Influence of the sterilization method on the binding capacity of bovine collagen for MMP-2 and MMP-13

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Introduction

The healing of acute and chronic wounds differs significantly. While normal wounds heal in a very orderly and efficient manner characterized by four distinct, but overlapping phases (homeostasis, inflammation, proliferation and remodeling) chronic wounds possess an elongated inflammatory phase that leads to severe tissue damage [1]. A lot of studies have shown that exudates from non-healing wounds contain elevated levels of proteolytic enzymes, like matrix metalloproteinases (MMP) and neutrophile elastase [2]. Therefore the reduction of these proteases seems to be a suitable way to promote normal wound-healing [3].

As sterilization is essential for medically applied products, the aim of this study was to investigate whether the sterilization of the wound dressing Suprasorb® C containing bovine collagen by treatment with ethylene oxide or γ- and β-irradiated (maximum doses of 20 kGy) has an influence on the binding capacity for MMP-2 and MMP-13 compared to the native collagen.

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 (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 (Quantikine Immunoassays for pro-MMP-13 and MMP-2 from R&D Systems, Minneapolis, USA).

Results

Suprasorb® C is able to bind MMP-2. Already after 1 h a highly significant ($p<0.01$) decrease of the MMP-2 concentration was observed. The wound dressings treated with γ- and β-radiation as well as the collagen sterilized with EO gas were also able to bind significant amounts of MMP-2 over the examined period (fig. 1).

In contrast to these results neither native collagen nor one of the sterilized collagen samples were able to bind MMP-13 (fig. 2).

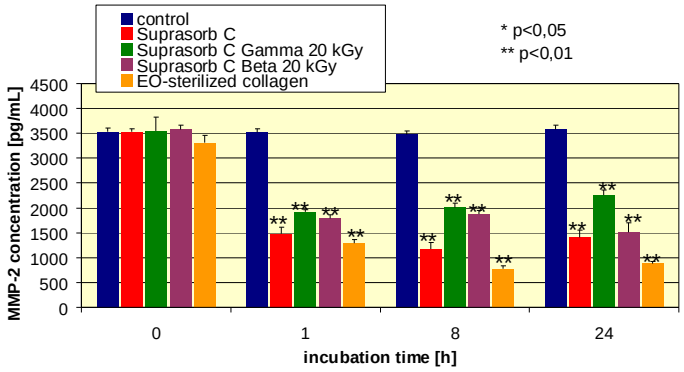


Fig. 1: Binding of MMP-2 by native, γ- and β-irradiated as well as EO-sterilized Suprasorb® C from MMP-2 solution (mean ± SE).

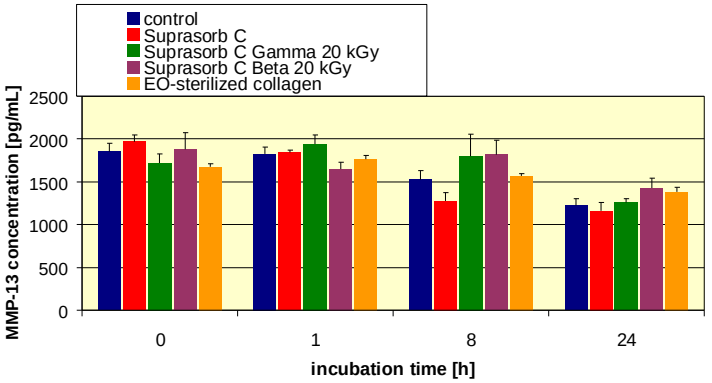


Fig. 2: Binding of MMP-13 by native, γ- and β-irradiated as well as EO-sterilized Suprasorb® C from MMP-2 solution (mean ± SE).

Conclusions

Suprasorb® C can absorb fluids efficiently because of its porous structure and capillary activity (fig. 3). Furthermore, it possesses a high binding capacity for MMP-2.

The exudates of chronic wounds contain elevated concentrations of MMPs, their activity keeps the chronic wound trapped in the inflammatory phase [1-3].

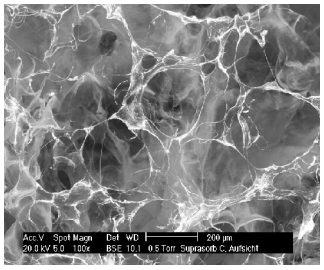
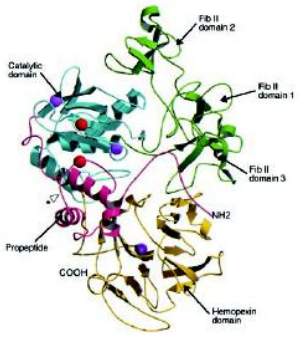


Fig. 3: SEM of native Suprasorb® C

Therefore the binding of the excessive proteases should establish a physiological wound environment and promote healing.

Thermal, physical and chemical methods are used for the sterilization of medical applied products, all three bearing advantage and disadvantage. Irradiation causes the formation of free radicals that can damage bio molecules like collagen fibers. EO gas is commonly used as sterilant in the medical field. However, a negative effect on active proteins (e.g. BMP) has been reported [4].



In this study was shown that γ- or β-irradiation of bovine collagen up to a maximum of 20 kGy has no influence on the binding affinity MMP-2. Only the binding capacity of γ-irradiated bovine collagen over a time period of 24 hours was lower compared to native collagen. The affinity of EO gas sterilized collagen for MMP-2 on the other hand was slightly higher than that of native collagen.

Fig. 4: Structure of pro-MMP-2. The prodomain, catalytic domain, fibronectin domains, and hemopexin domain are shown in red, blue green and yellow. Zn2+ ions are indicated in red, and Ca2+ ions are magenta [Science 1999; 284:1667-70]

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