. After coating 4.5 bilayers of collagen I/HA multilayers onto the payload region, we observed a thin-film structure that provides complete coverage of the underlying payload morphology, and presents its own unique micron scale roughness (Fig. 2b).

Dex release experiments were performed by immersing a substrate in a sealed vial containing 3 mL of buffered solution. At indicated time points, films were removed and reimmersed into appropriate buffer solution. Figure 3a shows the change in the total multilayer film thickness with time following immersion in PBS solution. The thickness of the portions of film remaining on the surface was determined to decrease linearly with respect to time of exposure to PBS solution over predetermined time periods. Furthermore, we expect that the large error bars in Fig. 3a are due to changes in the film's uniformity after significant swelling during PBS solution exposure. Films with different cross-linking densities demonstrate swelling to differing degrees and dissolution upon

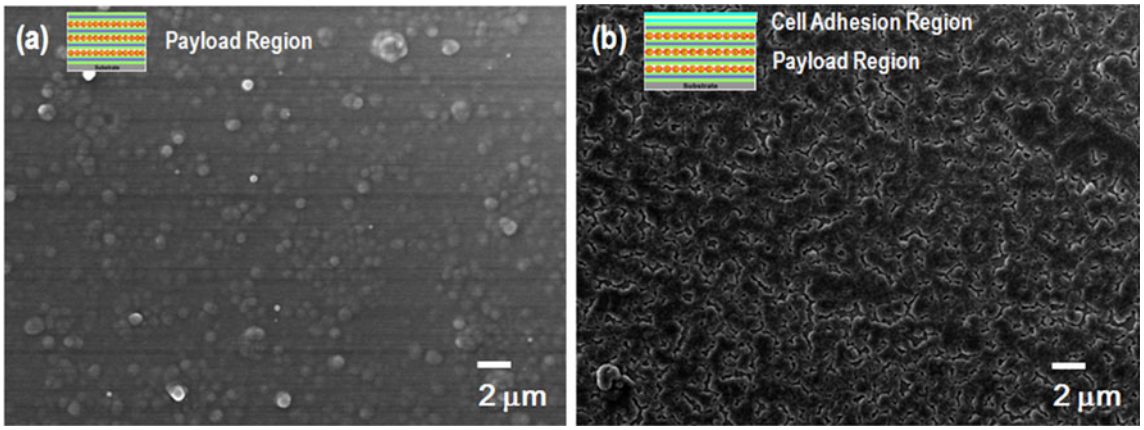

Fig. 2 SEM images of **a** substrate/$(PDAC/PSS)_{10.5}(PMAA/PAAm/PMAA/PEO\text{-}b\text{-}PCL^{dex})_{20}(PMAA/PAAm)_{3.5}$ and **b** substrate/$(PDAC/PSS)_{10.5}(PMAA/PAAm/PMAA/PEO\text{-}b\text{-}PCL^{dex})_{20}(PMAA/PAAm)_{3.5}$(collagen I/HA)$_{4.5}$. Both images are measured as prepared

submersion. Following a brief initial swelling period, hydrolysis of the anhydride cross-links emerges as the dominant factor for decreasing film thickness. In all cases, film thickness decreases at a constant rate: approximately 438, 433.6, 7.47, 3.63, and 1.88 nm/h for 0-, 3-, 6-, 9-, and 12-h heat treatment-based conditions, respectively.

Finally, to examine the potential of a drug payload region as a platform for hydrophobic therapeutic delivery the release profiles of Dex were evaluated by measuring the Dex release from the payload region after incubation in a model physiological solution (PBS buffer, 37 °C, 5 % CO_2). Dex was released over a course of two hours from untreated multilayer films, whereas the cross-linked film exhibited a significantly longer release of up to 72 h for 6 h thermally cross-linked films and 290 h for 12 h thermally cross-linked films (Fig. 3). The amount of Dex released was calculated on the basis of UV–vis measurements to be 18.1 μg/cm^2 for a $(PMAA/PAAm/PMAA/PEO\text{-}b\text{-}PCL^{dex})_{20}$ (ca. 0.7 × 0.7 cm^2), which is well above the known Dex concentration in osteogenic medium (typically 10 nM). This is a unique feature of the hydrogen-bonded system, for which simple postmodification to introduce internal cross-links within the LbL film enables the control of the release kinetics of active small, hydrophobic therapeutics from 3 up to 290 h. We expect that this fine control over the release profile will be highly advantageous for potential use in biological applications that require a controlled release of hydrophobic active therapeutics. For example, untreated, non-cross-linked films would be interesting for wound dressings and therapeutic surface coatings, where an immediate delivery of therapeutics is necessary upon contact with the film. In contrast, cross-linked film would be useful as a carrier for active therapeutics

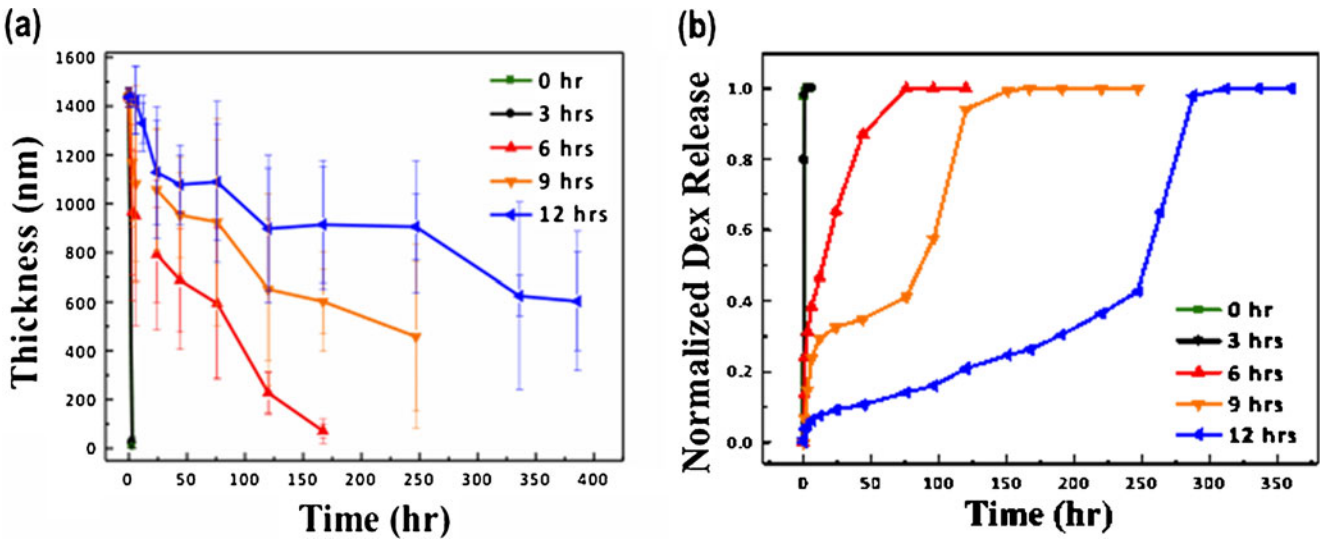

Fig. 3 **a** Film thickness as a function of release time following immersion in PBS at pH 7.4. *Error bars* represent the standard deviation of measured thickness values. **b** Cumulative normalized kinetics of Dex release from platform immersed in PBS (pH 7.4 at 37 °C), for films prepared with different cross-linking densities by heat treatment. In both (**a**) and (**b**), total film thickness includes payload and cell adhesive regions

on biomaterial surfaces for an extended period of time such as biomedical implants.

To assess the ability of Dex-loaded thin-film multilayers to exert a biological effect we examined the expression of early osteogenic markers in MSCs cultured on multilayers which produce a controlled Dex release over extended times. Cells were cultured for 14 days on three different kinds of multilayer films including cell adhesive region only (Fig. 4a), payload without dex/cell adhesive region (Fig. 4b) and payload with dex/cell adhesive region (Fig. 4c) with a Dex release profile matching the longest release time. As shown in Fig. 4d, MSCs cultured on Dex releasing surfaces exhibited a 2-fold increase in alkaline phosphatase activity when compared with MSCs cultured on control surfaces with no Dex payload. Likewise, mRNA for later markers of osteogenic differentiation such as osteopontin and osteocalcin were upregulated by 1.5- and 20-fold, respectively, when compared with control cultures, as indicated by quantitative real time PCR in Fig. 4e. The ability of thin films with a Dex payload to induce early and mid-point osteogenic markers in MSCs is indicative of the utility of these surfaces to bring about real biological effects over time spans that are therapeutically relevant. The induction of osteogenic markers in MSCs using only expansion medium and under conditions which derive osteogenic stimuli only from the payload release and cell adhesive properties of the thin-film substrate is a clear demonstration of the potential of this approach.

Furthermore, in this study both EGF and EGFR are used as a proxy for the differential regulation of proliferation-associated transcripts in response to Dex dosing and as a way of assessing the biological impact of Dex along a pathway independent of osteogenesis. As shown in Fig. 4e, EGF is slightly up regulated while there is no significant differential regulation of EGFR expression versus the control. These results may be interpreted to indicate that by day 14, there is relatively limited upregulation of proliferative signal along the EGFR pathway, consistent with lineage commitment and differentiation.

Conclusions

We have demonstrated a simple, tunable drug-release thin-film multilayer platform that is capable of producing significant biological effects over extended time periods. Using our approach, we demonstrated early osteogenic differentiation of mesenchymal stem cells induced through controlled release of Dex from a payload region as well as from surface adhesion

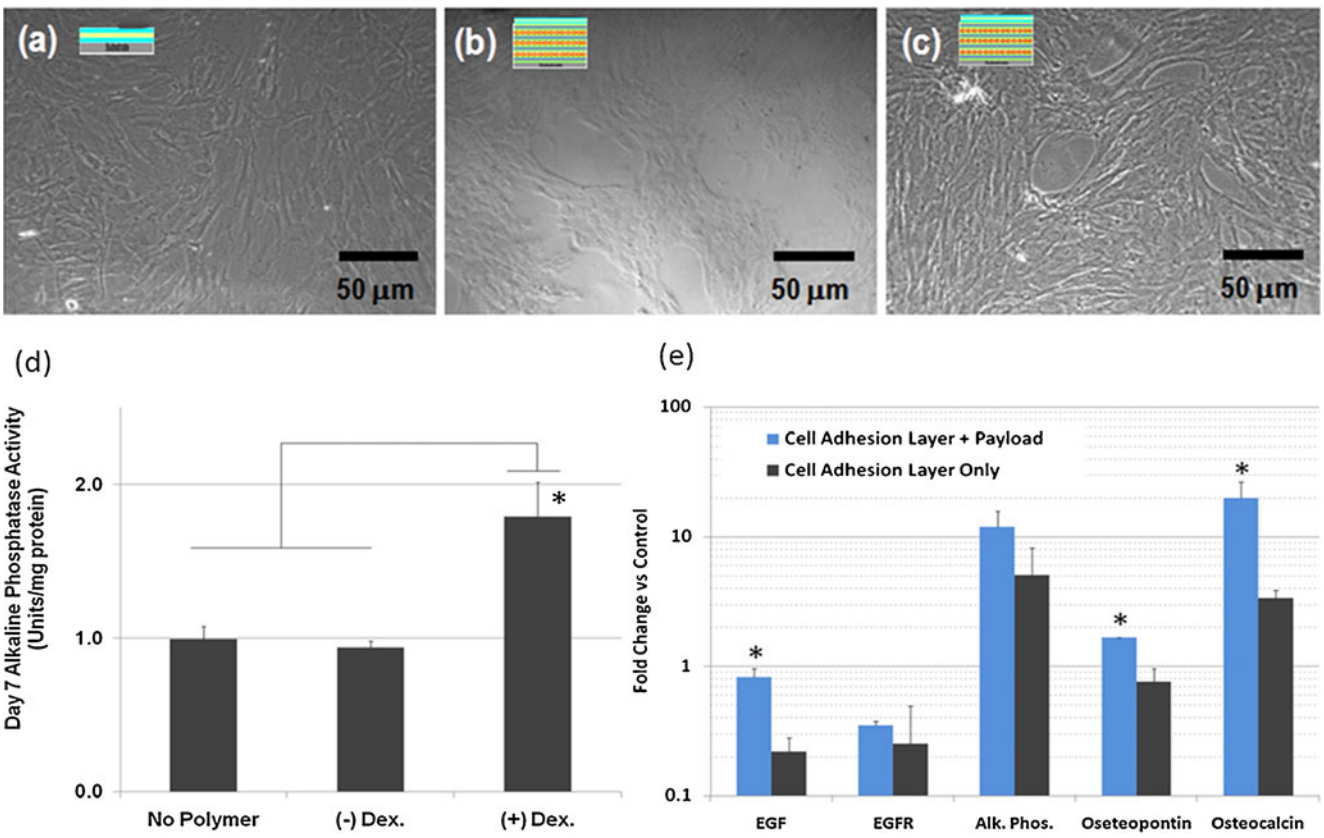

Fig. 4 Brightfield micrographs of MSCs after 14 days on **a** glass/$(PDAC/PSS)_{10.5}$(collagen I/HA)$_{4.5}$, **b** glass/$(PDAC/PSS)_{10.5}$(PMAA/PAAm/PMAA/PEO-*b*-PCL)$_{20}$(PMAA/PAAm)$_{3.5}$(collagen I/HA)$_{4.5}$, and **c** glass/$(PDAC/PSS)_{10.5}$(PMAA/PAAm/PMAA/PEO-*b*-PCL^{dex})$_{20}$(PMAA/PAAm)$_{3.5}$(collagen I/HA)$_{4.5}$. **d** Alkaline phosphatase activity on day 7 under the three culture conditions shown (n=3 replicates; "+/–" SD; *p< 0.01). **e** Fold change in gene expression levels of the genes shown on day 10 (n=3 replicates; "+/–" SD; *p<0.05)

cues. The simplicity of this design is attributable to the few functional layers required: a substrate base layer, a payload region, and a cell adhesion region. The best performing construct incorporating these layers consisted of (PMAA/PAAm/PMAA/PEO-*b*-PCL^{dex})$_{20}$(PMAA/PAAm)$_{3.5}$ and (collagen I/HA)$_{4.5}$, which exhibited highly tunable release profiles to complement the surface cues from collagen I and HA. The ability to tune drug release and surface properties to this extent opens additional applications of LbL technology to challenging problems in regenerative medicine.

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