Facilitation of β-Tricalcium Phosphate–Induced Alveolar Bone Regeneration by Platelet-Rich Plasma in Beagle Dogs: A Histologic and Histomorphometric Study

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Purpose: *The effects of 2 graft materials, β-tricalcium phosphate (Cerasorb) alone and a combination of Cerasorb and platelet-rich plasma (PRP), on the bone regeneration process were evaluated in the canine mandible.* ***Materials and Methods:*** *The mandibular premolars of 12 beagle dogs were surgically removed. The extraction sockets were filled with Cerasorb on the control side and a mixture of Cerasorb and PRP on the test side. Bilateral biopsy samples were taken from the graft insertion sites at 6, 12, and 24 weeks after surgery. Sections were prepared from the undecalcified resin-embedded samples.* ***Results:*** *Six weeks after grafting, the proliferation of cellular osteogenic mesenchyma was more abundant in the test group. The histomorphometric data revealed a significantly higher percentage of bone area in the test group (45.9%) than in the control group (30.8%) ($P < .05$). Twelve weeks after grafting, the test group still had some advantage over the control group in terms of bone regeneration (52.5% bone in the test group versus 49.4% in the control group, $P < .05$). Twenty-four weeks after grafting, bone-forming activity was nearly equal in the 2 groups, and the bone area in the 2 groups did not differ significantly (62.9% and 61.9%, respectively) ($P < .05$).* ***Discussion:*** *The histomorphometric results suggested more intensive bone regeneration in the early healing phase following the topical application of PRP.* ***Conclusion:*** *The increase in bone density facilitated by grafting with a combination of Cerasorb and PRP requires thorough study in humans.* INT J ORAL MAXILLOFAC IMPLANTS 2004;19:832–838

Key words: *animal research, β-tricalcium phosphate, bone regeneration, histology, histomorphometry, platelet-rich plasma*

Large bone defects and insufficient bone volume cause many difficulties in the application of dental implants. Restoration of defects and contour irregularities in the craniofacial region is difficult and often requires complex solutions.[1–4] The current trend in the reconstruction of jaw defects is directed toward the facilitation of bone regeneration so that the harvest of autogenous bone grafts can be avoided. These methods eliminate additional surgical procedures and the attendant risks.[5] Reports on a wide variety of materials used in bone regeneration procedures can be found in the literature.[2,4,6–10]

Histologic and histomorphometric studies in humans have revealed that new bone formation can vary considerably, depending on the graft type, the amount of time the defect is given to heal, and additional factors.[10–12] Combination of the graft material with various accessory factors (eg, venous blood, human osteogenic protein-1, osteoprogenitor cells)

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has also been studied by several authors.[10,12,13] The addition of platelet-rich plasma (PRP) to grafts in human studies resulted in earlier and more intense bone regeneration.[14] Platelets can release growth factors that provoke angiogenesis and mitogenesis, and thus capillary proliferation and the multiplication of osteoprogenitor cells, which are advantageous for bone regeneration.[15]

Tricalcium phosphate is a biomaterial that has been studied extensively.[16–18] Cerasorb (Curasan, Kleinostheim, Germany) is a pure β-phase tricalcium phosphate; granules of Cerasorb are spherical, with a porous structure. This material has been shown clinically to be a suitable bone substitute capable of biodegradation; it is gradually replaced by new mineralizing bone tissue.[1,16,19] The osteoconductive capacity of Cerasorb is also advantageous in maxillofacial surgery.[11,16,17,19]

Allogenic graft materials must be extensively studied in experimental models simulating relevant clinical situations. The effects of different environmental factors can be more thoroughly studied and compared in experimental situations than in clinical situations. In the present study, an experimental model was developed to evaluate bone healing in the extraction socket of the canine mandible after grafting. The objective of the study was to compare long-term bone healing with 2 different graft materials—Cerasorb alone and a Cerasorb-PRP combination. The effects of these 2 materials were compared using histologic and histomorphometric methods. The aim was to clarify whether the well-known advantageous attributes of Cerasorb could be enhanced by combining Cerasorb with PRP.

MATERIALS AND METHODS

Animals

Twelve beagle dogs between 2 and 3 years old and within a body weight range of 10 to 12 kg were selected for the study. Routine clinical veterinary examinations and radiographic evaluation excluded dental or bone pathology in the jaws. The animals had not previously participated in research. The procedures employed in the study did not require animal sacrifice, which allowed for long-term graft evaluation. The study was approved by the animal use committee of the Veterinary Faculty of the Szent István University.

Surgery

Analgesia was achieved by intramuscular administration of medetomidine hydrochloride (20 to 30 μg/kg body weight; Domitor; Novartis Animal Health, Basel, Switzerland) and butorphanol (0.2 mg/kg body weight; Butomidor; Richter Pharma, Wels, Austria). After bilateral extraction of the first, second, and third mandibular premolars, the extraction sockets were filled with Cerasorb alone on the control side and a mixture of Cerasorb and PRP on the test side. The granules ranged from 500 to 1,000 μm in diameter. The PRP was prepared from autologous blood obtained during surgery using a Curasan Separation Kit. It was added to a mixture of Cerasorb and autogenous blood taken from the bone defect. Trephine burs were used to take bilateral biopsy samples of the healing bone from each dog at 6, 12, and 24 weeks after graft insertion. A total of 72 tissue samples were taken.

Histology

Processing and staining of the bone samples were carried out as reported earlier.[11] Briefly, the samples were fixed in 4% formaldehyde in phosphate buffer, dehydrated in a graded series of alcohols, and embedded in methylmethacrylate resin at 4°C. Five-μm-thick histologic sections were cut in the longitudinal plane with a diamond knife and stained with toluidine blue and hematoxylin-eosin. They were also stained according to Goldner's trichrome method for light microscopy. Photomicrographs were taken by means of a digital camera. New bone and osteoid formation were identified visually.

Histomorphometry

Histomorphometric studies were performed according to the principles of Parfitt and associates.[20] Sections for histomorphometry were taken from 4 levels of each sample, with a space of 150 μm between them. Quantification procedures were performed after scale calibration (Calibration Grid Slide; MicroBrightField, Williston, VT) by 2 independent observers who were blinded with regard to the graft material used. The samples were measured semiautomatically by means of an Olympus BH2 microscope equipped with a drawing tube (Leitz, Wetzlar, Germany), cursor, and digitalizing table that were connected to a computer using Osteoplan software (Zeiss Kontron, Oberkochen, Germany). For each sample, the bone area, graft area, soft tissue area, and total biopsy area were calculated. Bone area, graft area, and soft tissue area were also calculated as percentages of the total biopsy area. The percentage of Cerasorb granules showing peri- or intragranular osteoid or bone deposition was also determined.

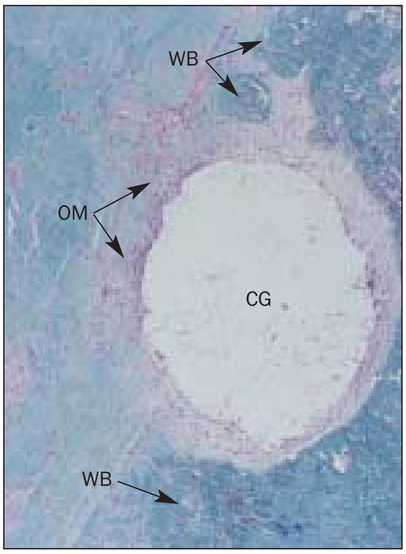
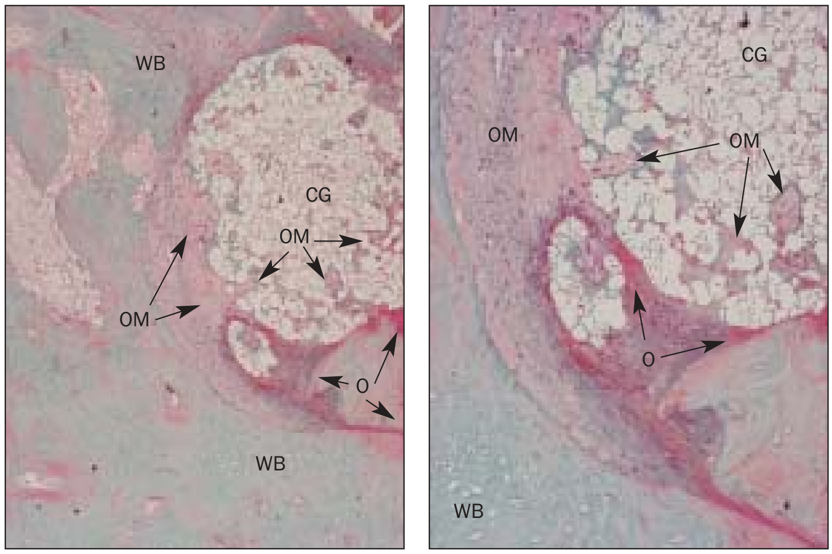


Fig 1 Control group, 6 weeks after grafting. Cerasorb granules are embedded in a cell-rich mesenchymal tissue. CG = Cerasorb granule, WB = woven bone, OM = osteogenic mesenchyma (Goldner's trichrome; original magnification ×10).



Figs 2a and 2b Test group, 6 weeks after grafting. Porous granules are densely packed with osteoprogenitor ingrowth. CG = Cerasorb granule, O = osteoid, WB = woven bone, OM = osteogenic mesenchyma (Goldner's trichrome; original magnifications ×10 and ×25, respectively).

The length of the tissue-graft interface was obtained by measuring the outline of the granules. Mean values and standard deviations were subsequently calculated for the samples.

Statistical Analysis

The Student *t* test was used to determine statistical significance. Values of $P < .05$ were considered to be significant.

RESULTS

Histology

Tissue responses, vascularization, ingrowth of osteogenic mesenchyma and bone into the porous Cerasorb granules, and signs of resorption of the graft material were investigated histologically. Bilateral biopsy samples were removed from all animals for examination at 6 weeks, 12 weeks, and 24 weeks (ie, 24 samples, 12 test and 12 control, were examined per time period).

6 Weeks. Six weeks after grafting, the porous granules in the control group were embedded in a cell-rich mesenchymal tissue. There was no significant ingrowth into the pores of the granule (Fig 1). The capillarization of the connective tissue was abundant, and the woven bone formation had resulted in acellular osteoid production and mineralization of the newly developing bone. Osteoid and new bone formation appeared as a thin peripheral rim surrounding some granules. The graft particles were partly dissolved by the acidic Goldner's trichrome stain, but their sites could easily be recognized by their characteristic form. No inflammatory cells or foreign body reactions were observed.

In the test group, abundant proliferation of a cellular osteogenic mesenchyma was a typical finding. The porous granules were densely packed with osteoprogenitor ingrowth (Figs 2a and 2b). This formed a network of tissue strands and bridges connecting the central islands to the cell-rich peripheral rim.

Signs of osteoid and woven bone production were also seen. Lacunar resorption and osteoclastic activity were not observed.

12 Weeks. Twelve weeks after grafting, a dynamic process of bone remodeling was conspicuous in the control group. A random arrangement of highly cellular osteogenic mesenchyma and woven bone in the vicinity of the graft material was characteristic (Fig 3a). Lacunar bone resorption by multinuclear osteoclasts resulted in the replacement of woven bone by a less cellular, more mineralized, and structurally more organized lamellar bone. The thin channels of the porous Cerasorb granules were partially filled by homogenous osteoid strands or small bony islands forming a fine network (Fig 3b). No signs of active bioresorption of the graft material were observed.

In the test group, the events of bone regeneration revealed some advantages compared with the control group. A noteworthy perigranular apposition of

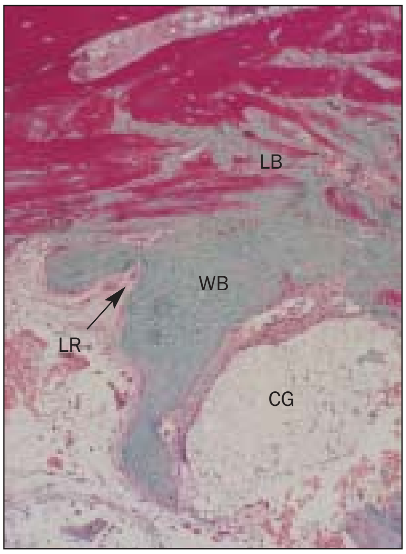


Fig 3a Control group, 12 weeks after grafting. Woven bone formation is evident in the vicinity of the granules, along with lacunar osteoclastic bone resorption. CG = Cerasorb granule, LR = lacunar resorption, WB = woven bone, LB = lamellar bone (Goldner's trichrome; original magnification ×10).

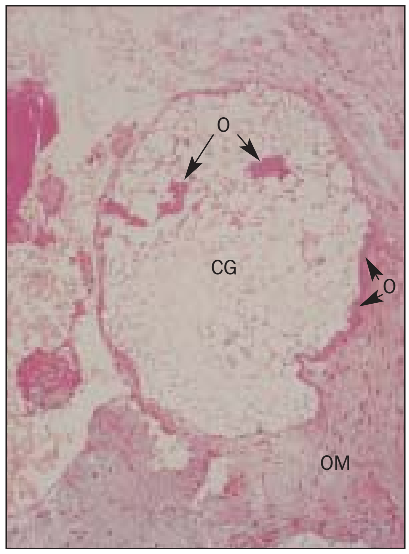


Fig 3b Control group, 12 weeks after grafting. Thin channels of the porous granules are filled by a fine osteoid network. CG = Cerasorb granule, O = osteoid, OM = osteogenic mesenchyma (hematoxylin-eosin; original magnification ×25).

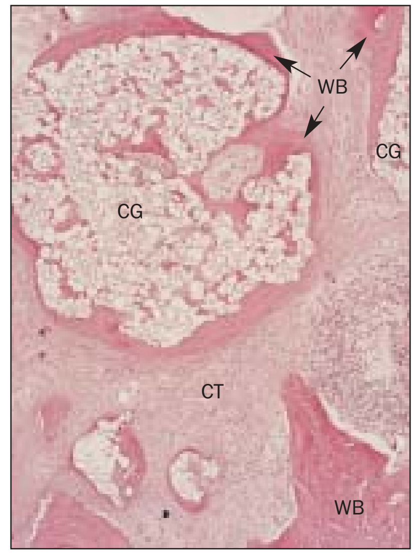


Fig 4 Test group, 12 weeks after grafting. Peri- and intragranular new bone formation is apparent. CG = Cerasorb granule, WB = woven bone, CT = connective tissue (hematoxylin-eosin; original magnification ×10).

osteoid and woven bone had penetrated the centers of the disintegrated graft particles (Fig 4). The intragranular budding of the richly capillarized osteogenic mesenchyma and the profusion of smaller bone islands suggested active replacement of the Cerasorb particles by newly formed bone. The concave surfaces of the resorbing granules and the osteoid or bone deposition along the graft-tissue interface also demonstrated active new bone formation. No signs of active osteoclastic resorption of the graft were detected.

24 Weeks. At 24 weeks, the regenerative process in the control group had caught up with that in the test group. The majority of granules were embedded in newly formed lamellar bone. Partial resorption of the granules was indicated by their scalloped surfaces, and perigranular osteoid or bone formation was a characteristic finding (Fig 5).

Histologic findings in the test group were similar. The newly formed bone trabecules and the graft remnants were closely intermingled with each other at the tissue-graft interface (Fig 6a). Mechanically stable lamellar bone and the remnants of partially resorbed Cerasorb particles produced a hard, safe bed suitable for dental implantation (Fig 6b). There were no inflammatory reactions. Resorbing Cerasorb particles were apparently being replaced continuously by the newly formed bone.

Histomorphometry

The areas of graft particle and newly formed bone were measured in each biopsy specimen, and mean percentages and standard deviations (SDs) for each group and time period were calculated.

Histomorphometry revealed that after 6 weeks the percentage of bone area was lower in the control group than in the test group (30.8% ± 18.8% versus 45.9% ± 20.6%, $P < .05$). This difference was less marked after 12 weeks, although the percentage of bone area was still greater in the test group (52.5% ± 18.4% versus 49.4% ± 17.7%, $P > .05$). After 24 weeks, the difference had virtually disappeared; the percentages of bone area in the test and control groups were nearly equal (61.9% ± 16.8% and 62.9% ± 22.4%, respectively, $P > .05$).

By 24 weeks, graft area as a percentage of the total biopsy reflected only moderate resorption; however, intragranular bone deposition and perigranular scalloping were histologically evident by this time. No relevant differences were found between the 2 groups in terms of the percentage of graft area. After 6 weeks, the percentage of the sample area accounted for by graft material was 7.1% ± 3.9% in the control group and 6.8% ± 3.7% in the test group ($P > .05$). After 12 weeks, there was a slight difference (6.9% ± 4.1% in the control group versus 6.3% ± 3.6% in the test group, $P > .05$). After 24 weeks, the graft area percentages (6.5% ± 3.8%

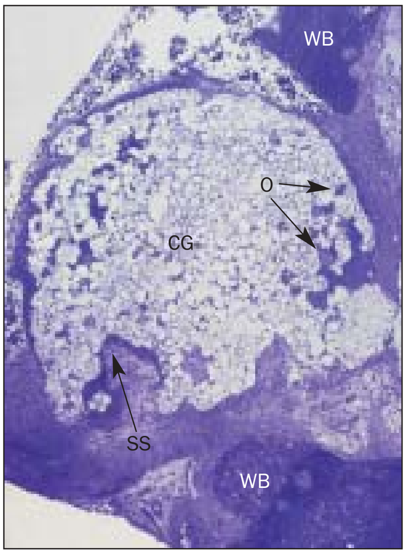


Fig 5 Control group, 24 weeks after grafting. Osteoid and bone formation can be seen. Note the scalloped surface, which suggests partial resorption of the granule. CG = Cerasorb granule, O = osteoid, WB = woven bone, SS = scalloped surface (toluidine blue; original magnification ×10).

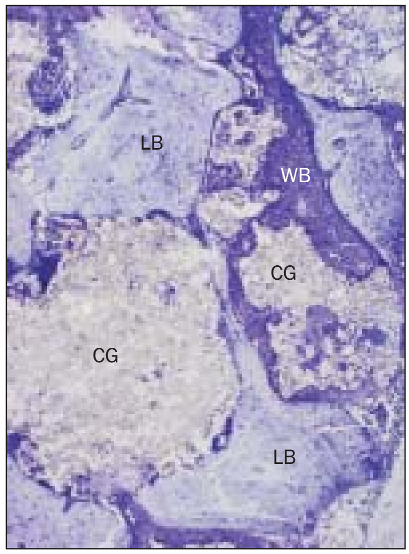


Fig 6a Test group, 24 weeks after grafting. Newly formed bone trabeculae and graft remnants intermingled with each other. CG = Cerasorb granule, WB = woven bone, LB = lamellar bone (toluidine blue; original magnification ×25).

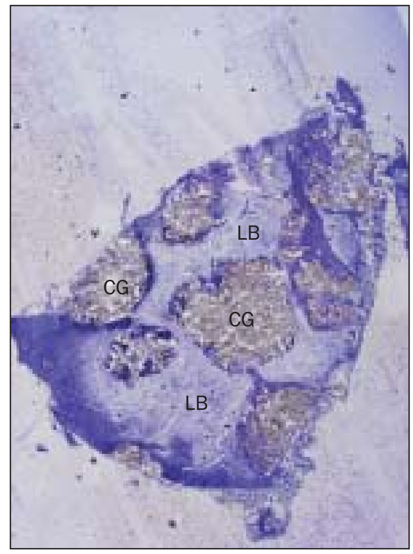


Fig 6b Test group, 24 weeks after grafting. The majority of the granules are embedded into newly formed bone. CG = Cerasorb granule, LB = lamellar bone (toluidine blue; original magnification ×2).

in the test group and 6.3% ± 2.9% in the test group, $P > .05$) had not changed markedly.

The percentage of the Cerasorb granules showing peri- or intragranular osteoid or bone deposition was also determined, and the means ± SDs were calculated.

Six weeks after grafting, bone-forming activity was conspicuously more marked in the test group. The percentage of granules showing osteoid or bone formation was 11.5% ± 5.8% in the control group versus 27.1% ± 12.6% in the test group ($P < .05$). After 12 weeks, the percentage of granules showing osteoid or bone formation was still greater in the test group than in the control group (49.6% ± 24.2% versus 36.5% ± 18.2%, $P > .05$). After 24 weeks, bone formation was at an advanced stage in both groups, and the percentages of the bone/osteoid–containing granules were quite similar: 70.9% ± 31.4% in the control group and 75.9% ± 41.5% in the test group ($P > .05$).

The bony fraction of the tissue-graft interface was histomorphometrically measured in the specimens and the means and SDs were calculated. Six weeks after grafting, the mean percentage of the outline of the granule in contact with bone was relatively low in the control group (15.4% ± 8.2%) but higher in the test group (31.2% ± 23.0%) ($P > .05$). Twelve weeks after surgery, the mean percentages were 26.3% ±15.0% in the control group and 39.3% ± 21.4% in the test group ($P > .05$). Twenty-four weeks after grafting, the percentage of the granule perimeter interfacing with bone was very high in both the control and test groups: 58.4% ± 24.7% and 61.2% ± 37.7%, respectively ($P > .05$).

The fact that the differences between the 2 groups proved to be insignificant can probably be explained by the low number of parallel samples and the relatively high SD values.

DISCUSSION

The question as to which material is most appropriate for the stimulation of bone regeneration is a subject of controversy. Excellent clinical results can be achieved in maxillofacial surgery by using autologous bone grafts.[3,4,11] Cancellous bone grafts meet the 2 basic requirements; they have both osteoconductive and osteoinductive capacities.[10] In osteoconduction, the graft material serves as a spacer and a conductive scaffold for newly formed bone. In contrast, in osteoinduction, the new bone formation is activated by inductive substances, such as special growth factors, which promote the differentiation of osteoprogenitor cells and the formation of a new capillary network.[15] However, the harvest of autogenous bone requires a second surgery, which increases the time demands and cost of the therapy and gives rise to considerable complications.[5]

Alloplastic materials, such as synthetically manufactured porous tricalcium phosphate, are available in unlimited supply. However, although they are osteoconductive, they lack osteoinductive properties. The basic demands made on bone substitutes are porosity and resorptive capacity; both are crucial for the success of these alloplastic substitutes.[8,10]

Cerasorb has a large-mesh, interconnecting pore system, which facilitates the spread of the osteogenic mesenchyma and the creeping of the otherwise nonmotile bone-forming osteoblasts. At the same time, the inner surface of the porous particles becomes greatly enlarged, which can positively influence the inward growth of the new bone.[11,13,16] In the present experiment, the histologic analysis revealed an intimate connection between the graft material and the newly formed bone.

The resorptive capacity or biodegradation of alloplastic materials is a further factor affecting the successful osseous penetration of the bone substitute material.[10] This so-called biodegradation should take place at the proper time, yielding space when new bone invades the granule. Long-lasting survival of the conducting graft material would hamper bone regeneration mechanically. Ideally, gradual graft resorption is followed by increased deposition of new bone. This was observed in the present study in both the test and control groups.

Cerasorb is capable of biodegradation by virtue of its chemical structure, though the mechanism at work is an unsettled question. Zerbo and associates[19] histologically observed an active osteoclastic resorption of this graft material. Further mechanisms, such as chemical dissolution, may also operate in the removal of these graft particles. The histologic findings of the present experiment did not support the active cellular biodegradation of Cerasorb particles.

The addition of certain growth factors has resulted in earlier bone regeneration and more mature bone production, even in cases of bone graft insertion.[15] Platelet-derived growth factor binds to endothelial cells to initiate the ingrowth of capillaries.[21] This is very advantageous, as sufficient vascularization is an absolute precondition to the osteogenetic process. Platelets can also release transforming growth factors (TGFβ1, TGFβ2), which bind to the endosteal osteoblasts and marrow stem cells to initiate their proliferation and to stimulate osteoid production.[15]

Cerasorb is an alloplastic material with no osteocompetent cells or stem cell population. In the present study, it was combined with autologous PRP to clarify whether the addition of PRP would enhance its advantageous properties. The present study justified the osteoconductive properties of the Cerasorb graft inserted alone, which led to the development of new bone formation both at the surface and in the pores of the granules. The results of the present study showed that this process could be facilitated by the addition of PRP, especially in the early stages of new bone formation. The histomorphometric data suggested earlier and more intensive bone regeneration in the test group in comparison with the control group. Six weeks after grafting, this difference proved to be significant. Proliferation of the osteogenic mesenchyma, the intragranular ingrowth of the capillaries, and the osteoid production in the early phases were more intensive in the test group because of the addition of autologous PRP. The percentage of the granules exhibiting peri- or intragranular bone deposition was significantly higher in the test group. The percentage of bone along the tissue-graft interface was also higher in the test group 6 and 12 weeks after surgery. However, after 24 weeks the quality and the measurable density of the bone in the graft insertion area were similar regardless of whether Cerasorb alone or Cerasorb with PRP had been applied. By this time, bone-formation activity was nearly equalized in the 2 groups. Similarly, Zechner and colleagues[22] found increased bone regeneration during early healing after PRP application in minipigs.

The rapidity of the graft resorption did not reveal measurable differences on the 2 sides. The histologic findings suggested early signs of resorption of the graft particles at 12 weeks postsurgery. New bone was formed both in the widened pore system of the granules and along their scalloped surfaces. After 24 weeks, high bone density in the grafted area was achieved through integration of the Cerasorb granules into the newly formed bone network. The graft resorption apparently occurred without any adverse biologic response.

These results confirm that the addition of PRP can be advantageous when grafting with Cerasorb. The combination of Cerasorb and PRP appeared to result in faster and more intensive new bone formation.

CONCLUSION

Conditions in animal experiments differ greatly from those encountered in humans.[1,6,8,13,22] The results of animal studies must therefore be treated with caution. A direct comparison of the bone regeneration process in canine and human mandibles is naturally impossible. The increase in bone density facilitated by a combination of Cerasorb and PRP grafting requires thorough study in humans.

However, the results of animal studies in this area are promising, as PRP can be obtained easily to enhance both the rate of bone formation and the stability of the new bone formed. In the future, such combined grafting can hopefully ensure earlier and more stable anchorage of dental implants.

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