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Tooth eruption through autogenous and xenogenous bone transplants: a histological and radiographic evaluation in beagle dogs

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SUMMARY. **The effect of implanting autogenous and xenogenous (Bio-Oss) bone transplants into metabolically active sites within beagle dog mandibles during permanent premolar tooth eruption was examined. Ten 14-week-old beagles were used. Before commencing the radiographic experiments, metal bone markers were placed in the caudal margin of the mandible at the age of 10 weeks. The deciduous first and third molar teeth were extracted and their sockets over the permanent second and fourth premolars were implanted with autogenous particulate enchondral iliac crest bone, autogenous particulate membraneous mandibular body bone, xenogenous bovine anorganic bone mineral spongiosa granules (1–2 mm^3) (Bio-Oss, Geistlich Pharma, Switzerland) or left empty. The third premolar served as a control site. Standardized oblique lateral radiographs were taken once a week. A number of coordinates of defined points and structures were determined by means of a coordinate digitizing system. Animals were killed 4, 10 and 16 weeks after bone transplantation for histological examination of the transplantation sites. All premolars showed no delay in eruption or disruption of crown and root development. On histology, the Bio-Oss particles were not resorbed or integrated in the alveolar bone but were pushed forward into the gingiva. We have demonstrated that there is no difference in the eruption curve of the permanent premolars in the four groups (ANOVA $P>0.5$) and that bone transplantation has no inhibitory effect on eruption (ANOVA $P>0.3$) and crown development of the underlying permanent premolar but that Bio-Oss does not have the same resorbable or integrating capability as autogenous bone grafts.**

INTRODUCTION

Secondary bone grafting of the residual alveolar cleft in patients with cleft lip and palate has become a well established procedure with well accepted advantages (*Freihofer* et al., 1993; *Kalaaji* et al., 1996). One of these advantages is to promote the eruption of the permanent canine into the alveolar defect (*Witsenburg* and *Remmelink*, 1993). Therefore, the bone grafting has to be performed prior to permanent canine eruption, at the stage in which this tooth has formed about one-half to two-thirds of its root. Usually, free autogenous bone from the anterior iliac crest, a rib or the chin is used (*Witsenburg* and *Remmelink*, 1993). The age of the patient by that time varies between 8 and 12 years. The bone graft stabilizes the dento-osseous segments, improves alveolar continuity, decreases tooth loss due to periodontal disease, increases alar cartilage support and allows any unerupted teeth in the area to erupt through osseous tissue (*Witsenburg* and *Remmelink*, 1993). These grafts can be autografts, allografts or xenografts. Different sources of autografts for closing palatal defects are described: chin, rib, calvarium and ilium. Chin bone is reported to give the best results (*Freihofer* et al., 1993). Advantages of the chin as a donor site are reduction in operation time, less morbidity, decreased admission time in hospital and an invisible scar (*Hoppenreijs* et al., 1992). Also, the manner of healing is in favour of the use of chin bone: intramembraneous bone, i.e. ectomesenchymal bone, maintains more of its volume (*Smith* and *Abramson*, 1974) because of less postoperative resorption (*Zins* and *Whithaker*, 1983; *Koole* et al., 1989). This is probably due to earlier vascularization of the transplant (*Kusiak* et al., 1985) in comparison with enchondral, i.e. mesenchymal, bone transplants.

Bone grafting also has disadvantages, such as the need for surgery at another site, extension of surgery time, increased possibility of infection and other complications (*Hoppereijs* et al., 1992; *Sugimot* et al., 1993). A special disadvantage of the chin as the donor site is its small volume: if the cusps of the mandibular permanent canines are not yet erupted, the space to take an explant is very small (*Bähr* and *Coulon*, 1996). Damage to these teeth is an unacceptable complication. So, if there is a chance of damaging the toothbuds, autografts taken from rib, ilium or calvarium are indicated (*Borstlap* et al., 1990; *Witsenburg* and *Freihofer*, 1990; *Cohen* et al., 1991; *Koole* et al., 1991; *Kortebein* et al., 1991).

Apart from autografts, xenografts taken from different species are also advocated, but they do not have extensive clinical application in palatal surgery. The use of xenografts has the potential to reduce morbidity, as the harvesting of autogenous bone is

unnecessary. The antigenic potential of xenografts can be diminished or eliminated by chemical treatment. Bio-Oss (Geistlich-Pharma, Wolhusen, Switzerland) is a natural material consisting of anorganic bovine bone matrix containing carbonate apatite. Electron microscopic examinations have shown that its macro- and microconfigurations closely parallel the structure of human bone. Bio-Oss, which is commercially available in both spongiosa and cortical blocks, has been completely deorganified by means of a proprietary extraction process that renders it free from antigenicity. This permits its implantation as a matrix that appears to stimulate all normal physiological responses closely, mimicking the stages of bone repair (*Klinge* et al., 1992). Previous clinical reports have demonstrated its efficacy in oral and maxillofacial surgery (*Boyne*, 1990; *Hislop* et al., 1993; *Wetzel* et al., 1995).

Before using xenogenous bone transplants in alveolar clefts, more has to be known about their effect on tooth eruption. Therefore, their effects on the eruption of permanent premolars were studied in beagle dogs after extraction of the deciduous predecessors. The extraction wounds were filled with different autogenous and xenogenous bone grafts, and the subsequent tooth eruption was studied by radiographic and histological techniques.

MATERIAL AND METHODS

Surgical procedures

The experiment was performed on 10 14-week-old female beagle dogs. They were housed in light- and temperature-controlled rooms and access to food and water was allowed ad libitum. Four experimental treatments (mandibular bone graft, iliac bone graft, Bio-Oss, sham) were carried out in the extraction sockets of the mandibular m1 and m3, the predecessors of P2 and P4. Treatment modalities were determined by random permutation in each dog. The eruption of the P3 served as the control.

Prior to the surgical procedures, the animals were premedicated with 0.5 ml Thalamonal (fentanyl 0.05 mg/ml plus droperidol 2.5 mg/ml; Janssen Pharmaceutica, Beerse, Belgium) and 0.5 ml atropine (atropine sulphate 0.5 mg/ml). Subsequently, they were anaesthetized with an intravenous injection of 30 mg/kg Narcovet (sodium pentobarbital 60 mg/ml; Apharmo, Arnhem, The Netherlands). After intubation, anaesthesia was maintained with Ethrane (enflurane 15 mg/ml; Abott, Amstelveen, The Netherlands). The oral mucosa and the dentition were cleaned with chlorhexidine digluconate 0.1% in water. In addition, approximately 6 ml Xylocaine (lidocaine hydrochloride 0.4 mg/ml; Astra Chemicals, Rijswijk, The Netherlands) were injected into the vestibular mucosa to avoid excessive bleeding during surgery. After routine aseptic measures, the anterior iliac crest was exposed by means of a 2–3 cm incision through the skin, subcutaneous tissue and periosteum. With a dental bur, a 0.5–1 cm^3 bone graft was harvested. A similar procedure was used to harvest a bone graft from the angle of the mandible. The bone grafts were placed in isotonic saline and finally cut into small chips of up to about 10 mm^3 with a bone grinder.

The m1 and m3, the predecessors of P2 and P4, of the right and left mandible were extracted. The extraction sockets were filled with either autogenous iliac crest bone chips, autogenous mandibular bone chips, bovine anorganic spongiosa granules (1–2 mm^3; Bio-Oss, Geistlich Pharma, Switzerland) or left empty. The mucosal defects were closed primarily by a mucosal flap and closed with a resorbable suture (Vicryl 3-0, Ethicon, Norderstedt, Germany). The m2 was left in place to evaluate the normal eruption pattern of its successor, the P3.

All animals were medicated prior to surgery with 1 ml of Albipen 15% (ampicillin anhydrate 150 mg/ml; Mycopharm, de Bilt, The Netherlands) and maintenance doses of 1 ml Albipen LA (ampicillin anhydrate 100 mg/ml; Mycopharm, de Bilt, The Netherlands) on days 2 and 4 postoperatively. All animals received a normal diet after surgery.

Radiographic procedures

Before the start of the study, under general anaesthesia, tantalum implants (Ole Dich, Hvidovre, Denmark), measuring 1.2 mm in length and 0.5 mm in width were inserted as bone markers in the lower margin of the mandible, deep to the m2, the m3 and as far distally as possible (*Björk*, 1955). This was done 4 weeks before the extraction of the m1 and m3, at the age of 10 weeks (Fig. 1). During that period, every week, standardized oblique-lateral radiographs of the right and left halves of the mandible of each dog were taken under general anaesthesia, to follow the normal eruptive movements. To that end, the animals were fixed in a specially constructed cephalostat (*Maltha*, 1982) with two ear rods and with a pin in the mid-sagittal

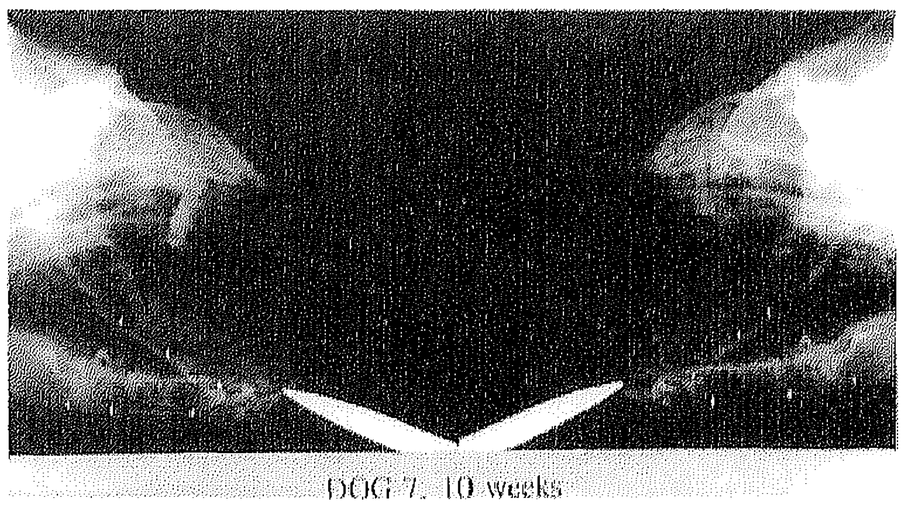

Fig. 1 – Radiograph of the mandible of dog 7, at the age of 10 weeks, after insertion of the tantalum bone markers.

plane. For the radiographs, Kodak X-ray speed photo (Kodak Nederland BV, Driebergen-Rijsenburg, The Netherlands) was used. Radiographs were taken with a Philips Practic X-ray Machine (Philips Nederland, The Hague, The Netherlands), set at 20 mA and 90 kV, the focus–film distance being 3.0 m, the object–film distance 7 cm and the exposure time 4 s. If a radiograph showed that a bone implant had become loose, a new one was inserted immediately, and the radiographic procedure was repeated.

Radiographic analysis

The definition of tooth eruption used in this study has been restricted to the vertical movement because only small mesio-distal movements were observed. Therefore the changes in the x-coordinates of the tips of the teeth during the eruption were all omitted.

For each of the radiographs, the points of the three metal bone markers and the tips of the decidous m1, m2, m3, P2, P3 and P4 were digitized (Fig. 2). The coordinates of these points were digitized with an electronic measuring table (*van der Linden* et al., 1972) with an accuracy of 0.01 mm. Due to the stable metal bone markers, the x-axis was defined as a straight line through the most distant bone markers, and the y-axis as a straight line perpendicular to the x-axis with the origin situated at a defined distance from the most proximal bone marker. The coordinate system was chosen in such a way that all the coordinates in the mandible were situated in the first quadrant, so they all had positive values (*Maltha*, 1982). To describe the eruption of the premolars, the y-coordinates of each measurement were plotted against the age of the animals.

Statistical analysis

The developmental stages for the P3 and the P4 are attained almost simultaneously (*Maltha*, 1982). Therefore the data from P3 could function as a control for the eruption of P4. The eruption of the P2 had no controlled data. The total vertical tooth displacement in the experimental, sham and control groups in the period from 18–23 weeks is compared, using a one-way analysis of variance (ANOVA). All recordings were performed in duplo by the first author, resulting in a measurement error of 0.007 mm for the bone markers and 0.11 mm for the tips of the teeth. Finally, the mean of both recordings was used, reducing the measurement error by $\sqrt{2}$.

Fig. 2 – Tracing of the mandible of dog 7, at the age of 10 weeks, with points digitized with an electronic measuring table.

Histological evaluation

For the histological study, the animals were killed: 1 animal at 4 weeks, 2 animals at 10 weeks, and 6 animals at 16 weeks after surgery. One animal had died during the surgical intervention at the beginning of the experiment. The animals were anaesthetized using 30 mg/kg Narcovet, after which 0.5 mg/kg heparin (Thromboliquine, Organon Tetnika, Boxtel, The Netherlands) was administered. After taking the final radiographs, a lethal dose of Narcovet was injected intravenously. The thorax of each animal was opened and the vascular system was perfused via the arch of the aorta with physiological saline followed by 4% neutral formaldehyde as a fixative. After perfusion the mandibles were dissected and immersed in 4% neutral formaldehyde, decalcified in 20% formic acid and 5% sodium citrate, dehydrated and embedded in Paraplast (Sherwood Medical, St Louis, MO) (*Wijdeveld* et al., 1991). Serial mesiodistal sections were cut at 7 μm and stained with haematoxylin and eosin.

RESULTS

Radiology

The eruption curves of P2, P3 and P4 in all experimental groups are comparable. In the first instance, the premolar moves slowly in an occlusal direction. At a certain age, the rate of movement increases, finally reaching a stable plateau after an abrupt slowing down of the vertical movement.

Test sites

Two weeks after transplantation (age 16 weeks), the permanent P3 and P4 started to erupt through the bone grafts, and they were seen intraorally at 21 weeks of age, without dysplasia or resorption of the crown (*Sugimot* et al., 1993) and without any difference between P3 and P4 (ANOVA, $P>0.3$). The mean vertical eruption from 18–23 weeks of age showed no difference between the four experimental groups (ANOVA, $P>0.3$) (Table). The mean vertical eruption in the sham group seemed to start 1 week earlier than in the other groups, without any statistical significance (Fig. 3).

The eruption curve of the experimental P2 is not as uniform as that of the P4, probably due to mesio-distal and linguo-buccal movements (Fig. 4). Although the vertical movement of P2 in the period 18–23 weeks is slower than P3–P4 (ANOVA, $P<0.001$), the eruption rate shows no significant difference between the four experimental groups (ANOVA, $P>0.3$).

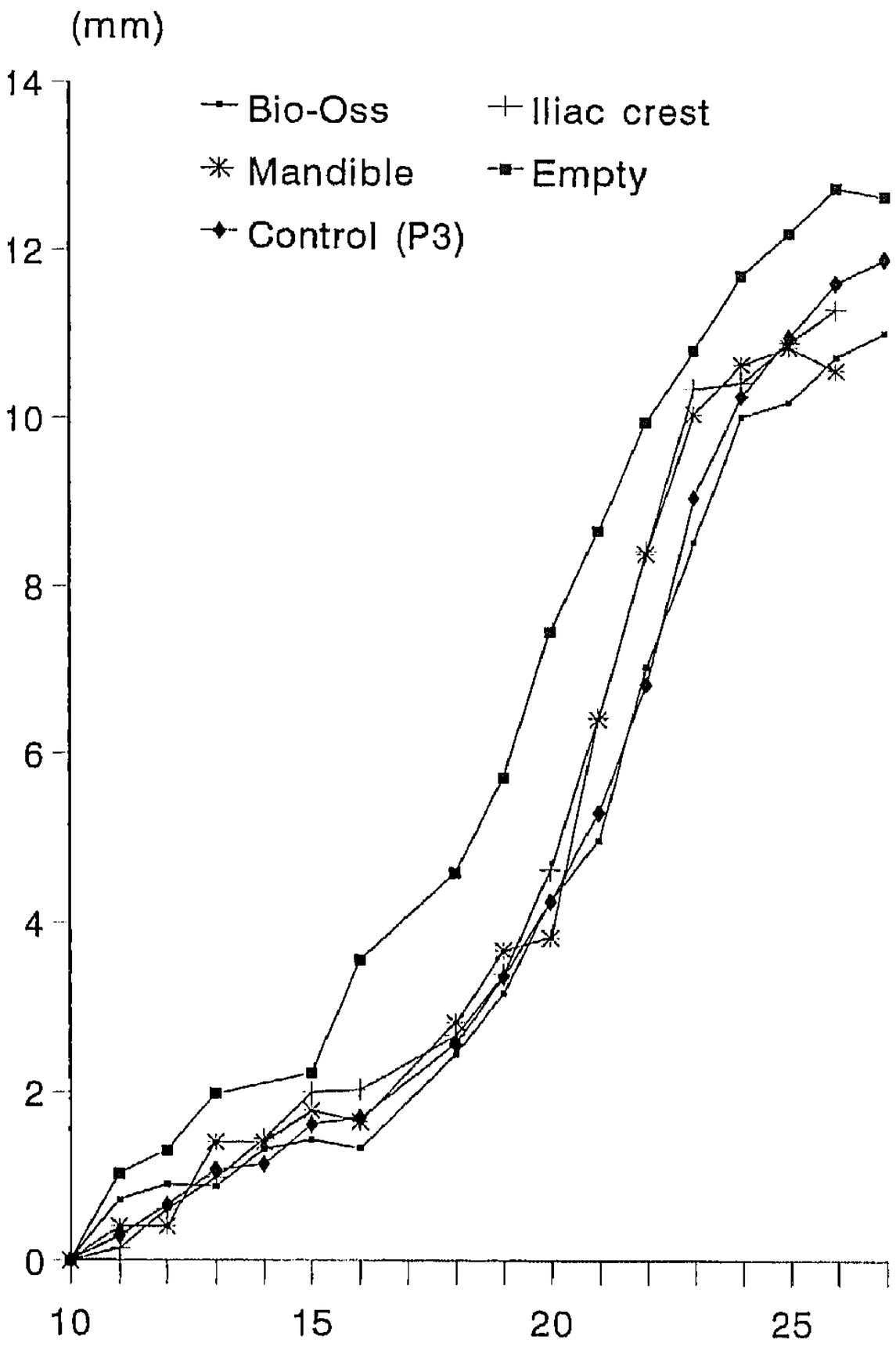

Fig. 3 – Mean relative vertical tooth displacement of P4 (experimental and sham groups) and P3 (control group).

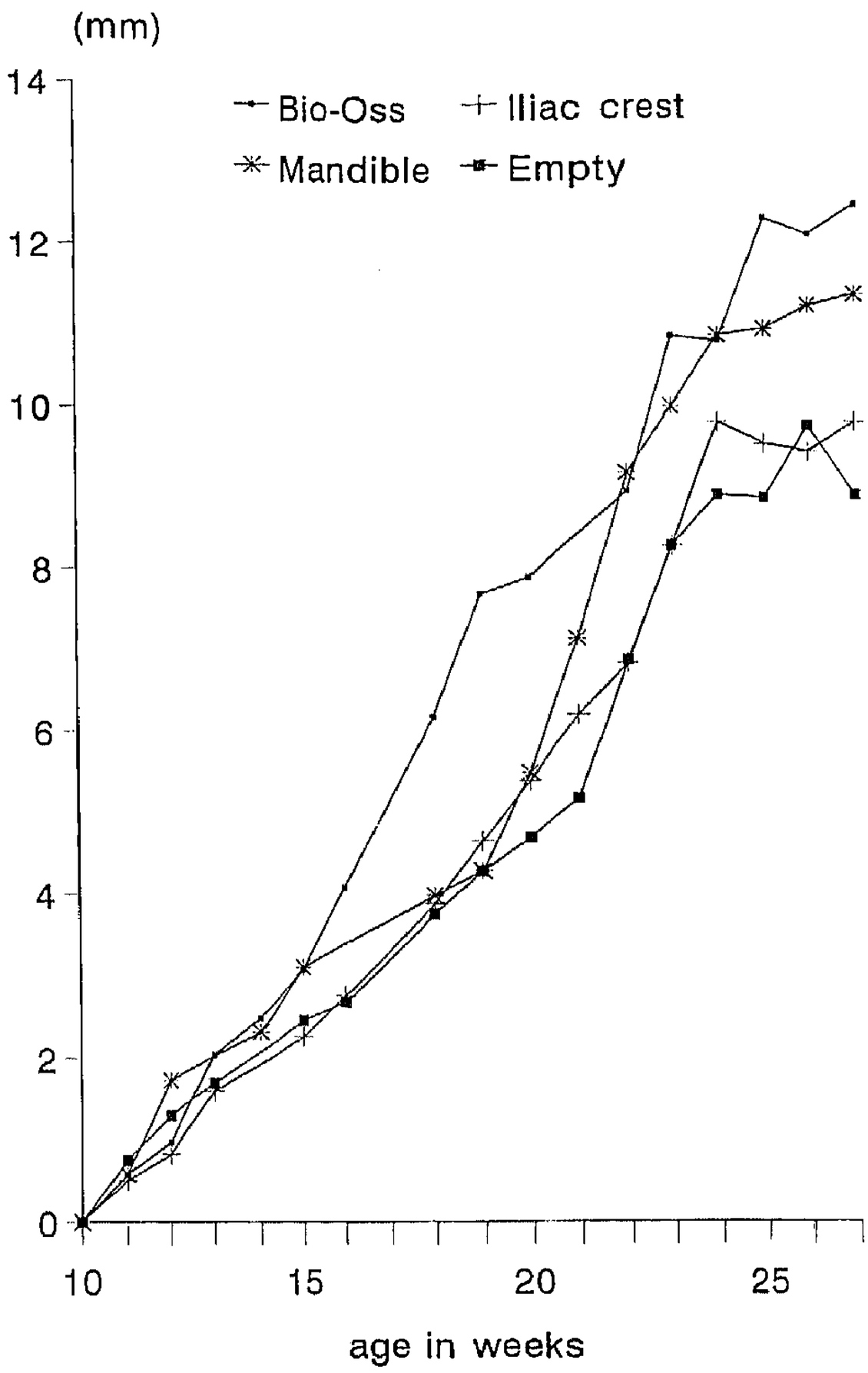

Fig. 4 – Mean relative vertical tooth displacement of P2 (experimental and sham groups).

Table – Mean vertical displacement (in mm) ± SD from 18 to 23 weeks of age

	P2	P3	P4
Control		6.59 ± 1.47	
Sham	5.78 ± 1.38		6.90 ± 1.23
Bio-Oss	4.40 ± 0.65		6.36 ± 1.49
Mandible	5.93 ± 1.44		7.40 ± 0.56
Iliac crest	4.35 ± 0.84		7.50 ± 0.92

P2: No difference between experimental and sham groups (ANOVA, $P > 0.3$).
P4: No difference between experimental, sham and control groups (ANOVA, $P > 0.3$).
P2 < P4 for experimental and sham groups (ANOVA, $P < 0.001$).

Control sites

In these sites, resorption of the m2 tooth roots and the eruption of the successive permanent P3 started 4 weeks after the beginning of the experiment. At the age of 21 weeks, P3 erupted into the oral cavity and its roots were almost completely formed. The eruption lasts for a mean age of 23.1 ± 1.0 weeks. From that point, the tip of P3 remains stable in nearly all animals.

Histology

Mandibular bone chips

In 18-week-old beagle dogs (4 weeks after transplantation) the membraneous bone chips had a non-vital appearance, with only some osteocytes in the lacunae and no signs of inflammation (Fig. 5). The surfaces of the grafts occlusal to the erupting tooth were integrated in the normal physiological process of resorption. The periodontal ligament was intact with a normal structure and orientation of the fibres. At the age of 24 weeks, the teeth showed no delay in eruption or disruption with attachment of the functional epithelium on the enamel–cemental border. The implanted bone grafts were incorporated in the developing alveolar bone. After 30 weeks, all premolars had erupted to their functional level with normal alveolar bone height, intact periodontal ligament, a normal crown and roots of a normal length. Remnants of bone grafts were no longer seen.

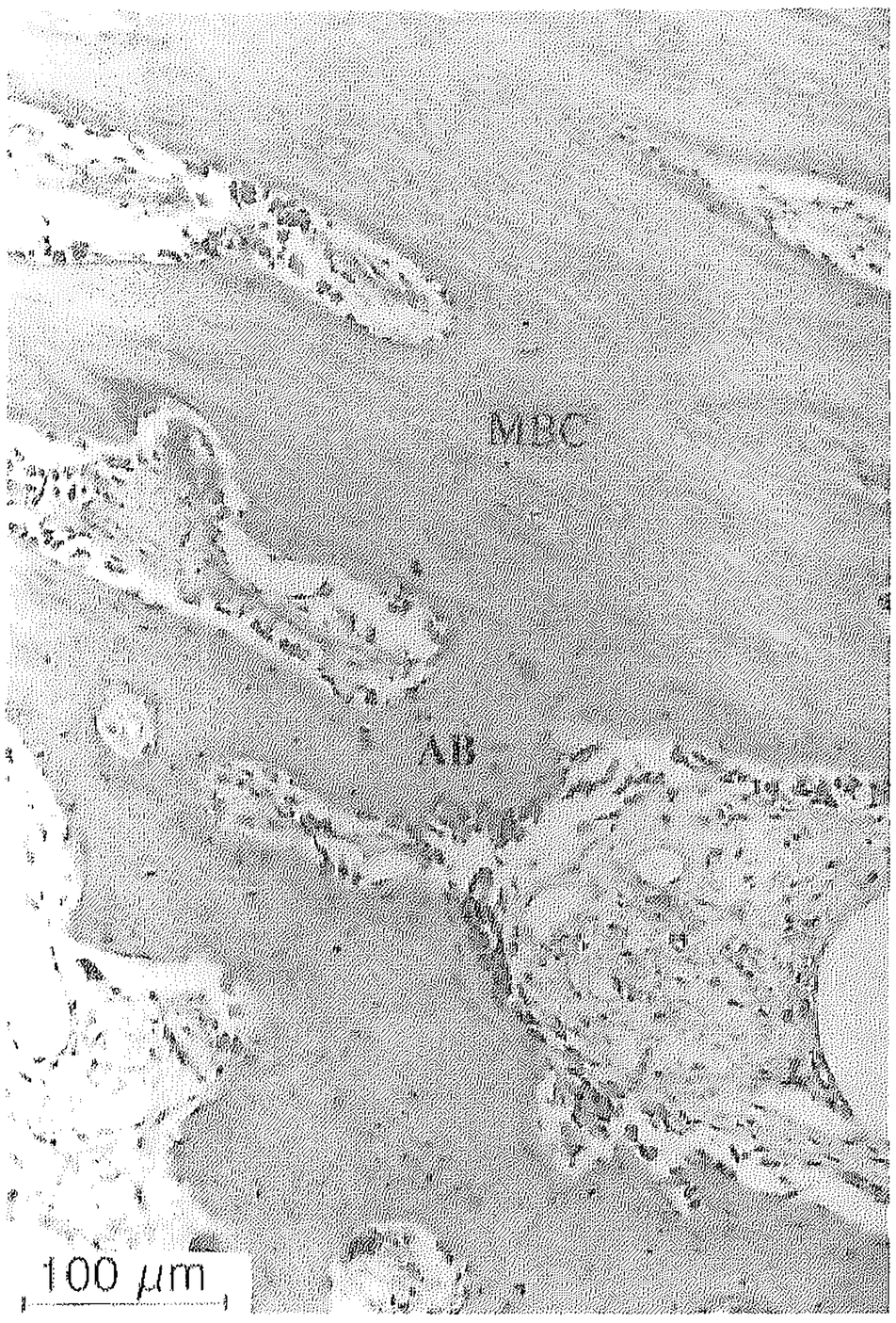

Fig. 5 – Photomicrograph of mandibular bone chips 4 weeks after transplantation at the age of 18 weeks, showing the non-vital appearance of the bone chips with only some osteocytes in the lacunae. Haematoxylin and eosin; neg. magn. ×40. MBC: mandibular bone chips, AB: alveolar bone.

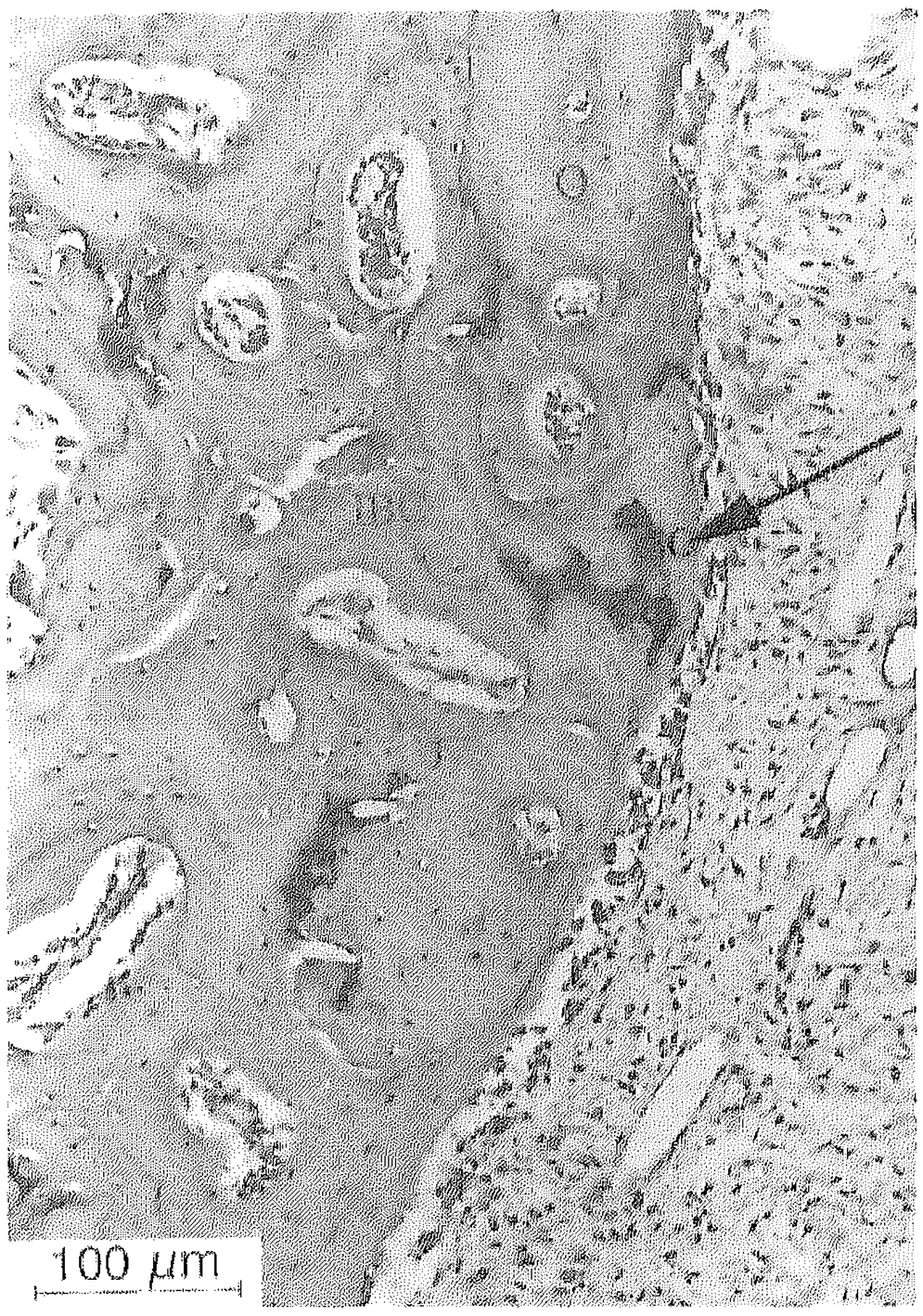

Fig. 6 – Photomicrograph of iliac crest bone chips 4 weeks after transplantation at the age of 18 weeks, showing the incorporated bone chips with osteons among them. Arrow indicates remnants o cartilage. IBC: iliac crest bone chips, O: new osteon. Haematoxylir and eosin; neg. magn. ×40.

Iliac crest bone chips

At the age of 18 weeks (4 weeks after transplantation) the iliac crest bone chips were fully incorporated with osteons among the whole transplant, without signs of inflammation (Fig. 6). The structure and orientation of the parodontal ligament of the erupting tooth was normal. Some cartilage remnants, probably from the iliac crest, were seen. The bone grafts were integrated in the physiological resorption process, occlusal to the erupting tooth. At the age of 24 weeks, no abnormalities were seen. After 30 weeks, the premolars had emerged, with intact periodontal ligament, crown and roots. Remnants of the bone chips were integrated in the alveolar bone.

Bio-Oss

The number of Bio-Oss granules was reduced at 18 weeks (4 weeks after transplantation) because connective tissue was lying between the particles and they were no longer compressed (Figs 7 and 8). The granules were not yet incorporated, lying in the empty alveolus as well as occlusal to the alveolar bone, within connective tissue. They were surrounded by macrophages and foreign body giant cells. Because no Howship's lacunae were seen, they were not interpreted as osteoclasts. The attachment of the fibres of the periodontal ligament of the erupting tooth was at a normal height at the enamel–cemental border. At 24 weeks, foreign body giant cells were

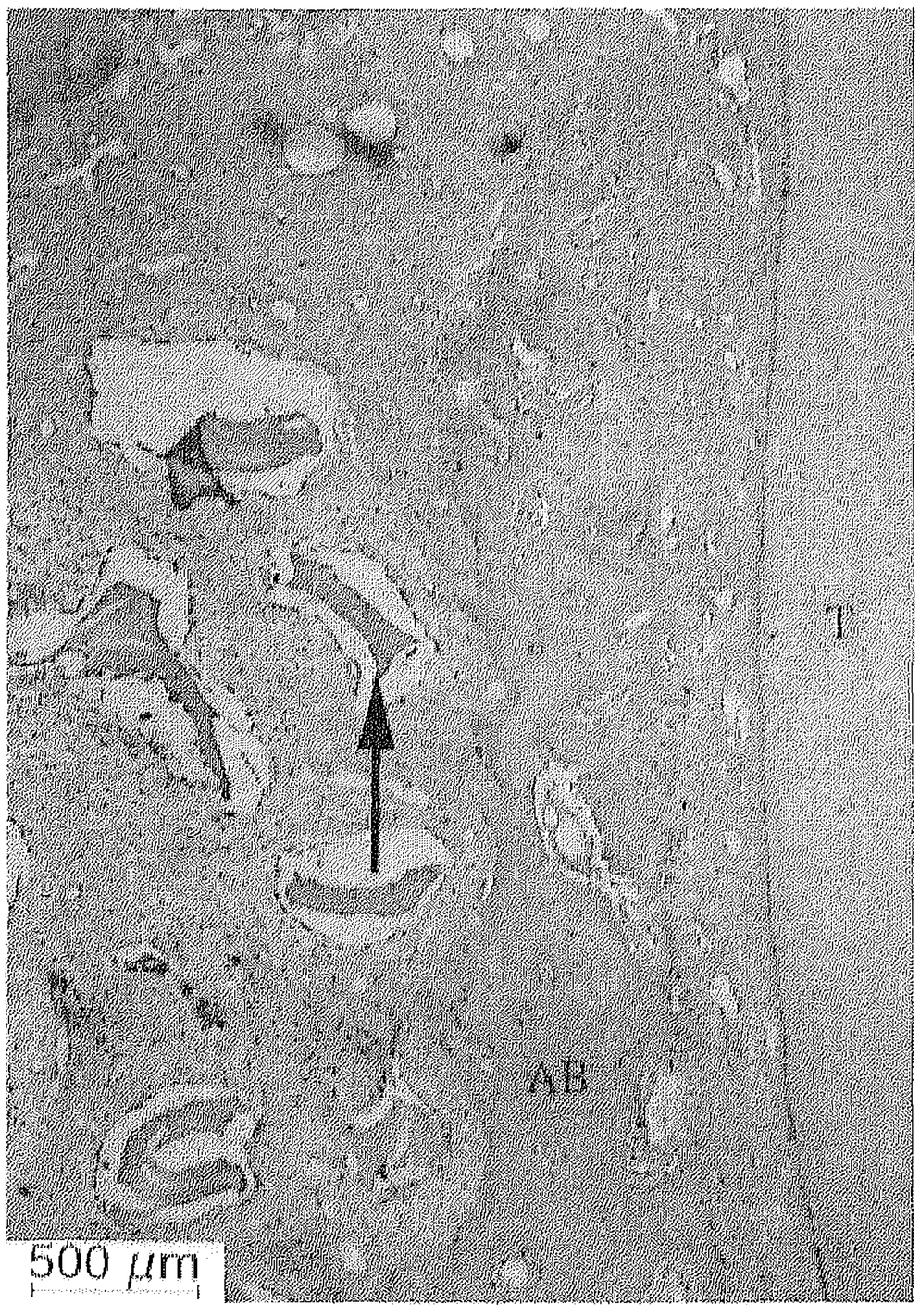

Fig. 7 – Photomicrograph of Bio-Oss particles 4 weeks after transplantation at the age of 18 weeks, showing the connective tissue layer around them without signs of inflammation. Arrow indicates Bio-Oss particle. T: tooth, AB: alveolar bone. Haematoxylin and eosin; neg. magn. ×10.

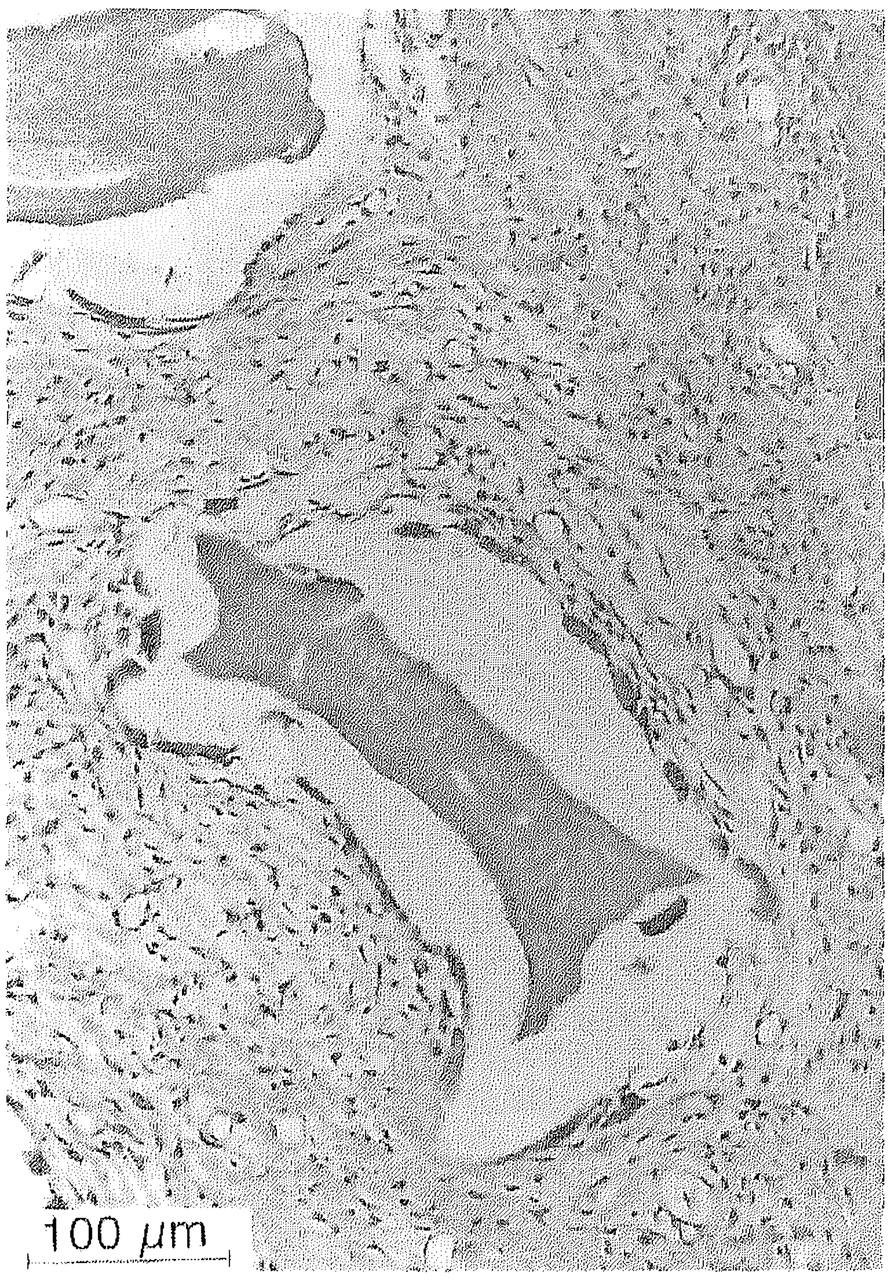

Fig. 8 – Detail of Figure 7. Macrophages and foreign body cells surrounding the particles. Haematoxylin and eosin; neg. magn. ×40.

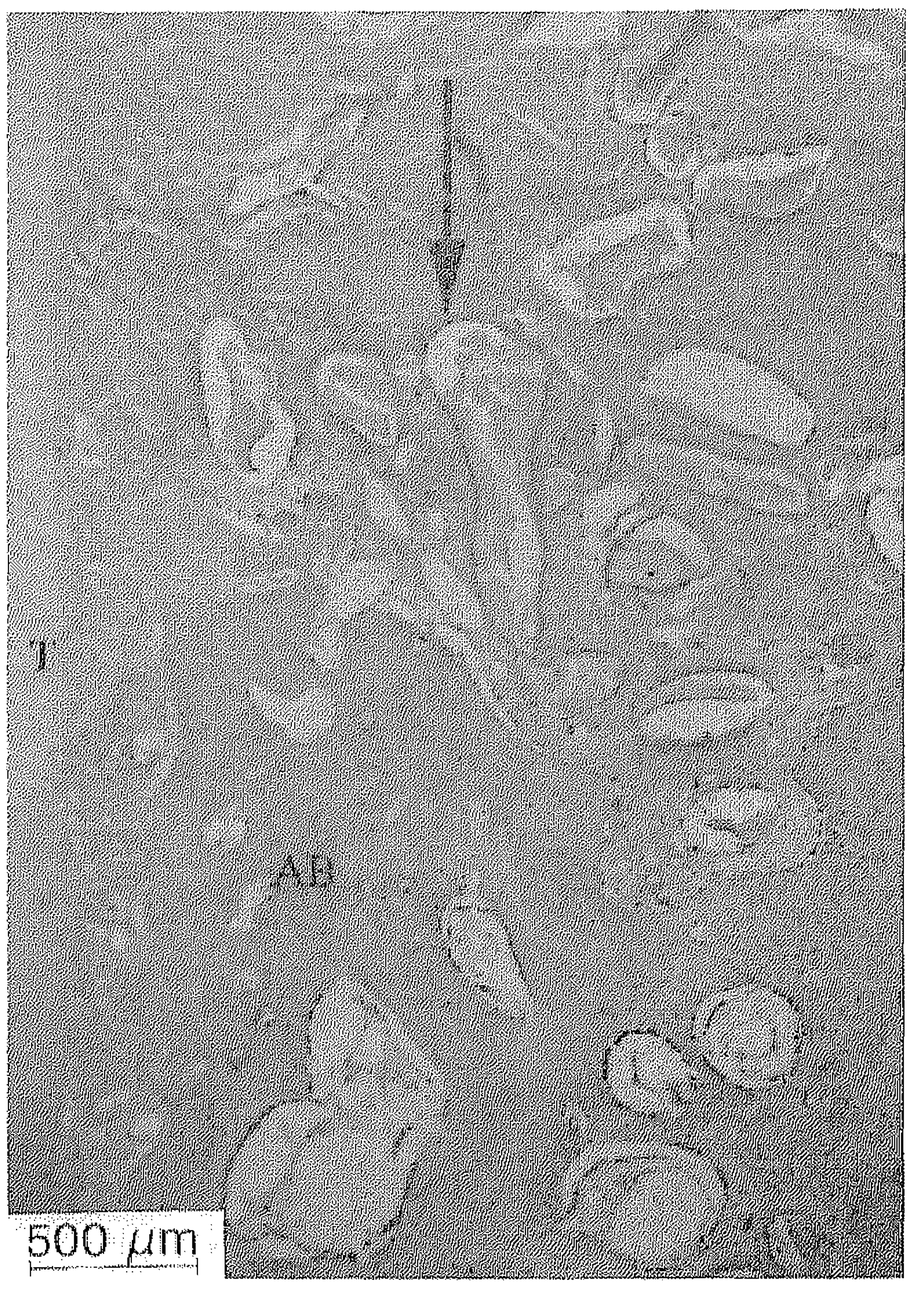

Fig. 9 – Photomicrograph of Bio-Oss particles 16 weeks after transplantation at the age of 30 weeks. Arrow indicates Bio-Oss particle. T: tooth, AB: alveolar bone. Haematoxylin and eosin; neg. magn. ×10.

seen in the vicinity of the particles without further signs of inflammation. The presence of the granules was restricted to the connective tissue occlusal to the enamel–cement border.

At the age of 30 weeks, most of the Bio-Oss particles were surrounded by a thin fibrous sheath without an inflammatory response or osteoclastic activity (Figs 9 and 10). Some of them were incorporated in the bone, but most were lying in connective tissue occlusal to the alveolar bone. The premolar eruption was undisturbed with a normal structure of the periodontal ligament, crown and roots.

Control

The eruption processes in the control group were not disturbed, with normal orientation and structure of the fibres of the periodontal ligament. In the period between extraction of the deciduous molar and the emergence of its successor, no alveolar bone was deposited in the empty alveolus.

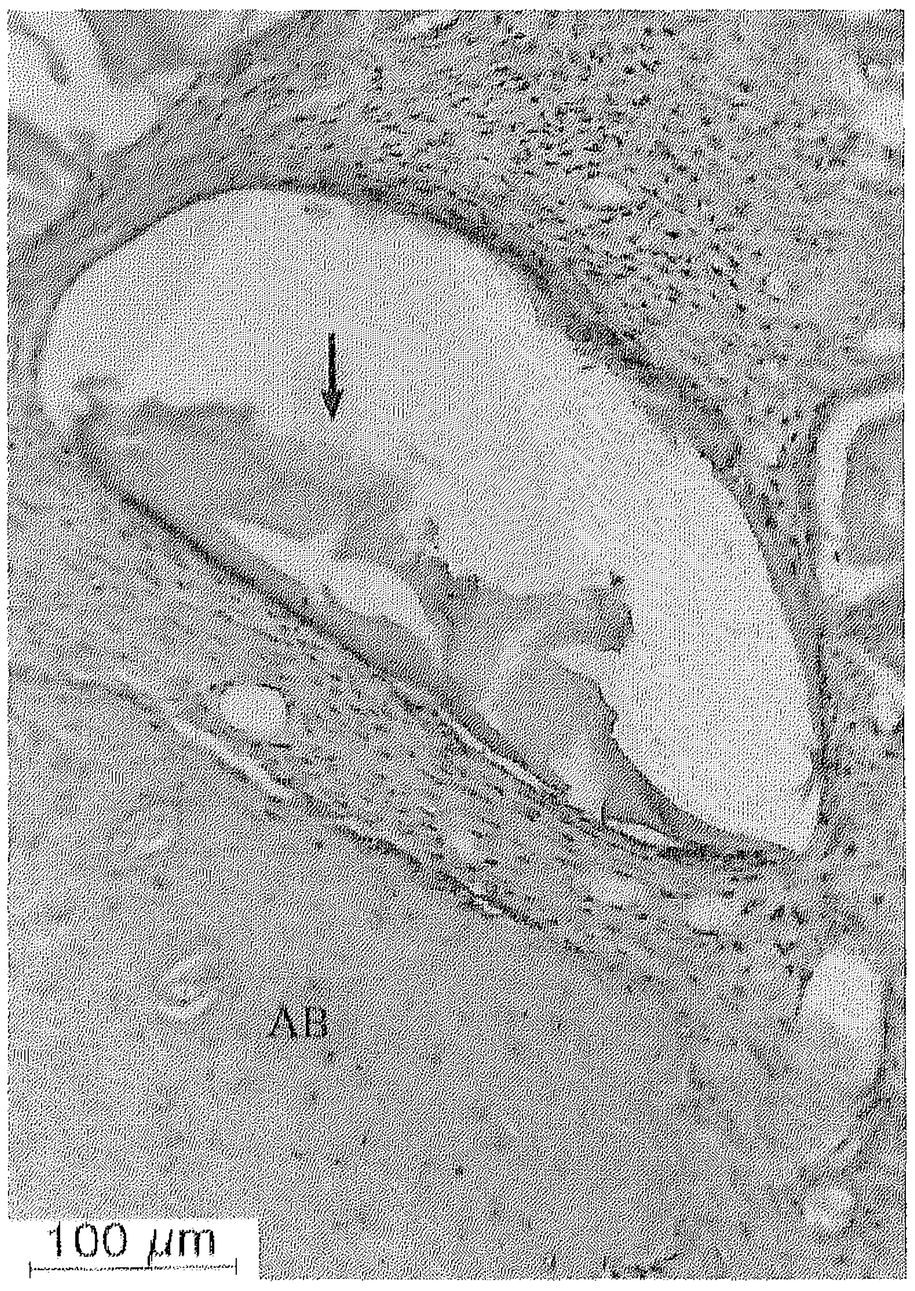

Fig. 10 – Photomicrograph of one Bio-Oss particle (arrow) 16 weeks after transplantation at the age of 30 weeks, showing the thin fibrous sheet around the particle lying in connective tissue, not integrated in the alveolar bone (AB). Haematoxylin and eosin; neg. magn. ×40.

DISCUSSION

There are still unanswered questions relating to the physiology of bone transplantation into the alveolar defect. One of these questions concerns the effect of the bone graft on the eruption of the permanent canine. In our institute, chin bone, intramembraneous in origin, is the grafting material of choice (*Freihofer* et al., 1993). Membraneous bone has several advantages over enchondral bone: it is usually removed with less donor site morbidity and less postoperative pain (*Tessier*, 1982; *Hoppenreijs* et al., 1992; *Roche* and *Schwartz*, 1993) and it has been shown that it is less subject to postoperative remodelling and resorp-

tion (*Smith* and *Abramson*, 1974; *Zins* and *Whithaker*, 1983; *Kusiak* et al., 1985). In this experiment there were no indications of a slower eruption through the mandibular bone graft. Only the eruption through the 'empty' alveolus of P4 seemed to start earlier than through the grafted alveolus.

Studies of premolar eruption in dogs have shown that it is accomplished primarily by localized resorption of alveolar bone on the coronal side of the tooth (*Cahill*, 1969; *Marks* and *Cahill*, 1986; *Marks* et al., 1993), that the osteoclasts are activated on bone surfaces just prior to eruption (*Marks* et al., 1983; *Wise* et al., 1985) and that eruption depends on the dental follicle proper, a thin collagenous investment of the crown of each developing tooth (*Marks* and *Cahill*, 1984; *Cahill* and *Marks*, 1980; *Marks* et al., 1993). This cell-mediated process results in a local tuning of the erupting tooth and the overlying alveolar bone.

In our experiment, the erupting premolars are able to resorb the iliac crest and mandible bone grafts without any difference from each other and to the control sites. The number of Bio-Oss granules is reduced, although we did not see resorption sites. The presence of osteoclasts on the surface of Bio-Oss granules at 4 and 10 weeks after surgery as observed by us and *Klinge* et al. (1990) only suggests resorption in a more physiological manner. Most of the Bio-Oss granules are pushed forwards into the soft tissues or are probably discharged into the oral cavity during the eruption. It is possible that more time for incorporation is needed in comparison with autologous bone transplants because of the lack of osteoinductive capacity (*Thaller* et al., 1994). The results of the present study are in accordance with a study which showed that the effect of four kinds of calcium phosphate ceramic particle insertion (hydroxyapatite [HA] dense and porous, and tricalcium phosphate [TCP] dense and porous) on tooth eruption in 20 3-month-old dogs produced no delay in tooth eruption, no dysplasia and no resorption of dental hard tissue (*Sugimot* et al., 1993). Studies on the effect of calcium phosphate on tooth eruption in 40 3- to 4-month-old kittens indicated that the use of non-resorbable HA for grafting, however, resulted in impediment to eruption and distortion of crown development. This was in contrast to the TCP, which had a minimal effect on tooth eruption and development (*Feinberg* and *Vitt*, 1988; *Feinberg* et al., 1989). So, the uniformity of eruption curves in our experimental (and control) groups indicates that Bio-Oss can be considered as a resorbable calcium phosphate.

The results of this study indicate that the use of mandible bone or iliac crest bone in residual alveolar clefts has no negative influence on the eruption of permanent teeth.

The number of Bio-Oss particles decreased during eruption. The time interval between insertion of the particles and the start of the eruption is probably too short for incorporation into the bone because of the lack of osteoinductive capacity. Bio-Oss particles separated from new bone are pushed into peripheral soft tissue or discharged into the oral cavity at the time of eruption. Iliac crest bone, mandibular bone and Bio-Oss, when implanted into extraction sockets of deciduous teeth, have no inhibitory effect on eruption and crown development of the underlying permanent mandibular premolars. Before using this xenogene bone mineral in alveolar clefts, more has to be known of the healing process in osseous defects resembling the volume of alveolar clefts.

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