Investigation of the interaction of mediators with matrix components and analysis of extracellular matrix by NM

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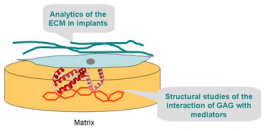

In the third funding period, the investigations of the interaction of regulatory proteins with components of the extracellular matrix will be continued and extended. Our main protein target is sclerostin, which is an important regulator in bone regeneration. Sclerostin is a well-known regulator of the Wnt signaling pathway and intensively studied in project B2. In particular, we are interested in the interaction of sclerostin with GAG. To study this interaction, we will recombinantly express the protein with NMR active isotopes (15N, 13C, 2H) and subject it to solution NMR studies. Once full NMR signal assignment is achieved, natural and artificial GAGs will be titrated to the protein in order to study its interaction with these molecules. In addition to the experimental determination of structural parameters and binding constants, we shall be carrying out computer simulations to dock the GAGs into the active protein structure in collaboration with A7. The goal of this research is the development of an atomistic model of the complex of sclerostin with various GAGs. As the influence of the Wnt signaling pathway is mediated via the interaction with the protein LRP5/6, we would like to study how GAG can modify the interaction between these proteins. To this end, we will express the first two propeller domains of LRP6 and carry out binding studies with sclersotin in the presence and absence of GAG. Studies of the diffusion of regulatory proteins in implant materials will also be continued in the next funding period. Finally, we would continue recording the formation of extracellular matrix in bone implants quantitatively. To this end, surface-modified artificial matrices developed by our collaboration partners will be implanted into animal bone models in collaboration with A1, B2, and B5. After explantation, we will carry out the quantitative monitoring of the de novo expression of collagen and bioapatite in the implants by MRI and NMR spectroscopy. We will study samples from a fracture model of the femur of mouse and rat. The incorporation of differently coated materials will be analyzed with respect to the specific chemistry of the surface coat and its influence of the bone healing process.

Project-related Publications

1. Panitz N, Theisgen S, Samsonov SA, Gehrcke JP, Baumann L, Bellmann-Sickert K, Köhling S, Pisabarro MT, Rademann J, Huster D, Beck-Sickinger AG. The structural

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11. Möbius K, Nordsieck K, Pichert A, Samsonsov SA, Thomas L, Schiller J, Kalkhof S, Pisabarro MT, Beck-Sickinger AG, Huster D. Investigation of lysine side chain interactions of interleukin-8 with heparin and other glycosaminoglycans studied by a methylation-NMR approach. **Glycobiology** . 2013;23:1260-9.

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13. Förster Y, Scheidt HA, Nimptsch A, Hacker MC, Schulz-Siegmund M, Ahnert P, Schiller J, Rammelt S , Huster D . The pore size of PLGA bone implants determines the de novo formation of bone tissue in tibial head defects in rats. **Magn Reson Med.** 2012;70:925-35 .

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