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pHEMA-nHA Encapsulation and Delivery of Vancomycin and rhBMP-2 Enhances its Role as a Bone Graft Substitute

Xinning Li MD, Jianwen Xu PhD, Tera M. Filion PhD, David C. Ayers MD, Jie Song PhD

Abstract

Background Bone grafts are widely used in orthopaedic procedures. Autografts are limited by donor site morbidity while allografts are known for considerable infection and failure rates. A synthetic composite bone graft substitute poly(2-hydroxyethyl methacrylate)-nanocrystalline hydroxyapatite (pHEMA-nHA) was previously developed to stably press-fit in and functionally repair critical-sized rat femoral segmental defects when it was preabsorbed with a single low dose of 300 ng recombinant human bone morphogenetic protein-2/7 (rhBMP-2/7).

Questions/purposes To facilitate clinical translation of pHEMA-nHA as a synthetic structural bone graft substitute, we examined its ability to encapsulate and release rhBMP-2 and the antibiotic vancomycin.

Methods We analyzed the compressive behavior and microstructure of pHEMA-nHA as a function of vancomycin incorporation doses using a dynamic mechanical analyzer and a scanning electron microscope. In vitro release of vancomycin was monitored by ultraviolet-visible spectroscopy. Release of rhBMP-2 from pHEMA-nHA-vancomycin was determined by ELISA. Bioactivity of the released vancomycin and rhBMP-2 was examined by bacterial inhibition and osteogenic transdifferentiation capabilities in cell culture, respectively.

Results Up to 4.8 wt% of vancomycin was incorporated into pHEMA-nHA without compromising its structural integrity and compressive modulus. Encapsulated vancomycin was released in a dose-dependent and sustained manner in phosphate-buffered saline over 2 weeks, and the released vancomycin inhibited *Escherichia coli* culture. The pHEMA-nHA-vancomycin composite released preabsorbed rhBMP-2 in a sustained manner over 8 days and locally induced osteogenic transdifferentiation of C2C12 cells in culture.

Conclusions pHEMA-nHA can encapsulate and deliver vancomycin and rhBMP-2 in a sustained and localized manner with reduced loading doses.

Clinical Relevance The elasticity, osteoconductivity, and rhBMP-2/vancomycin delivery characteristics of pHEMA-nHA may benefit orthopaedic reconstructions or fusions with enhanced safety and efficiency and reduced infection risk.

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Introduction

Autogenic and allogenic bone grafts [5] are used for surgical treatments of volumetric bone loss resulting from

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trauma or bone tumor resections [27, 29] and attempted arthrodesis of the spine [25], wrist [41], foot and ankle [39], and knee [38]. These methods are associated with patient donor site morbidity (autografts) and multiple complications (allografts), including delayed unions, poor graft incorporations, infections, and need for revision operations [3, 9, 10, 26]. Furthermore, the utilization of autografts and allografts can be limited by both volume and graft-defect size mismatches [23]. Adjuvant use of osteoinductive recombinant human bone morphogenetic protein-2 (rhBMP-2), along with the allograft, has been effective in augmenting graft healing. The clinical use of rhBMP-2, which was approved by the FDA, has been primarily limited to spine fusions and open tibial fractures [4, 16]. The negative side effects associated with high clinical doses of rhBMP-2 delivered by collagen sponge carriers have been major safety concerns. A recent review of the clinical uses of rhBMP-2 revealed risks for adverse events that were 10 to 50 times the original estimates reported in industry-sponsored studies [7]. Thus, the development of osteoconductive synthetic scaffolds capable of delivering safe doses of osteogenic growth factors and antibiotics in a localized and sustained manner, either as synthetic structural bone graft substitutes or as auxiliary delivery vehicles, to enhance the performance of allografts is highly desired [26].

A poly(2-hydroxyethyl methacrylate)-nanocrystalline hydroxyapatite (pHEMA-nHA) composite exhibiting a combination of osteoconductivity, elasticity for surgical press-fitting, and an attractive retention/release profile for protein therapeutics was recently developed [36]. It was able to encapsulate or release rhBMP-2/7 heterodimer and RANKL in a localized and sustained manner, while reducing the minimal effective loading doses by two to three orders of magnitude, compared to conventional ceramic carriers [1, 21, 42]. Furthermore, when preabsorbed with a single dose of 400 ng rhBMP-2/7 and press-fit in a 5-mm critical-sized rat femoral segmental defect, pHEMA-nHA led to the functional repair of the defect in 8 to 12 weeks [11].

pHEMA-nHA can also encapsulate and deliver vancomycin. Vancomycin is frequently used in orthopaedic surgery for the prevention and treatment of infections caused by gram-positive organisms and those unresponsive to other antibiotics (eg, methicillin-resistant *Staphylococcus aureus* [MRSA]) [20, 31]. Compared to systemic treatment, local antibiotics delivery can achieve considerably higher drug concentration at the infection site with reduced systemic toxicity [15, 22]. A substantial release of vancomycin from a synthetic carrier in the first few days postoperation, followed by a sustained release of lower yet meaningful doses over the course of a couple of weeks, would be highly desired. It was shown that pHEMA-nHA could encapsulate and locally deliver tetracycline in a sustained manner over 2 weeks [42]. Such a sustained local delivery of antibiotics may also reduce the need for prophylactic systemic antibiotic administration.

We examined the ability of pHEMA-nHA to encapsulate and deliver rhBMP-2 and vancomycin to facilitate its clinical translation as a synthetic bone graft substitute. Our specific aims were to determine (1) the appropriate encapsulation dose of vancomycin in pHEMA-nHA without compromising its structural and mechanical integrity, (2) the vancomycin release profile under physiologic conditions over 2 weeks and the antibacterial efficacy of the released vancomycin in vitro, and (3) the release of rhBMP-2 from this synthetic composite and the osteoinductivity of the released rhBMP-2 in culture. This is the first study evaluating the encapsulation and release characteristics of both rhBMP-2 and vancomycin from the elastomeric and osteoconductive pHEMA-nHA synthetic bone graft substitute.

Materials and Methods

Hydrogel monomer 2-hydroxyethyl methacrylate (HEMA) (Sigma-Aldrich Co, St Louis, MO, USA) was distilled under reduced pressure, and crosslinker ethylene glycol dimethacrylate (EGDMA) (Sigma-Aldrich) was stored with a 4-Å molecular sieve before use. Porous aggregates of hydroxyapatite (HA) nanocrystals (Alfa Aesar, Ward Hill, MA, USA) were used as received. We purchased the recombinant protein rhBMP-2 and an rhBMP-2 Quantikine® ELISA kit (R&D Systems, Minneapolis, MN, USA) and reconstituted it per the vendor's instructions. Vancomycin powder (99%; APP Pharmaceuticals, Schaumburg, IL, USA) and the alkaline phosphatase (ALP) staining kit (Leukocyte Alkaline Phosphatase Kit; Sigma-Aldrich) were used as received.

We prepared pHEMA-nHA-vancomycin composite grafts by crosslinking HEMA with 2 wt% EGDMA in the presence of 50 wt% HA and 2.4, 4.8, 9, or 13 wt% vancomycin powder. Specifically, vancomycin was dissolved in the mixture of HEMA, EGDMA, and viscous solvent ethylene glycol and thoroughly mixed with the HA powder before aqueous solutions of radical initiators ammonium persulfate (480 mg/mL) and sodium metasulfite (180 mg/mL) were added to initiate the polymerization. The mixture was solidified at room temperature overnight in borosilicate glass tubing with an inner diameter of 3.8 mm. The polymerized composites were retrieved from the glass tubing, cut into 4.0-mm-long cylindrical segments, and equilibrated in Type 1 Ultrapure Milli-Q® water (EMD Millipore, Billerica, MA, USA) for 24 hours to remove ethylene glycol and radical initiators before being subjected to mechanical testing and cell culture studies.

To pursue our first aim, we analyzed the compressive behavior of pHEMA-nHA-vancomycin composites under physiologic conditions as a function of the vancomycin incorporation dose on the Q800 Dynamic Mechanical Analyzer (TA Instruments, New Castle, DE, USA), equipped with a submersion compression fixture. An L-square was used to ensure the sanded top and bottom surfaces of the cylindrical specimen were parallel to the load cell before testing. The fully hydrated specimens (after 24-hour equilibration in water) were compressed in force-controlled mode in phosphate-buffered saline (PBS, pH 7.4) at 37°C, ramping from 0.03 N to 10.0 N and then back to 0.03 N at a rate of 3.0 N/minute for 10 cycles or until sample failure was detected (n = 3). All stress-strain curves presented were based on the engineering stress and strain recorded assuming a fixed cross section of the material as defined at the start of the test.

After the repetitive compressive loading-unloading, macroscopically intact specimens were bisected longitudinally for further examination of the microstructures at the cross section by a scanning electron microscope (SEM). Specimens with the same content of vancomycin that did not undergo compressive loading-unloading were also bisected for the examination of microstructures at the cross section. SEM micrographs were acquired on a Quanta™ 200 FEG MKII SEM (FEI Co, Hillsboro, OR, USA) under the environmental mode with an accelerating voltage of 5 kV.

To pursue our second aim, we monitored the release of vancomycin from each freshly prepared composite specimen (3.8 mm in diameter, 4.0 mm in height) in PBS (pH 7.4) at 37°C using ultraviolet-visible spectroscopy over 14 days, with replacement of fresh PBS (1 mL) for the same specimen at each prescheduled time point. Specifically, at 0, 1, 2, 4, 6, 12, 24, 48, 96, 168, and 336 hours, each specimen was retrieved from the 1-mL PBS solution and place into fresh 1-mL PBS solution for continued incubation. The PBS solutions collected at each time point for various specimens were quantified for vancomycin absorption at 280 nm. Three specimens (n = 3) were examined for each vancomycin encapsulation dose at each time point. A standard vancomycin concentration-absorption curve at 280 nm was generated for quantification.

The antibiotic activity of the vancomycin released from the pHEMA-nHA-vancomycin composites was evaluated by the ability to inhibit *Escherichia coli* culture. Warm Luria broth (LB) (25 g/L)-agar (15 g/L) solution was poured into a sterile plastic petri dish (100 × 15 mm) and cooled to room temperature. The surfaces of the LB-agar plates were coated with 100 μL *E coli* XL-2 solution (absorbance at 600 nm = 0.256) with glass beads and cultured at 37°C for 30 minutes before a composite graft (3.8 mm in diameter, 4.0 mm in height) encapsulated with 0 (negative control), 2.4, or 4.8 wt% of vancomycin was placed on the surface of the agar dish. The *E coli* culture was continued at 37°C in a humidified incubator, and pictures of the culture were taken at 6, 12, 24, and 48 hours to monitor the clear zone that developed around each graft. The diameters of the clear zones surrounding the grafts (n = 3) were measured at each time point and reported as mean ± SD.

To pursue our third aim, we determined the retention and release of rhBMP-2 from pHEMA-nHA-vancomycin grafts containing 2.4 or 4.8 wt% vancomycin using a BMP-2 ELISA kit. Ten microliters of rhBMP-2 solution (30 ng/μL) was loaded to each composite (n = 3) to achieve a final loading dose of 300 ng/graft carrier. Each specimen was placed in 1 mL Milli-Q® water and incubated at 37°C. The aqueous solution was collected at 0, 1, 2, 4, 6, 12, 24, 48, and 96 hours, and the BMP-2 released to the solution at each time point was quantified and calibrated by the ELISA kit following the vendor's instructions.

The bioactivity of the rhBMP-2 released from the pHEMA-nHA-vancomycin graft containing 2.4 wt% vancomycin over 8 days was examined by the ability to induce osteogenic transdifferentiation of myoblast C2C12 cells. C2C12 cells, seeded at 5000/cm^2 in a 24-well plate, were allowed to attach overnight in Dulbecco's Modified Eagle's Medium (DMEM; Mediatech Inc, Manassas, VA, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories, Inc, Logan, UT, USA) and 1% penicillin-streptomycin (Mediatech) before being switched to low mitogen medium (DMEM, 5% FBS, 1 mL/well; 24-well plate), to which a graft containing 2.4 wt% vancomycin and freshly loaded with 300 ng rhBMP-2 was placed (n = 3). The culture was continued for 4 days without medium changes or any additional supplements of growth factors. The graft was retrieved on Day 4 and the cells were fixed and stained for osteogenic marker ALP using the ALP staining kit. The retrieved graft was then placed in another fresh C2C12 culture without additional supplements of rhBMP-2 until the cells were stained for ALP after 4 days of culture.

Results

The content of vancomycin had a direct impact on the compressive behavior of the composite under physiologic conditions (in PBS, pH 7.4, 37°C), with the stiffness of the composites inversely correlated with the vancomycin content (Fig. 1A). The grafts containing 2.4 and 4.8 wt% of vancomycin could withstand the repetitive 0.675-MPa compressive stress (or 26%–30% compressive strains) with good shape recovery. SEM micrographs (Fig. 1B) of the

cross sections of the composites after repetitive compressive loadings did not reveal any microfractures. By contrast, the grafts encapsulating 9 to 13 wt% vancomycin were not able to recover their original dimensions after the same compressive loads (Fig. 1A), with major cracks developing within the composites leading to macroscopic failures (disintegration) of the specimens.

Vancomycin was released in a sustained and dose-dependent manner (Fig. 2). Whereas the 9- and 13-wt% composites released about 75% and 80% of the encapsulated vancomycin within the first 12 hours, respectively; only 25% to 35% of encapsulated vancomycin was released from the lower-content (2.4- and 4.8-wt%) composites within the same period of time. After the initial burst release, an additional 35% to 45% of vancomycin was released from the 2.4- and 4.8-wt% composites in a sustained manner over the course of 2 weeks. By contrast, the release of vancomycin from the 9- and 13-wt% composites reached 90% within the first 48 hours with little sustained release thereafter. The antibiotic activity of the vancomycin release was evaluated by its ability to inhibit the *E coli* culture. The mean diameters of the clear zone surrounding the 2.4- and 4.8-wt% grafts by 48 hours were 13.00 ± 0.20 mm and 18.60 ± 0.34 mm, respectively (Fig. 3). No clear zone developed surrounding the graft without pre-encapsulated vancomycin.

After an initial release of 5% and 10% of 300 ng rhBMP-2 in PBS from the 4.8- and 2.4-wt% composites in the first 12 hours, respectively, rhBMP-2 release plateaued, with greater than 89% of the rhBMP-2 stably retained on the 2.4-wt% composite by 24 hours and greater than 94% of the rhBMP-2 stably retained on the 4.8-wt% composite by 48 hours (Fig. 4A). An established BMP-2-induced osteogenic transdifferentiation of myoblast C2C12 culture model was used to evaluate the bioactivity of rhBMP-2 released from the grafts [17]. The BMP-2 released from the 2.4-wt% composite graft, preabsorbed with 300 ng rhBMP-2 when it was placed in the C2C12 cell culture, led to positive detections for ALP from cells surrounding the graft on Day 4 (Fig. 4B, middle). Furthermore, when this graft was retrieved and placed in a fresh C2C12 culture, the remaining rhBMP-2 retained on the graft was able to again

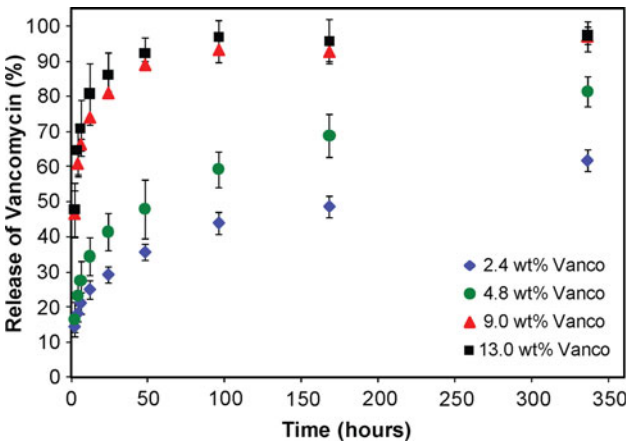
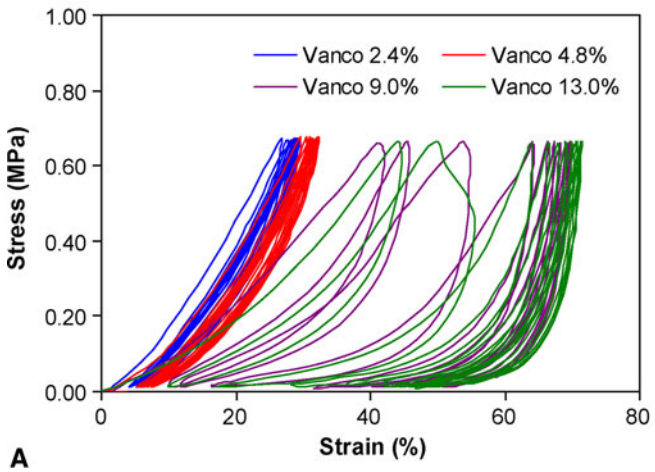


Fig. 2 A graph shows the release kinetics of vancomycin from pHEMA-nHA-vancomycin composites (Vanco) in PBS (pH 7.4) at 37°C as a function of time and vancomycin encapsulation content. Vancomycin was released in a sustained and dose-dependent manner.



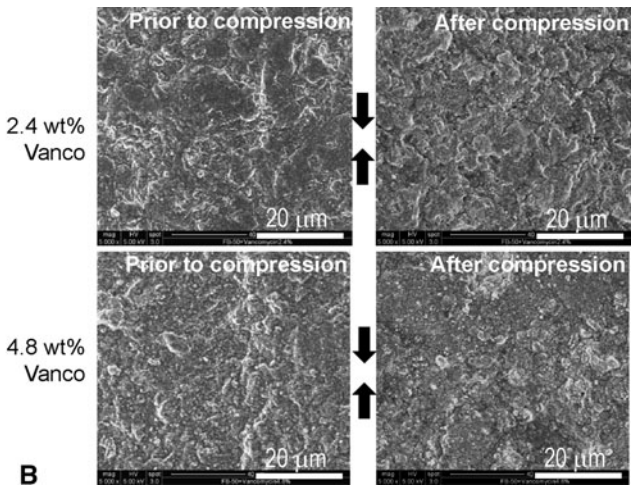


Fig. 1A–B (**A**) A graph shows the compressive stress-strain curves of pHEMA-nHA-vancomycin composites (Vanco) as a function of vancomycin encapsulation content. Ten continuous loading-unloading cycles (from 0.03 N to 10.0 N and back to 0.03 N, loading rate of 3.0 N/minute) were applied to the composite at 37°C in PBS (pH 7.4), using a submersion compressive fixture. The content of vancomycin had a direct impact on the compressive behavior of the composite, with the stiffness of the composites inversely correlated with the vancomycin content. (**B**) SEM micrographs (original magnification, ×5000) show cross sections of pHEMA-nHA-vancomycin composites containing 2.4 wt% vancomycin before (left) and after (right) the repetitive compressive loadings. The composites did not reveal any microfractures after the loadings.

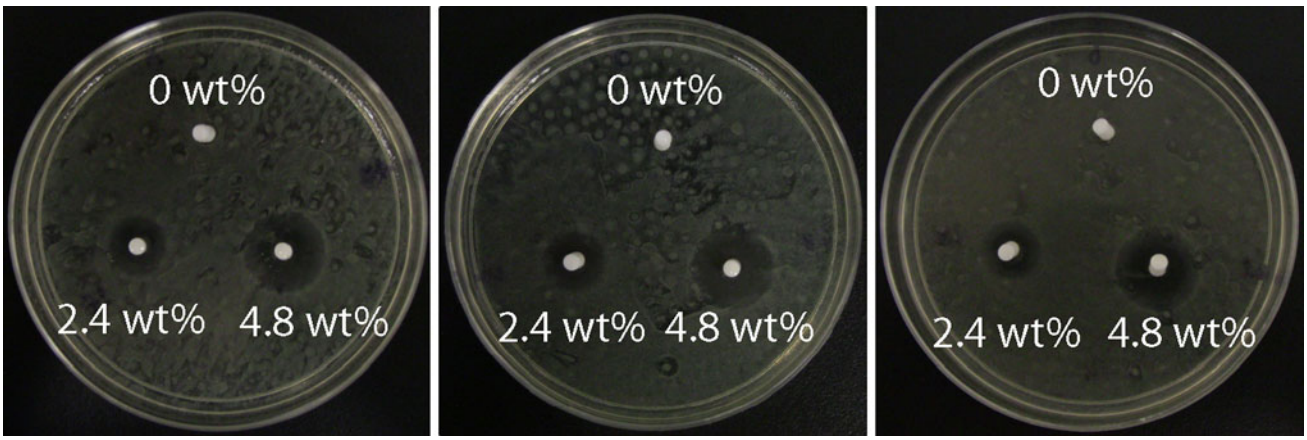


Fig. 3 Images show the development of clear zones in *E coli* culture surrounding pHEMA-nHA-vancomycin composites at 48 hours (triplicates). The composites placed on each plate of *E coli* culture contained 0, 2.4, or 4.8 wt% vancomycin. The mean diameters of the clear zone surrounding the 2.4- and 4.8-wt% grafts by 48 hours were 13.00 ± 0.20 mm and 18.60 ± 0.34 mm, respectively, whereas no clear zone developed surrounding the graft with 0 wt% vancomycin.

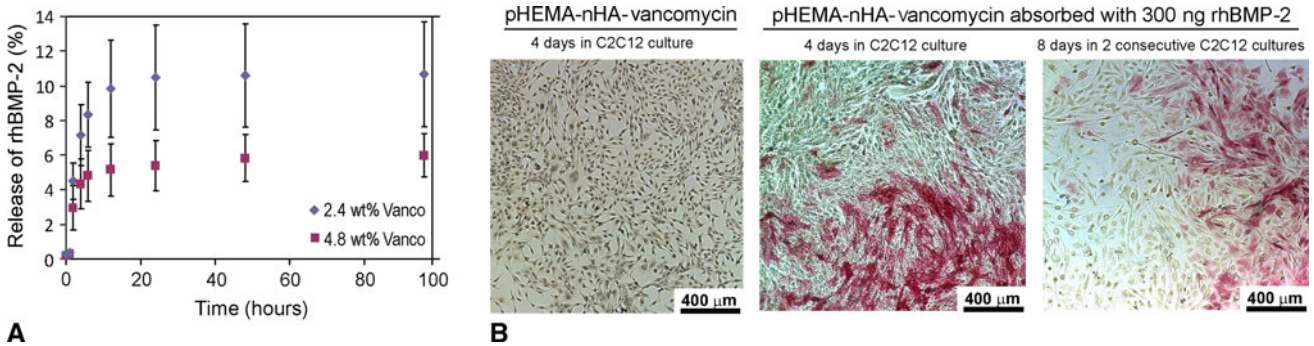


Fig. 4A–B (**A**) A graph shows the release kinetics of rhBMP-2 from pHEMA-nHA-vancomycin as determined by ELISA. After an initial release of 5% and 10% from the 4.8- and 2.4-wt% vancomycin composites (Vanco) in the first 12 hours, respectively, rhBMP-2 release plateaued (> 89% retained on 2.4-wt% composites by 24 hours, > 94% retained on 4.8-wt% composites by 48 hours). (**B**) Images (original magnification, ×100) show ALP staining of C2C12 cultures after placing 2.4 wt% pHEMA-nHA-vancomycin grafts preloaded with nothing (left) or 300 ng rhBMP-2 in culture for 4 days (middle) or 8 days (right). The staining was carried out on Day 4 for each culture. There are positive detections for ALP from cells surrounding grafts preloaded with rhBMP-2 on Days 4 and 8 but none from cells surrounding grafts without preabsorbed rhBMP-2.

induce the localized osteogenic differentiation of C2C12 cells in 4 more days (8 total days) (Fig. 4B, right). As expected, no ALP expression was detected from the culture treated with grafts without preabsorbed rhBMP-2 (Fig. 4B, left).

Discussion

Synthetic bone grafts that possess both the structural and biochemical microenvironments of natural bone, while having the ability to encapsulate and sustainably release antibiotics and protein therapeutic factors, are highly desired in orthopaedic care. We examined the ability of pHEMA-nHA to encapsulate and deliver rhBMP-2 and vancomycin to facilitate its clinical translation as a synthetic bone graft substitute. Our specific aims were to determine (1) the appropriate encapsulation dose of vancomycin in pHEMA-nHA without compromising its structural and mechanical integrity, (2) the vancomycin release profile under physiologic conditions over 2 weeks and the antibacterial efficacy of the released vancomycin in vitro, and (3) the release of rhBMP-2 from this synthetic composite and the osteoinductivity of the released rhBMP-2 in culture.

There are a number of limitations to our study. First, the release profiles established for both vancomycin and rhBMP-2 under static in vitro conditions will likely differ from those under in vivo scenarios where the specimens will be subjected to dynamic mechanical loadings. Second, we chose *E coli* for the examination of antibacterial activities of the vancomycin locally released from the

synthetic bone graft substitute. Although this is a good model for proof-of-concept evaluations, it is necessary to also address the efficacy of the locally released vancomycin in inhibiting more clinically relevant bacteria such as *S aureus* or MRSA in followup studies. Finally, the ultimate success of this synthetic bone graft substitute would be most appropriately evaluated using a suitable animal long-bone defect infection model.

We showed by way of direct encapsulation of up to 4.8 wt% vancomycin and 50 wt% nHA within crosslinked pHEMA hydrogel that pHEMA-nHA-vancomycin composites could be prepared with good structural integration and desirable elastomeric properties. The resulting composites were able to withstand repetitive 25% compressive stains under moderate compressive loads (0.675 MPa) with excellent shape recovery under physiologic conditions. Such a property represents an advantage for the surgical handling and manipulation of the synthetic bone graft to enable convenient and stable press-fitting within a defect. Unlike many existing drug delivery systems that either release encapsulated vancomycin too slowly (eg, 0.6% total release over 5 weeks, including a 0.2% burst release in the first hour from a 40-g packet of Palacos® R cement powder loaded with 1 g vancomycin) [30] or too quickly (80%–100% release within 5 days from pHEMA) [2], the pHEMA-nHA-vancomycin composites (2.4 and 4.8 wt% vancomycin) exhibited an attractive release profile, with 35% and 50% releases in the first 2 days followed by a steady, continued release that totaled 60% and 80% release over 2 weeks, respectively. This is consistent with a literature report wherein nHA-containing composite scaffolds led to a more sustained release of vancomycin (eg, a composite of calcium sulfate and nHA named PerOssal®) [33]; it does not, however, possess the surgical compressibility of pHEMA-nHA-vancomycin. In addition, we showed the released vancomycin remained bioactive and was able to inhibit the *E coli* culture in an encapsulated, dose-dependent manner. These combined properties would allow us to rationally select the initial vancomycin encapsulation dose to exert control over both the mode (burst release versus sustained release) and the overall quantity of the released antibiotics to meet the needs of both short-term higher-dose (eg, for acute osteomyelitis) and long-term sustained-dose (eg, for chronic infections) treatments.

The importance of sustained release of rhBMP-2 from a drug carrier in promoting more robust bone formation is well recognized [18, 19]. Clinically, rhBMP-2 delivered via collagen sponge carriers (INFUSE® Bone Graft) is FDA-approved for use in posterior-lateral spine fusion, open tibia fracture, and specific craniofacial applications to expedite bone fusion or healing [16]. In current clinical practice, however, rhBMP-2, in the order of milligrams, is typically used to induce fusion [13, 24]. The burst release of high-dose rhBMP-2 from collagen sponge carriers can lead to rapid diffusion of the proteins away from the defect site, increasing the risk for both systemic and local side effects, as well as wastefully inflating the cost of the treatment [15]. Such high initial dosage could also result in local bone overgrowth, inflammation response, periimplant bone resorption, and radically increased risks of postoperative complications [13, 35]. Complications related to high-dose rhBMP-2 (up to 2.1 mg/level) when used in anterior cervical fusion included postoperative edema, which resulted in an increased rate of readmission, dysphagia, and reintubations that resulted in tracheotomies [28, 35]. Recently, major osteolysis (69%) and back pain were also reported in patients undergoing transforaminal lumbar interbody fusion procedures utilizing higher dosages of rhBMP-2 (4.2–8.4 mg for single-level fusion, 12 mg for multilevel fusion [8]). Also, in patients undergoing anterior lumbar interbody fusion, heterotopic bone formations were reported [34, 40] that resulted in neurologic deficits. Therefore, it is highly desired to deliver rhBMP-2 via a synthetic bone graft with enhanced retention and more sustained release profile to reduce the minimal effective loading dose of the osteogenic therapeutic proteins. Here, we demonstrated the 2.4- and 4.8-wt% pHEMA-nHA-vancomycin composites could stably retain 89% and 94%, respectively, of 300 ng rhBMP-2 preabsorbed on the 64-mg graft, even after 48-hour incubation in PBS. This was accomplished by taking advantage of the large surface area of the nHA component, which is known to exhibit high affinity for a wide range of proteins [12, 37].

In addition, using a BMP-2-induced osteogenic transdifferentiation of a myoblast C2C12 cell culture model, we showed the preabsorbed, single dose of 300 ng rhBMP-2 can be released from the pHEMA-nHA-vancomycin graft in a localized and sustained manner over 8 days, a time frame critical for initiating effective injury repair and graft healing [6, 32]. The loading dose of 300 ng rhBMP-2/64-mg graft accomplished by the pHEMA-nHA-vancomycin was more than two orders of magnitude lower than those reported for other literature carriers [1]. For instance, a recently reported rhBMP-2 delivery system based on tricalcium phosphate-chitosan scaffolds required a loading dose of 50 μg rhBMP-2/graft to induce osteogenic transdifferentiation of C2C12 cells using the same cell culture model [1]. Reduction of the minimal effective loading dose, combined with the localized and sustained release profile accomplished by pHEMA-nHA-vancomycin, can potentially reduce both local and systemic side effects and the overall cost of the scaffold-assisted BMP-2 therapy.

In summary, we demonstrated up to 4.8 wt% of vancomycin can be encapsulated in the pHEMA-nHA without

compromising the structural integrity and elastomeric property of the synthetic bone graft. The resulting composites were able to release vancomycin in a sustained manner over 2 weeks and, when preabsorbed with a single dose of 300 ng rhBMP-2, could exert their osteoinductive properties in cultures in a localized and sustained manner over 1 week. The retention and localized release of osteoinductive protein factors can potentially lower the effective BMP-2 dosage needed for fusion, thereby lowering cost and minimizing complications associated with higher concentrations of BMP-2. These properties, combined with previously demonstrated in vivo efficacy of pHEMA-nHA in treating critical-sized femoral segmental defects in rats [11], open the possibility of the use of the synthetic graft in conjunction with vancomycin and low doses of rhBMP-2 to benefit a wide range of orthopaedic reconstruction procedures, including spine surgery, attempted arthrodesis, and tumor reconstruction.

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