

## Stimulation of bone formation by transfer of phVEGF in a gene activated matrix

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**INTRODUCTION:** The early vascular response is essential for the normal progress of fracture healing.<sup>1</sup> VEGF (Vascular Endothelial Growth Factor) is known to stimulate therapeutic vascular growth<sup>2</sup>. Direct application of VEGF plasmid has been used in clinical trials on leg ischemia with positive results.<sup>2</sup> Furthermore VEGF is known to influence osteoclasts, osteoblasts and bone remodelling at the growth plates. We wanted to show that the application of VEGF-plasmid as a gene activated matrix is able to promote angiogenesis in bones and prevent pseudarthroses.

**METHODS:** A 15mm segmental defect was created in the mid-part of the radial shaft of adult New Zealand White Rabbits and filled with a collagen sponge. 60 rabbits were operated in 5 groups. The sponge was either not loaded (group 1) or loaded with 100 or 1000µg of the naked plasmid pCR3.1 (group 2 and 3) or active plasmid with phVEGF<sub>165</sub> (group 4 and 5). After 6 or 12 weeks animals were sacrificed and bones harvested. The amount of new bone was evaluated by µCT scan with app. 32 slices/mm. Only bone that was formed in the bed of the removed bone was counted. Postoperatively every three weeks x-rays of the operated limb were taken in two perpendicular planes and the density and amount of new bone was evaluated semiquantitative. Following fixation and decalcification longitudinal sections were taken in three predefined layers. Semi-automatic image evaluation helped counting vessels in 9 predefined ROIs.

**RESULTS:** No bone healing occurred in the three control groups. X-ray controls showed essential osteogenesis in the two verum groups. Complete bridging was seen in 14 of 24 animals, 10 had major ossification with transfixation to the ulnar. None of the animals in the control group achieved a bridging ossification. There was a higher degree of bridging ossification in the VEGF 100µg group than in the VEGF 1000µg group after 6 weeks.



Fig 1: x-rays  
12weeks postoperative  
a) VEGF group  
b) control group

µCT scans showed a significant higher amount of new bone in the two VEGF groups. After 12 weeks

slightly more bone was seen in the VEGF 100µg group than in the VEGF 1000µg group. After 6 weeks no differences were measured.

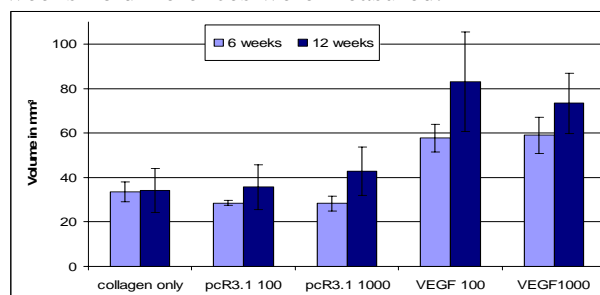


Fig. 2. Volume of new bone seen in µCT scans

More than twice as much vessels were counted in the two study groups compared to the control groups. Slightly more vessels were counted in the VEGF 1000µg group. After 12 weeks we found fewer vessels than after 6 weeks.

**DISCUSSION & CONCLUSIONS:** The GAM (gene activated matrix) gives us the possibility to sustain sufficient VEGF levels over the time needed.<sup>3</sup> Histological evaluation showed that higher amounts of VEGF plasmid led to higher vessel density after six weeks, while bone bridging was slightly higher in the 100µgVEGF group. It is possible that higher doses of VEGF reduce osteogenesis by activating osteoclasts and bone turnover. In X-ray evaluation after 6 weeks more bone bridging was seen in the 100µg group. After 12 weeks slightly more bone was found in the µCT scans. Differences between the two verum groups were not significant, whereas in all three examined criterions (amount of bone in µCT, bone bridging in x-ray and vessel formation) there were significant differences to the control groups. This study shows that VEGF has the potential to induce bone formation in situations that would otherwise lead to atrophic pseudarthroses.

**REFERENCES:** <sup>1</sup>Glowacki (1998) Angiogenesis in Fracture Repair. Clin Orthop. (355):82-89 <sup>2</sup>Nikol et al.(2001) J Invas Card: Preclinical and clinical Experience in Vascular Gene Therapy: Advantages over Conservative/Standard Therapy 13: 333-338 <sup>3</sup>Scherer et al.(2002) Nonviral vector loaded collagen sponges for gene delivery in vitro and in vivo. J Gene Med 4: 634-643