Irreversible binding and activity reduction of elastase by native collagen type I *in vitro*

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Introduction

Non-healing wounds represent a serious problem because degrading processes, like destruction of extracellular matrix and growth factors, prevent wound closure [1]. It has been shown that chronic wounds in contrast to acute wounds contain elevated levels of neutrophil elastase (fig. 1) which is responsible for most of the degradation of growth factors [2]. Binding or inactivation of elastase by wound dressings could be a promising way to contribute to the treatment of chronic wounds. The aim of this study was to investigate the influence of bovine collagen type I (Suprasorb® C, Lohmann & Rauscher) on elastase activity and concentration *in vitro*.

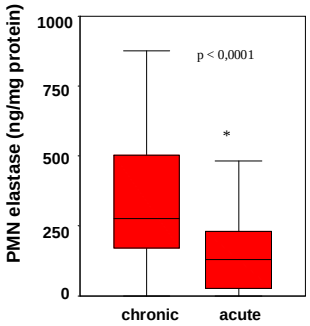

Fig. 1: Comparison of the PMN elastase concentration in the fluid of chronic and acute wounds [3]

Material and methods

Wound dressing samples were cut into equal pieces (0,5 cm²). Each specimen was taken in a final volume of 1 mL of elastase solution (250 ng/mL for elastase binding and 0.1 U/mL for the activity assay, respectively). Samples were incubated up to 24 h at 37°C on a plate mixer. Subsequent, the supernatants were collected and the wound dressing samples washed with PBS (+ 0.5 % BSA) for 1 h to recover bound elastase. In both, supernatant and eluate, elastase concentration was determined by means of a specific ELISA (Milenia biotec) and the activity was measured with the EnzChek Elastase Assay Kit (Molecular Probes).

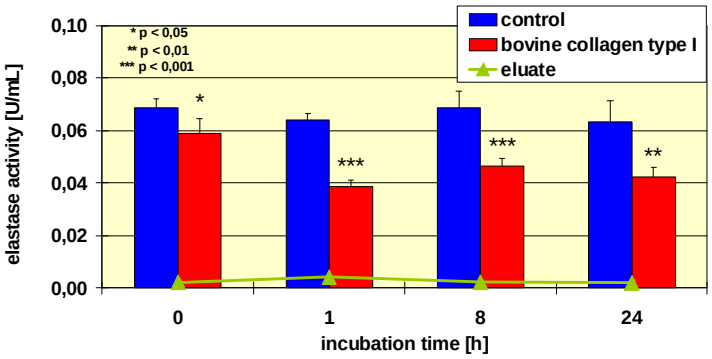

Fig. 2: Elastase activity determined in supernatants and eluates from bovine collagen type I after incubation with elastase solution (mean ± SE).

Results

Collagen type I reduced the activity of elastase already after an incubation of 1 h and the activity could not be recovered by elution of the wound material (fig. 2). Consistent with these results, the concentration of elastase was reduced after 8 and 24 h incubation with collagen and only a negligible amount of the enzyme was detected in the eluate (fig. 3).

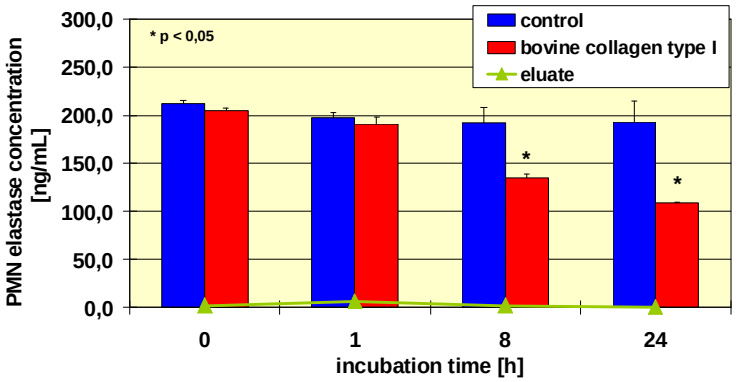

Fig. 3: Concentration of unbound elastase in supernatants and eluates from bovine collagen type I after incubation with PMN elastase (mean ± SE).

Conclusions

The physical properties of collagen type I such as porous structure (fig. 4) and capillary activity allow the absorption of large quantities of fluid. As the results of this study demonstrate, collagen is able to reduce the activity of elastase and to absorb considerable amounts of the enzyme *in vitro*. Elution of the wound dressing revealed a strong, possibly irreversible binding of elastase by collagen as reason for the diminished activity. Proteases like PMN elastase promote degrading processes and delay wound healing [1-2]. Irreversible binding of elastase could contribute to maintenance of growth factors and thus support the healing process.

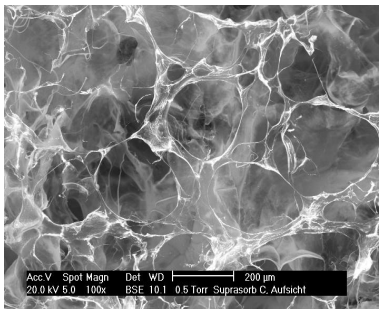

Fig. 4: SEM of native bovine collagen type I

References

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