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BASIC RESEARCH

Vancomycin-bearing Synthetic Bone Graft Delivers rhBMP-2 and Promotes Healing of Critical Rat Femoral Segmental Defects

Jordan D. Skelly ME, Jeffrey Lange MD, Tera M. Filion PhD, Xinning Li MD, David C. Ayers MD, Jie Song PhD

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Abstract

Background Bone grafts simultaneously delivering therapeutic proteins and antibiotics may be valuable in orthopaedic trauma care. Previously, we developed a poly(2-hydroxyethyl methacrylate)-nanocrystalline hydroxyapatite (pHEMA-nHA) synthetic bone graft that, when preabsorbed with 400-ng rhBMP-2/7, facilitated the functional repair of critical-size rat femoral defects. Recently, we showed that pHEMA-nHA effectively retains/releases vancomycin and rhBMP-2 in vitro. The success of such a strategy requires that the incorporation of vancomycin does not compromise the structural integrity of the graft nor its ability to promote bone healing.

Questions/purposes (1) To evaluate the ability of pHEMA-nHA-vancomycin composites in combination with 3-μg rhBMP-2 to repair 5 mm rat femoral segmental defects, and (2) To determine if the encapsulated vancomycin impairs the graft/rhBMP-2-assisted bone repair.

Methods pHEMA-nHA-vancomycin, pHEMA-nHA, or collagen sponge control with/without 3-μg rhBMP-2 were press-fit in 5 mm femoral defects in SASCO-SD male rats (289–300 g). Histology, microcomputed tomography, and torsion testing were performed on 8- and 12-week explants to evaluate the extent and quality of repair. The effect of vancomycin on the temporal absorption of endogenous BMP-2 and stromal cell-derived factor-1 was evaluated by immunohistochemistry. These factors are important for bone healing initiation and stem cell recruitment, respectively.

Results Partial bridging of the defect with bony callus by 12 weeks was observed with pHEMA-nHA-vancomycin without rhBMP-2 while full bridging with substantially mineralized callus and partial restoration of torsional strength was achieved with 3-μg rhBMP-2. The presence of vancomycin changed the absorption patterns of endogenous proteins on the grafts, but did not appear to substantially compromise graft healing.

Conclusions The composite pHEMA-nHA-vancomycin preabsorbed with 3-μg rhBMP-2 promoted repair of 5 mm rat femoral segmental defects. With the sample sizes applied, vancomycin encapsulation did not appear to have a negative effect on bone healing.

Clinical Relevance pHEMA-nHA-vancomycin preabsorbed with rhBMP-2 may be useful in the repair of critical-size long bone defects prone to infections.

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Introduction

Bone graft-assisted repair of traumatic orthopaedic injuries resulting in large volumetric bone loss face multiple clinical challenges. Synthetic bone graft substitutes hold the promise to overcome some of the important challenges associated with autografting (inadequate supply and donor site morbidity [13, 25, 26]) and allografting (graft rejection, nonunion, or graft instability [2, 5, 6]) procedures. As the incidence of orthopaedic infection increases [21], there is an increasing clinical need for the development of new grafts that can locally deliver therapeutic proteins and antibiotics simultaneously to treat traumatic defects prone to infections [29, 30]. Vancomycin, an antibiotic often used in orthopedics to treat gram-positive organisms and as the last line of defense in treating antibiotic resistant infections [15, 23], is an appealing candidate for incorporation with synthetic bone grafts [11, 18] to minimize the risks for infections while avoiding the negative effects associated with the systemic delivery of vancomycin. However, the success of such a strategy requires that the incorporation of vancomycin does not compromise the structural integrity of the graft nor its ability to promote bone healing. Previous in vitro studies suggest that the addition of antibiotics may inhibit osteogenesis [1, 12, 22, 24]. However, relatively few in vivo studies have directly examined the impact of vancomycin incorporation with synthetic bone grafts on bone healing [10, 12, 16].

We recently developed an elastic poly(2-hydroxyethyl methacrylate)-nanocrystalline hydroxyapatite (pHEMA-nHA) composite as an osteoconductive bone graft substitute [27]. When loaded with a single dose of 400 ng recombinant human bone morphogenetic protein-2/7 heterodimer (rhBMP-2/7) and press-fit into a 5-mm rat femoral segmental defect, the composite facilitated both the formation of fully bridged bony callus in 8 to 12 weeks and the restoration of torsional rigidity by 12 weeks [8]. Furthermore, up to 4.8 wt% vancomycin can be encapsulated within pHEMA-nHA without significantly compromising the structural integrity or the elasticity of the composite graft [17]. Recombinant human bone morphogenetic protein-2 (rhBMP-2), approved by the FDA for spine fusions and tibial fractures [3, 7, 9], can also be absorbed and released in a localized and sustained manner along with vancomycin with retained bioactivities in vitro [17].

In this study, we aimed to (1) evaluate the ability of pHEMA-nHA-vancomycin composite in combination with 3-μg rhBMP-2 to repair 5-mm rat femoral segmental defects, and (2) determine if the encapsulated vancomycin impairs the graft/rhBMP-2-assisted bone repair.

Materials and Methods

All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified. The pHEMA-nHA-vancomycin composite with 50 wt% nHA and 4.8 or 0 wt% vancomycin was prepared as previously reported [17, 27]. Briefly, distilled hydrogel monomer 2-hydroxyethyl methacrylate (HEMA) was crosslinked with 2 wt% ethylene glycol dimethacrylate and ethylene glycol by aqueous radical initiators ammonium persulfate and sodium metabisulfite in the presence of 50 wt% hydroxyapatite (Alfa Aesar, Ward Hill, MA, USA) and 4.8 wt% vancomycin powder (Hospira Inc, Lake Forest, IL, USA). Grafts without the addition of vancomycin were also fabricated. The hydrogel mixture was drawn into a glass tube with an inner diameter of 3 mm and allowed to polymerize overnight. The pHEMA-nHA-vancomycin or pHEMA-nHA composites were cut into 6-mm segments, and drilled with one longitudinal channel using a drill bit of 1.19 mm in diameter, and one intersecting orthogonal channel using a drill bit of 0.79 mm in diameter, to maximize potential bone marrow penetration from the adjacent marrow cavity (Supp. Fig. 1, supplementary material available in the online version of CORR®). The pHEMA-nHA-vancomycin composite was then equilibrated in deionized water (MilliQ) on an orbital shaker for 4 or 48 hours to remove residual radical initiators, unreacted monomers, ethylene glycol, and fragments from the drilling.

Our previous study showed that upon 4 and 48 hours equilibration in water, about 80% and about 50% of the preencapsulated vancomycin remains within the graft, respectively [17]. Grafts with 0 wt% vancomycin were equilibrated in water for 48 hours prior to use. The composites were sterilized overnight with UV (254 nm) and air-dried for storage. Sterile Helistat® Absorbable Collagen Sponge (Integra LifeSciences Corporation) cylinders of 3.2 mm in diameter and 5-mm in length were prepared using a sterilized hole punch. Prior to implantation, the grafts were hydrated with sterile saline for 30 minutes before 11.3-μL additional saline or reconstituted rhBMP-2 solution was uniformly applied to achieve a final loading dose of 0 or 3-μg rhBMP-2 per graft.

The ability of the pHEMA-nHA-vancomycin composite with or without 3-μg BMP-2, compared with the clinical standard of resorbable collagen sponge pre-absorbed with 3-μg rhBMP-2, to repair the rat femoral segmental defect was evaluated by a combination of microCT, torsion testing and histology at 8 and 12 weeks postop (Fig. 1A). A minimum sample size of 5 for quantitative analysis was chosen based on a power analysis as well as a prior study of similar nature [8]. Assuming the mean difference in MicroCT or torsion analysis outcomes between the experimental groups (pHEMA-nHA-vancomycin with versus without rhBMP-2, or pHEMA-nHA versus pHEMA-nHA-vancomycin both with rhBMP-2) at a

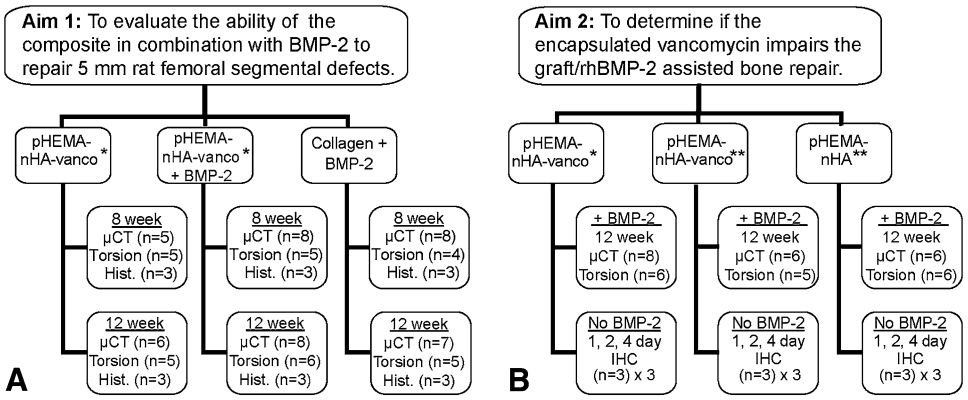

Fig. 1A–B The flow charts depict the treatment groups, outcome measures, and sample sizes of (**A**) aim 1 and (**B**) aim 2 studies. *Grafts equilibrated for 4 h prior to implantation (~80 % of the 4.8 wt% vancomycin remaining in the graft at the time of implantation). **Grafts equilibrated for 48 h prior to implantations (~50 % of the 4.8 wt% vancomycin remaining in the graft at the time of implantation).

given time is 33% and the standard deviation is 15%, a sample size of 5 would achieve a statistic power of 80% at the significance level of 0.05.

To determine if the presence of vancomycin impairs the outcome of graft-assisted bone healing, pHEMA-nHA-vancomycin equilibrated in water for 4 or 48 hours and the pHEMA-nHA (no vancomycin) control were press-fit within the rat femoral defect, either with 3-μg rhBMP-2 for quantitative microCT and torsion analyses at 12 weeks postop, or without rhBMP-2 for qualitative immunohistochemical (IHC) detection of the endogenous BMP-2 and stromal cell-derived factor 1 (SDF-1) absorbed on the grafts at 1, 2, or 4 days (Fig. 1B). Both BMP-2 and SDF-1 are known to play an important role in initiating fracture healing [28] and stem cell recruitment [4, 14].

All animal procedures were approved by the University of Massachusetts Medical School Animal Care and Use Committee. Sedated male Charles River SASCO-SD rats (289–300 g) were maintained by 2% isoflurane–oxygen throughout surgery. The periosteum of the entire diaphysis of the exposed femur was circumferentially removed to emulate a challenging clinical scenario where this important source of progenitor cells and signaling molecules is lost. A 5-mm femoral segmental defect was created and stabilized with a polyetheretherketone internal fixation plate as previously described [8]. The 5-mm segmental defect, along with the periosteum removal, is unable to heal on its own as shown previously, and is therefore considered a critical-sized defect [8]. The defect site was thoroughly irrigated with saline to remove bone debris and residue of detached periosteum before it was press-fit with a graft or collagen control with or without rhBMP-2. The wounds were closed with sutures and the rats were given cefazolin (20 mg/kg, SC, once a day) and buprenorphine (0.08 mg/kg, SC, every 8 hours) during the next 2 days. To ensure proper graft positioning, rats were radiographed immediately postoperation and every 2 weeks thereafter to monitor the mineralized callus formation.

Fresh 8- or 12-week explants, cut from both ends of the defect with the plate fixator intact, were scanned on a Scanco vivaCT 75 microCT system (SCANCO Medical, Switzerland). The effective voxel size of the reconstructed images was $30 \times 30 \times 30$ μm^3. The two internal screws attached to the plate fixator were used to help locate the center of the graft, from which 83 consecutive 30-μm slices from each side (~5 mm in total length) were included as the region of interest for analysis. A global threshold of 170 was applied to remove soft tissue background for three-dimensional (3-D) image reconstructions. For quantitative data analysis, a higher global threshold of 280 was applied to exclude the composite bone graft background. Bone volume fraction and bone mineral content within the region of interest were calculated using the distance transformation function of the SCANCO Medical's 3-D analysis software.

Following microCT scans, using a Dremel drill, the fixator plates attached to the explants were thinned to minimal attachment to facilitate the handling of the explants not encapsulated with a bridging calcified callus during the potting. The explants were then potted in a hex nut with bone cement [20]. After both ends of the explant were potted, the remainder of the uncut plate was carefully removed. The potted explants were torqued to failure at 1°/s on a mini-torsion tester (Admet, Norwood, USA). Maximum torque recorded was reported as the failure torque, and torsional stiffness was determined from the linear region of the torque-angular deflection curve. Intact femurs ($n = 7$) were also collected from healthy rats age matched to those 12-week postoperation and loaded to failure in torsion.

For histological analyses, fresh explants were fixed in a periodate-lysine-paraformaldehyde fixative [19] at 4°C for

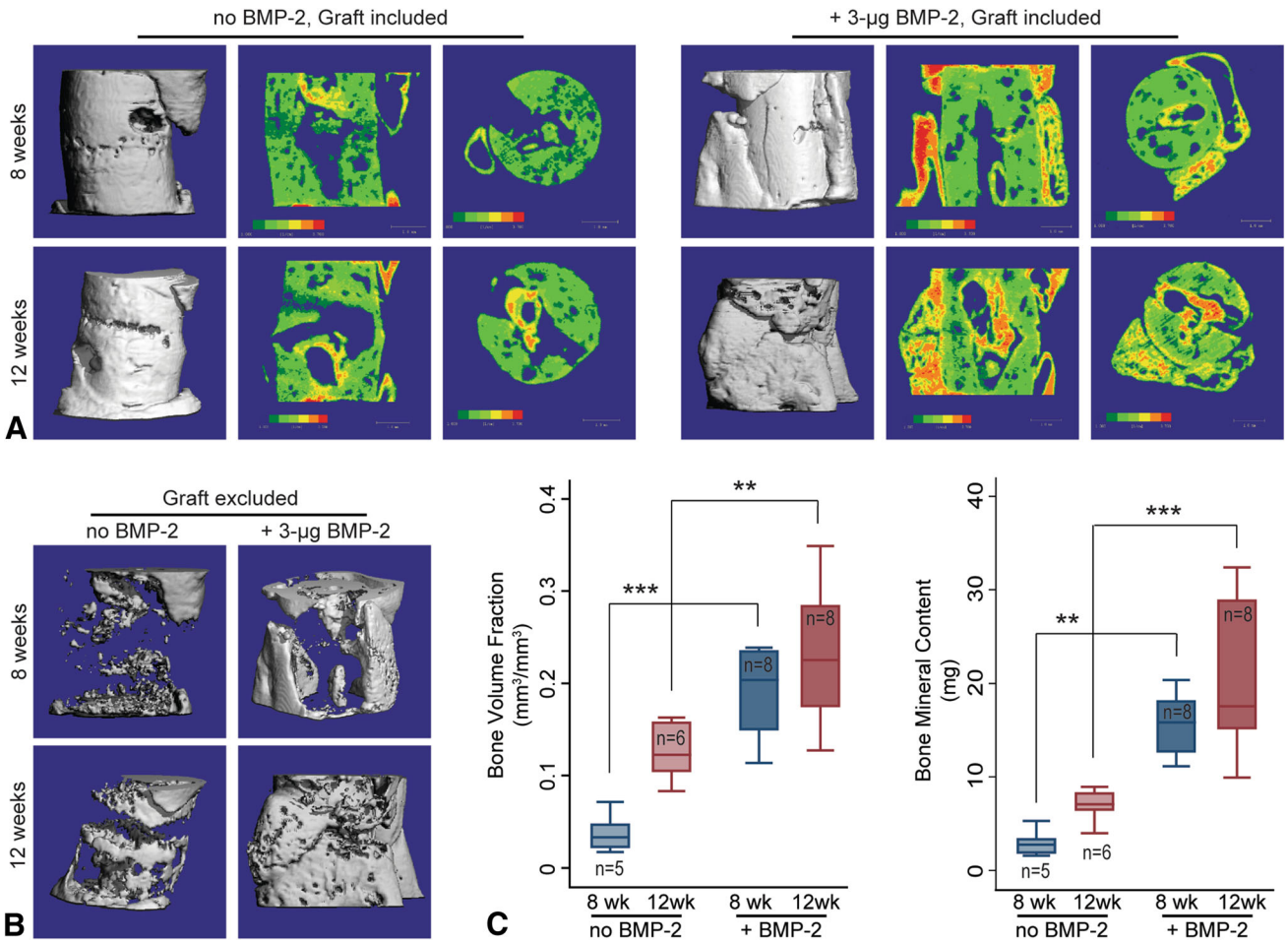

Fig. 2A–C MicroCT analyses of the 8 and 12-week explants press-fit with pHEMA-nHA-vancomycin grafts preabsorbed with 0 or 3-µg rhBMP-2 show different degrees of graft healing. (**A**) Reconstructed 3-D images and 2-D bone mineral density color maps are shown at a threshold that excludes soft tissue background but includes the grafts. Density color maps show center sagittal and axial slices of the region of interest with red representing the highest degree of mineralization. (**B**) Reconstructed 3-D images are shown at a threshold that excludes the grafts. (**C**) The boxplots of bone volume fraction and bone mineral content of the region of interest at a threshold that excludes the grafts reveal quantitative differences in bony callus formation. **$0.001 < p < 0.01$ ***$p < 0.001$.

2 days, followed by decalcification in 18% aqueous ethlenediaminetetraacetic acid (pH 8.0) for 4 weeks with frequent exchanges of fresh ethlenediaminetetraacetic acid solutions. The fixators were removed from the decalcified explants before they were subjected to serial dehydration, paraffin embedding and sectioning (6-µm sections), and stained by hematoxylin and eosin for cellularity, osteogenic marker alkaline phosphatase, and osteoclast lineage marker tartrate-resistant acid phosphatase for bone remodeling, and toluidine blue for cartilage formation [8]. Polarized light microscopy was used to evaluate the collagen fibril orientation/maturity of new bone formation. The 1-, 2-, and 4-day explants press-fit with pHEMA-nHA-vancomycin grafts without preabsorbed rhBMP-2 (with 0 or 4.8 wt% vancomycin, equilibrated for 4 hours or 48 hours prior to implantation) were fixed, paraffin-sectioned, and subjected to IHC detection of absorbed endogenous BMP-2 (primary antibody from Novus Biologicals) and SDF-1 (primary antibody from Santa Cruz) onto the respective scaffolds over time. Unimplanted composite grafts were stained by the same antibodies and rabbit immunoglobulin G isotype controls were included for all sections.

Statistical comparisons for quantitative microCT and torsion analyses were made using different methods based on the normality of data. For the metrics passing the normality test, multi-variant comparisons were carried out using two-way ANOVA. Otherwise, nonparametric Wilcoxon-Mann-Whitney ranked-sum test was used for pairwise comparisons while the Kruskal-Wallis rank test was used for multiple group comparisons. P values of < 0.05 were considered significant. All statistical analysis was performed using STATA 11.0 software (StataCorp LP, Collage Station, USA).

Results

Effect of BMP-2 on pHEMA-nHA-Vanco Graft-assisted Repair of 5 mm Rat Femoral Defects

The pHEMA-nHA-vancomycin grafts, with and without rhBMP-2, remained stably press-fit within the femoral defects as indicated by postoperative radiograph followup. Partial bridging of the segmental defect, with bony callus formation over the press-fit graft containing 4.8 wt% vancomycin without rhBMP-2, was observed at 12 weeks (Supp. Fig. 2). By contrast, complete bridging of the critical-size defect by bony callus was achieved with the addition of 3-μg rhBMP-2 within the same time frame.

More robust healing of the defect was found in the 8- and 12-week explants when BMP-2 was added to the grafts, as evident from qualitative microCT analysis. Two-dimensional density color maps of the center sagittal and axial cross-sections showed that the grafts were partially encapsulated with substantial, mature (higher bone mineral density indicated by the color mapping), and canalized calluses both within the drill hole and surrounding the grafts at 12 weeks with the addition of 3-μg rhBMP-2 (Fig. 2A). The robust external callus formation templated by the composite grafts (Fig. 2B) was in stark contrast to the disorganized new bone formation observed with the collagen sponge/rhBMP-2 control (Supp. Fig. 3, supplementary material available in the online version of CORR®), which were characterized with ectopic bone formations.

Bone volume fraction of the 8-week explants increased from 0.0384 ± 0.02173 to 0.1789 ± 0.05141 mm^3/mm^3 with the addition of BMP-2 ($p < 0.001$), while that of the 12-week explants increased from 0.1258 ± 0.03081 to 0.2308 ± 0.07588 mm^3/mm^3 with the addition of BMP-2 ($p < 0.01$) (Fig. 2C, left). Bone mineral content of the 8-week explants increased from 2.960 ± 1.475 to 15.59 ± 3.382 mg with the addition of BMP-2 ($p < 0.01$) while that of the 12-week explants increased from 6.969 ± 1.748 to 20.69 ± 8.333 mg with the addition of BMP-2 ($p < 0.001$) (Fig. 2C, right). Bone volume fraction and bone mineral content did not increase from 8 to 12 weeks within each treatment group. The collagen sponge/BMP-2 group was not quantitatively compared to the composite graft groups because the extensive ectopic bone formation beyond the defect site observed with the collagen/BMP-2-treatment made it impossible to consistently define the regions of interest for fair quantitative comparisons.

Torsional stiffness of the 12-week explants increased from $6.094 \pm 4.182 \times 10^{-4}$ Nm/deg to $1.815 \pm 1.011 \times 10^{-3}$ Nm/deg with the addition of BMP-2 ($p < 0.05$) (Fig. 2, left). The maximum torque of the 12-week explants also increased from 0.03454 ± 0.01735 to 0.0649 ± 0.01764 Nm with the addition of BMP-2 ($p < 0.05$) (Fig. 3, right). However, neither group was restored to the level of the unoperated control femurs which had a stiffness of 0.03566 ± 0.01042 Nm/deg and a maximum torque of 0.365 ± 0.08187 Nm.

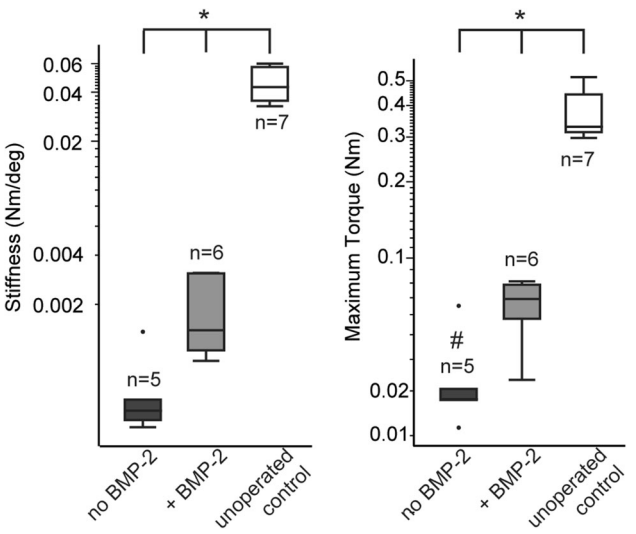

Fig. 3 The boxplots of torsional stiffness and failure torque of the explants press-fit with pHEMA-nHA-vancomycin grafts preabsorbed with 0 or 3-μg rhBMP-2 at 12-weeks postop reveal quantitative differences in the restoration of biomechanical integrity of the defect. #The explants without BMP-2 treatment did not fail by the time data recording was terminated at 150° due to lack of a fully bridged bony callus and the elasticity of the underlying graft composite. *$p < 0.05$ is considered significant.

The addition of rhBMP-2 led to the formation of a mostly calcified external healing callus bridging over the graft-filled defect by 12 weeks as revealed by the qualitative assessment of hematoxylin and eosin (Fig. 4A) and toluidine blue (Fig. 4B) stained longitudinal sections of the explants. Only a small region of the external callus near the right cortical bone-graft junction remained uncalcified as indicated by positive (purple) stains for cartilage by toluidine blue, supporting that the healing proceeded via endochondral ossification. Extensive new bone formation at the cortical bone-graft interface, particularly near the marrow cavity, was also observed, supporting good integration between the cortical bone and the new bone, as well as between the new bone and the graft. Polarized light microscopy (Fig. 4C) revealed the formation of oriented collagen fibrils, supporting significant maturation of the bony callus by 12 weeks. Alkaline phosphatase and tartrate-resistant acid phosphatase staining (Fig. 4D) revealed active remodeling, characterized with an intense line of osteoclast activity (red tartrate-resistant acid phosphatase stain) followed by a line of osteoblast activity (blue alkaline phosphatase stain), along the graft/callus interface and the mineralized/un-mineralized callus interface.

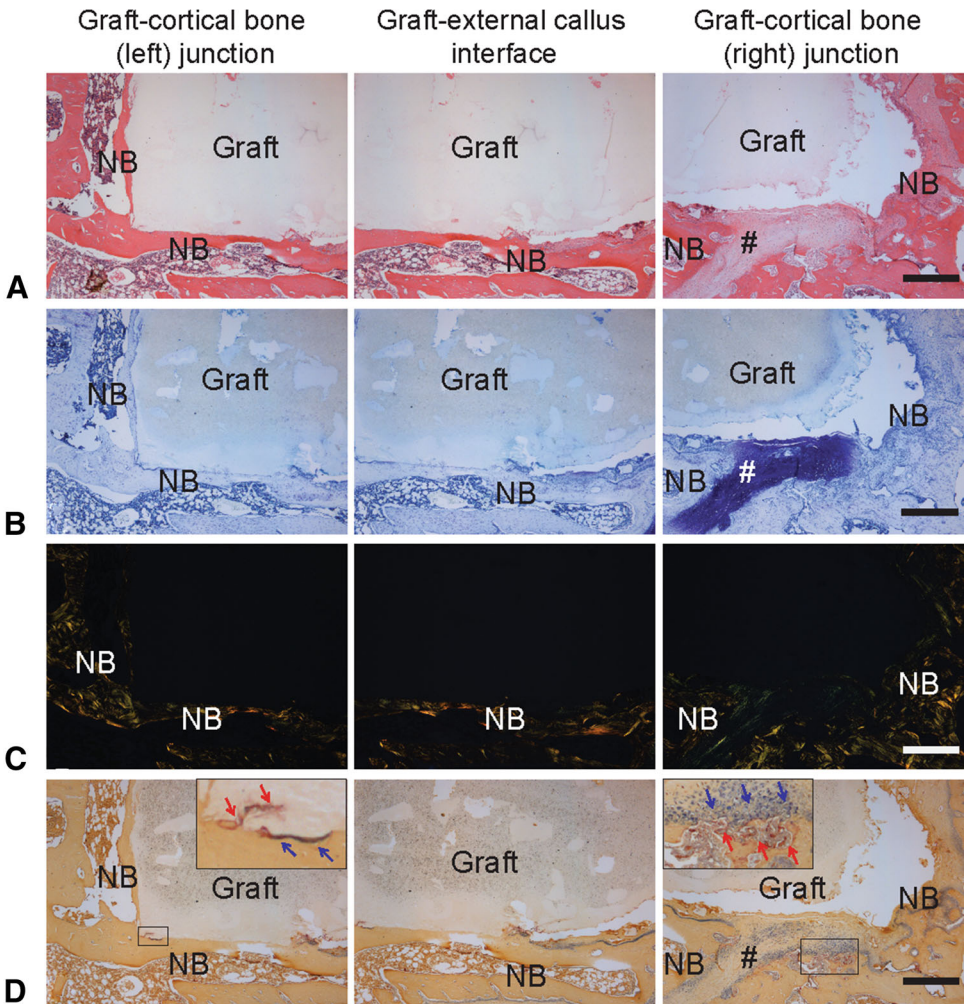

Fig. 4A–D Histological analysis of the longitudinal sections of 12-week explants press-fit with pHEMA-nHA-vancomycin grafts preabsorbed with 3-μg rhBMP-2 show the extent of graft/callus remodeling. (**A**) Hematoxylin and eosin staining reveals cellularity within the healing callus. (**B**) Toluidine blue staining reveals cartilage formation within the healing callus. (**C**) Polarized light (PL) microscopy observation reveals collagen fibril orientations (maturity) of callus. (**D**) Alkaline phosphatase (blue) and tartrate-resistant acid phosphatase (red) staining reveals bone remodeling within the healing callus and at the graft-callus interfaces. Insets show the magnified view of the boxed area revealing coordinated osteoclastic (red arrows pointing to tartrate-resistant acid phosphatase stains) and osteoblastic (blue arrows pointing to alkaline phosphatase stains) remodeling activities. NB = new bone, # = cartilage/unmineralized callus. Scale bars are 500 μm.

Effect of Vancomycin Incorporation on Bone Graft-mediated Healing

There was no change in the bone volume fraction and bone mineral content (Fig. 5A) or the stiffness and maximum torques (Fig. 5B) between the 12-week explants treated with pHEMA-nHA (without vancomycin) and rhBMP-2 and those treated with pHEMA-nHA-Vanco and rhBMP-2 regardless of their pre-equilibration time (4 h versus 48 h) prior to implantation. Immunohistochemical detection of endogenous BMP-2 and SDF-1 absorbed/sequestered by the synthetic grafts (without rhBMP-2) within the first few days of implantation revealed visually distinctive absorption patterns as a function of vancomycin content (Fig. 6). The pHEMA-nHA composites without vancomycin absorbed endogenous proteins primarily at the surface of the implant, with only slight penetration into the graft interior during 4 days. By contrast, the endogenous proteins initially enriched at the surface of the pHEMA-nHA-vancomycin graft (equilibrated for 48 h prior to implantation) were able to gradually penetrate throughout the graft over time. A similar absorption pattern was observed with the pHEMA-nHA-vancomycin graft equilibrated for a shorter period (4 h), although the endogenous proteins penetrated into the graft to a lesser extent.

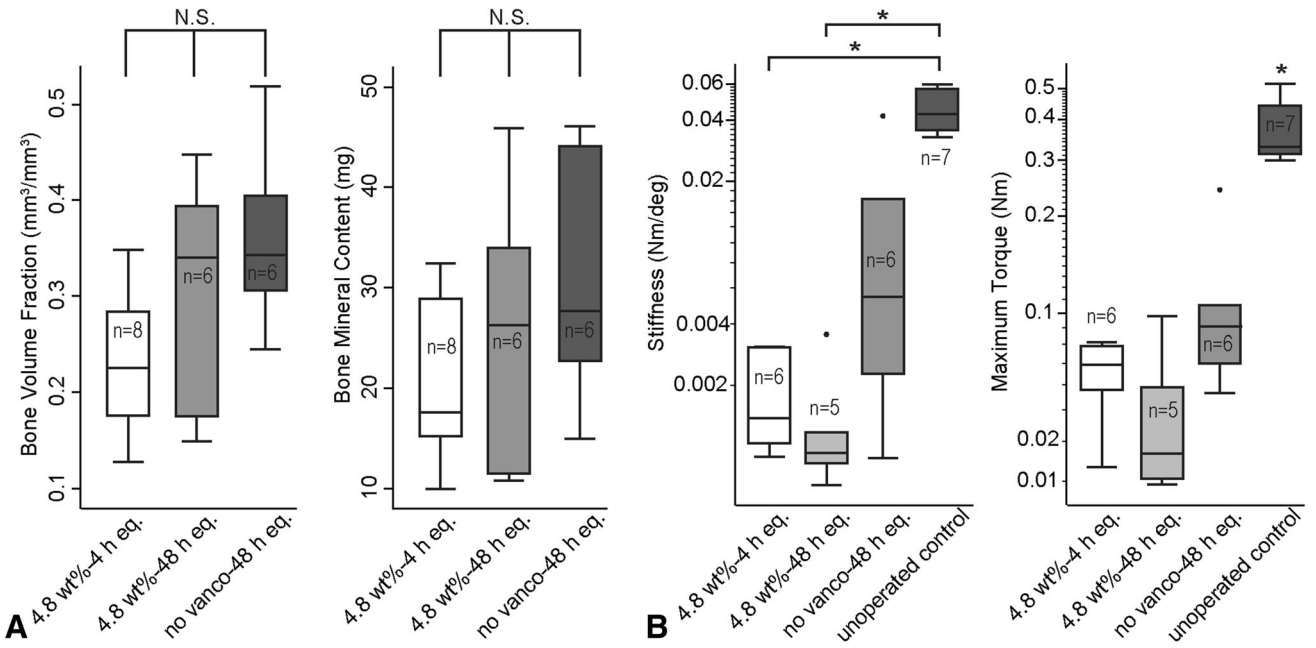

Fig. 5A–B MicroCT and torsional analyses of 12-week explants press-fit with pHEMA-nHA-vancomycin grafts equilibrated for 4 or 48 h versus the no-vancomycin pHEMA-nHA grafts, all preabsorbed with 3-μg rhBMP-2, reveal no differences. (**A**) Boxplots of bone volume fraction and bone mineral content of the region of interest at a threshold that excludes the graft show quantitative differences in bony callus formation among the explants. (**B**) Boxplots of the failure torque of the explants show quantitative differences in torsional strength among the explants. N.S. denotes a lack of statistical significance ($p > 0.05$).

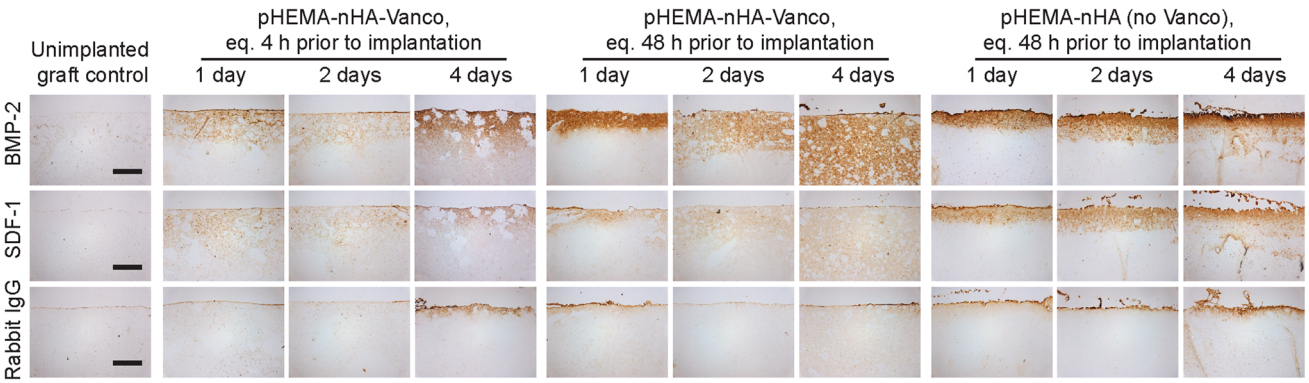

Fig. 6 IHC detection shows the absorption of endogenous BMP-2 and SDF-1 onto the composite grafts with or without 4.8 wt% vancomycin, equilibrated in water for 48 hours or 4 hours prior to implantation, at 1, 2, or 4 days postimplantation. Unimplanted pHEMA-nHA was stained as a negative control (left) and a rabbit immunoglobulin G was included for each composition and each time point (bottom panel). Scale bars are 150 μm.

Discussion

Synthetic bone grafts simultaneously delivering therapeutic proteins and antibiotics are desired for orthopaedic care. Previously, we developed a pHEMA-nHA composite graft that, when preabsorbed with 400-ng rhBMP-2/7, facilitated the functional repair of critical-size rat femoral defects [8]. Recently, we showed that pHEMA-nHA effectively retains/releases vancomycin and rhBMP-2 in vitro [17]. The purpose of this study was to determine whether the pHEMA-nHA-Vanco composite graft can promote the healing of critical rat femoral segmental defects with rhBMP-2 and whether the incorporation of vancomycin would compromise the graft healing. We found that pHEMA-nHA-Vanco promoted the healing of 5 mm rat femoral segmental defects with a single dose of 3-μg rhBMP-2, and that the incorporation of vancomycin did not compromise the bone healing outcome.

One limitation of this study is that the efficacy of the vancomycin-bearing composites in inhibiting infections was not examined. Such a question can be better addressed utilizing an established femoral infection model in future studies. Additionally, although this study was powered for comparing the efficacy of pHEMA-nHA-Vanco with/without BMP-2 in promoting the healing (Aim 1), it was not adequately powered to statistically determine the effect of vancomycin addition to graft healing (Aim 2). This was because, with the current sample size, the standard deviations in Aim 2 quantitative microCT and torsion analyses were larger than those reported in an earlier study utilized to guide the power analysis [8]. Central limit theory suggests that with a large enough sample size, the mean converges to the true value and the standard error will become smaller. Thus, one may speculate that with a larger sample size, given the same mean difference but a smaller standard deviation, it is possible there may be a statistical difference in graft healing as a function of vancomycin incorporation. However, future studies will have to be carried out to validate such a speculation.

In this study, we showed that without the addition of rhBMP-2, the pHEMA-nHA-vancomycin grafts only enabled the formation of bony callus partially bridging over the defects by 12 weeks, consistent with our prior findings with pHEMA-nHA composites [8]. However, full bridging of the critical-size defect with mineralized callus along with partial restoration of the torsional strength of the defect was consistently observed by 12 weeks when a single loading dose of 3-μg rhBMP-2 was applied to the pHEMA-nHA-vancomycin composite. No ectopic bone formation was observed surrounding the defect/graft. This observation is in stark contrast with the group treated with collagen sponges preabsorbed with 3-μg rhBMP-2, where the induced bony callus formation was characterized with significant undesired ectopic bone formation. These observations support that the sustained release of rhBMP-2 from pHEMA-nHA-vancomycin demonstrated in vitro [17] has translated into the in vivo benefits of preventing ectopic bone formation and facilitating more uniform callus formation and subsequent callus maturation. Furthermore, we observed coordinated osteoblastic/osteoclastic activities within the healing callus and at the composite graft-callus interface at 12 weeks, suggesting that the graft remodeling and the increase of torsional strength of the remodeled/more mature bony callus is likely to continue. Such continued remodeling at the graft-tissue interface is essential for long-term osteointegration of the graft and the success of graft-assisted repair of the critical bone defect.

In regard to our question of whether vancomycin adversely impacted graft healing, with the sample sizes applied, no difference in bone volume fraction or bone mineral content of the explants by 12 weeks was observed among these groups. In the presence of 3-μg rhBMP-2, with the sample sizes applied, there was no difference in torsional strength restored by 12 weeks between the groups with and without vancomycin. We speculate that the ability of the pHEMA-nHA graft to absorb and sequester a higher concentration of endogenous factors such as BMP-2 and SDF-1 near the graft surface, as opposed to the more even distribution of the sequestered proteins within the vancomycin-containing grafts (Fig. 6), may translate into more effective recruitment of stem/progenitor cells and/or initiation of the healing.

In summary, we showed that pHEMA-nHA-vancomycin bone grafts with a single loading dose of 3-μg rhBMP-2 promoted the healing of critical-size femoral segmental defects in healthy rats. With the sample sizes applied, the addition of 4.8 wt% vancomycin to the grafts appeared to have minimal impact on the quality of the bone healing, suggesting that the benefits of both the osteoinductive factor and the antibiotics may be delivered with a single off-the-shelf composite bone graft, though future studies will be needed to validate this concept.

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