Rabbit Bone Marrow Response to Bovine Osteoinductive Proteins and Anorganic Bovine Bone

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The effects caused by the implantation of bioabsorbable hydroxyapatite (HA) bound to a pool of bone morphogenetic proteins (BMPs) and other bone noncollagenous hydrophobic proteins mixed with anorganic bovine bone inside rabbit bone marrow were assessed. Within the interior of hollow cylindric titanium prototypes, the following biomaterials were inserted: (1) test group: HA containing a pool of BMPs and noncollagenous hydrophobic proteins mixed with anorganic bovine bone; (2) control group: HA without any protein mixed with anorganic bovine bone; and (3) negative control group: blood clot. The cylinders were placed surgically into the medial portion of the tibiae of 7 rabbits in a manner that allowed the biomaterials to contact just the bone marrow. Morphometric analysis showed that: (1) the biomaterials containing the protein mixture resulted in significantly less new bone than the biomaterials without such a mixture; (2) the group without the protein pool formed larger amounts of bone within the cylinder when compared to the negative control (blood clot only); and (3) the biomaterials containing the protein pool did not show any difference in relation to the negative control. It was concluded that a pool of BMPs and other bone noncollagenous hydrophobic proteins had an inhibitory effect on osteogenesis, and that the biomaterials without a protein pool formed a favorable substrate to bone formation. (INT J ORAL MAXILLOFAC IMPLANTS 2001;16:799–808)

Key words: *biomaterials, bone marrow, bone morphogenetic proteins, bone substitutes, hydroxyapatite, titanium*

Osseointegration, in terms of absolute numbers, has been reported to result in a high endosteal implant success rate, but in regions with poor bone quality (Type IV) the failure rate can reach up to 35%.[1] Three hypotheses have recently been investigated to decrease the failure rate in Type IV bone: *(1)* modification of the implant surface[2]; *(2)* improvement of primary stability of the implant[3]; and *(3)* the use of osteoinductive substances to improve bone quality.

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Bone morphogenetic proteins (BMPs) represent a promising modality for modifying bone quality, because such proteins are able to recruit new osteoprogenitor cells from undifferentiated stem cells, and, theoretically, to increase bone formation around the endosseous implant.[4] Rutherford and coworkers[5] showed that bovine extracted osteogenic protein 1 (bOP-1) was capable of improving bone contact with implants immediately placed in extraction sites in monkeys. Also, the bOP-1 was able to restore gaps up to 3 mm between the remaining bone and the titanium surface. Wang and associates[6] verified that bBMPs shortened the osseointegration period in dogs when used with titanium implants. Sailer and Kolb[7] treated hundreds of patients in whom implants were placed in critical situations for osseointegration to occur, and, with the association of bBMPs, the failure rate was reduced considerably.

Although some authors have reported using bBMPs in humans successfully,[7–10] there is the possibility that xenogenic BMPs, depending on the degree of purity, might cause immunologic reactions that would jeopardize the osteoinduction phenomenon.[11]

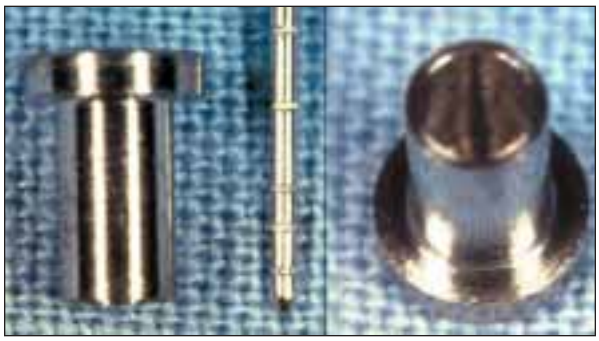


Fig 1 Titanium prototype (lateral view and view from the bottom).

The present trial sought to evaluate the effects of new biomaterials and osteogenic proteins within the bone marrow of the rabbit tibia, which simulates poor-quality bone.[12]

MATERIALS AND METHODS

Titanium Prototype Fabrication

Hollow cylindric prototype implants made from commercially pure titanium were machined with an electronic lathe (Schaublin 110CNC, Delemont, Switzerland) with the following characteristics (Fig 1): 4 mm of diameter at the top; 3 mm body diameter, and 6 mm length; and an opening for the hollow part located at the bottom of the prototype. The prototypes received surface treatment based on cleaning and etching to provide a rougher substrate. Such surface treatment consisted in removing titanium residue, cleaning for 10 minutes with ultrasound using ethyl alcohol 100%, cleaning 10 minutes more with ultrasound using a solution of trichloroethylene, and 2 more passes in ultrasound (10 minutes each pass) in absolute ethyl alcohol. After cleaning, the prototypes were allowed to dry passively in filtered air current. Once dried, the prototype surfaces were etched with a mixture of hydrofluoric acid 3% and nitric acid 30% for 30 minutes, and then the surface was cleaned with deionized water (obtained with Milli-Q academic, Millipore, Bedford, MA). After the etching process, the prototypes were cleaned again with deionized water and the ultrasound cleaning described above was repeated. After the surface treatment, the prototypes were placed in ampoules and passivated 30 minutes at 180ºC. Once passivated, 3 mL of ethyl alcohol 10% were added to the ampoules, and these were closed and autoclaved. At this point, the prototypes were ready for use surgically.

Animals

Seven male white New Zealand rabbits (approximately 4 months of age) between 3 and 3.5 kg were used. The animals were kept in the university animal care facilities, where they were fed ad libitum.

Test Materials

In the control group (without BMPs), anorganic bovine bone (Osseobond DC, Baumer SA, Mogi Mirim, SP, Brazil) was mixed in equal volumetric proportion to bioabsorbable hydroxyapatite (HA; Bioapatita, Baumer SA, Mogi Mirim, SP, Brazil). In the test group (with BMPs), anorganic bovine bone (Osseobond DC) was mixed in equal volumetric proportion to BMPs and other bovine hydrophobic noncollagenous proteins bound to bioabsorbable HA (BMP-HA). One rabbit received 6 negative control prototypes filled only with blood clot.

A protein pool was extracted with guanidine hydrochloride treatment in the laboratory of Biochemistry of Faculty of Odontology of Bauru, University of São Paulo, according to Urist and colleagues.[13] However, this pool was not subjected to any kind of chromatography to separate BMPs from other hydrophobic noncollagenous proteins. After the extraction, the pool was lyophilized and bound to bioabsorbable HA in a mass proportion of 20 (HA): 1 (pool).

Bioapatita (Ministry of Health approval #103.455.00004) is an absorbable HA that is completely absorbed in critical size defects of *Cavia porcellus* skull in 3 months (these studies were presented to the Brazilian Ministry of Health). This material is prepared from a chemical reaction between calcium chloride ($CaCl_2$) and sodium phosphate (Na_2HPO_4) with a posterior thermal treatment with sodium hydroxide and phosphate buffer. Its mean particle size is 5 μm (Fig 2).

Osseobond DC (Ministry of Health approval #103.455.00001) is a natural HA obtained from cortical bovine bone with particle size ranging from 250 μm to 1,000 μm. This material is deproteinized at a temperature of 250°C under pressure, and then receives chemical treatment for cleaning and neutralization (Fig 3).

Implantation of the Prototypes and Test Materials

The animal surgeries were conducted only after the protocol had been approved by the Faculty of Odontology of Bauru/University of São Paulo commission for animal experimentation. The animals were sedated with Rompun (Bayer, Belford Roxo, RJ, Brazil) intramuscular injection (0.25 mL/kg) followed by Ketalar (Pfizer/Parke-Davis, São Paulo,

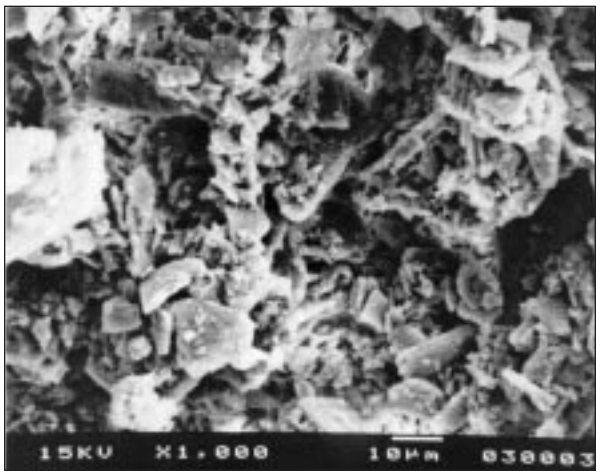


Fig 2 View of absorbable hydroxyapatite (scanning electron microscopy).

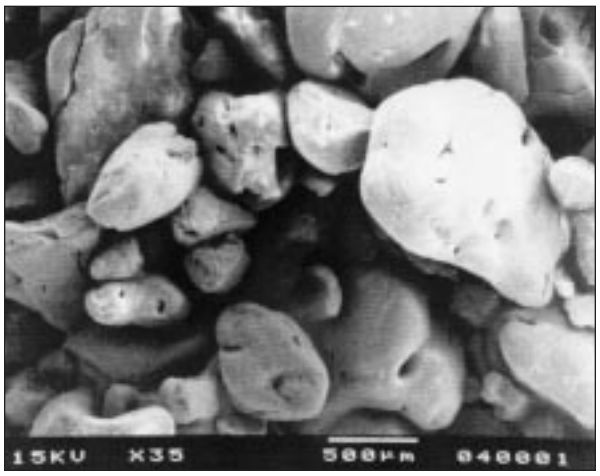


Fig 3 View of anorganic bovine bone (scanning electron microscopy).

Fig 4 Insertion of biomaterials inside the titanium cylinder.

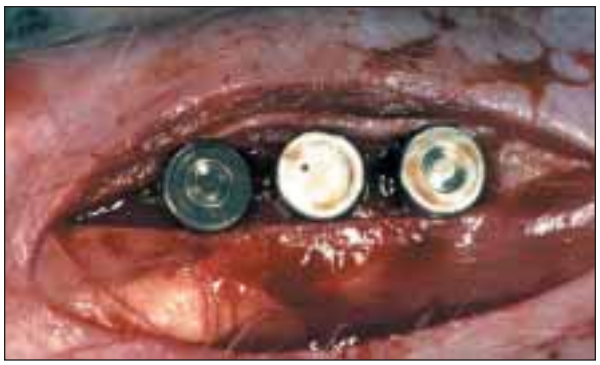


Fig 5 The titanium cylinders carrying biomaterials are placed in rabbit tibia.

SP, Brazil) intramuscular injection (0.35 mL/kg). Once the animal was sedated, a 3-cm incision was created at the flat portion of the tibial head near the medial joint, and a flap was raised for bone exposure. The bone was drilled sequentially with 2- and 3-mm cylindric burs with thorough saline irrigation. Three perforations were made in each tibia to receive the prototypes containing the test biomaterials. The biomaterials (described above) were agglutinated with blood from the perforations and inserted in the hollow portion of the prototype (Fig 4). The materials were inserted slowly with a blunt instrument (Fig 4) to avoid air bubbles inside the cylinder. The amount of material inserted was standardized by the size of the inner portion of the cylinders (which had been precisely machined in an electronic lathe, mentioned earlier). The animals received 3 prototypes in each tibia (Fig 5), with one side carrying the BMP group and the other side carrying the group without BMP. One animal received 6 prototypes containing blood clot only to serve as a negative control.

Blood was taken from the perforations instead of from another distant site, because one of the objectives of this study was to verify the effects of the test materials on potential osteogenic bone marrow cells. Blood from perforations might carry such cells.

After suturing, the surgical wounds were cleaned with saline and iodine. The postoperative procedure consisted of cleaning the wound with Lepecid BR spray (DowElanco Industrial, São Paulo, Brazil) over a 10-day period once daily. After this period the sutures were removed. The animals were sacrificed 67 days postsurgery and the tibiae were removed and placed in 10% phosphate-buffered formalin for 1 week. After fixation in formalin, the histologic processing was performed.

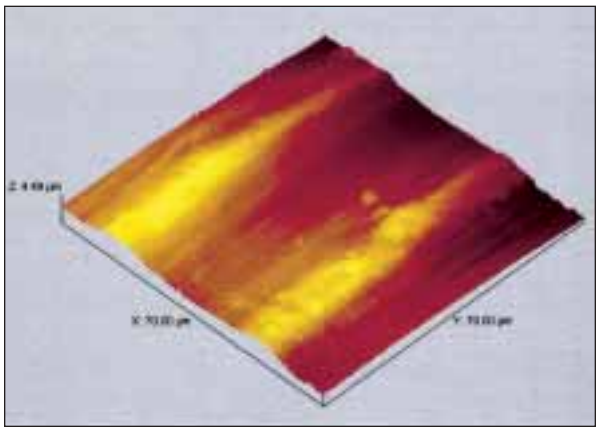


Fig 6a Implant surface before etching treatment (just machined; mean roughness 1.10 μm; atomic force microscopy).

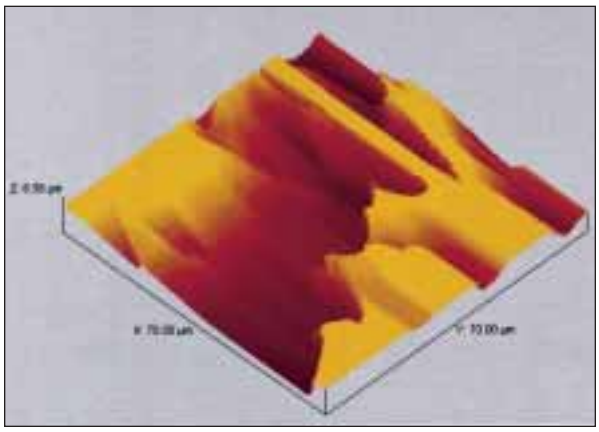


Fig 6b Implant surface after etching treatment used in the cylinders of this research (mean roughness 3.62 μm; atomic force microscopy).

Histologic Processing and Histomorphometric Analysis

The blocks were trimmed near each prototype and subsequently dehydrated and embedded with plastic resin (Technovit 7200, Kulzer, Wehrheim, Germany). The resin blocks were submitted to the process of cutting and grinding (Exakt system, Exakt Apparatebau, Norderstedt, Germany) until they reached a thickness between 80 and 100 μm. The sections were stained with toluidine blue, coded to ensure blindness, and analyzed. The histologic slides were analyzed with a Leica light microscope (Mannheim, Germany) using a 25× Leica objective lens and a Kpl 8× Zeiss eyepiece (Zurich, Switzerland) containing a Zeiss II integration graticule with 100 points. The whole area of the cylinder's midsection was analyzed. The relative point-counting plannimetry analysis consisted of counting the points under the following structures: anorganic bovine bone, newly formed bone, and connective tissue/HA (counted together); a count of points overlying one structure is proportional to their area. The structures inside the cylinders generated between 30 to 50 microscopic fields, depending on the area occupied by material inside the cylinder after the histologic processing. After the structures were counted in each slide, the results were analyzed statistically.

Statistical Analysis

All statistical analyses were performed with Sigma Stat 1.0 for Windows (1992–1994, Jandel Corporation, SPSS Science, Chicago, IL). For comparison between the groups with and without the protein pool, the Student *t* test or the Mann-Whitney test was used. The latter test was performed when the data did not pass the normality test or equivalent variance test. For comparison of the groups with and without BMPs against the negative control (blood clot only), 1-way analysis of variance was used with the Bonferroni *t* test against a control (blood clot only). For all cases, $P < .05$ was established as the level of significance.

The samples from the negative control (blood clot only) all came from the same animal, because this group was not introduced into the research since, theoretically, it was thought that this group would not form new bone. So the negative controls were placed in 1 animal simply to illustrate a lack of bone formation; surprisingly, however, there was bone formation in some specimens. Thus, it was decided that these results should be reported (that is now a subject of another investigation of implant surface treatments; see Figs 6a and 6b). Since the negative controls all came from the same animal, caution should be taken in the interpretation of the comparisons with them.

RESULTS

Histomorphometric Results

The grinding procedure associated with the histologic processing may result in loss of soft tissue or loss of tissue without proper resin infiltration. For this reason, it was decided to compare also the relationships among the remaining structures to check whether or not the absolute values of each structure were representative.

The relationships calculated for comparisons between the groups with and without BMPs were: *(1)* newly formed bone, divided by anorganic bovine

Table 1 General Results

Rabbit no.	Group	No. of points				Ratio		
		New bone	Bovine bone	Connective tissue and HA	Total	New bone/ bovine	New bone/ total	Connective tissue and HA/ bovine bone
1	Without BMP	443	1,676	667	2,786	0.2643	0.1590	0.3980
1	Without BMP	457	425	314	1,196	1.0753	0.3821	0.7388
1	Without BMP	316	1,157	1,060	2,533	0.2731	0.1248	0.9162
2	Without BMP	324	1,433	1,678	3,435	0.2261	0.0943	1.1710
2	Without BMP	268	1,064	310	1,642	0.2519	0.1632	0.2914
2	Without BMP	602	1,282	430	2,314	0.4696	0.2602	0.3354
3	Without BMP	99	807	551	1,457	0.1227	0.0679	0.6828
3	Without BMP	137	1,506	480	2,123	0.0910	0.0645	0.3187
4	Without BMP	526	1,435	348	2,308	0.3666	0.2279	0.2425
4	Without BMP	541	1,082	274	1,897	0.5000	0.2852	0.2532
4	Without BMP	382	1,004	162	1,548	0.3805	0.2468	0.1614
5	Without BMP	763	1,643	326	2,732	0.4644	0.2793	0.1984
5	Without BMP	886	1,684	680	3,250	0.5261	0.2726	0.4038
6	Without BMP	557	1,845	762	3,164	0.3019	0.1760	0.4130
1	With BMP	302	1,939	878	3,119	0.1558	0.0968	0.4528
2	With BMP	347	1,420	126	1,893	0.2444	0.1833	0.0887
2	With BMP	271	1,282	661	2,214	0.2114	0.1224	0.5156
2	With BMP	397	1,851	552	2,800	0.2145	0.1418	0.2982
3	With BMP	86	1,621	892	2,599	0.0531	0.0331	0.5503
3	With BMP	215	1,245	652	2,112	0.1727	0.1018	0.5237
3	With BMP	226	1,749	1,670	2,645	0.1292	0.0620	0.9548
4	With BMP	250	737	295	1,282	0.3392	0.1950	0.4003
4	With BMP	172	708	86	966	0.2429	0.1781	0.1215
4	With BMP	243	877	387	1,507	0.2771	0.1612	0.4413
5	With BMP	367	1,512	513	2,392	0.2427	0.1534	0.3393
5	With BMP	334	1,114	1,002	2,450	0.2998	0.1363	0.8995
5	With BMP	142	1,309	780	2,231	0.1085	0.0636	0.5959
7	Blood clot	630	—	139	769	—	0.8192	—
7	Blood clot	295	—	190	485	—	0.6082	—
7	Blood clot	209	—	144	353	—	0.5921	—
7	Blood clot	55	—	56	111	—	0.4955	—
7	Blood clot	205	—	149	354	—	0.5791	—
7	Blood clot	65	—	117	182	—	0.3571	—

bone (this material was chosen for the majority of the relationships because there were similarities in both groups and because the new bone usually was formed in direct apposition to this material); *(2)* newly formed bone, divided by the total count of structures inside the cylinder (new bone, anorganic bovine bone, HA, and connective tissue); and *(3)* HA and connective tissue, divided by the quantity of anorganic bovine bone. Counting of the structures inside the cylinder resulted in the data shown in Table 1.

Anorganic bovine bone was chosen for comparison of the relationships with bone or connective tissue plus HA because, as observed in Table 2, the total values of this material were not different between the groups with and without BMP proteins. The group without BMPs formed more bone than the group with BMPs, when the absolute values were considered (Table 3). The group without BMPs was also superior to the group with BMPs concerning the relationship between new bone formed with anorganic bovine bone (Table 4) and the proportion of new bone formed (new bone/total of structures) (Table 5). There was no difference between the groups with and without BMP when the relationship of connective tissue plus HA with anorganic bovine bone remaining was evaluated (Table 6).

A comparison of the absolute values of new bone formation of the groups with and without BMPs against a negative control (blood clot only) revealed that only the group without BMPs formed more bone than the negative control (Tables 7 and 8).

Table 2 Comparison of Groups With and Without BMPs Concerning Absolute Quantity of Anorganic Bovine Bone Remaining

Group	n	Mean	Standard deviation	Standard error
Without BMP	14	1288.8	392.3	104.8
With BMP	13	1335.7	402.5	111.6

P = .7617 (Student *t* test).

Table 3 Comparison of Groups With and Without BMPs Concerning Absolute Quantity of New Bone Formed

Group	n	Mean	First quartile	Third quartile
Without BMP	14	450.0	316.0	557.0
With BMP	13	250.0	204.3	337.3

P = .0125 (Mann-Whitney test; this was chosen because the data failed in equal variance test).

Table 4 Comparison of Groups With and Without BMPs Concerning the Relationship of New Bone to Anorganic Bovine Bone

Group	n	Mean	First quartile	Third quartile
Without BMP	14	0.334	0.252	0.470
With BMP	13	0.214	0.149	0.253

P = .0125 (Mann-Whitney test; this was chosen because the data failed in normality test).

Table 5 Comparison of Groups With and Without BMPs Concerning the Relationship of New Bone to Total Bone

Group	n	Mean	First quartile	Third quartile
Without BMP	14	0.202	0.125	0.273
With BMP	13	0.136	0.089	0.165

P = .0348 (Mann-Whitney test; this was chosen because the data failed in equal variance test).

Table 6 Comparison of Groups With and Without BMPs Concerning the Relationship of Connective Tissue and HA to Bovine Bone

Group	n	Mean	Standard deviation	Standard error
Without BMP	14	0.466	0.299	0.0799
With BMP	13	0.476	0.253	0.0701

P = .93 (Student *t* test).

Table 7 Comparison of Groups With and Without BMPs and Negative Control Concerning the Absolute Values of New Bone Formation

Group	n	Mean	Standard deviation	Standard error
Without BMP	14	450.1	220.1	58.8
With BMP	13	257.8	91.6	25.4
Negative control	6	243.2	210.8	86.0

P = .014 (1-way analysis of variance).

Table 8 Comparison of Groups With and Without BMPs Against a Negative Control Concerning the Absolute Values of New Bone Formation*

Group	Difference between the means	*t*	*P* < .05
Group without BMP × negative control	206.9	2.380	Yes
Group with BMP × negative control	14.7	0.167	No

*Bonferroni *t* test.

Histologic Observations

The samples without BMP frequently showed pronounced and well-defined bone formation (Fig 7a). The samples with BMP often resulted in weaker osteogenesis than those without BMP. In the BMP group, connective tissue formation was frequently seen (Fig 7b).

In the majority of the prototypes, the negative control presented minor osteogenesis because it was isolated from the phenomenon of osteoconduction derived from the tibia cortical bone (Fig 8a). In 1 of the prototypes of the negative control group, there was intense osteogenesis, even without migration of osteoblastic cells from the tibia cortical bone (Fig 8b).

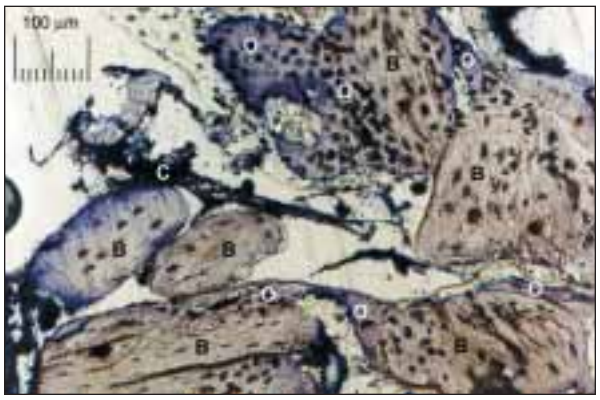


Fig 7a Group without BMP: Intense new bone (O) has formed around anorganic bovine bone particles (B). C = connective tissue.

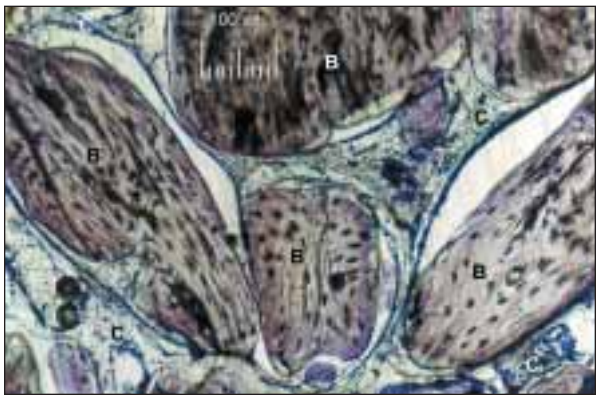


Fig 7b Group with BMP: Connective tissue (C) has formed around the anorganic bovine bone particles (B).

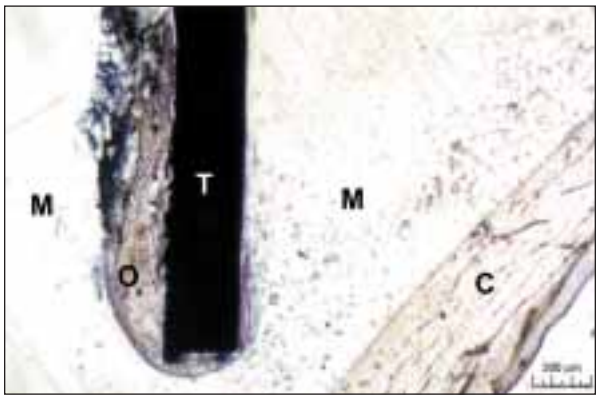
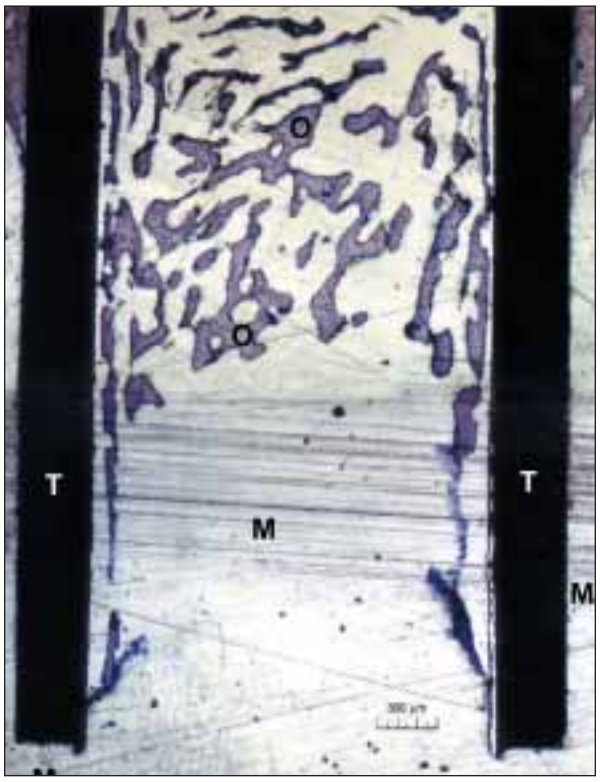


Fig 8a Negative control group (blood clot only). Typically, there is little new bone (O) formation in contact to titanium (T) surface without the migration of bone differentiated cells from the tibia's cortical bone (C). This picture shows that the bone marrow (M) contains cells with osteogenic potential.

Fig 8b In 1 of the prototypes of the negative control group, there was intense new bone (O) formation within the hollow portion of the titanium (T) cylinder. The new bone formation did not match the migration of differentiated cells from the tibial cortical bone; therefore, it was possible to prove the existence of cells with osteogenic potential within the bone marrow (M).



DISCUSSION

This work created a model that can be used to test the influence of biomaterials in contact with poor-quality bone. This animal model was designed considering the following details for objective accomplishment: *(1)* young adult animals were selected (4 months of age) because they have a great amount of mesenchymal stem cells and osteoprogenitor cells within the bone marrow (both cells are known to be the most responsive to BMP stimuli[14]); *(2)* 67 days elapsed before animal sacrifice, which, in the rabbit situation, is enough for new bone formation because the rabbit's sigma period (time wasted for a complete bone tissue turnover) is 6 weeks (42 days)[15]; and *(3)* the biomaterials were isolated from the osteoblastic cells derived from the tibia cortical bone. Such cells were unable to reach the test materials and produce bone by the osteoconduction phenomenon. In this model, the effects of biomaterials on the mesenchymal steam cells and osteoprogenitor cells from the rabbit tibia bone marrow were tested.

Many articles have reported positive effects concerning bone formation and repair in rabbits when

bovine extracted BMP (bBMP) was used.[14,16–19] Bovine extracted BMP also has proven its osteoinductive potential and bone repair properties in other animal species.[6,20–27] Many human clinical studies have also demonstrated improved osteogenic potential when bBMP are used bound to a myriad of carriers.[7–10]

In contrast to the above-mentioned work, the present investigation showed that a hydrophobic noncollagenous protein pool that contained BMPs impaired osteogenesis within the bone marrow. The samples without BMPs formed significantly more bone than the group with BMPs (Tables 3 to 5). The main difference between the present research and other investigations is the use of the whole hydrophobic noncollagenous protein extract, rather than purified or semi-purified BMP fractions.

The first hypothesis to explain the inhibitory phenomenon caused by the crude hydrophobic noncollagenous protein pool observed in the present trial is the existence of some proteins in this extract that could cause immunologic reactions sufficient to block the bone neoformation. Sampath and Reddi[28] observed that, in contrast to rat demineralized bone matrix, the bone matrix from humans, monkeys, and bovines did not show osteoinductive effects when implanted in rats' muscle pouches. But when the authors inactivated the xenogenic bone matrix with guanidine hydrochloride and reconstituted it with 50,000 Dalton or less hydrophobic noncollagenous protein fraction, the xenogenic bone matrices recovered their osteoinductive power. The authors suggested that the immunogenic components able to impair osteoinduction have molecular weights greater than 50,000 Dalton. The present work used the whole protein fraction; thus the protein with more than 50,000 Dalton may have interfered in the osteogenesis within the rabbit bone marrow.

Heckman and associates[29] showed that semipurified dog bone morphogenetic protein could repair dogs' fractures, in contrast to BMPs derived from bovines. Gao and coworkers[11] also verified that semipurified moose BMPs bound to HA evoke anti-moose BMP antibody production in sheep, and such an immune reaction results in the regeneration of poorer-quality bone than that caused by the use of HA without moose BMP. Nilsson and Urist[30] verified that bBMP can regenerate up to 96% of the original bone defect in critical-size defects in dog skulls. However, when bBMP was applied a second time, 3 weeks after the first application, only 34% of the original defects were regenerated due to humoral immune response. More strong evidence that immune events may impair osteoinduction resides in the fact that semipurified bBMP implanted in immunosuppressed rats' muscle pouches induces more bone formation than in normal (non-immunosuppressed) rats.[11]

The second hypothesis to explain the deleterious effects of the protein pool used here upon the osteogenesis (within the rabbits' bone marrow) is that this pool may contain osteogenic inhibitor proteins (OIPs).[31]

The third hypothesis is that the pool used in this research might have a low concentration of BMPs. Hollinger and coworkers[15] mentioned that low concentrations of BMPs may turn mesenchymal stem cells into fat cells instead of osteoblasts. Therefore the protein pool could increase the number of fat cells within the rabbits' bone marrow and, according to Benayahu and associates,[32] the fat cells exert inhibitory effects on alkaline phosphatase production by the osteoblastic cells.

The fourth hypothesis, and the least probable, is suggested by 2 studies of Jeppsson and colleagues,[33,34] who verified that recombinant human BMP-2 (rhBMP-2) and rhBMP-7 diminished osteogenesis within bone-harvesting chambers placed in rabbits. The authors speculated that rhBMPs could cause apoptotic events in this specific animal model, since rhBMPs increase osteogenesis in the mouse harvesting chamber model. However, the authors suggested that in addition to the species difference, the mice bone-harvesting chambers received rhBMPs at the time of their placement, and the rabbit bone-harvesting chambers received rhBMPs after their complete osseointegration. Such a difference renders the surgical trauma at the moment of the rhBMP insertion in the rabbit chambers negligible. The chemical mediators released by the surgical trauma might be important for the rhBMPs' osteogenic effects to occur. In the present study, the pool with BMPs was placed at the same time as the surgical trauma. However, Nilson and Urist[14] showed that semipurified bBMP inserted within rabbits' femur condyle bone marrow increased osteogenesis, which contradicts the idea of apoptosis caused by the protein pool.

One or more of the 4 presented hypotheses could be supporting the concept of the protein pool impairing osteogenesis within rabbit bone marrow. The protein pool should be tested in the same titanium prototype but in other animal species. The pool also should be well-characterized by high-performance liquid chromatography to ascertain which fractions might be impairing the osteogenesis or causing an immune response.

A positive aspect of the present study was the enhanced bone formation within the rabbits' bone

marrow when anorganic bovine bone mixed with absorbable HA without BMP was used, in comparison to the negative control (blood clot only). Moreover, the anorganic bovine bone proved to bind closely to the newly formed bone.

Rickard and associates[35] stated that the bone marrow stroma has special pluripotent cells, which are able to differentiate spontaneously in osteoblasts when cultivated in vitro. These cells with osteogenic potential present within the bone marrow would not have been expressing the osteoblast phenotype because, according to Benayahu and coworkers,[32] the adipocytes from the bone marrow exert an inhibitory effect on alkaline phosphatase expression by the osteogenic cells.

It is known that the chemical composition and surface characteristics of a substratum influence many steps of cellular biology (attachment, migration, differentiation, and matrix synthesis).[2] The anorganic bovine bone creates an excellent attachment substratum for the bone marrow's cells with osteogenic potential because, according to Spector,[36] this kind of biomaterial has a mineral surface identical to the surface of bone (because of the production process, which preserves the bone mineral microstructure). Once the cells with osteogenic potential attach to the anorganic bovine bone surface, such cells have potential to proliferate, to undergo terminal morphologic maturation into osteoblasts, and to produce bone matrix. The anorganic bovine bone without the protein pool seems to create ideal conditions for the osteoprogenitor cells to migrate (osteoconduction properties) and to mature (bone matrix synthesis).

According to Hollinger and coworkers,[15] cellular attachment induces changes in the cellular cytoskeleton that result in the exposition of receptors for BMPs and growth factors. These receptors have a fundamental role in the differentiation of cells with osteogenic potential, since such cells are exposed to the effects of latent BMPs and growth factors released by the surgical trauma and the repair process.[15,37] After the first incoming cells attach to the anorganic bovine bone surface, they differentiate toward osteoblastic cells and start to synthesize bone matrix and soluble factors capable of enhancing osteogenesis by autocrine and paracrine stimuli.

Another positive characteristic of anorganic bovine bone is that, as true bone mineral, it has mechanical properties that resemble living bone.[36] This fact favors new bone staying in close contact with the remaining anorganic bovine bone.

Another surprising aspect concerning the animal model used in this investigation was that, in spite of the isolation of the migration of differentiated bone cells from the tibia cortical bone, in some specimens, the negative control (blood clot only) formed well-differentiated new bone in contact with the acid-etched titanium surface. This fact corroborated the existence of cells with osteogenic potential within the rabbit bone marrow stroma, since the cells, once anchored to the titanium surface, started to synthesize bone matrix. The new experimental model was shown to be adequate for testing biomaterials or different titanium surface treatments in poor-quality bone (rich in bone marrow).

This research showed that anorganic bovine bone seems to improve poor-quality bone by serving as a good attachment substratum for cells with osteogenic potential, and pointed out that crude BMP extracts should be carefully investigated for potential adverse effects. It is also advisable to immunologically test all BMP extracts in various animal models before human use.

SUMMARY

This investigation demonstrated that anorganic bovine bone seems to improve poor-quality bone by serving as a good attachment substratum for cells with osteogenic potential, and suggested that crude BMP extracts should be carefully investigated for potential adverse effects. It would also seem advisable to immunologically test all BMP extracts in various animal models before human use.

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