

ASSESSMENT OF A MULTIFUNCTIONAL WOUND DRESSING USING AN EX VIVO WOUND INFECTION HEALING MODEL

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INTRODUCTION

Hard to heal wounds cause complications such as infections caused by microbial biofilms. Biofilms are difficult to eradicate since they resist both the host defenses and antibiotics. The microorganisms in the biofilm live as sessile organisms and can release planktonic cells that can spread and cause more complications. Therefore, it is of interest to evaluate if advanced wound dressings can inhibit biofilm formation and at the same time allow the wound to heal. There is an ongoing debate how to predicate reality and several *in vitro* as well as animal *in vivo* studies have been used. We propose to use a novel *ex vivo* model to analyze the effect on biofilm and surrounding tissue.

Aim

To assess the effect of a multifunctional polymeric membrane* dressing (PMD) and comparator dressings on bacterial biofilm burden and wound healing in a novel *ex vivo* infection wound healing model.

METHOD

A novel *ex vivo* infection wound healing model was established based on the reports of Steinstraesser *et al.*, Kratz, and Lipp *et al.* The novelty with this model compared with existing models is that the activity of dressings on both the biofilm and infected human tissue can be evaluated under conditions where the biofilm and tissue are subjected to continuous flow of medium. This biofilm reactor, where the skin and wound dressings are contained, consists of a base with inlet and outlet and a top that is attached to its base, (see figure 1).

A consistent flow is maintained with a pump that stands on top of the incubator and the medium is delivered through tubes that are drawn through a hole in the wall of the incubator to the reactors. Planktonic and dead cells are discarded through the outlet to a waste bottle. The skin itself was provided by healthy donors and then punched at a size of 12 mm in diameter whereupon 3 mm wide wounds were created. The skin was pre-conditioned for six days before a biofilm of a co-culture of *Pseudomonas aeruginosa* (CCUG 56489) and Methicillin resistant *Staphylococcus aureus* (CCUG 35600) at 10^6 cells/ml was formed on wounded skin and different dressings were applied; two made of polyurethane foam and one made of 100% cotton gauze. A control was included that was infected, wounded skin without any dressing. The assemblies were incubated in the biofilm reactors with a continuous flow of medium under 5% CO₂ at 37°C. After 72 hours the bioburden in the skin was evaluated using plate count and the re-epithelialization as well as presence of biofilm was assessed by histology.

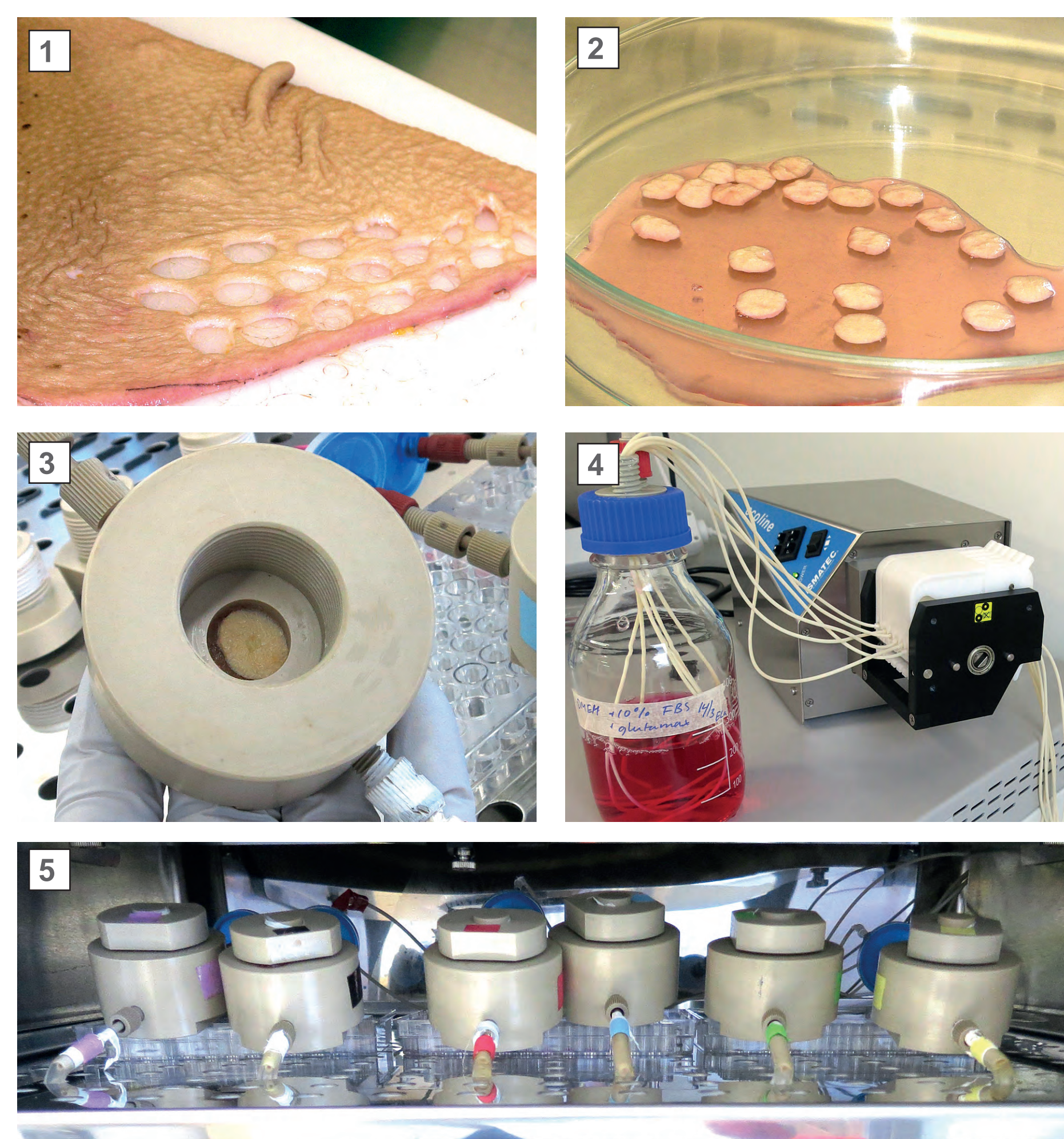


Figure 1. 1-2) 12 mm wide donated human skin is punched and 3 mm in diameter wounds are created. 3) The skin pieces are placed in the reactors and the dressings are placed on top. 4) The reactors are connected to a pump, creating a continuous flow of media and 5) placed in a CO₂ incubator.

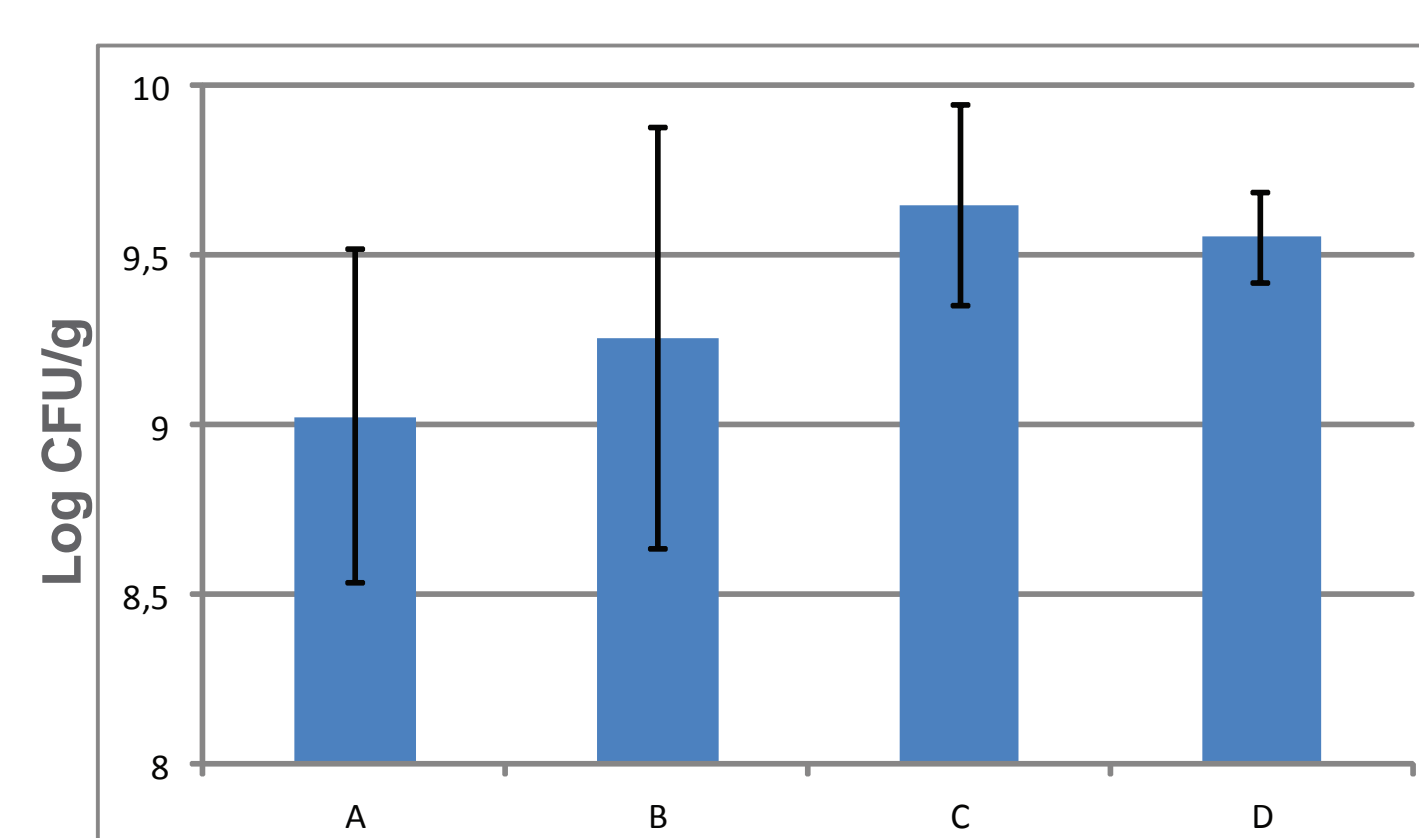


Figure 2. Bacterial biofilm burden per gram of tissue after 72 hours; n = 2, variations visualized by standard error of the mean (SEM).

A: PolyMem, Ferris Mfg.
B: Mepilex, Mölnlycke Health Care
C: Gauze, Mölnlycke Health Care
D: Control

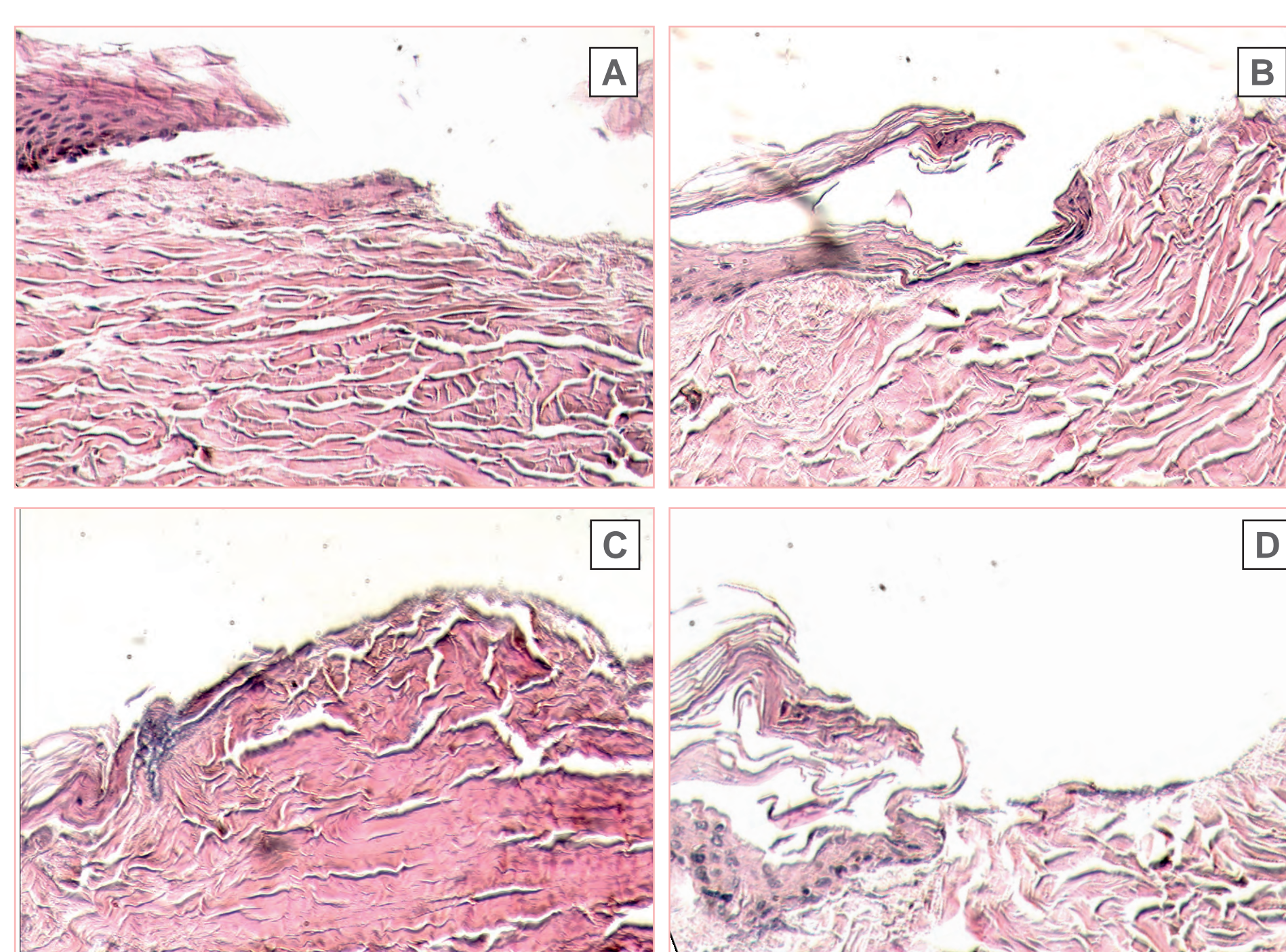


Figure 3. Photomicrographs taken at x 10 show histological images of the wounds stained with Hematoxylin & Eosin.

RESULTS

Plate count analysis revealed that the bioburden in the skin was lower when using the PMD than when the comparator dressings were used (Figure 2). This result was supported by the histological analyses, i.e. less bacterial cells were detected in the skin that had been exposed to PMD than with the other dressings (Figure 3). The histological analyses also revealed initiation of re-epithelialization for wounds treated with the PMD while wounds untreated (=control) or treated with the other dressings showed no evident wound healing. The physical texture of the skin that had been infected but not treated with any dressing had softened compared with the skins that had been treated with dressings. Equivalent observation was done also for skin that had been pre-incubated for two weeks instead of six days before infection and treated with PMD; i.e. wound healing processes were seen to have been initiated for wounds treated with PMD dressing which could not be detected for the comparator dressings or control (data not shown).

DISCUSSION

The novel *ex vivo* infection wound healing model indicated that the PMD had a stimulating effect on re-epithelialization despite the presence of biofilm composed of the pathogens *P. aeruginosa* and MRSA. This was even seen when the skin had been pre-incubated for two weeks instead of six days before infection, yet an aggravating condition in addition to the presence of biofilm for wound healing to proceed. The histological analyses demonstrated less bioburden in the wound exposed to PMD compared to the other investigated dressings. This observation was confirmed by plate count analyses where the tissue was homogenized and spread on plates and the bioburden calculated. The re-epithelialization of the wounded skin exposed to the comparator dressings may have been inhibited by the higher biofilm burden indicating that an important role of polymeric membrane dressings is to inhibit biofilm formation in order for wound healing to proceed.

References

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