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(RESEARCH ARTICLE)

The use of p-toluensolfonic acid to manage biofilm effectively (C-deb)

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Abstract

A biofilm can be described as a microbial colony encased in polysaccharide matrix which can become attached to a wound surface. This can affect the healing potential of chronic wounds due to the production of destructive enzymes and toxins which can promote a chronic inflammatory state within the wound. There appears to be a correlation between biofilms and non-healing in chronic wounds. It is suggested that biofilms are a major player in the chronicity of wounds.

Keywords: P-toluensulfonic acid: C-deb; Biofilm; Wound Healing; Diabetes; Chronic Wounds; Adjunctive debridement agent

1 Introduction

Biofilm plays a significant role in the pathogenesis of most chronic infections in humans, either tissue-specific or involving medical implants.[1] Biofilm-associated infections exhibit high resistance to host defenses, often contributing to an excessive or inappropriate inflammatory response leading to further tissue damage and spreading of the infection. [2] Biofilms are highly tolerant to antimicrobial therapy.[3] Biofilms can tolerate up to 100–1000 times higher minimal inhibitory concentration (MIC) than the same bacterial cells in planktonic growth.[4] Unfortunately, the effective antibiotic MIC in vivo for biofilm eradication may be impossible to reach due to the drugs' toxicity and side effects, including limitations imposed by renal and/or hepatic functions.[5]

Despite their importance, the early recognition of biofilm-associated infections still represents an unmet need in clinical microbiology.

Therefore, the development of new therapeutic strategies is needed to manage biofilm effectively.

Biofilm communities are highly resistant to host immune defenses and to conventional wound treatment modalities, including antiseptic cleansers and topical and systemic antibiotic therapies.

To effectively eradicate the biofilm and promote healing, an antimicrobial must be able to penetrate the EPS and attack the bacteria inside with a prolonged action that prevents the biofilm from reforming.

Biofilm is estimated to be present in 78% of chronic wounds and to delay them the healing.

P-toluensofonic acid (C-deb) is indicated for the topical local treatment of skin areas at high risk of bacterial development thanks to its ability to remove bacterial biofilm with a physical mechanism of action dependent on the dehydrating nature of the product and independent of the bacterial species present.

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A study has been done on the activity of p-toluensulphonic acid (C-deb) on the biofilm treatment.

Compounds have been provided by Dept. of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy and evaluated for the antimicrobial efficacy against bacterial strains grown in biofilms. The bacterial strains used in this study were: methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, and Proteus mirabilis, usually isolated from diabetic foot ulcers. Bacterial strains were cultured starting from clinical specimens and were characterized. Bacterial strains were grown at 37°C under standard conditions and $1x10^4$ CFU (colony-forming unit) were seeded into 96-well plates. Following 48 hours, bacteria were able to form pure culture biofilms. Then, bacterial biofilms were gently washed and incubated at room temperature for 5 seconds – 15 minutes with the tested compounds (80 µL, minimal volume ensuring biofilm coverage). At the end of incubation, samples were collected, washed, properly diluted, and seeded on agar plates (immediate effect). Bacterial cells possibly remaining in the well-plates were incubated with fresh culture medium for 72 hours and then seeded onagar plates as described above (over time effect). Agar plates were incubated at 37°C and check for bacterial colonies appearance up to 72 hours. Bacterial colonies were enumerated, and data are reported as geometric mean of bacterial CFU obtained from 3 independent experiments, each performed in triplicate. Statistical analysis was performed using non-parametric Kruskal Wallis test.

25 compounds were tested.

In vitro microbiological tests clearly shown compound C-deb as the most effective:

- *C-deb removes biofilms formed by the tested bacterial strains.*

In vitro microbiological assays were performed on biofilm formed by 3 bacterial strains (MRSA, P. aeruginosa, and P. mirabilis). Anti-biofilm activity of C-deb was comparable among the 3 different strains, resulting in a non-specie-specific antibacterial effect.

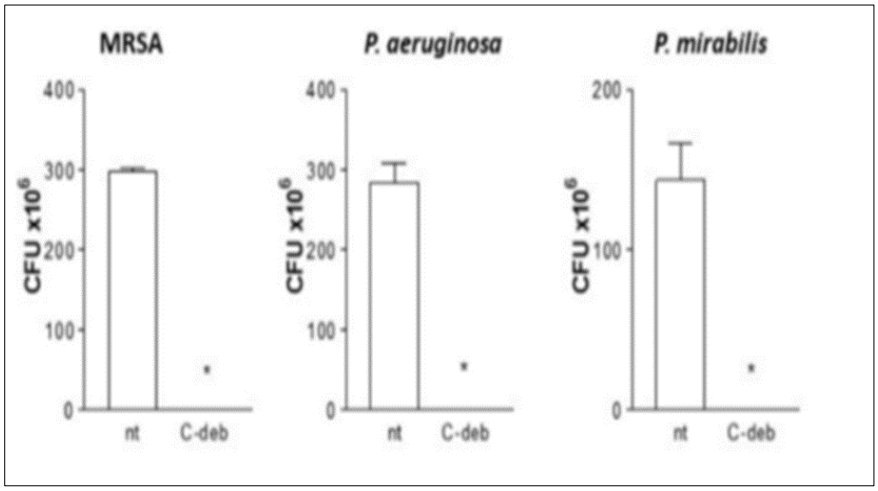


Figure 1 Effect of C-deb on bacterial biofilms following 5 mintues incubation

C-deb removes bacterial biofilms within few seconds of incubation.

Inhibition of bacterial growth is reported following only 5 seconds of C-deb incubation with bacterial biofilm (immediate effect).

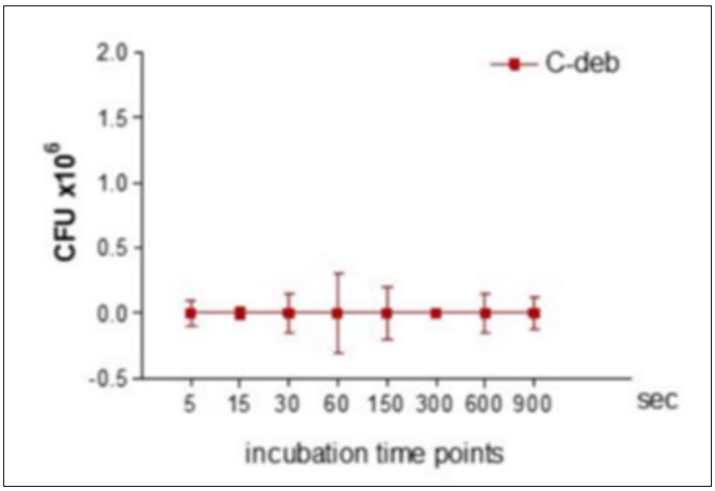


Figure 2 Time course effect of C-deb biofilm formed by MRSA

- Anti-biofilm effect of C-deb lasts over time.

In order to assess the long-lasting effect of C-deb, bacterial biofilms were incubated for 5 or 10 minutes with C-deb. Samples were then washed to remove the compound and incubated in fresh medium for 72 hours to replicate in vitro a possible reactivation of infection. As reported in Figure 3, ten minutes application of C-deb ensures the biofilm inactivation and prevents bacterial reactivation up to 72 hours.

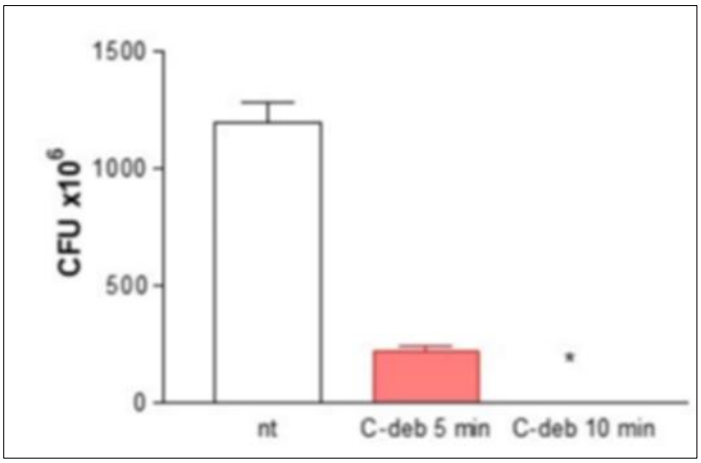


Figure 3 Effect of C- deb on MRSA biofilm at 72 hrs of incubation following initial treatment for 5 or 10 min

Overall, the study reports a remarkable anti-biofilm effect of C-deb.

The anti-bacterial activity is explained by the dehydrating effect of C-deb.

Biofilm plays a significant role in the progression and chronicity of diabetic foot ulcers. In their review article, Pouget et al. [7] discussed current knowledge and the contribution of biofilms on diabetic foot ulcers. In particular, the authors focused on preventive strategies to hinder the establishment of microbial biofilms and wound chronicity to support or eventually replace the current approach for managing diabetic foot ulcers. C-deb is a solution based of p-toluensulfonic acid which denatures, dehydrates and coagulates the biofilm matrix and microbes.

The cases presented represented a fraction of the applications for which the use of the C-deb proved to be useful.

The photographic documentation of the cases presented demonstrate the efficacy and types of changes in the ulcer after using C-deb: the absence of previous infections and formation of granulation tissue with an important improvement in the edges of the ulcer. It was noted that the use of C-deb after the initial debridement of the wound reported a rapid

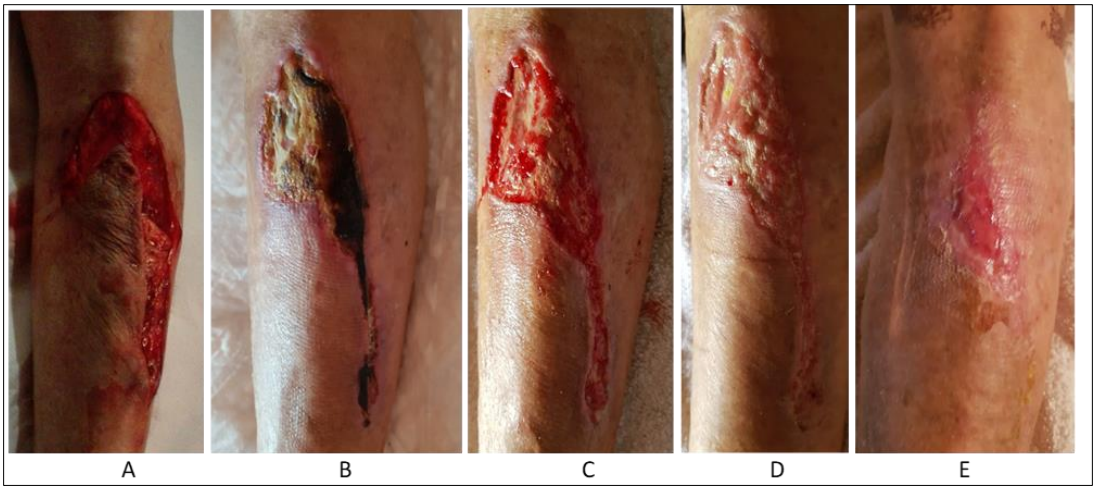
transition up to its coverage of 100% with granulation tissue. It should be noted that the inflammatory characteristics, including inflammation of the edges of the wound, excessive exudation, etc., resolved relatively quickly after application of C-deb.

The mechanisms of action of C-deb include anti-inflammatory mechanisms, denaturation, desiccation and removal of the biofilm, its bacterial population and the polysaccharide matrix that hosts pro-inflammatory cytokines and metalloproteases (MMS). The metalloproteases are attributed the ability to deteriorate fibroblastic proliferation and the deposit of collagen. Overproduction of these enzymes is characteristic of chronic ulcers.

2 Clinic

2.1 Case 1

Man, 58 yrs old patient; 2009 kidney transplant: lower leg trauma



(Authorized from: Mariana Peroni, Renzo Girardello, Ornella Pancheri, Stefano Bonvini and Giampietro Bertasi: "Hard-to-heal wounds: A new biofilm treatment with a novel desiccant". Magna Scientia Advanced Biology and Pharmacy, 2021, 03(01), 058–063) [6]

Figure 4 A: trauma – B: 1 week post-op; C: Applications C-deb time 0; D: 1 week second aplication; E: granulation tissue at 3rd week

2.2 Case 2

Man 68 years old: vascular ulcer

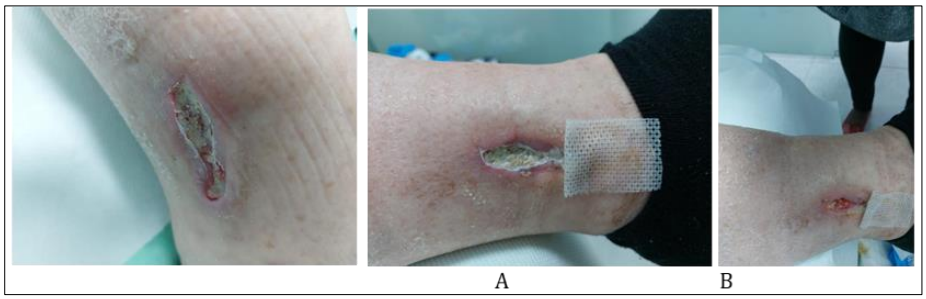


Figure 5 A: application of C-deb for 15'; B: after C-deb removal

2.3 Case 3

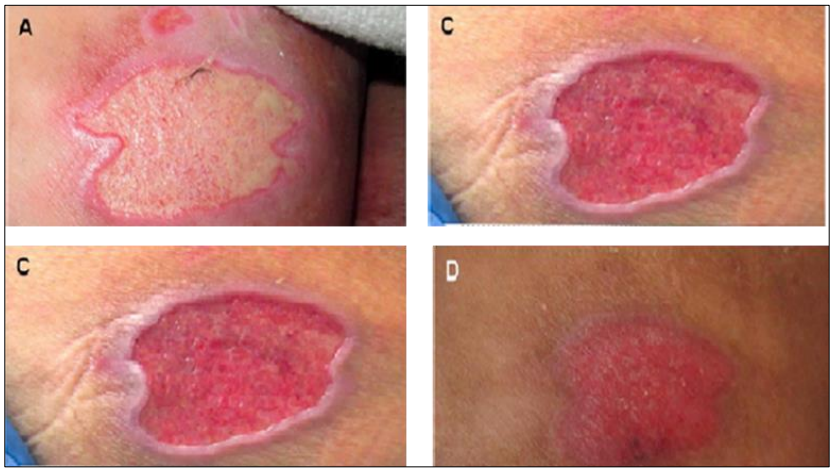


Figure 6 Pressure ulcer

Pressure ulcer on left buttock, a 40-year-old morbidly obese, non-diabetic male. A) Wound prior to HYB application. B) One week post C-deb, note granulation coverage. C) Two-week visit. D) 49 days post C-deb

2.4 Case 4

Man, 47 years old

Necrosis in type 2 diabetes woundP-toluensulfonic acid applications after surgical debridment

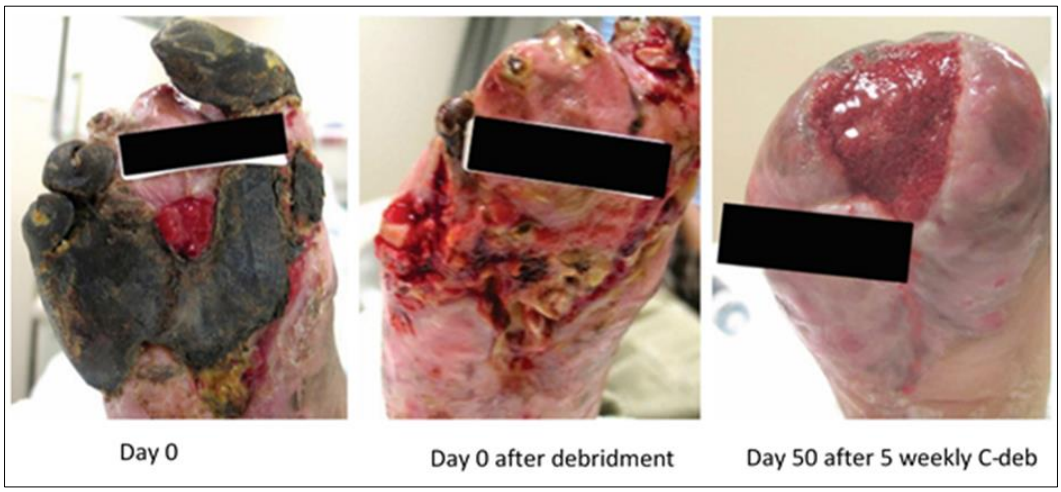


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Figure 7 Chronic ulcer in type 2 diabetes

3 Conclusion

C-deb represents an important innovation: it eliminates both the biofilm matrix and the microorganisms in a biophysical way: denaturation and desiccation. The development of bacterial resistance is unlikely as it leaves host tissue intact, including fibroblasts and the forming collagen matrix.

It also shows the agent's desiccating effect on the wound bed, its effect on progression to granulation.

Observations from a large group of patients have shown that C-deb treatment of patients with chronic ulcers represents an important step in their treatment: rapid progression of granulation tissue, which is necessary for healing. This is due to the ability of C-deb to eliminate biofilm where standard biofilm treatments have failed for the above reasons.

Compliance with ethical standards

Acknowledgments

DAPA srl, Verona Italy for providing C-DEB. C-deb is p-toluensulfonic acid, a dehydrating agent.

Disclosure of conflict of interest

The Authors declare that there is no actual or potential conflict of interest in relation to this case study.

Statement of ethical approval

The present research work does not contain any studies performed on animal subjects by of the author.

Statement of informed consent

Informed consent was obtained from the participants included in the study.

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