Influence of alginate and silver containing alginate on elastase and ROS activity as well as TNF-α concentration *in vitro*

Friedrich-Schiller-Universität Jena

seit 1558

C. Wiegand[1], M. Abel[2], P. Ruth[2], M.-Th. Gorka[2], P. Elsner[1], U.-C. Hipler[1]

[1]Department of Dermatology, University Medical Center Jena, Germany
[2]Lohmann & Rauscher GmbH & Co. KG, Rengsdorf, Germany

Introduction

The exudates of chronic wounds contain elevated levels of proteolytic enzymes, inflammatory cytokines, and reactive oxygen and nitrogen species (ROS/RNS). This overproduction results in severe tissue damage and impairs healing. Therefore, the reduction of these active species seems to be a suitable way to promote normal wound-healing [1]. Within the present study we investigated the influence of the alginate wound dressing Suprasorb® A (Lohmann & Rauscher) for TNF-α, elastase and ROS/RNS . As well as the effect of two alginate wound dressings containing ionic silver (Suprasorb® A+Ag, Lohmann & Rauscher) and nanocrystalline silver (Acticoat® Absorbent, Smith & Nephew), respectively.

Fig. 1: Light microscopy image of partially welled alginate containing silver

Material & Methods

The wound dressing samples were cut into equal pieces (0.5 cm^2). Each specimen was taken in a final volume of 1 mL of elastase solution (0.1 U/ml) or TNF-α solution (100 pg/ml). Samples were incubated up to 24 h at 37°C on a plate mixer. Supernatants were collected and stored at -20°C until testing. The activity of unbound protease in the supernatant was determined by means of substrate digestion (EnzChek® Elastase Assay Kit, Molecular Probes). The concentration of unbound cytokine was determined by means of specific ELISA (Mabtech AB, Sweden). Antioxidant potential was measured using the chemiluminescent ABEL® Antioxidant Test Kits containing Pholasin® specific for superoxide and peroxynitrite (Knight Scientific Limited).

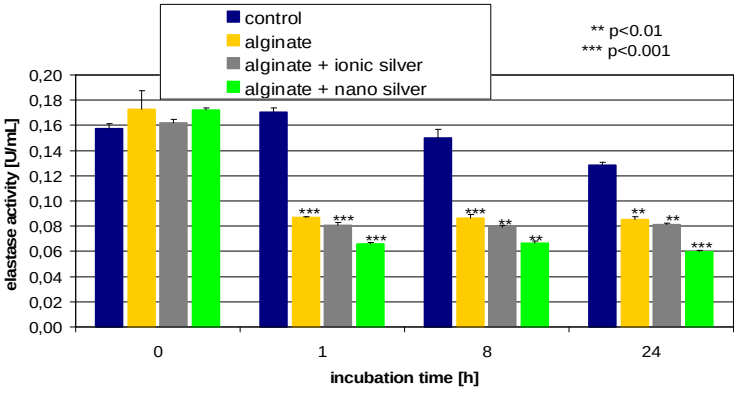

Fig. 2: Repression of elastase activity by alginate and alginate containing ionic silver or nano silver (mean ± SE).

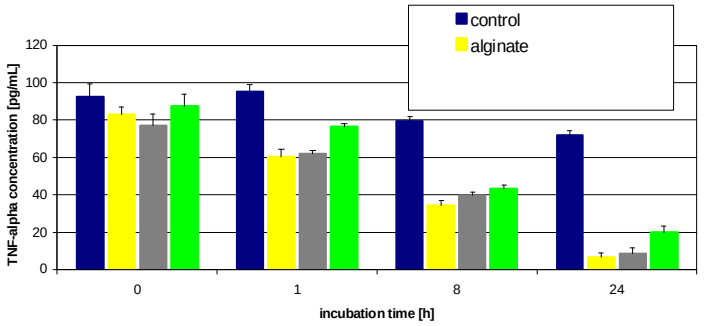

Fig. 3: Reduction of TNF-α concentration by alginate and alginate containing ionic silver or nano silver (mean ± SE).

Results

Alginate is able to bind elastase (fig. 2) and TNF-α (fig. 3). Already after 1 h a highly significant decrease of both the protease activity and the cytokine concentration was observed. The wound dressings of alginate + ionic or nano silver were also able to reduce the activity of elastase (fig. 2) and the level of TNF-α significantly over the examined period (fig. 3). Furthermore, all tested samples showed significant antioxidant capacity (fig. 4). The binding of ROS/RNS was significantly higher for silver containing products in contrast to alginate alone.

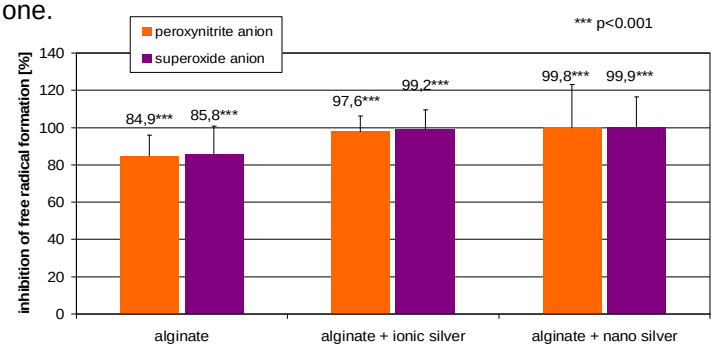

Fig. 4: Inhibition of the formation of free radicals by alginate and alginate containing ionic silver or nano silver after incubation (mean ± SE).

Conclusions

Alginate (fig. 1) can absorb fluids efficiently and the wetting results in the formation of a hydrated gel which promotes healing by retaining a moist environment [3]. Moreover, it possesses a high binding capacity for the pro-inflammatory protease elastase, is able to bind significant amounts of TNF-α, and inhibits the formation of free radicals. As our studies have shown, the combination of alginate wound dressings with ionic and nanocrystalline silver as anti-microbial agent did not affect the performance of the alginate wound dressing against TNF-α and slightly improved the effect of the wound dressing on reduction of elastase activity and free radical scavenging *in vitro*. The decrease of these pro-inflammatory mediators should aid the establishment of a physiological wound milieu *in vivo* and improve the healing outcome.

References

1. Trengove NJ, Bielefeldt-Ohmann H, Stacey MC. Mitogenic activity and cytokine levels in non-healing and healing chronic leg ulcers. Wound Rep Reg 2000; 8:13-25.
2. Tang P, Hung M-C, Klostergaard J. Human pro-Tumor necrosis Factor is a homotrimer. Biochemistry 1996; 35:8216-25.
3. Edwards JV, Bopp AF, Batiste SL, Goynes WR. Human neutrophil elastase inhibition with a novel cotton-alginate wound dressing formulation. J Biomed Mater Res 2003; 66A:433-40