

# The influence of radiation and EO-sterilisation on the binding capacity of bovine collagen for PMN elastase and its antioxidative potential *in vitro*

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## Introduction

Exudates from non-healing wounds contain elevated levels of proteolytic enzymes, like elastase from polymorphonuclear granulocytes (PMN elastase) and reactive oxygen and nitrogen species (ROS/RNS). The overproduction of proteolytic enzymes leads to reduced concentrations of growth factors and proteinase inhibitors resulting in an imbalance between degradation and remodelling processes. As a previous study has shown, the collagen wound dressing Suprasorb® C is able to bind significant amounts of PMN elastase from chronic wound fluid and to scavenge ROS/RNS, such as superoxide and peroxynitrite [1]. Within the presented study, we investigated whether  $\beta$ - and  $\gamma$ -irradiation or treatment with ethylene dioxide (EO) modify the binding capacity of the collagen wound dressing for elastase and ROS/RNS.

## Material and Methods

Wound dressing samples were irradiated with 20 kGy  $\beta$ - or  $\gamma$ -radiation or treated with EO. Afterwards, the collagen sponge was incubated up to 24 h at 37°C in 1 mL PMN elastase (250 ng/mL) solution. The supernatants were collected and the concentration of unbound elastase was determined by means of an ELISA (Milenia, Germany). The ability to scavenge ROS/RNS was assessed using the chemiluminescent ABEL® Antioxidant Test Kits containing Pholasin® specific for superoxide and peroxynitrite, respectively (Knight Scientific Limited, UK).

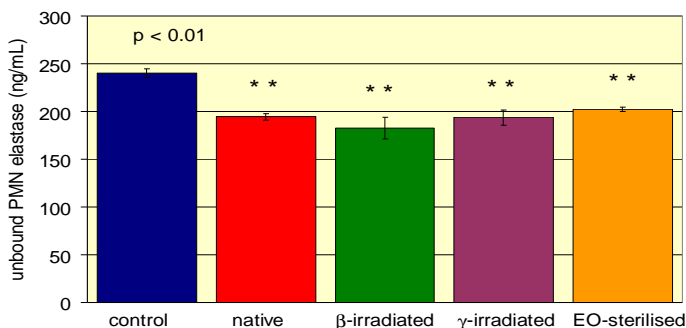


Fig 1: Binding of PMN elastase by Suprasorb® C after 24h

## Results

As figure 1 demonstrates, the collagen wound dressing Suprasorb® C binds considerable amounts of PMN elastase. After 24 h the concentration of unbound elastase was reduced to about 80 percent. Irradiated and EO-sterilised samples show nearly the same binding capacity for PMN elastase as the non-irradiated native collagen.

All tested samples showed antioxidant capacity as shown in figures 2 and 3. The ability to scavenge peroxynitrite of native,  $\beta$ -irradiated and EO-sterilised collagen was statistically significant (fig. 2). Additionally, native,  $\gamma$ -irradiated and EO-sterilised collagen samples inhibit the formation of superoxide radicals significantly (fig. 3).

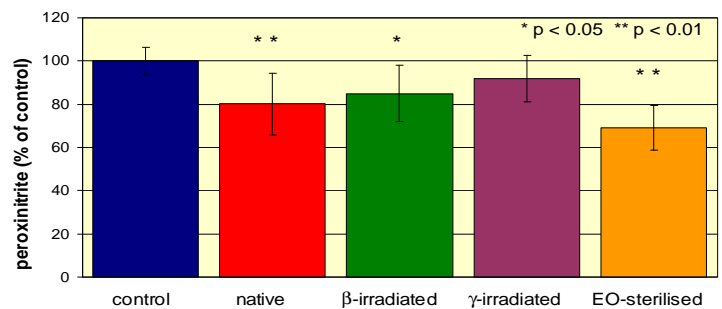


Fig 2: Inhibition of peroxynitrite formation by the collagen wound dressing Suprasorb® C.

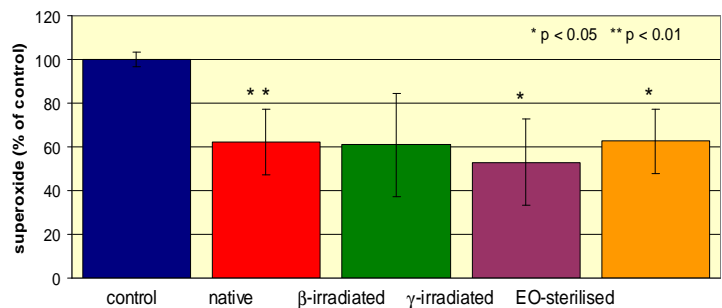


Fig 3: Inhibition of superoxide formation by the collagen wound dressing Suprasorb® C.

## Discussion

The physical properties of Suprasorb® C such as porous structure and capillary activity allow the absorption of large quantities of fluid. Furthermore, the collagen sponge takes up substantial quantities of PMN elastase and inhibits the formation of free radicals. Radioactive radiation is a common way to sterilise medical devices during the production process. As the presented data show, neither irradiation doses up to 20 kGy nor EO-sterilisation do alter the binding capacity of the tested collagen wound dressing for PMN elastase. In addition, none of the tested sterilisation methods has a significant effect on the antioxidative potential of the collagen samples.

## References

1. Schönfelder U, Abel M, Wiegand C, Klemm D, Elsner P, Hipler UC. The influence of selected wound dressings on PMN elastase in chronic wound fluid and their antioxidative potential *in vitro*. *Biomaterials* 2005;26:6664-6673.