Growth promoting in vitro effect of synthetic cyclic RGD-Peptides on human osteoblast-like and endothelial cells attached to cancellous bone

Peter Diehl¹,², Jörg Auernheimer³, Susanne Bierbraum², Horst Kessler³, Wolfram Mittelmeier¹, Ulla Magdolen⁴
¹Orthopedic Surgery, University of Rostock, Rostock, Germany; ²Biomet Deutschland GmbH, Berlin, Germany; ³Chemistry, Technical University Munich, Munich, Germany; ⁴Orthopedic Surgery, Technical University Munich, Munich, Germany

Introduction: In tissue engineering, the application of biofunctional compounds on biomaterials such as integrin binding RGD-peptides has gained growing interest. Anchorage-dependent cells like osteoblasts bind to these peptides, thus ameliorating the integration of a synthetic implant. In case sterilized bone grafts are used as substitutes for reconstruction of bone defects, the ingrowth of the implanted bone is often disturbed because of severe pretreatment such as irradiation or autoclaving, impairing the biological and mechanical properties. Here, we report for the first time on the in vitro coating of the surface of freshly resected, cleaned bone discs with synthetic, cyclic RGD-peptides.

Materials and Methods: The synthetic, cyclic RGD-peptides we have used display unique and enhanced stability in vivo compared to linear RGD-peptides and are not degraded by human proteases. Moreover, these synthetic RGD-peptides contain phosphonate anchor groups which efficiently react with the inorganic bone matrix to form stable bone-RGD-peptide constructs. We used two different RGD-peptides, one containing two phosphonate anchors, the other peptide four of these binding moieties. Human cancellous bone disks were cut from hip bone, cleaned and defatted by acetone, similar to the procedure applied for chemical sterilization of bone used in the preparation of allogenic and autologous bone grafts. These bone discs were subjected to coating with two types of cyclic RGD-peptides differing only in the number of phosphonate anchor groups, with the aim to enhance the rate of adhesion and proliferation of osteoblast-like cells and human endothelial cell (HUVEC). The cultivation of osteoblast-like cells and endothelial cells on RGD-coated bone discs was performed for 8 days before the number of attached cells was analyzed by determination of the lysosomal enzyme hexosaminidase, an indirect measure of cell number.

Results: We found a significantly higher growth of cells on RGD-coated bone discs with 18% more for RGD-1 and 40% more for RGD-2 for osteoblasts-like cells, and 25% and 52% for endothelial cells, compared to non-treated control bone discs. Interestingly, this enhanced settlement rate is not due to a higher initial adhesion rate, as the amount of attached cells at the beginning is not increased on RGD-coated bone discs compared to uncoated controls. This results are in line with immunohistological findings of the bone discs.

Discussion: Interaction of cells with ECM proteins or synthetic RGD-peptides is mediated by integrins, transmembrane protein receptors that control cell functions such as cell-matrix adhesion and cell proliferation. The synthetic, cyclic RGD-peptides we applied are selective for binding of αv-containing integrins; therefore, upon cell binding, they mimic the effects of the multifunctional RGD-peptide containing ECM proteins fibronectin and vitronectin. Evidently, the stimulation of long-term settlement of osteoblast-like cells and endothelial cells is more effective for RGD-2 than for RGD-1. In this respect it is worth mentioning that the peptide RGD-2 contains four phosphate anchors and therefore provides stronger anchoring to the inorganic bone matrix than RGD-1 which contains only two phosphonates. Though there was no effect of RGD-peptides in the initial phase of the adhesion experiments, we observed a long-term growth-stimulating effect on the osteoblast-like cells during the 8 day cultivation period if attached to an RGD-peptide-coated, fibronectin-mimicking surface in relation to the non-coated control bone discs.

Our data provide evidence that RGD-coating positively stimulates the growth of osteoblast-like cells attached to native human bone. This new finding opens a new vista to a convenient, practicable method of conditioning the surface of any kind of bone graft prior to implantation, thus promoting an improved and earlier integration of a resected bone implant in vivo. For an optimal accessibility of the inorganic bone matrix to the RGD-peptides, a cleaning step to remove tissue debris and fatty components from the bone graft surface is advisable. A gentle cleaning procedure for autologous bone grafts might be ultrasonication, which was shown to efficiently clean bone pieces before cultivation with osteoblast-like cells.

We would like to stress that endothelial cells bind to RGD-motifs as well. Therefore we expect that RGD-coating of bone matrices does promote neovascularization, a precondition for osteointegration after bone implantation.

We conclude that coating of native human bone with synthetic, cyclic small size RGD-peptides stimulates in vitro growth of human osteoblast-like cells attached to this modified bone surface. Therefore, it is intriguing to speculate that coating of bone implants with RGD-peptides prior to reimplantation could serve as a novel methodical approach in order to accelerate the in-growth and healing of a bone implant.