Evaluation of Autogenous Bone Grafts, Particulate or Collected During Osteotomy with Implant Burs: Histologic and Histomorphometric Analysis in Rabbits

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Purpose: *The aim of this study was to evaluate bone regeneration in bone cavities filled with particulate autogenous bone either harvest in blocks and subjected to milling procedures or collected during osteotomy with implant burs.* ***Materials and Methods:*** *In 12 rabbits, 3 noncritical unicortical cavities 7 mm in diameter were prepared with a trephine drill on the right tibia. The cavities were filled respectively with particulate autogenous bone achieved with a manual bone crusher (particulate group), with particulate autogenous bone obtained using bone collector during osteotomy (collected group), and with blood clot (control group). Animals were sacrificed at 7, 15, and 30 days after surgery (4 animals for each time period). The sections were examined by histologic and histomorphometric analysis.* ***Results****: At 7 days, the samples were filled by coagulum, and bone particles were observed only in the collected (24%) and particulate groups (44.75%). At 15 days, there was connective differentiation in all groups, with presence of grafted bone particles and onset of newly formed bone in the collected (38.88%) and particulate groups (46.0%). At 30 days, there was bone fill (immature trabecular bone) of the cavities in the control (50%), collected (64.63%) and particulate groups (66%).* ***Conclusion:*** *No significant difference was demonstrated between noncritical unicortical bone defects in rabbit tibiae filled with particulate bone harvested as a block and subjected to milling and those filled with bone collected during osteotomy with implant drills when the defects were observed up to 30 days following their creation.* Int J Oral Maxillofac Implants 2007;22:201–207

Key words: *bone regeneration, bone remodeling, bone transplantation, particle size, revascularization*

Because of its biocompatibility and biological properties, autogenous bone is considered the gold-standard graft material for the reconstruction of bone defects. In addition to its osteogenic and osteoinductive potential, it does not trigger immunologic response, and it is readily resorbed by osteoclasts.[1] It may be used in blocks or as particulate bone; the latter may be used in isolation or associated with allogenic and alloplastic materials.[2]

Autogenous particulate bone grafts are indicated to cover peri-implant[3,4] and periodontal fenestrations [5,6] for small alveolar reconstructions,[7] to fill spaces between block bone grafts, and to augment the maxillary sinus. [8] The main limitation of particulate graft material is its immediate instability; it requires protection by biological barriers or the presence of bone walls.[7] Bone graft particles hypothetically present osteoinductive activity and act as centers of ossification in the surrounding mineralized matrix, promoting new bone formation.[9,10] Particulate bone may be created by crushing autogenous bone blocks in manual or electric mills or by using bone scrapers and collectors.

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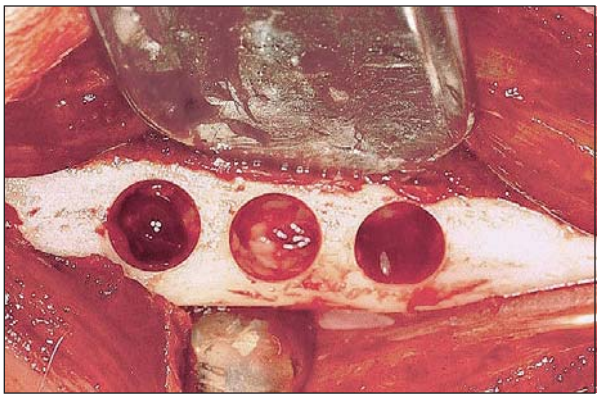

Fig 1 Experimental cavities filled with particulate autogenous bone, blood clot, and collected bone.

A simple and conservative method for achievement of bone particles comprises collection during preparation of bone sites for placement of osseointegrated implants. Graft material should be collected only from the surgical site with an aspiration protocol to avoid microbial contamination from buccal fluids.[11]

This material is a mixture of medullary and cortical bone and cellular components. It may be used directly under the periosteum or may be covered with biologic barriers.[3] Despite its routine utilization in implant dentistry, little is known on the osteogenic ability of these particles for the repair of bone defects.

The aim of this study was to evaluate the bone regeneration in bone cavities filled with particulate or collected autogenous bone obtained during osteotomy with implant burs.

MATERIALS AND METHODS

The study protocol was approved by the Animal Studies Review Committee of Araçatuba Dental School and Veterinary Medicine School (UNESP) and was conducted according to institutional guidelines (UNESP) and the standards of government authorities for the care and protection of animals.

Twelve adult male New Zealand rabbits, weighing 3.1 to 4.3 kg each, were used as experimental animals. Preanesthesia was performed with intramuscular injection of acepromazine (Acepran 0.2%, 0.12 mL/kg; Univet, São Paulo, Brazil) and ketamine hydrochloride (0.12 mL/kg). After 20 minutes, general anesthesia was induced with intravenous injection of sodium pentobarbital (Hypnol 3%, 0.6 ml/kg, Fontolever, Itapira, Brazil) on the right ear.

The anterior region of right tibia was shaved and submitted to antisepsis with polyvinylpyrrolidone-iodine (PVP-I 10%). An incision nearly 6 cm long was made to expose the internal side of the tibia in the diaphysis-proximal metaphysis. After cutaneous-periosteal detachment, 3 unicortical cavities 7 mm in diameter were created 2 mm from each other with a trephine drill (TRE 06; 3i; Palm Beach Gardens, FL), mounted in a dental handpiece (DU 300; 3i) at a speed of 1,700 rpm, continuously irrigated with sterile saline.

Each cavity was filled with a different material. Thus, there were 3 groups: the particulate group, the collected group, and the control group (Fig 1). The cavities in the particulate group were filled with particulate autogenous bone achieved by crushing the cortical bone removed with the trephine drill during preparation of the experimental cavities with aid of a manual bone crusher (Neodent, Curitiba, Brazil). The cavities in the collected group were filled with autogenous bone particles achieved with a bone collector (Neodent) connected to the blood-suction unit during osteotomy. The sequence of burs used consisted of a round 2-mm bur, a pilot bur, and a 3-mm bur (Neodent). The control group was filled only with blood clot. The soft tissue was repositioned and sutured at the muscular and cutaneous planes with nylon suture 5.0 (Superlon; Cirurmédica, São Paulo, Brazil).

For postoperative pain relief, an intramuscular injection of sodium dipyrone (1 mg/kg; Ariston Industrias Químicas e Farmacêuticas, São Paulo, Brazil) was given during the first 24 hours. Gentamicin sulfate (Garamicina, 3 mg/kg/d; Schering-Plough, Kenilworth, NJ) was administered for 5 days. Animals were sacrificed at 7, 15, and 30 days after surgery (4 animals per time period) by intravenous injection of sodium pentobarbital (Hypnol 3%, 1.2 mL/kg). The operated tibiae were dissected from soft tissues and fixed in 10% formalin for 24 hours. The specimens were demineralized and dehydrated with 18% EDTA solution (50 g EDTA and 6 g of sodium hydroxide diluted in 250 mL of distilled water) for 60 days and embedded in paraffin for the creation of semiserial longitudinal sections 6 μm thick. The sections were stained with Masson trichrome for microscopic and histomorphometric analysis.

Qualitative and quantitative analyses were performed under a light microscope. Images were acquired with the aid of a digital camera (JVC TK-1270 Color Video Camera, Germany) connected to a binocular light microscope (Carl Zeiss, Oberkochen, Germany). The microscope and camera were connected to a computer, and the images were projected onto a monitor screen (SyncMaster 3Ne, 15 inches; Samsung, Kyonggi, South Korea). Quantitative analysis was performed with utilization of the Merz

Table 1 No. of Points (of 100) Where Bone Tissue Was Observed

	Control		Particulate		Collected	
Animals	Border	Center	Border	Center	Border	Center
7 days						
1	0	0	38	31	25	18
2	0	0	52	48	27	25
3	0	0	65	45	34	26
4	0	0	45	34	23	14
15 days						
5	0	0	45	45	33	23
6	0	0	47	42	46	37
7	0	0	57	29	50	43
8	0	0	61	42	73	6
30 days						
9	60	41	76	45	61	61
10	45	43	65	60	67	65
11	54	43	85	56	73	62
12	63	51	72	69	64	64

grid,[12] with 100 equidistant points 10 mm apart. This grid was superimposed on the histologic images (160× magnification), comprising only the cortical portion of the experimental defects. The grid was superimposed on sections from the peripheral and central regions of each cavity, for a total of 200 points per cavity (Table 1).

Analysis of variance was performed, with due regard to pairing. A standard Student *t* test was performed for effects of interest. The level of significance used was 5%.

RESULTS

Histologic Results

At 7 days, in the control group, blood clot filled the entire experimental bone defect (Fig 2). In the particulate group there were autogenous bone particles of variable size, surrounded by a blood clot that filled the entire cavity (Fig 3). In the collected group there were groups of small autogenous bone particles, surrounded by blood clot, with absence of new bone formation close to the graft (Fig 4).

At 15 days, the bone defects in the control group were entirely filled by connective tissue and presented some organization (Fig 5). The particulate group exhibited mild resorption of the bone fragments and onset of new bone formation between the grafted bone particles and from the cavity walls; osteogenic cells surrounded the graft (Fig 6). In the collected group there was onset of connective maturation. Several blood vessels were observed as well as agglomerated and isolated autogenous blood particles surrounded by osteogenic cells (Fig 7).

At 30 days, the control group presented new formation of immature bone trabeculae from the cavity walls, with the presence of connective tissue and blood vessels toward the center of the cavity (Fig 8) and remarkable osteogenic activity close to the newly formed bone (Fig 9). In the particulate group, the experimental bone cavity was almost entirely filled with newly formed bone in final remineralization, and small nonresorbed particles of autogenous bone could be observed (Fig 10). There was presence of osteogenic activity within the newly formed bone; osteoprogenitor cells and osteogenesis were observed (Fig 11). In the collected group, the experimental cavities exhibited immature bone trabeculae with large medullary spaces (Fig 12). Thicker trabeculae and smaller medullary spaces were observed close to the bone walls (Fig 13).

Histomorphometric Results

The amount of bone tissue in the control, particulate, and collected groups at 7, 15, and 30 days is pre-

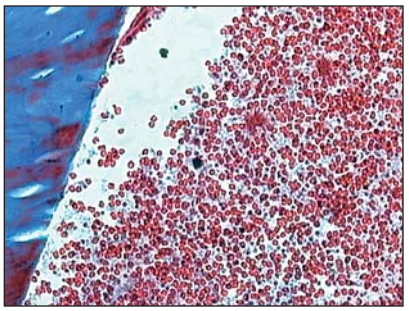

Fig 2 Control group at 7 days. Aspect of osteotomy close to the bone wall. Blood clot and lympho-plasma cells can be observed (Masson trichrome; original magnification ×250).

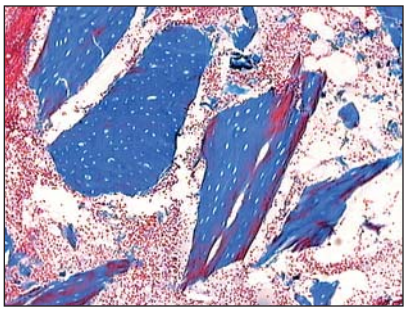

Fig 3 Particulate group at 7 days. Autogenous bone particles surrounded by blood clot (Masson trichrome; original magnification ×160).

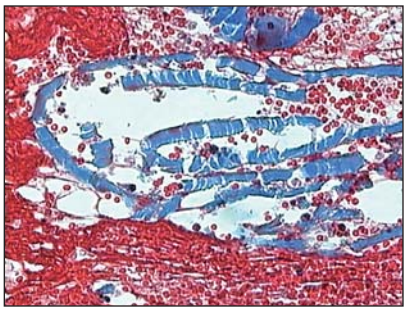

Fig 4 Collected group at 7 days. Central region of the experimental cavity, revealing small fragments of collected bone graft surrounded by blood clot (Masson trichrome; original magnification ×250).

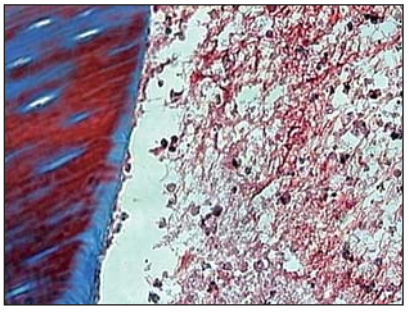

Fig 5 Control group at 15 days. Bone wall of the cavity with connective tissue (Masson trichrome; original magnification ×250).

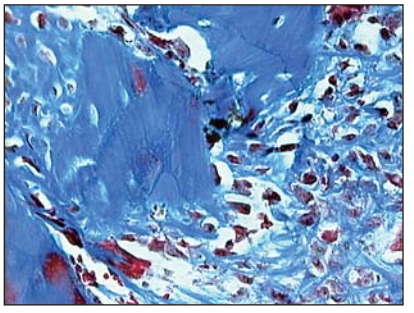

Fig 6 Particulate group at 15 days. Fragment of grafted bone surrounded by a large number of osteogenic cells (Masson trichrome; original magnification ×250).

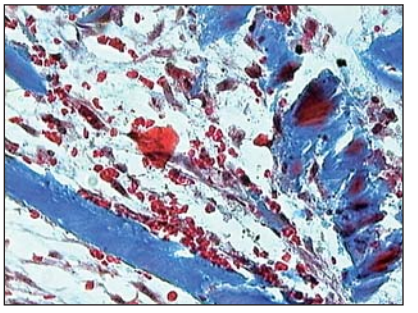

Fig 7 Collected group at 15 days. Small fragments of grafted bone surrounded by osteogenic cells (Masson trichrome; original magnification ×250).

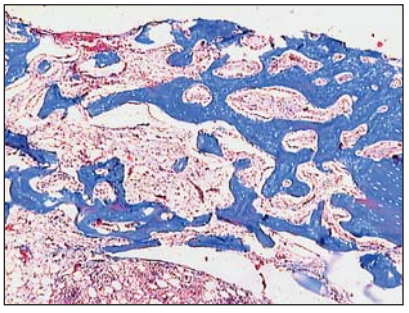

Fig 8 Control group at 30 days. Immature bone trabeculae with large marrow spaces at the bone wall and presence of connective tissue at the central region of the cavity (Masson trichrome; original magnification ×63).

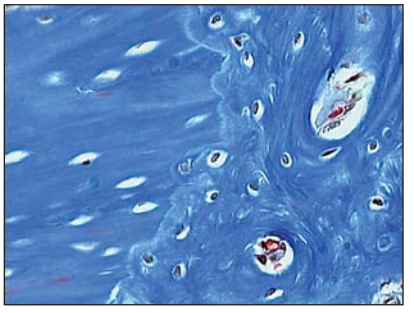

Fig 9 Control group at 30 days. Close contact between the newly formed bone and the bone defect wall, with immature bone trabeculae (Masson trichrome; original magnification ×250).

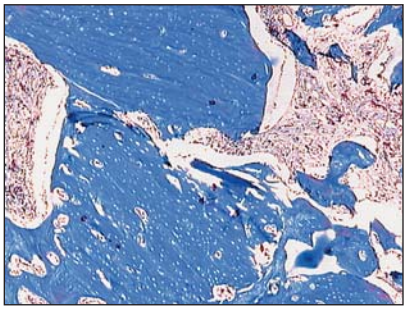

Fig 10 Particulate group at 30 days. Nonresorbed grafted autogenous bone particle surrounded by immature bone trabeculae (Masson trichrome; original magnification ×160).

sented in Fig 14. At 7 days, all groups were filled with blood clot; autogenous bone particles were present in the particulate (44.75%) and collected groups (24%). At 15 days, all groups exhibited connective tissue. Bone trabeculae were not observed in the control group (0%). Autogenous bone particles with newly formed bone trabeculae were observed in the particulate group (46%), and more intensive osteogenic activity, with some bone particles and newly formed bone, was observed in the collected group (38.88%). At 30 days, the control-group cavities were filled with connective tissue and newly formed bone trabeculae (50%); particulate-group cavities with connective tissue and bone trabeculae (66%); and collected-group cavities with connective tissue and newly formed bone trabeculae (64.63%).

Means, standard deviations, and *P* values are presented in Table 2.

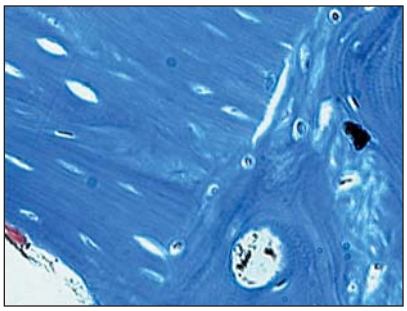

Fig 11 Particulate group at 30 days. Close contact between the newly formed bone and the bone defect wall, with immature bone trabeculae (Masson trichrome; original magnification ×250).

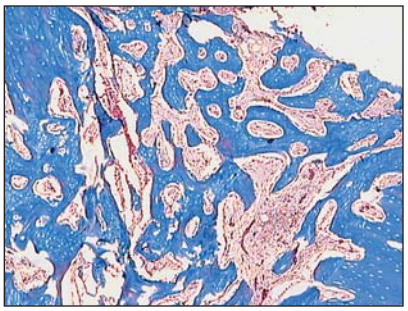

Fig 12 Collected group at 30 days. Central region of the cavity filled with newly formed bone tissue, with large marrow spaces and connective tissue (Masson trichrome; original magnification ×63).

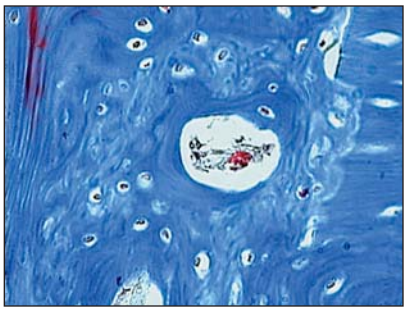

Fig 13 Collected group at 30 days. Close contact between newly formed bone and the bone defect wall, with immature bone trabeculae (Masson trichrome; original magnification ×250).

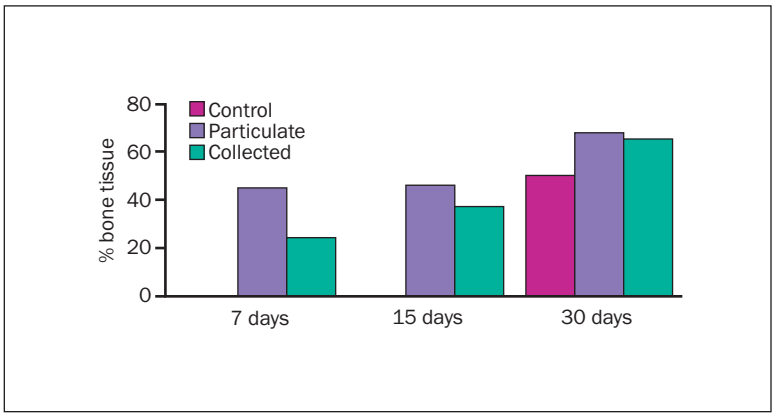

Fig 14 Percentage of bone tissue in each group at each time point based on the 100-point grid. The control group exhibited 0% bone tissue at 7 and 15 days.

Table 2 Mean Amount of Bone Tissue, Standard Deviation, and *P* Values

Compared groups	Mean	SD	*P*
7 days			
Control	0	0	.002
Particulate	44.7	9.40	
Control	0	0	.002
Collected	24.0	5.05	
Particulate	44.7	9.40	.005
Collected	24.0	5.05	
15 days			
Control	0	0	< .001
Particulate	46.0	3.76	
Control	0	0	.002
Collected	38.8	7.82	
Particulate	46.0	3.76	.217
Collected	38.8	7.82	
30 days			
Control	50.0	5.40	.009
Particulate	66.0	5.26	
Control	50.0	5.40	.025
Collected	64.6	2.81	
Particulate	66.0	5.26	.570
Collected	64.6	2.81	

Significant difference if $P < .05$ (Student *t* test; paired samples test).

DISCUSSION

This study evaluated 2 types of autogenous particulate bone at the initial periods of bone repair. The use of a mixture of cortical and cancellous bone (the collected sample) was compared with the use of primarily cortical bone (the particulate sample).

The samples were analyzed at 3 time points during resorption, replacement and new bone formation, and the parameters measured between the grafted groups and the control group could be compared and interpreted.

Particulate autogenous bone has cells with osteoinductive ability, which act as centers of ossification in the mineralized matrix.[9,10] When implanted, it is gradually resorbed and induces new bone formation. The size of the particles is fundamental for the occurrence of resorption.[13] Small particles and cancellous bone have resorbed faster[14] and may promote differentiation of a higher number of osteogenic cells at the area.[8]

Cortical bone particles of different shapes and sizes may be obtained with different types of bone mills (manual or electric) and scrapers. Bone collectors connected to a suction unit may be used to derive bone particles from cortical and cancellous bone during preparation of cavities for implants or during osteotomy with rotary instruments. It is a convenient method of obtaining autogenous bone without the need of a donor surgical site.[15]

Mailhot and Borke[16] conducted an in-vitro investigation in which they isolated and characterized the osteoblastic cells of human bone particles collected during osteotomy with implant burs and determined the ability of these cells to promote new bone formation. These particles should be obtained in ideal conditions, under thorough cooling, with sharp burs at low speed.[15,17] Bone harvest requires continuous irrigation with saline solution for cooling to avoid excessive heating of the bone tissue,[18,19] bone necrosis, and delayed repair.[20,21]

When manual bone crushers are used to achieve particulate bone, particles measuring nearly 1 mm in diameter are obtained[22]—larger particles than those collected from osteotomies with implant burs.[9] In the present study, the processes of resorption and osteoinduction occurred more rapidly in cavities in the collected group compared to those in the particulate group because these cavities were filled with cancellous bone mixed with smaller particles of cortical bone.

Pallesen et al[23] evaluated the initial stage of bone regeneration of bone defects in the skulls of rabbits. In their study, the defects were filled with autogenous bone particles obtained with an electric mill and autogenous bone fragments measuring 10 mm.[3] At 2 and 4 weeks, new formation of more mature and thicker bone trabeculae was observed in defects grafted with smaller particles. There was also more intensive resorption and osteogenesis in this group after 4 weeks, which corroborates the present findings. Isaksson and Alberius[24] reported more intensive new bone formation in bone defects 5 mm in diameter filled with particles of 0.5 to 1 mm^3 compared to bone microparticles after an initial period of 4 weeks. However, after 15 weeks, there were no significant differences between groups.

In the present study, osteoinductive activity occurred earlier and was more intensive in the collected group compared to the particulate group. These results are probably due to faster osteoclastic activity of the smaller cortical particles and cancellous bone, which may have released a larger amount of differentiation and bone growth factors. However, there was maintenance of a larger amount of bone graft particles in the particulate group compared to the collected group, probably because bone particles were larger and more stable inside the cavities, where they may have acted as a support for osteoinduction.

The more intensive bone regeneration observed in the grafted groups probably occurred due to the support provided by the larger bone particles in the particulate group and by the earlier bone resorption and induction of bone microparticles in the collected group.

CONCLUSION

The results of this animal study demonstrate no significant difference between noncritical unicortical bone defects in rabbit tibiae filled with particulate bone harvested as a block and subjected to milling and those filled with bone collected during osteotomy with implant drills when the defects were observed up to 30 days following their creation.

ACKNOWLEDGMENT

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