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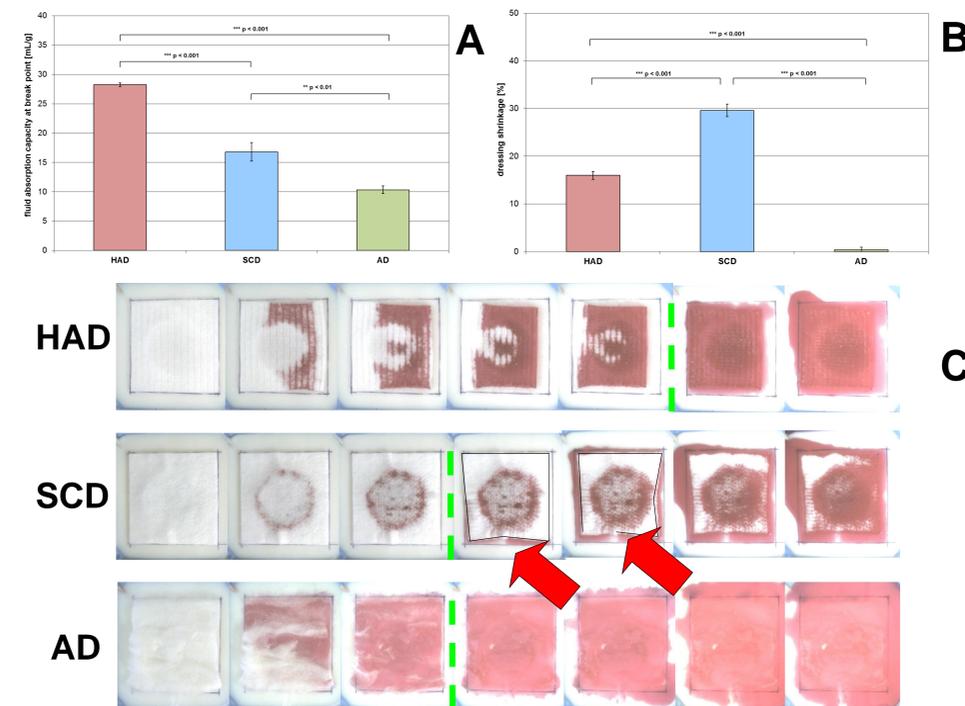
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## Introduction

Chronic wounds have become a rising charge to society as an increasing number of patients suffer from wounds that fail to heal. Stagnation in wound healing derives from an imbalance between degradation and remodeling as non-healing wounds contain elevated levels of proteases, e.g. polymorphonuclear elastase (PMN elastase), and inflammatory cytokines [1-3]. The excessive action of elastase leads to considerable reduced amounts of growth factors and proteinase inhibitors like TIMPs (tissue inhibitors of matrix metalloproteases) leading to the destruction of extracellular matrix (ECM). Furthermore, exposure of the peri-wound skin to exudate can subsequently damage the skin by maceration [4]. Maceration is the elixation of the skin by prolonged exposure to moisture that impedes healing due to failure of the skin protection and possible microbial infections [5]. Chronic wounds are often colonized by different kinds of microorganisms, the most prominent being *Staphylococcus aureus* and *Pseudomonas aeruginosa* [6]. An increased bacterial load on the surface of a wound amplifies and/or perpetuates a pro-inflammatory environment [7-8]. The presence of bacteria induces the immigration of monocytes, macrophages and leukocytes, whose inflammatory response exaggerates the tissue damaging processes. Hence, it is of interest elucidate dressing performance by a comprehensive *in vitro* approach including testing of the binding capacity for PMN elastase, determination of the antibacterial activity against *S. aureus* and *P. aeruginosa*, and assessment of the fluid handling capability.

## Material & Methods

A hydroactive dressing (HAD) consisting of cellulose/ethyl-sulfonate-cellulose fibres processed to a fleece compresses has been investigated. HADs are thought to absorb exudate directly into the fibres and lock it away to protect the surrounding skin. An *in vitro* maceration model was used to quantify and evaluate fluid uptake, fluid distribution in the dressing, and shape changes. For determination of the binding capacity for elastase, HAD samples (0.5 cm<sup>2</sup>) were taken in a final volume of 1mL of protease solution (0.2U/mL) and incubated up to 24h at 37° C. Activity of unbound protein in the supernatants was determined by specific protease activity assay. The determination of antimicrobial activity was performed according to the internationally recognized Japanese industrial standard (JIS L 1902:2008, „Testing method for antibacterial activity of textiles“) against *S. aureus* ATCC 6538 and *P. aeruginosa* DSM 1117.



**Figure 1:** (A) Assessment of the fluid absorption capacity at maceration breakpoint in [mL/g] for the different dressings. Results shown as mean  $\pm$  SE (n = 3). (B) Evaluation of the reduction of the covered area by determination of the dressing shrinkage in [%]. Results shown as mean  $\pm$  SE (n = 3). (C) Determination of the fluid management by HAD, SCD, and AD over time using video documentation (VF0700, Creative Labs, U.S.). The spread of the colored solution allows the measurement of the break point of maceration (green dotted line) at which the dressings stop to take up fluid and start to leak. HAD and AD exhibited an even fluid distribution while it was most notable that only the middle of SCD was wetted and that it started to leak before it was completely saturated with fluid (red arrows).

## References

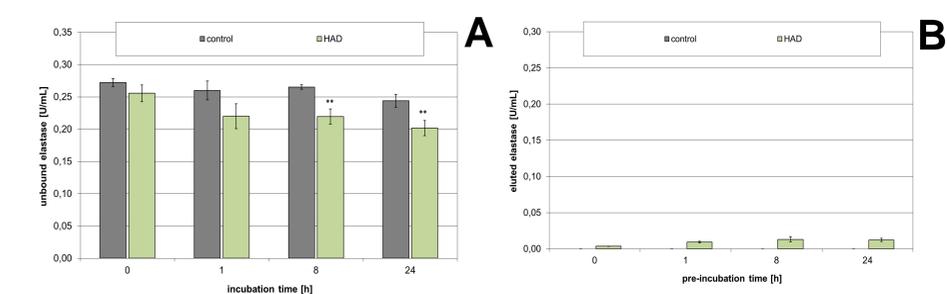
[1] Barrick et al. Wound Rep Reg 1999; 7:410-22 [2] Trengove et al. Wound Rep Reg 1999; 7:433-41 [3] Nwomeh et al. Clin Plast Surg 1998; 25(3):341-356 [4] White, Cutting . Br J Nurs 2003; 12:1186-201 [5] Cutting, White . J Wound Care 2002; 11:275-8 [6] Dissemond et al. Hautarzt 2004; 55:280-8 [7] Warriner , Burrell . Adv Skin Wound Care. 2005; 18(Suppl 1):2-12 [8] Wright et al. Wound Rep Reg 2002; 10:141-51

## Results

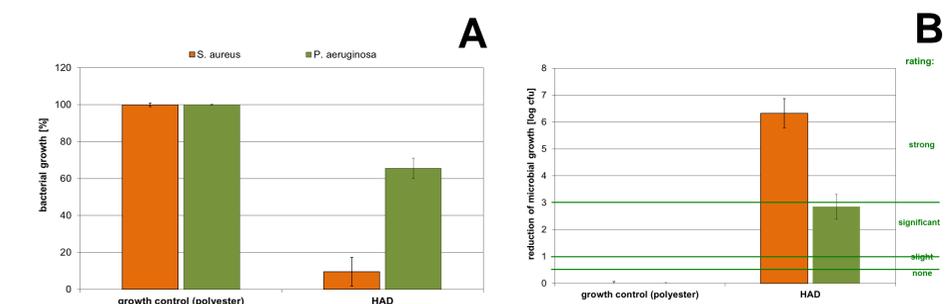
The HAD exhibited significantly higher fluid uptake (approx. 28mL/g) than an alginate dressing (AD, 10mL/g) or a sodium carboxymethylcellulose dressing (SCD, 17mL/g) (figure 1A). Moreover, it could be shown that it possess a distinctly higher form stability with only 16% shrinkage compared to the SCD with 29% shrinkage during fluid uptake (figure 1B). Moreover, the SCD already macerated before the dressing was completely soaked while leakage with HAD and AD was only observed after they were completely gelled (figure 1C). It could further be shown that HAD is able to slightly but statistically significantly reduce the activity of elastase *in vitro* (figure 2). In addition, only marginal residual elastase activity was detected in the eluate. Last but not least, HAD exhibited a strong antibacterial activity against *S. aureus* (log-reduction > 3) and a significant antibacterial activity against *P. aeruginosa* (log-reduction > 1) according to JIS L 1902:2008 (figure 3).

## Conclusion

Using a comprehensive *in vitro* approach, performance of dressings can be assessed and compared under standard conditions. Here, a cellulose/ethyl-sulfonate-cellulose hydroactive dressing was evaluated for binding of PMN elastase, antibacterial activity against *S. aureus* and *P. aeruginosa*, and fluid handling capability. It could be shown that the dressing is able to reduce elastase activity and inhibit bacterial growth. Furthermore, it was shown that the fluid handling capacity was superior to the alginate and a sodium carboxymethylcellulose dressings tested.



**Figure 2:** (A) Binding of elastase by HAD and inhibition of protease activity in the supernatant. (B) Only marginal release of active elastase was observed in a subsequent elution step. (n = 4, data presented as mean  $\pm$  SE)



**Figure 3:** (A) Effect of HAD on growth of *S. aureus* and *P. aeruginosa*. (B) Evaluation of the antibacterial activity of HAD according to JIS L 1902:2008. (n = 3, data presented as mean  $\pm$  SE)