Influence of irradiation on the binding capacity of bovine collagen for inflammatory proteases

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Introduction

In contrast to physiological wound healing chronic wounds are characterized by an imbalance of the underlying molecular processes resulting in an elongated inflammatory phase and severe tissue damage. A lot of studies have shown, hat exudates from non-healing wounds contain elevated levels of proteolytic enzymes and proinflammatory cytokines [1,2]. Therefore the reduction of these proteases seems to be a suitable way to promote normal wound-healing [3, 4]. The aim of this study was to investigate the binding capacity of the native as well as γ- and β-irradiated (maximum dosis of 20 kGy) wound dressing Suprasorb® C containing bovine collagen for neutrophil Elastase, MMP-2 and MMP-13.

Material and methods

The wound dressing samples were cut into pieces by means of punch biopsies (8 mm diameter, corresponding to 0.5 cm^2). Each specimen was taken in a final volume of 1 mL of protease solution (250 ng/mL neutrophil elastase, 4000 pg/mL MMP-2 and 2000 pg/mL MMP-13). Samples were incubated up to 24 h at 37°C on a plate mixer. After incubation supernatants were collected, immediately frozen and stored at -20 °C until testing. The concentration of unbound proteases in the supernatants were determined by means of specific ELISAs (neutrophil elastase ELISA from milenia biotec, Bad Nauheim, Germany and Quantikine Immunoassays for pro-MMP-13 and MMP-2 from R&D Systems, Minneapolis, USA, respectively).

Results

Suprasorb® C is able to bind neutrophil elastase. Already after 1 h a significant (p<0,05) decrease of the elastase concentration was observed. The wound dressings treated with γ- and β-radiation were also able to bind significant amounts of elastase over the examined period (fig. 1).

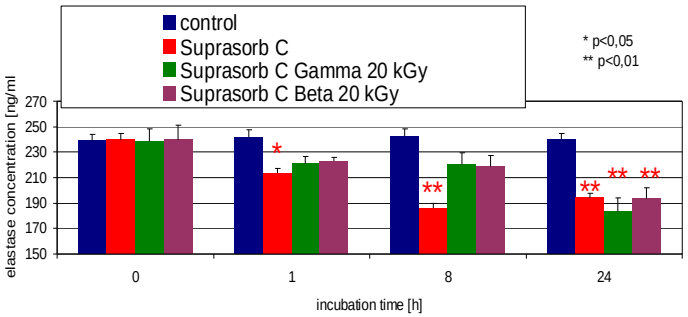

Fig. 1: Binding of neutrophil elastase by native as well as with γ- and β-irradiated Suprasorb® C from neutrophil elastase solution (mean ± SE).

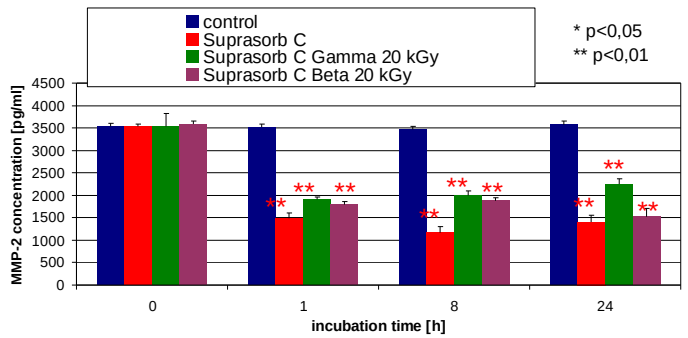

Fig. 2: Binding of MMP-2 by native as well as with γ- and β-irradiated Suprasorb® C from MMP-2 solution (mean ± SE).

Similarly native and irradiated bovine collagen can bind considerable amounts of MMP-2. A significant decrease ($p < 0,01$) of the MMP-2 concentration could be observed already after 1 h (fig. 2). In contrast to these results neither native nor irradiated collagen were able to bind MMP-13 (fig. 3).

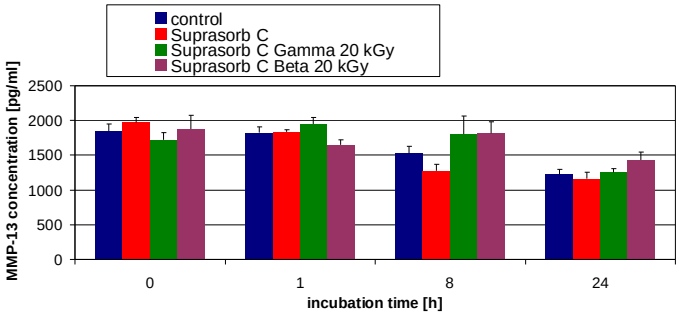

Fig. 3: Binding of MMP-13 by native as well as with γ- and β-irradiated Suprasorb® C from MMP-13 solution (mean ± SE).

Conclusions

Because of its porous structure (fig. 4), Suprasorb® C is able to bind proteases at different rates.
In particular, Suprasorb® C has a considerable binding capacity for MMP-2. It has the ability to reduce the concentration of neutrophil elastase in solution as well.

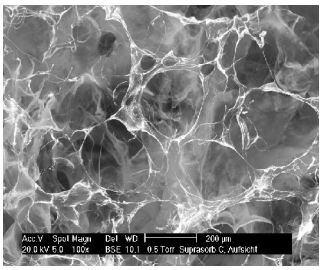

Fig. 4: SEM of native Suprasorb® C

The exudates of chronic wounds contain elevated concentrations of MMPs [1,2,3]. The level of neutrophil elastase is also higher than normal. Especially, the elastase activity keeps the chronic wound trapped in the inflammatory phase, because it not only cleaves collagen and other ECM proteins but also activates MMPs and destroys growth factors. Therefore the binding of the excessive proteases should establish a physiological wound environment and promote healing.
Irradiation is used to sterilise materials, but can influence the characteristics of bio molecules like collagen fibres. In this study was shown that γ- or β-irradiation of bovine collagen up to a maximum of20 kGy has no influence on the binding affinity for neutrophil elastase and MMP-2. Only a slightly lower capacity to bind MMP-2 was found for γ-irradiated bovine collagen.

References

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