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Original scientific paper

Treatment of Tibial Bone Defect with Rotational Vascular Periosteal Graft in Rabbits

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ABSTRACT

It is well known that the periosteum is capable of bone formation. In the present study, the value of the vascularized periosteal graft in healing the long-bone defect filled with the allogenous bone graft was studied. The aim of the study was to verify the efficiency of the rotational vascularized periosteal graft as a optional surgical method as well as to prove its advantages in comparison with the nonvascularized periosteal graft. The study was undertaken on 40 rabbits. Four rabbits served as allogenous bone graft donors, while the remaining 36 were divided into two equal groups. In the control group, the experimentally created bone defect on the junction of the tibial proximal and median thirds was filled with allogenous bone graft and then covered with avascular periosteal graft. In the experimental group, the allogenous bone graft filled defect was covered with the rotational vascular periosteal graft. Groups of 6 rabbits from each group were sacrificed after 2, 5 and 12 weeks following surgery. The results were evaluated with radiographical, histological and morphometric methods. The results obtained 2 and 5 weeks after surgery demonstrated better tibial bone defect healing in the experimental group. The defect was bridged in both analysed groups after 5 weeks and was completed after 12 weeks with no difference between the control and the experimental groups. The obtained results have confirmed the efficiency of the method using the rotational vascularized periosteal graft in the treatment of tibial bone defect in rabbits. The advantage of the vascularized periosteal graft as compared to the avascular one has been proved by better quality of bone healing in the early stage.

Key words: *periosteum, allograft, bone healing, rabbit*

Introduction

The incidence rate of fractured extremities appears today to have assumed epidemic dimensions. Yet, despite greatly improved surgical techniques and treatment quality, complications may still be expected during hospitalisation and rehabilitation on the way to a complete recovery. For example, the healing of bone diaphysis fracture may entail delayed union or development of pseudarthrosis. Therefore, it is necessary to search and develop improved methods for stimulating fracture healing based on latest achievements in the field of bone biology. Delayed bone healing may be the result of osteogenous, circulatory or mechanical disturbances at the fracture site[1]. Such disturbances may be associated with the fracture type and location, the size of the bone defect at the fracture site, the cause of the fracture (contusion, gun shot) patient's bad general condition, poor surgical technique and improper selection of the osteosynthetic material. There are various surgical techniques used in the healing of aseptic non-united fractures or defect pseudarthroses. Depending of the cause of delayed healing, these techniques include application of an altered type or form of osteosynthesis, spongioplastics (autogenous and allogenic), massive allogenic bone grafts and external fixation and/or translation of bone fragments – the Ilizarov method. In a certain number of cases healing may not be achieved by reosteosynthesis or spongioplastics applied either independently or in a combination and it becomes necessary then for the bone healing to be stimulated.

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Bone healing could be stimulated by growth factors such as bone morphogenetic proteins (BMPs)[2]. Application of the BMP-7 along with cancellous bone was proved to be successful in animal and in clinical studies as well[3]. Eventhough promising, growth factor applications have to be interconnecting with newly developed surgical treatment in order to improve bone healing. Massive allogenic bone grafts or external fixation by Ilizarov method are usually applied in cases of extremely large bone defects. Their application is rather complex in technical and surgical terms and more difficult for patients to handle[4]. The idea of the fracture healing enhancement using periosteum was born as early as the late 19th centurie, following experimental studies which confirmed osteogenic property of the periosteum cambial layer[5–8]. These studies are dealing with the osteogenous property of the isolated periosteum and proved mesenchymal origin of periosteal cells, capable of cartilage formation by proliferation and its subsequent transformation into bone[9,10]. In the studies that followed, periosteum was used as a free nonvascularized graft because of osteogenous property of its cambial layer in repairing bone defects. That was the base for the maxillary periosteal graft used by Skoog in overlying the palatine bone defect[11]. Later on, the free nonvascularized periosteal graft found its application in overlying congenital and traumatic defects of the calvary, tracheal and articular cartilage[12–15]. The evidence in support of the osteogenic property of the free nonvascularized periosteal graft has been supplemented by Ritsila with quantitative indicators showing a higher ossification rate within the profusely vascularized muscle as compared to the fascia. The bone formation process within the tube like closed periosteum as a tibial pseudarthrosis healing model is fundamental in the evaluation of the periosteal circulation[16]. Finley's achievement is regarded as the turn point in the application of periosteal grafts[17]. In overlying a tibial defect in dogs, he used a costal revascularized periosteal graft connected through intercostal blood vessels to the local muscular blood vessels. The separated half of the graft with no circulation compared to the revascularized one presented evidence in support of the advantages of the revascularized graft. Subsequent studies also used osteoperiosteal revascularized graft comprising a thin layer of cortical bone with the periosteum in order to preserve the cambial layer[18–20]. Jaroma and Ritsila applied a free periosteal graft in combination with an allogenic bone graft in the bone defect repair[21]. In their experiment, the allogenic spongious graft was overlaid with a nonvascularized periosteal graft to prove its capability of inhibiting the resorption of the spongiosa and stimulating the new bone formation. The assumption that the efficiency of the bone graft remodelling also depends on the local vascularization rate was confirmed by experimental studies using a vascularized periosteal graft[22–24]. However, taking into consideration that the quality of the bone tissue remodelling depends on the quality of the periosteum, the periosteal osteoinduction rate thus depends on the underlying material. The highest osteoinduction rate is obtained with spongiosa, followed immediately by corticalis, whereas it is almost completely inhibited where synthetic material is applied[25]. Any of the above mentioned methods using vascularized periosteal grafts is quite complex in technical terms, requiring perfect microsurgical skill and more time for the operation itself. This is why notwithstanding the theoretical excellence and fair experimental results those methods are hardly applicable in daily practice. This experimental study applied a rather simple surgical technique. The study builds on the idea that the healing and remodelling process of the bone defect filled with a spongious allogenic graft is faster and better where overlaid with a vascularized periosteal graft. The osteogenesis at the fracture site is thus stimulated from two directions: partly from the donor's bone and partly from the autogenous vascularized periosteal graft.

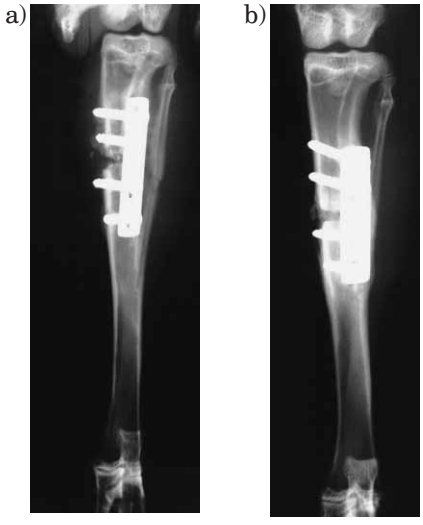

Fig. 1. Radiographic findings from the control (a) and experimental animals (b) 2 weeks after surgery.

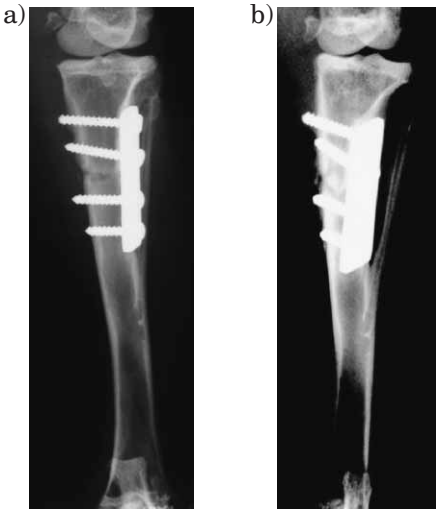

Fig. 2. Radiographic findings from the control (a) and experimental (b) animals 5 weeks after surgery.

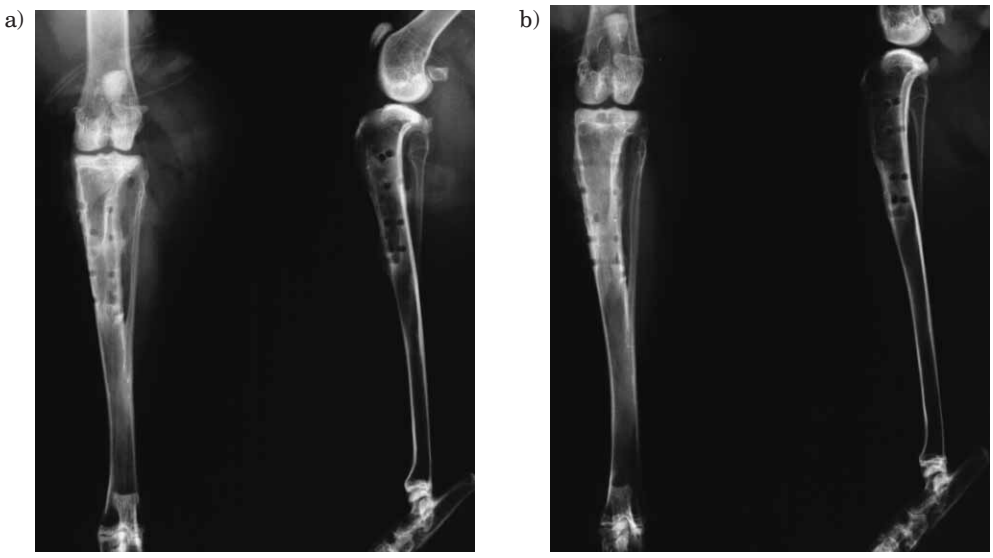

Fig. 3. Radiographic findings from the control (a) and experimental animals (a) 12 weeks after surgery.

Materials and Methods

Experimental animals

40 fully grown male rabbits, 4 months old, weighing 3–4 kg were hosted in standard cages in controlled laboratory conditions. Free access to water and standard pellet food (MK Moslavka, Kutina, Croatia) was allowed. The animals were randomly divided into 2 groups of 18 animals, control and experimental, each of them undergoing different experimental treatments. All procedures performed in the study were carried out in accordance with Medical school policies and Guidelines for the care and use of laboratory animals. All efforts were made to minimize the animals suffering.

Bone allografts

Four bone donor animals were sacrificed and their femur, tibia and coxes were isolated under aseptic conditions. The marrow from the bones was cleaned up with saline and the periosteum and articular cartilage were removed. Thereafter, the bones were mechanically ground into small particles and stored in sterile tubes. These bone allografts were kept in sterile conditions at –80 °C (Bone bank from the Clinic for Orthopedic Surgery Lovran). They were thawed out at room temperature 30 minutes before used.

Surgery

All surgical procedures were performed in the laboratory of the Department of Anatomy, Medical School in Rijeka. Rabbits were anesthetized with ketamine hydrochloride 50 mg/mL-Ketalar® via the marginal ear vein. The skin of the animal was shaved and disinfected. Thereafter, the skin was cut lengthwise in the anterior part of the rabbit hind limb and the tibia was exposed. The bone transverse osteotomy, 3 mm in width, was created approximately between the proximal and middle thirds of the bone shaft. The muscles lying on the tibial bone lateral side were lifted up and a plate with four cortical screws was placed on the tibial bone shaft. The bone defect was filled with bone allografts from the bone bank. After that, in the control animals, bone defect was covered with avascular periosteal flap which originated from the proximal tibial part. In the experimental group of animals, the bone defect was covered with rotational vascular periosteal flap from proximal tibial part achieved by cutting the periosteum with an »L« cut and 90° rotations. The periosteal flap was carefully sutured using polyglactin 910 4/0 (Vicryl®) without tension, to avoid circulation insufficiency. After surgery, the wound was washed with saline and sutured. Postoperatively, all animals received antibiotic cefazolinum (Kefzol®). All surgical procedures were performed by one of the authors (V.Š.) for

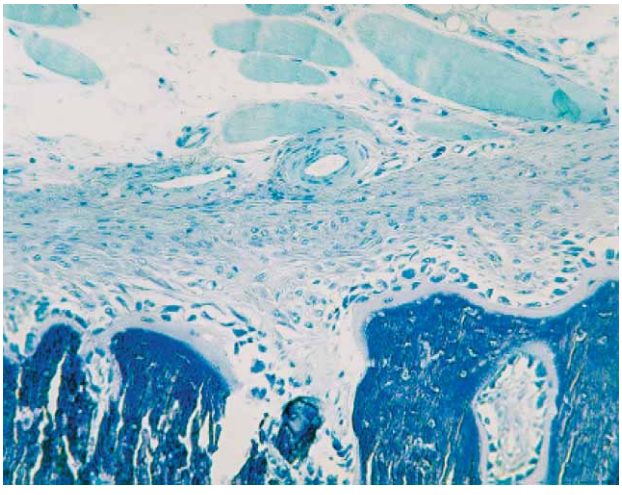

Fig. 4. Microphotograph of the periosteal membrane from the experimental animal 2 weeks after surgery (toluidine blue, magnification 400 XD.)

the sake of the uniform technique. After a certain duration of the experiment (2, 5 and 12 weeks after surgery), animals were sacrificed by overdose of anaesthetic (6 animals at each time point from both groups). A segment of rabbit tibia (1 cm), beneath the plate for osteosynthesis, was taken and prepared for histological and histomorphometrical analyses.

Methods for Bone Defect Healing Evaluation

Radiology

The animals were radiographed at the end of experimental periods (2, 5 and 12 weeks respectively). Radiographs in two planes were taken of every tibia that had been operated. Thereafter, the scoring system for bone defect healing evaluation (Table 1.) was used in order to quantify differences between the control and experimental groups[26].

Histological and histomorphometric examination

Tissue samples (tibial segment) were rinsed with saline, fixed in 4% paraformaldehyde, dehydrated in ethanol, cleared in xylene and embedded undecalcified in methyl metacrylate. Tissue samples were cut in 7 μm thick serial slices with microtome (Leica RM 2155) equipped with tungsten carbite knife. Each tissue sample was cut in 500 μm intervals through the whole volume of the tissue sample. Within each interval, 10 tissue slices were taken and mounted on silanated glass slides (Silane-Prep slides, Sigma S 4651), dried at 37 °C and stained with hematoxyline-eozin, toluidin blue, Von Kossa and Goldner trichrom.

The tissue morphology during the bone healing processes experimentally induced in rabbits and treated with two types of periosteal flaps, was analysed. Histology revealed clear differences between bone healing in the experimental and control groups of animals. In order to compare these morphological differences objectively, the authors applied a semi quantitative scoring system (Table 2) which allows for a statistical analysis of the differences in bone healing processes between two groups of animals. The evaluation was carried out by two independent observers (SZ, VŠ). The maximum quality of the bone healing was evaluated by score 15[27,28].

Tissue sections were examined under an Olympus BHA microscope (Olympus, Tokyo, Japan) and photographed under the magnification of 40X using a Pulnix digital camera (Pulnix, Yokohama, Japan) connected to a personal computer. The digital images were captured under the 40X magnification and analyzed using a semiautomatic image analysis system that employs Issa software (VAMS, Zagreb, Croatia). The parameters which allow for evaluation of the bone turnover at the sites of bone healing were measured as follows:

1. bone volume at the site of tibial defect (BV/TV (%));
2. bone volume in the medular cavity round cortical screws (BV/TV (%));
3. osteoblasts number (Nob (/mm^2)),
4. thickness of the periosteal layer above the fracture callus (μ),
5. periosteal cells number above the fracture callus (Npc (/mm^2)),

TABLE 1
SCORING SYSTEM FOR BONE DEFECT HEALING EVALUATION ON RADIOGRAPHS

Radiographic appearances of the bone healing evaluated in study	Grade
No change from immediate postoperative appearance	0
Trace of radiodense material in defect	1
Flocculate radiodensity with flecks of calcification and no defect bridging	2
Defect bridged in at least one point with material of nonuniform radiodensity	3
Defect bridged on median and lateral sides with material of uniform radiodensity, cut ends of cortex remain visible	4
Same as grade 3, at least one of four ends of cortex remain visible	5
Defect bridged by uniform new bone, cut ends of cortex not seen	6

TABLE 2
BONE HEALING SCORING SYSTEM BASED ON MORPHOLOGICAL CRITERIA

Morphological parameters		Grade
Bone allografts incorporation	Intact, no sign of cellular activity	0
	Resorptive activity	1
	New bone formation	2
	Fully incorporated in new bone	3
Cortex	No sign of cellular activity	0
	Resorptive activity	1
	New bone formation	2
	Bridging to surrounding tissue	3
Callus	fibrous	0
	cartilaginous	1
	bony	2
Bridging of the bone defect	No bridging of the bone defect	0
	Bridging with fibrous tissue	1
	Bridging with fibrous and cartilaginous tissue	2
	Bone defect closure	3
Newly formed bone tissue	No newly formed bone tissue	0
	Osteoblasts accumulation	1
	Woven bone	2
	Lamellar bone	3
Periosteum	avascular	0
	vascular	1

Statistical evaluation

The results of radiological evaluation were analysed using Fischer's Exact Test. Histological and histomorphometric results were evaluated using Students t-Test.

Results

Radiographic findings

The efficiency of the bone healing of experimentally created bone defect was evaluated by two independent observers (VS, BN) on the basis of radiographic findings (Table 3). The bone healing of experimentally created tibial defect demonstrated different results between the control and experimental groups after two and five weeks. Differences in bone defect healing between two groups is statistically significant after two weeks (Fischer's Exact Test, $p<0.05$).

Histological analysis

The bone healing after 2 weeks was evaluated with a mean score 5 (± 0.9) in control group and with mean score 9 (± 1.2) in experimental group. After 5 weeks, bone healing was advanced and was evaluated with higher mean score than after 2 weeks and still there was a significant differences between mean score in two groups of animals (8 (± 0.85) in control group; 14 (± 2.8) in experimental group). After 12 weeks of experimentally induced bone healing, there was no difference in bone healing. At that time point, in both groups complete bone healing and remodelling at the site of osteotomy occurred and all animals from both groups achieved the maximal grade 15. Results are shown in Table 4.

TABLE 3
THE RESULTS OF THE RADIOLOGICAL EVALUATION

Duration of bone healing	Control group		Experimental group	
	Number of animals	Grade	Number of animals	Grade
2 weeks	13	0	6	1 (*)
	5	1	12	2
5 weeks	6	4	5	4
	6	5	7	5
12 weeks	6	6	6	6

(*$p < 0.05$, Fisher's Exact Test)

TABLE 4
THE RESULTS OF SCORING SYSTEM BASED ON MORPHOLOGICAL CRITERIA FOR BONE DEFECT HEALING EVALUATION

	Scoring system based on morphological criteria for bone defect healing evaluation		
	2 weeks	5 weeks	12 weeks
Control group	5 ± 0.9	8 ± 0.85	15 ± 0
Experimental group	9 ± 1.2 (*)	14 ± 2.8 (**)	15 ± 0

The results are present as a mean ± St error. (T-Student Test* vs. control group,** vs. control group; $p<0.05$).

Histomorphometric analysis

During the experimental period of the bone healing process, new bone formation occurred within the bone defect filled with bone allografts. New bone formed at that site showed after 2 weeks a higher volume of bone healing in the experimental group as compared to the bone volume in the control group. The results of the measured bone volume (BV/TV) in the experimental group showed that 25.66 % of the total tissue volume in the bone defect was newly formed. In the control group of animals, a smaller proportion of the total tissue volume was occupied by newly formed bone (12.22 % ± 4.5). After 5 weeks, the quantity of the newly formed bone in the bone defect was approximately the same in the experimental and control groups, and after 12 weeks, there could be detected no remainders of the regenerative bone in either of the groups. Medullar cavity was formed and the bone healing process was completed in both groups at that point of time. The newly formed bone in the bone defect was woven and characterized by very high cellularity rate. The activity of osteoblasts in the bone healing process was measured by the number of active osteoblasts on surfaces of the newly formed bone trabecula. As expected, the number of osteoblasts was smaller in the control group of animals in relation to the experimental group (924.2 ± 20.2; 1028.7 ± 18.3 respectively) after 2 weeks, but after 5 weeks approximately the same value in both groups was reached. During the course of the experiment, new bone formation occurred in the vicinity of the cortical screws. This phenomenon is linked with the osteoconductive property of the screws. The newly formed bone volume round the cortical screws did not differ between the two groups of animals in either of the 3 time points studied. Two types of periosteal membrane transplanted in two groups of animals were measured. The results of morphometric measurement for the periosteal membrane showed that there were initial differences in periosteal thickness and periosteal cell number after 2 weeks of bone healing in favour of the experimental group. These parameters were higher in the experimental group of animals showing higher cellular activity involved in the bone healing process. After 5 and 12 weeks of bone healing, these parameters did not differ significantly in either group. The results of histomorphometry were shown in Table 5.

Discussion

The aim of the experimental study was to examine the efficiency of surgical treatment of the tibial bone defect using a rotational vascularized graft combined with an allogenic bone graft. The experimental rabbit model was selected for several reasons. First of all, rabbits have proven to be suitable animals for osteoinduction and osteoconduction models and that is why they have been used in most similar experimental studies so far[29–31].

TABLE 5
THE RESULTS OF HISTOMORPHOMETRY

Histomorphometric parameter	Duration of bone healing	Control group	Experimental group	T-Student test
BV/TV in defect (%)	2 weeks	10.12 ± 4.5	28.21 ± 5.2	SS
	5 weeks	49.00 ± 7.5	50.60 ± 2.1	NS
	12 weeks	/	/	/
Osteoblasts number (/ mm^2)	2 weeks	912.2 ± 20.2	1038.9 ± 18.3	SS
	5 weeks	880.40 ± 20.8	911.7 ± 10.3	NS
	12 weeks	/	/	/
BV/TV in medullar cavity (%)	2 weeks	26.84 ± 4.5	32.45 ± 5.6	NS
	5 weeks	50.89 ± 4.8	51.00 ± 4.8	NS
	12 weeks	4.5 ± 2.3	2.5 ± 1.4	NS
Periosteal thickness (μm)	2 weeks	201.3 ± 29.3	248.7 ± 19.6	SS
	5 weeks	98.2 ± 12.5	95.8 ± 15.9	NS
	12 weeks	42.3 ± 12.6	45.9 ± 14.9	NS
Periosteal cell number (/mm^2)	2 weeks	2890.4 ± 211.3	3300.9 ± 189.6	SS
	5 weeks	2320.5 ± 268.5	2520.3 ± 362.2	NS
	12 weeks	405.0 ± 29.6	380.0 ± 45.9	NS

Surgical approach and the technique applied to the tibia in rabbits are also applicable to the tibia in humans both in hypothetical and technical terms. The earlier workers confirmed the presence of an normal periosteal circulation in vascularized periosteal graft covering the site of the bone defect from the very first day. Besides that, the vascularized periosteal graft is liable to the well known fracture site vascular hyperaemia curve. Approximately three days after the injury, the local circulation rate goes up to reach the maximum at the end of the second week and then to decrease gradually down to the normal value as before the injury[32–35]. By contrast, the control group which was implemented a nonvascularized periosteal graft resulted in the interrupted periosteal circulation during two weeks. These facts are extremely important because the bone healing in initial stage depends of the interaction between the fracture gap haematoma and the periosteum, which stimulated chondrogenesis and endochondral bone formation[36]. Owing to the use of the allogenic spongious graft, the surgery is made shorter and an additional surgical section is avoided. Since the allogenous spongiosa only induces the osteoconductive activity, the osteoinductive activity at the bone healing site originates mostly from the periosteum[37,38]. In addition, by overlying the bone defect with a periosteal graft, the concurrent growing in of the surrounding fibrous tissue is inhibited. Thus, the results obtained may be compared with objectivity because the quantity of the spongiosa used to fill up the bone defect was quite the same in any of the cases. Stability of the bone fragments being one of the basic factor for normal bone healing. Thus, the selected osteosynthetic plate and cortical screws ensured sufficient stability of bone fragments in the experiment.

The animals were sacrificed after 2, 5 and 12 weeks from the surgery because of expected time of bone healing and presence of differences between the control and experimental groups. The results obtained from similar experimental studies have been checked and evaluated in different ways. Based on such experience, the results obtained from this study were subject to analyses by radiological and histological methods.

The radiological evaluation of the efficiency of bone healing varied between the control and experimental groups after 2 and 5 weeks from the surgery. Better healing efficiency results attaching to the experimental group resulted statistically significant ($p<0.05$) after 2 weeks, whereas the difference after 5 weeks was not statistically significant. After 12 weeks, in both groups complete bone healing occurred, yet the experimental group showed in all 6 animals radiologically visible medullar cavity. This could be indicative of the earlier adaptational bone remodelling within the experimental group.

Histological and histomorphometric analyses and evaluations of bone healing results are more sensitive and more objective than the radiological ones. The interpretation of those results is therefore more reliable.

The study presented here, used the qualitative and semi-quantitative scoring method as well as the histomorphometric analysis. Changes were observed on the examined tibial bone cross-section samples in rabbits both within and round the bone defect. Within the experimentally created defect, our observation was focused on the resorption of the allogenic bone graft and the quantity of the new bone formation round the graft and/or within the gap. Particular attention was paid to various periosteal graft quality analyses within both the control and experimental groups. The site of the experimentally created defect overlaid by a periosteal graft was observed for thickness and vascularity, as well as the endosteal reaction in the proximity of the experimentally created defect. Qualitative and quantitative analyses of the common features substantiated certain differences in the samples of different animal groups.

The scoring system used for evaluation of histological efficiency in bone healing highlighted the difference between the test groups. Thus, the results of histological efficiency of the bone defect healing were significantly better in the experimental group than in the control group, showing a statistically significant difference ($p<0.05$) after 2 and 5 weeks from the surgery, whereas after 12 weeks from the surgery they became levelled.

After 2 weeks, the analysed tissues samples showed that the experimental group had higher resorption of the allogenic bone graft, larger quantity of the new bone formation and more profuse periosteal and endosteal reactions as compared to the control group. After 5 weeks, both the control and experimental groups showed remodelling of the newly formed bone within repairing tissue, although in some tissue samples in the control group a few non-resorbed allogenic bone graft patches could be still found. The periosteal thickness became almost equal in the experimental and control groups, yet still differing from the normal periosteum. After 12 weeks from the surgery, the site of the experimentally created defect was almost invisible in both groups of test animals. The periosteal membrane was thin and with no signs of any cellular activity deep in the cambial layer, although there were found some bony callus residues, on the external side of the corticalis. There was no endosteal reaction and cortical bone was reestablished at the defect site. Yet, the cortical bone did not appear to be completely compact because of tiny patches of spongious lamellar bony tissue still present.

The results of the histomorphometric analysis showed that the larger quantity of the new bone formation observed in the experimental group after 2 weeks was statistically significant ($p<0.05$). Whilst there were also differences between other results obtained for the control and experimental groups, but they did not result statistically significant.

Relying on the histological method as the more objective and more precise evaluation method in respect of the experimentally created bone defect healing efficiency, there is a distinct difference to be observed in respect of the bone healing dynamics between the control and experimental groups, although the final healing results level up after 12 weeks. Sooner and better bone repair allows possibility of sooner and heavier loading of extremities. The application of this healing method might enhance the efficiency of the healing bone fractures within the zones with primarily poor circulation, fractures with the surrounding soft tissue seriously damaged and in delayed bone healing.

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LIJEČENJE DEFEKTA TIBIJE ROTACIJSKIM VASKULARIZIRANIM PERIOSTALNIM PRESADKOM U ZEČEVA

S A Ž E T A K

Poznata je činjenica da periost ima osteogenu moć. U ovome radu istraživana je vrijednost vaskulariziranog periostalnog presadka u cijeljenju defekta duge kosti ispunjenog alogenim koštanim presadkom. Glavni cilj istraživanja bio je procijeniti efikasnost primjene vaskulariziranog periostalnog presadka kao opcije kirurške metode liječenja kao I dokazati prednosti njegove primjene u odnosu na primjenu slobodnog nevaskulariziranog periostalnog presadka. Istraživanje je provedeno na 40 zečeva. Četiri životinje služilo je kao donori alogenog koštanog presadka, dok je ostalih 36 životinja podijeljeno u dvije jednake skupine. U kontrolnoj skupini životinja, ekspermentalno je učinjen defekt tibije na granici prve I srednje trećine trupa kosti I ispunjen alogenim koštanim presadkom koji je potom prekriven avaskularnim slobodnim periostalnim presadkom. U eksperimentalnoj skupini životinja istim je postupkom učinjen I ispunjen defekt trupa ali je prekriven rotacijskim vaskulariziranim periostalnim presadkom. Skupine od po 6 životinja žrtvovano je nakon 2, 5 I 12 tjedana od učinjenog zahvata. Ishod cijeljenja kosti analiziran je radiološkom metodom, histološkom I morfometrijskom metodom. Rezultati navedenih analiza ukazuju na na bolju kvalitetu cijeljenja kosti u eksperimentalnoj skupini i nakon 2 i nakon 5 tjedana od zahvata u odnosu na kontrolu. Koštani defekt je premošten u obje analizirane skupine nakon 5 tjedana, a nakon 12 tjedana je potpun bez razlike između kontrolne I eksperimentalne skupine. Dobiveni rezultati potvrđuju veću efikasnost primjene vaskulariziranog periostalnog presadka u cijeljenju koštanog defekta tibije u zečeva u odnosu na nevaskularizirani periostalni presadak što je dokazano bržim I kvalitetnijim cijeljenjem.