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EXPERIMENTAL STUDY ON GUIDED BONE REGENERATION IN CANINE SEGMENTAL ULNAR DEFECTS

M. PASKALEV[1], S. KRASTEV[1], SV. MECHKARSKI[2], J. PHILIPOV[1] & SV. DYAKOVA[2]

[1] Department of Veterinary Surgery, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, [2] Institute of Kryobiology and Food Technologies, Sofia; Bulgaria

Summary

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The present study aimed to determine the possibility of application of guided bone regeneration in experimental non-fixed ulnar defects in dogs. In 24 dogs, a total of 48 ulnar bone defects were created (24 left and 24 right). The dogs were allotted to 4 groups (6 animals each). The right defects were treated as followed: group I – the bone defects were filled with autocancellous bone; in group II – with allocancellous bone; in group III – with xenocancellous bone and in group IV – with xenocancellous bone and bioceramics. The defects of left ulnas were treated similarly and isolated from adjacent soft tissues with enzymatically processed and freeze-dried canine dura mater. Both ends of the ulna were not fixed by osteosynthesis. In the post operative period, radiological and clinical surveys were performed immediately after the surgery and 1, 2, 4 and 6 months after treatments. The guided bone regeneration using processed dura mater slowed down the osteogenesis in segmental non-fixed canine ulnar defects regardless of the type of used osteoinductor graft.

Key words: bone defects, bone regeneration, bone transplants, dog

INTRODUCTION

The primary aim of guided bone regeneration (GBR) is to reduce as much as possible the involvement of adjacent tissues in the process of bone healing. This is achieved via the so-called biological membranes (natural or artificial).

The most extensively studied artificial membrane material is expanded polytetrafluoroethylene (ePTFE) (Nyman *et al.,* 2001). Its principal disadvantage is its non-absorbability. This necessitates the creation of synthetic (polylactate, polyglycolate, carboxymethylcellulose, silicone etc.) or natural absorbable materials (collagen membranes, dura mater, fascia lata etc.) (Barboza, 1999; Bohning *et al.,* 1999; Zhang *et al.,* 1999; Fowler *et al.,* 2000; Giardino *et al.,* 2002). The natural membranes are however characterized by a rapid absorption that does not provide the necessary time for bone tissue ingrowth in the empty space.

The isolation of bone defects could be combined with autotransplantation or application of other bone replacing materials (Marti *et al.,* 2001; Nociti *et al.,* 2001a; Nociti *et al*., 2001b).

The guided bone regeneration in animals is well studied in maxillar or mandibular defects in dogs (Zhang *et al.,* 1999; Nociti *et al.,* 2001a; Nociti *et al.,* 2001b; Borissov *et al.,* 2004). The literature data reporting the effect of GBR in tubular bone defects are however few.

The aim of the present study was the investigation of application of GBR in experimental non-fixed ulnar defects in dogs.

MATERIALS AND METHODS

Animals

Twenty four mixed-breed dogs aged 2–7 years and weighing 12–20 kg were used. The animals were obtained from a licensed kennel and after the end of the experiment, were returned back to it. The dogs were male, intact and previously freed from parasites. They were housed in individual boxes and fed with a commercial dry canine food for adult dogs (Jambo-dog, Gallisman-94 S.A., Bulgaria) and received water *ad libitum*. Prior to the experiment, all dogs were treated against ecto- and endoparasites.

The experiment was approved by the Committee on Animal Experimentation at the Trakia University, Stara Zagora, Bulgaria and was performed according to Animal Welfare Act № 25/10.06.05 and the The Law on Veterinary and Medical Activities.

Materials

Collection of fresh autocancellous bone (AuCB). Fresh AuCB was obtained during the operation from the proximal tibial metaphyse using a medial approach and bone trepane.

Collection of allogeneic canine bone, dura mater and xenogeneic human bone. Six dogs, road accidents victims, were euthanized with the consent of their owners and examined for acute infectious diseases. After dissection of skulls, dura mater was removed, put into physiological saline and then, in individual bags, deeply frozen until the subsequent processing. The vertebral bodies of the same dogs were collected. Vertebral cancellous bone was removed in the form of blocks that were also deeply frozen until processing. Human bones were obtained from cadavers, that did not suffer from any infectious disease. The donor blood was preliminarily tested for AIDS, lues and hepatitis.

Preparation of bone allogeneic and xenogeneic transplants. The technology was adapted from human medicine (Mechkarski, 1991) using canine allocancellous and human xenocancellous bone processed as followed:

- Stage 1: defattening with chloroform and ethanol;
- Stage 2: partial demineralization with 1% HCl;
- Stage 3: freezing at –20 ^{o}C;
- Stage 4: freeze drying;
- Stage 5: grinding to particles of 0.6 mm;
- Stage 6: dosing and packing in 1 g flasks;
- Stage 7: sterilization with gamma rays.

Preparation of xenotransplants and bioceramics (BC). BC was obtained as followed:

- Stage 1: Preparation of source material. Cancellous bone from healthy calves was used. The bone was mechanically cleaned from muscles and bone marrow and cut into 1×1×2 cm pieces. The latter were abundantly

washed with distilled water until the complete removal of blood.

- Stage 2. Defattening. Bone material was extracted three times with chloroform:ethanol (1:1) and washed with distilled water until the complete removal of chloroform.
- Stage 3. Deproteinization. Defattened cancellous bone was processed with 20% and 10% hydrogen peroxide solutions at 35–37°C for 24–48 h.
- Stage 4. Thermal processing. The bone material was heated to 800°C for 1 h for the complete elimination of proteins and other organic substances and to obtain low crystalline natural hydroxyapatite.
- Stage 5. Grinding and mixing. Deproteinized bovine bone was grinded to 0.2–0.8 mm particles and was mixed with previously prepared partially demineralized xenogeneic human cancellous bone.
- Stage 6 . Dosing and packing in 1 g flasks.
- Stage 7. Gamma ray sterilization (25 kGy, 17–18 °C).

Preparation of the biological membrane (canine dura mater, CDM). The procedure included the following stages:

- Stage 1. Washing, mechanical cleansing and formation of transplants into desired dimensions.
- Stage 2. Enzymatic processing of transplants with 0.4% tripsin solution (Fluka, Germany) in phosphate buffer (pH 7.2–7.4) at 35–38 °C for 3 h. Then the material was profusely washed with distilled water. Thus, the globular proteins were removed.
- Stage 3. Chemical processing of transplants. The processed dura mater was immersed in citric acid (pH=3.0) for neutralization and additional tripsin removal. Afterwards, the transplants were washed with distilled water to neutral pH and treated with 3% hydrogen peroxide solution for 1 h at room temperature. Transplants were thrice washed with distilled water and deeply frozen at –35 to –40 °C.
- Stage 4. Vacuum sublimation drying.
- Stage 5. Packing. The processed dura mater was packed in paper in the form of white freeze-dried rectangle stripes with dimensions of 4 × 5; 6 × 8 and 4 × 10 cm.
- Stage 6. Gamma ray sterilization (^{137}Cs source, 25 kGy, 17–18 °C).

Experimental design

All dogs were submitted to double transperiosteal osteotomy of both ulnar diaphyses for creation of defects whose length was 1.5 times bigger than the bone width. The anaesthetic protocol included premedication with 0.02 mg/kg s.c. atropine sulfate (Sopharma, Bulgaria) and 0.05 mg/kg i.m. acepromazine maleate (Neurotranq, Alfasan, Woerden, Holland), induction with 6 mg/kg i.v. 2.5% thiopentone sodium (Thiopental, Biochemie GmbH, Kudl, Austria), intubation and maintenance of general anaesthesia with 2.5 vol% halothane (Narcotan, Spofa, Czech Republic).

The dogs were allotted to 4 groups (6 animals each). The bone defects in the different groups were treated according the scheme presented in Table 1.

In all groups, the aim was to achieve a good contact between transplants and host bone edges. In treatments where dura mater was used, it isolated the defects beyond both host bone edges at a distance of 0.5 cm apart from the osteotomy site. The soft tissues were sutured routinely. Postoperatively, the animals were managed for pain, as required, with butorphanol tar-

Table 1. Experimental approach and bone replacing materials applied in the treatment of segmental ulnar bone defects in dogs from the different groups

Groups	Treatment	
	Right ulnar defect	Left ulnar defect
Group I	*AuCB* fresh autocancellous bone (AuCB), obtained from the proximal tibial metaphyse, medially to the tibial crest (n=6)	*AuCB+CDM* the defect was isolated from adjacent soft tissues with enzymatically processed and freeze-dried canine dura mater (CDM) and then filled with fresh AuCB (AuCB+CDM) (n=6)
Group II	*AlCB* demineralized and freeze-dried allocancellous bone (AlCB) (n=6)	*AlCB+CDM* the defect was isolated from adjacent soft tissues with enzymatically processed and freeze-dried canine dura mater (CDM) and then filled with AlCB (AlCB+CDM) (n=6)
Group III	*XeCB* demineralized and freeze-dried xenocancellous bone (XeCB) in the form of 0.2-0.6 mm granules (n=6)	*XeCB+CDM* the defect was isolated from adjacent soft tissues with enzymatically processed and freeze-dried canine dura mater (CDM) and then filled with XeCB (XeCB+CDM) (n=6)
Group IV	*XeCB+BC* demineralized and freeze-dried xenocancellous bone (XeCB) and bioceramics (BC) (n=6)	*XeCB+BC+CDM* the defect was isolated from adjacent soft tissues with enzymatically processed and freeze-dried canine dura mater (CDM) and then filled with XeCB and BC (XeCB+BC+CDM) (n=6)

trate (Torbutrol, Fort Dodge,USA) at 0.2 mg/kg, s. c. Intramuscular antibiotic combination lincomycin 50 mg and spectinomycin 100 mg per mL (Linco-Spectin, Pharmacia N.V./S.A., Puurs, Belgium) was administered at 1 mL/5 kg for four days following surgery.

During the first ten post operation days, a general physical examination of dogs was performed and the surgical status with regard to the condition of the wound, the type and degree of lameness was monitored on a daily basis. In the consequent period to the end of the study, these examinations were done once weekly.

The bone union (BU) and bone formation (BF) were radiographically assessed using the scoring system of Johnson *et al.* (1996). The bone union of each transplant to the proximal and distal host bone edges was evaluated with 0 to 3 points each, so that the maximum value of this parameter ranged between 0 and 6 points. The bone

formation was evaluated with 0 to 4 points. The maximum points of both parameters (BU+BF) was from 0 to 10.

Data were statistically processed by one-way ANOVA.

RESULTS

Clinical observations

During the postoperative period, dogs did not show any deviations in parameters characterizing the general condition. Body temperature, respiratory and heart rates were within the reference range. The appetite was normal.

In the region of both forearms there was a moderate, slightly painful and temperated oedema that disappeared within 4–6 days. In 4 dogs however (2 from group I and 2 from group IV), a superficial suppuration of one suture (in 3 dogs) and 3 sutures (in 1 dog) was noticed. During the first postoperative days the dogs

Table 2. Bone union and bone formation scores assessed on radiographs of dogs with segmental bone ulnar defects following different treatments. Values are expressed as means ± SEM from six experiments

	Bone union				Bone formation			
	post operative months				post operative months			
	1	2	4	6	1	2	4	6
AuCB	3.33 ± 0.50	5.17± 0.40	5.83± 0.17	6.00 ± 0.00	3.83± 0.17	3.83± 0.17	2.33± 0.50	1.17± 0.40
AuCB+ CDM	0 ± 0***	1.67± 0.62***	2.50± 0.99*	3.67 ± 1.2	1.17± 0.48**	1.50± 0.62*	1.17± 0.40	0.83± 0.40
AlCB	1.33 ± 0.50	2.33± 0.33	2.50± 0.84	2.67± 0.33	2.17± 0.17	2.50± 0.34	1.67± 0.42	1.83± 0.40
AlCB+ CDM	0 ± 0$^{\&}$	0.83± 0.65$^{\&}$	0.83± 0.65$^{\&}$	0.83± 0.65	0± 0$^{\&\&\&}$	0.67± 0.33$^{\&\&}$	0.67± 0.33	0.50± 0.34$^{\&}$
XeCB	0.67± 0.21	1.50± 0.56	1.50± 0.56	1.83± 0.54	1.33± 0.50	1.33± 0.50	1.33± 0.33	1.17± 0.31
XeCB+ CDM	0±0 ^{a}	0 ± 0 ^{a}	0 ± 0 ^{a}	0 ± 0 ^{a}	0±0 ^{a}	0±0 ^{a}	0±0 ^{b}	0±0 ^{a}
XeCB+ BC	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0±0	0±0	0±0	0±0
XeCB+ CDM+BC	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2.33± 0.21$^{\#\#\#}$	2.50± 0.22$^{\#\#\#}$	2.33± 0.21$^{\#\#}$	2.33± 0.21$^{\#\#}$

AuCB = autocancellous bone; AlCB = allocancellous bone; XeCB = xenocancellous bone; CDM= canine dura mater; BC = bioceramics; * P<0.05; ** P<0.01; ***P<0.001 between AuCB and AuCB+ CDM; $^{\&}$ P<0.05; $^{\&\&}$ P<0.01; $^{\&\&\&}$ P<0.001 between AlCB and AlCB+CDM; ^{a} P<0.05; ^{b} P<0.01; ^{c} P<0.001 between XeCB and XeCB+CDM; $^{\#}$ P<0.05; $^{\#\#}$ P<0.01; $^{\#\#\#}$ P<0.001 between XeCB+BC and XeCB+BC+CDM.

remained recumbent, then manifested a moderate to acute weight-bearing lameness that was present for one week and then, the function of limbs was restored.

Radiological survey

The results of serial radiography are presented in Table 2 and Fig. 1-4.

Application of autocancellous bone vs autocancellous bone and canine dura mater (AuCB vs AuCB+CDM). By postoperative months 1 and 2, the bone healing score was significantly higher in defects filled with AuCB compared to AuCB+CDM ($P<0.001$). The same was true for postoperative month 4 ($P<0.05$), but by month 6 the difference was not statistically significant. All six defects filled with AuCB were completely healed by the end of the experiment, whereas 3 defects treated with AuCB+CDM healed both proximally and distally, 1 defect only distally and another 2 showed no healing at all. Bone formation was more expressed in AuCB defects, where the difference vs AuCB+CDM was statistically significant in months 1 and 2. In both treatments, new bone formation was the most obvious up to the end of month 2. Thereafter an initiation of an inverse resorption and remodelling of new bone could be seen radio-

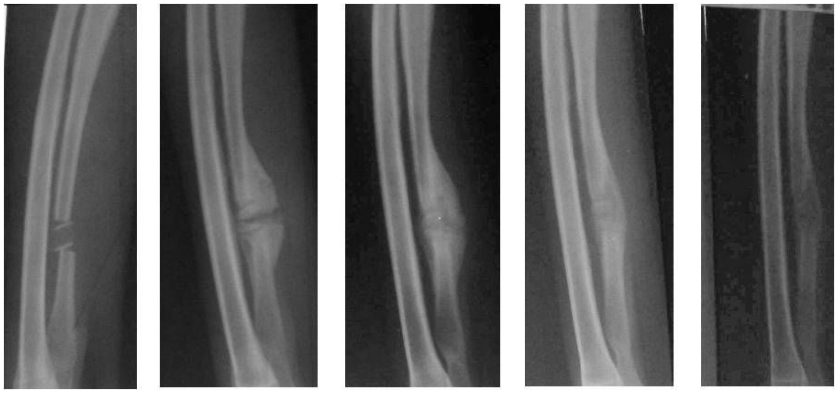

Fig. 1. (from left to right): Radiographs of canine ulna immediately after transplantation of autocancellous bone (AuCB) and by post transplantation weeks 4, 8, 16 and 36.

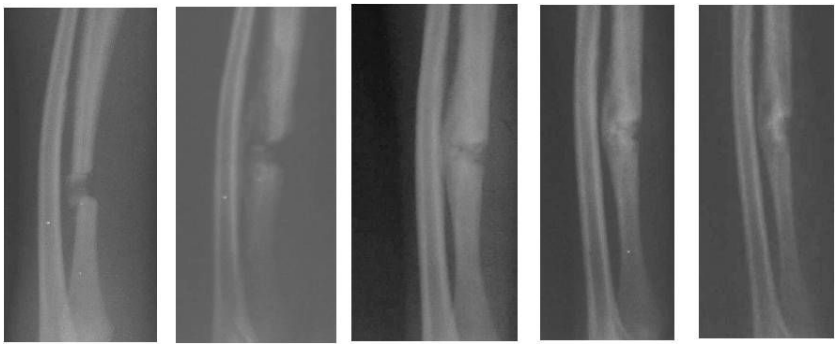

Fig. 2. (from left to right): Radiographs of canine ulna immediately after transplantation of autocancellous bone and dura mater (AuCB+DM) and by post transplantation weeks 4, 8, 16 and 36.

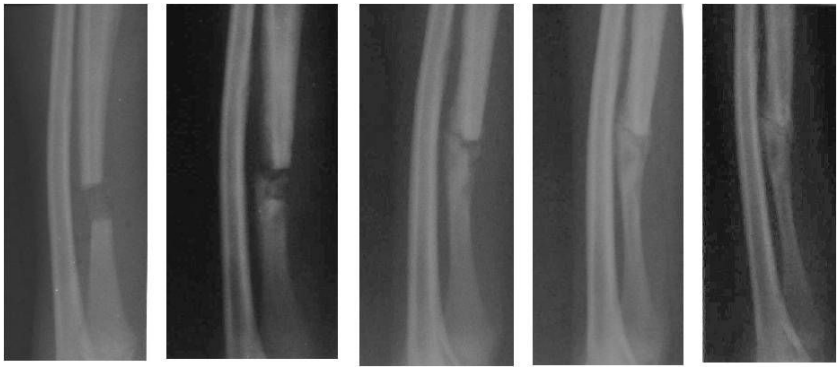

Fig. 3. (from left to right): Radiographs of canine ulna immediately after transplantation of allocancellous bone (AlCB) and by post transplantation weeks 4, 8, 16 and 36.

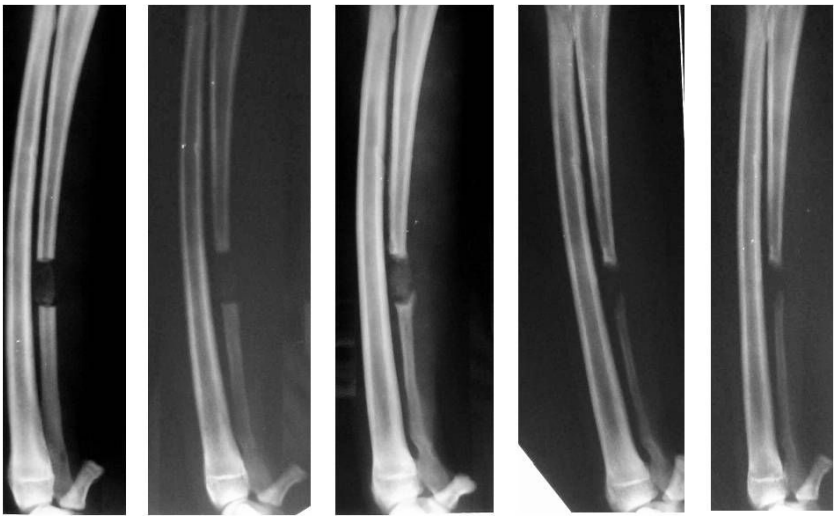

Fig. 4. (from left to right): Radiographs of canine ulna immediately after transplantation of allocancellous bone and dura mater (AlCB+DM) and by post transplantation weeks 4, 8, 16 and 36.

graphically (Fig. 1 and 2).

Application of demineralized and freeze-dried allocancellous bone vs demineralized and freeze-dried allocancellous bone and canine dura mater (AlCB vs AlCB+CDM). Bone healing was better in AlCB group compared to the AlCB+CDM group by post operative months 1, 2 and 6 ($P<0.05$). By the end of the 6th month, 5 out of 6 transplants in the AlCB group were only distally healed, the proximal osteotomy line was open while in the AlCB+CDM group none of transplants was healed (Fig. 3 and 4). Bone formation scores were also higher in AlCB group by months 1 ($P<0.001$), 2 ($P<0.01$) and 6 ($P<0.05$).

Application of demineralized and freeze-dried xenocancellous bone vs demineralized and freeze-dried xenocancellous bone and canine dura mater (XeCB vs XeCB+CDM). The bone healing and

bone formation scores for defects treated with XeCB+CDM were equal to zero. In bone defects that were not isolated with dura mater, bone formation was induced adjacently to host bone edges. The differences between both treatmen groups were significant ($P<0.05$), but the newly formed bone was not sufficient for healing of defects. The partially demineralized xenotransplants were radiologically visible at the beginning of the trial and in isolated defects were visible up to the end of the 6^{th} month, whereas in the XeCB group, they were gradually resorbed and by the end of the experiment completely dissolved.

Application of demineralized and freeze-dried xenocancellous bone and bioceramics vs demineralized and freeze-dried xenocancellous bone, bioceramics and canine dura mater (XeCB+BC vs XeCB+BC+CDM). In both groups, there were no signs of bone healing of transplant to host bone up to the end of postoperative month 6. The bone formation was significantly better in the XeCB+BC+DM group ($P<0.001$). However, the observed bone ingrowth was not sufficient for an adequate filling of defects and bone edge healing and clear atrophy was noticed.

DISCUSSION

The bone defect model on canine ulna is frequently used for studies on bone regeneration. The ulna is a tubular bone, that is indispensable from a biomechanical point of view because the radius provides a stability of the antebrachium and is the principal weight-bearing bone. The dimensions of defects used in our study (1.5 times bigger than bone diameter) are critical for tubular bones. It is shown that they could not heal spontaneously if left empty. Such defects have been used in various studies with bone transplants and bone replacement materials (Cook *et al.,* 1998; Itoh *et al.,* 1998). In some studies, various methods of ulna fixation were applied and good and excellent results were reported (Cook *et al.,* 1998). Tuominen *et al.* (2000) used two techniques of fixation (intramedullar osteosynthesis and plate and screw osteosynthesis) but with a contradictory effect because the fixation devices broke several weeks later. In all cases, however, the stability during the first weeks post osteosynthesis was crucial. In our experiments, we did not fix the bone edges and this was probably the cause for the unfavourable effect upon transplants with a minor osteogenic effect. The locomotion in the region of bone defect, although minimal, could also compromise bone healing. Moreover, the dogs included in our study were not haused in cages, but were allowed to move freely in big boxes.

In order to achieve more differentiated interpretation of radiological findings various scoring systems are used. Although not ideal, they allow to perform a better comparison using figures instead of a mere description. The Johnson's *et al.* (1996) scoring system used in this study, ensured the possibility bone healing and bone formation to be scored separately and then summed up.

It is known that the osteogenic effect of fresh cancellous autografts is the best, because cellular elements (osteoblasts and osteoclasts) survive the operation and combine with those of the host bone and non-differentiated mesenchymal cells of the adjacent soft tissues, thus producing a new bone relatively early (Wippermann *et al.,* 1999; Touminen *et al.,* 2000). The isolation of defects from soft tissues with dura mater, however, limits those opportunities. Furthermore, the ulna, being one

of the thinner tubular bones, provided the least contact of transplants with bone marrow elements of bone defect edges. This hypothesis was confirmed in all groups treated with CDM, compared to groups without CDM. In the former, the osteogenesis was very low or lacking completely, with the exception of groups where BC was added. In them, the opposite effect was observed, but only with regard to bone formation.

Demineralized bone tissue participates in osteogenesis via osteoinduction (Cho *et al.,* 1998; Barboza, 1999). However, biological membranes (dura mater in our case) restrict the contact of transplant and mesenchymal cellular elements of soft tissues. Those cells take an insignificant part in bone ingrowth from the host bone because in canine ulna the contact area is very small (Itoh *et al.,* 1998). Thus, this explains the poorer results obtained in groups where demineralized cancellous allo- and xenografts combined with CDM were applied. In jaw bone defects, this phenomenon was not reported. In all reported cases, jaw bone defects were filled faster and with a better quality bone after isolation with biological membranes (Stentz *et al.,* 1997; Caplanis *et al.,* 1998; Zhang *et al.,* 1999), with the exception of xenogeneic demineralized bone matrix (Cho *et al.,* 1998). In jaw bones, the site of contact of transplants with fully cancellous host bone is much larger, so the osteoinduction could occurs far more easily.

Hydroxyapatite ceramics is used as bone replacement material and is reported to stimulate osteogenesis via osteoconduction, to be compatible with host bone (Johnson *et al*., 1996; Marcacci *et al.,* 1999) and not to induce inflammation (Johnson *et al.,* 1996; Marcacci *et al.,* 1999). Wippermann *et al.* (1999) observed that the addition of hydroxyappatite granules to autogenous bone marrow enhances bone ingrowth in a 3.5 cm defect in ovine tibias. The mix of hydroxyapatite bioceramics with autocancellous bone or autologous bone marrow in the same model of bone defects resulted in considerable improvement of radiological and biomechanical parameters of ingrown bone (Johnson *et al.,* 1996). In our studies, the combination of demineralized and freeze-dried XeCB+BC showed that both bone replacement materials did not complement one another, although they participated in osteogenesis via two different mechanisms. Moreover, the results obtained in the XeCB and XeCB+BC groups suggested that hydroxyapatite BC inhibited or slowed down osteoinductive properties of xenografts in our model of bone defects.

It is known that dura mater is a good membrane material. It is resorbed relatively slowly and could be used for study of guided bone regeneration in bone defects where the processes occurred more slowly. Canine dura mater is thinner than human, but our technology, adapted from human medicine, allowed its processing. The end product permitted isolation of tubular bone defects after rehydratation and suturing. Being an allogenous graft, it is well tolerated, without signs of inflammation or rejection.

It could be summarized that in jaw bones, the filling of defects isolated with biological membranes occurred more rapidly and with a better quality bone (Zhang *et al.,* 1999). When guided bone regeneration was applied in tubular bones, as in our study, bone marrow must be allowed to grow freely into the defect. It is a source of polypotent cells (Nyman *et al.,* 2001). This condition was present in our study, but the amount of canine ulnar bone marrow was relatively low and thus it was

probably one of the causes for worse results in groups, where the GBR technique with dura mater tolerated was used. Another cause could be the granulated composition of transplants. In previous studies of ours using the same experimental design, xenocancellous grafts were used under the form of demineralized xenocancellous pieces and the results observed for the same time period were better (Paskalev, 1997). Most probably tolerated, in bones with a larger bone marrow channel the GBR technique would yield more promising results. The application of xenograft and GBR in segmental canine ulnar defects in our studies corresponded to the results of Cho *et al.* (1998) in mandibular canine defects, where osteogenesis was lacking or very weak.

In conclusion, it could be assumed that guided bone regeneration using processed dura mater impeded osteogenesis in segmental non-fixed canine ulnar defects regardless of the type of used osteoinductor graft.

REFERENCES

Barboza, E. P., 1999. Clinical and histologic evaluation of the demineralized freeze-dried bone membrane used for ridge augmentation. *International Journal of Periodontology and Restorative Dentistry,* **19**, 601–607.

Bohning, B. P., W. D. Davenport & B.G. Jeansonne, 1999. The effect of guided tissue regeneration on the healing of osseous defects in rat calvaria. *Journal of Endodontics,* **25**, 81–84.

Borissov, I., N. Uzunov & M. Paskalev, 2004. Clinical and radiological studies upon a combined method for guided bone regeneration in experimental mandibular defects in dogs **–** a preliminary communication. *Bulgarian Journal of Veterinary Medicine,* **7***,* 35–44.

Caplanis, N., M. B. Lee, G. J. Zimmerman, K. A Selvig & U. M. Wikesjo, 1998. Effect of allogenic freeze-dried demineralized bone matrix on guided tissue regeneration in dogs. *Journal of Periodontology*, **69,** 851–856.

Cho, K. S., S. H. Choi, K. H. Han, J. K. Chai, U. M. Wikesjo & 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***,* 419–428.

Cook, S. D., G. S. Baffes, M. W. Wolfe, T. K. Sampath & D. C. Rueger, 1998. Recombinant human bone morphogenic protein-7 induces healing in a canine long-bone segmental defect model. *Clinical Orthopaedics and Related Research,* **301***,* 302–312.

Fowler, E. B., L. G. Breault & G. Rebitski, 2000. Ridge preservation utilizing an acellular dermal allograft and demineralized freeze-dried bone allograft. *Journal of Periodontology,* **71***,* 1360–1364.

Giardino, R., N. Aldini, M. Fini, G. Giavaresi & P. Torricelli, 2002. Enhanced guided bone regeneration with a resorbable chamber containing demineralized bone matrix. *Journal of Trauma,* **52**, 933–937.

Itoh, T., M. Mochizuki, R. Nishimura, S. Matsunaga, T. Kadosawa, S. Kokubo, S. Yokota & N. Sasaki, 1998. Repair of ulnar segmental defect by recombinant human bone morphogenetic protein-2 in dogs. *Journal of Veterinary Medical Science,* **60**, 451–458.

Johnson, K. D., K. E. Frierson, T. S. Keller, C. Cook, T. Scheinberg, J. Zerwekh, L. Meyers & M. F. Sciadini, 1996. Porous ceramics as bone graft substitutes in long bone defects: A biomechanical, histological and radiographic analysis. *Journal of Orthopaedic Research,* **14**, 351–356.

Marcacci, M., E. Con, S. Zaffagnini, R. Giardino, M. Racca, A. Corsi, A. Benvenuti, P. Bianco, R. Quarto, I. Martin, A. Muraglia & R. Cancedda, 1999. Recon-

struction of extensive long-bone defects in sheep using porous hydroxyapatite sponges. *Calcified Tissue International,* **64**, 83–90.

Marti, J. M., W. S. Nalley & D. R. Wallace, 2001. Guided bone regeneration in the treatment of a compound odontoma in a dog. *Veterinary Comparative Orthopaedics and Traumatology,* **13**, 204–207.

Mechkarski, Sv., 1991. Creation, experimental study and clinical application of Bulgarian biopreparations for the medical practice. PhD thesis. National Institute of Cryobiology and Liophylization, Sofia, Bulgaria.

Nociti, F. H., R. G. Caffese, E. A. Sallum, M. A. Machado, C. M. Stefani & A. W. Sallum, 2001a. Clinical study of guided bone regeneration and/or bone grafts in the treatment of ligature-induced periimplantitis defects in dogs. *Brazilian Dentistry Journal,* **12**, 127–131.

Nociti, F. H., M. A. Machado, C. M. Stefani & E. A. Sallum, 2001b. Absorbable versus nonabsorbable membranes and bone grafts in the treatment of ligature-induced periimplantitis defects in dogs – a histometric investigation. *International Journal of Oral and Maxillofacial Implants,* **16**, 646–652.

Nyman, R., L. Sennerby, S. Nyman & D. Lundgren, 2001. Influence of bone marrow on membrane-guided bone regeneration of segmental long-bone defects in rabbits. *Scandinavian Journal of Plastic Reconstructive Surgery and Hand Surgery,* **35**, 239–246.

Paskalev, M. D., 1997. Studies on fractures in dogs and cats and the treatment of postfracture complications by bone grafting. PhD thesis, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria.

Stentz, W. C., B. L. Mealey, P. V. Nummikoski, J. C. Gunsolley & T. C. Waldrop, 1997, Effect of guided bone regeneration around commercially pure titanium and hydroxyapatite-coated dental implants. I. Radiographic analysis. *Journal of Periodontology*, **68**, 199–208.

Touminen, T., T. Jasma, T. Tuukkanen, T. S. Lindholm & P. Jalovaara, 2000. Fresh tubular long bone autografts and allografts in the healing of a canine ulnar defect fixed with intramedullary Kirschner wire. *Journal of Musculoskeletal Research,* **4**, 55–62.

Wippermann, B., C. Donow, H. Schratt, F. den Boer, T. Blokhuis & P. Patka, 1999. The influence of hydroxyapatite granules on the healing of a segmental defect filled with autologous bone marrow. *Annales Chirurgiae et Gynaecologiae,* **88**, 194–197.

Zhang, Q., K. Yao, L. Liu, Y. Sun, L. Xu, X. Shen & Q. Lai, 1999. Evaluation of porous collagen membrane in guaded tissue regeneration. *Artificial Cells, Blood Substitutes and Immobilization Biotechnology,* **27**, 245–253.

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