Two in vitro studies on the micro-organism absorption capacity and influence on fibroblasts of TenderWet active

Summary of two publications:

**Background:** Chronic wounds covered with slough and necrotic tissue require effective debridement and a moist wound environment for healing. Removing slough and necrotic tissue reduces the bacterial bioburden, and in a physiological moist wound environment the wound can exert its own debriding capacity.

**Objective:** The efficiency of the wound dressing TenderWet active, marketed under the brand name Hydroclean in France, to absorb and retain bacteria and the influence of the dressing on fibroblasts were investigated in two in vitro studies.

**Methods:** Quantity of S. aureus, S. epidermidis, P. aeruginosa and C. albicans and the behaviour of fibroblasts – the key cells in granulation tissue formation and wound contraction – were investigated in the presence and absence of TenderWet active.

**Results:** TenderWet active significantly reduced the quantity of all micro-organisms tested. At the same time, the dressing exerted a positive effect on fibroblast behaviour.

**Conclusion:** TenderWet active reliably removes und retains pathogenic bacteria from the wound, recovers the biological behaviour of fibroblast and supports the healing process in chronic ulcers. Clinical studies and experiences confirm these in vitro results and underline the fast cleansing and debriding effect of TenderWet active in patients with chronic wounds of different aetiologies.

**Introduction**
Chronic wounds, particularly those covered with slough and necrotic tissue, provide a favourable environment for microbial growth. The most frequently detected bacteria in chronic ulcers are Pseudomonas aeruginosa and Staphylococcus aureus. Colonisation alone without any clinical signs of infection does not impair wound healing. But if a critical colonisation or infection develops and clinical signs of infection can be diagnosed, the exudative phase is prolonged and wound healing is further delayed. Especially multi-morbid and immunocompromised patients are at risk, suffering increased pain, and it may take months or sometimes even years until the lesion has healed.

Chronic wounds with slough and necrotic tissue require effective debridement and a moist wound environment which supports the wound healing process. Debriding slough and necrotic tissue reduces the bacterial bioburden. The physiological moist wound environment enables the wound to exert its own debriding capacity. During this autolytic debridement, cells in the wound area release endogenous proteolytic enzymes and activate phagocytes. Thus, necrotic tissue and slough are digested and separated from healthy tissue, promoting the formation of granulation and epithelial tissue. Fibroblasts and myofibroblasts are key cells in the formation of granulation tissue and wound contraction. During the process of cutaneous repair, the dermal fibroblasts migrate to the area of the lesion where they proliferate, synthesise the constituents of the extracellular matrix and differentiate into myofibroblasts. Myofibroblasts, the fibroblasts of granulation tissue, play an active role in the process of wound contraction and are characterised by the presence of bundles of actin-filaments in the cytoplasm.

The moisture-activated polyacrylate dressing pad TenderWet active supports the autolytic debridement of the wound itself. The dressing pad attracts and permanently removes proteins from necrotic tissue, attracts and retains toxins and bacteria and provides a moist and physiological environment for enzymatic autolysis.

Two in vitro studies have now investigated the bacterial absorption and retention properties of TenderWet and the resulting effects on fibroblasts.
Material and Methods

Behaviour of fibroblasts in contact with TenderWet active

The French study of Courderot-Masuyer et al. analysed the in vitro behaviour of two different kinds of fibroblasts in the presence of Pseudomonas aeruginosa and TenderWet active. Normal fibroblasts and fibroblasts obtained from incipient venous ulcers in a 75-year-old female patient were used. In the in vitro model investigated in the study was a stretched skin equivalent known as tense collagen lattice (10). This model can be likened to granulation tissue which is involved in cutaneous scar formation. In this situation, cell growth is increased and the extracellular matrix is non-retractable and under tension (11). In a collagen matrix, fibroblasts exhibit behaviour resembling their in vivo behaviour (12). Courderot-Masuyer et al. therefore used this in vitro stretched skin equivalent model to evaluate the effect of TenderWet active on the behaviour of healthy and venous ulcer fibroblasts. The tense collagen lattices were cultured for 4 days, and on the 5th day TenderWet active dressing was placed on the surface of the tense collagen lattice in the presence or absence of P. aeruginosa.

Viability of fibroblasts was measured on:
- normal fibroblasts or fibroblasts from venous ulcer not infected by P. aeruginosa;
- normal fibroblasts or fibroblasts from venous ulcer infected with P. aeruginosa;
- normal fibroblasts or fibroblasts from venous ulcer not infected but on which TenderWet active was applied;
- normal fibroblasts or fibroblasts from venous ulcer infected with P. aeruginosa and on which TenderWet active was applied.

Additionally, the myofibroblast differentiation was investigated by immunohistochemical analysis of alpha-smooth muscle actin (alpha-SM-actin, specific for myofibroblast) and F-actin (specific for the cytoskeleton of fibroblasts and myofibroblasts).

Micro-organism absorption properties

The in vitro study of Bruggisser demonstrated the bacterial and fungal absorption and retention capacity of TenderWet. Overall, four organisms were analysed:
- Staphylococcus aureus,
- Staphylococcus epidermidis,
- Pseudomonas aeruginosa and
- Candida albicans.

Bruggisser assessed the growth of micro-organisms in a suspension before and after placing the dressing in the cell culture flask. To visualise the absorption properties, TenderWet contaminated with S. aureus was photographed using a scanning electron microscope. Additionally, the behaviour of the micro-organisms under the dressing was studied. For that purpose, samples of the microbial film on the agar were collected and the number of micro-organisms in the border area and in the area below the dressing was counted.

Results

Behaviour of fibroblasts in contact with TenderWet active

As Courderot-Masuyer et al. report in their publication, a significant decrease in the number of P. aeruginosa could be detected in the presence of TenderWet active in the culture medium of normal fibroblasts and fibroblasts from venous ulcer

![Figure 1](image1.png)

**Figure 1** Number of colony forming units of P. aeruginosa detected in the culture media of normal fibroblasts and fibroblasts from venous ulcer in the absence and presence of TenderWet (*p<0.01 versus groups: fibroblasts infected with P. aeruginosa)

![Figure 2](image2.png)

**Figure 2** Study of the viability of fibroblasts after 24 hours of culturing in tense collagen lattices in the absence and presence of TenderWet and P. aeruginosa (JPC 2005; 51: 3-7). There is no statistically significant difference between the viability of the fibroblasts before and after application of TenderWet active. The application of TenderWet does not adversely affect the viability of the fibroblasts (*p < 0.05 versus control; *p < 0.05 versus Fibroblasts + P. aeruginosa)
blasts from venous ulcer (Figure 1). The use of TenderWet did not cause a significant decrease in the viability of the normal fibroblast or fibroblasts from venous ulcer cultured in tense collagen lattices. In contrast, the presence of P. aeruginosa significantly reduced the viability of the fibroblasts. The use of TenderWet in the presence of P. aeruginosa was associated with a considerable number of viable fibroblasts (Figure 2).

**TenderWet did not affect the organisation of cytoskeleton of fibroblasts**

The presence of P. aeruginosa in the culture medium resulted in the death of the fibroblasts. The use of TenderWet active in the presence of P. aeruginosa allowed the fibroblasts to survive and the organisation of the cytoskeleton (F-actin) to be preserved. Additionally, the use of TenderWet did not impair myofibroblast differentiation (detection of alpha-SM-actin fibres) of fibroblasts. The fibroblasts cultured in the presence of P. aeruginosa and TenderWet active exhibited normal morphological and functional (presence of alpha-SM-actin) characteristics of myofibroblast differentiation.

**Micro-organism absorption properties**

Bruggisser underlines in her publication that for all investigated strains, the number of micro-organisms in the suspension without a wound pad or with gauze as control was significantly higher than in the suspension containing TenderWet active. Figure 3 illustrates the results for S. aureus, S. epidermidis and P. aeruginosa. In the electron microscopy
pictures, the adherence of bacteria to the superabsorber polymer core of the dressing was obvious (Figure 4).

Figure 5 depicts the number of different micro-organisms under the wound dressing and on the non-covered border area. Compared with the border area, a one to three log\textsuperscript{10} reduction in the number of viable germs was assessed under TenderWet. As the author points out, the number of micro-organisms in the non-covered border area remained constant over the investigated period of time.

Discussion
Both in vitro investigations clearly demonstrate that TenderWet active was capable of absorbing and retaining bacteria such as S. aureus, S. epidermidis, and P. aeruginosa which often cause clinically relevant infections in chronic wounds and delay the healing process (2). Bruggisser emphasised in her publication that a traditional wound dressing such as gauze was not able to achieve this significant reduction of micro-organisms. Furthermore, TenderWet did not re-contaminate the wound surface or the nutrient broth within 24 hours, the clinically recommended time interval for changing the hydrogel dressing. The reduction of micro-organisms by the wound dressing helps to avoid or reduce excessive bacterial burden on wounds, according to author’s statement.

However, the effective absorption and retention capacity of TenderWet did not impair the viability of fibroblasts, the key cells in the wound healing process, and re-epithelialisation of the wound surface. Courderot-Masuyer and colleagues could not observe in their study any disruption of the cytoskeleton organisation (detection of F-actin) and myofibroblast differentiation (detection of alpha-SM-actin) due to the action of TenderWet active in the presence or absence of the bacteria. Even after P. aeruginosa had decreased the viability of fibroblasts, TenderWet removed the bacteria from fibroblasts and retained them, resulting in a recovery of the biological behaviour of fibroblasts.

The fact that the results of these in vitro assays can be transferred into clinical practice had been shown previously in clinical studies, in which TenderWet provided a fast cleansing and debriding effect and supported the wound healing process in patients with chronic wounds of different aetiologies (9, 13).
Conclusion
TenderWet active removes und retains pathogenic bacteria from the wound but also exerts a positive effect on fibroblast and supports the healing process of chronic ulcers. In both in vitro studies the wound dressing exhibited a high absorbent and retention capacity for all bacterial and fungal strains tested. At the same time, TenderWet active did not adversely affect the viability of the fibroblasts and their myofibroblastic differentiation necessary for the process of wound closure. Clinical studies and experiences underline this effective cleansing and debriding effect of the wound dressing. Thus, TenderWet is suitable for all wounds requiring a therapy that both provides a moist wound environment and also absorbs and retains microorganisms. The absorbed bacteria are removed when the dressing is changed.

References

Mode of action
TenderWet supports autolytic debridement

TenderWet active is a ready-to-use dressing already pre-activated with Ringer’s solution; it is available in two versions, one for flat wounds, and the other for cavity wounds (TenderWet active cavity). The dressing consists of three parts: a cover of non wound adherent polypropylene knitted fabric, a moisture repellent layer, and a superabsorbent polyacrylate pad pre-activated with Ringer’s solution (a biocompatible sterile isotonic solution based on sodium chloride, potassium and calcium). Applied to the wound, TenderWet continuously releases Ringer’s solution up to over 24 hours and supports autolytic debridement of the wound. During this process, necrotic tissue and coatings are softened, loosened and rinsed out. At the same time, the wound dressing pad absorbs bacteria-laden wound exudate into its absorbent core and binds it there. This exchange – Ringer’s solution is released and proteins are taken up – functions because the superabsorber of the wound dressing pad has a greater affinity for the protein-containing wound exudate than for the salt-containing Ringer’s solution. As the wound is being cleansed of necrotic tissue and other slough, an environment conducive to cellular migration, angiogenesis and the development of granulation tissue is provided.

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