A Systemic Study on Key Parameters Affecting Nanocomposite Coatings on Magnesium Substrates

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Abstract

Nanocomposite coatings offer multiple functions simultaneously to improve the interfacial properties of magnesium (Mg) alloys for skeletal implant applications, e.g., controlling the degradation rate of Mg substrates, improving bone cell functions, and providing drug delivery capability. However, the effective service time of nanocomposite coatings may be limited due to their early delamination from the Mg-based substrates. Thus, the objective of this study was to address the delamination issue of nanocomposite coatings, improve the coating properties for reducing the degradation of Mg-based substrates, and thus improve their cytocompatibility with bone marrow derived mesenchymal stem cells (BMSCs). The surface conditions of the substrates, polymer component type of the nanocomposite coatings, and post-deposition processing are the key parameters that contribute to the efficacy of the nanocomposite coatings in regulating substrate degradation and bone cell responses. Specifically, the effects of metallic surface versus alkaline heat-treated hydroxide surface of the substrates on coating quality were investigated. For the nanocomposite coatings, nanophase hydroxyapatite (nHA) was dispersed in three types of biodegradable polymers, i.e., poly(lactic-co-glycolic acid) (PLGA), poly(L-lactic acid) (PLLA), or poly(caprolactone) (PCL) to determine which polymer component could provide integrated properties for slowest Mg degradation. The nanocomposite coatings with or without post-deposition processing, i.e., melting, annealing, were compared to determine which processing route improved the properties of the nanocomposite coatings most significantly. The results showed that optimizing the coating processes addressed the delamination issue. The melted then annealed nHA/PCL coating on the metallic Mg substrates showed the slowest degradation and the best coating adhesion, among all the combinations of conditions studied; and, it improved the adhesion density of BMSCs. This study elucidated the key parameters for optimizing nanocomposite coatings on Mg-based substrates for skeletal implant applications, and provided rational design guidelines for the nanocomposite coatings on Mg alloys for potential clinical translation of biodegradable Mg-based implants.

Keywords: magnesium; hydroxyapatite (HA) nanoparticles; nanocomposites; poly(lactic-co-glycolic acid) (PLGA); poly(L-lactic acid) (PLLA); poly(caprolactone) (PCL); biodegradable polymers; revised simulated body fluid (rSBF); bone marrow derived mesenchymal stem cells (BMSCs); internal stress; residual stress; bioresorbable skeletal implants.

1. Introduction

1.1. Magnesium for Biodegradable Implant Applications - Promise and Challenge

Biodegradable materials such as magnesium (Mg) [1-3] provide an attractive alternative to non-degradable materials for skeletal implant applications when the implants (*e.g.*, internal fixation devices) only need to serve their functions in the body for a set period. Mg implants naturally degrade in the body and thus do not require surgical removal. In addition, Mg has excellent mechanical [2, 4, 5] and biological [6-8] properties, and can bond strongly to bone tissue *in vivo* [1].

One of the major challenges for clinical translation of Mg alloys is that they degrade faster than the typical clinical requirement for bone repair [9, 10] due to the abundant aggressive ions such as chloride (Cl⁻) [11, 12]. The degradation rate of Mg-based implants may be controlled by adjusting alloy composition and processing, or by modifying the surface properties through coatings and surface treatments. This article focuses on the coating approach for controlling the degradation of Mg-based substrates.

1.2. Surface Coatings for Controlling Magnesium Degradation - Promise and Challenge

Surface coatings have many advantages as a means of controlling Mg degradation, because coatings can be combined with other degradation control mechanisms such as alloy composition and processing [13, 14]. As the Mg substrates support the load-bearing requirements, we designed nanocomposite coatings for the dual purposes of reducing Mg degradation and improving interfacial bioactivity.

Hydroxyapatite [HA; Ca₁₀(PO₄)₆(OH)₂] is the predominant mineral component in natural bone and has excellent osteoconductivity [15-17], especially when it is in the form of nanophase HA (nHA) [18]. HA or its derivative phases, when used alone, may not be ideal for load-bearing applications due to their inherent brittleness. Thus, HA-based calcium phosphates are often used as coating materials on metallic implant substrates, such as Ti or Mg [19, 20]. However, the brittleness of HA and its mismatch

with metallic substrates in thermal expansion coefficient often lead to cracking in HA coatings [20]. Moreover, when the HA coatings have weak adhesion to the substrates, the implant stability becomes a serious concern [21]. These drawbacks of using HA alone may be mitigated by compositing HA with a polymer of complementary properties.

Polymers have also been studied as coating materials for controlling Mg degradation, especially biodegradable poly(lactic-co-glycolic acid) (PLGA) [22-24], poly(L-lactic acid) (PLLA) [25-27], and poly(caprolactone) (PCL) [25, 26, 28-30]. These polymers are biocompatible and their degradation products are naturally occurring metabolites, e.g., lactic acid, glycolic acid, and 6-hydroxyl caproic acid [31]. The microstructure and properties of polymers can be further tailored during post-deposition processing. Heat treatments, such as annealing or melting, can reduce the permeability of the polymers by increasing their crystallinity and sealing pores [32-34]. However, PLGA, PLLA, and PCL have low mechanical properties [35] and bioactivity [36-39]. Skeletal implants with low bioactivity may have little bone tissue ingrowth [40-42] and likely leave an empty socket in the bone after the implants degrade [36, 41, 43]. Thus, the biodegradable polymers should be combined with bioactive ceramics to achieve desirable mechanical properties and bioactivity for implant applications.

The integration of nHA, biodegradable polymers, and Mg-based biodegradable metals addresses the key challenges encountered when using each material alone and provides synergistic properties for skeletal implant applications. The presence of a flexible polymer phase could prevent crack propagation through HA coatings [30]. Three types of biodegradable polymers, i.e., PLGA (85:15), PLLA, and PCL, were investigated in this study to elucidate the effects of a broad spectrum of polymer properties on nanocomposite coatings. The nHA particles were dispersed into the polymer matrix because they increased the mechanical properties and bioactivity of polymers *in vitro* [44, 45], and improved bone ingrowth *in vivo* [37]. The ratio of 30 wt.% nHA and 70 wt.% polymer was used in the nanocomposites because it provides a balanced mechanical and biological properties for bone regeneration [44, 46-48]. The nHA/polymer coatings can increase the surface osteoconductivity and reduce the degradation rate of

Mg substrates, while the Mg substrates provide mechanical properties similar to cortical bone for load bearing. The synergistic properties of these three components (nHA, polymer, Mg) are ideal for skeletal implant applications.

1.3. Optimization of Nanocomposite Coatings on Mg Substrates - Objectives of This Study

The objective of this study was to optimize the design and processing of nanocomposite coatings to maximize the synergy between the three components (i.e., nHA, polymer, Mg). To achieve this, we first screened different combinations of the coating parameters for surface hydrophobicity and the lowest corrosion rates using potentiodynamic polarization (PDP) curves. Secondly, we characterized the slowest degrading samples using immersion degradation and coating adhesion strength tests. Lastly, we investigated *in vitro* responses of bone marrow derived mesenchymal stem cells (BMSCs) to the samples with the slowest immersion degradation rate. This study elucidated the interactions among different parameters of nanocomposite coatings (i.e., substrate surface conditions, polymer types, and post-deposition processing) and their effects on the degradation of Mg substrates, thus providing valuable design guidelines for polymeric and nanocomposite coatings on Mg-based substrates for skeletal implant applications.

2. Materials and Methods

2.1. Preparation of Magnesium Substrates

Mg-based bars (97% Mg, 3% Al, 1 mm thick; Minisci, Cat# MGFLAT) were ground with 600 grit silicon carbide paper (SiC; Ted Pella) while using ethanol (EtOH; Koptec) as a lubricant. The grinded Mg bars were then cut into 10 x 10 mm squares using a notcher (no. 100, Whitney Metal Tool Co). The Mg substrates were degreased in acetone (Sigma Aldrich) in an ultrasonic bath (VWR Symphony) for 30 minutes, and then cleaned in ethanol in the same ultrasonic bath for another 30 minutes. These Mg substrates had a metallic surface and were designated as M_Mg.

A separate group of M_Mg substrates had their surface modified using an alkaline heat treatment procedure to create a surface rich in $\text{Mg}(\text{OH})_2$ [19]. The alkaline heat treatment was performed by immersing the M_Mg substrates in 1 M NaOH (Strem Chemicals) at 80 °C for 2 hours. After immersion, the substrates were gently rinsed with deionized (DI) water (Millipore Milli-Q® Biocel System) for three times and dried in air at room temperature. These alkaline-heat-treated substrates were named as A_Mg.

Before the coating deposition, the M_Mg or A_Mg substrates were cold mounted into epoxy resin (MG Chemicals, Cat # 832HT) with a diameter of 18 mm and a thickness of 2 mm. Only a single face of 10 x 10 mm of the Mg substrates was exposed for coating. Prior to mounting, a copper wire was secured underneath each Mg substrate using a copper tape for PDP measurements. The copper wire was encased in epoxy until it exited the epoxy disk at one side, which insulated the copper wire from electrolyte when the exposed surface of the Mg substrate was completely immersed in rSBF.

2.2. Preparation of Nanocomposite Coatings on the Mg Substrates

The nHA was prepared using a wet chemistry precipitation method followed by hydrothermal treatment, as described previously [22]. The nHA was added to (85:15) PLGA (200 kDa, Cat# AP52), PLLA (230 kDa, Cat# AP65), or PCL (200 kDa, Cat# AP09) at a ratio of 30 wt.% nHA to 70 wt.% polymer. All polymers were procured from Polysciotech. The nHA/polymer mixtures were then suspended in a 10 wt./vol. % concentration in chloroform (Sigma Aldrich), and mixed in an ultrasonic bath (VWR Symphony) at 40 °C for 1 hour. The suspensions were dried in air for 1 hour on polytetrafluoroethylene (PTFE) dishes, and then re-suspended in chloroform again in the same ultrasonic bath at 40 °C for 2 hours, to reduce the settling of the nHA particles. To further improve the dispersion of nHA nanoparticles, the nanocomposites were sonicated at high power (9 W, 20 kHz; Misonix Sonicator S-4000) for 10 min.

The nHA/polymer suspensions were deposited onto the Mg substrates using a spin-coater (Smart Coater; Best Tools, LLC). The spin-coating speed was adjusted such that all the coatings had a similar thickness in the range of 30-40 μm .

The coatings deposited on the Mg substrates were subjected to one of four different post-deposition processing routes: (1) no post-deposition processing, (2) melting the coating for 5 minutes, (3) annealing the coating for 30 minutes, and (4) melting the coating for 5 minutes followed by annealing the coating for 30 minutes. The nHA/PLGA and nHA/PLLA coatings were melted by heating in an oven (HeraTherm; Thermo Scientific) at 200°C for 5 minutes, and/or annealed by heating at 115°C for 30 minutes. The nHA/PCL coatings were melted by heating at 80°C for 5 minutes, and/or annealed by heating at 50°C for 30 minutes.

2.3. Measurement of Surface Hydrophobicity

The surface hydrophobicity influences water permeability and cell activities on the surfaces. The polymer types and post-deposition processing could significantly alter the hydrophobicity of the nanocomposite coatings. To determine the surface hydrophobicity, the contact angle on the sample surface was measured using a goniometer (EasyDrop; Krüss). Specifically, the advancing contact angles were measured 20 seconds after depositing 2 μ L of deionized (DI) water on the surfaces. The contact angles of three replicate samples were measured for each sample type; and each sample was measured three times in three different areas. The average and standard error of the measurements were calculated and plotted. The contact angle measurements were performed for all different combinations of variable parameters of Mg substrate surface conditions (M_Mg and A_Mg), polymer types in the nanocomposite coatings (PLGA, PLLA, and PCL), and post-deposition processing (None, Annealed, Melted, and Melted then Annealed), as listed in Table 1.

2.4. Potentiodynamic Polarization Measurements for Corrosion Rates

The potentiodynamic polarization (PDP) study was performed according to ASTM standard G 102-89. The PDP tests were conducted in rSBF using a Potentiostat/galvanostat (model 273A; EG&G Princeton applied research) to screen the corrosion rates of all the samples prepared using different parameters. The rSBF was used because it represents the same ionic concentration as in human blood

plasma [49]. Each sample served as the working electrode, with an Ag/AgCl electrode as the reference electrode (part # CHI111, CHI Instruments) and a Pt foil as the counter electrode (part # CHI 115, CHI Instruments). The electrodes were immersed in rSBF at 37 °C for the PDP measurements. For all the PDP measurements, the potential ranged from +1 V to -3 V, with a 10 mV step size and a 0.5-second step time at a 100 mV/s scan rate. On the PDP curves, straight lines were drawn along the linear portion; the corrosion current (I_{corr}) and corrosion potential (E_{corr}) were extrapolated from the intersection of these straight lines. The corrosion rate (CR) (in mm/year) of the samples was calculated using the following equation from ASTM standard G 102-89.

$$CR = \frac{I_{corr} \times K_1 \times EW}{\rho \times A} \quad \text{Eq. 1}$$

Where I_{corr} is the corrosion current, K_1 is the constant for unit conversion, EW is the equivalent weight of Mg, ρ is the density of Mg, and A is the area of the sample submerged in rSBF. EW and ρ were calculated for the Mg substrates with 97 wt.% Mg and 3 wt.% Al. Specifically, $K_1=3.27 \times 10^{-3}$ (mm•g)/($\mu\text{A} \cdot \text{cm} \cdot \text{year}$), $EW=12.05$, $\rho=1.76 \text{ g/cm}^3$, and $A=1 \text{ cm}^2$. PDP measurements were performed in triplicate for each sample type listed in Table 1.

2.5. Degradation Measurements via Immersion in rSBF

For each combination of Mg surface condition and polymer type of the nanocomposite coatings, the post-deposition processing that resulted in the slowest corrosion rates in the PDP measurements were selected for the subsequent immersion study in rSBF, as shown in Table 1.

For the immersion study, each sample was sterilized in ethanol (Koptec) for 1 hour, and then immersed in 4 mL of rSBF for 7 days under standard cell culture conditions (that is, at 37 °C, 5% CO_2 /95% air, humidified, sterile environment). After each day of incubation (i.e., every 24-hour incubation period), each sample was collected, dried for at least 24 hours until reaching constant mass, and photographed under sterile condition. After every 24 hours of incubation, the rSBF was collected for pH and ionic concentration measurements. After the rSBF was completely removed from each incubation

well, fresh sterile rSBF of the same volume was added to each well of the samples for each incubation period. The pH of rSBF was measured immediately after collection. The ionic concentration of rSBF was measured using inductively coupled plasma - optical emission spectrometry (ICP-OES; Optima 8000, Perkin-Elmer). The rSBF collected at every 24 hours was diluted to 1/100 in DI water for measuring $[Mg^{2+}]$ and $[Ca^{2+}]$ using the ICP-OES. The ICP-OES system was calibrated using 0.5-5.0 mg/L Mg^{2+} standards and 0.1-1.0 mg/L Ca^{2+} standards.

After 7 days of immersion in rSBF, the samples were examined using scanning electron microscopy (SEM; Nova NanoSEM 450, FEI Co.) under low vacuum conditions with a 5 kV accelerating voltage. The surface elemental composition of the samples was measured using energy dispersive x-ray spectroscopy (EDX) under low vacuum conditions with a 15 kV accelerating voltage. All samples for SEM and EDX analyses were sputter-coated with Pt/Pd at 10 mA for 30 seconds (Cressington; Sputter Coater 108 Auto). The samples before the immersion study was also analyzed using SEM and EDX for comparison.

2.6. Measurements of Coating Adhesion Strength

Tensile testing was used to measure the coating adhesion strength before and after immersion. A mechanical testing system (Model #5969, Instron) was used to perform the tensile testing at an extension rate of 1 mm/minute. The coated samples were mounted for tensile testing following a procedure modified from the literature [26].

2.7. BMSC Study *In Vitro*

Bone marrow derived mesenchymal stem cells (BMSCs) were harvested from the marrow cavity of the femur and tibia of Sprague-Dawley rat weanlings for culturing with the best sample that showed the slowest degradation and the best coating adhesion, i.e., nHA/PCL coated M_Mg with the post-deposition processing of melting and then annealing. Non-coated M_Mg, nHA/PCL films without Mg substrates, epoxy mounts without Mg substrates, glass, and polystyrene-tissue culture plate (PSTC) were

used as controls and references. All samples were run in triplicate. The rat weanlings were euthanized by CO₂ asphyxiation. The distal ends of the femur and tibia were removed so that bone marrow could be flushed out with Dulbecco's Modified Eagle Medium (DMEM; Sigma Aldrich) supplemented with 10% fetal bovine serum (FBS; HyClone) and 1% penicillin/streptomycin (P/S; Invitrogen). The DMEM supplemented with 10% FBS and 1% P/S will be referred to as DMEM. A 70 µm nylon strainer was used to separate cell aggregates and remove tissue debris. The harvested BMSCs were cultured in DMEM under standard cell culture conditions to reach 90% confluency.

The samples were sterilized in ethanol for 1 hour before BMSC culture, placed into the wells of a 12-well tissue culture treated plate, and washed with 2 mL of DMEM to prepare the local environment for cell seeding. The BMSCs were detached using Trypsin (Invitrogen), seeded onto the samples at a concentration of 10,000 cells/cm², and incubated for 24 hours under standard cell culture conditions. After 24 hours of culture, each sample was washed with PBS to remove non-adherent cells. The adherent cells were fixed with 4% paraformaldehyde, and stained with Alexa Fluor[®] 488-phalloidin for F-actin and 4',6-diamidino-2-phenylindole (DAPI; Invitrogen) for nucleic acids. Five random locations on each of the triplicate samples were imaged using a fluorescence microscope (Eclipse Ti; Nikon) with a 10x objective lens. The DAPI stained nuclei were counted in each image to calculate the average cell adhesion density (cells/cm²). The stained F-actin was used to calculate the cell spreading area, maximum Feret diameter, and minimum Feret diameter using ImageJ. The aspect ratio of the BMSCs was calculated by dividing the maximum Feret diameter by the minimum Feret diameter. The euthanasia of the rats and harvesting of cells from the rats were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California at Riverside. The BMSC culture method was established previously [50].

2.8. Statistical Analyses

R was used to perform the statistical analyses. The normal distribution of the data was checked using the Shapiro-Wilks test. Homogeneity of variance was checked using the Bartlett test. The distribution and variance of the data were used to determine the suitable statistical tests.

The corrosion rates of the coated Mg substrates from the PDP measurements were analyzed using factorial ANOVA, after a cubic root transformation was applied to the data to ensure normal distribution and homogeneous variance. Factorial ANOVA was used to reveal the interactions between experimental parameters. The non-coated Mg substrates were not included in the factorial ANOVA analysis because their corrosion rates were much greater than the coated Mg substrates and did not have a normal distribution or homogeneous variance. An unpaired two-tailed t-test was thus used to compare the corrosion rates of the non-coated M_Mg and A_Mg substrates.

A wild bootstrap test with Bonferroni correction was used to analyze the effects of the coating polymer component, the post-deposition processing, the substrate surfaces, and the immersion time on the $[Mg^{2+}]$ measured in the immersion study, the key indicator of the sample degradation rate. The wild bootstrap was used because the $[Mg^{2+}]$ data did not have normal distribution or homogeneous variance. The Bonferroni correction was used to control familywise error. The data was stratified for each time point of immersion study in the wild bootstrap test.

The coating adhesion strengths before immersion and after immersion were separately examined using the Kruskal-Wallis test, and the results did not show statistically significant difference. This was mainly due to outliers that increased the standard deviation, and the small number of samples tested.

The Kruskal-Wallis test was used to analyze the data from the *in vitro* BMSC study to determine the statistical significance of sample type on cell adhesion density, cell spreading area, and cell aspect ratio. The Kruskal-Wallis test was used because the data did not have normal distribution or homogeneous variance. Post-hoc comparisons were made using the Dunn test with the Bonferroni correction.

3. Results

3.1. The Effects of the Coating Parameters on Surface Hydrophobicity

Surface hydrophobicity has been known to affect water permeability [51] and bioactivity [52-54]. Figure 1 shows the contact angles of deionized water on the nHA/polymer nanocomposite coatings on Mg substrates and non-coated Mg control substrates. The greater contact angle indicates the surface is more hydrophobic. The contact angles of the nanocomposite coated Mg substrates ranged from 74-86, and the M_Mg and A_Mg control substrates had a respective contact angle of 57 and 78. The contact angles of the nanocomposite coated Mg substrates varied in a relatively narrow range, suggesting the coating hydrophobicity would not be a significant variable during the degradation tests.

The nHA/PLGA coatings on M_Mg without post-deposition processing showed a slightly lower contact angle than the rest of nHA/PLGA coatings. For the nHA/PLLA coatings, the post-deposition processing of melting then annealing slightly increased the coating contact angles on both M_Mg and A_Mg substrates. Melting caused slight increase in the contact angles of nHA/PLLA as compared with its corresponding coatings without heat treatments. For the nHA/PCL coatings, the post-deposition processing of melting then annealing slightly increased the average contact angle on both M_Mg and A_Mg substrates.

3.2. The Effects of the Coating Parameters on Corrosion Rates Calculated from PDP Testing

The representative PDP curves for the nanocomposite coated and non-coated Mg substrates with different post-deposition processing were plotted (Figure 2). The nHA/PLGA and nHA/PLLA coatings reduced the I_{corr} , but had little effect on the E_{corr} . In contrast, the nHA/PCL coatings reduced the I_{corr} , and some also had significant effect on E_{corr} . Post-deposition processing reduced the I_{corr} for the nHA/PLGA and nHA/PLLA coated A_Mg substrates, but showed little effect on the respective coatings on M_Mg substrates. The nHA/PCL coated M_Mg and A_Mg had similar E_{corr} and I_{corr} before post-deposition processing. Post-deposition processing reduced I_{corr} more on the nHA/PCL coated A_Mg substrates than

the M_Mg substrates. Post-deposition processing also increased the E_{corr} for the nHA/PCL coated A_Mg substrates but had little effect on the E_{corr} for the M_Mg substrates.

The corrosion rates were calculated based on the corrosion current density extrapolated from PDP curves, as shown in Figure 3. All of the nanocomposite coatings reduced the corrosion rates as compared with the non-coated Mg substrates. When comparing the non-coated M_Mg with A_Mg substrates using a two-tailed t-test, their corrosion rates did not show statistically significant difference ($p = 0.24$). However, Mg substrate surface conditions did show statistically significant effects on the corrosion rates of the nanocomposite coated samples in factorial ANOVA, because the other parameters (polymer types, and post-deposition processing) interacted with Mg substrate surface conditions (i.e., M_Mg versus A_Mg). Specifically, the post-deposition processing increased the corrosion rates of nHA/PLGA and nHA/PLLA coated M_Mg substrates, but the post-deposition processing of melting then annealing reduced the corrosion rates of the respective coatings on A_Mg substrates. Post-deposition processing reduced the corrosion rates of nHA/PCL coated M_Mg and A_Mg. The nHA/PCL coated A_Mg substrates that underwent post-deposition processing showed the lowest corrosion rate among all of the sample types.

The interactions of the coating parameters (polymer component of the nanocomposite coating, substrate surface, and post-deposition processing) were analyzed using factorial ANOVA, as summarized in Figure 4. Based on the p values in Figure 4D, most parameters showed statistically significant interactions, except the interaction between polymer component and substrate surface. In Figure 4A, the lines in the interaction plot for polymer component and substrate surface were parallel or near-parallel, indicating no or little interactions detected. Furthermore, all three lines for the polymer components sloped downwards from the M_Mg to A_Mg substrates, indicating slower corrosion rates on the A_Mg substrates. The line for the PLGA component was slightly below the line for the PLLA component, indicating that PLGA component had a slightly slower average corrosion rate. The line for the PCL component was far below the other two lines, indicating that nHA/PCL coating reduced the corrosion rate

more significantly than the other two polymer types. In Figure 4B, some lines in the interaction plot for post-deposition processing and substrate surface were intersected, indicating statically significant interactions between them. The two lines for the annealing and the melting then annealing treatments were near parallel to each other, indicating these two heat treatments had similar effects on corrosion rates; their steeper slopes indicate that they significantly decreased the corrosion rates on the A_Mg substrates. The line for the melting treatment was nearly horizontal, suggesting the melting treatment had similar effects on the corrosion rates for M_Mg and A_Mg substrates. In Figure 4C, the interaction plot for post-deposition processing and polymer component showed that post-deposition processing affected PCL differently than the other two polymer types. That is, the post-deposition processing increased the corrosion rates for the PLGA and PLLA polymer types, but decreased the corrosion rates for the PCL polymer type. The three lines for the post-deposition processing of annealing, melting, and melting then annealing were nearly parallel to each other, and showed a slight upward slope from the PLGA to PLLA polymer types and a steep downward slope to the PCL polymer type. The fourth line for no post-deposition processing was nearly straight, showed a slight upward slope from the PLGA, PLLA to the PCL polymer types, and intersected with the post-deposition processing lines at the positions above PCL but below PLGA and PLLA.

3.3. Degradation Results from the Immersion Study

The immersion study was performed in rSBF for 7 days to investigate the surface changes during immersion, and ion release from the sample degradation, which complemented the PDP measurements.

3.3.1. Surface Changes during Immersion Degradation

For each type of nHA/polymer nanocomposite coating on M_Mg or A_Mg substrate, the post-deposition processing that provided the slowest corrosion rate (Figure 3) was selected for further investigation of the sample degradation in the immersion study, as summarized in Table 1. That is, no post-deposition processing was used for the nHA/PLGA and nHA/PLLA coatings on M_Mg substrates,

and the post-deposition processing of melted and then annealed was used on A_Mg substrates. The post-deposition processing of melted and then annealed was used for the nHA/PCL coatings on both M_Mg and A_Mg substrates. All of these nanocomposite coatings significantly reduced the degradation rate of Mg substrates without delamination after 7 days of immersion in rSBF (Figure 5). During the 7 days of immersion, the samples were observed and photographed every 24 hours, but only the images of 1, 3, 5, and 7 days were included in Figure 5 to compare with the respective sample before immersion (day 0). In general, all the coated or non-coated Mg samples showed some degree of change during the 7 days, when compared with the coated or non-coated Ti control. The epoxy that was used as the control for the mounting material showed no visible changes throughout the entire 7 days of immersion. During the immersion, small blisters were observed on the coated Mg substrates, but not on the coated Ti substrates. After 5 days of immersion, only the nHA/PLLA coatings on the A_Mg substrates showed a large blister emerged from small blisters. After 7 days of immersion, the nHA/PCL coatings on the M_Mg substrates showed much less blisters than the respective coatings on A_Mg substrates.

3.3.2. Analyses of the pH and Ion Concentrations in rSBF during Immersion Degradation

Figure 6 shows the pH measurements and ion concentrations in rSBF during immersion degradation. As shown in Figure 6A, all of the nanocomposite coatings significantly reduced the alkaline pH in the rSBF as compared with the non-coated Mg substrates. The non-coated Mg substrates showed the highest pH in rSBF throughout the immersion, while the controls without Mg substrates, i.e., rSBF only, epoxy mount, non-coated Ti, and nHA/PLGA coated Ti, had negligible effect on the pH of the immersion media. Generally, the nHA/PCL coated Mg substrates showed lower pH than the nHA/PLGA and the nHA/PLLA coated Mg substrates. The nHA/PCL coated M_Mg and A_Mg had similar pH on day 1, but later the nHA/PCL coated M_Mg showed lower pH than the same coating on A_Mg on day 2, 3, 4, and 7. Figure 6B shows the Mg^{2+} ion concentrations [Mg^{2+}] in rSBF during immersion, the key indicator of Mg degradation. All of the nanocomposite coatings reduced the release of Mg^{2+} ions when compared with the non-coated Mg substrates. In consistent with the pH results, the nHA/PCL coated Mg substrates

showed much lower $[\text{Mg}^{2+}]$ in rSBF than the nHA/PLGA and nHA/PLLA coated Mg substrates. Moreover, the nHA/PCL coated M_Mg showed lower $[\text{Mg}^{2+}]$ in rSBF than the same coating on A_Mg. Remarkably, the nHA/PCL coated M_Mg showed very similar $[\text{Mg}^{2+}]$ as the controls that did not contain Mg and the baseline $[\text{Mg}^{2+}]$ in rSBF. Figure 6C shows the Ca^{2+} ion concentrations $[\text{Ca}^{2+}]$ in rSBF during immersion, which ranged from 1.6 to 2.3 mM in average for all the sample types, a much narrower distribution than $[\text{Mg}^{2+}]$. In average, the $[\text{Ca}^{2+}]$ decreased for all Mg containing samples, but did not change much for the Ti-based and epoxy controls, when compared with the baseline $[\text{Ca}^{2+}]$ in rSBF. Among the Mg-based samples, non-coated Mg samples showed the lowest $[\text{Ca}^{2+}]$, nHA/PCL coated Mg samples showed the highest $[\text{Ca}^{2+}]$, and nHA/PLGA and nHA/PLLA coated Mg samples were in between.

The statistical analyses of the parameters that influenced Mg degradation in immersion study are summarized in Table 2. The interactions among the parameters of substrate surface conditions, the types of polymer component in the nanocomposite coatings, and immersion time showed statistically significant effect on Mg degradation, i.e., $[\text{Mg}^{2+}]$ in rSBF, except the interactions of substrate surface:polymer component:time. This corroborates the statistical analyses of the corrosion rates reported in Figure 4D. Moreover, the substrate surface alone (M_Mg versus A_Mg) did not show any significant effect on Mg degradation in rSBF in terms of pH and $[\text{Mg}^{2+}]$, although non-coated M_Mg showed less release of Mg^{2+} ions in average than the non-coated A_Mg after 5 days of immersion in rSBF.

3.3.3. Surface Characterization Before and After Immersion Degradation

Figure 7 shows the SEM images of the nanocomposite coated and non-coated Mg samples before and after immersion in rSBF for 7 days. The nHA/PLGA coatings and nHA/PCL coatings remained intact on both M_Mg and A_Mg substrates. The nHA/PCL coated M_Mg and A_Mg attracted significant deposition after immersion, and the deposition appeared to be more abundant on the nHA/PCL coated M_Mg than the same coating on A_Mg. However, the nHA/PLGA coatings on the A_Mg substrates showed large cracks and craters after 7 days of immersion, which were not observed for the same coatings

on the M_Mg substrates. Figure 8 shows the craters formed in the nHA/PLGA coating on A_Mg after 7 days of immersion in rSBF due to H₂ gas evolution from Mg degradation.

Figure 9 shows the surface elemental compositions of the nanocomposite coated and non-coated Mg samples before and after immersion in rSBF for 7 days. All of the nanocomposite coated Mg samples showed C, O, Ca and P as the main elements on the surface before and after immersion in rSBF. Mg was not detected on any of the nanocomposite coated Mg samples before immersion and only small amounts of Mg were detected after immersion, which demonstrated the effectiveness of the coatings in reducing Mg degradation. The amount of Ca and P increased on the surface of nHA/PLGA and nHA/PCL coated Mg but not on nHA/PLLA coated M_Mg. The non-coated Mg substrates did not show Ca and P on the surface before immersion, but showed Ca and P on the surface after immersion, which demonstrated Ca and P deposition from the rSBF. Non-coated M_Mg and non-coated A_Mg showed increasing O after immersion when compared with before immersion.

3.4. Coating Adhesion Strength

Figure 10 shows the coating adhesion strength before and after immersion in rSBF for 7 days. Before immersion, the nHA/PLGA and nHA/PLLA coatings showed similar adhesion strengths around 9 MPa, and the nHA/PCL coatings showed slightly higher adhesion strengths at 11-12 MPa. The Mg substrate surface conditions (M_Mg versus A_Mg) had negligible effect on the coating adhesion strength before immersion. After 7 days of immersion, all of the nanocomposite coatings showed significantly reduced adhesion strength. After immersion, the nHA/PCL coated M_Mg showed the greatest adhesion strength among all the sample types, with the average adhesion strength of 0.9 MPa. In contrast, the nHA/PCL coated A_Mg showed the average adhesion strength of 0.13 MPa after immersion. The nHA/PLGA coated M_Mg and A_Mg showed similar coating adhesion strength at 0.14-0.15 MPa after immersion. The nHA/PLLA coated M_Mg showed slightly higher coating adhesion strength than the same coating on A_Mg after immersion, 0.15 MPa versus 0.05 MPa, respectively.

3.5. BMSC Adhesion and Morphology

Figure 11 shows the representative fluorescence images of BMSCs directly cultured on the samples and controls for 24 hours. All the samples and controls showed the attachment of BMSCs, but the cell morphology varied depending on the sample type. Figure 12 shows the quantified results of BMSC adhesion density, spreading area, and aspect ratio. Kruskal-Wallis tests showed that the sample type had statistically significant effects on BMSC adhesion density ($p=2.2 \times 10^{-16}$), spreading area ($p=2.2 \times 10^{-16}$), and aspect ratio ($p=1.2 \times 10^{-4}$). Pairwise comparisons using the Dunn test showed that the nHA/PCL coatings minimized the negative effects of rapid Mg degradation on the BMSCs (Figure 12).

In average, the nHA/PCL coated M_Mg substrates showed the greatest BMSC adhesion density, the non-coated M_Mg showed the least, and the control samples of nHA/PCL film, epoxy, glass, and PSTC were in between (Figure 12A). Statistically, the nHA/PCL coated M_Mg showed significantly greater BMSC adhesion density than the non-coated M_Mg, nHA/PCL film, and glass reference. The BMSCs on non-coated M_Mg, nHA/PCL coated M_Mg, nHA/PCL film, and epoxy had average spreading areas that were below $3000 \mu\text{m}^2$ per cell (Figure 12B). The BMSCs on glass and PSTC controls had higher spreading areas of over $4000 \mu\text{m}^2$ per cell. Statistically, the BMSCs on the nHA/PCL coated M_Mg showed significantly greater spreading area per cell than the non-coated M_Mg and nHA/PCL film. The BMSCs had a much higher average aspect ratio of 3.7 on non-coated M_Mg, and the rest of samples and controls had an aspect ratio of 1.6-1.9 (Figure 12C). Statistically, the aspect ratio of BMSCs on the non-coated M_Mg was significantly greater than the rest of samples and controls.

4. Discussion

4.1. A Defined Model System for Studying the Key Parameters Affecting Nanocomposite Coatings on Magnesium

Nanocomposite coatings provide synergistic properties at the tissue-implant interface by decreasing the degradation rate of Mg alloys and improving cellular interactions; however, delamination

remains a key challenge because it limits the functional lifetime of the coatings in mediating the degradation of Mg alloys [55]. The objective of this study was to optimize the nanocomposite coatings to address the challenge and to elucidate the engineering guidelines for improving the nanocomposite coatings on Mg substrates for skeletal implant applications. The key parameters, *i.e.*, Mg substrate surface conditions (M_Mg versus A_Mg), the polymer component type in the nanocomposite coatings (PLGA, PLLA versus PCL), and the post-deposition processing (melting, annealing, the combination of the two versus none), were investigated to determine their effects on the sample degradation, coating adhesion strength, and BMSC responses. For this purpose, a model system based on spin-coating was established and used for the systemic study of the key parameters that affect nanocomposite coatings on Mg substrates.

The spin-coating process was revised based on our previous investigation in order to provide a more accurate model system for characterizing the material properties of nanocomposite coatings on Mg substrates. In our previous study, the substrates were spin-coated on both faces and the edges of the substrates were dip coated; as a result, the edge had greater coating thickness than substrate faces, which promoted delamination because internal stress is concentrated at edges and thicker coatings are more likely to delaminate [56]. Furthermore, the boundary regions between the spin and dip coatings may have created additional internal stress, and delamination often initiated at the edges of the samples and then propagated inwards, which damaged the coating-substrate interface within 8 hours of immersion [55]. The rapid delamination caused by the combination of spin and dip coatings made it difficult to characterize how the key parameters of material and surface affect the performance of the nanocomposite coated Mg substrates. Therefore, the model coating system was modified by mounting the Mg substrates in epoxy so that only one surface of a Mg substrate would be exposed and coated with the nanocomposites, which eliminated the edge effects and other irrelevant factors from the key material parameters of interest. It is worth mentioning that the spin-coating process serves as a well-defined model system for studying the coating materials and their interactions with the substrates, but other coating

methods for conformal coatings on three-dimensional skeletal implants are still needed for clinical translation.

4.2. The Polymer Component in the Nanocomposite Coatings Influenced the Coating Permeability and Interface with the Mg Substrate

Permeability was one of the key differences among the polymer components (PLGA, PLLA, or PCL) in the nanocomposite coatings. PCL had the lowest water diffusion coefficient among the three polymers, and PCL and PLLA had much less water absorption than PLGA. Table 3 lists a summary of relevant properties of PLGA, PLA, and PCL [57-62]. The slow water transport through PCL is one of the reasons that the nHA/PCL coatings reduced Mg degradation more effectively than the nHA/PLGA and nHA/PLLA coatings.

Another key difference among the polymer components studied in the nanocomposite coatings is their viscosity. The nHA/PCL suspension had a lower viscosity than the nHA/PLGA or nHA/PLLA suspensions when the nHA/polymer weight ratio (30/70) and the nanocomposite/solvent ratio (10 wt./vol. %) were fixed. This required slower spin-coating speeds for the nHA/PCL suspensions than the nHA/PLGA and nHA/PLLA suspensions to achieve the same coating thickness. The combination of lower viscosity and slower spin-coating speed enabled the nHA/PCL suspension to fill in the grinding grooves on M_g substrates better than nHA/PLGA and nHA/PLLA suspensions that had higher viscosity and faster spin-coating speed. Filling in the grinding grooves with the nanocomposite suspension increased the adhesion strength of the nHA/PCL coatings due to improved physical interlocking. The nanocomposite suspensions solidified in less than 1 minute during spin-coating, which limited the time for more viscous suspensions to flow into the grinding grooves. If the grinding grooves were not filled with the nanocomposite suspension due to the higher viscosity or faster spin-coating speed (in the cases of nHA/PLGA and nHA/PLLA suspensions), there would be less physical interlocking and the possible micro-spaces at the coating-substrate interface would act as reservoirs for water to promote degradation of Mg substrates.

4.3. The Post-Deposition Processing and Substrate Surface Conditions Influenced the Coating Permeability

The melting treatment for the nHA/PCL coatings reduced their permeability because the melted PCL was able to flow and seal residual voids in the coating or at the interface, while the annealing treatment for the nHA/PCL coatings reduced their permeability due to the increase of PCL crystallinity [63]. In contrast, the effects of melting and annealing treatments on the nHA/PLGA and nHA/PLLA coatings were dependent on both the polymer component type in the nanocomposite coatings and the substrate surface conditions. Specifically, on the A_Mg substrates, the post-deposition processing of melting then annealing for the nHA/PLGA and nHA/PLLA coatings reduced the sample corrosion rates; however, on the M_Mg substrates, the same post-deposition processing increased the sample corrosion rates, when compared with no post-deposition processing (Figure 3). This indicated that the effects of the post-deposition processing on the sample corrosion were more than just altering the coating permeability, because similar melting and annealing treatments reduced the permeability of PLGA [32] and PLLA [64] films. It is speculated that the post-deposition processing might have affected the coating-substrate interface as well.

The interaction plots in Figure 4 indicated that the three key parameters (the polymer component in the nanocomposite coatings, the post-deposition processing, and the substrate surface conditions) interacted with one another; i.e., one parameter could increase or decrease the corrosion rates depending on the other parameters. The substrate surface condition alone did not have any statistically significant effect on the corrosion rates of the samples without coatings (t-test, p value = 0.24, Figure 3), but the substrate surface did have statistically significant effect on the corrosion of the samples with coatings (factorial ANOVA, p value = 5.83×10^{-7} , Figure 4). This suggested that the substrate surface conditions (A_Mg versus M_Mg) did not have any significant direct impact on the corrosion of Mg substrates, but instead indirectly affected the corrosion of Mg substrates by influencing the permeability of the coatings and the coating-substrate interface.

The alkaline heat treatment enriched the Mg substrate surfaces (A_Mg) with Mg(OH)_2 [65], which is a known nucleating agent for polymers [66-69]. The abundance of nucleating agents increased the number of polymer crystals formed on the surface and decreased the grain size, which correspondingly reduced the permeability of the polymer matrix. The smaller crystals also reduced the crack propagation in the polymer matrix in comparison with the larger crystals [70], which further increased the durability and protectiveness of the coatings. Furthermore, increased nucleation of polymer crystals directly on the Mg substrates could increase the interfacial strength between the coating and substrate due to more sites of interactions [71]. The abundance of nucleating agents on the A_Mg substrate surfaces was especially beneficial for the nHA/polymer coatings with the post-deposition processing.

4.4. Interactions among the Key Parameters and their Effects on Internal Stress and Corrosion

Figure 13 illustrates the interactions among the key parameters (polymer component in the nanocomposite coatings, substrate surface, post-deposition processing) and their effects on the sample performance, e.g., corrosion. The mechanical properties of the polymer components in the nanocomposite coatings, the post-deposition processing, and the substrate surface conditions affected the internal stress in the coatings. One of the most common sources of internal stress in coatings is shrinkage after deposition. Polymers generally expand when they undergo solid-liquid phase transition, and shrink when they solidify after deposition. The polymers in coatings are constrained by the substrate surface when they solidify, which creates internal stress. The volume changes of material during solidification and crystallization were reported to induce internal stresses in the range of 10-30 MPa [72, 73], which is significant as compared with the coating adhesion strengths. Internal stresses increase sharply at the edges and discontinuities, which explains why the coating delamination often initiates from the edges [56]. The stress could also accelerate the degradation of polymers [74] and reduce the adhesion strength of coatings [75]. Internal stress could cause the crack propagation in the coatings, which impairs the barrier properties of the coatings. Swelling is another source for internal stress. Polymers could swell as they absorb water,

and the coatings have a limited ability to accommodate this volume change due to the substrate constrain; for the same reason, the coatings also have a limited ability to accommodate shrinkage.

As described in the equation 2, the residual stress (internal stress locked in the material) could emerge from the deposition of dissolved coating polymers (Eq. 2A) [76], deposition of melted coating polymers (Eq. 2B) [76], and/or hygroscopic swelling of the coating polymers (Eq. 2C) [77], all of which share striking similarities with one another. Specifically, the residual stress is directly proportional to the two key parameters, i.e., the elastic modulus and the volume change of the coating material, as shown in Eq. 2 A, B, and C. According to the elastic moduli listed in Table 3, PLA has the highest stiffness, PCL has the lowest stiffness, and PLGA is in between PLA and PCL; thus, the stiffer PLA has greater internal stress from the parameter of elastic modulus. It is also important to know that minimizing the volume changes of the coating materials could significantly reduce the internal stress. For example, reducing the glass transition temperature (T_g) of the polymer can minimize the shrinkage originated from depositing dissolved polymers. This is because the difference in the volume fraction of the solvent ($\phi_s - \phi_r$) is reduced in the coating, as ϕ_s is delayed to the later stages of the drying process when there is less solvent [76, 78]. According to Table 3, PCL has lower T_g and thus has less internal stress generated by shrinkage. For depositing melted polymers on Mg substrates, using a polymer that has a lower thermal expansion coefficient (i.e., reducing $\Delta\alpha$) and allows a lower processing temperature (i.e., reducing ΔT) can minimize the shrinkage. The internal stress is generally lower for the polymer coatings annealed or cured at the lower temperatures than at the higher temperatures [79]. The parameters describing volume change in Eq. 2C are dependent on water uptake (i.e., water absorption) and relative humidity. The polymers with greater water uptake such as PLGA (Table 3) have higher internal stress according to Eq. 2C. Collectively, the polymers with lower elastic modulus, lower T_g , lower thermal expansion coefficient, and less water uptake will have less internal stress. For these reasons, the nHA/PCL coatings had less internal stress than the nHA/PLGA and nHA/PLLA coatings.

$$\sigma = \frac{E}{1-\nu} \frac{\phi_s - \phi_r}{3} = \frac{E \Delta \alpha \Delta T}{1-\nu} = \frac{E \alpha_f^h}{1-\nu} \Delta RH$$

Shrinkage after Deposition		
(A)	(B)	(C)
Dissolved	Melted	Hygroscopic
Coating	Coating	Swelling of
Polymers	Polymers	Coating
		Polymers

σ = Residual Stress ν = Poisson Ratio E = Elastic Modulus

ϕ_s = Volume fraction of solvent at solidification point

ϕ_r = Volume fraction of solvent in dry coating

$\Delta \alpha$ = Difference in thermal expansion coefficients between coating and substrate

ΔT = Difference between processing and operating temperatures

α_f^h = Function derived from the ratio of water uptake volume and polymer volume

ΔRH = Change in relative humidity

The substrate surface affected the distribution of internal stress in the coatings. The grinding grooves on the M_Mg substrates came from 600 grit SiC paper that had a feature size of 15 μm , similar to the size range of the crystals formed in the polymers studied in the nanocomposite coatings. The surface grooves acted as discontinuities in the coatings [80], and internal stress rises sharply at edges and discontinuities[56]. The abundance of discontinuities on the M_Mg surfaces required significant accommodation from the polymer component of the coatings after deposition and post-deposition processing. The more elastic PCL were able to compensate the discontinuities better than PLLA and PLGA. Furthermore, the coating material may have been pulled out of the grinding grooves during the polymer shrinkage since the coating could only shrink perpendicular to the plane of the substrate. Polymer crystals may also have grown larger than the width of the grinding grooves during post-

deposition processing and been physically excluded, as illustrated in Figure 14A. PLLA is amorphous after spin-coating because the solidification occurred much faster than the crystallization [26], but annealing could induce the formation of crystals larger than 15 μm [80]; which explains why annealing nHA/PLLA coatings on M_Mg increased the corrosion rates (Figure 3). In contrast, PCL is semi-crystalline after spin-coating [26], and annealing led to the formation of crystals smaller than those in PLLA [81]; which explains why annealing nHA/PCL coatings on M_Mg reduced the corrosion rates (Figure 3). Additionally, the smaller crystals restricted crack propagation more effectively than the larger crystals [70]. PLGA is an amorphous polymer, and annealing nHA/PLGA coatings on M_Mg increased the corrosion rates (Figure 3). After alkaline heat treatment, hydroxides filled the grinding grooves and reduced their sharp edges, which reduced the concentration of internal stress, as illustrated in Figure 14B. The abundant nucleating agents on A_Mg surfaces might have also reduced crystal growth and prevented the coating material from being pulled out of the grooves as the polymer shrinks. These are the possible reasons why the alkaline heat treatment of the Mg substrates reduced the corrosion rates of some nanocomposite-coated samples (Figure 3).

4.5. The Key Parameters Affecting the Coating Integrity and Adhesion Strength before and after Immersion Degradation

The immersion degradation study was performed to complement the PDP measurements of corrosion, because the immersion study takes into account how gas evolution and long-term exposure to body fluids would affect the coating properties and sample degradation.

The nanocomposite coatings maintained their structural integrity and adhesion throughout the 7-day immersion study (Figure 5). This is a significant progress considering the rapid onset of coating delamination from Mg substrates in the published literature [82, 83] and our previous results [55]. The microstructure of all the nanocomposite coatings was thoroughly examined using SEM (Figure 7) and showed that the coatings remained intact after 7 days of immersion, except that the nHA/PLGA coated A_Mg showed the cracks and holes and exposed the underlying substrate (Figure 7 and 10). Notably, the

edges around the holes on the the nHA/PLGA coated A_Mg (Figure 8) were still attached to the Mg substrate, indicating good coating adhesion. The cracks and holes were only found in the nHA/PLGA coated A_Mg sample, possibly because PLGA had the highest permeability that increased Mg degradation, and the highest water absorption and hygroscopic swelling that increased internal stress in the coating (Table 3). The holes only formed on the A_Mg substrates because they were less stable than the M_Mg substrates at later immersion time points, as shown by the higher pH and $[Mg^{2+}]$ of the non-coated A_Mg substrates (Figure 6).

Theoretically, nHA/polymer nanocomposite coatings on Mg-based substrates provide attractive properties for bone repair; however, in practice, there are significant engineering challenges yet to be addressed, e.g., delamination of the nanocomposite coatings due to H_2 evolution and reactive Mg surfaces [55]. As reported previously, nHA/PLGA coatings did reduce degradation of Mg substrates and alkalization of the local environment, but the coating delamination significantly shortened the service lifetime of the coatings [55]. Based on the failure mode and effects analysis (FMEA), we determined that H_2 gas evolution, as a result of Mg degradation, was the major factor that led to delamination of the nanocomposite coatings. The holes in the nHA/PLGA coatings on A_Mg were similar to those observed by Oosterbeek *et al*, and occurred for the same reasons described in their work [84]. Unlike Oosterbeek *et al*, the PLLA-based coatings in this manuscript did not develop these holes due to differences in sample preparation. One of the main differences is that the Mg substrates in this manuscript were mounted in epoxy and then spin-coated, which created coatings with solid and uniform structures; whereas Oosterbeek *et al* dip coated their Mg substrates, which created porous coatings that expedited water transport to the Mg substrate. This confirmed the importance of creating a solid and uniform structure within the coatings in order to minimize Mg degradation.

The polymer component of the nanocomposite coatings and Mg substrate surface conditions had significant effects on the coating adhesion strength, especially after immersion (Figure 10). The nHA/PCL coatings had the greatest average adhesion strength before and after immersion. In contrast,

other published results showed that pure PLLA coating had stronger adhesion to Mg than pure PCL coating [26]. The presence of dispersed nHA in the polymer matrix in this study might have contributed to this difference. Before immersion, the nHA/PCL coatings on the M_Mg had slightly greater average adhesion strength than on the A_Mg substrates, possibly because the nHA/PCL coating physically interlocked with the grinding grooves that were deeper in the M_Mg than in the A_Mg substrates. After 7 days of immersion in rSBF, the nHA/PCL coatings on the M_Mg substrates retained their average adhesion strength significantly more than the nHA/PLGA and nHA/PLLA coatings did. After immersion, the nHA/PCL coatings also retained their average adhesion strength significantly more on the M_Mg than on the A_Mg substrates, possibly because the M_Mg substrate surface was less susceptible to aggressive ions at later immersion time points.

4.6. The Most Effective Nanocomposite Coatings in Reducing Degradation and Improving BMSC Adhesion

Overall, the nHA/PCL coatings on the M_Mg substrates with the post-deposition processing of melted then annealed provided the best combination in reducing sample degradation and improving BMSC adhesion. Specifically, the nHA/PCL coatings on the M_Mg substrates showed the least release of Mg^{2+} ions and the least pH increase during immersion degradation, and the highest average adhesion strength before and after immersion. This is most likely because PCL had a combination of desirable mechanical, thermal, and transport properties that minimized internal stress and water permeability when compared with the other polymers studied, as illustrated in Figure 15. Generally, the coating materials should have low elastic modulus, high fracture toughness, low T_g , low thermal expansion coefficient, low water uptake, and low water permeability to achieve the best outcome in reducing Mg substrate degradation. PLGA and PLLA had some of the desirable properties, but not an optimal integration of all. The nHA/PCL coatings on the M_Mg performed better than the same coatings on the A_Mg substrates because the combination of nHA/PCL coatings with the M_Mg substrates provided desirable physically interlocking between the coatings and the grinding grooves of the substrates, and became less susceptible

to degradation at later time points of immersion. Although the nHA/PLGA and nHA/PLLA coatings were not the optimal, they still significantly reduced the degradation of their Mg substrates.

The nHA/PCL coated M_Mg was selected for the cytocompatibility study in BMSC culture because it showed promising results in immersion degradation and coating adhesion testing. The nHA/PCL coated M_Mg showed the highest BMSC adhesion density among all the samples tested (Figure 11, 14A), most likely because the synergy between Mg^{2+} and nHA was beneficial for bone cell adhesion. It has been known that both Mg^{2+} and nHA could increase bone cell adhesion [6, 85-87]. The non-coated Mg showed lower BMSC adhesion density and less spreading area per cell than the nHA/PCL coated Mg (Figure 12A and 14B) because of the higher pH and unstable surface conditions caused by rapid degradation. The nHA/PCL films also showed lower BMSC adhesion density and less spreading area per cell than the nHA/PCL coated Mg, possibly due to the lack of Mg^{2+} . The epoxy and PSTC controls showed similar BMSC adhesion densities (Figure 12A), indicating the epoxy mounts for the Mg substrates did not have significant effects on BMSC adhesion. Interestingly, the aspect ratios of BMSCs on the non-coated M_Mg (~4) were significantly higher than the BMSCs on all the other sample types (~2) (Figure 12C), possibly because the M_Mg had a surface feature size of around 15 μm (similar to the size range of BMSCs) from grinding with 600 grit SiC paper. It has been reported that supplementing the cell culture media with Mg^{2+} ions did not significantly affect the cell aspect ratio (~2), and non-coated pure Mg and Mg-Zn-Ca alloys did not significantly alter the cell aspect ratio either (~2) when they were polished with 0.25 μm diamond paste [88]. The difference in surface feature size due to polishing might be the reason for the observed difference in the aspect ratio of BMSCs.

5. Conclusion

The results of this study demonstrated that the nanocomposite coatings could provide dual functions of controlling Mg degradation and improving bone cell responses simultaneously, both important for clinical translation of Mg-based biomaterials. The nHA/polymer nanocomposite coatings

significantly reduced the degradation of Mg substrates during immersion in rSBF. Optimization of the key parameters (i.e., substrate surface conditions, polymer component types in the nanocomposite coatings, and the post-deposition processing) further improved the sample performance, i.e., slower degradation and better BMSC adhesion. The nHA/PCL coated M_Mg with the post-deposition processing of melting then annealing provided the best overall results in decreasing Mg degradation and improving BMSC adhesion density, due to reduced internal stress and water permeability. Post-deposition processing could improve or impair the barrier properties of the nanocomposite coatings depending upon the properties of the polymer component in the nanocomposites and the substrate surface conditions, suggesting these key parameters should be considered collectively rather than separately due to their interactions. This systemic investigation of nanocomposite coatings on Mg substrates provided valuable design guidelines for polymeric and nanocomposite coatings on Mg-based substrates for potential skeletal implant applications.

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Captions of Tables and Figures

Table 1: List of nHA/polymer nanocomposite coated Mg samples with all the processing parameters that were investigated, screened, and compared for optimal outcome. Contact angle was measured to compare surface hydrophobicity. Potentiodynamic polarization (PDP) curve was measured to

select the samples with the lowest corrosion current. For each pair of polymer component and substrate surface type, only the prescribed post-deposition processing that resulted in the lowest corrosion current were included in the subsequent studies. Only the best combination of all the variables (substrate surface type, polymer component, and post-deposition processing) that reduced Mg degradation most effectively was selected for further studies with BMSCs. All tests were performed in triplicate; except for the coating adhesion strength tests, which were performed in duplicate.

Table 2: Statistical analysis of the parameters that affected Mg degradation in immersion study using the Wild Bootstrap method. The $[Mg^{2+}]$ concentration was measured as the key indicator of Mg degradation after immersion in the rSBF.

Table 3: The properties of each polymer type used in the nanocomposite coatings on the Mg substrates. The polymer properties play important roles on the properties of the nanocomposite coatings on Mg substrates. The first values for thermal expansion coefficient that are denoted with a “#” occur below the T_g , while the second values occur above the T_g . PCL has such a low T_g that only the thermal expansion coefficient above the T_g was given. Values with an asterisk* are the median values based on the data found on the Polymer Database (Polyinfo: http://polymer.nims.go.jp/index_en.html). The calculation of median values for each polymer type included different variations of that polymer.

Figure 1: Contact angles of deionized water on the nHA/polymer coatings on Mg substrates and non-coated Mg control substrates. Values are mean $\pm$ standard error; n=9. The small changes to the contact angles of nanocomposite coatings suggested that the coatings hydrophobicity was not a significant variable during degradation testing.

Figure 2: Representative potentiodynamic polarization curves of the nHA/polymer coated and non-coated Mg substrates with different post-deposition processing for the nanocomposite coatings on M_Mg and A_Mg substrates.

Figure 3: Corrosion rates of the nHA/polymer coated and non-coated Mg substrates that were calculated based on corrosion current density extrapolated from PDP curves. All coatings significantly reduced corrosion rates as compared with the non-coated Mg substrates. The post-deposition processing had different effects on corrosion rates, depending upon polymer component and substrate surface. Values are mean $\pm$ standard error; n=3.

Figure 4: Interaction plots illustrating the effects of polymer component type of the nanocomposite coating, substrate surface conditions, and post-deposition processing on corrosion rates. (A) Parallel or near-parallel lines indicate that no or little interactions were detected between polymer component and substrate surface. (B) Intersecting lines indicate statistically significant interactions between the post-deposition processing and substrate surface. (C) Intersecting lines indicate statistically significant interactions between the post-deposition processing and polymer component of the nanocomposite coating. These complex interactions suggested that post-deposition processing should be optimized for each specific pair of polymer component and substrate surface.

Figure 5: Photographs of sample surface morphology after immersion in rSBF for the prescribed period.

Figure 6: Characterization of rSBF immersion media. (A) pH of the rSBF, (B) $[Mg^{2+}]$, and (C) $[Ca^{2+}]$ in the rSBF after sample immersion under standard cell culture conditions for the prescribed period. Values are mean $\pm$ standard error; n=3. The nanocomposite coatings reduced the changes to pH and $[Mg^{2+}]$, but had little effect upon $[Ca^{2+}]$. This demonstrated that all nanocomposite coatings were effective in reducing degradation during immersion in rSBF.

Figure 7: SEM images of the samples before and after immersion in rSBF for 7 days. Scale bars = 20 μm .

Figure 8: SEM image of craters in the nHA/PLGA coating on A_Mg after 7 days of immersion in rSBF. The craters formed due to hydrogen gas evolution from Mg degradation.

Figure 9: Surface elemental compositions of the nanocomposite coated Mg and non-coated Mg before and after immersion in rSBF for 7 days, according to EDX analyses. This graph showed that all of the nanocomposite coatings prevented significant accumulation of Mg degradation products at the surface, which demonstrated the effectiveness of the nanocomposite coatings on protecting Mg substrates from degradation.

Figure 10: Coating adhesion strength measured before and after immersion in rSBF for 7 days. Values are mean $\pm$ standard error; n=2.

Figure 11: Fluorescence images of BMSCs after direct culture on the samples and controls for 24 hours. Blue indicates DAPI stained nuclei and green indicates Alexa Fluor[®] 488 stained F-actin. Scale bars = 100 μ m.

Figure 12: Quantitative analyses of BMSC adhesion and morphology using ImageJ. (A) Average BMSC adhesion density directly on the sample surface. (B) Average spreading area of BMSCs directly on the sample surface. (C) Average aspect ratio of BMSCs directly on the sample surface. Values are mean $\pm$ standard error; n=15. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

Figure 13: Interactions among the key parameters (polymer component in the nanocomposite coatings, substrate surface, post-deposition processing) and their effects on the sample performance. These interactions affected the permeability of the coatings and interfacial properties, and thus the overall sample performance.

Figure 14: (A) The grinding grooves on the M_Mg substrate surfaces negatively interacted with PLGA and PLLA during post-deposition processing. The larger size of individual crystal led to physical exclusion from the grinding grooves. (B) The A_Mg substrate surfaces had less steep slopes along the grinding grooves and had abundant nucleating agents. Impingement of the crystals limited their size and prevented their physical exclusion from the grinding grooves.

Figure 15: Comparison of the mechanical, thermal, and transport properties of the polymer components used in the nanocomposite coatings. PCL reduced Mg degradation more effectively than

PLGA and PLLA because its lower elastic modulus, glass transition temperature (T_g), and thermal expansion coefficient (α) minimized internal stress; while its lower water permeability and uptake reduced the amount of water available for Mg degradation reactions.

7. Supplementary Material

7.1. Spin-Coating Procedure and Coating Thickness

The spin-coating duration was always 60 seconds, and the spin -coating speed always increased by 1000 revolutions per minute for each second of acceleration until reaching the desired speed. The only variable changed during spin-coating was the spin speed. Generally, the higher viscosity nHA/PLLA suspension required a higher spin-coating speed to achieve the desired coating thickness, the lower viscosity nHA/PCL suspension required a lower spin-coating speed, and the nHA/PLGA suspension with an intermediate viscosity had an intermediate spin-coating speed. For the same polymer component, the A_Mg substrates required a higher spin-coating speed than the M_Mg substrates to obtain the same coating thickness.

A digital micrometer (Marathon; Marathon Watch Company LTD) was used to measure the sample thickness before and after spin-coating. The difference between the two measurements was the coating thickness. These coating thickness measurements were confirmed using optical images of the sample cross-sections. ImageJ was used to measure the thickness of the coating cross-sections in the optical images. To obtain the cross-section images, the Mg substrates were cut in half using a notcher and then mounted in epoxy side-by-side in their original orientation for spin-coating. After spin-coating, a razor was used to cut the coating in-between the two halves of the substrates, and one of those halves was removed from the the epoxy mount so that its cross-sections could be imaged using an optical microscope (SE305R-P; AmScope) at an original magnification of 30x. The details of the spin-coating parameters and coating thickness measurements are summarized in Table 4. It is important to mention that the coating

thickness can be adjusted to control the degradation of Mg substrates if needed. However, the objective of this study was to elucidate the effects of the parameters of nanocomposite coatings (i.e., substrate surface conditions, polymer types, and post-deposition processing) on the degradation, thus, the coating thickness was not treated as a variable. Instead, the coating thickness was controlled to be similar for all of the nanocomposite coatings (Figure 16).

7.2 Temperature Considerations for Post-Deposition Processing

A melting temperature of 200 °C was chosen because it is frequently used to melt PLLA [89, 90]. Melting temperatures are generally not given for PLGA because it is amorphous. The same 200°C melting procedure was used for nHA/PLGA because its polymer component was 85% PLA, and glycolic acid has a much higher thermal degradation temperature than PLA. The nHA/PLGA was viscous and sticky after the melting process. The annealing procedure for PLGA was chosen because it reduced water uptake by the polymer the most effectively [32]. The annealing temperature for PLLA was chosen because PLLA formed smaller crystals at 115°C as compared with higher temperatures [34] and developed higher crystallinity at 115°C as compared with lower temperatures [91]. The melting and annealing temperatures for nHA/PCL were chosen based on the literatures [92, 93]. Annealing PCL at 50°C is known to reduce the diffusion of water soluble drugs more efficiently than lower annealing temperatures, which demonstrates a greater reduction to water diffusion rates [33]. All samples were cooled in air at room temperature for 10 minutes after post-deposition processing. The non-coated Mg controls did not go through the post deposition processing since there were no polymers involved.

7.3 Tensile Testing for Measuring Coating Adhesion Strength

The tensile testing setup for measuring coating adhesion strength is shown in Figure 17. Specifically, two wooden rods with a dimension of 5 x 5 x 50 mm were mounted onto the top and bottom of the samples, which were then secured into the Instron grips. One of the wooden rod was attached to the surface of the nanocomposite coatings using cyanoacrylate-based adhesive (Loctite) and allowed to cure

for 24 hours at room temperature. This wooden rod was then fixed into the upper Instron grip for moving upwards during tensile testing. Another wooden rod was embedded in the cylindrical epoxy (Jetset; MetLab, catalog # M135A) jigs to secure the bottom of the samples, and then fixed into the lower Instron grip that remained stationary during tensile testing. It is important to mention that the coating was outlined around the perimeter of the attached wooden rod using a sharp blade, to ensure that it was the coating adhesion strength being measured, not the tensile strength of the coating material. The tensile testing was set to stop when the measured load dropped by 40%. The stress-strain curve was plotted to calculate the coating adhesion strength.

Captions for Supplemental Tables and Figures

Table 4: Spin-coating speed and coating thickness for three nanocomposites with different polymer components on the Mg substrates with metallic surface (M_Mg) or alkaline heat treated surface (A_Mg). The thicknesses of nanocomposite coatings on the Mg substrates were quantified based on optical images of sample cross sections using ImageJ.

Figure 16: Optical images of cross-sections of nHA/polymer nanocomposite coatings on M_Mg and A_Mg substrates. The red dashed line indicates the interface of the coating (top) and the Mg substrate (bottom). Scale bars = 100 μm .

Figure 17: Experimental setup for measuring coating adhesion strength. Tensile testing was performed to measure the maximum force needed to detach the coating from the substrate. The maximum force was used to calculate the coating adhesion strength.

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***Statement of Significance**

This manuscript describes the systemic optimization of nanocomposite coatings to control the degradation and bioactivity of magnesium for skeletal implant applications. The key parameters influencing the integrity and functions of the nanocomposite coatings on magnesium were identified, guidelines for the optimization of the coatings were established, and the benefits of coating optimization were demonstrated through reduced magnesium degradation and increased bone marrow derived mesenchymal stem cell (BMSC) adhesion in vitro. The guidelines developed in this manuscript are valuable for the biometal field to improve the design of bioresorbable implants and devices, which will advance the clinical translation of magnesium-based implants.

Table 1: List of nHA/polymer nanocomposite coated Mg samples with all the processing parameters that were investigated, screened, and compared for optimal outcome. Contact angle was measured to compare surface hydrophobicity. Potentiodynamic polarization (PDP) curve was measured to select the samples with the lowest corrosion current. For each pair of polymer component and substrate surface type, only the prescribed post-deposition processing that resulted in the lowest corrosion current were included in the subsequent studies. Only the best combination of all the variables (substrate surface type, polymer component, and post-deposition processing) that reduced Mg degradation most effectively was selected for further studies with BMSCs. All tests were performed in triplicate; except for the coating adhesion strength tests, which were performed in duplicate.

Parameters of Interest			■ Indicates the samples that were included in respective study		
Substrate Surface	Polymer Types	Post-Deposition Processing	Contact Angle	Immersion Degradation	BMSC Adhesion
			PDP Curve	Coating Adhesion	
M Mg	nHA/PLGA	None	■	■	
M Mg	nHA/PLGA	Annealed	■		
M Mg	nHA/PLGA	Melted	■		
M Mg	nHA/PLGA	Melted then Annealed	■		
A Mg	nHA/PLGA	None	■		
A Mg	nHA/PLGA	Annealed	■		
A Mg	nHA/PLGA	Melted	■		
A Mg	nHA/PLGA	Melted then Annealed	■	■	
M Mg	nHA/PLLA	None	■	■	
M Mg	nHA/PLLA	Annealed	■		
M Mg	nHA/PLLA	Melted	■		
M Mg	nHA/PLLA	Melted then Annealed	■		
A Mg	nHA/PLLA	None	■		
A Mg	nHA/PLLA	Annealed	■		
A Mg	nHA/PLLA	Melted	■		
A Mg	nHA/PLLA	Melted then Annealed	■	■	
M Mg	nHA/PCL	None	■		
M Mg	nHA/PCL	Annealed	■		
M Mg	nHA/PCL	Melted	■		
M Mg	nHA/PCL	Melted then Annealed	■	■	■
A Mg	nHA/PCL	None	■		
A Mg	nHA/PCL	Annealed	■		
A Mg	nHA/PCL	Melted	■		
A Mg	nHA/PCL	Melted then Annealed	■	■	

Table 2: Statistical analysis of the parameters that affected Mg degradation in immersion study using the Wild Bootstrap method. The $[Mg^{2+}]$ concentration was measured as the key indicator of Mg degradation after immersion in the rSBF.

Statistical Significance of the Parameters and their Interactions that Affected Mg Degradation	
Parameter	Bonferroni Corrected <i>p</i> Values
Substrate Surface	1
Polymer Component	5.97×10^{-5}
Time	5.97×10^{-5}
Substrate Surface : Polymer Component	3.9×10^{-3}
Substrate Surface: Time	1.49×10^{-2}
Polymer Component : Time	3.9×10^{-4}
Substrate Surface : Polymer Component : Time	0.6

Table 3: The properties of each polymer type used in the nanocomposite coatings on the Mg substrates. The polymer properties play important roles on the properties of the nanocomposite coatings on Mg substrates. The first values for thermal expansion coefficient that are denoted with a “#” occur below the T_g , while the second values occur above the T_g . PCL has such a low T_g that only the thermal expansion coefficient above the T_g was given. Values with an asterisk* are the median values based on the data found on the Polymer Database (Polyinfo: http://polymer.nims.go.jp/index_en.html). The calculation of median values for each polymer type included different variations of that polymer.

Polymer	T _g (°C)	T _m (°C)	Thermal Expansion Coefficient (1/°K)	Elastic Modulus (MPa)	Ultimate Tensile Strength (MPa)	Elongation at Break (%)	Water Absorption (wt %)	Water Diffusion Coefficient (cm ² /s)
Reference	[79*]	[79*]	[79*, 83]	[79*]	[79*]	[79*]	[79*, 84]	[80, 81, 82]
PLGA	48	N/A	2.14x10 ⁻⁴ #/ 0.134	431	17	3.6	20	8.49x10 ⁻⁹
PLA	57	163	1.08x10 ⁻⁴ #/ 2.12x10 ⁻²	4614	60.6	4.4	0.27	1.17x10 ⁻⁸
PCL	-62	55	7x10 ⁻⁴	245	12.3	336	0.32	9.11x10 ⁻¹⁰

Figure 1: Contact angles of deionized water on the nHA/polymer coatings on Mg substrates and non-coated Mg control substrates. Values are mean $\pm$ standard error; n=9. The small changes to the contact angles of nanocomposite coatings suggested that the coatings hydrophobicity was not a significant variable during degradation testing.

Figure 2: Representative potentiodynamic polarization curves of the nHA/polymer coated and non-coated Mg substrates with different post-deposition processing for the nanocomposite coatings on M_Mg and A_Mg substrates.

Figure 3: Corrosion rates of the nHA/polymer coated and non-coated Mg substrates that were calculated based on corrosion current density extrapolated from PDP curves. All coatings significantly reduced corrosion rates as compared with the non-coated Mg substrates. The post-deposition processing had different effects on corrosion rates, depending upon polymer component and substrate surface. Values are mean $\pm$ standard error; $n=3$.

(D)	Sample Type	<i>p</i> Value
Polymer Component		1.89x10 ⁻⁷
Post-deposition Processing		0.04
Substrate Surface		5.83x10 ⁻⁷
Polymer Component : Post-deposition Processing		3.39x10 ⁻³
Polymer Component : Substrate Surface		0.35
Post-deposition Processing : Substrate Surface		3.98x10 ⁻³
Polymer Component : Post-deposition Processing : Substrate Surface		4.90x10 ⁻³

Figure 4: Interaction plots illustrating the effects of polymer component type of the nanocomposite coating, substrate surface conditions, and post-deposition processing on corrosion rates. (A) Parallel or near-parallel lines indicate that no or little interactions were detected between polymer component and substrate surface. (B) Intersecting lines indicate statistically significant interactions between the post-deposition processing and substrate surface. (C) Intersecting lines indicate statistically significant interactions between the post-deposition processing and polymer component of the nanocomposite coating. These complex interactions suggested that post-deposition processing should be optimized for each specific pair of polymer component and substrate surface.

Coating	Post-deposition Processing	Substrate Surface	Immersion Time (Days)				
			0	1	3	5	7
Non-coated	No Processing	M_Mg
		A_Mg
nHA/PLGA	No Processing	M_Mg
	Melted then Annealed	A_Mg
nHA/PLLA	No Processing	M_Mg
	Melted then Annealed	A_Mg
nHA/PCL	Melted then Annealed	M_Mg
		A_Mg
Non-coated	No Processing	Ti
nHA/PLGA	No Processing	Ti
Non-coated	No Processing	Epoxy

Figure 5: Photographs of sample surface morphology after immersion in rSBF for the prescribed period.

Figure 6: Characterization of rSBF immersion media. (A) pH of the rSBF, (B) [Mg²⁺], and (C) [Ca²⁺] in the rSBF after sample immersion under standard cell culture conditions for the prescribed period. Values are mean $\pm$ standard error; n=3. The nanocomposite coatings reduced the changes to pH and [Mg²⁺], but had little effect upon [Ca²⁺]. This demonstrated that all nanocomposite coatings were effective in reducing degradation during immersion in rSBF.

Figure 7: SEM images of the samples before and after immersion in rSBF for 7 days. Scale bars = 20 μm .

Figure 8: SEM image of craters in the nHA/PLGA coating on A_Mg after 7 days of immersion in rSBF. The craters formed due to hydrogen gas evolution from Mg degradation.

Figure 9: Surface elemental compositions of the nanocomposite coated Mg and non-coated Mg before and after immersion in rSBF for 7 days, according to EDX analyses. This graph showed that all of the nanocomposite coatings prevented significant accumulation of Mg degradation products at the surface, which demonstrated the effectiveness of the nanocomposite coatings on protecting Mg substrates from degradation.

Figure 10: Coating adhesion strength measured before and after immersion in rSBF for 7 days. Values are mean $\pm$ standard error; n=2.

Figure 11: Fluorescence images of BMSCs after direct culture on the samples and controls for 24 hours. Blue indicates DAPI stained nuclei and green indicates Alexa Fluor® 488 stained F-actin. Scale bars = 100 μm .

Figure 12: Quantitative analyses of BMSC adhesion and morphology using ImageJ. (A) Average BMSC adhesion density directly on the sample surface. (B) Average spreading area of BMSCs directly on the sample surface. (C) Average aspect ratio of BMSCs directly on the sample surface. Values are mean $\pm$ standard error; $n=15$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

Interactions Among Polymer Component, Post-Deposition Processing, and Substrate Surface Influenced the Sample Performance

Figure 13: Interactions among the key parameters (polymer component in the nanocomposite coatings, substrate surface, post-deposition processing) and their effects on the sample performance. These interactions affected the permeability of the coatings and interfacial properties, and thus the overall sample performance.

Figure 14: (A) The grinding grooves on the M_Mg substrate surfaces negatively interacted with PLGA and PLLA during post-deposition processing. The larger size of individual crystal led to physical exclusion from the grinding grooves. (B) The A_Mg substrate surfaces had less steep slopes along the grinding grooves and had abundant nucleating agents. Impingement of the crystals limited their size and prevented their physical exclusion from the grinding grooves.

Integrated Desirable Properties of PCL Reduced Coating Internal Stress and Water Permeability

Figure 15: Comparison of the mechanical, thermal, and transport properties of the polymer components used in the nanocomposite coatings. PCL reduced Mg degradation more effectively than PLGA and PLLA because its lower elastic modulus, glass transition temperature (T_g), and thermal expansion coefficient (α) minimized internal stress; while its lower water permeability and uptake reduced the amount of water available for Mg degradation reactions.

Supplementary Materials

Supplementary table and figure

Table 4: Spin-coating speed and coating thickness for three nanocomposites with different polymer components on the Mg substrates with metallic surface (M_Mg) or alkaline heat treated surface (A_Mg). The thicknesses of nanocomposite coatings on the Mg substrates were quantified based on optical images of sample cross sections using ImageJ.

Spin-coating Parameters and Coating Thickness			
Substrate Surface	Polymer Component in the Nanocomposite Coatings	Spin Speed (RPM)	Average Coating Thickness (μm)
M_Mg	PLGA	400	34 $\pm$ 1
A_Mg	PLGA	700	39 $\pm$ 4
M_Mg	PLLA	700	34 $\pm$ 2
A_Mg	PLLA	800	39 $\pm$ 4
M_Mg	PCL	375	41 $\pm$ 1
A_Mg	PCL	600	33 $\pm$ 1

Polymer Type of the Nanocomposite Coatings	Substrate Surface	
	M_Mg	A_Mg
PLGA
PLLA
PCL

Figure 16: Optical images of cross-sections of nHA/polymer nanocomposite coatings on M_Mg and A_Mg substrates. The red dashed line indicates the interface of the coating (top) and the Mg substrate (bottom). Scale bars = 100 μm .

Figure 17: Experimental setup for measuring coating adhesion strength. Tensile testing was performed to measure the maximum force needed to detach the coating from the substrate. The maximum force was used to calculate the coating adhesion strength.