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Healing of bone defect by application of free transplant of greater omentum

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ABSTRACT

In this study the process of the bone healing was observed. As a model the femoral bone of New Zealand rabbit was used. An identical bone defect was created on the left and right femur of the each experimental animal. On the right side the defect was covered with the corresponding of muscle tissue, and with free transplant of the greater omentum on the left side. The healing of the defect was observed by X-ray and histological examination. The results of the investigations showed that the process of the callus formation and its mineralisation are much quicker and thicker on the defect that was covered with the free transplant of the greater omentum.

Key words: bone healing, free transplant, greater omentum, rabbit

Introduction

Disturbances in the healing of fractures occur more often when the circulation is severely damaged by trauma (FROST, 1989a; HULTH, 1989; McKIBBIN, 1978). Therefore the necrobiotical changes in the bone tissue and the corresponding structures are caused by lack of circulation (FROST, 1989a). In this process the part of the osteogenic cells, which are necessary for the reparatory process also, perish (EINHORN, 1995).

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Sources for the osteogenic cells are: 1 - determinate bone cells that are precursors to the periost, endost and the bone medulla and which produce bone tissue without previous stimulation (DIAZ-FLORES et al., 1992); 2 - inductible bone cell precursors of which the main part is made from the unidentified mesenchymal cells of the corresponding muscle tissue and their cover. Those cells under the influence of the local growth factors and the mechanical factors metaplasticly change into osteogenic cells, which afterwards participate in bone tissue construction (BAX et al., 1999; BOSTORM, 1998; FROST, 1989b; REDDI, 1998; ROSS et al., 1986; SCHMITT et al., 1999; TRIPPEL et al., 1996; TASHIJAN et al., 1985).

For the healing of such fractures a method was needed which could improve the circulation of the bone tissue and increase the quantity of the osteogenic cells in the fracture zone. For this purpose, numerous methods are used in practical clinical work and application of bone morphogenetic protein and homologous bone transplant is most certainly one of most efficient methods for healing of complicated fractures (VUKIČEVIĆ et al., 1995; PECINA et al., 2001; DJAPIĆ et al., 2003). However, in this work we decided that the free omentum graft could be more suitable because of the following qualities: 1 - its vitality (HOSGOOD, 1990); 2 - its capability for the spontaneous formation of anasthomosys and revascularisation with the tissues it contacts (KARASAWA et al., 1993; MASUMORI et al., 1992; PIAFARRE and HUFNAFEL, 1968; VINEBERG et al., 1968); 3 - its high content of mesenchymal cells with metaplastic capacity (KARASCH, 1975); 4 - its content of cells which possess fagocitosis possibility, and its very feasibility for immunological reactions (KARASCH, 1975; HODEL, 1970; MESTNER et al., 1996).

The objective of our investigation was to estimate possibilities for application of free transplant of the greater omentum in improving the bone defect healing process.

Materials and methods

In this work, six-month-old male New Zealand rabbits (Institute of Immunology, Zagreb, Croatia) with a body mass of 1.9 - 2.5 kg (average of 2.1 kg) were used. The 36 experimental animals were divided in to 6 groups of 6 animals each, according to the forward planning sacrificial pattern. The experiment was performed in 3 phases: operation, X-ray examination, and histological examination.

The operation. Experimental animals were inducted into general anaesthesia by using ketamine (Narketan, Vetoquinol, Germany) in doses of 30 mg/kg of body mass IM. Three operations were then performed on each animal.

First, medial laparotomy was carried out and tissue of the greater omentum was explanted in a dimension of 5 × 2 cm. After the closure of the laparotomic wound the left femoral bone was explored and a bone defect was made in the middle section of the diaphisis. The defect was performed in the lateral cortex a 3.2 mm dia. drill and a hole was made into the medullar channel (Fig. 1). The defect was covered with the free transplant of the greater omentum and stitched to the corresponding muscles by Vycril 4-0 (Ethicom LtD, Belgium). After the closure of the wound on the left leg the procedure was repeated on the right leg (Fig. 2). The only difference was that corresponding muscle tissue covered the second defect.

The experimental animals were sacrificed as mentioned previously on the 7th, 14th, 21st, 28th, 35th and 42nd days after the operation. After euthanasia, both femoral bones were prepared for X-ray and histological examination.

All femoral bones were radiologically observed by X-ray using films for mammography.

The parts of the femoral bones with the defect were firstly decalcified, embedded in paraffin and cut into 5-micrometer slices. They were coloured using the haematoxilin-eosin method.

All procedures were performed according European standards with regard to keeping and handling laboratory animals (86/609/EEC) (GREEVE et al., 1993).

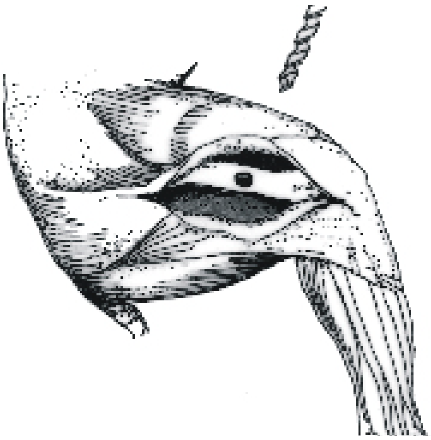

Fig. 1. Scheme of trepanation of femoral bone

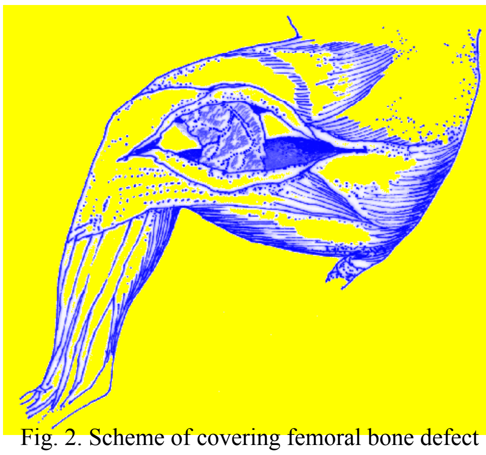

Fig. 2. Scheme of covering femoral bone defect with omentum

Results

Visual evaluation of the radiographs. In the 1st group of animals sacrificed on the 7th day post-operation there were no observed differences on the radiographs of the left and right femoral bones.

On the 14th day the shadows of paraosal mineralisation could be seen on both femoral bones. On the right bone, those shadows were close to the bone defect, but on the left bone they covered the whole diaphisal circumference area to a width of the 2-3 cm, including the bone defect (Figs. 3 and 4).

On the 21st day X-ray findings were similar to those of the 14th day (Figs. 5 and 6).

On the 28th day the shadow of paraosal mineralisation on the right bone had mostly disappeared, while on the left bone the volume was increased but of higher intensity (Figs. 7 and 8).

On the 35th day regression of paraosal mineralisation could be noticed on both bones, and periostal thickening of the diaphisis of the femoral bones could be easily observed at points of contact with the free transplant of the greater omentum.

On the 42nd day X-ray findings of both femoral bones were similar to those of the 35th day (Figs. 9 and 10).

Histological findings. Of the histological specimens the most noticeable differences were in the groups of animals sacrificed on the 7th, 14th, 21st, 28th, 35th and 42nd day post-operation.

On the 7th day fibroblastic proliferation was clearly noticeable on the specimen from the right femoral bone, while on the left femoral bone specimen the incalcination zone is visible, as well as strongly expressed fibroblastic proliferation (Figs. 11 and 12).

On the 14th day osteoid was observed in the fibroblastic proliferation zone on the right specimen, while on the left femoral bone specimen the formation of immature bone tissue could be noticed (Figs. 13 and 14).

On the 21st day mineralisation of osteoid was observed on the right specimen, while on the left specimen a stronger and wider spread of formation of immature bone tissue (wool bone) was observed (Figs. 15 and 16).

On the 28th day the formation of immature bone tissue could be confirmed on specimens of both femoral bones, although the tissue from the left femoral bone was wider spread and stronger in its mineralisation.

On the 35th day findings are very similar to that on the 28th day post-operation.

On the 42nd day immature bone tissue callus was formed in the zone of the defect on the specimen of the right femoral bone, while in the zone of the defect of the left femoral bone mature bone tissue had formed (Figs.17 and 18).

Fig. 3. Radiograph of left femoral bone on the 14th day post-operation

Fig. 4. Radiograph of right femoral bone on the 14th day post-operation

Fig. 5. Radiograph of right femoral bone on the 21st day post operation

Fig. 6. Radiograph of left femoral bone on the 21st day post-operation

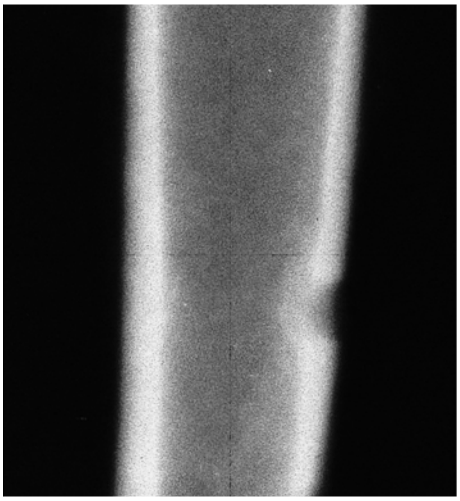

Fig. 7. Radiograph of right femoral bone on the 28th day post-operation

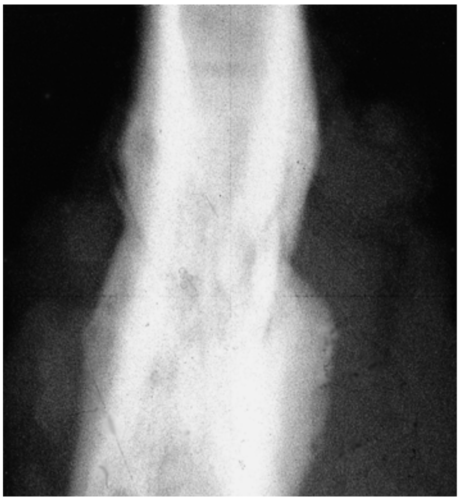

Fig. 8. Radiograph of left femoral bone on the 28th day post-operation

Fig. 9. Radiograph of right femoral bone on the 42nd day post-operation

Fig. 10. Radiograph of left femoral bone on the 42nd day post operation

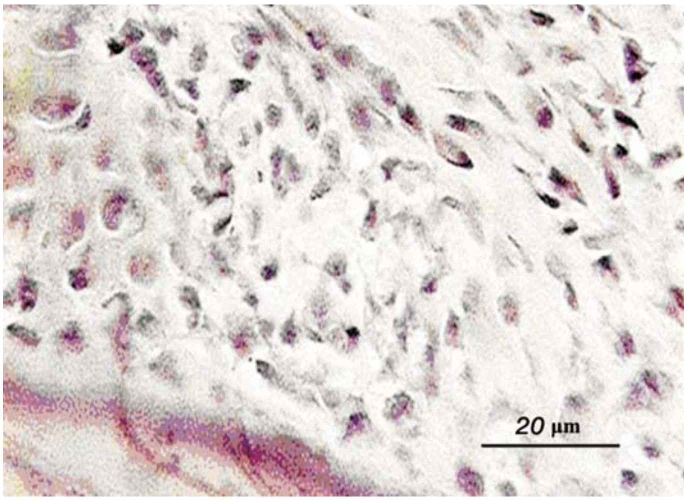

Fig. 11. Fibroblastic proliferations on the right femoral bone on the 7th day post-operation. H&E; × 250; scale bar = 20 µm.

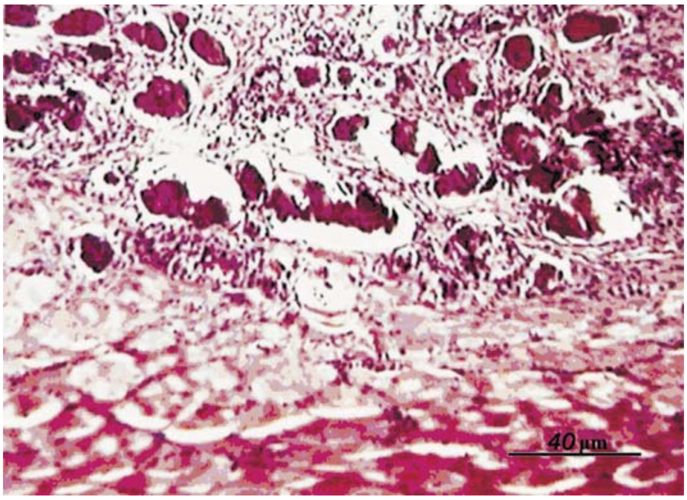

Fig. 12. Fibroblastic proliferation on the left femoral bone with incalcification on the 7th day post-operation. H&E; × 125; scale bar = 40 µm.

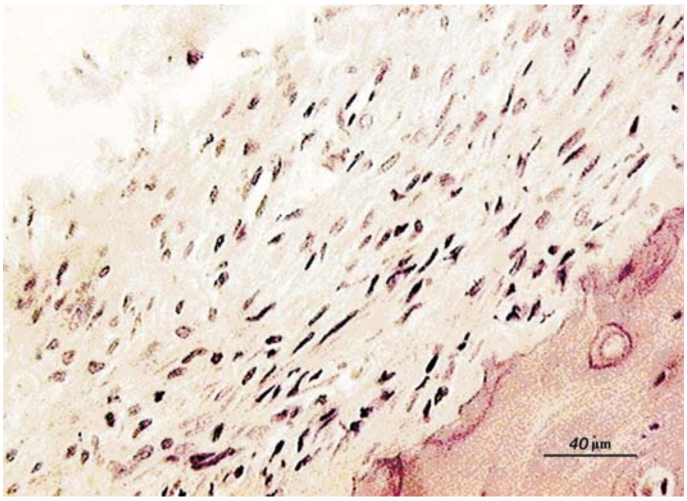

Fig. 13. Fibroblastic proliferation zone with osteoid on the right femoral bone on the 14th day post-operation. H&E; × 125; scale bar = 40 µm.

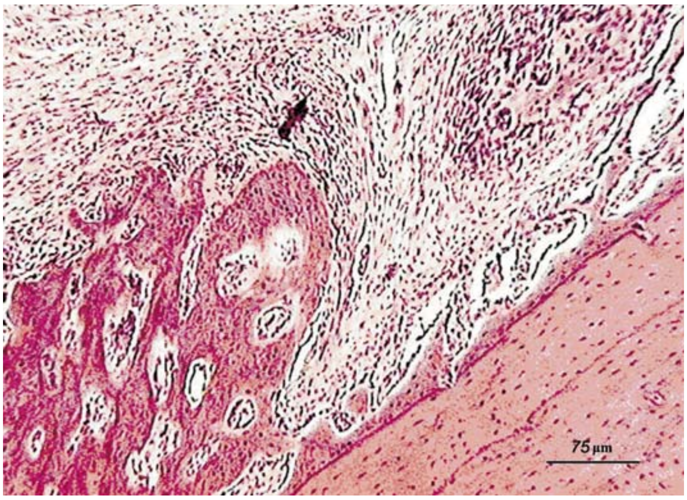

Fig. 14. Immature bone tissue on the left femoral bone on the 14th day post-operation. H&E; × 75; scale bar = 75 µm.

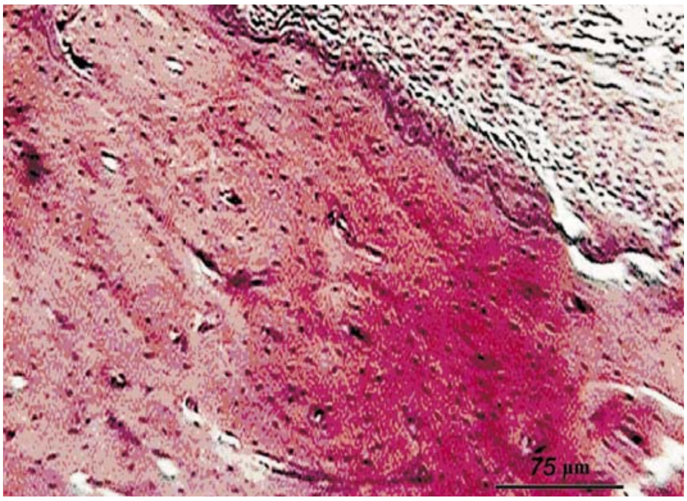

Fig. 15. Mineralisations of osteoid on the right femoral bone on the 21st day post-operation. H&E; × 75; scale bar = 75 µm.

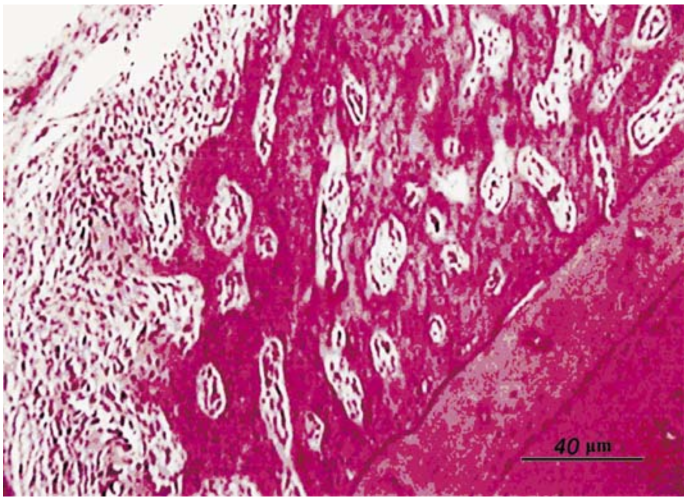

Fig. 16. Strong, wide formations of immature bone tissue (wool bone) on the left femoral bone on the 21st day post-operation. H&E; × 125 ; scale bar = 40 µm.

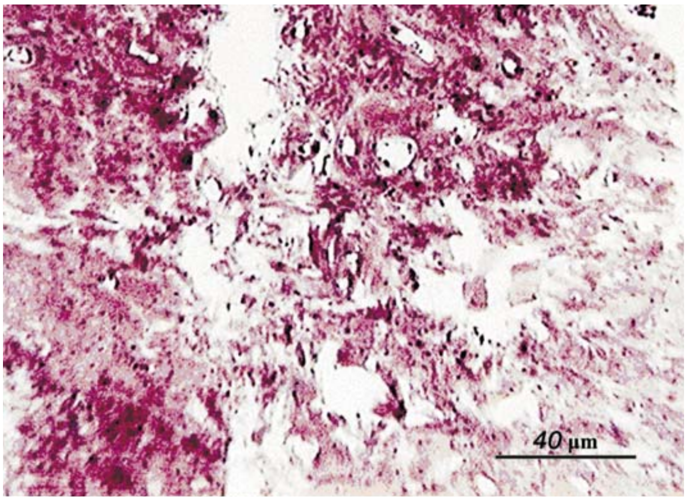

Fig. 17. Immature bone tissue callus on the right femoral bone on the 42nd day post-operation. H&E; × 125; scale bar = 40 µm.

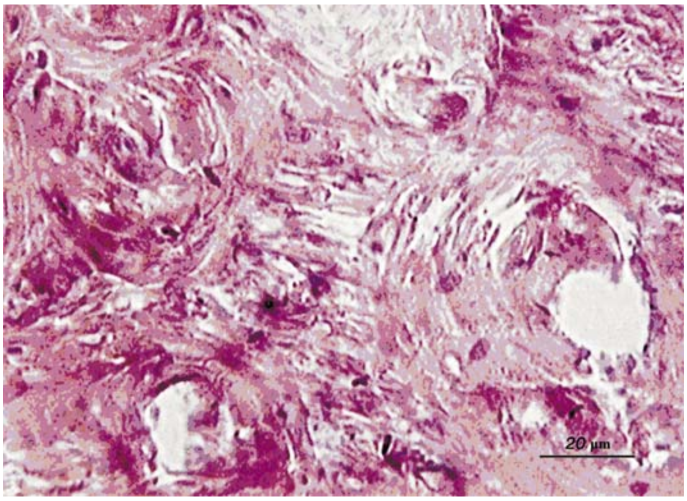

Fig. 18. Immature bone tissue on the left femoral bone on the 42nd day post-operation. H&E; × 250; scale bar = 20 µm.

Discussion

In this research the healing of bone defect was parallely observed. Defects were made on both femoral bones of 36 New Zealand rabbits. The corresponding muscle tissue covered the defect the right femoral bone, and the defect on the left femoral bone was covered with free transplant of the greater omentum.

Our presumption is that free transplant of the greater omentum implanted at the ectopic site between muscle tissue and bone will maintain its vitality and results by the forming of anasthomosys between the muscle and the defected bone tissue.

Angyogenesis and revascularisation were provoked and the undifferentiated mesenchymal cells were influenced by local growth factors metaplastically altered in the osteogenic cells. Thus, the absolute number of osteogenic cells in the zone of the bone defect increased. Radiological and histological examinations of the bone defect area were performed on the 7^{th}, 14^{th}, 21^{st}, 28^{th}, 35^{th} and the 42^{nd} day post-operation.

On radiological findings a visually significant difference was observed in the quantity of the callus and the intensity of the mineralisation in the groups of the experimental animals elaborated on the 14^{th}, 21^{st}, 28^{th}, 35^{th} and the 42^{nd} day post-operation. In all groups the quantity of callus formation and the intensity of its mineralisation were more strongly distinct on specimens where the bone defect was covered with the free transplant of the greater omentum. Also, those specimens had histologically noticeable earlier formation of the bone tissue that fulfilled the bone defects.

On the 42^{nd} day post-operation the bone defects of the right thigh bone were fulfilled with immature bone callus tissue while those of the left thigh bone with mature bone callus tissue.

The influence of mechanical factors was kept to the minimum, because drilling only the cortex of one side created the bone defect. The periostal callus, which covered the circumference the diaphisis of the femoral bone, which defect was covered by free transplant of the greater omentum, proved that the bone healing process was faster and more effective that in the control group.

All the aforementioned could lead in to the conclusion that greater omentum tissue has well-developed osteogenic potential. This potential could have certain implications in human medicine.

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SAŽETAK

U istraživanju su promatrani procesi cijeljenja koštanih oštećenja na bedrenim kostima novozelandskih kunića. U svake pokusne životinje učinjeno je po jedno istovjetno koštano oštećenje na desnoj i lijevoj bedrenoj kosti. Na desnoj bedrenoj kosti oštećenje je prekriveno priležećim mišićjem, a na lijevoj slobodnim presatkom velikog omentuma. Procesi cijeljenja koštanih oštećenja praćeni su rendgenološkim i histološkim pretragama. Rezultati istraživanja pokazuju da su procesi tvorbe kalusa i njegova mineralizacija brži i većeg opsega u području koštanih oštećenja prekrivenih slobodnim presatkom velikoga omentuma u odnosu na koštana oštećenja prekrivena priležećim mišićjem

Ključne riječi: cijeljenje kostiju, slobodni presadak, veliki omentum, kunić

Book review

RENATE WERNERY, ULRICH WERNERY, JÖRG KINNE, JAIME SAMOUR: **Colour Atlas of Falcon Medicine**. Schlütersche Verlaggesellschaft mbH & Co. KG, 2004. Nine chapters, 144 pages, 378 illustrations, 31 tables. Hard cover. ISBN 3-89993-007-X. Price: € 94.- / CHF 151.-

Finally an excellent book about falcons and falcon medicine is overcoming a gap in until now missing collected knowledge. A group of five not only enthusiasts but more over well-known experts managed to put together many years of their experience in avian medicine and specific field of birds of pray. Colour Atlas of Falcon Medicine has nine chapters covering the history and actual significance of falcons and falconry in Middle East. Systematically in the next eight chapters described are hematology and blood chemistry, the most important viral and bacterial diseases, fungal diseases and parasitic diseases. Diseases of unknown etiology and those caused by some errors in feeding, applied technology and by toxins are also included. The recent knowledge in specific immunoprotection is also included. The Atlas is illustrated with 380 color photos of excellent quality, related to the details of diseases described. For diagnostic purposes the relevant hematology and biochemistry values of healthy and diseased falcons are given in 30 tabular surveys. Written in an easy understandable mode it is dedicated to all those who has an interest in falcons and falcons medicine, primarily to students of veterinary medicine, veterinarians specializing in avian medicine and also biologists, zoologists as well as ecologists.

Hrvoje Mazija