Adaptive growth factor delivery from a polyelectrolyte coating promotes synergistic bone tissue repair and reconstruction

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Adaptive growth factor delivery from a polyelectrolyte coating promotes synergistic bone tissue repair and reconstruction


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Edited by Kristi S. Anseth, Howard Hughes Medical Institute, University of Colorado, Boulder, CO, and approved July 23, 2014 (received for review May 1, 2014)

Traumatic wounds and congenital defects that require large-scale bone tissue repair have few successful clinical therapies, particularly for craniofacial defects. Although bioactive materials have demonstrated alternative approaches to tissue repair, an optimized material system for reproducible, safe, and targeted repair remains elusive. We hypothesized that controlled, rapid bone formation in large, critical-size defects could be induced by simultaneously delivering multiple biological growth factors to the site of the wound. Here, we report an approach for bone repair using a polyelectrolyte multilayer coating carrying as little as 200 ng of bone morphogenetic protein-2 and platelet-derived growth factor-BB that were eluted over readily adapted time scales to induce rapid bone repair. Based on electrostatic interactions between the polymer multilayers and growth factors alone, we sustained mitogenic and osteogenic signals with these growth factors in an easily tunable and controlled manner to direct endogenous cell function. To prove the role of this adaptive release system, we applied the polyelectrolyte coating on a well-studied biodegradable poly(lactic-co-glycolic acid) support membrane. The released growth factors directed cellular processes to induce bone repair in a critical-size rat calvaria model. The released growth factors promoted local bone formation that bridged a critical-size defect in the calvaria as early as 2 wk after implantation. Mature, mechanically competent bone regenerated the native calvaria form. Such an approach could be clinically useful and has significant benefits as a synthetic, off-the-shelf, cell-free option for bone tissue repair and restoration.

Significance

A critical challenge in the field of tissue repair is effective bone repair and reconstruction. The clinical standard of extracting bone from another area in the body or from donors is severely hampered by short supply, pain, and concerns about disease transmission. In this study, we developed a polymer-based nanolayered coating that carries active biological drugs in physiologically relevant amounts for tissue repair, with tunable release properties to induce bone repair. Using a rodent model, we observed that these coatings yield mature, mechanically stable bone that bridges large defects and restores the native form. This system is a potent strategy for safe and precise tissue repair and has the potential to significantly boost successful outcomes for bone repair.


Conflict of interest statement: H.J.S. is an employee at Bioventus LLC, owns stock in, and is a paid consultant for, Pfizer, Inc.

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Because the requirements for bone regeneration are difficult to achieve with a single material system, scaffolds with the appropriate physicochemical properties have been investigated for their potential in bone regeneration (7). Structurally, the pore morphology defines the interaction between the scaffold and the host environment and directly affects bone formation. Pores allow nutrient flow and migration of progenitor cells, and they support vascularization. Small pores can limit cell migration and can result in the formation of a cellular capsule around the scaffold, which can hinder diffusion processes and result in necrotic regions. Conversely, pores that are too large have a reduced surface area for cell adhesion and may allow prolapse of soft tissue in a bone wound. Various methods of scaffold fabrication yield different pore size distributions from a few nanometers to hundreds of micrometers and have been examined for bone regeneration. On the materials side, tricalcium phosphate (TCP)-based scaffolds loaded with biologics have been examined, but have not yet provided a highly controlled release pattern of growth factors and loading amenable to clinical implementation (8). Hybrid materials, including various polymer/calcium phosphate composites, have been explored with tunable degradation properties. Extracellular matrix-based materials, such as collagen–glycosaminoglycan scaffolds and hyaluronic acid hydrogels, have also been employed with cells that are seeded before grafting (9, 10). Softer materials, including hydrogel-based delivery systems, can effectively present biological cues, such as growth factors at low doses (11, 12). A range of BMP-2 doses have been explored from biomaterial carriers—from 100 ng to 2 mg in rats—and bone regeneration was observed at all of these doses (13). In general, next-generation biomaterial delivery vehicles: (i) aim to cover large defects in such a way as to maintain a bony contour; (ii) provide a controlled tunable release of the growth factors; (iii) enable the use of a safe, low dose of the growth factor without reducing the osteogenic effectiveness of the device; and (iv) sometimes require the addition of progenitor cells, which are expanded ex vivo.

We focused on incorporating BMP-2 and PDGF-BB in a nanolayer coating that could be applied to a broad range of scaffolds, from metals to degradable plastics and TCP constructs, including highly porous and geometrically complex shapes and contours that might be used for craniofacial reconstruction. The coating consists of a micron-scale poly(ethylene-co-vinyl alcohol) multilayer (PEM) thin film composed of layers of BMP-2 and PDGF-BB growth factors and may be adapted to release nanograms of growth factor per square millimeter for extended and physiologically meaningful time periods for bone healing by endogenous progenitor cells. Our previous studies have demonstrated that we can easily apply the PEM coatings to any substrate of choice, which can be other orthopedic polymers such as poly-caprolactone and polyether ether ketone, metals such as titanium, and calcium phosphates (14–17). To demonstrate the efficacy of this approach, we chose to coat a well-studied biodegradable porous poly(lactic-co-glycolic) acid (PLGA) membrane with well-understood physicochemical properties. This coated membrane was generated by using a simple phase-inversion casting method, cut, and customized to the size of the wound before application, allowing it to induce targeted bone repair. The polymer membrane had microstructures with interconnected pores that allowed for deposition and sequestration of active biologics and bone repair materials within the poly-electrolyte coatings (Fig. 1A). The nanolayer coating that we designed for this system causes PDGF-BB to release quickly, along with a more sustained release of BMP-2.

The use of a highly tunable release system that is independent of the underlying substrate allowed the investigation of the impact of controlled staggered release of key growth factors. We hypothesized that time-dependent growth factor delivery from the membrane would (i) recapitulate cellular-regenerative processes and substantially enhance bone formation by inducing a mitogenic and osteogenic response; and (ii) promote rapid bone repair and provide a supporting structure to guide the regenerative process where needed. To potentially enhance bone formation by modulating bone resorption, we also explored the use of alendronate, a bisphosphonate that binds to the mineral phase of osteous tissue, and an inhibitor of osteoclast resorption of bone. We demonstrated that growth factors released from the PEMs allowed direct control of the bone-regenerative process to rapidly induce repair in a critical-size rat calvaria defect with mechanically competent bone.

Results
Tunable Growth Factor Deposition in PEM Coatings. We used PEMs, which are nanostructured coatings formed by a layer-by-layer (LbL) technique of iterative adsorption of alternately charged materials, to design a tunable hydrodynamically degrading system (18–20). PEMs can sequester and elute multiple biologic cargos in a controlled, preprogrammed manner over several weeks; the release profiles can be tuned by modifying the multilayer architecture. We used Poly2, with an aliphatic backbone and a known hydrolytic degradation profile, as the cationic species in the PEM coating. The LbL coating composition consisted of Poly2, poly(acrylic acid) (PAA), and a growth factor (PDGF-BB or BMP-2) in a tetralayer repeat unit: Poly2/PAA/PDGF-BB/PAA or Poly2/PAA/BMP-2/PAA, denoted as P and B, respectively. The subscripts indicate the total dose per implant of each growth factor in micrograms. To create a gradient concentration of growth factors, the BMP-2–containing layers were deposited directly on the membrane surface. Subsequently, the PDGF-BB–containing layers were deposited on top of the BMP-2–containing layers.

Modification of PLGA Support Membrane. We created a porous, degradable PLGA membrane using a solvent-induced phase
inversion technique from a ternary system of PLGA-DMF-water (21). The resulting membrane had a hierarchical architecture and a tunable surface chemistry (Fig. 1B). The side in direct contact with the glass plate (bottom surface) during phase inversion had a broad pore size distribution, that spanned ~1.5 to ~20 μm (Fig. 1 C–F). The top surface (away from the glass plate) had much smaller pore sizes that were <500 nm. The data indicate that there is a general trend of smaller pore sizes on the top surface and larger pore sizes on the bottom surface. Scanning electron micrographs of PLGA membranes coated with growth factors revealed a conformal, single coating on the membrane and within the internal structure (Fig. 1D); typically, the coating thickness was ~0.5 μm for single growth factor and ~1 μm for dual growth factor coatings. The thickness of the PEM coatings reduces pore size and shifts the pore size distribution, as expected. More than 95% of the nanoscale pores on the top surface, smaller than the thickness of the coating, were covered. The porosity was estimated by dividing the total area of the pores by the total area of the image. As anticipated, the porosity of the uncoated and coated bottom surface remained between 30% and 40%, whereas porosity of the top surface was 26% and 8% for the uncoated and coated top surface, respectively—a consequence of reduced pore area due to the PEM coating.

We explored the use of PLGA with end groups conjugated with alendronate (Fig. S1). The reaction placed negatively charged phosphonate end groups at the end of the hydrophobic PLGA backbone, at 2.07 ± 0.33 μg (SEM) of alendronate per mg of polymer, to generate an amphiphilic molecule. We hypothesized that, as the membrane degrades, the released alendronate is able to bind to hydroxyapatite, thus inhibiting bone resorption and potentially leading to rapid bone formation. Unmodified membranes and alendronate-conjugated PLGA membranes are denoted as M and MA₂, respectively.

**Bone Repair in a Critical-Size-Defect Model.** A relevant model to illustrate the clinical translational potential for treating CMF bone defects is a critical-size calvarial defect in a skeletally mature rat, corresponding to an 8-mm circular wound (22). Calvarial defects can answer questions about the biocompatibility and the biological functions of bone repair materials and morphogens before putting them into a clinical setting. It has been demonstrated that the rate of scaffold degradation is critical to bone healing (23). We held the membrane thickness constant at 120 ± 10 μm (SEM) and monitored in vivo degradation of a P₀₂ + B₀₂-coated membrane of 8-mm diameter as a function of the lactic acid to glycolic acid ratio in the PLGA copolymer. The objective was to select a ratio that would yield a degradation half-life of ~4 wk to coincide with bone growth. The mass and diameter of the uncoated membranes placed in the rat cranial defect were monitored at predetermined time intervals to determine a relationship between copolymer ratio and rate of degradation. We observed that PLA:PGA (50:50) yielded the desirable degradation profile for cranial defect healing (Fig. S2). The end-functionalization of alendronate to the PLGA (50:50) backbone did not noticeably alter the in vivo degradation kinetics. Each implant was ~5 mg, and the dose of alendronate per implant was ~10 μg.

Activation of progenitor cells is highly sensitive to growth-factor dose and its local availability. To induce the desired biological response for bone tissue repair, we examined the effect of growth-factor combinations released from the PEM coating. We applied 40 layers of each growth factor in a B or P tetralayer repeat unit. Single-growth-factor PEM coatings contained 40 layers of BMP-2 or PDGF-BB. Dual-growth-factor coatings contained 40 layers of each growth factor, for a total of 80 growth factor layers. Loading per layer was proportional to growth-factor concentration and was used to control the amount of growth factor that was incorporated in the PEM coating. In dual-growth-factor releasing PEMs, the growth factors were arranged so that BMP-2 was incorporated in the bottom 40 layers closest to the membrane and the PDGF-BB was incorporated in the subsequent 40 layers. The arrangement resulted in a concentration gradient of growth factors within the film and allowed for different rates of growth factor release in vivo (Fig. 2A). P₀₂ + B₀₂ coatings were applied on PLGA membranes, and PDGF-BB and BMP-2 were tracked simultaneously by using near-IR dyes in the same animal. PDGF-BB was detectable for ~11 d after surgery, whereas BMP-2 was detected for 20 d. The duration of in vivo growth factor release corresponded to similar in vitro release profiles from the P and B single-growth-factor coatings (Fig. 2B).

Importantly, burst release of either growth factor was not observed; rather, the release was sustained over different times, as intended. In vitro, ~20% of growth factor from the single-factor PEM eluted within ~24 h after release. Within this 24-h time period, the release rate was approximately constant in this time period (R² = 0.951). In vivo, we observed a decrease in the fluorescence signal of ~22% and 6% for the PDGF-BB and BMP-2, respectively, over the same time period. The release reported in this study is an order of magnitude lower than what has typically been reported for single-growth-factor burst release systems, in which 40–60% of the growth factor is released within 3 h after release, with low therapeutic effect (24, 25).

We systematically investigated the effect of growth-factor formulations on inducing tissue repair (Table S1). Bone healing in this model is characterized by new bone tissue deposition and coverage of the defects. We monitored the healing process temporally using microcomputed tomography (μCT) (Fig. 3A). As anticipated, no bone healing was observed in an untreated defect. Spicules of bone were observed with an uncoated membrane, which did not integrate with the parent bone. B and P+B layers induced a potent bone-healing response and induced closure within 4 wk after treatment. Defects reconstructed with growth factor-loaded PLGA membranes exhibited multifocal bone formation, where new bone formation initiated at the margins and gradually filled in the defect. Repair initiated by P+B layers together resulted in a smaller defect after 2 wk compared with single-factor BMP-2–induced repair (Fig. 3B).

Increasing the total dose of BMP-2 to 2 μg did not appear to
membrane resulted in a larger defect compared with the unmodified membrane, likely owing to the inhibition of bone remodeling and migration of new bone into the defect. However, at the end of 4 wk, the defect completely bridged with new bone that had a significantly higher bone volume (BV) and bone mineral density (BMD) than the single- and dual-growth-factor groups. New bone formed as a result of B layers alone lacked mineralization and compact bone formation. The osteoid layer had wide borders, indicating that rapid tissue deposition preceded mineralization. Qualitatively, the bone formed by P+B layers had a greater number of vascular channels and a higher cell density within the bone, indicating the mitogenic role of PDGF-BB in the bone formation process. We observed that the growth-factor-coated PLGA polymer membrane resulted in bone repair via intramembranous ossification preceded by highly cellular granulation tissue supported by the membrane (Fig. 4B). We observed that, as new bone filled the gap, the tissue layer remodeled and reduced in thickness from 1 to 2 wk, eventually reducing to a one-cell-thick layer form after bone had completely filled the gap at ~4 wk after surgery. The thick tissue layer was a rich source of progenitor cells for bone formation in the uncoated membrane group was sparse and structurally immature and lacked connection with the existing bone. Loosely arranged collagen fibers were present with only partial bony ingrowth at the wound margins. Outer and inner cortical tables were variably present. In contrast, bone formed under the influence of growth factors in the treatment groups was trabecular, with evidence of remodeling and maturation with extensive bone development in a hypercellular environment that is characteristic of bone wound healing. In all growth-factor-treated groups, the defect was completely bridged within 4 wk with bone that exhibited ongoing active remodeling processes for all growth-factor-treated groups. New bone formed as a result of B layers alone lacked mineralization and compact bone formation. The osteoid layer had wide borders, indicating that rapid tissue deposition preceded mineralization. Qualitatively, the bone formed by P+B layers had a greater number of vascular channels and a higher cell density within the bone, indicating the mitogenic role of PDGF-BB in the bone formation process. We observed that the growth-factor-coated PLGA polymer membrane resulted in bone repair via intramembranous ossification preceded by highly cellular granulation tissue supported by the membrane (Fig. 4B). We observed that, as new bone filled the gap, the tissue layer remodeled and reduced in thickness from 1 to 2 wk, eventually reducing to a one-cell-thick layer form after bone had completely filled the gap at ~4 wk after surgery. The thick tissue layer was a rich source of progenitor cells for bone

Histological Evaluation of Regenerated Bone. A histological examination revealed the underlying cellular processes involved in bone repair (Fig. 4A). There were no indications of adverse foreign-body reactions as evidenced by the lack of foreign body giant cells, long-term inflammation, or infection. Bone formation processes were completely absent in the untreated defect. Tissue

Fig. 3. µCT imaging of bone repair in live animals. (A) Representative radiographs of bone formation around drilled implants with different coatings at 1, 2, and 4 wk. Red broken circle indicates the location of the defect in each radiograph and has an 8-mm diameter. Defect closure was achieved in all animal groups with different treatment conditions within 4 wk. n = 5 per group. (B) The images in A were used to quantify BV and BMD at 2 and 4 wk within the regions of interest marked by dotted red circles. Each point represents an individual animal. Data are means ± SEM (n = 5 or 6 per group). *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant (ANOVA with Tukey post hoc test). All groups are compared with the mechanical properties of the M + B0.2 + P0.2 group.

alter the rate of bone repair. Using the MAl membrane resulted in a remarkable difference in the rate and quality of bone repair. At 2 wk, single-growth-factor BMP-2 release from the MAl membrane appeared to reduce the rate of bone repair and resulted in a larger defect compared with the unmodified membrane, likely owing to the inhibition of bone remodeling and migration of new bone into the defect. However, at the end of 4 wk, the defect completely bridged with new bone that had a significantly higher bone volume (BV) and bone mineral density (BMD) than the single- and dual-growth-factor groups. Together, these observations suggest that the alendronate binds with high affinity to newly formed bone tissue and prevents remodeling, a known physiological effect of bisphosphonates. The action of BMP-2 caused osteoblasts to continue bone deposition; thus, significantly more bone tissue is present throughout the repair site. At 4 wk, the BMD of bone formed by B layers alone was lower than that of native calvaria and bone formed by P+B layers. However, these groups had comparable BV, suggesting that BMP-2 delivery alone resulted in less mature bone.

Histological Evaluation of Regenerated Bone. A histological examination revealed the underlying cellular processes involved in bone repair (Fig. 4A). There were no indications of adverse foreign-body reactions as evidenced by the lack of foreign body giant cells, long-term inflammation, or infection. Bone formation processes were completely absent in the untreated defect. Tissue

Fig. 4. Histology of new tissue formed with various coating formulations. (A) Each image is a cross-section of the calvarial defect after 4 wk, at which time different levels of bone-tissue morphogenesis was observed at the defect site. The broken lines indicate the position of the defect site and are 8 mm apart. Collagen is represented by blue, and osteocytes (mature bone) are represented by red. Sections were stained with Masson’s trichrome stain and viewed under bright-field microscopy. (B) Granulation tissue layer at 1, 2, and 4 wk during bone repair in the M + B0.2 + P0.2 treatment group. The tissue gradually reduces in thickness from 1 to 4 wk as bone repair is completed. Pieces of the PLGA membrane were observed in some section. (Scale bars, 30 μm.) Arrowheads: red, PLGA membrane; yellow, granulation tissue layer.
repair and helped nucleate the repair machinery. Bone formation under the influence of the M_{Al} membrane bridged the gap with excess bone that lacked specific orientation and was less compact compared with bone formed under the influence of B layers alone. These observations are consistent with a lack of remodeling behavior in the presence of alendronate.

**Comparison of Bone Mechanical Properties.** We performed compression tests to investigate the mechanical integrity of the reconstructed region and obtain a measure of the mechanical properties of the repaired bone. The stiffness and compressive failure force for the regenerated bone formed in different groups at the 4 wk endpoint and compared it to native calvaria bone that was not injured. Tissue regenerated with the uncoated PLGA membrane lacked a cohesive bone structure and thus had low stiffness and a very low resistance of 16.9 ± 3.8 (SEM) MPa to compressive load. Bone formation was significant, organized, and cohesive with B layers alone and thus had a higher stiffness of ~82 MPa, independent of BMP-2 dose. This value was ~27% lower than the stiffness of native calvaria bone. However, bone formation with P+B layers was comparable with that of native bone. These observations correspond well to the disparate histological observations. Bone formed with B layers alone was less mature and had a lower BMD, which resulted in a lower stiffness compared with native calvaria bone. Conversely, PDGF-BB and BMP-2 codeelivery resulted in mature bone formation with mechanical properties that closely matched those of the native calvaria. A similar structure–property relationship existed for bone formed with the M_{Al} membrane. As noted, the excess bone present was not compact, and we observed that the bone was ~43% stiffer than the native calvaria bone. Loose tissue formed by uncoated PLGA membrane had very low resistance to compressive loads and fractured easily. Bone formed by BMP-2 alone had ~14% lower compressive strength than native calvaria bone and was dose-independent, owing to a lack of maturation, and corresponded with the observation of lower stiffness. BMP-2 and PDGF-BB acted in concert to induce bone with the same mechanical loading behavior as that of the native calvaria. M_{Al} + B_{0.2} resulted in stiffer bone, and the mechanical failure load was significantly lower. The lower failure load is explained by the lack of cohesiveness in brittle bone and the consequent nonuniform load distribution.

**Discussion**

The search for new bone regeneration strategies has emerged as a key priority fueled by the increasing medical challenges of a burgeoning aging population. In this study, we have used materials for directing bone-tissue repair processes by the fine-tuned and robust tunable spatiotemporal control of biologics from a thin film coating. Recent work has demonstrated the benefit of delivering multiple growth factors for bone-tissue engineering (26). Dose tunability and delivery of these potent biologics in a manner that can be adapted for clinical application is critical to the success of this strategy. In this study, we have demonstrated that the release rates of the growth factors can be tailored by using the PEM coatings. Typically, PEM coatings have characteristics of both a stratified and a blended film. There is a concentration gradient of materials in the film, in the order in which they are deposited. In these films, the BMP-2 is enriched in bottom layers of the film, and the PDGF-BB is in the top. When the film surface degrades from the top down, the growth factors elute, in which the PDGF-BB elutes faster than the BMP-2. In addition, the pores in the membrane provide an additional means to sequester the BMP-2–enriched PEM coating—further contributing to a more sustained release. Particle systems or scaffolds that persist in the wound, in some cases, may even hinder formation of cohesive, mechanically competent bone that also recapitulates geometry (27). The time taken to induce repair is significantly longer, and the reported bone strength with these permanent systems is often lower than native bone (28). Our studies suggest that the release of specific known growth factors, BMP-2 and PDGF-BB, either individually or in combination, is critical to enhanced bone regeneration. This combination of growth factors has been reported to induce rapid and successful bone-tissue regeneration (29). Both PDGF-BB and BMP-2 are growth factors that participate in the bone-healing cascade. It is known that introducing PDGF-BB expands the number of progenitor cells available to induce bone repair. We have demonstrated in this study that having an early, sustained signal of this growth factor directly increases the rate of repair, at levels that could not be achieved even by 10-fold increase in the dose of BMP-2. This strategy of delivering multiple growth factors with tunable control is particularly crucial in higher-order animals with slower rates of bone repair, including humans. The PEM coating can be applied, even if the membrane itself were modified to tune the degradation kinetics for adoption to higher animals. In this study, through the combination of specific materials known to play a role in bone formation, we developed an understanding to control the rate, amount, and quality of bone to repair a defect and provided structure–function relationships.

The composite PEM coating can be scaled to complex surfaces with large dimensions. Importantly, PEM assembly uses mild, aqueous conditions that preserve the activity of fragile biologics. We have previously demonstrated the importance of controlling the release of biologics from multilayer films by introducing the therapeutic as a layer during the PEM assembly process (15). Lack of toxicity is critical for materials used in implantable devices, and the long-term host response to permanent implants continues to be a concern. In this work, all of the components were selected with biocompatibility in mind: PLGA is a biodegradable polymer with a long history of clinical use in drug-delivery devices and has been used in bone-fixation systems with no adverse immunologic responses (30). In our work, the surface of the PLGA membrane with the smaller pores and lower porosity (polymer dense) surface faced outward, toward the skin. We used the different pore sizes on the PLGA membrane surface; and (i) form a temporary barrier with nanoscale pores and prevent soft-tissue prolapse into the wound; (ii) allow progenitor-cell infiltration in the less-polymer-dense, microporous surface; and (iii) achieve adaptable, controlled growth-factor release. We observed that the membrane remained intact and structurally competent over the timescale of bone formation. The use of the PEM was essential, because the uncoated microporous membrane resulted in the formation of a fibrous tissue layer. Furthermore, the same approach with PEM coatings could be applied to other biodegradable membranes and scaffolds, as we have described previously. Previous studies have demonstrated the compatibility of the poly(β-amino ester) family in vitro and in vivo (31, 32). PAA is a well-characterized weak
polynomial with a high charge density distributed over a noncrucible backbone that has been listed as an approved excipient in the FDA’s Inactive Ingredient Database (www.accessdata.fda.gov/scripts/cder/ig/index.Cfm) in oral and topical drug-delivery formulations. Therefore, there is a path to regulatory approval for its use in a degradable implant. The amount of alendronate (~10 μg per implant) is several orders of magnitude lower than the levels that are known to cause side effects. Consistent with these expectations, we observed no local toxicity in any of the animals treated throughout these studies. Importantly, this strategy is cell-free and does not rely on the extraction and ex vivo expansion of progenitor cells for reimplantation in the body. In effect, these nanolayered coatings could be adapted on demand to induce repair in a variety of bone defect types by recruiting endogenous progenitor cells. This approach provides an alternative to autologous bone grafts for CMF bone repair and reconstruction. Although the true potential of any bone regeneration strategy can only be realized through large animal preclinical studies and, ultimately, human clinical trials, the data shown here suggest that bone healing using an engineered regenerative surface is a potent strategy for safe, precise, and targeted tissue repair and demonstrate the use of alternating nanolayer assembly as a platform technology with the potential to be applied universally in regenerative medicine.

Methods

Materials. Alendronate sodium trihydrate (Alfa-Aesar), PLGA (50:50 (Mn ~ 38,000–54,000)), PAA (M, ~ 450,000), Sigma, and PDGF-BB (Osteohealth) were purchased. Poly2 (Mn ~ 12,000) was synthesized by using a reported method (33). BMP-2 (Pfizer) was obtained through a materials-transfer agreement.

PLGA Membrane Preparation. The PLGA membrane was prepared by using the diffusion-induced phase-separation process. A homogenous 20% (wt) solution of PLGA in DMF was prepared at room temperature and degassed. By using a doctor blade knife, the polymer solution was cast on a glass plate to yield a uniform polymer solution film. After immersing it in deionized water at room temperature for 48 h, it was thoroughly rinsed and dried at ambient conditions. A micrometer was used to determine the composite membrane thickness by measuring at least 10 different locations, including the center. Alendronate conjugation is described in SI Methods.

PEM Deposition, Characterization, and Release. Multilayer coatings were deposited by using the LBL method. Polyelectrolyte solutions were prepared at a concentration of 1 mg/mL (PAA, Poly2) in sodium acetate buffer. Concentrations of PDGF-BB and BMP-2 dipping solutions were adjusted to control the total loading in the PEMs. PLGA membranes were plasma sterilized, and layers were deposited by using a Carl Zeiss HMI-DS50 slide stainer. The substrate was immersed alternatively in Poly2 (5 min), PAA (5 min), either BMP-2 or PDGF-BB (5 min), and PAA (5 min), with a wash step in between. Additional details can be found in SI Methods.

In Vivo Critical-Size Defect Studies. All animal work was performed in accordance with protocols approved by the Committee on Animal Care at the Massachusetts Institute of Technology. The animal model, μCT imaging protocol, and histological methods have been described in SI Methods.

Mechanical Testing of Calvaria. Explanted calvaria were stored in PBS for immediate mechanical compression testing (Instron 5943). The thickness of the calvaria was measured by using a set of calipers before and after applying a constant force of 10 N for 60 s to measure stiffness. The compressive failure force, perpendicular to the regenerative calvarial bone, was the maximum load achieved before compressive fracture.

Statistical Analysis. Prism 5 (GraphPad) was used for all analyses. Results are presented as means ± SEM. Data were analyzed by ANOVA, and comparisons were performed with a Tukey post hoc test (multiple groups). P < 0.05 was considered significant.

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