

# INCREASED BONE REMODELING AROUND HYDROXYAPATITE COLLAGEN COMPOSITE BONE SUBSTITUTES IN THE RAT TIBIA

[S. Rammelt](#)<sup>1</sup>, E. Schulze<sup>1</sup>, M. Witt<sup>2</sup>, E. Petsch<sup>1</sup>, M. Holch<sup>1</sup>, W. Pompe<sup>3</sup>, H. Zwipp<sup>1</sup>

<sup>1</sup>*Department of Trauma and Reconstructive Surgery,* <sup>2</sup>*Department of Anatomy,* <sup>3</sup>*Institute of Material Sciences, Technical University of Dresden, Germany*

**INTRODUCTION:** Hydroxyapatite (HA) as an organic bone mineral is frequently used as bone substitute in Trauma and Reconstructive Surgery. Among the problems encountered with HA implants are the formation of fibrous membranes around the implant, foreign body reactions to HA particles and the absence of complete remodelling. In order to find a more biologic bone substitute we used a synthetic HA-collagen composite that was mineralised in vitro under physiologic pH and temperature (1).

**METHODS:** A standardized rat tibia model was used for in vivo assessment of the bone substitutes. Cylinders of 6 mm in length and 1.5 mm in diameter either of pure HA and HA containing 3 vol % bovine collagen (HAK) were implanted into the metaphyseal portion of intact proximal rat tibiae under ketamine anaesthesia. The animals were sacrificed after 2, 4, 6, 14 and 28 days. The tibiae were excised, fixed in buffered formalin, decalcified for 8h, dehydrated, embedded in paraplast and sectioned at 6 µm thickness. For immunohistochemical characterization of the interface region, we used antibodies against the prevalent intermediate filaments of mesenchymally derived cells (vimentin), inflammatory cell receptors (Galectin 8) bone-specific ECM products (osteopontin, osteocalcin, osteonectin), cell adhesion molecules (CD44). Furthermore antibodies against smooth muscle actin and v. Willebrand-factor were used. The macrophage marker ED1 and the cysteine proteinase Cathepsin D that is predominantly seen in osteoclasts (2) but also in fibroblasts and epitheloid cells, were assessed numerically in the cells at the interface. Significance was tested with the Mann-Whitney and Wilcoxon test and assumed with  $P < 0.05$ .

**RESULTS:** Two days after surgery a thin cell layer consisting of osteoclasts exhibited an increasing reactivity for CD44 and vimentin. At this stage Cathepsin D in multinucleated cells (osteoclasts) was increased significantly around

HAK as compared to HA. Osteogenetic capacity of the new interface was demonstrated at day 4 by immunostaining against osteopontin, a ligand for CD44 and osteonectin. Around HAK both ED1 and Cathepsin D were increased significantly. After 6 days, cells of the macrophagic lineage were still present in close contact to the implant. In later stages (day 14), neovascularization was demonstrated by a strong immunostaining against von-Willebrand-factor. After 28 days both bone substitutes were surrounded completely by new trabecular bone without being invaded. At 6, 14 and 28 days Cathepsin D was significantly increased around HAK as compared to HA.

**DISCUSSION & CONCLUSIONS:** The rat tibia model has proved to be a useful tool for the detection of early osteogenesis in the interface region between bone and bone substitutes with immunohistochemical characterization. Since cathepsins play an important role in bone remodelling both under physiologic and pathologic conditions, the significant increase around collagen-containing HA suggests a higher rate of bone turnover. To further promote invasion and complete restructuring of the bone substitute, further investigation is needed concerning the porosity of the bone substitute and the possible role of other molecules in the extracellular matrix, like osteopontin.

**REFERENCES:** <sup>1</sup> Bradt et al. (1999) *Chem Mater* **11**, 2694-2701, <sup>2</sup> Blair et al. (2000) *J Cell Biochem* **78**, 627-637

**ACKNOWLEDGEMENTS:** This study is supported by grant FOR 308/2-1 from the Deutsche Forschungsgemeinschaft (DFG)