Bulgarian Journal of Veterinary Medicine (2004), 7, No 1, 35–44

CLINICAL AND RADIOLOGICAL STUDIES UPON A COMBINED METHOD FOR GUIDED BONE REGENERATION IN EXPERIMENTAL MANDIBULAR DEFECTS IN DOGS – A PRELIMINARY COMMUNICATION

I. BORISSOV[1], N. UZUNOV[2] & M. PASKALEV[1]

[1]Faculty of Veterinary Medicine, Trakia University, Stara Zagora;
[2] Faculty of Dentistry, Medical University, Plovdiv; Bulgaria

Summary

Borissov, I., N. Uzunov and M. Paskalev, 2004. Clinical and radiological studies upon a combined method for guided bone regeneration in experimental mandibular defects in dogs – a preliminary communication. *Bulg. J. Vet. Med.*, **7**, No 1, 35–44.

Investigations were carried out on 12 mixed-breed dogs aged 18–24 months with an uniform body weight and gender, put under similar conditions of housing and feeding. In all dogs, 36 mandibular bone defects were produced by extraction of the second premolar and the first molar. Thus, 3 bone defects were created in each dog: one from the first premolar alveolus and two – corresponding to alveoli of both roots of the second molar. The defects were randomly assigned to 4 groups (groups A, B, C and D). Groups C and D were further divided into subgroups (C_1,C_2 and D_1,D_2). The first group of defects (group A) were covered only with mucoperiosteal flaps (MPF) whereas in groups B, C and D, several modifications of the combined method for guided bone regeneration were applied.

The treated bone defects were compared clinically and radiologically. After a 3-month period of survey, the best results were obtained in the group treated with partially demineralized bone matrix (pDBM), enzymatically processed and lyophilized dura mater (EPLDM) and mucoperiosteal flap (MPF). The placement of titanium screw (TS) dental implants did not impair the guided bone regeneration, that was confirmed by their good osteointegration.

Key words: clinical studies, dogs, guided bone regeneration, radiological studies, titanium screw dental implants

INTRODUCTION

The terms "guided bone regeneration" and "osteopromotion" are used for designation of the application of physical means over an anatomical zone in order to prevent the ingrowth of other tissues (especially new connective tissue) and their involvement in reparative bone regeneration (Linde *et al.*, 1991).

The concept of guided bone regeneration (GBR) is known since the 50-ties of the last century (Murray *et al.*, 1957). It is generally accepted that the formation of a new bone requires three factors: 1) a blood clot; 2) vital osteoblasts and 3) contact with vital tissues. The various fenestrated plastic membrane are used for GBR to cover and protect the blood clot, whose inside is filled with new bone.

The efficacy of the membrane technique was confirmed in rats and monkeys (Dahlin *et al.*, 1988, 1990). In those experiments, expanded polytetrafluoroethylene (ePTFE) is used as membrane material. Linde *et al.* (1991) and Schmidt *et al.* (1991) suggested that the osteopromotive technique had the capacity to create new bone in anatomical zones where it was not present, i.e. to induce neoosteogenesis. *Dura mater* is a natural material characterized with a slow resorption (over 6 months) and good fibroblastic adhesion and epithelial inhibition. It is dense enough and could be used for providing an extra space and increasing the alveolar ridge dimensions (Tsvetkov and Mechkarski, 1988).

Now, the membrane technique is used for bone formation around screw dental implants, for alveolar ridge restoration and in reconstructive cranioplastics (Hopper *et al.*, 1996; Eppley, 1997).

The most efficient method for recipient zone augmentation in screw dental implantation is the use of fresh autografts (Buser *et al.*, 1995; Fowler *et al.*, 2000). During the last years, however, their application is restricted due to several disadvantages and the implementation of osteoconductive and osteoinductive techniques for bone regeneration via application of allo- and xenografts.

The immediate application of screw dental implants in fresh postextraction alveoli, combined with osteopromotive technique, has considerable advantages from a clinical point of view. Thus, the alveolar ridge is protected from resorption, that would probably allow the later implantation of longer dental implants and the adjacent supporting bone is increased. Moreover, the immediate placement of screw dental implants with barrier membranes shortens the treatment time.

All those facts suggest that the application of methods based on the combination of the osteoinductive, osteoconductive and osteopromotive features of various transplants and implants is a valuable approach for the treatment of jaw defects and missing teeth restoration. Nevertheless, their practical implementation is not developed enough and not fully elucidated. That is why our study aimed to compare the effect of application of various elements of the combined technique for guided bone regeneration on experimental mandibular defects in dogs via radiography.

MATERIALS AND METHODS

The experiments were performed on 12 dogs weighing 16–18 kg, at the age of 18–24 months. Two weeks prior to the experiment they were treated against parasitic infections, housed and fed in individual cages under uniform conditions. In all animals, the second mandibular premolar and the first mandibular molar were unilaterally extracted. Thus, 3 post extraction bone defects were created in each dog: one from the first premolar alveolus and 2 – corresponding to alveoli of both roots of the second molar. The defects in all dogs (a total of 36 defects) were assigned to 4 groups (groups A, B, C and D).

Enzymatically processed and lyophilized *dura mater* (EPLDM) was used as membrane material, and partially demineralized bone matrix (pDBM) – as bone replacing material. They were prepared according to the original technologies of Tsvetkov and Mechkarski (1988) and Mechkarski (1991) in the Laboratory of Biopreparations for the Medical Practice at the Institute of Nutritional Technologies and Lyophilization, Sofia. The transplants were of human origin and xenogeneic for the experimental animals. Twelve standard titanium screw implants,Tapper-Lok type (Paragon - Sulzer-Dental, USA) were also used.

The different groups were treated as followed:

- Group A (n=6): untreated (control) post extraction dental alveoli. After the clotting of blood, the mucoperiosteal flap (MPF) was replaced and hermetically sutured.
- Group B (n=6): experimental defects, filled with pDBM and covered with MPF (pDBM+MPF).
- Group C (n=12): experimental defects, isolated with EPLDM and MPF, sutured over it. In half of those defects, the interradicular septum was evened out and a titanium screw (TS) was implanted. Thus, two subgroups were formed: C_1 (n=6) – treated with EPLDM+MPF and C_2 (n=6) – treated with EPLDM+MPF+TS.
- Group D (n=12): Experimental defects treated by the combined method of GBR. This group was also divided into two subgroups: D_1 (n=6) – where the defects were filled with pDBM and covered with EPLDM. The MPF was replaced and hermetically sutured (pDBM+EPLDM+MPF). Into the defects of the D_2 subgroup (n=6), titanium screws were placed into the alveolus or into the interradicular septum, then filled with pDBM, covered with EPLDM. The MPF was replaced and hermetically sutured (TS+pDBM+ EPLDM+MPF).

During the postoperative period, the animals were fed with soft food "Pleasure"- BG for a week, and thereafter – with commercial dry food "Canil Social Gouomarc H" – Brasilia and water *ad libitum*. The physical examinations (changes in body temperature, heart and respiratory rates) were performed daily. The local changes were observed via inspection and palpation. The changes in mandibular bone and bone defects were followed out through lateral radiography by post operative weeks 2, 4, 8 and 12 with a TUR – 800 X-ray unit.

RESULTS

General condition of experimental animals during the post operative period

From all 12 animals, 11 had no complications during the postoperative period. During the first week, the dogs accepted voluntarily the soft food and the water and afterwards – the dry food. During the first 3–4 days, the body temperature varied from 39.1 to 39.5 ^{o}C but the differences vs baseline values were not statistically significant. The heart and respiratory rates were within the normal ranges. In one dog from group D_2, mandibular osteomyelitis occurred.

Evaluation of operative wound status and the recovery

Hyperaemia and oedema. As early as the first post operative day, moderate to severe hyperaemia and oedema were observed. They persisted for 5-9 days, then gradually decreased and disappeared completely. The surrounding soft tissues and the gingiva were with a normal colour and structure.

Dehiscences. The overall number of dehiscences was 7 (19.4%). All dehiscences were partial. They were treated with conservative methods and healed secondarily within the 27th post operative day. Two of them were in the control group A. One dehiscence was observed in group B (pDBM+MPF); 2 – in group C_2 (TS+EPLDM) and 3 – in group D (2 in subgroup D_1 and 1 in subgroup D_2).

Six of all dehiscences epithelized completely. In one, a small fistula in the region of the implanted titanium screw occurred following the secondary epithelization (group D_2).

Suppurations. One suppuration was observed in group D_2. It appeared on the 4th post operative day and was present up to 18th day after the operation.

*Osteomyelitis***.** Clinically, osteomyelitis was seen in one of defects from group D_2. It was confirmed by the pathoanatomical and histopathological study.

Rejection of the bone xenograft. A partial rejection of the xenograft was observed in one post extraction alveolus from group D_2, where the osteomyelitic focus has then developed.

Loosening and rejection of titanium screws were not observed.

Radiological results

The radiographs obtained by post operative weeks 2, 4 and 8 did not exhibit any significant differences. By the 3rd post operative month, the radiological picture was as followed:

Group A (MPL). Three months after the operation, the defects in the MPF group were present or were filled with fibrous tissue, had irregular borders and were characterized with an insignificant new bone ingrowth from the interradicular septum (Fig. 1 and 2).

Group B (pDBM+MPF). Here, 90% of the defects were filled with newly formed bone tissue. The boundary between the interradicular septum and the new bone was hardly visible (Fig. 3).

Group C_1 (EPLDM+MPF). Seventy percentages of the postextraction defects were filled with a well visible connection of the new bone with the interradicular septum, both cranially and caudally (Fig. 4).

Group C_2 (EPLDM+MPF+TS). In this subgroup, the placement of TS did not result in any considerable difference in osteogenesis. Into the defects, an osteoinductive factor was lacking.

Group D_1 (pDBM+EPLDM+MPF). The defects, isolated with EPLDM were completely filled with newly formed bone.

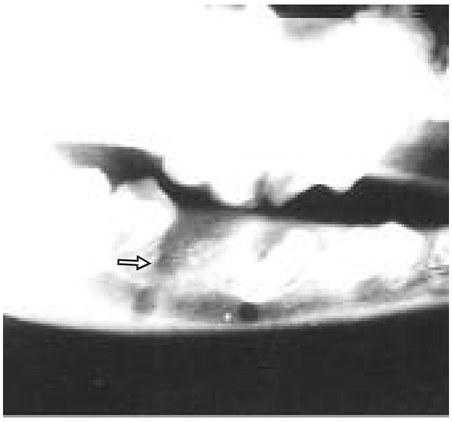

Fig. 1. Radiograph of the mandible in dog No 1, 3 months following the operation. The defect (arrow) is covered only with mucoperiosteal flap. No significant amount of newly formed bone is visible.

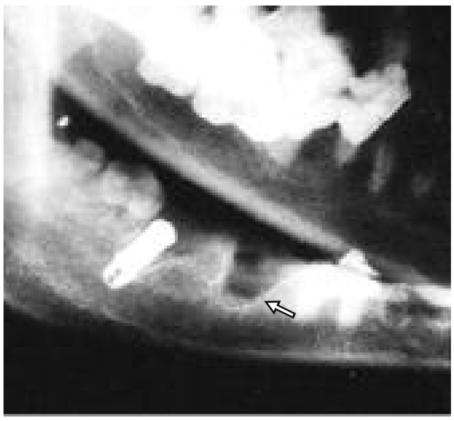

Fig. 2. Radiograph of the mandible in dog No 2, 3 months following the operation. The defect (arrow) is covered only with mucoperiosteal flap. No significant amount of newly formed bone is visible.

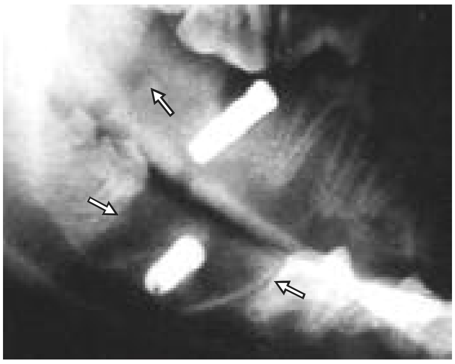

Fig. 3. Radiograph of the mandible in dog No 4, 3 months following the operation. The defect is treated with partially demineralized bone matrix and mucoperiosteal flap (upper arrow) and is almost entirely filled with newly formed bone tissue. Lower arrows show defects covered only with MPF with no signs of osteogenesis inside.

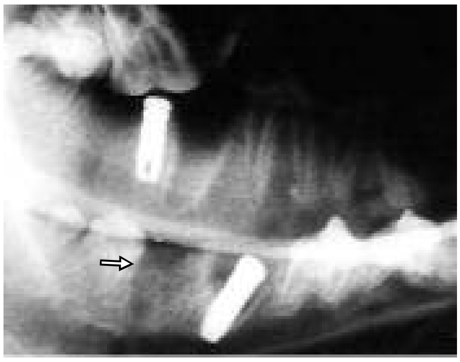

Fig. 4. Radiograph of the mandible in a dog, 3 months following the operation. The defect (arrow) is covered with enzymatically processed lyophilized *dura mater* and mucoperiosteal flap. The newly formed bone tissue is visible inside the defect.

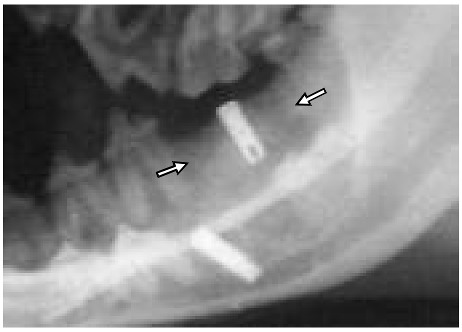

Fig. 5. Radiograph of the mandible in dog No 9, 3 months following the operation. The defects are treated with partially demineralized bone matrix, enzymatically processed and lyophilized *dura mater* and mucoperiosteal flap (arrows). They are entirely filled with new bone and the alveolar ridge is flattened.

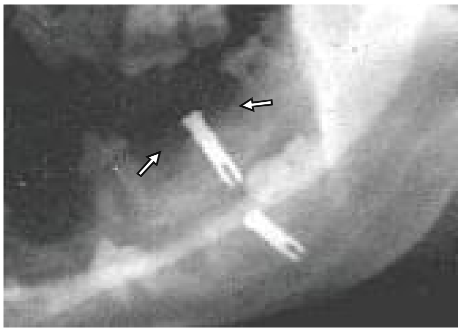

Fig. 6. Radiograph of the mandible in dog No 3, 3 months following the operation. The post extraction defects are treated with partially demineralized bone matrix and mucoperiosteal flap (arrows). They are filled almost entirely with new bone tissue.

The boundary between the host bone and the pDBM transplant was hardly visible. The alveolar ridge was well delineated and aligned to the adjacent one (Fig. 5). At the same time, the defects without EPLDM were with irregular alveolar ridge and were filled almost entirely with bone tissue (Fig. 3 and 6).

Group D_2 (TS+pDBM+EPLDM+ MPF). The placement of titanium screws imme-

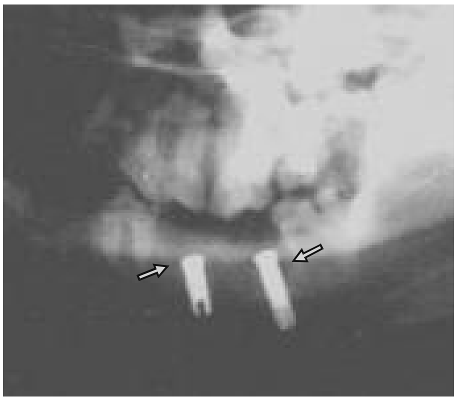

Fig. 7. Radiograph of the mandible in dog No 11, 3 months following the operation. The defects are treated with titanium screws, partially demineralized bone matrix, enzymatically processed and lyophilized *dura mater* and mucoperiosteal flap (arrows). Implants are well integrated into the newly formed bone tissue.

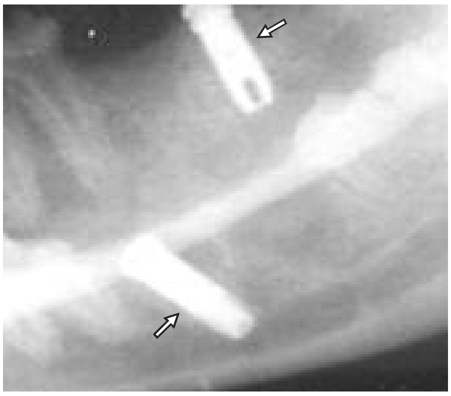

Fig. 8. Radiograph of the mandible in dog No 12, 3 months following the operation. The upper arrow shows a titanium implant placed into the interradicular septum and the lower arrow – a titanium implant placed into the dental alveolus. All defects are treated with partially demineralized bone matrix, enzymatically processed and lyophilized *dura mater* and mucoperiosteal flap. A good osteointegration (without peri-implant osteolysis zones) is visible.

diately after the teeth extraction and defect formation did not influence the new bone ingrowth regardless of the place of its implantation – into the alveolus or into the interradicular septum (Fig. 7 and 8). The osteointegration of the implant into the new bone could not be differentiated from that in the host bone (Fig. 8). The boundary between bone regenerate and the host bone was not visible.

On the basis of the clinical and radiological studies we established a good compatability of both screws and transplants in 11 of all dogs (91.6%).

DISCUSSION

Considering the general condition and the local clinical signs in the early and the late post operative period, the dogs supported well the surgical intervention. Despite that the experimental model for the formation of mandibular bone defects was very offensive, serious complications were not observed. The partial dehiscences and suppurations of buccal wounds were easily overcome, evidenced from the high percentage of successful results after the application of our combined method for GBR and TS implantation. The occuring complication in one dog from group D_2 (osteomyelitis) could be due to operative manipulations. This inflammation has not, however, resulted in screw loosening. Similarly, Persson *et al.* (2001) reported that after experimentally provoked peri-implantitis, titanim implants did not lose their stability. Therefore, our results showed a high efficacy of the treatment of mandibular bone defects with combined method of guided bone regeneration.

The best studied artificial membrane material is expanded polytetrafluoroethylene (ePTFE) (Dahlin *et al.*, 1990; Dahlin, 1993; Buser *et al.*, 1995; Nyman *et al.*, 1995, 2001). Its principal disadvantage is that it is not absorbable. This necessitated the development of synthetic (Becker *et al.*, 1996; Etienne *et al.*,1996; lu *et al.*,1996; Caffesse *et al.*,1997) or natural absorbable materials (Zhang *et al.*, 1999; Bohning *et al.*, 1999; Barboza, 1999; Fowler *et al.*, 2000; Giardino *et al.*, 2002). A disadvantage of natural membranes is their rapid absorption that does not allow enough time for bone tissue ingrowth in the empty space.

The human *dura mater* used by us was a good membrane material for guided bone regeneration of canine mandibular defects. In all defects where *dura mater* was placed under the mucoperiosteal flap, it provided the time required for new bone ingrowth. When the membrane was combined with pDBM, this occurred more rapidly and more efficiently.

The combined method for guided bone regeneration, applied by us, resulted in filling of bone defects and preservation of mandibular shape and dimensions. The newly formed bone tissue was of good quality and allowed a good osteointegration of titanium screws.

The loading of oral implants in dogs, when they are used as anchors of orthodontic materials results in a significant increase in hardness and density of alveolar bone ridge near the implants. The degree of osteointegration according to our studies however seemed not to be related to the load degree. According to Melsen and Lang (2001) and Gotfredsen *et al.* (2001) unloaded implants often preserve the bone features of the alveolar ridge.

Electron microscopic studies of titanium implants in dogs did not exhibit a statistical difference in the peri-implant bone volume due to compression and tension influences. This is an evidence for the maintenance of a rigid osteointegration that makes titanium implants suitable for orthodontic units (Saito *et al.*, 2000). The studies of Gotfredsen *et al.* (2001) hoever showed a better mineralization around the loaded titanium implants compared to unloaded ones. In our studies, the implants were not submitted to any load, but the results suggest that the good osteointegration would probably justify their application as a base of dental prostheses and orthodontic materials.

The titanium implants used by us, showed a good stability even in cases with clinically manifested inflammation. In none of them, loosening and rejection was observed. The placement of absorbable and non-absorbable biological membranes after surgical debridement and bone grafting in induced peri-implantitis with bone defect do not play a significant role upon bone-to-implant contact (Nociti *et al.*, 2001a).

In peri-implant bone defects, the vertical bone fill is better when treatment methods are combined with bone grafting (Nociti *et al.*, 2001b). Our studies also confirmed this fact. As to the osteointegration of titanium implants in newly formed bone, there is no difference between ceramic transplant-induced bone fill and that, induced by other forms of bone grafting – the osteoinductive properties of the graft and the fill of mandibular defects is thought to be sufficient (von Arx *et al.*, 2001). In all cases, the defects were filled more rapidly and with superior quality bone after isolation via biological membranes, with the exception of xenogeneic demineralized bone matrix (Cho *et al.*, 1998). The bone graft, tested in our experiment – pDBM, although xenogeneic, resulted in a good osteointegration of implants.

In conclusion, the approach of guided bone regeneration, applied independently or in combinations with implantation of titanium screw in dogs with experimental mandibular bone defects, tested in our studies, was efficient, well tolerated by animals, stimulated the osteogenesis and was characterized with an excellent osteointegration of implants in newly formed bone. It is promising and could be applied in veterinary dentistry and maxillo-facial surgery.

REFERENCES

Barboza, E. P., 1999. Clinical and histologic evaluation of the demineralized freeze-dried bone membrane used for ridge augmentation. *International Journal of Periodontics and Restorative Dentistry*, **19**, No 6, 601–607.

Becker, W., B. E. Becker, J. Mellonig, R.G. Caffesse, K. Warrer, J. G. Caton and T. Reid, 1996. A prospective multi-center study evaluating periodontal regeneration for Class II furcation invasions and intrabony defects after treatment with a bioabsorbable barrier membrane: 1-year results. *Journal of Periodontology*, **67**, No 7, 641– 649.

Bohning, B. P., W. D. Davenport and B. G. Jeansonne, 1999. The effect of guided tissue regeneration on the healing of osseous defects in rat calvaria. *Journal of Endodontics*, **25**, No 2, 81–84.

Buser, D., K. Dula, U. C. Belser, H. P. Hirt and H. P. Berthold, 1995. Localized ridge augmentation using guided tissue regeneration. II. Surgical procedure in the mandible. *International Journal of Periodontology & Restorative Dentistry*, **15**, 11–29.

Caffesse, R. G., L. F. Mota, C. R. Quinones and E. S. Morrison, 1997. Clinical comparison of resorbable and non-resorbable barriers for guided periodontal tissue regeneration. *Journal of Clinical Periodontology*, **24**, No 10, 747–752.

Cho, K. S., S. H. Choi, K. H. Han, J. K. Chai, U. M. Wikesjo and C. K. Kim, 1998. Alveolar bone formation at dental implant dehiscence defects following guided bone regeneration and xenogeneic freeze-dried demineralized bone matrix. *Clinical Oral Implants Research*, **9**, No 6, 419–428.

Dahlin, C., A. Linde, J. Gottlow, and S. Nyman, 1988. Healing of bone defects by guided tissue regeneration. *Plastic and Reconstructive Surgery,* **71,** 672–678.

Dahlin, C., J. Gottow, A. Linde and S. Nyman, 1990. Healing of maxillary and mandibular bone defects using a membrane technique. An experimental study in monkeys. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery*, **24,** 13–18.

Dahlin, C., 1993. Osteopromotion: Regeneration of bone by a membrane technique. PhD thesis, Department of Radiology, University Hospital of Gothenburg, Sweden.

Eppley, B. L., 1997. Potential for guided bone regeneration and bone graft fixation with resorbable membranes in pediatric craniofacial surgery. *Journal of Craniofacial Surgery*, **8,** 127–128.

Etienne, D., M. Davarpanah, C. Mattout, M. Kebir, P. Mattout, J. L. Giovannoli and P. Bouchad, 1996. Guided tissue regeneration: Advantage of resorbable synthetic membrane (Resolut) in the treatment of intraosseus defects. *Journal de Parodontologie & d'Implantologie Orale*, **15,** 1–11.

Fowler, E. B., L. G. Breault and G. Rebitski, 2000. Ridge preservation utilizing an acellular dermal allograft and demineralized freeze-dried bone allograft. *Journal of Periodontology*, **71,** No 8, 1360–1364.

Giardino, R., N. N. Aldini, M. Fini, G. Giavaresi and P. Torricelli, 2002. Enhanced guided bone regeneration with a resorbable chamber containing demineralized bone matrix. *Journal of Trauma*, **52,** No 5, 933–937.

Gotfredsen, K., T. Bergundh and J. Lindhe, 2001. Bone reaction adjacent to titanium implants subjected to static load. A study in the dog. *Clinical Oral Implants Research*, **12,** No 1, 1–8.

Hopper, R. A., J. H. Phillips and L. Hughes, 1996. Use of a fibrillar polylactic acid homopolymer in sheep cranial defects. *Journal of Craniofacial Surgery*, **7,** No 1, 32–35.

Linde, A., P. Alberius, C. Dahlin, K. Bjurstam and Y. Sundin, 1991. Osteopromotion: A soft-tissue exclusion principle using a membrane for bone healing and bone neogenesis. *Journal of Periodontology*, **8**, 16–23.

Lu, S., Z. Zhang and J. Wang, 1996. Guided bone regeneration in long bone. An experimental study. *Chinese Medical Journal*, **109,** No 7, 551–554.

Mechkarski, S. S., 1991. Creation, experimental evaluation and clinical application of Bulgarian biopreparations for the needs of medicine. PhD thesis, Research Institute of Cryobiology and Lyophilization, Sofia (BG).

Melsen, B. and N. P. Lang, 2001. Biological reactions of alveolar bone to orthodontic loading of oral implants. *Clinical Oral Implants Research*, **12,** No 2, 144–152.

Murray, G., R. Holden and W. Roachlau, 1957. Experimental and clinical study of new growth of bone in a cavity. *American Journal of Surgery*, **93,** 385–389.

Nociti, F. H., M. A. Machado, C. M. Stefani and E. A. Sallum, 2001a. Absorbable versus nonabsorbable membranes and bone grafts in the treatment of ligature-induced peri-implantitis defects in dogs – a histometric investigation. *International Journal of Oral and Maxillofacial Implants*, **16,** No 5, 646–652.

Nociti, F. H., R. G. Caffese, E. A. Sallum, M. A. Machado, C. M. Stefani and A. W. Sallum, 2001b. Clinical study of guided bone regeneration and/or bone grafts in the treatment of ligature-induced peri-implantitis defects in dogs. *Brazilian Dentistry Journal*, **12,** No 2, 127–131.

Nyman, R., L. Sennerby, S. Nyman and D. Lundgren, 2001. Influence of bone marrow on membrane-guided bone regeneration of segmental long-bone defects in rabbits. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery*, **35,** No 3, 239-246.

Nyman, R., M. Magnusson, L. Sennerby, S. Nyman and D. Lundgren, 1995. Membrane-guided bone regeneration. Segmental radius defects studied in rabbits. *Acta Orthopaedica Scandinavica*, **66,** No 2, 169–173.

Persson, L.G., I. Ericsson, T. Berglundh and J. Lindhe, 2001. Osseintegration following treatment of peri-implantitis and replacement of implant components. A experimental study in the dog. *Journal of Clinical Periodontology,* **28,** No 3, 258–263.

Saito, S., N. Sugimoto, T. Morohashi, M. Ozeki, H. Kurabayashi, H. Shimizu, K. Yamasaki, A. Shiba, S. Yamada and Y. Shibasaki, 2000. Endosseous titanium implants as anchors for mesiodistal tooth movement in the beagle dog. *American Journal of Orthodontics and Dentofacial Orthopedics*, **118,** No 6, 601–607.

Schmidt, J., C. H. F. Hammerle, H. Stich and N. P. Lang. 1991. Supraplant R, a novel implant system based on the principle of guided bone regeneration. *Clinical Oral Implants Research*, **2,** 199–204.

Tsvetkov, T. and S. Mechkarski, 1988. Tissue Transplants. Zemizdat, Sofia.

von Arx, T., D. L. Cochran, J. S. Hermann, R. K. Schenk, F. L. Higgimbottom and D. Buser, 2001. Lateral ridge augmentation and implant placement: an experimental study evaluating implant osseointegration in different augmentation materials in the canine mandible. *International Journal of Oral and Maxillofacial Implants*, **16,** No 3, 343–354.

Zhang, Q., K. Yao, L. Liu, Y. Sun, L. Xu, X. Shen and Q. Lai, 1999. Evaluation of porous collagen membrane in guided tissue regeneration. *Artificial Cells, Blood Substitutes and Immobilization Biotechnology,* **27,** No 3, 245–253.

Paper received 15.05.2003; accepted for publication 04.02.2004

Correspondence:
Assoc. Prof. Ivan Borissov
Department of Surgery,
Faculty of Veterinary Medicine,
Trakia University,
6000 Stara Zagora, Bulgaria