Bacterial and fungal absorption properties of a hydrogel dressing with a superabsorbent polymer core

- **Objective:** To study *in vitro* the micro-organism absorption properties of a hydrogel wound dressing, TenderWet.
- **Method:** Microbial films on agar plates and suspensions with common wound bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*) and a fungal strain (*Candida albicans*) were studied.
- **Results:** The hydrogel dressing reduced the number of micro-organisms significantly, both on the agar plate and in suspension. The *in vitro* data show that the hydrogel dressing absorbed the micro-organisms from the environment. Electron microscopic imaging clearly demonstrated that the germs were attached to the surface of the dressing's superabsorbent polymer core.
- **Conclusion:** *In vitro* data show that the hydrogel dressing TenderWet attracts and retains micro-organisms and reduces the number of viable germs. Clinical experience underlines this fast cleansing and debriding effect of the hydrogel wound dressing.

- **Declaration of interest:** This study was supported by IVF HARTMANN AG, Neuhausen, Switzerland.

hydrogel dressing; absorption of micro-organisms; wound infection; moist wound management

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An adequate oxygen supply, the release of cytokines and normal functioning of the immune system all have a positive influence on wound-repair mechanisms. In contrast, microbial infections, dryness and excessive cooling will delay healing.[1-4]

Modern wound dressings such as hydrogels, foams, hydrocolloids and alginates are designed to promote healing by providing a moist wound environment.[5,6] Such an environment allows phagocytic cells to liquify necrotic tissue, thereby promoting the formation of granulation tissue.[7]

Chronic wounds, especially when covered with necrotic material, provide a favourable environment for microbial growth.[7] Many micro-organisms found in infected wounds exist as harmless normal flora on intact skin. However, in injured skin, particularly that of an immunocompromised host, they are potentially pathogenic and thus detrimental to healing. In chronic leg ulcers, for instance, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the two most frequently isolated organisms.[8,9] The use of antibiotics or antimicrobials for infected wounds, however, may be toxic to fibroblasts[10] and contribute to an increase in multi-resistant bacteria.

Hydrolytic enzymes secreted by bacteria degrade extracellular components, such as collagen.[11] The formation of a fibrous network with collagen is necessary to trap blood cells and regenerate blood vessels in the wound, so any degradation of collagen will be detrimental to the healing process.[12] The presence of bacteria also increases the inflammatory response and thus the concentration of matrix metalloproteases (MMPs), which inhibit wound healing.[13,14]

A wound dressing must therefore provide both a moist wound environment to promote debridement of necrotic tissue and prevent or manage an infection. One such dressing is TenderWet (Paul Hartmann), a hydrogel dressing comprising a knitted polypropylene fabric cover and an absorbent core containing a superabsorbent polymer, polyacrylate. Before use, the dry dressing is activated with a pre-measured volume of Ringer's solution. Studies have

Table 1. Micro-organisms used for the *in vitro* investigations

Micro-organism	ATCC
Staphylococcus aureus	25923
Staphylococcus epidermidis	13518
Pseudomonas aeruginosa	27853
Candida albicans	2091

All micro-organisms were supplied by Microbiologics, USA-St. Cloud

Table 2. Agar plates used for micro-organisms

Micro-organism	Agar
Staphylococcus aureus	CASO
Staphylococcus epidermidis	CASO
Pseudomonas aeruginosa	Cetrimid
Candida albicans	Sabouraud-4%-glucose

demonstrated that the dressing maintains a moist wound environment and facilitates debridement of necrotic tissue.[15,16]

As TenderWet does not contain an antimicrobial agent, this study set out to investigate its bacterial and fungal absorption properties using two *in vitro* model systems.

Method

Four organisms were selected (Table 1). Lyophilised cultures were obtained from the American type culture collection (ATCC).

The cultures were revived by overnight growth in casein-peptone soymeal-peptone (CASO) broth USP (Merck KGaA, D-Darmstadt) (one pellet in 9ml CASO broth at 37°C) and tested for culture purity by streaking on CASO agar (Merck KGaA, D-Darmstadt).

Colonies of the micro-organisms were then collected and incubated in 9ml CASO broth.

Stock solutions with 200 organisms/ml were prepared in CASO broth. This concentration assures an exponential growth over 24 hours at 25°C. To control the exponential growth, the number of micro-organisms was monitored over 26 hours.

The number of organisms was determined by colony forming unit (CFU) assay. Aliquots of 1ml of the well-mixed suspension were taken, diluted in Ringer's solution (1:10–1:10^6), and 25µl of every dilution was streaked on agar plates (Merck KGaA, D-Darmstadt). Different agar plates were chosen for the different micro-organisms (Table 2). The number of micro-organisms was determined by counting the single colonies of the suitable dilution (10–100 colonies).

Microbial absorption properties of the hydrogel dressing

To investigate the microbial absorption properties of the test dressing, the growth of micro-organisms in the suspension was investigated by placing the hydrogel dressing in a cell culture flask containing 20ml CASO broth with suspended micro-organisms (200 organisms/ml) (control). Added to this were:

- 8ml Ringer's solution (control with Ringer's solution)
- TenderWet (4cm diameter) activated with 8ml Ringer's solution (wound dressing)
- 8ml Ringer's solution and gauze (MediSet 5 x 5cm, eight-times folded).

Triplicates of every suspension and every micro-organism were prepared.

The suspensions were maintained by shaking for 24 hours at 25°C. The number of micro-organisms in the suspension was then determined by CFU assay.

To visualise the absorption properties of the hydrogel dressing, the samples were studied using

Fig 1. Number of viable germs in suspension with or without the hydrogel dressing pad (TenderWet) or gauze

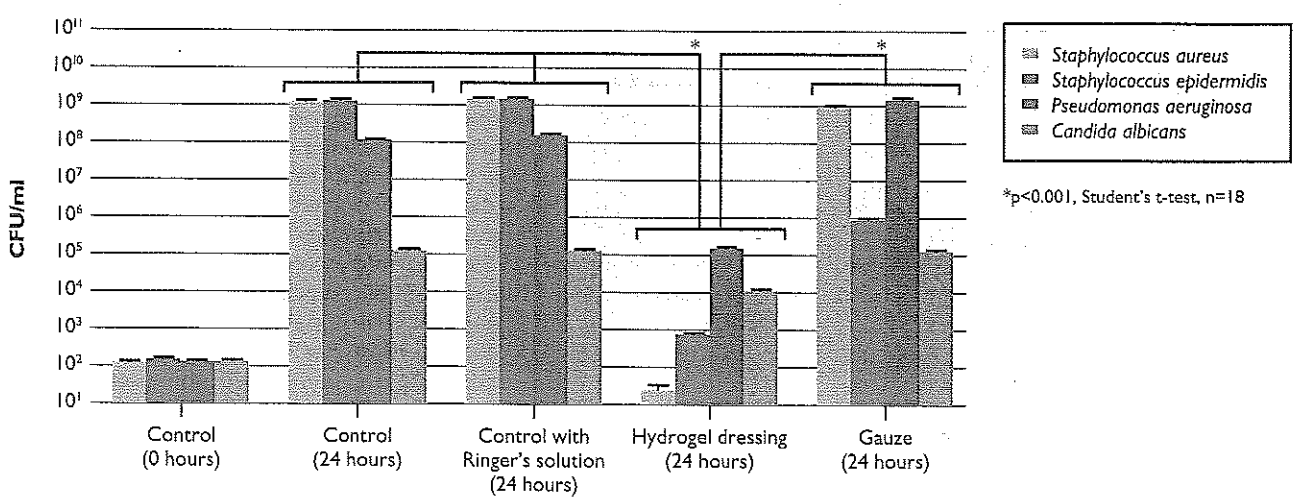
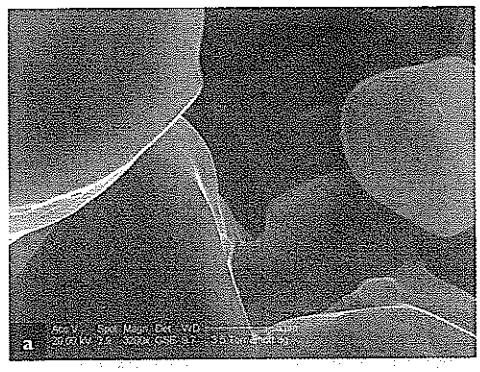
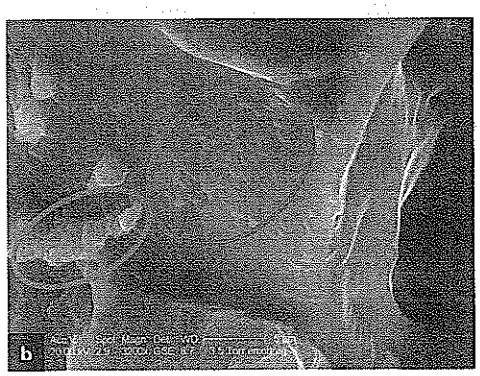


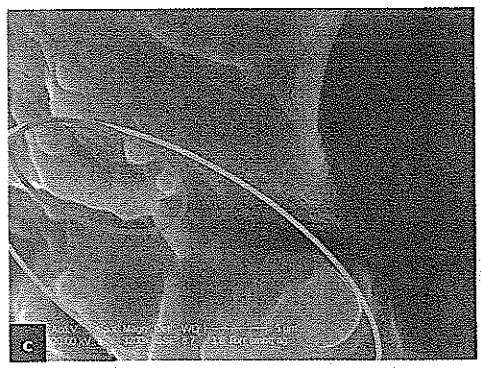
Fig 2. Electron microscopic images (ESEM LX40) of superabsorbent polyacrylate without (a) and with (b) *Staphylococcus aureus*. The bacteria adhere to the surface of the superabsorbent polymer and remain attached even after the addition of water by condensation (c)



Clean surface of single particles of the superabsorbent polyacrylate (extension 2067:1)



Single bacteria (red area) and clusters of bacteria (blue area) (*Staphylococcus aureus*) adhere to the surface of the superabsorbent polyacrylate (extension 2067:1)



The superabsorbent polymer swells following the addition of water (yellow area). The single bacteria and clusters of bacteria are absorbed into the swollen hydrogel (blue area) (extension 2067:1)

an environmental scanning electron microscope (ESEM LX40) under 1.6–3.8 Torr H2O and -3 to +2°C (EMOTT AG, University of Zurich, CH-Zurich). Samples of interest were the superabsorbent polymer and the polymer contaminated with *Staphylococcus aureus*.

Number of micro-organisms under the hydrogel dressing

The behaviour of the micro-organisms under the hydrogel dressing was also studied. One ml of the stock solutions (200 organisms/ml) was streaked out on appropriate agar plates and incubated at 37°C until confluent microbial films covered the agar (three to four days).

Additionally, two samples from patients with infected wounds were collected, cultured and streaked out on CASO agar plates.

The TenderWet dressing (art. no. 609 466, 5.5cm diameter) was incubated on the microbial film surface for 24 hours at 35°C and replaced at 24, 48 and 72 hours of incubation.

Samples of the microbial film on the agar were collected at each change in order to study the number of micro-organisms in the border area (the surrounding microbial film not covered by the wound dressing pad) and in the area below the moist wound pad.

To generate the samples, defined areas (1.33cm^2) of the agar were punched out under aseptic conditions and the micro-organisms were suspended in 20ml CASO broth. The number of suspended micro-organisms was determined by CFU assay using the appropriate agar (Table 2).

Results

Microbial absorption properties of the hydrogel dressing and gauze

For all investigated strains, the number of micro-organisms in the suspension without a wound pad or with gauze was higher than in the suspension containing the hydrogel dressing (Fig 1).

Using Ringer's solution to activate the hydrogel dressing had therefore not inhibited microbial growth. The smaller number of micro-organisms in the environment around the hydrogel dressing pad indicates that organisms had been absorbed into the core of the dressing.

Electron microscopy was used to investigate the absorption of a bacterium (*Staphylococcus aureus*) into the core material of the dressing.

Fig 2a illustrates the superabsorbent polymer before and after contact with the bacterium. In Fig 2b the adherence of the bacterium to the superabsorbent polymer is obvious. The addition of water did not wash out the bacteria. Indeed, the superabsorbent polyacrylate had swelled further and thus absorbed the bacteria (Fig 2c).

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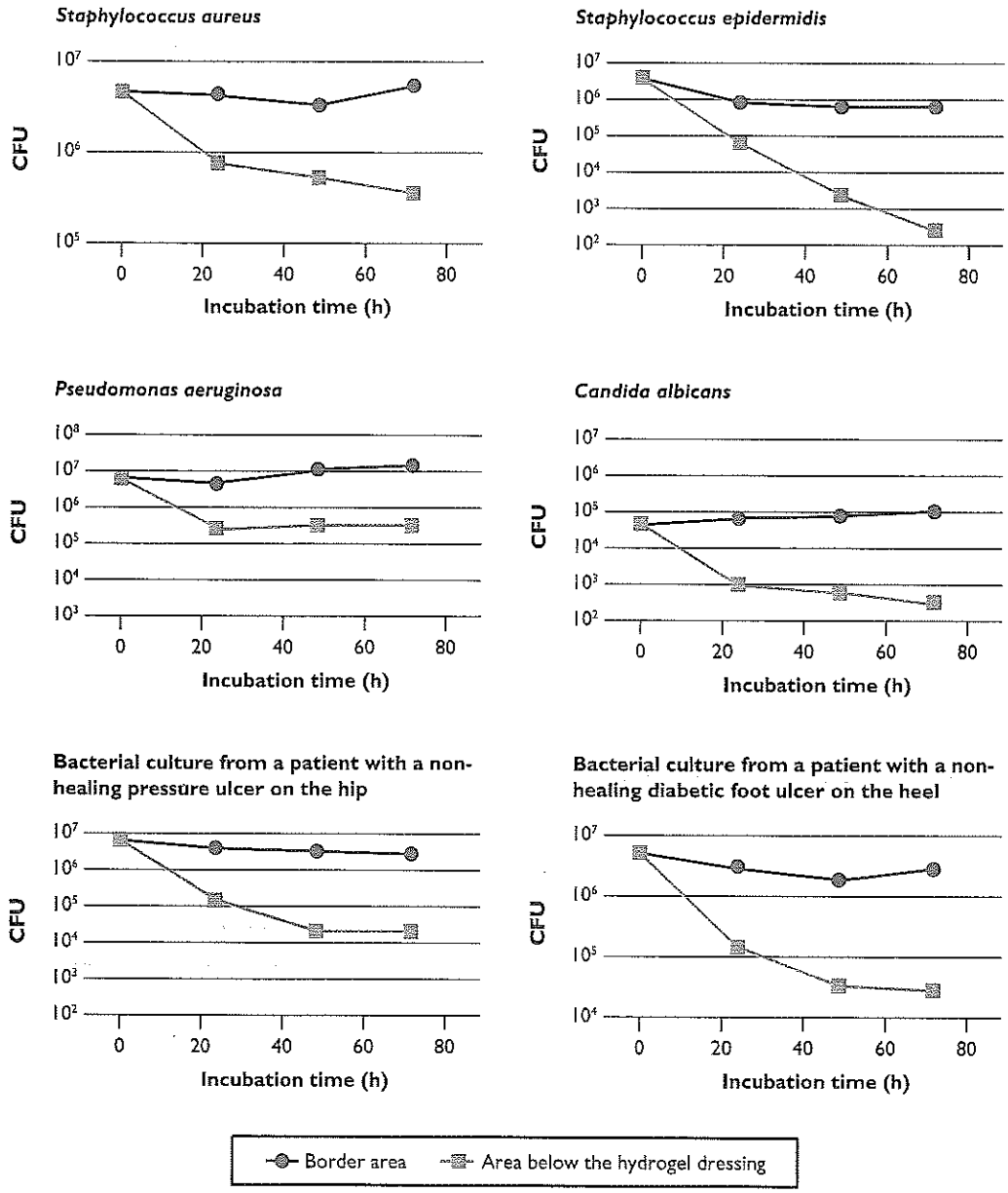
Fig 3. ***In vitro*** **comparison of the number of colony forming units (CFUs) of selected micro-organisms under the hydrogel dressing and of the number of CFUs in the margin region over a 72-hour time period**



The CFU was determined on an area of 1.33cm²

Number of micro-organisms under the hydrogel dressing

The number of different micro-organisms under the hydrogel dressing was compared with the number of organisms on the non-covered border area. Compared with the border area, a one to three $\log_{10}$ reduction in the number of viable organisms was measured under the wound dressing pad (Fig 3).

The number of micro-organisms from the samples of the two patients with infected wounds was also clearly reduced under the moist wound dressing pads (two $\log_{10}$ reductions in the number of viable organisms).

The number of organisms in the non-covered border area remained constant over the investigated time period.

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Discussion and limitations

These investigations demonstrated the bacterial-absorbing properties of the hydrogel wound dressing pad using two different assays.

The number of bacteria was reduced both in the suspension and on a film on the surface of an agar plate. Both *in vitro* assays showed there was a reduction in the number of micro-organisms on the dressing interface and not just a simple uptake of some micro-organisms into the hydrogel dressing.

A two to four $\log_{10}$ reduction of bacterial growth, as shown in Fig 1, would not be sufficient in a clinical situation. However, in an *in vitro* assay, where growth conditions for the organisms are optimised, this reduction can be considered meaningful. Clinical experience supports this fast cleansing and debriding effect of the hydrogel wound dressing.[16-19] The reduction of micro-organisms at the dressing interface helps to avoid or reduce excessive bacterial burden on wounds — one of the aims of modern wound dressings.

Gauze was unable to achieve this reduction of organisms by means of absorption.

The hydrogel wound dressing pad was able to attract and retain bacteria, even though the polyacrylate polymer was activated by Ringer's solution.

It can be assumed that the micro-organisms interacted with the hydrophobic and not the hydrophilic regions of the polymer (they did not interact with the water-binding side).

This is supported by the fact that condensation of water on the polymer surface did not rinse the bacteria from the surface. In addition, the bacteria are attracted by the hydrophobic surface of the wrapping knitted fabric.

The data also show that bacteria did not recontaminate the wound surface or the nutrient broth within 24 hours, the clinically recommended time interval for changing the hydrogel dressing.

Conclusion

The hydrogel dressing is suitable for all wounds that require a therapy that both provides a moist wound environment and also absorbs and retains micro-organisms. Furthermore, the absorbed bacteria will be removed when the dressing is changed. ■

Bulletin board

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Conformable dressing for hard-to-reach wounds

Mölnlycke has produced a thinner and more conformable version of its Mepilex range of dressings. Mepilex Border Lite is designed for wounds that are hard to reach and so hard to treat. It can be used on awkward sites such as fingers and toes, and also on children.

Mepilex Border Lite comes in a wide range of sizes including two small ones (4 x 5cm and 5 x 12.5cm). It will be available on the Drug Tariff from November.

- For more information on Mepilex Border Lite and the Mepilex range, email Mölnlycke Health Care at info.uk@molnlycke.com. Tel: 0800 731 1876; fax: +44 (0)800 608 1888. Or visit: www.tendra.

Advanced pressure relief

A silent air-loss bed that combines the use of Gore-Text and pulsation therapy has been produced by KCI Medical.

TheraKair Visio is claimed by its manufacturers to be as quiet as an alternating mattress. The pulsation therapy lightly massages the patient's skin to stimulate blood flow and reduce oedema. The Gore-Tex material, which is waterproof, breathable, vapour-permeable and has a bacterial barrier, helps prevent the skin getting hot and wet.

The mattress is controlled by a simple touch screen.

The bed is priced £5500 plus VAT, and can be rented on a weekly basis.

- Tel: 0800 980 8880.

Superglue product for closing keyhole surgical wounds

A skin-closure glue, LiquiBand Laparoscopic, has been developed for use after keyhole surgery.

The glue is an alternative to sutures and staples. Its manufacturer, Advanced Medical Solutions, says the advantages of this include faster wound closure and that it avoids the need for a secondary dressing, reduces the risk of trauma and infection, and increases the likelihood of a good cosmetic outcome.

LiquiBand is sold directly to hospitals priced at approximately £4 per unit (prices may vary).

- For more information contact Don Evans, Chief Executive, Advanced Medical Solutions on tel: 01606 545508.

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