

BONE FORMATION IN GLASS-CELLULOSE COMPOSITE IMPLANT IN RAT FEMORAL DEFECT FOLLOWS WOLFF'S LAW

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INTRODUCTION: Different methods have been described to create, fill and follow bone formation in osseous defects using biomaterial implants. For practical reasons (large number of samples, economical tests, etc.) small animals such as rats are favourable despite of the fact that a critical size defect is difficult to achieve. We developed a standardised rat femur model to study bone formation in various biomaterials. The method allows biological (histology), biochemical (protein and DNA content, Northern hybridization and immunohistochemistry) and biomechanical (bending tests, etc.) analyses^{1,2}.

Using this model we detected that the femur reacted by producing new bone opposite to the defect site.

MATERIALS AND METHODS: Young adult male Sprague-Dawley rats were used in these experiments. A full-thickness cortical defect in the antero-lateral aspect of *trochanter major* was made by a series of dental drills and extended to 2.4 x 8 mm using a special device. After evacuation of debris and bone marrow the implants were inserted and bone formation was followed up to 24 weeks. Some defects were also left empty to follow spontaneous healing. Tetracycline and calcein fluorescence were used to monitor the growth of mineralized bone by subcutaneous injections (tetracycline 25 mg/kg and calcein 7.5 mg/kg) at three different time points (*Figure 1*). The labelled animals were killed at 1, 4, 8, 12 and 24 weeks, femurs were prepared and fixed either in 4% paraformaldehyde, embedded in hydroxyethyl-methacrylate (HMA) and 6 µm sections were stained by van Gieson or in ethanol, embedded in isobornyl-methacrylate and sectioned to 20 µm slices by a cutting-grinding method. Several other animals were kept until 60 weeks for routine histology.

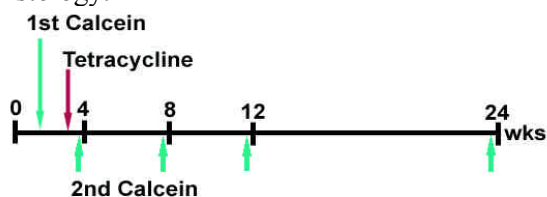


Fig. 1: Scheme for labeling with calcein and tetracycline.

RESULTS AND DISCUSSION: Histological sections showed that bone growth started from the implant ends, progressed into the matrix as a cancellous bony bridge, which was remodelled gradually to the centre of the implant and formed an almost complete tubular bone (*Figure 2*). There was still implant material left in the defect at 60 weeks after implantation. Thus, the defect never healed completely and was observed as a depression in the bone (*Figure 3*). Tetracycline and calcein labelling demonstrated new bone growth endosteally into the implant.

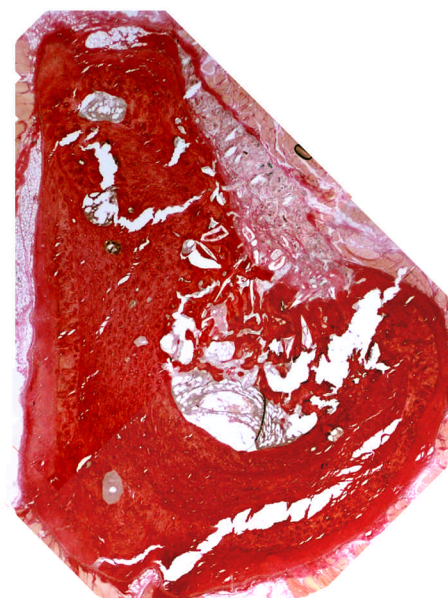


Fig. 2: van Gieson stained cross section of bone at 24 weeks.

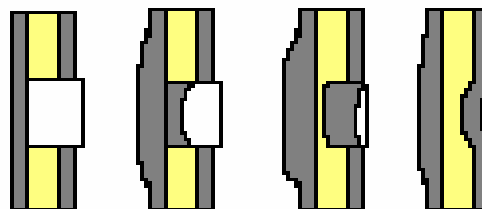


Fig. 3: Schematic picture of bone healing in the cortical defect model.

There was also a strong periosteal thickening of bone mainly opposite to the defect that supported the tubular bone structure (*Figure 4*). This bone

growth was active during the first four weeks and did not progress much thereafter. The Wolff's law determines how much and where bone will be formed to maintain bone strength. This fact and the availability of periosteum, which is active in bone healing, explains why new bone appeared first periosteally opposite to the defect.

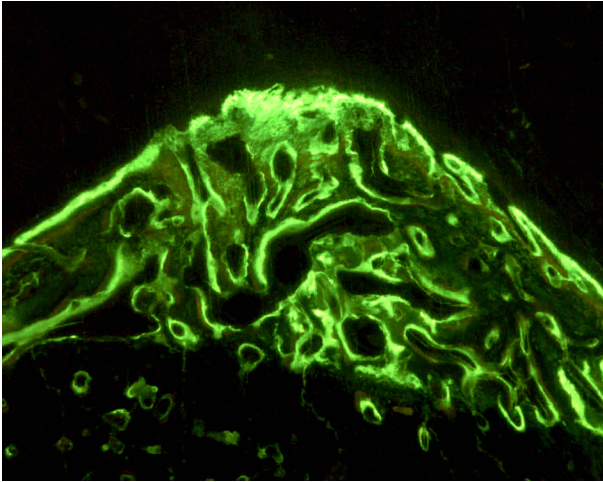


Fig. 4: Fluorescence labelling of the periosteal growth of bone.

This method produces sufficient sample size to allow histology, scanning electron microscopy, biochemical methods and biomechanical tests. Bending strength measurements of the bone containing the implant showed no differences between femurs with or without the implants. The bone was strengthened according to Wolff's law throughout the periost but especially at the opposite site already before new bone was formed into the implant. This response was rapid and might explain why appearance of new bone in the implant occurred at a rate less than expected.

CONCLUSIONS: We developed a method to evaluate implant materials in rat femur defects. The healing of the defect occurred according to Wolff's law. Much of the strength was achieved by thickening of bone opposite to the defect and later also by growth of new bone through the implant. Periosteal thickening thus allows achievement of normal bone strength quicker than new bone growth into the implants. This effect allows a clear-cut comparison of osteoconductive/osteopromotive properties of the implant materials.

REFERENCES: ¹M. Märtson, S. Mansikka-Savolainen, A. Kuusilehto, J. Holmbom, P. Saukko, R. Penttinen, J. Salonen Bioactive glass-cellulose composite as osteoconductive material. Follow-up of tissue growth into a bone defect in

the rat. Submitted. ² Ekholm, M. Tommila, A-P. Forsback, M. Märtson, J. Holmbom, V. Ääritalo, C. Finnberg, A. Kuusilehto, J. Salonen, A. Yli-Urpo, R. Penttinen Hydroxyapatite Coating of Cellulose Sponge Favors Fibroblastic Expression in Bone. Submitted.

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