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An amphiphilic degradable polymer/hydroxyapatite composite with enhanced handling characteristics promotes osteogenic gene expression in bone marrow stromal cells

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Abstract

Electrospun polymer/hydroxyapatite (HA) composites combining biodegradability with osteoconductivity are attractive for skeletal tissue engineering applications. However, most biodegradable polymers such as PLA are hydrophobic and do not blend with adequate interfacial adhesion with HA, compromising the structural homogeneity, mechanical integrity, and biological performance of the composite. To overcome this challenge, we incorporated a hydrophilic polyethylene glycol (PEG) block to poly(D,L-lactic acid) to improve the adhesion of the degradable polymer with HA. The amphiphilic triblock copolymer PLA-PEG-PLA (PELA) improved the stability of HA-PELA suspension at 25 wt% HA content, which was readily electrospun into HA-PELA composite scaffolds with uniform fiber dimensions. HA-PELA was highly extensible (failure strain >200% vs. <40% for HA-PLA), superhydrophilic (~0° water contact angle vs. >100° for HA-PLA), and exhibited an 8-fold storage modulus increase (unlike deterioration for HA-PLA) upon hydration, owing to the favorable interaction between HA and PEG. HA-PELA also better promoted osteochondral lineage commitment of bone marrow stromal cells in unstimulated culture and supported far more potent osteogenesis upon induction than HA-PLA. We demonstrate that the chemical incorporation of PEG is an effective strategy to improve the performance of degradable polymer/HA composites for bone tissue engineering applications.

Keywords

bone tissue engineering; composite materials; electrospinning; hydroxyapatite; amphiphilic polymer; biodegradable; stem cell lineage commitment

1. INTRODUCTION

Synthetic polymeric, ceramic, or polymer-ceramic composite scaffolds, when engineered with proper physical, chemical, and biomechanical cues, can play an important role in directing cellular fate/functions in scaffold-assisted regeneration of bone, cartilage, tendon, or their respective interfaces.[1–5] An ideal scaffold would recapitulate some key structural and biological properties of the extracellular matrices of the tissues of interest while

exhibiting unique features important for its clinical translation such as scalability and ease of use.[6–9] Achieving this delicate balance requires thoughtful selection and integration of biomaterial building blocks, which remains a fundamental challenge in the design of synthetic tissue scaffolds.

Bone is a structural composite mainly of type-I collagen and calcium phosphate minerals (50–70% dry weight), of which nanocrystalline hydroxyapatite (HA) is the main component, that is hierarchically organized from nanoscopic to macroscopic length scales.[10,11] HA is an osteoconductive bioceramic that has been shown to support bone cell attachment, growth factor binding and release, and expedite healing of bone defects *in vivo*. [12–14] Characterized with its high stiffness and brittleness, however, HA alone is not well suited for broad orthopedic applications beyond as a non-weight bearing bone void filler. Consequently, HA has been incorporated with synthetic polymers as 2- or 3-dimensional, dense or porous composite scaffolds using a wide range of fabrication techniques including but not limited to electrospinning, phase separation, injection molding, and 3-D prototyping. [15–19] The integration of these discrete structural components can be accomplished by either direct mixing/blending or by subjecting the polymer scaffolds to HA-mineralization, [20,21] where interfacial adhesion/affinity between HA and the polymeric component is the key to achieving structural and mechanical integrity of the final composite.[22,23] In the case of fabricating HA-polymer composite scaffolds by electrospinning, a process that uses electrical charge to form non-woven fibrous meshes with fiber dimensions in the nano to micro scale,[24–26] additional considerations for the stability and electrospinnability of the polymer-HA solution need to be taken into account.

Poly(lactic acid) (PLA) is one of the most widely used biodegradable polymers in synthetic tissue scaffolds. It is readily electrospinnable, and its *in vitro* and *in vivo* degradation profiles are well established. The intrinsic hydrophobicity of PLA, however, results in its poor mixing and adhesion with hydrophilic HA[27], making it difficult to electrospin HA-PLA composites with uniform fiber structures and adequate mechanical properties.[22,28–30] HA-PLA composites often exhibit inferior handling properties (e.g. brittleness) and inconsistent biological performance. For instance, the addition of even low contents of HA (~ 6%) results in the deterioration of the tensile modulus and impact strength of poly(L-lactic acid) (PLLA) films.[31] Scanning electron microscopy (SEM) examination of composite membranes following tensile loading reveals failure at the polymer-ceramic interface.[23] The addition of HA to PLA has led to minor improvements in cell adhesion and proliferation.[31–33] The impacts of HA-PLA composites on stem cell differentiation reported in literature have been mixed, with some concluding that HA promotes osteogenesis[34] or chondrogenesis[35] while others observing little impact of the addition of HA on differentiation.[36] We hypothesize that strengthening the interfacial adhesion between HA and PLA-based polymers could produce composite materials with greater consistency in structural, mechanical, and biological performance.

Previous attempts to strengthen the interfacial adhesion between HA and PLA include the addition of amphiphilic surfactants or modifying HA with surface-grafted polymers to improve interactions with the hydrophobic polyesters.[28,31,32,34,37,38] Such approaches, however, often introduce additives with ill-defined biological consequences. An alternative approach is to chemically integrate non-anionic structural motifs exhibiting adequate bonding affinity to HA into the degradable polymer. Our group and others have demonstrated favorable blending of HA with hydrophilic polymers such as poly(hydroxyethyl methacrylate) (pHEMA) and poly(ethylene glycol) (PEG).[12,39,40] In these hydrophilic composites, HA reinforces the mechanical properties of the polymers, with a freeze-dried pHEMA-HA composite containing 50% HA withstanding >80% compressive strains and 450MPa loads without exhibiting brittle fractures,[40] while PEG-HA

composites containing 15% HA withstand strains of ~2000% without breaking.[12] These remarkable mechanical properties are a result of strong interfacial adhesion between HA and the hydrophilic polymers.[39] In addition, the osteoconductivity of HA has endowed the otherwise bioinert pHEMA hydrogel the ability to enrich endogenous bioactive factors *in vivo*, promoting the healing of rat critical-size femoral defects.[14] Unfortunately, hydrophilic pHEMA and PEG themselves lack biodegradability and are not stable in aqueous environments without chemical crosslinking, making underivatized pHEMA or PEG unsuitable for fabricating degradable HA-polymer composites by electrospinning.

Here we report the design and characterization of an electrospun, biodegradable amphiphilic polymer/hydroxyapatite composite based on tri-block copolymer poly(D,L-lactic acid)-poly(ethylene glycol)-poly(D,L-lactic acid) (PELA). Although PELA of different molecular weights or block compositions (e.g. diblock or triblock copolymer) have been previously developed, they were intended for drug delivery applications,[41–43] modulating the physical properties of PLA blends,[44,45] or reducing non-specific protein adsorption on PLA-based tissue scaffolds.[46] The rational design of PELA to improve the interfacial binding with HA and optimize the physical and biological performance of degradable polymer-HA composite scaffolds for bone tissue engineering applications has not been previously explored. This design combines the degradability and aqueous stability of the poly(D,L-lactic acid) (PLA) block with the HA-binding capability of the poly(ethylene glycol) (PEG) block and the electrospinnability of both. We hypothesize that the addition of a hydrophilic PEG block to PLA can facilitate HA binding to the polymer, resulting in improved handling characteristics and more consistent biological activity. We tested this hypothesis by comparing the impact of HA incorporation on the performance of electrospun PELA and PLA with similar molecular weights and polydispersity. We examined their structural integration (fiber morphology, HA distribution), surface properties (water contact angle), tensile elasticity and storage modulus, *in vitro* degradation, and impact on the attachment, proliferation, lineage commitment and differentiation of bone marrow stromal cells.

2. MATERIALS AND METHODS

2.1 Materials

3,6-Dimethyl-1,4-dioxane-2,5-dione (D,L-lactide) was purchased from Sigma-Aldrich (St. Louis, MO) and purified by recrystallization twice in anhydrous toluene and dried under vacuum prior to use. Poly(ethylene glycol) (BioUltra, 20,000 Dalton) was purchased from Fluka (Switzerland). Polycrystalline hydroxyapatite powder was purchased from Alfa Aesar (Ward Hill, MA). All other solvents and reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used as received.

2.2 Polymer synthesis and characterization

Poly(D,L-lactic acid)-poly(ethylene glycol)-poly(D,L-lactic acid) (PELA) tri-block copolymer, was synthesized by melt ring opening polymerization. Briefly, poly(ethylene glycol) (20,000 Dalton, 4 g, 0.2 mmol) was heated to 100 °C in a Schlenk flask and stirred under vacuum for 1 h to remove residual water. The melt was cooled to room temperature before Tin(II) 2-ethylhexanoate (~95%, 24.18 mg, 0.06 mmol) in anhydrous toluene was introduced by syringe. The toluene was removed by heating the mixture under vacuum at 100 °C for 15 min. The mixture was cooled to room temperature before D,L-lactide (17.295 g, 0.12 mol) was added under argon purge. The melt polymerization proceeded at 130 °C for 5 h under argon. PLA was synthesized in the same manner with anhydrous ethylene glycol (99.8%, 0.87mg, 0.1407 mol) as the initiator. Briefly, D,L-lactide (15 g, 0.1038 mol) was added to the ethylene glycol in a Schlenk flask and heated to 130 °C. Tin(II) 2-

ethylhexanoate (21 mg, 0.05mmol) was introduced by syringe and the melt polymerization proceeded for 5 h. The crude PELA and PLA were dissolved in chloroform, purified by precipitation in methanol, and dried under vacuum.

Molecular weights and polydispersity of PELA and PLA were determined by gel permeation chromatography (GPC) on a Varian Prostar HPLC system equipped with two 5-mm PLGel MiniMIX-D columns (Agilent, Santa Clara, CA) and a PL-ELS2100 evaporative light scattering detector (Polymer Laboratories, UK). THF was used as an eluent at 0.3 mL/h at room temperature. Molecular weight and polydispersity calculations were calibrated with EasiVial polystyrene standards (Agilent, Santa Clara, CA).

^1H NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer at 298K using CDCl_3 containing tetramethylsilane as the solvent. ^1H NMR (400 MHz, CDCl_3) for PELA: 5.19 (m, 1126H), 3.65 (m, 1816H), 1.5 (m, 3921H) ppm.

2.3 Electrospinning

HA-PELA and HA-PLA composite scaffolds with 0 and 25 wt% HA were prepared by electrospinning. HA powder was sonicated in 5-mL 1:4 (v/v) dimethylformamide/chloroform for 30 min to break up aggregates before PELA or PLA (1.25g, 25% w/v) was added. The mixture was stirred overnight at room temperature and loaded into a 5-mL syringe. A high voltage power supply (Gamma High Voltage Research, Ormond Beach, FL) delivered a voltage of 12kV between a 22G ejection needle and an aluminum collection plate set at 15 cm away. The polymer solution was fed through the needle at rate of 1.7mL/h with a syringe pump (Orion Sage M361, Thermo Scientific, Billerica, MA), and the fibers were collected on the aluminum collector plate. The electrospinning proceeded for 2 h, with the collecting plate rotated by 90° every 15 min to ensure the homogeneity of the fibrous scaffold. The scaffolds (0.1–0.2 mm final thickness) were dried in a vacuum oven for 48 h to remove any residual solvent and stored in a desiccator prior to use.

2.4 Differential scanning calorimetry (DSC)

Thermal transitions of PELA and PLA were determined by conventional DSC and modulated DSC (MDSC) on a Q200 MDSC (TA Instruments, New Castle, DE). For DSC experiments, specimens (~6 mg) were scanned twice from $-90\text{ }^{\circ}\text{C}$ to $150\text{ }^{\circ}\text{C}$ ($20\text{ }^{\circ}\text{C}/\text{min}$). A constant nitrogen flow of 50 mL/min was applied. Temperature was calibrated with indium, gallium, and tin standards. T_g was defined as the midpoint of the inflection tangent from the second heating curve. MDSC was used to further analyze endothermic transitions in electrospun PELA. Samples (3–6mg) were held isothermal at $-40\text{ }^{\circ}\text{C}$ for 15 min and then heated at $2\text{ }^{\circ}\text{C}/\text{min}$ to $110\text{ }^{\circ}\text{C}$ with a 60-sec temperature modulation period and an amplitude of $0.318\text{ }^{\circ}\text{C}$, as described elsewhere.[47]

2.5 Scanning electron microscopy (SEM)

As-spun and vacuum-dried scaffolds were sputter coated with Au (~4 nm thick) and imaged on a Quanta 200 FEG MKII SEM (FEI Inc., Hillsboro, OR) under high vacuum at 5 kV. Fiber diameter was quantified from the SEM micrographs by measuring 100 random fibers, from micrographs of five different areas, with ImageJ software (National Institutes of Health).

2.6 Thermo-gravimetric analysis (TGA)

TGA was used to determine the actual percentage of HA in the as-spun and vacuum-dried scaffolds. The samples were heated at a rate of $20\text{ }^{\circ}\text{C}/\text{min}$ from room temperature to $500\text{ }^{\circ}\text{C}$ and the mass changes were recorded on a TGA Q50 (TA Instruments, New Castle, DE). HA powder was used as a control.

2.7 Ultimate tensile strain and ultimate tensile stress

HA-PELA and HA-PLA scaffolds were strained to failure in order to evaluate the effect of PEG on the extensibility and strength of the HA composites. Tensile tests were carried out according to ASTM D882-97. Scaffolds (13-mm wide, 75-mm long and 0.1–0.14 mm thick) were ramped to failure on a MTS Bionix 370 testing system equipped with a 500-N load cell. A 25-mm initial grip separation and 250-mm/min grip separation rate were applied. Ultimate tensile strain and stress were recorded at the peak of the stress/strain curve.

2.8 Water contact angle measurements

The wettability of the scaffolds was examined by the sessile drop technique. Five-microliter droplets of deionized water were deposited onto as-spun and vacuum-dried scaffolds or scaffolds freeze-dried following 24 h equilibration in 37 °C deionized water. The water contact angle was recorded using a CAM 200 goniometer (KSV Instruments, Finland). The droplet was imaged at 30 sec and the average contact angle from the left and right side of the drop was recorded. Five randomly selected areas per scaffold were used for each water contact angle measurement.

2.9 Dynamic mechanical analysis (DMA)

The tensile storage moduli of dry and hydrated (deionized water) scaffolds (n=3) were determined on a Q800 DMA (TA Instruments, New Castle, DE). Specimens ($5.3 \times 20\text{-mm}^2$) were cut with a parallel blade cutter and loaded onto a film tension fixture with a grip separation of 10 mm. Dry and hydrated samples were applied with a 0.001-N and 0.05-N pre-load force, respectively. Samples were equilibrated at 37 °C and held isothermal for 10 min prior to initiating 0.02% strain at a frequency 1 Hz and the tensile storage moduli were recorded. The 0.02% strain chosen falls within the linear viscoelastic region of the scaffolds.

2.10 In vitro hydrolytic degradation

In vitro hydrolytic degradation of PELA, HA-PELA, and HA-PLA were determined by monitoring the mass loss of the scaffolds upon incubation in $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free phosphate-buffered saline (PBS) (pH 7.4) at 37 °C. As-spun scaffolds were cut into $20 \times 20\text{-mm}^2$ squares (n=3 for each time point), weighed on an analytical balance (XS105, Mettler Toledo, Columbus, OH), and placed in conical tubes containing 20 mL of PBS and incubated at 37 °C for up to 12 weeks. At each time point, 3 specimens were removed, washed 3 times with deionized water, and lyophilized. The mass of each retrieved and dried specimen was recorded.

2.11 Rat bone marrow stromal cells (rMSCs) isolation

The rat bone marrow stromal cells (rMSC) were isolated from the long bones of a 4-week old male Charles River SASCO SD rat according to the procedure approved by the University of Massachusetts Medical School Institutional Animal Care and Use Committee and described previously.[40] Briefly, whole bone marrow was flushed from the marrow canal of dearticulated rat femur with minimal essential medium (α MEM without ascorbic acid, Life Technologies, Grand Island, NY). After lysing red blood cells with sterile water, the cells were centrifuged, re-suspended in α MEM (without ascorbic acid) containing 20% FBS, 1% penicillin-streptomycin, and 2% L-glutamine, passed through a sterile strainer, and cultured in the same media. Non-adherent cells were aspirated 4 days after initial plating and the remaining adherent cells were cultured until 70% confluence before being trypsinized and seeded on various scaffolds.

2.12 Attachment and proliferation of rMSCs on scaffolds

MTT cell viability assay (Roche, Indianapolis, IN) was performed to quantify cell attachment and early proliferation on the electrospun scaffolds. Scaffolds (n=3 per time point) were cut into 6.35-mm diameter circles using a hole punch, sterilized under UV for 1 h each side, and equilibrated in MSC expansion media (MEM without ascorbic acid, 20% FBS, 2% L-Glutamine, 1% penicillin-streptomycin) at 37 °C overnight. Passage 1 rMSCs (15,625/cm²) were seeded on the scaffolds placed in ultra-low attachment 96-well plates (Corning Inc., Corning, NY) and cultured in expansion media for 24 or 96 h. The MTT assay was performed according to manufacturer's instructions. Absorbance of the MTT product was read on a Multiskan FC microplate photometer (Thermo Scientific, Billerica, MA) at 570 nm with a 690-nm background correction.

2.13 Spontaneous lineage commitment of rMSCs on scaffolds

rMSCs were cultured on the scaffolds in expansion media to determine the effect of scaffold composition on their un-stimulated lineage commitments. Scaffolds were sterilized under UV for 1 h each side and equilibrated in MSC expansion media at 37 °C overnight. The scaffolds were placed in ultra-low attachment 24-well plates (Corning Inc., Corning, NY) and seeded with passage 1 rMSCs (50,000/cm²). Following 7 and 14 days in culture, total RNA from the MSCs adhered on the scaffolds and those from rMSCs prior to seeding on scaffolds (time 0 control) was isolated using TRIzol (Invitrogen, Carlsbad, CA) and purified by Direct-Zol miniprep (Zymo Research, Irvine, CA). RNA was reverse transcribed into cDNA with SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA) according to manufacturer's instructions on a GeneAmp 2700 PCR system (Applied Biosystems, Foster City, CA). qPCR was performed on an Applied Biosystems 7500 Fast Real-Time PCR system with TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA) and inventoried TaqMan probes for SOX9, osteocalcin, PPARG, and housekeeping gene GAPDH. All reactions were performed in triplicates and the gene expression was quantified using the delta-delta Ct method. Expression of each gene of interest at each time point was normalized using GAPDH and plotted as expression relative to that of rMSCs at time 0.

2.14 Osteogenic differentiation of rMSCs on scaffolds

PELA, HA-PELA, and HA-PLA scaffolds were sterilized and seeded with Passage 1 rMSCs (50,000 cells/cm²) as described above. The rMSCs were allowed to attach to the scaffolds for 24 h before the media were replaced with osteogenic media (MSC expansion media supplemented with 10 nM dexamethasone, 20 mM β -glycerol phosphate, 50 μ M L-ascorbic acid 2-phosphate). The scaffolds were cultured in the osteogenic media for 14 days with medium changes every 3–4 days. Total RNA was isolated, purified, and reverse transcribed to cDNA as described above. qPCR was performed with TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA) and inventoried TaqMan probes for Runx2, osteopontin, osteocalcin, and housekeeping gene GAPDH. All reactions were performed in triplicates and gene expression was quantified using the delta-delta Ct method. Expression of each gene of interest at each time point was normalized using GAPDH and plotted as expression relative to that of undifferentiated rMSCs at time 0.

2.15 Statistical analysis

All data are presented as mean $\pm$ standard deviation. Statistical significance was determined by one-way ANOVA with Tukey post-hoc testing. Differences were considered significant when $p < 0.05$.

3. RESULTS

3.1 Synthesis of PELA and PLA

PELA and PLA control were synthesized with similar molecular weights (116,298 and 126,139 Dalton, respectively) and polydispersity (PDI=1.44) by melt ring-opening polymerization of cyclic D,L-lactide, using PEG and ethylene glycol as initiators, respectively, under the catalysis of Tin(II) 2-ethylhexanoate (Scheme 1). DSC analyses revealed glass transitions at 17.73 °C and 44.77 °C for PELA and PLA, respectively.

3.2 Electrospinning HA-PELA and HA-PLA composites

The improved bonding affinity of HA with the amphiphilic PELA over PLA was manifested by the significantly more stable HA-PELA suspension in 1:4 (v/v) dimethylformamide/chloroform. No obvious settling of HA from the HA-PELA suspension (at HA content as high as 33 wt% relative to polymer weight) was detected upon storage at ambient conditions for days whereas the HA settled from the HA-PLA suspension by 24 h (Supplemental Fig. 1). The HA-PELA suspension could be readily electrospun without noticeable blockage of the needle tip by aggregated HA that was often observed during the electrospinning of HA-PLA. HA-PELA and HA-PLA composite scaffolds (average thickness of 0.1–0.2 mm) with 0 and 25 wt% HA were obtained after 2-h electrospinning and dried in a vacuum oven.

3.3 Microstructural and compositional analyses of electrospun HA-PELA vs. HA-PLA composites

SEM and subsequent image analysis was used to quantify the fiber uniformity of the electrospun scaffolds. While the mean diameter of the HA-PLA fibers more than doubled from that of PLA, there was no significant increase in fiber diameter of HA-PELA from that of PELA (Fig. 1a and b). The HA-PELA composites also exhibited a narrower distribution of fiber dimensions and fewer defects than HA-PLA.

Thermogravimetric analysis (TGA) of the electrospun PELA revealed a transition at approximately 83% weight loss, which closely correlated with the weight percentage of PLA in the PELA scaffolds. The PLA blocks, with a lower decomposition temperature (onset ~297 °C) than PEG (onset ~340 °C), were decomposed first. This step-wise decomposition feature, however, was not observed in the HA-PELA composite, suggesting an insulating effect of HA incorporation. The percentage of HA that remained after complete PELA thermal decomposition (>400 °C) matched precisely with the weight percentage of HA in the electrospinning solution (25%). By contrast, the actual HA content in the electrospun HA-PLA composite as determined by TGA was 28.4%, >3% higher than that of the HA-PLA suspension prior to electrospinning. Homogeneous HA distribution within the HA-PELA fibrous matrix was further confirmed by von Kossa staining for calcium[48] where intense positive von Kossa stain was detected uniformly within all fibers (Supplemental Fig. 2).

3.4 Tensile compliance of electrospun HA-PELA vs. HA-PLA composites

Tensile testing was performed to assess the extensibility and in turn the interfacial HA-polymer adhesion in the electrospun scaffolds. Electrospun HA-PELA was highly extensible, achieving ultimate tensile strains of over 200% (Fig. 2). By contrast, the HA-PLA was characterized with failure strains below 40%. HA-PELA also exhibited a failure stress of 2.5 MPa, 5 fold that of the HA-PLA.

3.5 Hydration-dependent surface properties of HA-PELA vs. HA-PLA composites

The hydrophilic PEG block in PELA significantly reduced the water contact angle (increased the wettability) of the scaffolds when compared to PLA (Fig. 3a). HA incorporation slightly increased the water contact angle of the PELA while exerted no significant effect on the wettability of PLA. To capture the polymer chain arrangements at the fully hydrated state, scaffolds were equilibrated in water at 37 °C for 24 h, and then freeze-dried before performing water contact angle measurement. The prior hydration significantly reduced the water contact angle of PELA from 71° to 54° and that of HA-PELA from >85° to 0°, revealing superhydrophilicity of the latter upon water equilibration. By contrast, prior hydration only reduced the contact angle on the HA-PLA scaffolds by 11°. The contact angle of HA-PELA dense films decreased by 17° following hydration (Supplemental Fig. 3).

3.6 Hydration dependent mechanical properties of HA-PELA vs. HA-PLA

The differences in surface properties between HA-PELA and HA-PLA upon hydration were also accompanied by drastic mechanical property alterations. Dynamic mechanical testing was used to assess the tensile storage modulus of both dry and hydrated samples at 37 °C (Fig. 3b). The storage modulus of the dry PELA increased from 1.1 MPa to 2.99 MPa with the incorporation of HA. By contrast, addition of HA significantly deteriorated the tensile storage modulus of dry PLA scaffolds from 110 MPa to 43 MPa. Strikingly, the amphiphilic PELA and HA-PELA scaffolds exhibited 75-fold and 8-fold increases in storage modulus upon hydration, respectively. As expected, hydration of PLA and HA-PLA resulted in significant storage modulus reductions.

3.7 DSC and MDSC analysis of the hydration dependent thermal behavior of PELA

Conventional and modulated DSC was carried out on scaffolds with and without prior hydration history to assess possible hydration-induced thermal behavior changes and the underlying structural rearrangements. Conventional DSC revealed an endothermic peak around 50 °C only with previously hydrated PELA specimens (Fig. 4a). To assess the nature of this transition, whether a reversible crystalline melting or an irreversible transition such as polymer aging, MDSC was carried out. It confirmed that this sharp transition was present in the reversible heat flow, consistent with a typical crystalline melting (Fig. 4b).

3.8 In vitro hydrolytic degradation of HA-PELA and HA-PLA composites

The *in vitro* hydrolytic degradation behavior of the HA-PELA and HA-PLA scaffolds (n=3) was examined by monitoring mass loss upon incubation in PBS at 37 °C over a period of 12 weeks (Fig. 5a). The PELA and HA-PELA scaffolds lost ~20% of their mass over 12 weeks whereas HA-PLA only lost ~3% of its mass during the same period. ¹H NMR analysis showed a strong PEG peak in the PELA degradation solution (Supplementary Fig. 4). SEM monitoring of structural changes of the incubated scaffolds revealed the least change in HA-PELA fiber morphology by 3 weeks while PELA fibers fused at their contact points and HA-PLA fibers aggregated together (Fig. 5b). The addition of HA effectively reduced the degree of fusing of the PELA fibers.

3.9 The role of PEG and HA on rMSC proliferation

rMSCs were seeded onto the PELA and HA-PELA scaffolds (n=3) and the viability of adherent cells was quantified by MTT assay at 24 and 96 h and compared with those adhered to HA-PLA (Fig. 6). More viable cells were adhered to HA-PELA than to PELA at 24 h. rMSCs adhered on all substrates were able to proliferate to some degree as indicated by MTT cell viability assay at 96 h. However, the increase in MTT absorbance from 24 h to 96 h was only significant for HA-PELA.

3.10 The role of PEG and HA on the lineage commitment and induced osteogenic differentiation of rMSCs

To examine the effects surface chemistry and HA incorporation on the spontaneous lineage commitment of MSCs, we cultured rMSCs on PELA and HA-PELA scaffolds along with HA-PLA control in expansion media without differentiation inducing supplements. Gene expression of typical osteoblast, chondrocyte, and adipocyte markers was analyzed by qPCR at 7 and 14 days to determine the temporal nature of the rMSC response to the PELA, HA-PELA, and HA-PLA scaffolds. Data were normalized to those obtained from the rMSCs prior to seeding on the various substrates (time 0). For the PELA scaffolds, HA incorporation resulted in significantly increased expression of chondrogenic marker Sox9 and the osteogenic marker osteocalcin as early as 7 days (Fig. 7a). Expression of the osteochondral lineage markers Sox9 and osteocalcin were significantly higher for MSCs cultured on HA-PELA scaffolds than for those cultured on HA-PLA. Although the expression level of osteocalcin by MSCs cultured on HA-PELA decreased from day 7 to day 14 (Fig. 7b), it was still significantly higher than those observed with MSCs cultured on HA-PLA. The differences in the expression of Sox9 by MSCs cultured on HA-PELA vs. HA-PLA, on the other hand, was even more pronounced by day 14. The expression of adipogenic maker PPARG was significantly higher at day 7 on PELA than on HA-PELA, but the significance of the difference was not sustained at day 14.

Finally, we examined the impact of HA and PEG on the potency of induced osteogenic differentiation of rMSCs for potential bone tissue engineering applications. PELA, HA-PELA, and HA-PLA scaffolds were cultured with rMSCs for 14 days in osteogenic media and total RNA was isolated. We used qPCR to quantify the expression of RUNX2, osteopontin, and osteocalcin (Fig. 8). rMSCs adhered on HA-PELA expressed significantly higher levels of all these marker genes than those adhered on PELA or HA-PLA. The gene expression of osteopontin and osteocalcin was over 2 orders of magnitude higher for MSCs adhered on HA-PELA than on either PELA or HA-PLA on day 14.

4. DISCUSSION

PLA is a widely used material in the field of tissue engineering due to favorable properties such as biocompatibility, biodegradability, and ease of processing into diverse architectures. However, the intrinsic hydrophobicity of PLA compromises its aqueous dimensional stability and its ability to support cell seeding, growth factor and hydrophilic drug loading, and direct blending with hydrophilic materials such as HA.[27] In this study, we tested the hypothesis that by chemically inserting a hydrophilic PEG block within PLA, the resulting amphiphilic triblock co-polymer PLA-PEG-PLA (PELA) will favorably interact with HA and be readily electrospun into HA-PELA composite scaffolds with improved physical and biological properties than HA-PLA composites or PELA alone.

We chose racemic D,L-lactide over L-lactide due to its accelerated degradation profile and the intent to avoid crystalline degradation by-products which can elicit adverse tissue responses during potential *in vivo* applications.[49,50] By simply changing the initiator (PEG vs. ethylene glycol), we were able to synthesize both the PELA and PLA with similar molecular weights and polydispersity using the same polymer chemistry. This allowed us to investigate the effect of polymer chemistry on the HA-composite scaffold properties without additional confounding factors. The facile synthesis and purification of PELA will also be advantageous for subsequent scale-ups for clinical uses.

In order to reproducibly fabricate a uniform polymer/HA composite scaffold by electrospinning, the HA needs to be homogeneously dispersed in the electrospinning solution. A manifestation of poor adhesion between a polymer and HA is that HA would rapidly settle

out of the suspension, as was observed with HA-PLA. We demonstrated that with PELA, a stable HA suspension could be achieved even at HA content as high as 33% w/w, in the absence of any surfactants. Such stability is one manifestation of the improved interfacial adhesion between PELA and HA. Consequently, electrospinning of the stable HA-PELA suspension resulted in a scaffold with more uniform fiber morphology and more predictable HA content than HA-PLA (Fig. 1b & c).

The robust blending and interfacial adhesion between HA and PELA was further manifested in the storage modulus enhancement of the HA-PELA composite over PELA, in contrast to the mechanical strength deterioration of HA-PLA compared to PLA (Fig. 3b). In addition, HA-PELA exhibited a 5-fold higher ultimate tensile strain and a 10-fold higher ultimate tensile stress than HA-PLA, respectively (Fig. 2). The far more brittle nature of the conventional HA-PLA composite reflects the inadequate interfacial adhesion between PLA and HA.[27] The significantly enhanced extensibility and ultimate tensile strength of electrospun HA-PELA compared to those of HA-PLA is advantageous for potential surgical manipulation of the composite scaffolds.

The amphiphilic nature of PELA is expected to lead to hydration-induced structural rearrangement of the polymer chains [51,52] and corresponding changes in surface wettability and mechanical properties, which are important for tissue engineering applications. As expected, the chemical incorporation of PEG with PLA reduced the water contact angle of the scaffold compared to PLA. A striking observation was that prior hydration conferred superhydrophilicity to electrospun HA-PELA (Fig. 3a), with its water contact angle approaching 0°, pointing to a possible hydration-induced structural rearrangement of the amphiphilic polymer that exposes the hydrophilic PEG to the surface (Scheme 2). The water contact angle of PELA was also reduced upon hydration, although the effect was less dramatic than that observed with HA-PELA, suggesting that HA plays a role in stabilizing the hydration-induced structural rearrangement of the amphiphilic polymer and/or affecting the morphology of the scaffold. We found that the nanofibrous morphology of the HA-PELA scaffolds was indeed important for acquiring hydration-induced superhydrophilicity as the water contact angle decrease was far less dramatic on solvent cast dense HA-PELA films (Supp Fig. 3). Contact angle measurement of the PLA fibrous scaffold after the water equilibration could not be obtained because the hydrophobic scaffold shrank dramatically upon hydration. Such an observation is consistent with previously reported dimensional instability of electrospun poly(D,L-lactic acid) scaffolds in an aqueous environment.[53–55] The extent of the shrinkage of the HA-PLA scaffolds was milder, likely due to the stabilizing effect of the HA within the composite scaffold. The superhydrophilicity combined with the dimensional stability of HA-PELA is advantageous for the loading of cells and aqueous therapeutics, allowing them to be readily absorbed into the scaffold.

We also observed striking storage modulus increases in the PELA and HA-PELA scaffolds upon hydration (Fig. 3b). The storage moduli of the electrospun PLA and HA-PLA scaffolds, on the other hand, expectedly reduced upon hydration, likely due to a combination of the plasticizing effect of water and the amorphous nature of PLA.[56] We hypothesize that the hydration-induced structural rearrangement of the amphiphilic polymer chains that exposes the hydrophilic PEG blocks to the surface, thereby reducing interfacial energy and lowering the water contact angle, may have also led to the local crystallization of PEG blocks, resulting in the reinforcement of the storage modulus of the material (Scheme 2). Indeed, using conventional DSC, we showed that while the as-spun dry PELA specimen only possessed a single glass transition, the specimen with prior hydration history was characterized with an extra endothermic peak in the DSC spectrum around 50 °C (Fig. 4a). This thermal transition, also present in the reversible MDSC heat flow (Fig. 4b), is

consistent with a crystalline melting temperature (T_m) attributable to PEG, the only crystalline component in the PELA triblock copolymer. It has been previously reported that the T_m for PEG in a PEG-PLA diblock polymer was 50 °C as opposed to 60 °C for pure PEG.[47] The hydration-induced stiffening of the HA-PELA scaffolds is potentially advantageous for applications such as scaffold-guided skeletal/dental bone regeneration. The elastic membrane, upon surgical delivery to fill in or cover an osseous defect, would stiffen within the physiological environment of the defect to prevent soft tissue collapse, thereby maintaining adequate space for bone regeneration.

As the HA-PELA scaffolds are designed to be biodegradable, we evaluated their *in vitro* degradation in PBS over 12 weeks (Fig. 5a). HA-PELA exhibited an accelerated degradation compared to HA-PLA, resulting in ~20% mass reduction in 3 months. Such a degradation profile is desired for scaffold-assisted repair of long bone fractures where the maturation/remodeling of bony calluses typically takes place 3–4 months following injury in adults.[50] It ensures that the scaffold fully exerts its templating role during the early stage of scaffold-assisted repair and only degrades at the desired later stage of callus remodeling. The accelerated degradation of PELA and HA-PELA compared to HA-PLA can be attributed to a combination of the more effective water penetration throughout the amphiphilic scaffold enabled by the hydrophilic PEG blocks, and the clearance of the cleaved water-soluble PEG segments from the polymer, as previously described [57–59]. It was also supported by our ^1H NMR monitoring of the degradation products (Supp. Fig. 4). Monitoring of microstructural changes of the scaffolds by SEM upon incubation in PBS for 3 weeks revealed that the HA-PLA scaffolds suffered from hydrophobicity-driven collapse of the fibers while the HA-PELA fiber morphology was largely unchanged (Fig. 5b). The PELA scaffold without HA exhibited some degree of fiber fusions upon incubation in PBS, although to a much lesser extent compared to HA-PLA. The PEG blocks in PELA have likely prevented more dramatic hydrophobicity-driven fiber aggregation observed with PLA or HA-PLA, while the dynamic rearrangement and crystallization of the PEG blocks in aqueous buffer may have resulted in the fusing of the amphiphilic fibers around their physical contact points. The maintenance of HA-PELA fiber morphology upon PBS incubation further demonstrates the structural stabilizing effect of HA on the amphiphilic scaffold. This combination of dimensional stability, superhydrophilicity, and superior mechanical integrity of HA-PELA composites in an aqueous environment establish HA-PELA composites as far more advantageous scaffolds for tissue engineering applications than the conventional HA-PLA composites.

To further evaluate the applicability of HA-PELA composites for skeletal tissue engineering applications, we compared the ability of HA-PELA and HA-PLA composite scaffolds to support the attachment and guide lineage-specific differentiation of mesenchymal stem cells (MSCs) for the regeneration of bone or cartilage tissues. MSCs residing in the bone marrow are capable of differentiating into a variety of cell types including osteoblasts, chondrocytes, adipocytes, and myoblasts.[60] They can be readily isolated and enriched as adherent bone marrow stromal cells, and expanded and used as a progenitor cell source for musculoskeletal tissue engineering.[61]

The non-fouling PEG block and the osteoconductive HA component of the HA-PELA composites are expected to exhibit opposite effects on protein and cell adhesion. For instance, un-mineralized di-block or tri-block PELA have been used as anti-adhesion membranes,[46] with their low protein adsorption characteristics attributed to the PEG exposed on the surface in the aqueous environment.[62,63] Meanwhile, HA is known for its ability to absorb a wide range of proteins due to its dynamic surface properties (e.g. pH-dependent zeta potential) and large surface area (for HA nanocrystals), promoting cell attachment.[64–66] We hypothesized that the incorporation of HA could improve rMSC

attachment on PELA. We showed by MTT assay that the addition of HA to PELA indeed increased the attachment of rMSCs (Fig. 6). HA also supported the rMSC proliferation with a significant increase in cell viability from 24 h to 96 h on HA-PELA. It has been reported previously that rMSC proliferation is reduced on PEG-PLA materials.[62] In our study, this effect was offset by the incorporation of HA. It is also worth noting that high standard deviation was observed for the cell viability at 96 h in the HA-PLA group, reflecting the poor HA dispersion within the inhomogeneous HA-PLA scaffold.

The addition of HA to synthetic scaffolds has been shown to promote osteochondral lineage commitment of MSCs in un-stimulated culture[34,35,67] or more potent chondrogenesis/osteogenesis of MSCs in response to chondrogenic/osteogenic inductions in culture and skeletal tissue repair *in vivo*. [14,68–71] However, such an effect has not been consistently established, potentially due to the inhomogeneous distribution of HA within many of the hydrophobic degradable polymer scaffolds utilized for such investigations. Further, how amphiphilic polymer-HA scaffolds impact the gene expression of MSCs under un-stimulated culture conditions is largely unknown.

We cultured rMSCs in expansion media on the PELA, HA-PELA, and HA-PLA scaffolds and used qPCR to quantify changes in gene expression of early (7 d) and later (14 d) stages of osteogenesis, chondrogenesis and adipogenesis markers. By using the expansion media free of differentiation inductive agents, we can examine the effect of scaffold composition on driving rMSC differentiation towards particular lineages. We showed that the HA-PELA scaffold supported consistently higher expression of the chondrocyte marker Sox9 and the osteoblast marker osteocalcin of the adherent MSCs than HA-PLA throughout the 14-day culture (Fig. 7). The addition of HA to PELA also increased the expression of these genes at day 7 but the difference was not sustained at day 14, possibly due to the unstimulated culture conditions being insufficient to sustain the spontaneous osteogenic differentiation over time. The increased osteochondral gene expression at day 7 with the addition of HA to PELA is in contrast with prior reports using materials such as electrospun poly(caprolactone)-HA or poly(ethylene oxide terephthalate)-poly(butylene terephthalate)-HA, where the addition of HA was shown to have little or no effect on MSC gene expression.[67,72] Another observation is that the expression of adipogenic marker PPARG also significantly decreased upon the addition of HA to PELA at 7 days. Furthermore, the expression of PPARG trends higher at 7 days for rMSCs cultured on HA-PLA than those cultured on the HA-PELA. These results suggest that the osteochondral-inductive properties of HA are more effectively manifested on HA-PELA where polymer-HA interfacial adhesion and dispersion is improved than on HA-PLA. Whether the HA-PELA scaffold can lead to sustained lineage selection favoring osteochondral differentiation of MSCs *in vivo* is the subject of an ongoing investigation.

To examine the role of polymer chemistry and HA incorporation in promoting osteogenesis of MSCs upon culture induction, we quantified the osteogenic gene expression of rMSCs cultured on PELA, HA-PELA and HA-PLA in osteogenic media. The expression of early (Runx2), middle (osteopontin), and late stage (osteocalcin) osteogenesis markers were all significantly up-regulated on HA-PELA compared to PELA or HA-PLA scaffolds (Fig. 8). [73,74] In particular, the expression of osteopontin and osteocalcin was increased over two orders of magnitude on HA-PELA. Prior work on MSCs attached to electrospun PLLA-HA (calcium deficient) scaffolds showed increased osteogenic gene expression with increasing HA content in expansion media while the effects were abrogated in osteogenic media.[34] By contrast, here we show that the effect of HA in promoting osteogenic gene expression of MSCs was consistently manifested by the HA-PELA scaffolds in both stimulated and unstimulated cultures. More homogenous HA distribution within the amphiphilic HA-PELA scaffold as opposed to the uneven distribution of aggregated HA within the HA-PLA

scaffold may translate into a higher surface area of the osteoconductive HA interacting with the adherent MSCs. Furthermore, prior work has shown that PEG/PLA diblock co-polymers or PEG/PLA blends promote the osteogenic differentiation of MSCs.[63,75] Here we show that the synergy between HA and PEG supported an even more potent osteogenic MSC response. The sensitized response of MSCs to osteogenic induction[73] enabled by the HA-PELA scaffold makes it possible to deliver exogenous osteogenic inductive agents such as bone morphogenetic protein (rhBMP-2) with reduced loading doses for enhanced safety and cost effectiveness of such therapeutic strategies. Taken together, HA-PELA composites exhibit unique handling properties (elasticity, superhydrophilicity, hydration-induced stiffening) and bioactivity (rMSC attachment, osteochondral gene expression) ideal for skeletal tissue engineering application. The *in vivo* efficacy of the electrospun HA-PELA scaffolds, with and without pre-seeded rMSCs or osteoinductive therapeutics, in promoting the healing of critical size bone defects are being examined in rodents.

5. CONCLUSIONS

A biodegradable electrospun HA-poly(D,L-lactic acid)-poly(ethylene glycol)-poly(D,L-lactic acid) (HA-PELA) composite was designed for skeletal tissue engineering applications. We showed that favorable interactions between HA and the PEG component of the amphiphilic block copolymer dictated the physical and biological performance of the composites. The hydrophilic PEG block improved HA blending and adhesion to the polymer, facilitating the preparation of composite fibrous scaffolds with uniform fiber dimensions and HA distribution by electrospinning. The incorporation of well integrated HA with PELA improved the dimensional stability and reinforced the tensile storage moduli of the electrospun composites while maintaining impressive tensile elasticity. The hydrophilicity and mechanical integrity of HA-PELA further improved in aqueous environment, likely resulting from hydration-induced structural rearrangement and crystallization of the PEG domains that may have been more effectively stabilized by HA. The combination of dimensional stability, mechanical integrity, superhydrophilicity, and tensile elasticity exhibited by HA-PELA can be exploited to facilitate cell seeding, drug delivery and convenient surgical manipulations of the composite scaffold. Finally, HA incorporation improved cell attachment to the relatively low-fouling PELA, and the HA-PELA composite, unlike HA-PLA, promoted osteochondral lineage commitment of MSCs and supported more potent osteogenic gene expression upon induction. The combination of improved handling characteristics and *in vitro* biological performance positions HA-PELA as a more suitable bone tissue engineering scaffold than HA-PLA.

Indeed, the facile synthesis of PELA, the consistent properties of electrospun HA-PELA, and the established safety profile of the constituents of the composite scaffold are appealing characteristics for successful clinical translation. On-going work includes the application of HA-PELA scaffolds pre-seeded with rMSCs or pre-absorbed with low-doses of rhBMP-2 in promoting the healing of critical-sized long bone defects in rats. Overall, this study demonstrates the importance of rational selection and assembly of key structural components with synergistic interactions in the design of effective tissue engineering scaffolds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA, Rivera-Feliciano J, Mooney DJ. *Nat Mater*. 2010; 9:518. [PubMed: 20418863]
2. Engler AJ, Sen S, Sweeney HL, Discher DE. *Cell*. 2006; 126:677. [PubMed: 16923388]
3. Lutolf MP, Gilbert PM, Blau HM. *Nature*. 2009; 462:433. [PubMed: 19940913]
4. Singh A, Zhan J, Ye Z, Elisseeff JH. *Adv Funct Mater*. 2012
5. Yang PJ, Temenoff JS. *Tissue Eng Pt B-Rev*. 2009; 15:127.
6. Lutolf MP, Hubbell JA. *Nat Biotechnol*. 2005; 23:47. [PubMed: 15637621]
7. Place ES, Evans ND, Stevens MM. *Nat Mater*. 2009; 8:457. [PubMed: 19458646]
8. Langer R, Tirrell DA. *Nature*. 2004; 428:487. [PubMed: 15057821]
9. Huebsch N, Mooney DJ. *Nature*. 2009; 462:426. [PubMed: 19940912]
10. Weiner S, Wagner HD. *Annu Rev Mater Sci*. 1998; 28:271.
11. Mann, S. *Biomaterialization: Principles and Concepts in Bioinorganic Materials Chemistry*. Oxford University Press; 2001.
12. Gaharwar AK, Dammu Sa, Canter JM, Wu C-J, Schmidt G. *Biomacromolecules*. 2011; 12:1641. [PubMed: 21413708]
13. Xu J, Li X, Lian JB, Ayers DC, Song J. *J Orthop Res*. 2009; 27:1306. [PubMed: 19350632]
14. Filion TM, Li X, Mason-Savas A, Kreider JM, Goldstein SA, Ayers DC, Song J. *Tissue Eng Pt A*. 2011; 17:503.
15. Thomson RC, Yaszemski MJ, Powers JM, Mikos aG. *Biomaterials*. 1998; 19:1935. [PubMed: 9863527]
16. Holzwarth JM, Ma PX. *Biomaterials*. 2011; 32:9622. [PubMed: 21944829]
17. Ma PX, Zhang R, Xiao G, Franceschi R. *J Biomed Mater Res*. 2001; 54:284. [PubMed: 11093189]
18. Wang M. *Biomaterials*. 2003; 24:2133. [PubMed: 12699650]
19. Rai B, Oest ME, Dupont KM, Ho KH, Teoh SH, Guldberg RE. *J Biomed Res A*. 2007; 81:888.
20. Rezwani K, Chen QZ, Blaker JJ, Boccaccini AR. *Biomaterials*. 2006; 27:3413. [PubMed: 16504284]
21. Palmer LC, Newcomb CJ, Kaltz SR, Spoerke ED, Stupp SI. *Chem rev*. 2008; 108:4754. [PubMed: 19006400]
22. Supová M. *J Mater Sci - Mater Med*. 2009; 20:1201. [PubMed: 19225871]
23. Kim H-W, Knowles JC, Kim H-E. *J Biomed Res*. 2004; 70A:467.
24. Pham QP, Sharma U, Mikos AG. *Tissue Eng*. 2006; 12:1197. [PubMed: 16771634]
25. Reneker DH, Chun I. *Nanotechnology*. 1996; 7:216.
26. Filion TM, Kutikov A, Song J. *Bioorg Med Chem*. 2011; 21:5067.
27. Neuendorf RE, Saiz E, Tomsia aP, Ritchie RO. *Acta Biomater*. 2008; 4:1288. [PubMed: 18485842]
28. Qiu X, Hong Z, Hu J, Chen L, Chen X, Jing X. *Biomacromolecules*. 2005; 6:1193. [PubMed: 15877333]
29. Wei J, Liu A, Chen L, Zhang P, Chen X, Jing X. *Macromol Biosci*. 2009; 9:631. [PubMed: 19165825]
30. Wang Y, Dai J, Zhang Q, Xiao Y, Lang M. *Appl Surf Sci*. 2010; 256:6107.
31. Hong Z, Zhang P, He C, Qiu X, Liu A, Chen L, Chen X, Jing X. *Biomaterials*. 2005; 26:6296. [PubMed: 15913758]
32. Kim H, Lee H, Knowles JC. *J Biomed Res A*. 2006; 79:643.
33. Jeong SI, Ko EK, Yum J, Jung CH, Lee YM, Shin H. *Macromol Biosci*. 2008; 8:328. [PubMed: 18163376]

34. D'Angelo F, Armentano I, Cacciotti I, Tiribuzi R, Quattrocchi M, Del Gaudio C, Fortunati E, Saino E, Caraffa A, Cerulli GG, Visai L, Kenny JM, Sampaioles M, Bianco A, Martino S, Orlacchio A. *Biomacromolecules*. 2012; 13:1350. [PubMed: 22449037]
35. Spadaccio C, Rainer A, Trombetta M, Vadalá G, Chello M, Covino E, Denaro V, Toyoda Y, Genovese JA. *Ann Biomed Eng*. 2009; 37:1376. [PubMed: 19418224]
36. Peng F, Yu X, Wei M. *Acta Biomater*. 2011; 7:2585. [PubMed: 21333762]
37. Kim H. *J Biomed Res A*. 2007; 83:169.
38. Yang F, Both SK, Yang X, Walboomers XF, Jansen Ja. *Acta Biomater*. 2009; 5:3295. [PubMed: 19470413]
39. Song J, Saiz E, Bertozzi CR. *J Eur Ceram Soc*. 2003; 23:2905.
40. Song J, Xu J, Filion T, Saiz E, Tomsia AP, Lian JB, Stein GS, Ayers DC, Bertozzi CR. *J Biomed Res A*. 2009; 89:1098.
41. Yang D-J, Xiong C-D, Govender T, Wang Y-Z. *J Biomat Sci-Polym E*. 2009; 20:1321.
42. Zhou S, Liao X, Li X, Deng X, Li H. *J Controlled Release*. 2003; 86:195.
43. Zhou S, Deng X, Yuan M, Li X. *J Appl Polym Sci*. 2002; 84:778.
44. Rathi SR, Coughlin EB, Hsu SL, Golub CS, Ling GH, Tzivanis MJ. *Polymer*. 2012; 53:3008.
45. Rathi S, Chen X, Coughlin EB, Hsu SL, Golub CS, Tzivanis MJ. *Polymer*. 2011; 52:4184.
46. Yang D-J, Chen F, Xiong Z-C, Xiong C-D, Wang Y-Z. *Acta Biomater*. 2009; 5:2467. [PubMed: 19427825]
47. Lucke A, Tessmar J, Schnell E, Schmeer G, Göpferich A. *Biomaterials*. 2000; 21:2361. [PubMed: 11055283]
48. Meloan SN, Puchtler H. *J Histotechnol*. 1985; 8:11.
49. Filion TM, Xu J, Prasad ML, Song J. *Biomaterials*. 2011; 32:985. [PubMed: 21040968]
50. Baroli B. *J Pharm Sci*. 2009; 98:1317. [PubMed: 18729202]
51. Von Burkersroda F, Gref R, Göpferich a. *Biomaterials*. 1997; 18:1599. [PubMed: 9613807]
52. Younes H, Cohn D. *Biomed Mater*. 1987; 21:1301.
53. Cui W, Zhu X, Yang Y, Li X, Jin Y. *Mat Sci Eng C*. 2009; 29:1869.
54. Jose MV, Thomas V, Johnson KT, Dean DR, Nyairo E. *Acta Biomater*. 2009; 5:305. [PubMed: 18778977]
55. Xie Z, Buschle-Diller G, DeInnocentes P, Bird RC. *J Appl Polym Sci*. 2011; 122:1219.
56. Steendam R, Van Steenberghe MJ, Hennink WE, Frijlink HW, Lerk CF. *J Control Release*. 2001; 70:71. [PubMed: 11166409]
57. Cohn D, Younes H. *Biomaterials*. 1989; 10:466. [PubMed: 2804234]
58. Hu D, Liu H. *J Appl Poly Sci*. 1994; 51:473.
59. Li S, Rashkov I, Espartero J, Vert M. *Macromolecules*. 1996; 29:57.
60. Caplan AI. *Journal of orthopaedic research*. 1991; 9:641. [PubMed: 1870029]
61. McCullen SD, Chow AGY, Stevens MM. *Curr Opin Biotech*. 2011; 22:715. [PubMed: 21646011]
62. Göpferich A, Peter SJ, Lucke A, Lu L, Mikos AG. *J Biomed Mater Res*. 1999; 46:390. [PubMed: 10397997]
63. Lieb E, Tessmar J, Hacker M, Fischbach C, Rose D, Blunk T, Mikos AG, Göpferich A, Schulz MMB, Tessmar J, Göpferich A. *Tissue Eng*. 2003; 9:71. [PubMed: 12625956]
64. Gorbunoff M, Timasheff S. *Anal Biochem*. 1984; 136:440. [PubMed: 6721144]
65. Webster TJ, Celaletdin E, Doremus RH, Siegel RW, Bizios R. *J Biomed Mater Res*. 2000; 51:475. [PubMed: 10880091]
66. Lee JH, Rim NG, Jung HS, Shin H. *Macromol Biosci*. 2010; 10:173. [PubMed: 19685498]
67. Polini A, Pisignano D, Parodi M, Quarto R, Scaglione S. *PloS one*. 2011; 6:e26211. [PubMed: 22022571]
68. Fischer EM, Layrolle P, Van Blitterswijk CA, De Bruijn JD. *Tissue Eng*. 2003; 9:1179. [PubMed: 14670105]
69. Zhao F, Grayson WL, Ma T, Bunnell B, Lu WW. *Biomaterials*. 2006; 27:1859. [PubMed: 16225916]

70. Xue D, Zheng Q, Zong C, Li Q, Li H, Qian S, Zhang B, Yu L, Pan Z. J Biomed Res A. 2010; 94:259.
71. Kim S-S, Sun Park M, Jeon O, Yong Choi C, Kim B-S. Biomaterials. 2006; 27:1399. [PubMed: 16169074]
72. Nandakumar A, Fernandes H, De Boer J, Moroni L, Habibovic P, Van Blitterswijk Ca. Macromol Biosci. 2010; 10:1365. [PubMed: 20799255]
73. Lian J, Stein G. Crit Rev Oral Biol M. 1992; 3:269. [PubMed: 1571474]
74. Lian JB, Stein GS, Stein JL, Van Wijnen aJ. Biochem Soc T. 1998; 26:14.
75. Ni P, Fu S, Fan M, Guo G, Shi S, Peng J, Luo F, Qian Z. Int J Nanomedicine. 2011; 6:3065. [PubMed: 22163160]

Fig. 1. Microstructural and compositional properties of electrospun HA-PELA and HA-PLA composite scaffolds. a) SEM micrographs of electrospun scaffolds. Scale bars = 50 μm . b) Fiber diameters ($n=100$, mean $\pm$ standard deviation) of electrospun scaffolds as determined from SEM micrographs using ImageJ. * $p<0.05$ (One-way ANOVA with Tukey post-hoc). c) TGA plots of electrospun scaffolds and HA powder control.

Fig. 2.
Tensile stress-strain curves of HA-PELA and HA-PLA composites.

Fig. 3.

Surface and mechanical property changes of electrospun PELA and PLA composite scaffolds upon hydration. a) Water contact angle (n=5) of as-spun and lyophilized scaffolds following water equilibration. #, water fully absorbed (~0°). n.a., no contact angle obtained due to scaffold shrinkage. * p<0.05 (One-way ANOVA with Tukey post-hoc). b) Storage modulus (n=3) of dry and hydrated scaffolds at 37 °C determined by dynamic tensile mechanical analysis (0.02% strain, 1 Hz). * p<0.05 (One-way ANOVA with Tukey post-hoc).

Fig. 4.

Crystallization behavior of PELA scaffolds upon hydration. (a) DSC curves of electrospun PELA scaffolds with or without hydration history. Hydrated PELA samples were equilibrated in deionized water @ 37 °C for 24 h and lyophilized. (b) MDSC curves of lyophilized electrospun PELA after water equilibration depicting reversible heat flow (green) and total heat flow (blue).

Fig. 5. (a) Hydrolytic degradation of HA-PELA and HA-PLA scaffolds in PBS (pH 7.4, Ca²⁺/Mg²⁺-free, 37 °C) measured by mass loss over 12 weeks. (b) SEM micrographs of PELA, HA-PELA, and HA-PLA scaffolds following a 3-week incubation in PBS (pH 7.4, Ca²⁺/Mg²⁺-free) at 37 °C. Scale bar = 50 μm.

Fig. 6. rMSC attachment and early proliferation on electrospun PELA, HA-PELA, and HA-PLA scaffolds (n=3) as determined by MTT cell viability assay at 24 and 96 h after initial cell seeding. * $p < 0.05$ (One-way ANOVA with Tukey post-hoc).

Fig. 7.

Effect of scaffold composition (polymer chemistry and HA) on the lineage commitment of rMSCs in unstimulated culture. Expression of Sox9, osteocalcin, and PPARG following (a) 7 or (b) 14 days culture in expansion media, as determined by qPCR. Data are normalized with housekeeping gene GAPDH and plotted as expression relative to that of rMSCs at time 0 (prior to seeding onto scaffolds). * $p < 0.05$ (One-way ANOVA with Tukey post-hoc).

Fig. 8.

Effect of scaffold composition (polymer chemistry and HA) on the osteogenic differentiation of rMSCs under induced differentiation culture conditions. Expression of RUNX2, osteopontin, and osteocalcin following a 14-day culture in osteogenic media. Data are normalized with housekeeping gene GAPDH and plotted as expression relative to that of rMSCs at time 0 (prior to seeding onto scaffolds). * $p < 0.05$ (One-way ANOVA with Tukey post-hoc).

Scheme 1.

Synthetic schemes for PELA (1) and PLA (2). $x=454$, $n=608$, $m=750$.

Scheme 2.

Depiction of a proposed structural rearrangement of the PELA block copolymer chains within HA-PELA composites upon hydration.