# Alveolar Ridge Repair Using Resorbable Membranes and Autogenous Bone Particles with Simultaneous Placement of Implants: An Experimental Pilot Study in Dogs

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*The aim of this experimental study was to evaluate the use of autogenous bone harvested during preparation of implant sites in combination with resorbable membranes for vertical ridge augmentation under 2 different defect site conditions. Combined vertical/horizontal alveolar bone defects were created by experimentally induced periodontal infections around all premolar teeth in the mandibles of 3 dogs (group 1). In another 3 dogs, fresh surgical defects were created after extraction of all premolar teeth in the mandibles (group 2). In all dogs, 2 implants were placed on each side of the mandible into the defect areas. One implant on each side of the mandible received augmentation with autogenous bone particles, and both implants on one side of the mandible were covered with polylactic acid membranes. After 5 months, the material was evaluated histologically. There was a small but significant increase in bone regeneration in the defects augmented with bone particles with and without membrane coverage in group 1. In group 2, no significant difference was seen between the controls and the augmented sites. The major limiting effect for bone regeneration appeared to be insufficient stability of the bone material to withstand the overlying soft tissue pressure. It was concluded that the placement of autogenous bone particles, either with or without membrane coverage, had little effect on the regeneration of peri-implant bone defects.* (Int J Oral Maxillofac Implants 2000;15:364–373)

**Key words:** *artificial membranes, bone regeneration, bone transplantation, dental implants, guided tissue regeneration, membranes, polymers*

Vertical ridge augmentation using guided bone regeneration is frequently compromised by a collapse of the barrier membrane, owing to pressure of the overlying soft tissues. To present this decrease in contour restoration, the use of augmentation material underneath the membrane has been advocated.[1–4] The material used for augmentation should be osteoconductive and must provide enough stability to resist soft tissue pressure above the membrane. For these purposes, autogenous bone is still considered to be the material of choice because of its unique biologic properties.[5] The major drawback of autogenous bone, however, is the additional surgical effort and increased morbidity of the harvesting procedure. Therefore, it is tempting to avoid problems with the procurement of autogenous bone by recovering bone particles from the drilling procedure of the alveolar bone during preparation of the implant site.[6] Currently, a number of filtering devices are available to collect alveolar bone powder and particles for augmentation of peri-implant bone dehiscences. The efficacy of these devices appears to provide a convenient solution for harvesting autogenous bone material, within the range of 0.8 to 1.5 g, for the repair of minor alveolar defects without additional morbidity.[6]

However, little is known about the efficacy of this procedure with regard to the restoration of ridge height and bone-implant contact in the augmented area. Currently, it is unclear whether the recovered

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bone particles will behave as small bone grafts with an active cellular component, or whether they are only "dead" matrix particles, albeit with a biologic function to enhance bone formation by providing osteogenic matrix proteins. Some authors have shown marrow stem cells to be present in these bone particles,[7] and others have been able to cultivate osteoblast-like cells from this material.[8]

Since many alveolar defects result from infectious bone loss related to periodontal disease, it is important to evaluate the performance of these autogenous bone particles in previously diseased sites in the alveolar process. Previous studies have shown that pathologic conditions of the alveolar process may have a negative effect on the outcome of guided bone regeneration procedures around implants, presumably because of decreased regenerative capacity in the alveolar bone and the overlying soft tissues.[9] Therefore, it was considered desirable to evaluate the reconstructive potential of autogenous bone particles derived from bone preparation for endosseous implants, in combination with resorbable barrier membranes under different defect site conditions, in an experimental pilot study.

## MATERIALS AND METHODS

The experiments were carried out on 6 adult female beagle dogs (average weight 13.9 kg). The animals were kept in groups according to the standards of government authorities for the care and protection of animals. All surgical procedures were performed under general anesthesia.

The animals were divided into 2 groups of 3 animals each. In group 1, defects of the alveolar ridge were produced in the mandible by ligature-induced periodontitis around all premolars during a 3-month period, described previously.[9] The marginal bone around the premolars was removed with a small rose bur down to the level of the bifurcation (approximately 1 to 2 mm) and ligatures were placed around the teeth. After 3 months, a decrease in alveolar bone height by approximately 4 mm was produced, as determined from the distance between the crestal margin of the defect and the bifurcation of the premolar roots. At this time, all mandibular premolars were extracted, and the infected sites were curetted. This resulted in a combined vertical and horizontal defect approximately 5 mm in height extending across the entire premolar area. This defect was left to heal for 3 months (Fig 1a). At the same time, all mandibular premolars in the dogs of group 2 were extracted, and the alveolar ridges were left to heal for 3 months with no defects created.

Subsequently, in group 1, the alveolar ridge was exposed from a vestibular incision and the bone surface in the defect area was perforated with a small rose bur. Two titanium screw implants (Nobel Biocare, Göteborg, Sweden) were placed into the edentulous area on both sides of the mandible. The oblique vertical alveolar bone defects resulted in exposure of implant threads for approximately 5 mm (Fig 1b). During bone preparation, bone "slurry" and particles from the alveolar ridge were recovered using a filter device connected to the suction tube (Friatec, Mannheim, Germany). The recovered autogenous bone material was used to reconstruct the alveolar bone contour around 1 implant on each side of the mandible (Fig 1c). On one side of the mandible, both implants were covered with a porous polylactic acid (PLA) membrane (L/DL 70/30, inh. viscosity 1.8 g/dL, pore size 1 to 6 μm, thickness 180 to 200 μm; ITV, Denkendorf, Germany) (Fig 1d), while on the opposite site no membrane coverage was provided. Thus, each dog carried 1 implant with bone particles and a resorbable barrier membrane, 1 implant with a resorbable barrier membrane only, 1 implant with bone particles only, and 1 control implant.

In group 2, oblique vertical defects were created surgically after exposure of the alveolar ridge bilaterally in the edentulous areas of the mandible (Fig 2a). The mandibles were then treated with implants, membranes, and autogenous bone identical to group 1 (Fig 2b). The difference between group 1 and group 2 was in the timing and means of defect creation: defects in group 2 were created at the time of implant placement, corresponding to fresh surgical bone wounds in a clinical situation, while the defects in group 1 were created by infection and had healed for 3 months before implant placement, corresponding to old periodontal bone loss in a clinical situation.

Following wound closure, the animals were examined once a week. In 10 implant locations, dehiscence occurred during the observation period. These sites were meticulously cleaned with chlorhexidine solution. No clinical signs of acute or chronic purulent infection were observed. Fluorochrome labels were administered during the first postoperative week (tetracycline, 12 mg/kg body weight; Fluka Chemie, Neu-Ulm, Germany) to label the starting point of bone regeneration; this was also done during the 5th week (calcein blue, 30 mg/kg, Fluka Chemie); the 12th week (alizarine, 30 mg/kg, Fluka Chemie); and the 18th week after the operation (calcein green, 20 mg/kg, Fluka Chemie). At the end of a 20-week observation period, the implants were removed from the mandibles together

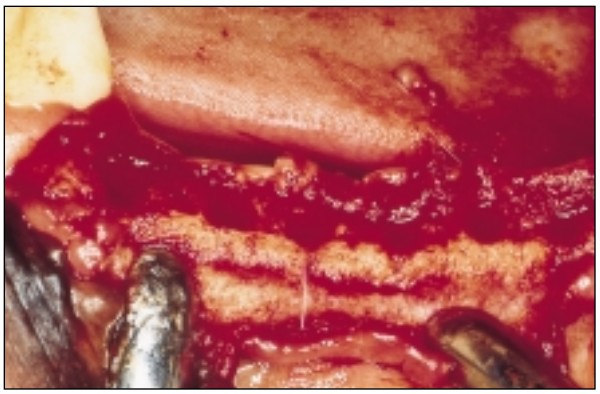

**Fig 1a** Vertical and horizontal defects are created in the alveolar ridge after extraction of periodontially diseased teeth.

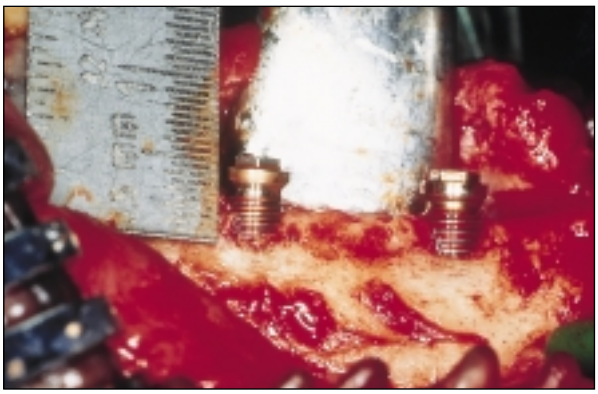

**Fig 1b** Two screw-type implants are placed.

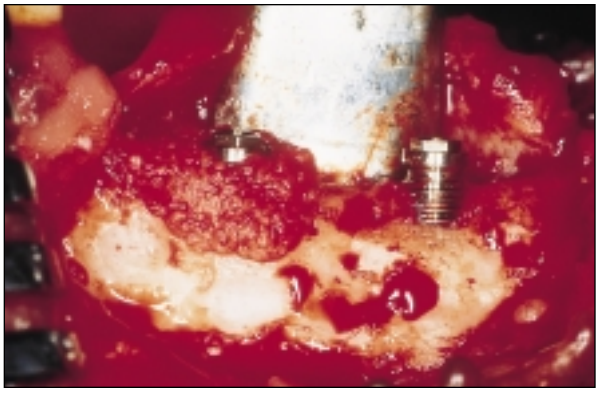

**Fig 1c** The alveolar ridge is reconstructed with recovered bone particles around 1 implant on each side of the mandible.

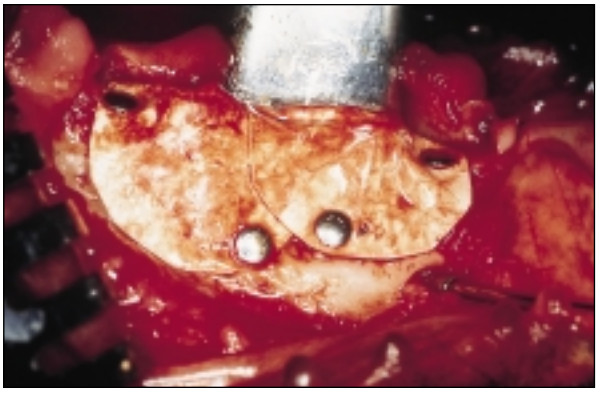

**Fig 1d** The reconstruction is covered by a polylactic acid membrane. The non-augmented implant was covered by a separate membrane to avoid contamination of this site by bone particles from the adjacent augmented site under a common membrane.

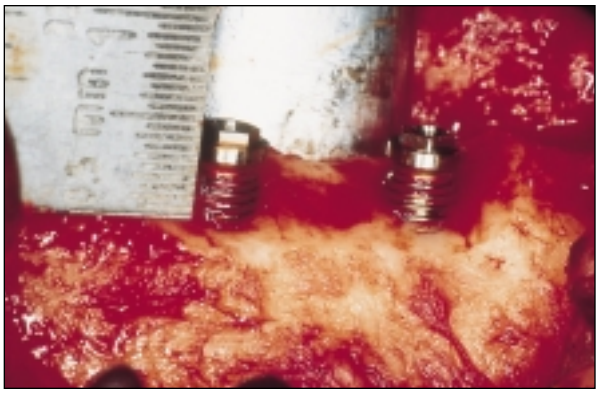

**Fig 2a** Two screw-type implants are placed in a surgically created vertical and horizontal defect of the alveolar ridge.

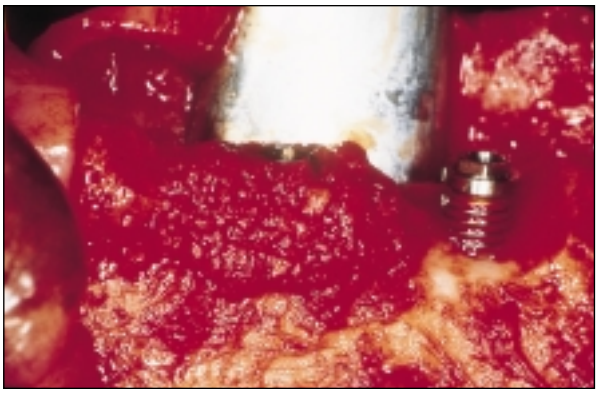

**Fig 2b** The alveolar ridge is reconstructed with autogenous bone.

with the surrounding bone and immediately fixed in buffered formalin (4%). The specimens were dehydrated and embedded into methylmethacrylate resin, and undecalcified thick sections (30 to 70 µm) were obtained from the tissue blocks parallel to the axis of the implants in a labiolingual plane using a saw with a diamond-edged blade (Fa. Leika, Hamburg, Germany). Every other thick-section specimen was surface-stained with alizarin-methylene blue, while the remaining specimens were used for fluorescence microscopy.

The quality of the regenerated bone was evaluated from the surface-stained specimens, while metric evaluations were carried out on the unstained specimens under the fluorescence microscope. The height of the regenerated bone was determined from the vertical distance between the surface of the newly formed bone and the tetracycline label on the buccal and the lingual side on each specimen. A mean value was calculated for each implant (Fig 3). The values of the individual implants were averaged for each group and were compared by Mann-Whitney tests within each group at a significance level of 5%.

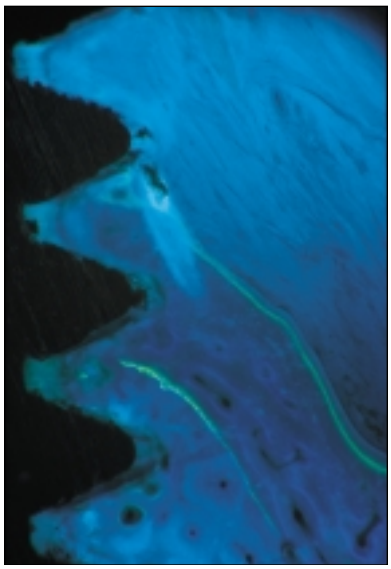

**Fig 3** Fluorescence microscopic image showing the labels of the original and the regenerated marginal bone level (original magnification ×40).

## RESULTS

Healing was uneventful in 14 of the 24 implant sites, whereas dehiscence was encountered in 10 locations. Some of the dehiscences appeared after 2 weeks, while others were seen only after 3 months. As there was no evidence of purulent suppuration and/or chronic infection, it was decided to leave the material and the implants in place and keep the mucosal sites as clean as possible until the end of the 5-month observation period. The occurrence of dehiscences was not significantly associated with the use of a barrier membrane, bone particles, or a combination of both (Chi-square test; $P = .3331$). In 3 cases of dehiscence, the membranes had ruptured over the buccal side of the implant head and collapsed, reducing the space available for bone regeneration (Fig 4a).

### Group 1

In the defects produced by experimental infection, implants without membrane or bone particles showed little regeneration of alveolar bone height and little remodeling activity of the old peri-implant bone by newly formed bone (Figs 4b and 4c). Those implants that were augmented with bone particles and no membrane coverage showed more bone formation, with immature bone structure and remodeling activity next to the implant surface. Most of the regenerated height, however, was gained on the lingual side (Figs 4d and 4e). Implants with the barrier membrane alone exhibited a variable degree of regeneration, ranging from moderate to very little bone formation. Regeneration of bone in peri-implant defects with bone augmentation and additional membrane coverage was also quite variable. Some of the defects showed formation of bone to a considerable extent very early (Fig 4f), while others exhibited only poor regeneration underneath the membrane. This early bone formation underneath the PLA membrane was not observed with the use of membranes alone, but it also did not occur regularly with the use of bone particles. In general, quantity and quality of alveolar ridge repair by regenerated bone tissue around the implants with barrier membranes and/or bone particles were very similar. In some of the specimens, dislocation of bone particles had occurred in an apical direction underneath the membrane, leading to formation of bone that was in direct contact with the membrane (Fig 4g).

The porous structure of the membranes used for coverage of the defects and/or augmentation had a feathered appearance in the histologic specimens. The membranes were incorporated into the fibrous soft tissue without evidence of inflammatory reactions around implants with a closed soft tissue layer. In one case, mineralization inside the porous structure of the membrane had occurred (Fig 4g). In 2 of the 4 cases with soft tissue dehiscence, however, cellular infiltration around the membrane was observed (Fig 4h).

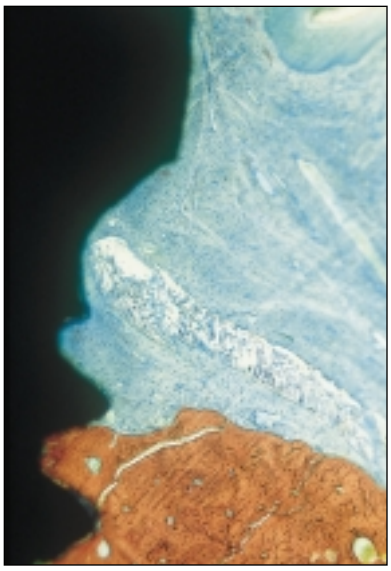

**Fig 4a** Micrograph showing membrane rupture under a soft tissue dehiscence with subsequent collapse (alzarine-methylene blue, original magnification ×40).

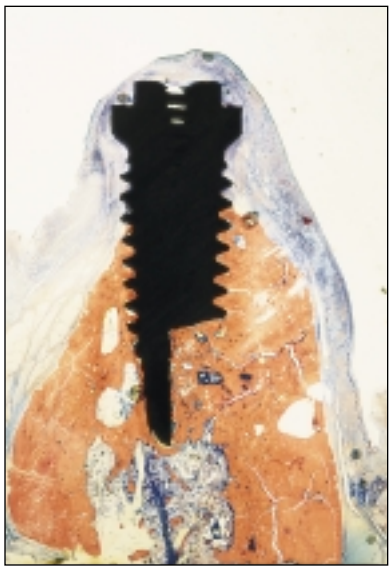

**Fig 4b** Micrograph of a control implant in group 1, showing no repair of the peri-implant bone defect (alzarine-methylene blue, original magnification ×7).

**Fig 4c** Higher-power view of same specimen shown in Fig 4b showing old peri-implant bone remodeled by newly formed bone (*arrows*) without an increase in height (alzarine-methylene blue, original magnification ×40).

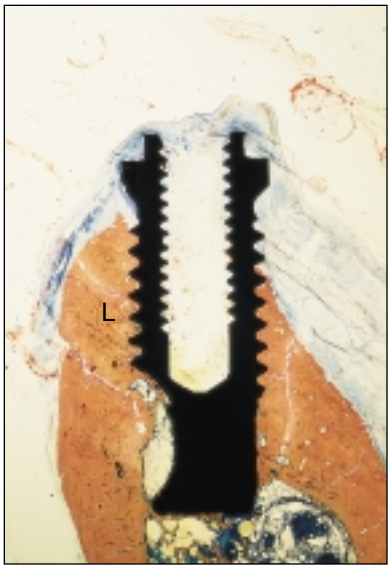


**Fig 4d** Micrograph of an implant in group 1 treated with bone augmentation only, showing an increase in bone height mostly on the lingual side (L) of the defect (alzarine-methylene blue, original magnification ×7).

**Fig 4e** Higher-power view of specimen shown in Fig 4d, showing an increase in bone height on the buccal side by formation of immature new bone in direct contact with the implant surface (arrows delineate old bone) (alzarine-methylene blue, original magnification ×40).

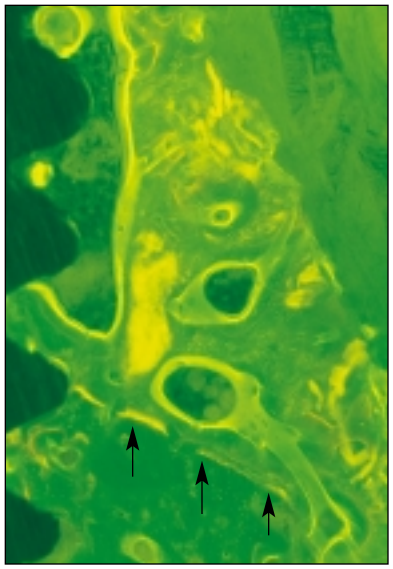

**Fig 4f** Fluorescence microscopic image of an implant in group 1 treated with bone augmentation and membrane coverage, showing early regeneration of bone to a considerable extent, as indicated by the extensive labeling of tetracycline. Note the clearly marked tetracycline level of the pre-existing bone peri-implant bone (*arrows)* (blue light excitation, original magnification ×40).

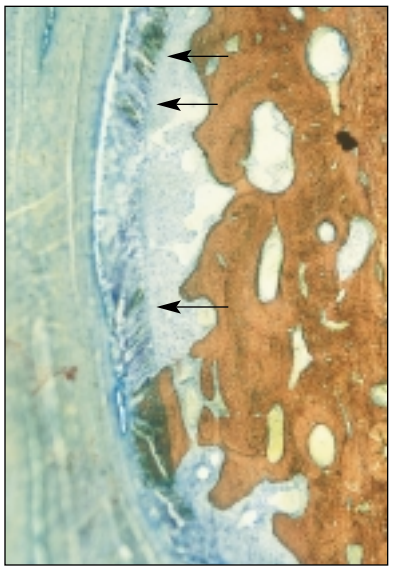

**Fig 4g** (*Left*) Micrograph of an implant in group 1 treated with bone augmentation and membrane coverage, showing dislocation of bone particles underneath the membrane in apical direction, with bone formation in immediate contact with the membrane penetrating into the porous structure. Note additional calcification of the membrane within the pores independently from adjacent bone (*arrows*) (alizarine-methylene blue, original magnification ×40).

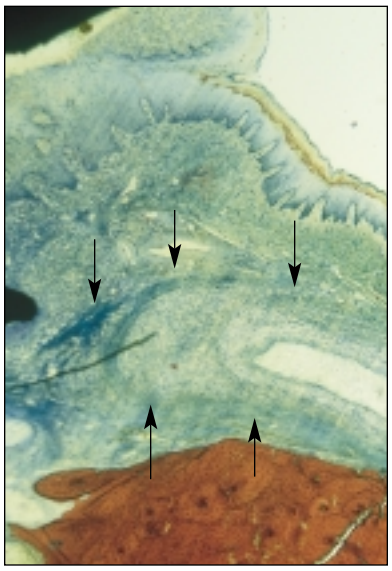

**Fig 4h** *(Right)* Micrograph showing infiltration by inflammatory cells (*arrows*) around the membrane under a soft tissue dehiscence (alzarine-methylene blue, original magnification ×40).

## Group 2

The control implants that were placed into surgically created defects showed limited bone repair on the lingual side. On the buccal side, the soft tissue had collapsed onto the implant surface and thus largely obstructed bone regeneration (Fig 5a). Implants with bone particle augmentation exhibited considerable restoration of alveolar ridge height and width in 2 cases, with bone formation protruding into the soft tissues on the buccal side (Fig 5b). One implant with bone augmentation showed only a moderate amount of regeneration owing to a mucosal dehiscence over the implant head. The defects that were augmented with barrier membranes alone showed incomplete regeneration of bone in all 3 cases, partly because of the dehiscence of overlying soft tissues and subsequent membrane collapse. Defects with membrane coverage plus bone particle augmentation showed complete regeneration around one implant (Fig 5c) but incomplete repair around another implant because of soft tissue dehiscence.

As in Group 1, apical dislocation of bone particles underneath the membrane was observed occasionally, with early bone formation in the resulting bulge (Fig 5d). The histologic appearance of the membrane material was identical to that of the Group 1 specimens. In one case, mineralization inside the porous structure was found. The histologic appearance and the structure of newly regenerated bone was very similar to that seen in Group 1. However, peri-implant augmentation using bone particles with and without membrane coverage tended to show increased regeneration of ridge width compared to the specimens in Group 1 (Fig 5e).

## Quantitative Results

In Group 1, the increase in alveolar ridge height was lowest in the control specimens, with an increase of only 0.29 mm (SD 0.21) (Fig 6). Those defects treated with bone augmentation had an average increase in bone level of only 0.87 mm (SD 0.40). The "membrane-only" defects showed vertical bone growth of 1.08 mm (SD 0.41), and the combination of bone particles and membranes yielded an increase in bone regeneration of 0.87 mm (SD 0.16). The difference between the control implants and those in which bone particles with and without membrane coverage were used was significantly different ($P$ = .0451 and $P$ = .0463, respectively). There was no significant difference between the different techniques of augmentation. Membrane coverage alone did not yield significantly different results versus the controls ($P$ = .1374).

In Group 2, the increase in bone height around the control implants was 0.50 mm (SD 0.18). Those defects augmented with bone particles alone showed bone formation to an average of 0.77 mm (SD 0.13) above the pre-existing bone level. The increase in bone height in the "membrane-only" defects was

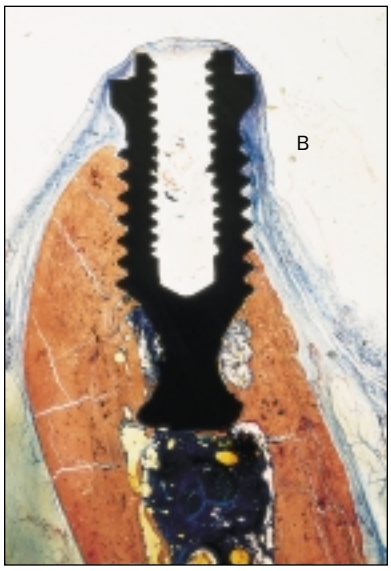


**Fig 5a** Micrograph of a control implant in group 2 showing lack of bone regeneration on the buccal side (B) resulting from collapse of soft tissues onto the implant surface (alzarine-methylene blue, original magnification ×7).

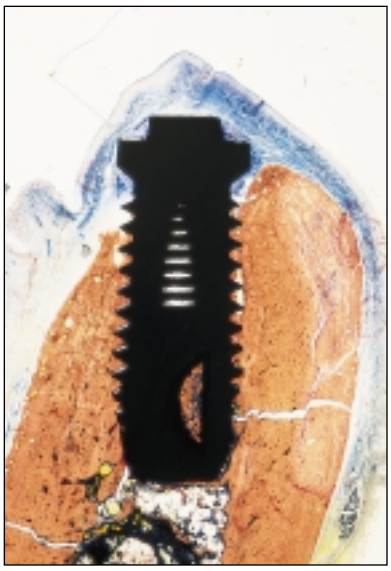

**Fig 5b** Micrograph of an implant in group 2 treated with bone augmentation only, showing substantial regeneration of peri-implant bone (alzarine-methylene blue, original magnification ×7).

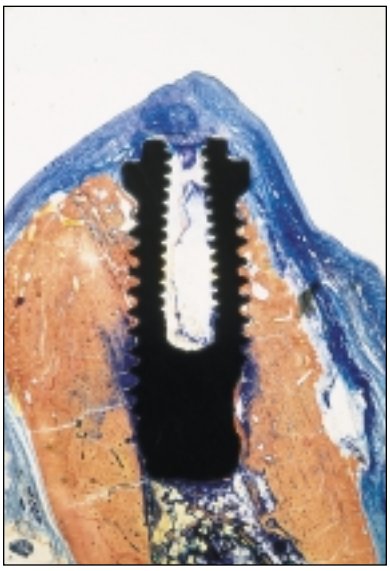

**Fig 5c** Micrograph of an implant in group 2 treated with bone augmentation and membrane coverage, showing complete regeneration of the alveolar crest around the implant (alzarine-methylene blue, original magnification ×7).

**Fig 5d** Fluorescence microscopic image of an implant in group 2 treated with bone augmentation and membrane coverage, showing apical dislocation of bone underneath the membrane. Early formation of bone can be seen from the extensive tetracycline labeling (ultraviolet light excitation, original magnification ×40).

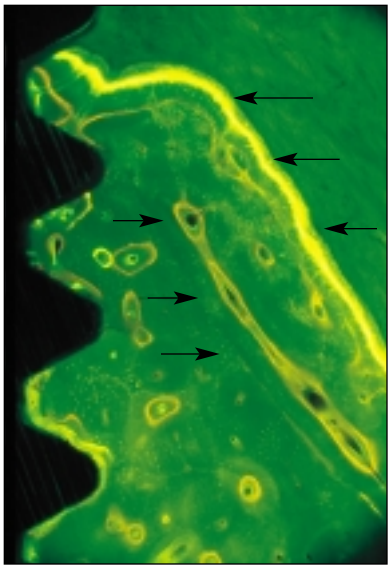

**Fig 5e** Fluorescence microscopy of an implant in group 2 treated with bone augmentation only, showing increased width of the alveolar process (*arrows*) but little increase in height (blue light excitation, original magnification ×40).

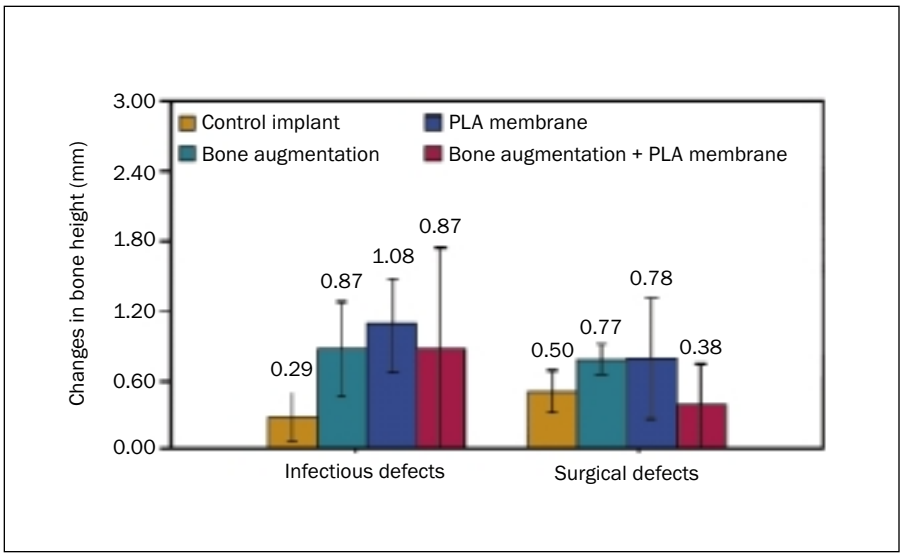


**Fig 6** Graph showing changes (mean + standard deviation) in marginal bone height. PLA = polylactic acid.

0.78 mm on average (SD 0.51), and the sites treated with both bone and membranes showed an average increase in bone height of 0.70 mm (SD 0.35). In contrast to Group 1, there was no significant difference between the different modes of augmentation and the controls.

## DISCUSSION

The treatment of alveolar bone defects using barrier membranes for guided bone regeneration has evolved as one of the standard therapies performed in conjunction with implant placement.[10–17] Although the necessity of complete buccal bone coverage of the implant surface as a precondition for implant success appears to be questionable from a clinical and biomechanical point of view,[18,19] increased esthetic demands for implant-supported restorations render it necessary to reconstruct the buccal alveolar bone contour. However, the collapse of barrier membranes frequently encountered under pressure of the overlying soft tissues often requires additional support via fillers placed underneath the membrane.[20] Since bone is removed during preparation of the implant site, it is useful to recover this bone for the augmentation of deficient areas of the alveolar crest. The idea that there is biologic activity beyond the passive filling of bone defects, exerted through osteogenic growth factors released from the extensively increased surface of these bone particles, is also attractive. Furthermore, there are reports suggesting that these bone particles contain vital osteoprogenitor cells,[7,8] so that, from a theoretical point of view, a positive effect on bone formation in peri-implant defects could have been expected.

However, the results of the present study suggest that the use of these bone particles for restoration of the alveolar bone contour had only a small enhancing effect on the results of the reconstruction. In the group of animals with defects resulting from experimentally induced infection, the increase in alveolar bone height was significantly greater in the defects augmented with bone particles with and without membrane coverage than in the controls, but the absolute differences were small and exhibited great variation. Although some individual implants showed quite extensive repair, in most cases the bone particles used for augmentation were not sufficiently stable to resist the soft tissue pressure, particularly on the buccal side, and therefore could not preserve enough space to allow for substantial bone formation.

The difference in the resulting bone height between the augmented defects and the controls was even smaller in the group of animals with surgically created defects (group 2), because the controls in group 2 showed a higher increase in bone height

than the controls in the defects resulting from infection (group 1). This suggests that the experimentally induced pathologic condition of the defect sites in group 1 had decreased the regenerative capacity of the alveolar bone and that, conversely, the use of bone slurry and particles from the alveolar crest compensated for this effect, as the metric result of augmentations was very similar in both groups. However, it is difficult to draw definite conclusions from the present results because of the relatively small number of implants and large variation of the results.

One reason for the large variation of the results may be the occurrence of soft tissue dehiscences over some of the implants, which was followed by membrane breakdown in three cases. The relatively high frequency of implants with dehiscence (10 of 24 implants) may be accounted for by the experimental defect model. As there was no correlation between the different experimental variations of the model and the occurrence of dehiscence, the general design of a combined vertical/horizontal defect model itself may be the reason. Other experimental designs with merely horizontal defects and preservation of ridge height did not encounter any dehiscence of this kind.[21] On the other hand, the frequency of dehiscence has been considerably decreased compared to a previous study in dogs[9] by the avoidance of immediate implant placement after the extraction of previously diseased teeth and by the placement of the incision line into the vestibular sulcus.

The membranes used were well-tolerated but were unable to preserve the shape of the reconstructed alveolar ridge after wound closure. Since all membranes were clearly visible in microscopic images and exhibited structural integrity, it is very likely that they provided a barrier function throughout the entire observation period. However, mechanical strength was apparently insufficient, and a space-making effect was completely lost in those cases where soft tissue dehiscences had led to rupture of the membrane and subsequent collapse into the defect. Since the bone particles used for augmentation provided little additional support to the membrane, only minor repair of the defects was accomplished in these cases.

The use of autogenous bone particles derived from bone preparation during implant placement for repair of alveolar bone defects therefore appears to not be very reliable. A substantial bone-enhancing effect of the material recovered from the drilling procedure of the alveolar crest was not clearly appreciable. One reason for the lack of a biologic effect might be that the release of a small percentage of non-collagenous proteins with osteoinductive properties from the mineralized collagen bone matrix does not occur rapidly enough and is not sufficiently strong to exert a substantial effect on bone formation. However, some of those cases in which larger portions of bone particles had been preserved by the membrane showed fairly extensive bone formation during the first week, suggesting that some kind of inductive enhancement has occurred. The major reason for the lack of a general increase in bone formation seems to be that the space available for bone regeneration was insufficient in most cases to restore the alveolar bone volume recontoured by bone particles. Thus, the results concur with those of experimental and clinical studies using bone morphogenetic protein preparations for the repair of alveolar bone defects which have shown unreliable results in cases where carriers with low mechanical strength such as collagen were used.[22]

## CONCLUSION

The present study showed that bone particles recovered from drilling during preparation of implant sites have insufficient stability to ensure the restoration of contours around implants in combined vertical/horizontal ridge defects. Augmentation using these bone particles with and without membrane coverage resulted in only a minor increase in bone height. The major limiting factor for bone regeneration appears to be compression of the augmented areas because of the instability of the bone material. Under these conditions, possible biologic activity of this material cannot be reliably assessed, since it is overridden by the effect of mechanical compression.

## ACKNOWLEDGMENTS

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