Effect of hyperbaric oxygen on demineralized bone matrix and biphasic calcium phosphate bone substitutes

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Objectives. The aim of this study was to assess the possible effect of hyperbaric oxygen (HBO) on the healing of critical-sized defects that were grafted with demineralized bone matrix (DBM) combined with Pluronic F127 (F127) to form a gel or putty, or a commercially available biphasic calcium phosphate (BCP), mixed either with blood or F127 to form a putty.

Study design. Twenty New Zealand White rabbits were randomly divided into 2 groups of 10 animals each. Bilateral 15-mm calvarial defects were created in the parietal bones of each animal, resulting in 40 critical-sized defects. Group I defects were grafted with either DBM putty or DBM gel. Group II defects were grafted with either BCP or BCP putty. Five animals from each group received HBO treatment (100% oxygen, at 2.4 ATA) for 90 minutes per day 5 days a week for 4 weeks. The other 5 animals in each group served as a normobaric (NBO) controls, breathing only room air. All animals were humanely killed at 6 weeks. The calvariae were removed and analyzed by micro computed tomography (mCT) and histomorphometry.

Results. mCT analysis indicated a higher bone mineral content (BMC, $P < .05$), bone volume fraction (BVF; $P < .001$), and bone mineral density (BMD; $P < .001$) of the defects grafted with BCP rather than DBM. Furthermore, the voxels that were counted as bone had a higher tissue mineral density (TMD) in the BCP- than in the DBM-filled defects ($P < .001$).

Histologically complete bony union over the defects was observed in all specimens. Histomorphometric analysis showed that DBM-filled defects had more new bone ($P < .007$) and marrow ($P < .001$), and reduced fibrous tissue compared with the BCP defects ($P < .001$) under NBO conditions.

HBO treatment reduced the amount of fibrous tissue in BCP filled defects ($P < .05$), approaching levels similar to that in matching DBM-filled defects. HBO also resulted in a small but significant increase in new bone in DBM-grafted defects ($P < .05$).

Conclusion. Use of DBM or BCP promoted healing in these critical-sized defects. Hyperbaric oxygen therapy resulted in a slight increase in new bone in DBM-grafted defects and much larger reduction in fibrous tissue and matching increases in marrow in BCP-grafted defects, possibly through increased promotion of angiogenesis. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:59-66)**

Vascular disruption and hypoxia are consequences of the creation of bony defects.[1] Although hypoxia has shown to stimulate vascular in-growth, extended hypoxia will blunt the healing process.[2-4] Hyperbaric oxy-

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gen (HBO) has been successfully used to improve the healing of bony defects that will not heal spontaneously (critical-sized defects).[5] The mechanism by which HBO is believed to work is that it increases the amount of oxygen dissolved in the blood (oxygen tension), which in turn can increase the amount of oxygen delivered to the hypoxic wound site, stimulating angiogenesis and osteogenesis.[6,7]

The current standard for the treatment of critical-sized defects is the use of autogenous bone grafts[8]; however, studies have shown that bone graft substitutes can be used to promote bony regeneration in critical-sized defects.[9] Demineralized bone matrix (DBM) has been shown to induce bony in-growth to critical-sized defects; however, this material lacks rigidity because of lack of mineralized components in its matrix. Alternatively, ceramics such as hydroxyapatite have been shown to have good rigidity but they take longer to resorb and be replaced by bone. Ceramics also have a tendency to integrate by fibrous union. HBO has been demonstrated to reduce bone mineral density (BMD) in particulate autogenous bone grafts possibly by activating angiogenesis and resorption of the grafted matrix.[10,11] These results suggest that HBO may promote resorption and minimize fibrous union when ceramics are used.

The aim of this study was to determine if the use of HBO in combination with DBM or bone ceramic enhances osseous healing and whether HBO prevents fibrous union when bone ceramics are used.

MATERIALS AND METHODS

The surgical protocol for this study was approved by the University of Toronto Animal Care and Ethics Committee (Protocol number 20005145). The surgical technique, anesthetics, and prophylactic antibiotics were described in a previous publication.[6] In brief, bilateral 15-mm full thickness osseous defects were created surgically in the parietal bones of 20 adult, skeletally mature, male New Zealand White rabbits weighing 3 to 4 kg. Bone graft substitutes were placed randomly on the right or left side of each animal. Animals were randomly divided into 2 groups of 10 subjects.

Experimental design

Group I (DBM) n = 10. Allogeneic demineralized bone matrix was prepared from rabbit long bones by treatment with 0.6 M HCl for 48 hours followed by extensive washing and lyophilization.

Pluronic F-127 (F127; poloxamer 407, BASF Canada Inc., Toronto, Canada) was prepared by slowly adding 33g F127 to 100 mL MilliQ water held at 4°C. The F127 was then autoclaved for sterility.

Animals of Group 1 were grafted with either DBM putty or DBM gel, both of which were prepared with allogeneic rabbits' long bones. DBM putty was prepared as a mixture of 70% DBM and 30% Pluronic F-127 gel. DBM gel was prepared as a mixture of 40% DBM and 60% Pluronic F-127 gel. The main differences between the gel and the putty are the consistency of the mixture and the amount of DBM in the compound.

Group II (BCP) n = 10. Animals of Group II were grafted with either BCP or BCP putty. Biphasic calcium phosphate (Straumann AG, Basel, Switzerland) is a commercially available synthetic bone substitute. It is made of a mixture of 60% hydroxyapatite and 40% tricalcium phosphate. BCP putty was prepared as a mixture of 70% BCP with 30% Pluronic F-127 gel. In defects where BCP was used without the gel, it was mixed with the animal's own blood.

Hyperbaric oxygen therapy. Five animals from each group underwent HBO. Rabbits were placed in a hyperbaric chamber and exposed to 100% oxygen at 2.4 atmospheres absolute for 90 minutes per day for 5 days a week for 4 weeks (20 treatments total). Five animals in each group served as normobaric controls (NBO) and were left to heal at room air without any further intervention. HBO treatment was initiated 24 hours after surgery.

Rabbits were humanely killed 6 weeks postoperatively. The parietal bones were harvested using an oscillating saw. Care was also taken to preserve the pericranium, the sagittal, coronal, and the lambdoid sutures because they served as a reference to the circumference of the defects. The final harvested specimens measured 30 × 25 × 12 mm in the greatest dimensions. Specimens were fixed in 10% formalin before analysis by micro computed tomography (mCT).

Micro-computed tomography

This study used an Explore Locus SP micro CT scanner (GE medical systems, London, Ontario, Canada). Before scanning the specimens, a calibration scan was performed using the manufacturers' standard, which includes synthetic bone, water, and an air sample.

Calvarial specimens were scanned at a resolution of 28 μm. Reconstruction of scanned images was done using the manufacturer's software after calibration using the bone, water, and air standard values. The reconstructed 3-dimensional image was then analyzed using MicroView software (v2.1.2 GE Medical Systems, London, Ontario, Canada). Before analysis, threshold values must be determined that permit the software to distinguish bone and ceramic from soft tissue. A lower threshold was selected that would count bone + ceramic by identifying areas of bone within the defects tracing regions of interest that incorporated only

Table I. MicroCT analysis

	DBM				*BCP*				
	GEL		*PUTTY*		*Blood*		*PUTTY*		*ANOVA*
	NBO	*HBO*	*NBO*	*HBO*	*NBO*	*HBO*	*NBO*	*HBO*	*P*
Vol (mm^3)	186 ± 103	161 ± 42	177 ± 95	179 ± 18	162 ± 56	180 ± 30	143 ± 14	128 ± 28	.096
BV (mm^3)	67 ± 36	62 ± 8	64 ± 36	65 ± 10	103 ± 33	111 ± 29	91 ± 9	72 ± 21	.017
BVF (%)	35 ± 7	40 ± 7	37 ± 8	34 ± 7	64 ± 4	61 ± 6	64 ± 3	56 ± 8	<.001
BMC (mg)	46 ± 23	38 ± 7	41 ± 22	41 ± 5	110 ± 36	116 ± 27	84 ± 7	66 ± 19	<.001*
TMC (mg)	29 ± 16	25 ± 4	27 ± 16	26 ± 5	107 ± 35	113 ± 29	80 ± 7	61 ± 19	<.001*
BMD (mg/mL)	223 ± 34	244 ± 28	232 ± 36	233 ± 27	682 ± 43	641 ± 81	594 ± 52	512 ± 72	<.001
TMD (mg/mL)	416 ± 30	410 ± 20	410 ± 4	401 ± 11	563 ± 23	547 ± 21	530 ± 23	506 ± 10	<.001

DBM, demineralized bone matrix; *BCP,* biphasic calcium phosphate; *GEL,* the DBM granules were mixed with Pluronic F127 at 40% DBM to 60% F127 (vol/vol); *PUTTY,* the DBM or BCP granules were mixed with F127 at 70% DBM/BCP to 30% F127 (vol/vol); *NBO,* normobaric oxygen; *HBO,* hyperbaric oxygen; *BV,* bone volume; *BVF,* bone volume fraction; *BMC,* bone mineral content; *TMC,* tissue mineral content; *BMD,* bone mineral density; *TMD,* tissue mineral density.
*Data were not normally distributed and groups were compared using analysis of variance (ANOVA) on Ranks.

bone and then determining the bone volume fraction (BVF) at a range of threshold values. The "bone + ceramic" threshold was determined to be the highest threshold value that gave a BVF greater than 0.95 for all bone ROIs (regions of interest).

The threshold value for the "ceramic only" was determined in a similar manner by tracing 8 particles in each defect that could be clearly identified. The highest threshold that resulted in every particle having a BVF (which in this case is the ceramic volume fraction) was greater than 0.95.

After determination of the threshold values, the margins of the defect were traced in 3 dimensions resulting in an ROI that incorporated the entire defect.

The ROI of each specimen was analyzed for total volume (TV), bone volume (BV), bone volume fraction (BVF = BV/TV), bone mineral content (BMC), and bone mineral density (BMD). The software also permitted measurement of the mineral content and mineral density of the voxels. which are counted as "bone" based on the threshold setting selected. These measures are called the tissue mineral content (TMC) and tissue mineral density (TMD). The measurements were made at both the bone + ceramic and ceramic only threshold values.

To correct for the presence of BCP in the defects corrected values for BV, BMC, BMD, TMC, TMD, and BVF were determined by subtracting the values obtained at the ceramic only threshold from those for the bone + ceramic threshold and recalculating.

Histomorphometry

Upon completion of mCT scanning, specimens were decalcified using 45% formic acid and 20% sodium citrate for 4 weeks. Each bone was sectioned into 2 portions: an anterior and posterior portion. Both portions were embedded in paraffin and 7-μm sections were prepared and stained with hematoxylin and eosin. Sections in the middle of the defects, representing the greatest cross-sectional dimension, were examined under the light microscope. Ten randomized sections within the middle of defects were digitized using a digital camera (RT Color; Diagnostic Instruments Inc., Sterling Heights, MI). A blinded investigator analyzed each section for the amount of new bone, marrow, fibrous tissue, and residual graft using Image ProPlus software (Media Cybernectics, Bethesda, MD).

Statistical analysis

Results were tested for normality and equal variance. Each parameter was compared across all groups using 1-way Analysis of Variance (ANOVA) for normal data and Kruskal-Wallis 1-way ANOVA on RANKS for non-normal data, followed by Student-Newman-Keuls (SNK) post hoc testing. Statistical significance was considered to be *P* less than .05. All analyses were done using Sigma Stat v3.5 (Systat Software, San Jose, CA).

RESULTS

Micro-computed tomography

All parameters were examined for significant differences between the different groups using ANOVA (Table I). Four different experimental conditions were compared in this study: BCP versus DBM, HBO versus NBO, and 2 different formulation comparisons of blood versus F127 putty within the BCP group and F127 gel versus F127 putty within the DBM group. Following a significant ANOVA result, the matched pairs that were exposed to these experimental conditions were tested for significant differences using a post hoc test (ie, for comparisons of BCP and DBM, the NBO-BCP-putty was compared with the NBO-DBM-putty and the

Table II. Post hoc *P* values for mCT parameters by matched groups

	Post hoc P values					
	BV[1]	*BVF*	*BMC*[2]	*TMC*[2]	*BMD*	*TMD*
BCP vs DBM						
Putty-HBO	.638	<.001	<.05	<.05	<.001	<.001
Putty-NBO	.098	<.001	<.05	<.05	<.001	<.001
Blood vs F127 putty						
BCP-HBO	.024	.294	<.05	<.05	<.001	.012
BCP-NBO	.459	.991	<.05	NSD	.026	.045
F127 gel vs putty						
DBM-HBO	.718	.718	NSD	NSD	.729	.506
DBM-NBO	.522	.829	NSD	NSD	.775	.693
NBO vs HBO						
BCP-blood	.661	.671	NSD	NSD	.206	.232
BCP-putty	.249	.152	<.05	<.05	.015	.087
DBM-gel	.661	.611	NSD	NSD	.905	.233
DBM-putty	.896	.896	NSD	NSD	.968	.087

Following a significant result in the 1-way ANOVA, the matched groups within each of the 4 different experimental conditions (BCP versus DBM, HBO versus NBO and 2 different formulation comparisons blood versus F127 putty with the BCP group and F127 gel versus F127 putty within the DBM group) were tested for significant differences using a post hoc test.

ANOVA, analysis of variance; *BV,* bone volume; *BVF,* bone volume fraction; *BMC,* bone mineral content; *TMC,* tissue mineral content; *BMD,* bone mineral density; *TMD,* tissue mineral density; *BCP,* biphasic calcium phosphate; *DBM,* demineralized bone matrix; *NBO,* normobaric oxygen; *HBO,* hyperbaric oxygen; *NSD,* not significantly different.

[1]Following a significant ANOVA test, none of the group comparisons for the BV were found to be significant by Student-Newman-Keuls post hoc testing. We therefore used Fisher's least significant difference (LSD) method to identify differences.

[2]*P* values were not obtained from post hoc testing of non-normal data and are reported as <.05 if they were found to be significantly different, or NSD if there were not.

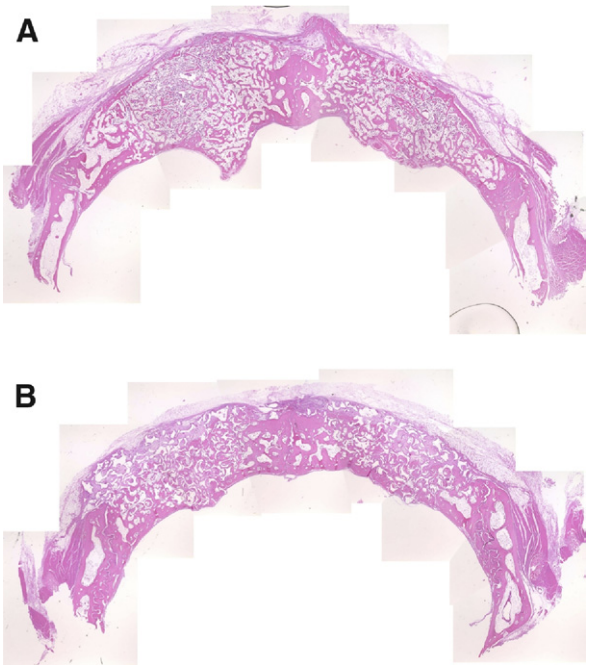

Fig. 1. **A,** Montage of photomicrographs covering an entire calvaria grafted with DBM gel and putty. Rabbit was exposed to HBO treatment. Bone bridges the entire defect resulting in complete union. (Hematoxylin and eosin, [H&E], original magnification of each photomicrograph ×4.) **B,** Montage of photomicrographs covering an entire calvaria grafted with BCP and BCP putty from a rabbit exposed to HBO treatment. Because of decalcification, the residual graft has been removed and so appears as voids in the defect. The defect is completely bridged by bone along its dural aspect. New bone and marrow elements are most common at the margins and dural aspect, with the tissue along the periosteal aspect being predominantly fibrous lighter. (H&E, original magnification of each photomicrograph ×4.)

HBO-BCP-putty was compared with the HBO-DBM-putty) (Table II).

ANOVA of the mCT results demonstrated significant differences between the groups for all parameters except total volume (see Table I). Identification of the groups that had significantly different bone volumes (BV) required use of the Fisher least significant differences (LSD) post hoc testing, as SNK was unable to do so. The bone volume was higher in the defects grafted with BCP mixed with blood compared with those mixed with F127 when exposed to HBO ($P = .024$) but not for the matching grafts under NBO. No other significant differences were seen in this parameter between the various matched groups.

Defects containing biphasic calcium phosphate had significantly higher BVF, BMC, TMC, BMD, and TMD than those containing demineralized bone matrix under the same experimental conditions (ie, NBO-putty and HBO-putty) ($P < .05$).

HBO reduced BMC, TMC, BMD and TMD in the BCP-putty treated defects, but not the defects filled with BCP mixed with blood. HBO did not alter any of the other parameters in any of the other groups.

Defects grafted with BCP mixed with blood compared with BCP mixed with F127 had significantly higher BMC, BMD, and TMD under NBO and HBO conditions. The amount of F127 mixed with DBM had no significant effects on any of the parameters measured.

Histological analysis

The DBM- and BCP-filled defects each had their own unique histological appearance (Fig. 1, *A* and *B*). The DBM gel- and putty-filled defects revealed the same microscopic features. All defects were bridged with a mixture of bone and residual bone substitute (Fig. 1, *A*).

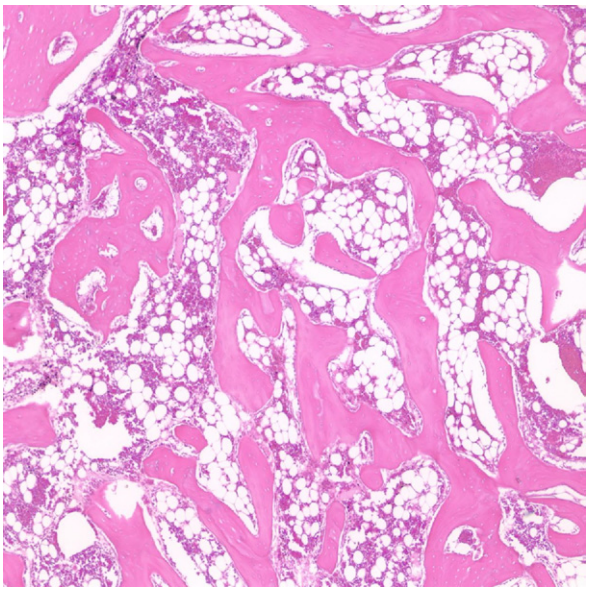

Fig. 2. Photomicrograph of a defect of a rabbit filled with DBM putty from the NBO group. Extensive marrow (M) and new bone (NB), which is often apposed to residual DBM, can be seen throughout the field. (H&E, original magnification ×20.)

DBM-filled defects contained bone, marrow, and some fibrous tissue as well as residual DBM particles (Fig. 2). Residual DBM contained empty lacunae (Fig. 3). Woven bone bounded the residual DBM and was lined by cuboidal cells that appeared morphologically to be osteoblasts (Fig. 3, *A* and *B*). New bone formation was also noted within the DBM particles.

BCP defects revealed similar microscopic features to DBM defects (Fig. 1, *B*), although fibrous tissue was more pronounced and could be found throughout the defect (Figs. 4 and 5, *A* and *B*), while it was limited to the margins in the DBM groups.

Histomorphometry

One-way ANOVA analysis revealed that there were significant differences within all parameters measured (Tables III and IV). Histomorphometric analysis demonstrated that DBM-filled defects had significantly more new bone ($P < .008$) and less residual graft ($P < .001$) than matching BCP-filled defects. Although DBM-filled defects exposed to NBO also had increased marrow and reduced fibrous tissue ($P < .001$ for both), when the BCP defects had been exposed to HBO these differences were abolished. These results also correlated with an observed increase in marrow and reduction in fibrous tissue in BCP defects exposed to HBO compared with BCP-grafted defects under NBO conditions.

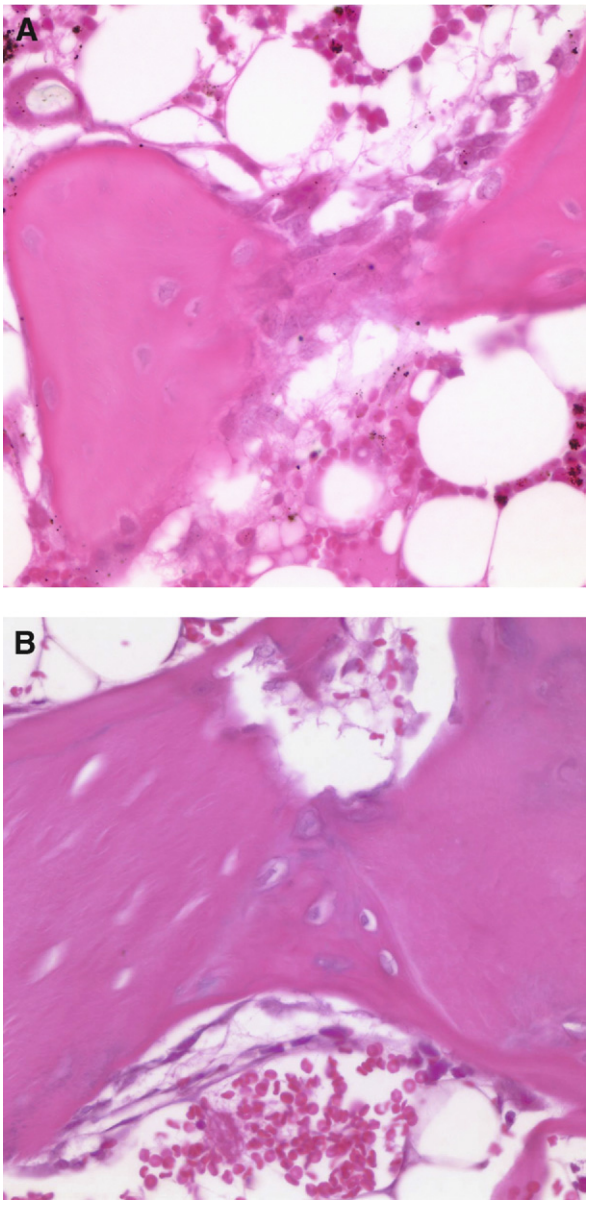

Fig. 3. **A,** Photomicrograph of a defect of a rabbit filled with DBM gel and treated with hyperbaric oxygen. Deposits of new bone are associated with cuboidal osteoblastlike cells some of which appear to be becoming entrapped in the bone matrix. (H&E, original magnification ×40.) **B,** Photomicrograph of a defect from rabbit filled with DBM putty and treated with hyperbaric oxygen. The new bone formed on the residual DBM can clearly be distinguished from the DBM by the presence of larger rounded lacunae that contain cells compared to the elliptical empty lacunae present in the DBM (H&E, original magnification ×40).

HBO was also seen to lead to a small but significant increase in the amount of new bone in the DBM-grafted defects ($P < .04$). Both marrow and fibrous tissue were

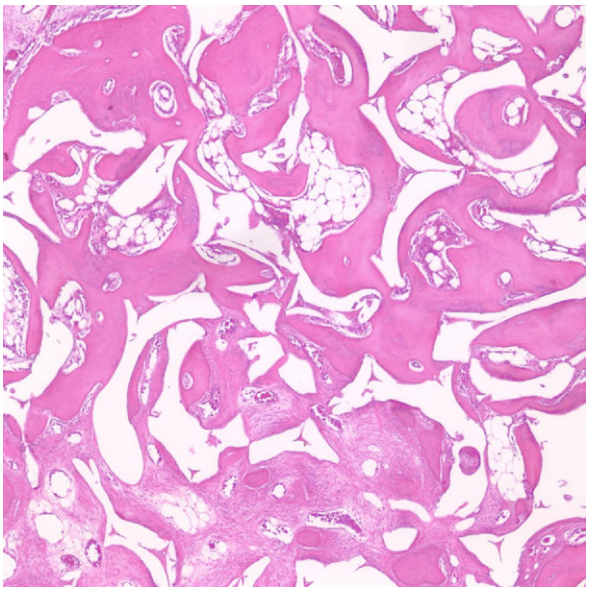

Fig. 4. Photomicrograph of a defect filled with BCP putty from a rabbit in the NBO group. The large empty voids are where the biphasic calcium phosphate particles were present. They were lost during decalcification. New bone, marrow, and fibrous tissue with a thick cellular infiltration can been seen apposed to the BCP particles (H&E, original magnification ×20).

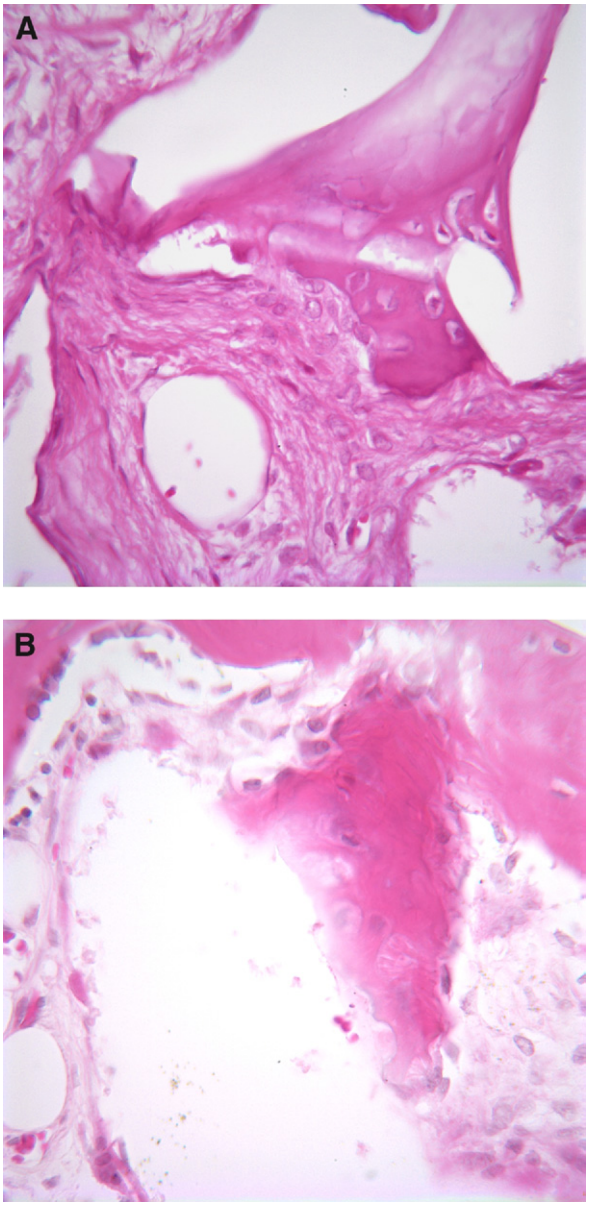

Fig. 5. **A,** Photomicrograph of a defect filled with BCP Putty in rabbits kept under normobaric conditions. A small island of bone can be seen apposed to the missing BCP particle and surrounded by the highly cellular fibrous tissue (H&E, original magnification ×40). **B,** Photomicrograph of a defect filled with BCP Putty in rabbits kept under hyperbaric conditions. A small island of bone can be seen apposed to the missing BCP particle and surrounded by marrow and new bone (H&E, original magnification ×40).

reduced in DBM-grafted defects exposed to HBO; however, this did not reach significance for either tissue type.

Histomorphometry was unable to note any significant differences between groups grafted with different formulations of BCP (blood versus F127) or DBM (gel versus putty) in any of the measured parameters.

DISCUSSION

Bone graft substitutes have been successfully used to treat defects, avoiding some of the limitations associated with autogenous bone including donor site morbidity, blood loss, and extended time in surgery. HBO has been shown to enhance the bony healing of critical-sized defects in rabbits without bone grafting and may be useful as an adjunct to bone graft substitutes in smaller defects.[12,13] The use of HBO as a testing modality may also allow the detection of differences between bone and various bone substitute materials that are not evident using other testing methods.

The aim of this study was to evaluate the effect of HBO on the healing of critical-sized defects in the presence of 2 commonly used types of bone graft substitutes, demineralized matrix (DBM) and biphasic calcium phosphate (BCP). We also investigated whether different preparations of these substitutes affected their performance.

Both bone substitute materials were able to promote healing of the critical-sized defects. HBO did not significantly increase the amount of bone present in the

Table III. Histomorphometric analysis

	DBM				*BCP*				
	GEL		*PUTTY*		*Blood*		*PUTTY*		*ANOVA*
	NBO	*HBO*	*NBO*	*HBO*	*NBO*	*HBO*	*NBO*	*HBO*	*P*
NB	36.3 ± 3.0	43.7 ± 4.9	35.1 ± 5.5	44.3 ± 2.9	22.0 ± 6.9	23.9 ± 3.7	23.6 ± 5.7	24.8 ± 6.6	<.001
M	47.0 ± 5.2	41.7 ± 7.8	50.6 ± 8.7	43.6 ± 5.6	28.6 ± 4.9	39.1 ± 4.1	29.4 ± 5.5	37.4 ± 5.1	<.001
NB+M	83.3 ± 6.1	85.5 ± 3.6	85.8 ± 4.4	87.8 ± 4.4	50.6 ± 8.9	63.0 ± 4.9	53.0 ± 5.5	62.2 ± 7.6	<.001
FT	13.7 ± 5.1	9.6 ± 3.3	9.8 ± 2.5	7.1 ± 3.0	25.0 ± 6.4	12.9 ± 5.0	23.1 ± 5.4	13. ± 4.7	<.001
RG	2.9 ± 1.8	5.0 ± 1.2	4.4 ± 2.7	5.1 ± 2.5	24.3 ± 3.0	24.1 ± 5.9	23.9 ± 3.2	24.4 ± 5.4	<.001

All results are reported as percentage area of defect occupied by tissue type.
DBM, demineralized bone matrix; *BCP,* biphasic calcium phosphate; *GEL,* the DBM granules were mixed with Pluronic F127 at 40% DBM to 60% F127 (vol/vol); *PUTTY,* the DBM or BCP granules were mixed with F127 at 70% DBM/BCP to 30% F127 (vol/vol); *ANOVA,* analysis of variance; *NBO,* normobaric oxygen; *HBO,* hyperbaric oxygen; *NB,* new bone; *M,* marrow; *NB+M,* new + marrow; *FT,* fibrous tissue; *RG,* residual graft.

Table IV. Post hoc *P* values for histomorphometric parameters by matched groups

	Post Hoc P-values				
	NB	*M*	*NB+M*	*FT*	*RG*
BCP vs DBM					
Putty-HBO	<.001	.391	<.001	.223	<.001
Putty-NBO	.007	<.001	<.001	<.001	<.001
Blood vs F127 putty					
BCP-HBO	.782	.666	.843	.868	.991
BCP-NBO	.630	.834	.537	.513	.977
F127 gel vs putty					
DBM-HBO	.877	.633	.642	.415	.978
DBM-NBO	.706	.346	.800	.547	.515
NBO vs HBO					
BCP-blood	.831	.047	.013	.002	.908
BCP-putty	.926	.045	.021	.006	.996
DBM-gel	.030	.365	.577	.611	.637
DBM-putty	.039	.169	.590	.630	.958

Following a significant result in the 1-way ANOVA, the matched groups within each of the 4 different experimental conditions (BCP versus DBM, HBO versus NBO and 2 different formulation comparisons blood versus F127 putty with the BCP group and F127 gel versus F127 putty within the DBM group) were tested for significant differences using a post hoc test.
NB, new bone; *M,* marrow; *NB+M,* new + marrow; *FT,* fibrous tissue; *RG,* residual graft; *BCP,* biphasic calcium phosphate; *DBM,* demineralized bone matrix; *NBO,* normobaric oxygen; *HBO,* hyperbaric oxygen.

BCP-grafted defects compared with defects in animals exposed to normal air. This result matches our previous observations where HBO promoted healing and bone formation in unfilled defects, but provided no further increase in bone when used in conjunction with autogenous bone grafts.[6,10] We did, however, observe a small but significant increase in new bone with DBM-grafted defects exposed to HBO.

Exposure to HBO reduced the amount of fibrous tissue in the BCP-grafted defects, and showed a matching increase in the marrow component of the repair tissue. We have previously demonstrated that HBO treatment results in prolonged increases in vascular endothelial growth factor (VEGF) expression,[7] whereas others have demonstrated VEGF promotes angiogenesis, bone formation, and remodeling,[13-15] suggesting that that HBO may potentially reduce fibrous tissue formation through promotion of angiogenesis, which could result in increased marrow.

Comparison of the amounts of new bone in the DBM-filled defects and the BCP-filled defects indicated more bone and marrow formation in the DBM group irrespective of HBO or NBO therapy. DBM-filled defects also revealed less fibrous tissue formation when compared with BCP-filled defects when treated under NBO conditions. We did not see any differences in the repair of defects by different preparations of DBM (gel versus putty), whereas the different preparations of BCP (blood versus F127) showed some differences in mineralization as assessed by the mCT, but not by histology.

In conclusion, HBO resulted in small increases in new bone formation in defects grafted with DBM. In defects grafted with BCP, HBO resulted in a large reduction in fibrous tissue and an increase in its replacement with marrow.

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