This paper reports on an experimental animal study evaluating a method of mandibular reconstruction in dogs using autogenous corticocancellous bone graft and fibrin glue. Eight animals underwent a resection at the mandibular body and primary reconstruction was carried out using osteosynthesis plates and screws. The defect was bridged with an autogenous particulate bone graft from the anterior iliac crest. To accelerate bone healing, fibrin glue (FG) was mixed with particulate bone graft. The hypothesis of this study was based on the presumption that bone healing, in segmental reconstruction of the dog mandible with a particulate cancellous bone graft mixed with FG, would be successful. All dogs had eventful healing. The histological results were less favorable with regard to bone remodeling than the results obtained in similar experiments with a particulate corticocancellous bone graft.

Key words: corticocancellous bone graft, fibrin glue, experimental animals, mandibular reconstruction

INTRODUCTION

The present study is part of an ongoing project in which we wish to achieve reconstruction of the mandible that comes as close as possible to the original contour of the resected part of the bone. The study on the use of a cortical scaffold filled with a particulate cancellous anterior iliac crest bone graft in combination with platelet rich plasma (PRP), provided insight to the contribution of PRP to the healing of the bone graft. Radiological and histological evaluation showed that addition of PRP considerably improved bone-healing (Fennis et al., 2002, Fennis et al., 2004). Extensive bone-union, more intensive callus formation along the grafted segment, a higher amount of vital bone surface and a higher amount of capillaries within the cancellous bone graft confirmed this observation. It was also found that in the animals treated with PRP, there was a smaller tendency to form a
fibrous capsule around the grafted segment. Maturation and remodelling of the bone graft appeared to continue between 6 and 12 weeks after surgery. There is no data in literature about usage of FG and autogenous corticocancellous bone graft taken from the iliac crest in mandible defects reconstruction. The aim of this experimental animal study was to evaluate the efficiency of this method in mandible defects reconstruction with autogenous corticocancellous bone graft taken from the iliac crest mixed with FG. We postulated that bone grafts will have satisfactory healing.

**MATERIAL AND METHODS**

The study was performed at the Institute for Experimental Medicine Investigations Medical Military Academy (MMA), Institute for Transfusiology MMA, Institute for Pathology and Forensic Medicine MMA and Central Clinical Hematological Laboratory MMA. In the prospective study we have included 8 experimental animals (dogs of German shepard race, weighing appr. 20 kg and 3 years old). Defects on the one side of mandible were reconstructed with with autogenous corticocancellous bone graft taken from the iliac crest mixed with FG. Research methods enclosed: preoperative methods, operative methods in multiple phases, postoperative follow up of bone graft healing up to the time of its consolidation.

**Preoperative methods**

Experimental animals underwent medical examination and dental status checking. The number of eritrocytes, their sedimentation rate, leukocyte formula, serum protein, immunoglobuline and ionic cooper concentrations along with the alkaline phosphatase activity were determined in the blood samples

**Operative method**

**First phase of experiment**

Premedication consisted of Acepromazin (Combistress or Combelen) iv. in a dose of 0.03 mL/kg and atropin s.c. in a dose of 0.01 mg/kg. Ketamin chloride (5%) in a dose of 0.3 mL/kg was administered 15 minutes following premedication. All surgical procedures were performed in dissociative anesthesia with ketamine chloride and tiletamine chloride in such a way as to preserve normal respiratory and cardiovascular functioning. Before surgery, the operating areas (mandibular region and iliac crest region) were shaved. One side mandibular defect (5x1x1 cm) was performed by extroral approach. Skin incisions were made 2 cm below the mandible inferior edge in a length of 8 cm. After removing the periost, the mandibular defect (5x1x1 cm) was created with an electric bandsaw, a chisel and a hammer, moving from the inferior ridge toward the mandibular canal, taking care to preserve the neurovascular structures. Following this, autogenous corticocancellous bone graft from iliac crest (6x3x1 cm) for mandible defect reconstruction was detached. The bone graft was comminuted in 2 mm thick parts and preserved in an isotonic solution at 4°C. Bone defects were bridged with
one titanium plate on each side. Bone defect was replenished with autogenous corticocancellous bone graft and FG (in a dose of 3 mL, provided by Institute for Transfusion MMA). Wounds were closed by standard suturing.

Antibiotics (Penicillin 1600000 I.U. x 2 im and Streptomycin 1.0 im) have been administrated during seven days. Animals consumed mushy food and water starting from 12 h after surgery and sutures were removed after 10 days.

Second phase of the experiment
Three weeks after replenishing the bone defect on the same way as in the first phase we approached the mandible and measured width, height and thickness of bone graft on it's end points and in the middle. We used these results to calculate the volume of the bone graft. Specimens for pathohistological examination (5x5x5 mm) were taken from the end points and from the middle of the graft and fixed in 10% formalin. Wounds were closed by anatomical layers. Tissue specimens were decalcinised, cut down to 5-7 μm, stained by H&E - hematoxylin-eosin, Masson trichrom, Paff-Halmi and Van Gieson methods and analysed under light microscope (Leitz.) at the Institute for Pathology and Forensic Medicine MMA.

Third phase of the experiment
Six weeks after replenishing the bone defect on the same way as in the first and second phase we approached the mandible, performed biopsies and removed titanium plates and screws.

Fourth phase of experiment
After nine weeks we have repeated the same procedure as in the third phase.

Postoperative follow up
Occurrence of most common complications, such as infection and fistula, have been followed up by physical examination, as well as by blood tests. Pathohistological examination was performed at the Institute for Pathology and Forensic Medicine MMA.

RESULTS
Pathohistological studies of the bone graft in combination with FG three weeks after transplantation.
We have analyzed 8 (eight) specimens from the end points of the graft and 8 (eight) specimens from the middle of the graft. Mostly, bone transplants were necrotic without osteocytes in the bone lacunas. There was extended young connective tissue with fibroblasts and newly arisen blood vessels with thin walls and a dilated lumen (Fig. 1). In the new connective tissue, bigger or smaller fragments of necrotic bone were evident. Some bone fragments in the connective tissue had preserved substance with osteocytes in the lacunas. Beside the young connective tissue we were able to notice, in the form of bigger or smaller islands, the formation of mature connective tissue in the area of former bone tissue.
Formation of osteoid tissue with strong osleoblastic reaction and newly formed thin bone trabeculas in the form of a mesh was evident (Fig. 2). Young connective tissue was dominant instead of former bone tissue.

Figure 1. Bone bars in the stadium of necrosis, and between them newly arisen young connective tissue, rich in vascularization and with a strong osteoblastic reaction (Van Gieson, 10x)

Figure 2. Newly arisen thin bone bars in a mesh-like layout with some osteoid and a strong osteoblastic reaction (H&E, 10x)

Figure 3. Bone is mostly in the stage of necrosis, with remaining of thin bone bars and formation of connective tissue, without osteocytes and osteoblastic reaction

Figure 4. Instead of former bone tissue there is newly formed connective tissue, well vascularized, with strong osteoblastic reaction and newly formed bone bars distributed in a meshy layout (H&E, 10x)
Patohystological analysis of the bone graft in combination with FG six weeks after transplantation.

We have again analyzed 8 (eight) specimens from the end points of the graft and 8 (eight) specimens form the middle of the graft six weeks after transplantation. Mostly, bone transplant was necrotic without osteocytes in bone lacunas (Fig. 3). In the preserved bone tissue there was an obvious osteoclastic reaction. Instead of the former bone tissue, a young connective tissue, rich in fibroblasts and newly arisen blood vessels with thin walls and a dilated lumen (Fig. 4) was evident. In newly formed young connective tissue there were osteoid islets.
and thin bars that form a net with a strong osteoblastic reaction. In young connective tissue we were able to notice islands with parts of necrotic bone, but also some preserved bone tissue with an osteoclastic reaction (Fig. 5).

Pathohistological analysis of the bone graft in combination with FG nine weeks after transplantation.

After nine weeks, specimens (n=8) from the end points of the graft and 8 (eight) specimens form the middle of the graft were analysed again. Instead the former necrotic bone there was multiplicated connective tissue, partly young and rich with fibroblasts and partly mature. In newly formed connective tissue, in the form of bigger or smaller patches, parts of necrotic bone and parts of preserved bone with osteoclastic reaction (Fig. 6) were seen. A marked formation of osteoid islands, as well as newly arisen thin bone bars with signs of osteoblastic reaction (Fig. 7) were evident. Somewhere, bone bars were thick and formed lamellar bone structures. Connective tissue was present in a higher amount and was more mature then in specimens taken after three and six weeks. Also we noted formation of osteoid tissue with strong osteoblastic reaction (Figure 8).

![Figure 8](image_url)

Volume (resorption or amplification) of bone graft

Figure 8 represents degree of resorption (or amplification) of the bone graft after three weeks following transplantation.

DISCUSSION

German shepherds were chosen because they belong to large breeds so we were able to create bone defects of 5 cm. Their mandible is morphologically, functionally and physiologically most similar to the same bone in humans.

We are now aware of the fact that bone healing is the result of the activity of survived bone graft cells and induction of undifferentiated mesenchymal cells at the recipient area. Both types of osteogenesis (remodelling of bone graft with its own cells or with induction of undifferentiated mesenchymal or osteoprogenic cells) must be considered as integrated factors in graft healing (Axhausen, 1951).
More significance is attributed to graft healing by preserved cells. Value of the bone healing in this way was confirmed in many experiments and in clinical practice with superiority of monocortical iliac bone graft usage. Successfulness of graft healing depends on the vascularization of the recipient region, good immobilization and type of a vascular bone graft used for reconstruction (Boyne, 1969, Mrazik et al, 1980, Williams, 1985). In 1974, Seward and other authors quoted that corticocancellous bone grafts of the iliac bone are the most appropriate grafts for compensation of mandibular bone defects (Seward, 1974; Marx, 1993; Schliephake et al, 1994; Merkx et al, 1999; Mirković, 2000; Merkx et al, 2004). This was the main reason for conducting our trial in this way.

New insights on the effects of growth factors on bone healing using autogous corticocancellous bone grafts, have drawn the attention of clinicians who now tend to use growth factor preparations (BMP, IGF, TGFβ) in their clinical practice. Growth factors used in bone engineering are the bone morphogenic proteins (BMPs). Nowadays they are in the focus of interest in experimental studies, as well as in the compensation of jaw and facial bone defects (Boyne et al, 1999; Terheyden et al, 1999; Schliephake, 2002). BMPs cannot be isolated as an autogenous substance in high concentration. Scientists have made recombinant forms from bovine sources. More than 15 recombinant forms of BMPs have been developed using this method (Terheyden et al, 1999). Till now, the greatest comercial interest was achieved by recombinant BMP (rhBMP2 and rhBMP7). Efforts are being made to define if the purified human forms are alike bovine ones (Schliephake, 2002). The probable reasons for delay in routine clinical use of BMPs are insufficiency of clinical data, ongoing researches in finding optimal carrier and considerations about the role of this factor in bone tissue embryogenesis and regeneration. Isolated application of individual growth factors may provoke unrationak, uncontrolled tissue overgrowth (Hock and Cannalis, 1994; Schliephake, 2002). New insights in the mode of action of FL in healing of avascular bone grafts, led us to use FL in combination with corticocancellous iliac bone grafts to compensate mandibular defects.

Schliephake and Langer (1997), Aghaloo et al. (2004), Witfang et al (2004), Fennis et al (2005), Gerard et al (2006) claim that there is no significant justification for PRP usage in combination with bone grafts, and that results are not better when compared with isolated use of bone grafts alone. Another group of authors, especially Marx (2004) indicate that PRP usage results in accelerated bone graft healing. Fennis et al. (2005) pointed to the negative effect of fibrose tissue presence on healing of the bone grafts, in their experimental work. Authors proved that usage of PRP with bone grafts reduces fibrose tissue formation. Histology analysis indicated that there is no fibrose tissue formation in the bone graft if PRP was added (Fennis et al., 2005). Results of pathohistology analysis, in our experiment, showed the presence of fibrous tissue in those bone grafts in which the mandibular bone defect was compensated with corticocancellous iliac bone grafts in combination with FL.

Schliephake et al. (1994), Robiony et al (2002), Merkx et al. (2004), and Marx (1993) showed that usage of PRP with bone grafts increases proliferation of undifferentiated mesenchimal cells, as well as the number of osteoprogenitor
cells in the bone graft. Increase in young bone cells in bone grafts was not noticed, in all phases of the research, in those groups of experimental animals in which we had used corticocancellous iliac bone grafts in combination with FL for compensation of mandibular bone defects.

Marx (1993, 2004), Marx et al. (1998), Aghaloo et al. (2004) and Roldan et al. (2004) showed in their studies existence of histological reduction of bone graft necrosis when they were used in combination with PRP. In our experiment results of the pathohistological analyses have showed that bone graft necrosis was evident in half of the experimental animals in all stages of the trial. Marx (2004) indicated great histological increase in the number of newly formed blood vessels in bone grafts with PRP. In our experiment we have not achieved beneficial effect of FL on increasing the number of early appearing and newly formed blood vessels in bone grafts.

Marx 1993 and Roldan et al. 2004. showed in their studies histological reduction of bone graft necrosis when they had been used in combination with PRP. In our experiment, results of pathohistology analysis have showed that bone graft necrosis was present in half of the experimental animals in all phases of the research. Marx (2004) noted great histological increase in number of newly formed blood vessels in bone grafts with PRP but in our experiment we did not achieve this beneficial effect of FL.

Marx (2004) believe that bone graft maturing begins with osteoid remodelling. They also quotes that this process is substantially accelerated when PRP is applied and the course of bone remodelling achieves growth of about 0.7% per day (percentage of the graft size) and can be further accelerated by 5-8%. Gerard et al. (2006) did not find statistically significant differences in bone graft maturation with or without PRP, respectively. Mature bone is formed after three months, remodelling processes are identical either with or without usage of PRP, and yield was $1.82 \pm 0.3 \mu m/day$ (Gerard et al., 2006). Results of pathohistological analysis of bone grafts in our experiment indicate that the usage of FL alone with bone grafts does not result in a quicker formation of Havers’ canals.

The bone graft resorption represents a frequent complication of mandibular bone defect compensation performed with avascular bone grafts. After tooth loss, even in healthy mandible, mandibular height is reduced in the first year by 1.5 mm, and by 0.1 mm each subsequent year (Buchbinder 1991, Mowelem, 1963; Stošić, 1998). Almost all studies indicate that there is an increase in volume of newly formed bone if PRP was used in combination with corticocancellous parts (Malcom, 1964; Garg, 2000, Aghaloo et al, 2002, Schliephake, 2002; Marx, 2004, Witfang et al. 2004, Fennis, 2005). In our research, we noticed an increase in bone graft volume in 4 experimental animals, and bone graft resorption in other 4 animals, also. Resorption of the avascular bone graft can be up to 50% (Stošić, 1998). We have achieved better results using FL with avascular bone grafts, compared with similar studies in wich avascular bone grafts were used alone.

Robiony et al. (2002) and Fennis (2005) cite positive effect of PRP usage on bone rebuilding, and this effect is especially evident within 6 and 12 weeks after osteotransplantation, both histologically and radiologically as also noted by Terheyden et al, 2001 and Aghaloo et al, (2004). Marx in 2004. and Fennis in 2005.
indicated that PRP in combination with bone grafts from the iliac crest promotes bone graft fusion at the end points of the bone, increases the callus formation, accelerates callus maturation, increases vascularisation and decreases fibrose tissue formation inside the bone graft. Maturation and remodelling of the bone graft was seen 6 to 9 weeks after osteotransplantation. Investigating the effect of PRP on cortical grafts in bone defect reconstruction of the palatinal cleft, Oyama et al. (2003) also indicated a substantial increase of bone volume. Roldan et al. (2004) and Wiltfang et al (2004) did not achieve positive effects in bone rebuilding using PRP in their experiments. Based on time of appearance of Havers canals in bone grafts, number of osteocytes and bone graft vascularisation, rate and quality of bone rebuilding were assessed. In our researches we did not gain amelioration of velocity and quality of bone graft rebuilding using FL, compared to similar experiments in which FL was not used (Havers canals were not formed in any phase of the experiment).

There was no infection in neither early nor late phases of our experiment, as proved with results of laboratory blood analysis. Wound dehiscency, as an early complication, is almost always a consequence of an infection. This complication was not evident in our experimental animals. Other authors also indicate similar findings in their experiments with animals (Garg, 2000; Marx, 2004).

Absence of bone graft fusion with mandibular bone at the end points, as a late complication, was present in three experimental animals. This was the consequence of fibrose tissue formation at the junction site, which was also indicated by Fennis (2005) in his studies. Boyne (1999) believes that rigid osteosynthesis with plates, with or without intermaxilllar immobilisation, provides adequate bone fragments stability, and ensures favorable conditions for callus formation. Insufficiency of this method is that osteosynthetic plates have to be surgically removed later (Boyne, 1999). On the other side, rigid fixation has no advantages, compared to wire fixation (Boyd, 1990). In our experiment we used one plate for mandibular ends fixation, and we did not have subsequent fractures neither of the mandibula nor the plate.

The size of mandibular bone defect does not influence the decision if the defect would be compensated or not, but only determine selection of the kind of bone graft which would be used. Boyd (1990) and Stošić (1998) analysed the use of vascularised bone grafts depending on the size of mandibular bone defect. In clinical practice, microvascular bone grafts are established for the compensation of bigger defects, and success of their usage is even greater if they were applied in primary reconstruction (McCarthy et al, 1999; Schliepahake and Langer, 1997). Nevertheless, these bone grafts most often do not give satisfactory shape and volume that are needed in dental rehabilitation. Unvascularised bone grafts provide better profile and volume, as well as better conditions for subsequent dental rehabilitation, and represent grafts of choice for the compensation of mandibular defects of 5 cm or less (Mirković, 2000).

Whitman et al. (1997) and Marx et al. (1998) introduced a new concept in the reconstruction of mandibular defects. They reported that parts of corticocancellous graft with PRP can entirely compensate the mandibular bone defect, in shape as well as in size. That is very important, both estetically and
functionally, and also provides better conditions for subsequent dental implantation. In this way, better chances for a good prosthetic rehabilitation are provided (Marx, 2004). In our research we achieved a satisfactory shape of the mandible.

Complications in the donor's and recipient's regions (hematoma, infection, necrosis, dehiscency, disturbance of the bone graft position, resorption, arterial trombosis) are significantly less common when these bone grafts are used, compared to the vascularised bone grafts and composite grafts (Mirković, 2000).

Yamada et al. (2003) experimentally managed to provide formation of autogenic bone on the back of a rat, using a mixture of mesenchimal precursor cells, fibrin glue and 3-calcium-phosphate, but this product of genetic engineering is far away from its possible use in clinical practice. Many authors indicate the efficacy of FL usage in cardio surgery, abdominal surgery, neurosurgery, oral surgery etc. (Balint, 1996, Balint, 2002). Tayapongsak et al. (1994) described the use of fibrin glue in healing of jaws and facial soft tissues and bone tissues, and in 50% of the cases he achieved earlier wound healing and less scar tissue formation. This effect of FL is achieved by action of fibronectin, Factor XIII and fibrin, that provide optimal substrate for the mesenchimal cells migration. In our study with experimental animals use of FL with corticocancellous bone grafts resulted in decreased graft resorption, actually in some cases it even resulted in increased graft volume. Usage of FL did not result in a quicker or improved bone graft rebuilding, compared to similar studies in which bone graft was used without FL.

Usage of FL with corticocancellous bone grafts did not provide expected results in the compensation of mandibular defects of 5 cm or less.

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REFERENCES

REKONSTRUKCIJA DONJE VILICE KORTIKOSPONGIOZNIM KOŠTANIM TRANSPLANTATOM I FIBRINSKIM LEPKOM: EKSPERIMENT NA PSIMA

MIRKOVIĆ Z, LONČAREVIĆ S, DURBEVIĆ D, BUBALO MARIJA, JOVIĆ S i GARDAŠEVIĆ MILKA

SADRŽAJ

uobičajenim tokom. Rezultati histoloških ispitivanja nisu ukazali na kvalitetniju pregradnju koštanog transplantata u odnosu na slične eksperimente u kojima su defekti donje vilice premošćavani samo delićima kortikospongijoznog transplantata sa ilijačne kosti.