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ADDITION OF OSTEOCALCIN TO HA/COLLAGEN COMPOSITES

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INTRODUCTION: The filling of large osseous defects still poses a major problem in reconstructive surgery. While the ostoeconductive properties of hydroxyapatite (HA)-collagen composites are well recognised [1], the goal of the present study was to investigate, if the addition of osteocalcin, an extracellular matrix protein, enhances bone healing around such implants. Osteocalcin is expressed by osteoblasts and is thought to play a role in the early stages of bone healing. It is crucial in regulating osteoblast activity and binding of hydroxyapatite.

METHODS: Cylindrical implants of 2.5 mm diameter containing 97.5% HA and 2.5 vol% collagen type I were introduced into the tibial head of male adult Wistar rats. 10µg/g of osteocalcin (OC) was added to one group. 6 animals of each group were killed at 2, 7, 14, 28 and 56 days. Specimens were fixed with 4% buffered formalin, washed, decalcified in EDTA, dehydrated and embedded in paraplast. For Immunohistochemistry 2? m sections were dewaxed, dried, washed and incubated with primary antibodies that were detected with biotinylated secondary antibodies followed by a streptavidin/biotin-peroxidase complex. Peroxidase activity was visualised with 3´-3´-diaminobenzidine. We used antibodies against the prevalent intermediate filaments of mesenchymally derived cells (vimentin), bone-specific extracellular matrix products (osteopontin, bone sialoprotein), cell adhesion molecules (CD44s), macrophages (ED1 Cathepsin D) and osteoclasts (TRAP).

RESULTS: 7 days after implantation the interface around the OC implants was rich in mononuclear cells, while around the controls there was a more fibrous interface. Small newly formed osteoid seams were observed around both implants. At 14 days newly formed osteoid reached the implant surface in both groups, the contact area being greater in the OC group. At 28 days the newly formed woven bone around OC implants was replaced partly by lamellar bone (Fig. 1). Inflammatory cells, fibroblasts and mesenchymal stromal cells were observed abundantly around both implant types at day 2 and decreased more rapidly around the OC implants than in the controls. Staining for CD 44, indicating interaction of fibroblasts and osteoblasts, was seen as a small seam around the implants at day 2 and increased until day 14, more markedly around OC implants. Macrophages, specified with Cathepsin D and ED 1, were abundant at day 7 through 14 and decreased thereafter around the OC implants, while the reaction for ED1 and Cathepsin D increased until day 28 in the controls. Total cell numbers per low power field differed significantly at days 7, 14 and 28 ($p<0.05$). Both osteogenic progenitor cells and osteoblasts, staining for osteopontin and bone sialoprotein, were observed earlier (day 7) at the interface around OC implants than around the controls (day 14).

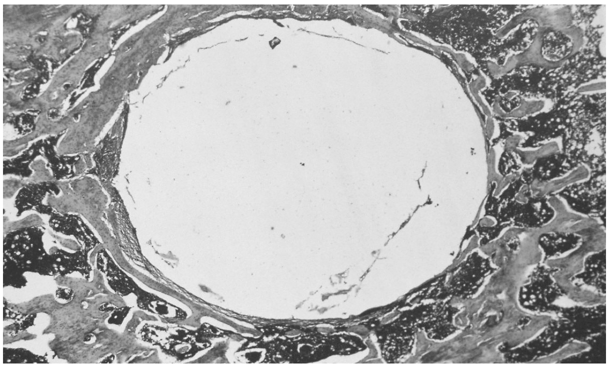

Fig. 1: Newly formed lamellar and woven bone around OC implant at 28 days (HE, x10).

DISCUSSION & CONCLUSIONS: The addition of osteocalcin enhances bone formation around HA-collagen composites in the rat tibia. The earlier observation and increased number of mononuclear phagocytozing cells at the interface around the OC implants suggests an earlier onset and higher rate of bone remodeling. The higher osteogenic capability of the bone-implant interface was demonstrated by the earlier and increased expression of bone-specific matrix proteins (osteopontin, bone sialoprotein) around the OC implants. The addition of further matrix components to HA-collagen composites appears to be an attractive step in the attempts to achieve a better osteogenetic potential of bone substitute materials to enhance defect healing.

REFERENCE: [1] S. Rammelt, E. Schulze, M. Witt, E. Petsch, M. Holch, W. Pompe, and H. Zwipp (2002) *Chir Forum*, **31**: 411-414

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