Comparison of the antimicrobial effect of PHMB- and silver-containing wound dressings using different *in vitro* test methods

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Introduction

Colonization with micro-organisms and infection are a common complication during the treatment of chronic wounds. Therefore, wound dressings combined with antimicrobial agents such as PHMB or silver are increasingly utilized in the management of critical colonized or infected chronic wounds. The antibacterial activity of these dressings is mostly evaluated using *in vitro* tests. This allows a direct comparison of the effects of the dressings on the micro-organisms. However, various test methods are available which significantly differ in their properties and hence in their outcome. Thus, we have measured the antibacterial effect of several PHMB- and silver-containing dressings* using different *in vitro* methods such as the agar diffusion test (ADT), contact tests like the JIS L 1902:2002 or the AATCC100, as well as new methods such as microplate-laser nephelometry (MLN) and luminometric quantification of bacterial ATP (LQbATP).

Material & Methods

Dressing samples of 0.6cm^2 were prepared for the ADT using a 6mm biopsie punch. ADT was then performed according to DIN 58940-3. In accordance with the AATCC100, dressing samples of 18cm^2 were cut using a circle cutter. Meeting the requirements of the JIS L 1902:2002 norm samples of 400 mg of the dressings were used for testing and polyester was utilized as reference material. Both, MLN and LQbATP, are performed in suspension and thus, extracts of the dressings have been prepared corresponding to the DIN EN ISO 10993-12 using an extraction ratio of 1g:50mL. *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 27853) were used in the tests.

***dressings:** (1) Suprasorb X + PHMB, Lohmann & rauscher; (2) Kerlix AMD gauze dressing, Covidien; (3) Kendall AMD foam dressing, Covidien; (4) Acticoat Absorbent, Smith & Nephew; (5) Supraosrb A, Lohmann & Rauscher; (6) Suprasorb A + Ag, Lohmann & Rauscher; (7) Aquacell, ConvaTec; (8) Aquacell Ag, ConvaTec; (9) Urgocell, Urgo Medical; (10) Urgocell silver, Urgo Medical

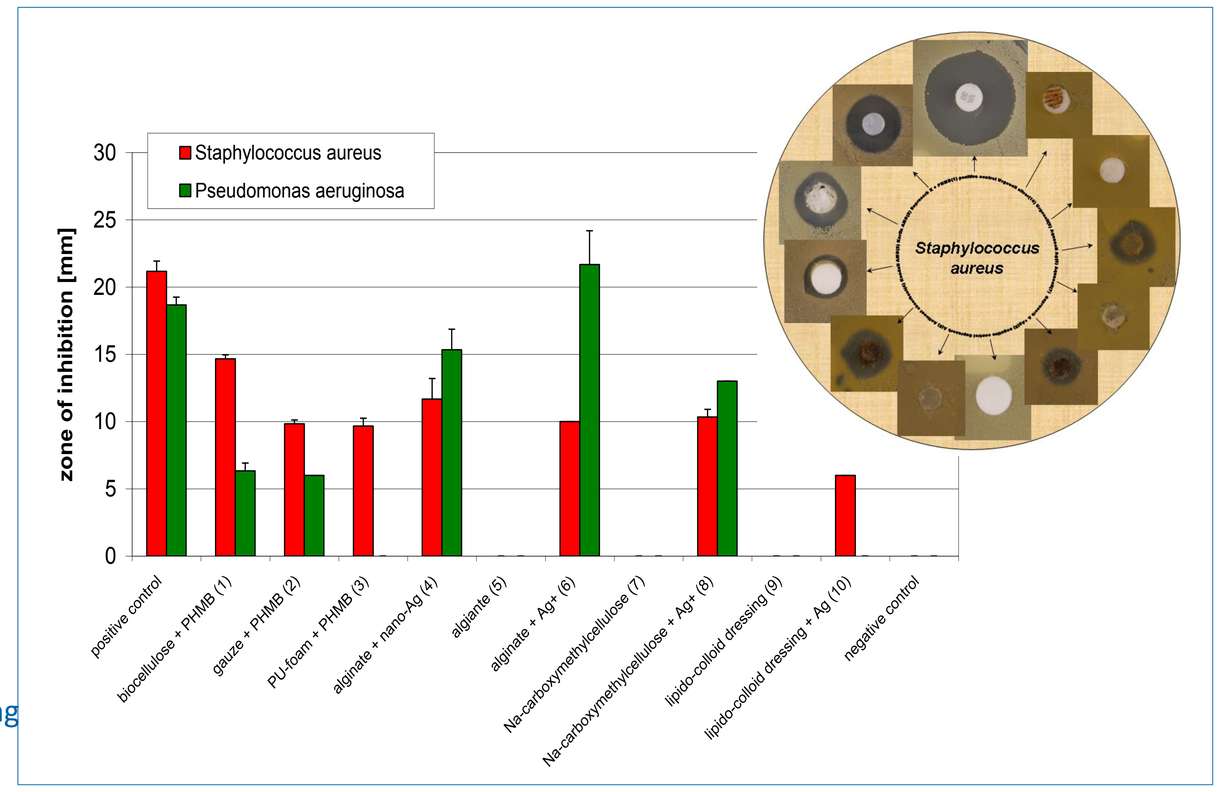

Figure 1: Results for the agar diffusion test (ADT) for PHMB- and silver-containing dressings. Only the dressings with a diffusible active agent were able to inhibit microbial growth resulting in measurable zones of inhibition. Vancomycin-containing disks were used as positive controls for *S. aureus* and gentamycin-containing discs for *P. aeruginosa*. For negative controls agent-free discs were used.

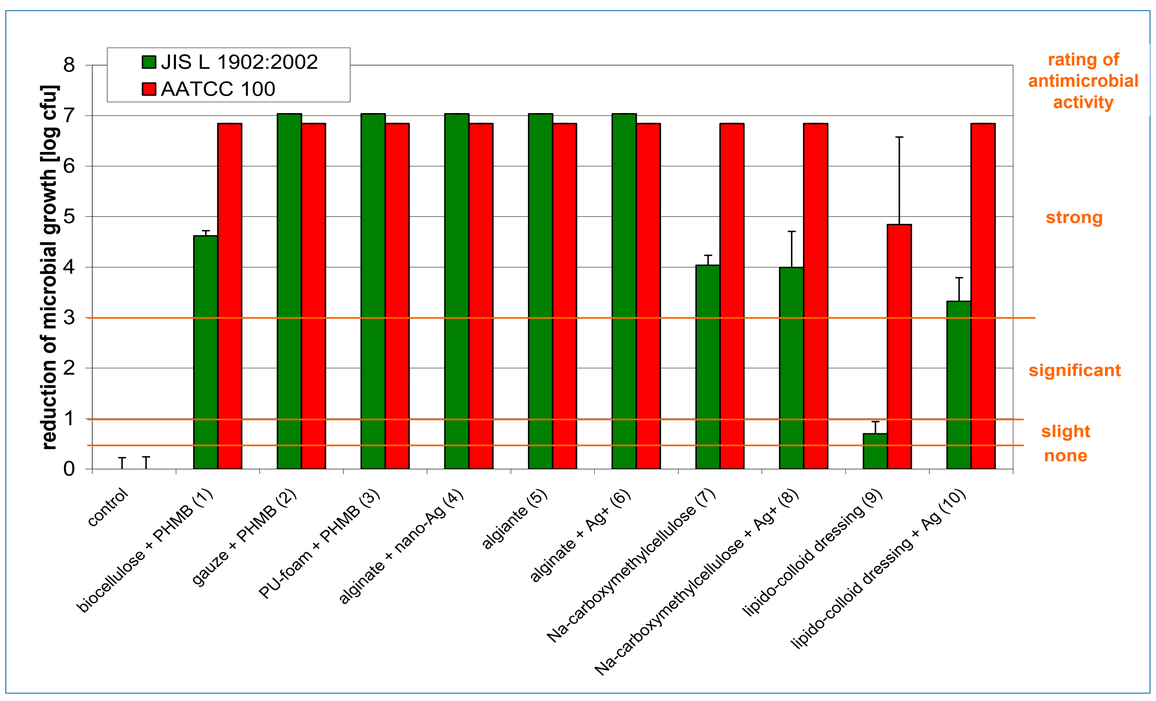

Figure 2: Comparison of JIS L 1902:2002 and AATCC 100. In these contact tests all dressings exhibited antimicrobial activity against *S. aureus* and *P. aeruginosa* (data not shown). A higher antibacterial effectiveness was commonly observed in the AATCC 100 compared to the JIS L 1902:2002 because more material is employed in this test.

Results

PHMB- and silver-containing dressings showed antibacterial activity in all *in vitro* tests used. Yet, total effectiveness varied between the single test methods as well as the properties of the basic dressing materials without the active agent. ADT depends on the diffusion capacity of the active agent tested; large molecules such as PHMB may have a reduced ability to disperse through the agar compared to small molecules like silver ions. Surprisingly, no significant difference was detected between the efficacies of PHMB- and silver-containing dressings in the ADT (Figure 1). Dressings without active agent had no effect in the ADT. In contrast, the basic alginate material showed a strong antibacterial activity comparable to agent containing dressings in the contact tests JIS L 1902:2002 and AATCC100 (Figure 2), because it is able to sequester bacteria during the gel formation. In accordance to the ADT, the new methods MLN (Figure 3A) and LQbATP (Figure 3B) determined a bactericidal effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa* only for the agent-containing dressing samples which further depended on the extractability of the antibacterial active agent. However, both were found to be superior to the ADT in sensitivity and quantification of the results.

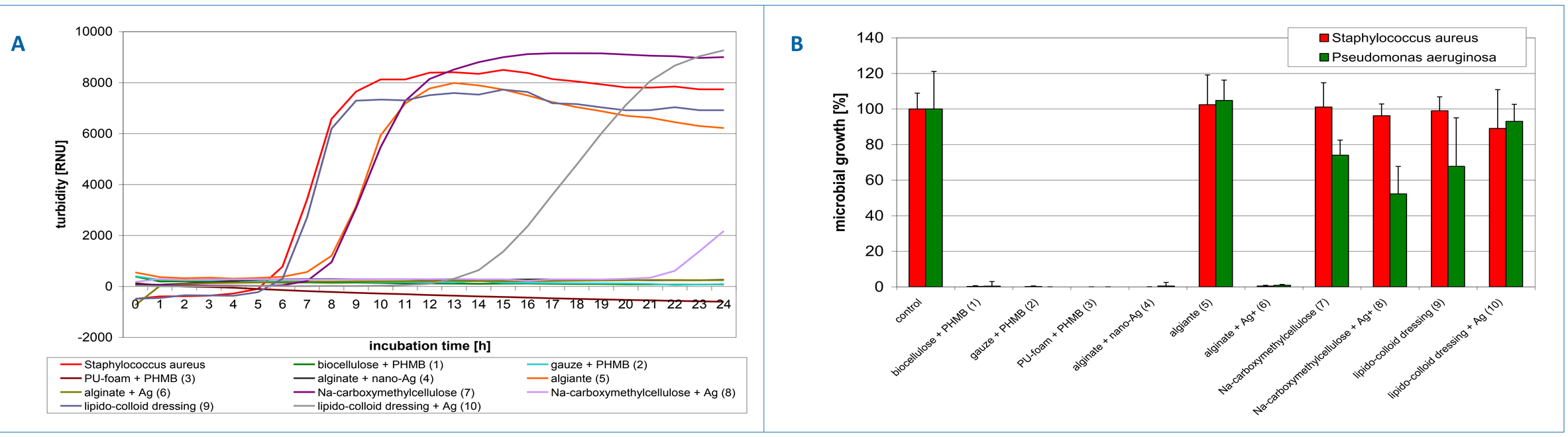

Figure 3: (A) Growth curves of *S. aureus* under the influence of the various dressing extracts Only the PHMB-containing dressings and Acticoat Absorbent as well as Suprasorb A + Ag were able to completely inhibit bacterial growth. Similar results were obtained for *P. aeruginosa* (data not shown). (B) All PHMB-containing dressings achieved almost complete reduction of the bacterial growth according to the LQbATP. Results for the silver-containing dressings varied, high amounts of active silver could be extracted from the dressings Acticoat Absorbant and Suprasorb A + Ag, lesser amounts from Aquacell Ag and none from Urgocell silver.

Discussion

Using *in vitro* tests for the evaluation of the antibacterial activity allows quantification and direct comparison of dressings' effectiveness under standard conditions. Various test methods are available as they differ in their properties and hence in their outcome, this has to be taken into account for selection of a specific test and interpretation of the results.

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