Evaluation of bone substitutes and gene expression pattern in a tibia defect model in the rat

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Introduction: A bovine bone substitute Bio-Oss® (BO) and the two full synthetic nanostructured bone substitutes NanoBone® (NB) and NanoBone®-S (NB-S) have been tested by implantation into rat tibia defects. NanoBone® consists of nanocrystalline hydroxyapatite, which is embedded in a highly porous matrix of silica gel. The initial phase of implantation is commonly marked by promotion of bone regeneration and partially degradation of biomaterials by osteoclast. Nevertheless, little is known about the pathways and molecular mechanisms generated by constituent parts of biomaterials. One aim of this study was the comparison of newly formed bone and material degradation after implantation of these three biomaterials. The other aim was the determination of specific biomaterial-initialized genes, which have been expressed in rats during bone formation.

Materials and Methods: The nanocrystalline hydroxyapatite are mixed with a silica sol at the ratio of 76:24 (wt%) HA:SiO_2 (NanoBone®) and alternatively of 61:39 (wt%) HA:SiO_2 (NanoBone®-S). The structures of NanoBone®, NanoBone®-S and Bio-Oss® were determined by transmission and scanning electron microscopy, powder diffraction and mercury porosimetry. Male Wistar rats (body weight 300–400 g; Charles River Laboratories, Sulzfeld, Germany) were used for experiments and kept on water and standard laboratory chow ad libitum. The rats were anaesthetized with an intraperitoneal injection of 60 mg/kg of 6% sodium pentobarbital (Sigma, Deisenhofen, Germany) and a monocortical defect (3,5 mm diameter) was created on the proximal tibia diaphysis. These defects were refilled with biomaterials (see above). Animals were sacrificed at day 3, 6, 9, 12, 21, 42, 63 and 84 (n=7 for each time point). Decalcified tissue samples were embedded in paraffin, 3µm sections were cut and stained with hematoxylin-eosin (HE) for routine histological analysis. Furthermore, after 12 days gene expression pattern induced by the different biomaterials was determined. Using microarray technology, differentially expressed bone specific genes were analyzed. The sample labelling, hybridization and staining have been carried out according to Protocol for Affimetrix® GeneChip® Rat Gene 1.0 ST Array System.

Results: All biomaterials comprised nano-crystallites of hydroxyapatite. NanoBone®-S was the biomaterial, whose morphology of the crystallites was identical to biological apatite. The nanoporous structure was contained in all samples. In comparison to the biomaterial Bio-Oss® NanoBone® was quickly degraded, whereas autologous proteins were incorporated into nanopores. New bone formation in NanoBone®-S was significantly higher in comparison to BO at day 84 after implantation. The presence of osteoclasts in tissue sections was demonstrated by AP-histology and ED1-immuno-histology.

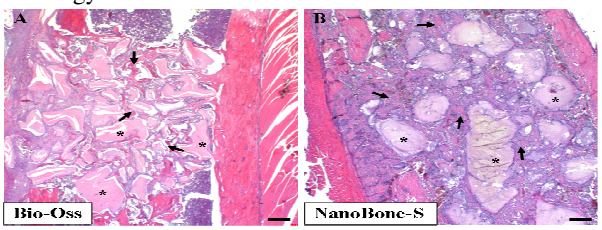

Figure 1: Histological HE-stained specimen of rat tibia defects (on day 12). Asterisks indicate nanoporous structure of biomaterials and arrows the newly formed bone. Scale bar: 400µm

Data from all microarray chips have been analysed and many bone specific genes have been identified, whereas the expression profiles were similar in all used biomaterials. Osteoblastic (e.g. Osteoactivin, Fibromodulin) as well as osteoclastic genes (e.g. Cathepsin K, Chemokines, Matrixmetallopepdidases) and genes encoding extracellular matrix components (e.g. Collagenases, Fibonectin, Laminin) could be detected. Furthermore, specific genes for osteoclasts and osteoblasts particularly showed expression pattern in dependence of biomaterials.

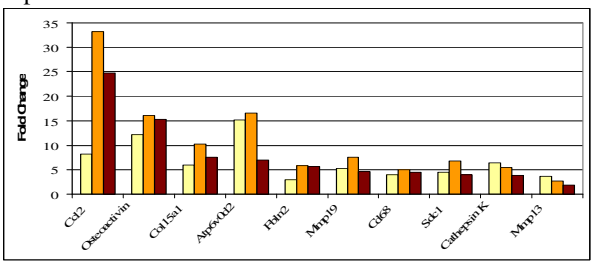

Figure 2: Gene expression pattern of osteoblastic and osteoclastic genes (BO:yellow; NB: orange; NB-S: brown)

Conclusions: The composition and structure of bone substitutes play an important role for the osteoconduction in bone defects. NB39 has good osteoconductive properties and shows adequate resorption process. Therefore, a clinical application of this biomaterial for the treatment of bone defects is conceivable. To understand the molecular mechanisms of bone formation using different bone substitute materials microarray analyses are important measurements for the identification of single genes of the differentiation processes of osteoblasic and osteoclastic progenitor cells. For investigations of their functions in bone formation/ remodelling and all interactions with other proteins, these genes can be analysed now. This study provides the basis for further analysis.