

## Modulation of VEGF Release from Functionalized Bone Graft Material

[U. Koenig<sup>1</sup>](#), [A. Guenther<sup>2</sup>](#), [O. Kreft<sup>2</sup>](#), [Th. Hanke<sup>1</sup>](#), [M. Yamamoto<sup>3</sup>](#), [M. Gelinsky<sup>1</sup>](#),

<sup>1</sup> [Max Bergmann Center of Biomaterials, Technical University Dresden, Institute for Material Science, Tissue Engineering & Biomineralization, Dresden, Germany](#); <sup>2</sup> [Max-Planck Institute of Colloids and Interfaces Research, Potsdam, Germany](#); <sup>3</sup> [Institute for Frontier Medical Sciences, Kyoto University, Department of Biomaterials, Kyoto, Japan](#)

**INTRODUCTION:** The current work is focused on the sufficient functionalization of biomimetic bone substitute material with growth factors. Thereby the applied material has been produced in our laboratory by the process called synchronous biomineralization. The developed composite material is a porous 3D-scaffold consisting of collagen I and hydroxyapatite – the main components of natural bone [1]. Different strategies have been established and investigated in order to optimize and regulate the delivery of hrVEGF (human recombinant vascular endothelial growth factor) from the mineralized collagen matrices as growth factor carriers. The cytokine VEGF is known as heparin-binding protein supporting the initiation of angiogenesis – the promotion of blood vessel formation in regenerative tissue. Therefore the aim of this present approach is to design a VEGF release system in a controlled and sustained manner using heparin as a key substance for the carrier modification. The possibility to enhance the angiogenic properties of the bone-like material gives a chance to assist the survival of ingrowing cells.

**METHODS:** The ongoing study is divided into two principal strategies. We want to compare the release characteristic of hrVEGF bonded to the mineralized collagen matrices *directly* via physical adsorption or covalently incorporated by using SS-PEG-SS [2] with the *indirect* functionalization by means of heparin. The heparin modification of scaffolds has been carried out after or during the manufacturing of the mineralized collagen matrices. The influence of heparin-crosslinking with sulfo-NHS and EDC as crosslinking agents has been observed. Additionally, polyelectrolyte microcapsules have been developed for hrVEGF encapsulation. The fabrication of microcapsules has been done according the electrostatic layer-by-layer technology [3], whereas heparin has been applied as one of the polyanion and calcium carbonate as the core template (Fig. 1). To characterize the properties of the scaffolds as well as of the microcapsules SEM, cLSM and fluorescence microscopy have been utilized. The indirect spectrophotometric

dimethylmethylene blue assay for proteoglycans has been used to examine the heparin binding efficiency. The *in vitro* rhVEGF release experiments have been evaluated by enzyme-linked immunosorbent assay (ELISA). Furthermore, *in vivo* release experiments have been done in cooperation and corresponding to the method described in detail in reference [4].

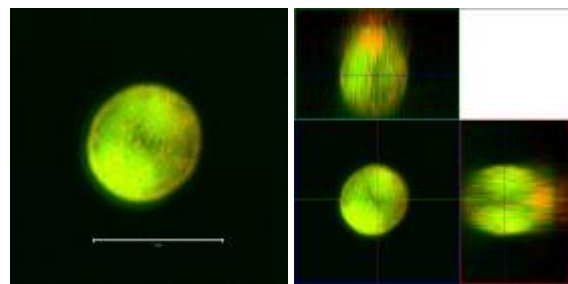


Fig. 1: cLSM image: polyelectrolyte microcapsule made of polyarginine, polyglutamate and DTAF-labeled-heparin; the calcium carbonate core was loaded with TRITC-BSA as model protein

**RESULTS:** It has been shown that the heparin immobilization to the mineralized collagen scaffolds resulted in a significant decrease of the initial burst during hrVEGF delivery. Both the amount of heparin and its crosslinking affected the binding affinity of hrVEGF. Similar data has been found by comparing the *in vitro* and the *in vivo* release kinetics.

**DISCUSSION & CONCLUSIONS:** Thus, this research was undertaken to examine the integrity of hrVEGF released from bone graft material which has been modified by different methods with several variables.

### REFERENCES:

- <sup>1</sup> M. Gelinsky, U. König, A. Sewing, W. Pompe (2004) *Materialwiss. Werkstofftech.* **35**, 229-233.
- <sup>2</sup> S. Koch, Ch. Yao, G. Grieb, E. Noah, G. Steffen (2006) *J. Mater. Sci. Mater. Med.* **17**, 735-741.
- <sup>3</sup> D. Volodkin, N. Larionova, G. Sukhorukov (2004) *Biomacromolecules* **5**, 1962-1972.
- <sup>4</sup> M. Yamamoto, Y. Ikada, Y. Tabata (2001) *J. Biomater. Sci. Polymer Edn.* **12**, 1, 77-88.