

RABBIT BONE TISSUE RESPONSE TO THE DEFECTS TREATED WITH DIFFERENT FIXATION METHODS

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Blood supply and stabilization at the fracture site are two essential factors in fracture healing. Different fixation methods may enable different conditions of stability needed for fracture healing.

The aim of this study was to determine, by histological tissue analysis, whether different methods of fixation and biomechanical characteristics of osteosynthetic material have an effect on the bridgeable tissue characteristics and the rate of the healing of defects of long bones in experimental animals.

Experimental procedure was performed on twenty-one Chinchilla rabbits. Artificially created bone defects were treated in one of the following ways: with a plate, an internal fixator and an external fixator.

Six weeks after surgery, the results of histological analysis showed that union was started in all examined samples. When a plate was used for fixation, fibro-cartilaginous healing stage was marked clearly with high levels of activity. In the defects treated with selfdynamisable internal fixator, the woven bone tissue was seen indicating the bony callus formation. When external fixator was used for fixation, the effect was comparable with that seen in the defect side treated with selfdynamisable internal fixator.

Based on the results obtained in our study, we can conclude that biomechanical characteristics of internal and external fixators are more superior than biomechanical characteristics of a plate in the treatment of bone defects in experimental animals.

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Key words: bone defects, plate, selfdynamisable internal fixator, external fixator, bone healing

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Introduction

At the moment of fracture, physiological load of the bone is completely damaged, whereby mechanical feedback for adaptive response of the bone on load is also disrupted.

After formation of callus, its remodeling demands reestablishment of normal cortical architecture

with the help of common adaptive mechanisms. This load through fracture is important for stimulation of a adaptive response and complete healing of the fracture.

The union of bone fractures means a complicated cascade of processes on cellular and biochemical levels, which ends with a complete structural and functional recovery of a damaged bone. Although the potential of bone tissue regeneration is exceptionally high, the process of bone healing is disrupted in 5 to 10 percent of cases, which results in delayed healing or the lack of it (1). If we take such a big number of fractures into account, this high incidence of healing dysfunction surely poses a significant problem in modern traumatology.

Damage of local blood vessels is a consequence of the fracture, especially in periosteum (2, 3). Considering that forming of callus depends on vascularization of periosteum as well as surrounding soft tissue, damage of these structures delays normal formation of callus (4, 5).

Natural process of fracture healing, including formation of stabilizing callus on the healing site, is stimulated by load and movements, although exces-

sive movement may cause damage of bone union on the fracture site (6, 7). Movements and load stimulate inflammatory phase of bone healing and especially revascularization (8). This biomechanical appearance is responsible for differentiation of healing direction, between primary and secondary bone healing and it leads to right differentiation of tissue into callus (9). If movements between fragments are eliminated and if load is transferred only via applied internal fixation, union of bone can be disrupted. In the presence of axial micro movements and rigid fixation via rigid fixation apparatus, biological and mechanical bone healing can be improved, which leads to faster and histological more qualitative healing (10). In practice, this means that greater rigidity of applied osteofixation material, which disables axial micro movements, may reduce the size of the healing process (11). Also, reduction of fixation rigidity especially after first 4 weeks of healing may fasten and cause greater healing (12). This idea enabled rigidity decrease of the external fixator frame and invention of new osteofixation materials, which have been applied in practice in the process of dynamisation at the fracture site (13).

The aim of this work was to establish, by analysis of histological preparations, whether different methods of fixation and biomechanical characteristics of osteosynthetic materials have influence on bridgeable tissue characteristics and dynamics of union of long bone defects in experimental animals.

Material and methods

Animals

Twenty-one Chinchilla rabbits were used in this study with average weight of 3.3 kg (3.2-3.5 kg). All the rabbits were sexually mature and all were parasite-free and had normal hematological profiles. All animals were acclimated to the environment for two weeks before the operation. Ethical committee of The Faculty of Medicine, the University of Niš, approved the study design.

Osteosynthetic material

As osteosynthetic material we used:

1) A plate with 4 screws which was made to resemble the classic AO plate used for fixation of long bone diaphysis.

2) A model of Mitkovic Selfdynamisable internal fixator with 4 clamps and 4 screws (14). The constituent parts of a mini internal fixator are: an oval clamp, a bar bearing the clamp and pins. Clamps with pins can be positioned in parallel position or under 90° angle.

3) A model of Mitkovic external fixator with 4 pins (15). The parts of a mini external fixator are: an oval clamp, the carrier of the clamp, the bar and the pin for fixation 2 mm in diameter. The fixator enables putting pins in parallel and convergent position up to 90 degrees.

All osteosynthetic material was made in "Trafix" Company, Niš, Serbia and all was made of 316L steel.

Surgical procedure

The surgery was performed according to principles of asepsis and antisepsis. Anesthesia of experimental animals was performed by giving Zoletil 50R (Virbac, France) in dosage of 10 mg/Kg of weight. Before the surgery Gentamicin (Galenika, Serbia) in dosage of 2 mg/Kg of weight was applied.

Each experimental animal had its right femur operated. Incision was made by lateral approach of the thigh 5 centimeters long. In the middle third of femoral diaphysis osteotomy was done by electric saw and a bone defect 2 mm big was created. At the ends of proximal and distal fragment, 1 mm in length, electrocauterization was performed to destroy osteoblasts and create conditions for nonunion of osteotomy. In six rabbits, the wound was closed with Vicryl 3-0 string and the extremity left without immobilization.

In other experimental animals the bone defect of 2 mm was fixed alternately by application of the plate, internal fixator and external fixator so that each of them was applied in five experimental animals, respectively. In the case where plate was applied, the periosteum was removed to ensure appropriate support of the plate. Axial reposition of proximal and distal femoral fragments was performed so that already created bone defect was lapsed. The hole for screws was done by Kirschner wire, 1 mm in diameter, and two distal and two proximal screws were placed and fixed tightly. Internal fixator was placed without removing of the periosteum. After the holes were made by K-wires, 1 mm in diameter, the pins were placed through the clamps of internal fixator under 90 degrees angle and tightly fixed at the bone and at the bar of the fixator. Two pins were placed in the proximal and two in the distal fragment of the femur.

To apply the external fixator, two convergent oriented pins were put into proximal and two pins into distal fragment of the femur after holes drilling by K-wires 1 mm in diameter. The created bone defect was also exposed in the length of skin incision in order to provide identical condition in experiment. Afterwards, clamps and bar of the external fixator were placed. The pins were fixed to the clamps and clamps were fixed to the bar as well. (Fig. 1)

After the surgery, the wound was closed in layers and sterile gauze and bandage were used. During three days post-surgery, injection of Gentamicin was given in dosage of 2 mg/body weights every twelve hours and to eliminate pain, 1 ml injection of Novalgetol (metamizole sodium) was given every twelve hours. The rabbits were kept in special cages, given standard food and water ad libitum. Support on to operated extremity was allowed.

Six weeks after surgery, material for histological analysis was taken from the place of previously created bone defect using the same skin incision.



Figure 1. Surgical procedure of creating defects and placing fixators.

Preparation for histological examination

All animals were sacrificed six weeks after surgery. After disarticulation in the hip joint the right femurs were harvested and the tissues in the bone defect side, as well as the bone at either end of the site, were processed for histological analysis. The tissue was fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 24 h at 4 °C. After fixation and decalcification, this tissue was processed with use of graded concentrations of alcohol and then it was embedded in paraffin, sectioned and stained with Hematoxylin and Eosin (HE). Tissue samples were cut at 2 microns on a microtome (Hisorange). Bone tissue sections were made in both longitudinal and transversal planes in relation to the place of osteotomy, owing to which the whole healing surface was obtained for examination.

Histological preparations were prepared and analyzed at the Scientific Research Center for Biomedicine at the Faculty of Medicine, University of Niš. Microscopic analysis was performed on light microscope Olympus BH, at 20x and 40x objective magnification.

Results

Six weeks after surgery the results of histological analysis showed that in all examined samples union was started.

In the group in which osteosynthesis was not used, material forming in the site of the osteotomy consisted largely of a fibrovascular collagenous connective tissue presenting fibrocartilaginous union (Fig. 2).

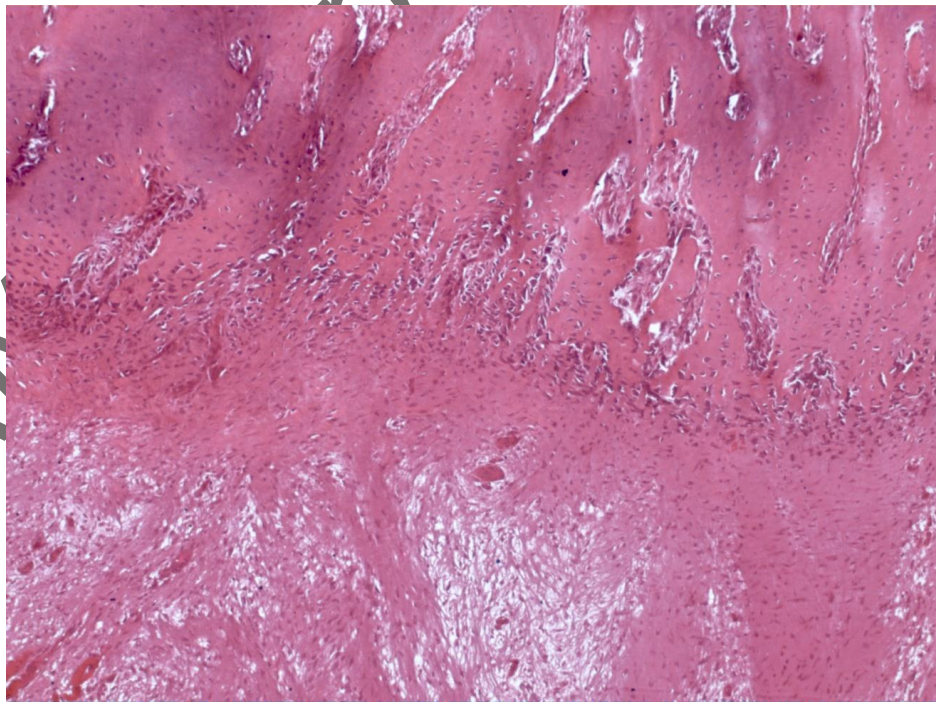


Figure 2. Histological images of the bone defect six weeks after the surgery in the group when osteosynthesis was not used. H&E staining; 20x objective magnification.

Fibrous tissue bridged residual part of osteotomy. At histological preparations where plate with screws was applied in experiments, all stadiums of tissue differentiation were visible within created defect such as granulation tissue, fibro-granulation tissue and highly differentiated fibrocartilaginous tissue

in less amount. Fibrocartilaginous union was marked clearly with high levels of activity and more brisk than was noted in the absence of fixation (Fig.3). Residual part of the osteotomy was bridged from fibrous tissue with vascular capillary inside the tissue.

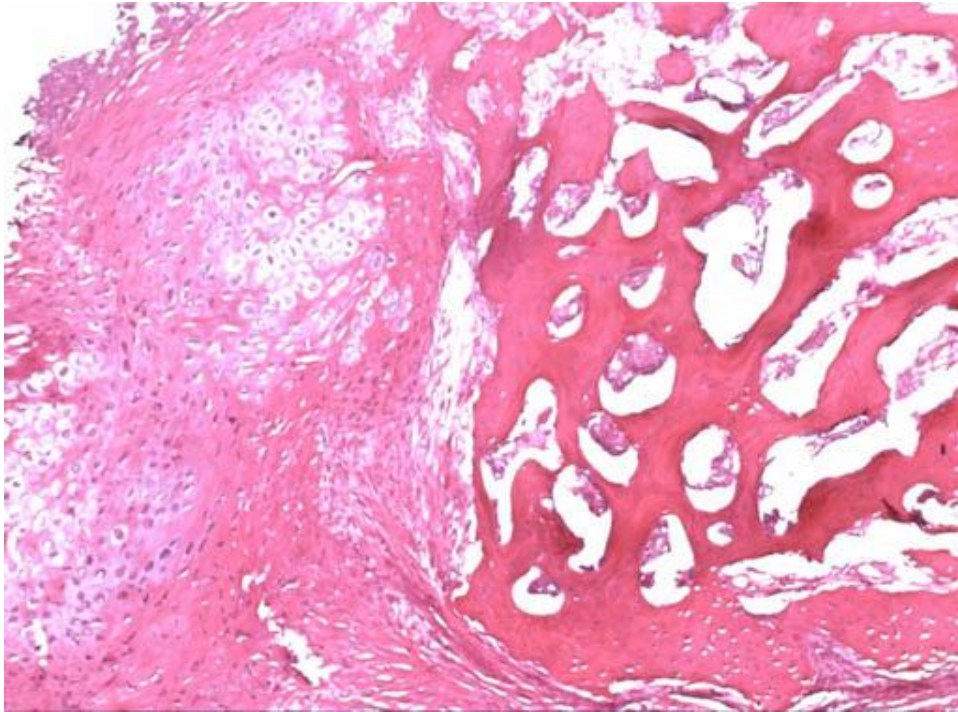
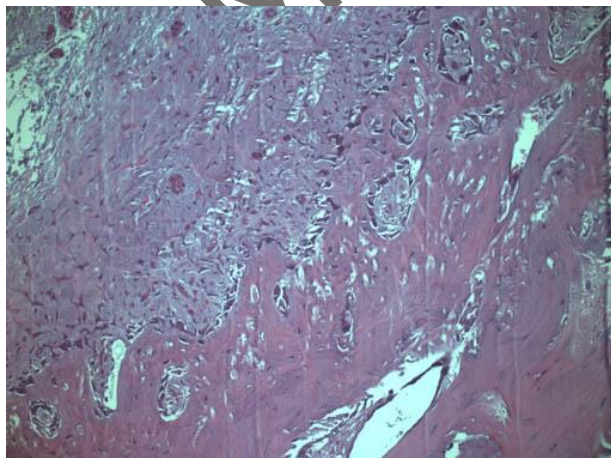


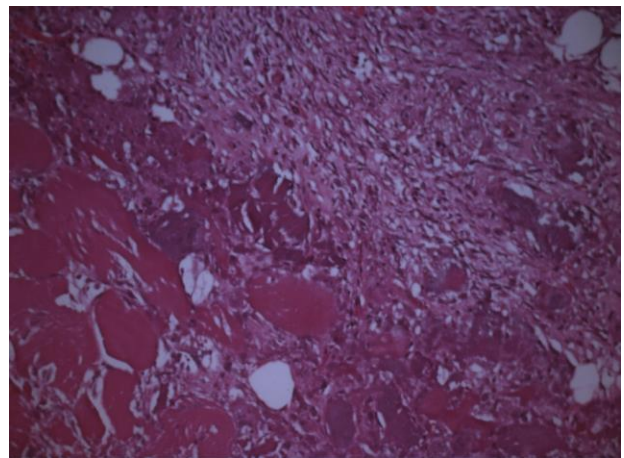
Figure 3. Histological images of the bone defect treated with plate osteosynthesis, six weeks after the surgery. H&E staining 20x objective magnification.

Histological preparations of the created defect site when internal fixator was applied showed the presence of newly formed woven bone with process of ossification and resorption (Figs. 4a and 4b). This

showed that osteogenic process in this case was much faster and more abundant in relation to osteogenic process with application of plate with screws.



a



b

Figure 4. Histological images of the bone defect treated with internal fixator, six weeks after the surgery. H&E staining; a) 20x objective magnification; b) 40x objective magnification

Histological preparations of the created defect fixed with external fixator showed the presence of the woven bone with resorption of old and ossification of woven bone (Figs. 5a and 5b). The effect was comparable to the one seen in defect

treated with self-dynamisable internal fixator. In two rabbits, disintegration of fixation was seen, in one case from the distal screws of the plate and in another from the distal pins of the external fixator.

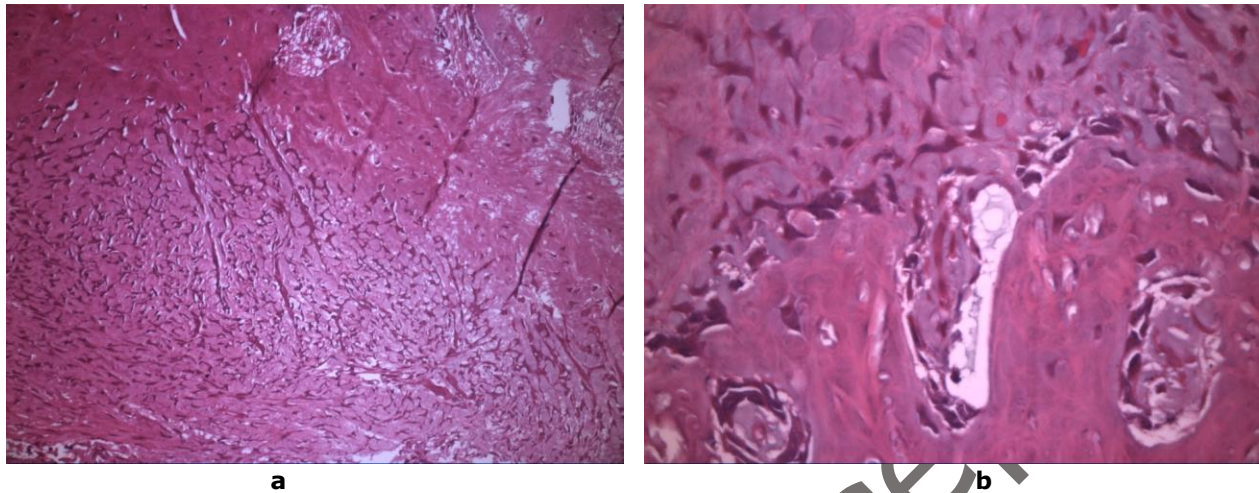


Figure 5. Histological images of the bone defect treated with external fixator, six weeks after the surgery. H&E staining; a) 20x objective magnification; b) 40x objective magnification

Discussion

Blood supply and stabilization at the fracture site are two essential factors in fracture healing. Each of them can be partially damaged but both of them must be present to enable healing. If these factors are absent nonunion and pseudoarthrosis appear. Different fixation methods may enable different conditions of stability needed for fracture healing. However, each method damage blood supply of the bone in some degree, which has to be considered when choosing adequate method for treatment of specific fractures.

Vascular response of the bone to all biocompatible implants mainly depends on: the site of implants' placing, effect of implantation on normal blood supply, stability of implants and characteristic of their surface as well as their functions. Bone physiology must be primarily taken into account by bioengineers who design implants and surgeons who use them.

Using a strong plate and screws for the fracture treatment anatomical reparation, faster recovery, mobilization of the joint and, to some degree, better bone healing could be achieved. Furthermore, it is known that drilling of holes, placing of screws, and application of plate and longer presence of the plate at the fracture site has a role in the development of bone osteopenia in different degree and different time (16). Plate fixation demands surgical incision through soft tissue, great deperiosting of the bone and drilling holes for the screws through cortex and medulla. All this leads to additional damage of already disturbed bone vascularization (17, 18). Stabilization of fracture with adequately placed compressive plate leads to transfer of compressed forces from proximal onto

distal part of broken bone through fracture. When the bone is directly connected to the plate with screws, then the plate and the bone have the same division of load. The plate suffers the same amount of axial shortage as the bone. Fixation with plate and screws represents firm fixation on the fracture side without presence of axial or lateral micro movements (19).

The pins of external fixator lead to limited damage of bone at the spot of placing but they cannot damage blood supply of the site of healing. Placed vertically in relation to longitudinal bone axis, they don't damage primary or secondary vascularization of broken bone significantly. Reparative processes at the fracture site are determined only by primary damage of bone vascularization that has happened at the fracture site. Hematoma at the fracture site is small, which enables easier and faster healing of blood vessels through it. External fixation, although not sufficiently stable at the beginning of healing, afterwards enables dynamization at the nonunion site and finally adequate healing. Less rigid external fixation enables interfragmentary mobility and union of the fracture through the process of secondary healing.

The pins and screws of internal fixator also damage the bone in a limited way, as in the case of external fixator. The bar of internal fixator itself does not damage periosteal vascularization since it does not lean on the periosteum as tightly as in the case of plate application. Placing internal fixator prevents the damage of periosteum necessary for the process of bone reparation (14). Placing of internal fixator minimally damages the soft tissue as well, because it is applied through two minimal skin incisions (14).

The internal fixator minimally damages not only the soft tissue and the bone vascularization, it

also has specific biomechanical characteristics in that it behaves as "intelligent implant". This means that at the beginning of union, internal fixator provides stable fixation of fracture or the site of nonunion. Later, as the healing process develops, osteolysis appears around the pins, and clamps fixing the pins for the fixator bar give way, which enables compression at the fracture site along the fixator bar. Rotational movements which can damage vascularization at the fracture site are disabled by convergent orientation of pins (14). This enables dynamisation along fixator bar, the load of the bone gradually increases and the union process advances (14).

According to histological changes at created defect sites under different conditions of fixation, it has been shown that biomechanical characteristics of internal and external fixator are much better than biomechanical characteristics of plate with screws. Since stability among fragments is much better if they are fixed with a plate in comparison to fixation with external fixator, it can be said that this stability possibly has influence on healing at the beginning of osteogenesis. In later stages, dynamization at the site of union and minimal damage of the bone vascularization are much important, and that can be

reached by application of external and internal fixator.

Conclusion

Histological analysis of tissue samples from experimental animals, points out that stabilization of bone fragments by internal and external fixators allows the bony callus formation six weeks after surgery. Fixation by plate resulted in early stages of fracture healing and fibrocartilaginous tissue formation. Based on the results obtained in our study we can conclude that biomechanical characteristics of internal and external fixators are more superior than biomechanical characteristics of the plate in the treatment of bone defects in experimental animals.

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References

1. Einhorn TA. Enhancement of fracture-healing. *J Bone Joint Surg Am* 1995; 77:940-56. [[CrossRef](#)] [[PubMed](#)]
2. Schweiberer L, Baumgart R, Deiler S. Die biologischen Bedingungen atropher und hypertropher Pseudoarthrosen der Schaftknochen. Ursachen und Erscheinungsbild. *Chirurg* 1999; 70:1193-201. [[CrossRef](#)] [[PubMed](#)]
3. Postacchini F, Gumina S, Perugia D, De Martino C. Early fracture callus in the diaphysis of human long bones. Histologic and ultrastructural study. *Clin Orthop Relat Res* 1995; 310:218-28. [[PubMed](#)]
4. Hulth A. Current concepts of fracture healing. *Clin Orthop Relat Res* 1999; 249:265-84. [[PubMed](#)]
5. Landry PS, Marino AA, Sadasivan KK, Albright JA. Effect of soft-tissue trauma on the early periosteal response of bone to injury. *J Trauma* 2000; 48:479-83. [[CrossRef](#)] [[PubMed](#)]
6. Sarmiento A, Sobol PA, Sew Hoy AL, Ross SD, Racette WL, Tarr RR. Prefabricated functional braces for the treatment of fractures of the tibial diaphysis. *J Bone Joint Surg J Am* 1984; 66:1328-39. [[CrossRef](#)] [[PubMed](#)]
7. Cornell CN, Lane JM. Newest factors in fracture healing. *Clin Orthop Relat Res* 1992; 277:297-311. [[CrossRef](#)] [[PubMed](#)]
8. Wallace AL, Draper ER, Strachan RK, McCarthy ID, Hughes SP. The effect of devascularisation upon early bone healing in dynamic external fixation. *J Bone Joint Surg Br* 1991; 73:819-25. [[CrossRef](#)] [[PubMed](#)]
9. Cheal EJ, Mansrann KA, DiGioia AM, Hayes WC, Perren SM. Role of interfragmentary strain in fracture healing: ovine model of a healing osteotomy. *Orthop Res* 1991; 9(1):131-42. [[CrossRef](#)] [[PubMed](#)]
10. Kenwright J, Richardson JB, Cunningham JL, White SH, Goodship AE, Adams MA, et al. Axial movement and tibial fractures. A controlled randomised trial of treatment. *J Bone Joint Surg Br* 1991; 73:654-9. [[CrossRef](#)] [[PubMed](#)]
11. Goodship AE, Watkins PE, Rigby HS, Kenwright J. The role of fixator frame stiffness in the control of fracture healing. An experimental study. *J Biomech* 1993; 26(9):1027-35. [[CrossRef](#)] [[PubMed](#)]
12. Eggers EL, Histan MB, Norrdin RW, Konde LJ, Schwarz PD. Canine osteotomy healing when stabilised with decreasingly rigid fixation compared to constantly rigid fixation. *Vet Comp Orthop Traumatol* 1993; 6:182. [[CrossRef](#)]
13. Behrens F, Searls K. External fixation of the tibia. Basic concepts and prospective evaluation. *J Bone Joint Surg Br* 1986; 68(2):246-54. [[CrossRef](#)] [[PubMed](#)]

14. Mitkovic M. New biological method of internal fixation of the femur. *Facta Universitatis* 2001; 8(1):50-3.
15. Mitkovic M. *New Concepts in External Fixation*. Nis: Prosveta; 1993.
16. Terjensen T, Norby A, Amulf V. Bone atrophy after plate fixation. Computed tomography of femoral shaft fractures. *Acta Orthop Scand* 1985; 56:416-8. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Barron SE, Robb RA, Taylor WF, Kelly PJ. The effect of fixation with intramedullary rods and plates on fracture-site blood flow and bone remodeling in dogs. *J Bone Joint Surg* 1977; 59:376-85. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Lewallen GD, Chao EY, Kasman RA, Kelly PJ. Comparison of the effects of compression plates and external fixators on early bone-healing. *J Bone Joint Surg Am* 1984; 66:1084-91. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Perren SM. The biomechanics and biology of internal fixation using plates and nails. *Orthopedics* 1989; 12(1):21-34. [\[PubMed\]](#)

AMM Paper Accepted

ODGOVOR KOŠTANOG TKIVA KUNIĆA NA DEFEKTE TRETIRANE RAZLIČITIM METODAMA FIKSACIJE

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Vaskularizacija i stabilizacija preloma su dva najvažnija faktora za zarastanje. Različite metode fiksacije daju različitu stabilnost potrebnu za zarastanje preloma.

Cilj rada bio je utvrditi da li različite metode fiksacije i biomehaničke karakteristike osteosintetskog materijala imaju uticaja na karakteristike premošćavajućeg tkiva i brzinu zarastanja na mestu defekata dugih kostiju kod eksperimentalnih životinja metodom histološke analize tkiva.

Eksperimentalno istraživanje je vršeno na Chinchilla kunićima. Veštački kreirani koštani defekti kod 21 kunića tretirani su na jedan od sledećih načina: pločom, unutrašnjim fiksatorom i spoljnim fiksatorom. Histološka analiza tkiva je nakon 6 nedelja pokazala da je u slučajevima fiksacije pločom prisutno fibro-hrskavičavo zarastanje sa visokom aktivnošću. Na mestu defekata tretiranih unutrašnjim fiksatorom uočava se formiranje mlade zrele kosti kao i u slučajevima primene spoljašnjeg fiksatora. Na osnovu dobijenih rezultata možemo zaključiti da biomehaničke karakteristike unutrašnjeg i spoljašnjeg fiksatora omogućavaju brže zarastanje defekata unutar 6 nedelja kod eksperimentalnih životinja.

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Ključne reči: koštani defekt, ploča, samodinamizirajući unutrašnji fiksator, spoljni fiksator, zarastanje preloma