B|BRAUN
SHARING EXPERTISE

PRONTOSAN®
MANAGING BIOFILMS

THE IN-VITRO EVIDENCE

Up to 90 percent of chronic wounds have biofilms[1]. Biofilm is defined as an aggregate of micro-organisms (e.g. bacteria or fungi) invisible to the naked eye and tolerant to treatment and the host defense[2]. The aggregate of microorganisms is encased in a thick, glue-like matrix of "extracellular polymeric substance" (EPS), which is composed of water, polysaccharides (sugars), nucleic acid (extracellular DNA) and proteins[3].

Why are biofilms difficult to manage and to treat? The microorganisms are in a "sessile" state (attached to surfaces or other microbes and metabolically inactive), which has been shown to survive concentrations of antibiotics up to 1,000 times higher than microorganisms in a "planktonic" state (free-floating and metabolically active); sessile bacteria also have an increased tolerance to the host's immune system. In addition, the EPS limits the diffusion of antibiotics, resulting in only sub-therapeutic levels reaching the microbes[3].

Effective alternatives to antibiotics are therefore sorely needed, in particular in view of the substantial problem of chronic wounds worldwide: In developed countries, it has been estimated that 1-2% of the population will experience a chronic wound during their lifetime[4].

What new evidence is there for the efficacy of in-vitro Prontosan®?

Presented here are the results of various test models conducted by two laboratories that investigated the antibiofilm activity of

- **Prontosan®** Wound Irrigation Solution
- **Octenilin®** Wound Irrigation Solution and
- **Microdacyn60®** Wound Care

Lab tests performed by the 5D Health Protection Group at the Center of Excellence in Biofilm Science (Steven Percival) investigated the products' activity against

1. *Pseudomonas aeruginosa* and *Staphylococcus aureus*[5]
2. Mono and mixed species biofilm[5].

The Lukasiewicz Research Network-PORT Polish Center For Technology Development compared the three products on their activity against

3. *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*[6].

1. *P. AERUGINOSA* AND *S. AUREUS*

MBEC

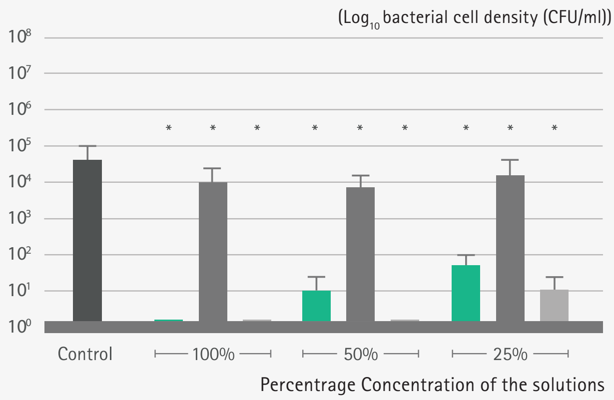
Method

The "Minimum Biofilm Eradication Concentration" (MBEC) assay is used to determine the efficacy of antimicrobials against biofilms. Biofilm is placed on pegs protruding from a plastic lid and placed into the wells filled with a culture medium (ASTM standard E2799-17).
In all methods, bacteria were counted before and after exposure in "CFU", short for "colony forming units", which are used to estimate the number of viable (= able to multiply) bacteria in a sample.

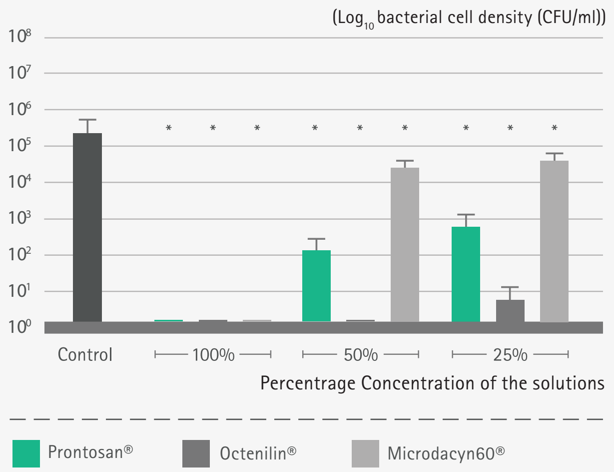
Results

- Treatment with 100% Prontosan® resulted in eradication of both the *P. aeruginosa* and the *S. aureus* biofilm:
- Treatment with 100% and 50% Octenilin® resulted in eradication of the *S. aureus* biofilm but only a 1.5-log reduction in bacterial cell density of the *P. aeruginosa* biofilm.
- Treatment with 100% Microdacyn60® resulted in eradication of both biofilms.

P. aeruginosa



S. aureus



CDC Bioreactor Model

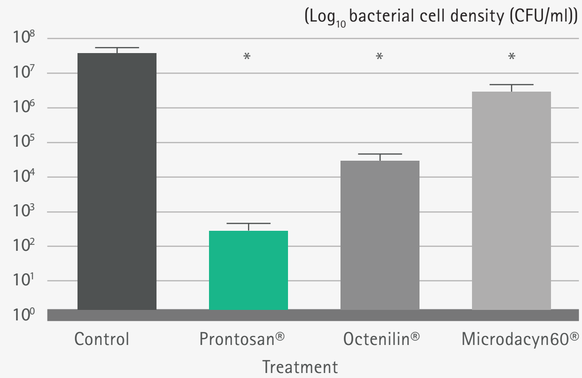
Method

The CDC (Centers for Disease Control and Prevention) biofilm reactor is used to assess biofilm formation and prevention on surfaces and devices (ASTM standard E2871-13). It consists of a bioreactor vessel with eight holders that can accommodate sample coupons suspended from the reactor lid.

Results

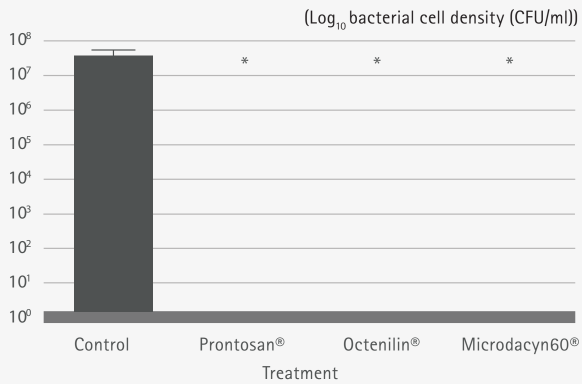
P. aeruginosa biofilm

- Treatment with Prontosan® resulted in a 5-log reduction of the *P. aeruginosa* biofilm
- Treatment with Octenilin® resulted in a 3-log reduction of the *P. aeruginosa* biofilm
- Treatment with Microdacyn60® resulted in a 1-log reduction of the *P. aeruginosa* biofilm (Fig.):



S. aureus biofilm

All treatments resulted in eradication



2. MONO AND MIXED SPECIES BIOFILM

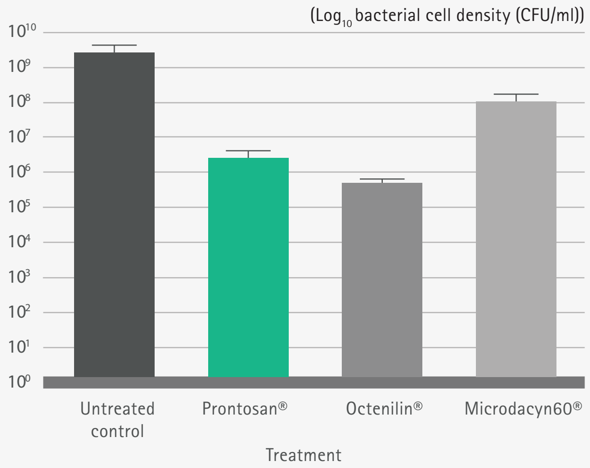
Drip Flow Bioreactor

Method

In this test method, a single colony of P. aeruginosa forms a biofilm on a glass slide coupon while a medium drip-flows over the growing biofilm (ASTM standard E2647-13).

Results

- Treatment with the Prontosan® resulted in a 3-log reduction in *P. aeruginosa* biofilm cell density.
- Treatment with Octenilin® resulted in a 4.5-log reduction in *P. aeruginosa* biofilm cell density.
- Treatment with Microdacyn60® resulted in a 1-log reduction in *P. aeruginosa* biofilm cell density.



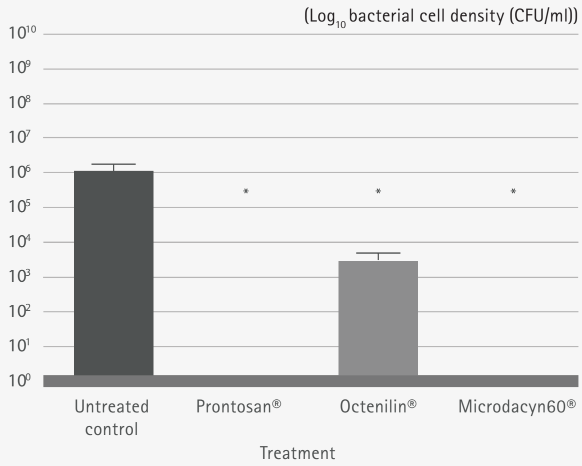
Multispecies Biofilm Model

Method

A "Multispecies Biofilm Model" was set up to grow a biofilm on a culture of P. aeruginosa, S. aureus and E. faecalis, which was subsequently exposed to the solutions.

Results

- Treatment with the Prontosan® resulted in a 6-log reduction
- Treatment with Octenilin® resulted in a 3-log reduction
- Treatment with Microdacyn60® resulted in a 6-log reduction (fig.)



3. *P. AERUGINOSA*, *S. AUREUS* AND *C. ALBICANS*

Biofilm-orientated antimicrobial test (B.O.A.T)

Method

Isolated colonies of microbial strains are incubated and then tested for biofilm formation. This method allows the quick and reliable estimate of the in vitro activity of working solutions of antimicrobial solutions in real contact times against bacteria in the biofilm form.

Results

- Prontosan® and Octenilin® were able to completely eradicate biofilms in 24h of contact time.
- Anti-biofilm activity of Prontosan® and Octenilin® is species and time dependent.[7]
- Microdacyn60® Solution was unable to completely eradicate biofilms within contact time range < 24h.

Number of survived strains in biofilm form (%)	Prontosan® / contact time				
	1 min	15 min	30 min	1 h	24 h
C. albicans	100	100	100	66.6	**0**
S. aureus	100	77.7	77.7	66.6	**0**
P. aeruginosa	100	88.8	77.7	44.4	**0**

Number of survived strains in biofilm form (%)	Octenilin® / contact time				
	1 min	15 min	30 min	1 h	24 h
C. albicans	100	100	66.6	0	**0**
S. aureus	100	66.6	55.5	55.5	**0**
P. aeruginosa	100	88.8	77.7	66.6	**0**

Number of survived strains in biofilm form (%)	Microdacyn60® / contact time				
	1 min	15 min	30 min	1 h	24 h
C. albicans	100	100	100	100	**100**
S. aureus	100	100	100	100	**100**
P. aeruginosa	100	100	100	100	**100**

Cellulose-Based (3D) Biofilm Model

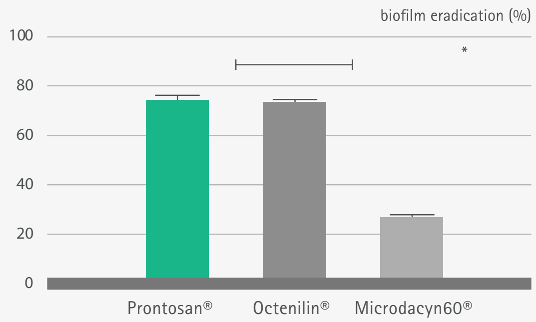
Method

In this biofilm model, the biofilm is cultivated on a cellulose carrier to reflect the 3D spatial surface of the biotic elements in a wound environment, in contrast to "classical flat biofilm" in vitro models that only reflect biofilms formed on abiotic surfaces.

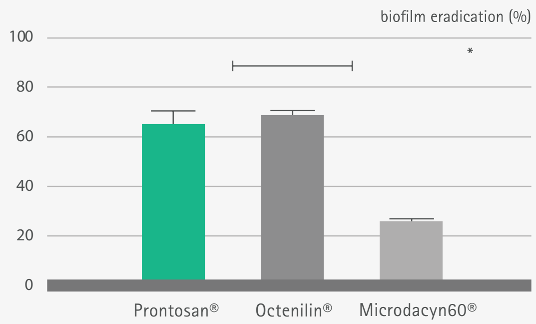
Results

- With 1h exposure time, Prontosan® and Octenilin® were able to eradicate 60-80% of *Candida albicans*, *Pseudomonas aeruginosa* and *S. aureus* biofilms.
- Prontosan® and Octenilin® eradicated biofilms formed by *C. albicans*, *S. aureus* and *P. aeruginosa* significantly better than Microdacyn60®.

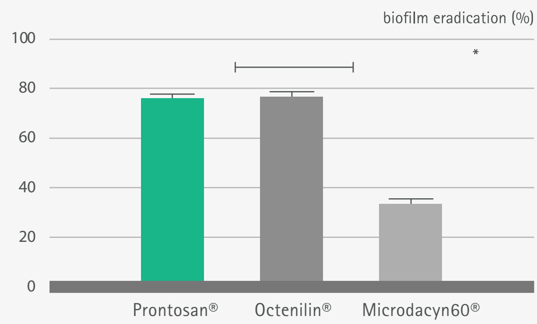
- *C. albicans*



- *S. aureus*



- *P. aeruginosa*



SUMMARY

This report presents six test methods, which revealed the antibiofilm activities of three wound irrigation solutions. The results show that the respective activity varies both between the test setups and the microorganisms tested. Now an overview of the total performance of each product can be provided. To transform the different results into comparable figures, a performance rating scheme was developed.

The products' performance in the **MBEC assay model** was rated according to its capability to either eradicate or at least significantly reduce (by a minimum of 4-log) the bacterial cell density of each tested biofilm respectively. The lower the concentration of the solution showed a notable effect, the higher the rating.

- The performance of the 25 % concentration was rated as 10 (full eradication) or 9 (significant log-reduction).
- The performance of the 50 % concentration was rated as 8 (full eradication) or 7 (significant log-reduction).
- The performance of the 100 % concentration was rated as 6 (full eradication) or 5 (significant log-reduction).
- The performance of the 100 % concentration was rated from 1 (very little log-reduction) to 4 (some log-reduction).

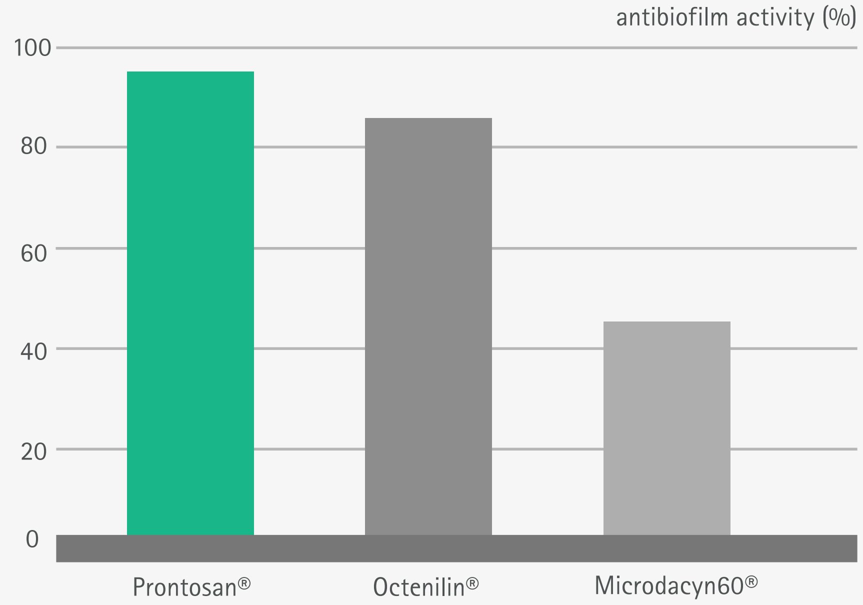
The performance of the products in the **CDC Bioreactor Model, the Drip Flow Bioreactor** and the **Multispecies Biofilm Model** was rated according to the following reduction factors:

- A log-reduction of 1 was rated as 2
- A log-reduction of 3 was rated as 4
- A log-reduction of 4 was rated as 5
- A log-reduction of 5 was rated as 7
- A log-reduction as of 6 was rated as 10

The products' performances in the test model **B.O.A.T.** and **cellulose-based (3D) biofilm** was calculated by dividing the percentages of biofilm eradication by 10.
The ratings of these calculations are presented in the following table. The product with the highest total score was Prontosan®.

	Prontosan®	Octenilin®	Microdacyn60®
MBEC Assay P. aeruginosa	7	1	9
MBEC Assay S. aureus	6	9	5
Bioreactor P. aeruginosa CDC	7	4	2
Bioreactor S. aureus CDC	10	10	10
Drip Flow Bioreactor P. aeruginosa	4	5	2
Multispecies Biofilm Model	10	4	10
B.O.A.T. C. albicans	10	10	0
B.O.A.T. S. aureus	10	10	0
B.O.A.T. P. aeruginosa	10	10	0
3-D Biofilm Model C. albicans	7.5	7.4	2.6
3-D Biofilm Model S. aureus	6.4	6.8	2.5
3-D Biofilm Model P. aeruginosa	7.5	7.8	3.2
Total Score	**95.4**	**85.0**	**46.3**

TOTAL SCORE



TAKE-HOME MESSAGE

The effectiveness of wound irrigation solutions on biofilms varies depending on the test model and microorganism. Prontosan® is effective in all models and is therefore the solution with the most versatile potential for wound irrigation in the fight against biofilm in chronic wounds.

Sources:

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