The Release of VEGF from Heparinized Collagen Matrices is controlled by Proteinase Induced Matrix Degradation

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Introduction: The *in vivo* application of engineered matrices in human wound healing processes is often hampered by the slow rate of vascularization. Therefore much research is directed towards enhancing the angiogenic properties of such matrices. One approach for enhancing the vascularization is the incorporation of angiogenic growth factors. Recently, we and others have reported on immobilizing such factors into collagen matrices by physical binding to covalently incorporated heparin. In this report, we investigated the release of vascular endothelial growth factor (VEGF) from collagen matrices under conditions which mimic potential *in vivo* situations. Relevant proteinase concentrations were deduced from *in vitro* experiments in which we evaluated the secretion of selected matrix metalloproteinases from fibroblasts upon contact with collagen.

Results: We investigated the release of VEGF from collagen matrices which were either non-modified (H0E0), cross-linked with EDC/NHS (H0E1) and cross-linked in the presence of heparin (H1E1)[1].

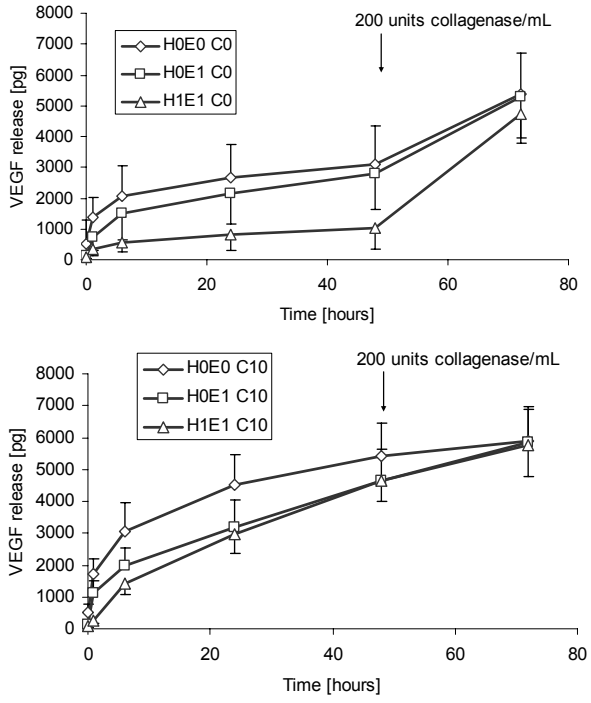

Fig.1 VEGF release from non-modified (H0E0), cross-linked (H0E1) and heparinized (H1E1) collagen matrices in the absence (C0) and presence (C10, 10 units collagenase/mL) of collagenase. Matrices were loaded with 10 ng rh $VEGF_{165}$. After 48 hours the collagenase concentration was raised to 200 units collagenase/mL.

Initially we studied the release behaviour with ELISA. Figure 1 shows that the release depends on two major factors: i. the presence of heparin and ii. the presence of proteinases (MMPs). In order to obtain information about in vivo levels of MMPs we exposed our collagen matrices to fibroblasts and evaluated the secretion of MMPs by zymography and ELISA. These studies showed that concentrations up to 3 units collagenase/mL are realistic figures for potential in vivo concentrations.
In an attempt to better understand the release behaviour we produced matrices with incorporated radioactively labelled ^{35}S-heparin and loaded them with ^{125}I-VEGF. Figure 2 demonstrates that i. The major part of the VEGF molecules co-elutes with heparin (see inset in Figure 2)and ii. the release only corresponds to a superficial degradation of the collagen matrix. The overall structure of the matrix remains intact.

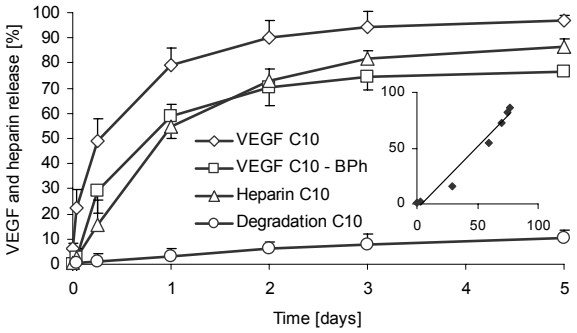

Fig. 2 ^{125}I-VEGF and ^{35}S-heparin release from ^{35}S-H1E1 matrices loaded with ^{125}I-VEGF in the presence of 10 units collagenase/mL (C10). Square symbols refer to the VEGF-release corrected for the burst phase release (Bph).

Conclusions: the release of VEGF from heparinized collagen matrices is rather complex. The release both depends on the presence of heparin and MMPs, the explanation of the release behaviour requires the presence of three species of VEGF molecules: i. non-bound molecules which are responsible for the burst-phase release, ii. non-specifically adsorbed VEGF-molecules and iii. VEGF-molecules specifically immobilized to heparin. The controlled release of these latter molecules is most likely responsible for the increase of the angiogenic potential of heparinized collagen matrices loaded with VEGF described earlier in in vitro and in vivo experiments [2].

References:

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