

Biofilm prevention through irradiation with visible light?

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INTRODUCTION & AIM

- biofilms are communities of microorganisms on wet surfaces, which are difficult to remove or disinfect [1-3]
- they do not only grow on teeth (plaque), but also on other natural and technical materials in the human body, such as catheters, implants, wounds, or bones [2,5-7]
- the release of potentially pathogenic microorganisms by biofilms can cause deadly infections [2,4-7]
- the total global economic damage caused by biofilms in the medical field alone is estimated at \$387 billion annually [8]
- there are studies on biofilm reduction or prevention by UV radiation; however, UV should not be applied inside or near human patients [9]
- visible blue and violet light is known for its antimicrobial properties on planktonic bacteria – caused by natural photosensitizer generating reactive oxygen species – and is less dangerous for humans [10-11]

⇒ **this study investigates the application of visible blue (450 nm) and violet light (405 nm) to prevent or reduce biofilm formation**

METHOD

- bacterial suspensions of *Bacillus subtilis*, *Pseudomonas stutzeri*, *Pseudomonas syringae*, and *Streptococcus cristatus* in tryptic soy broth were filled in the wells of polystyrol MTPs (micro titer plates) and cultivated on a shaker @ 100 rpm for up to 6 days
- during this period the MTP was irradiated with blue (450 nm) or violet (405 nm) light from below at intensities of 0, 10 and 20 mW/cm² (Fig. 1)

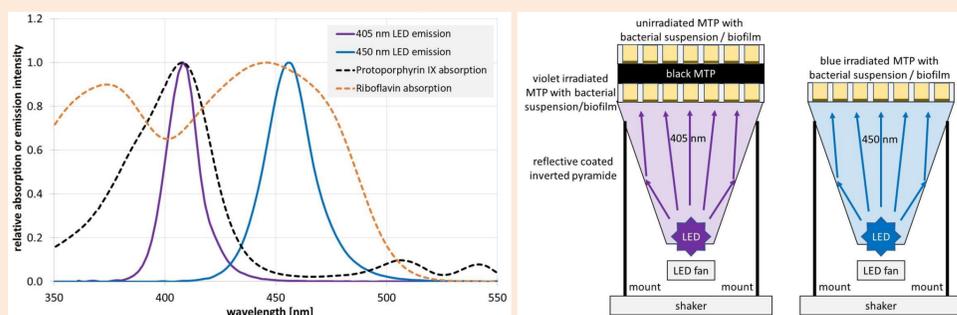


Fig. 1. left: LED emission and photosensitizer (porphyrin/flavin) absorption spectra; right: MTP irradiation setup with one violet, one blue and one unirradiated MTP

- all experimental runs were performed at least three times
- biofilm formation was judged by 3 optical techniques:
 - 1) photograph of the whole MTP in front of dark background (Fig. 2)
 - 2) microscopic images of a phase contrast microscope (VWR)
 - 3) biofilm absorption measurement by an MTP reader (BMG Labtech) at 405 nm



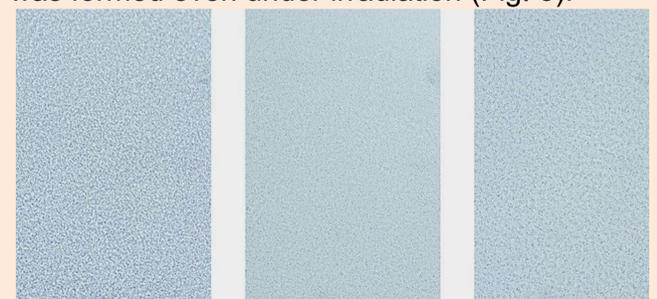
Fig. 2. Example photo of an unirradiated MTP after 3 days

- to adjust for potential different bacterial starting concentrations all biofilm absorptions were normalized to the absorption of the unirradiated MTPs after 24 h (for each bacterium and each run)

RESULTS & DISCUSSION

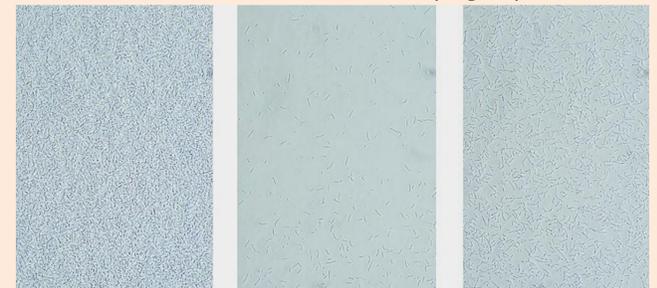
- biofilms were recognizable under the microscope
- in most cases biofilm was formed even under irradiation (Fig. 3):

Fig. 3. Microscopic images after 24 h of the bottoms of wells with *P. stutzeri*. Left: unirradiated, middle violet 405 nm@20 mW/cm² and right blue 450 nm @ 20 mW/cm²



- only for *B. subtilis* no biofilm formed under irradiation (Fig. 4):

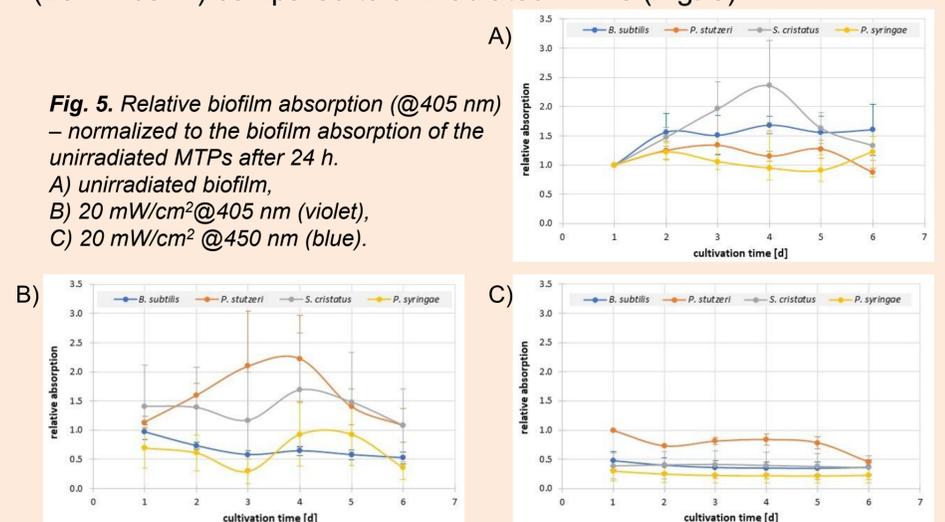
Fig. 4. Microscopic images after 24 h of the bottoms of wells with *B. subtilis*. Left: unirradiated, middle violet 405 nm@20 mW/cm² and right blue 450 nm @ 20 mW/cm². No biofilm is visible in irradiated wells



- the absorption measurements revealed reduced biofilm formation under blue (405 nm @ 10 and 20 mW/cm²) and violet irradiation (20 mW/cm²) compared to unirradiated MTPs (Fig. 5):

Fig. 5. Relative biofilm absorption (@405 nm) – normalized to the biofilm absorption of the unirradiated MTPs after 24 h.

A) unirradiated biofilm, B) 20 mW/cm²@405 nm (violet), C) 20 mW/cm²@450 nm (blue).



- a study limit is that the absorption measurements cannot distinguish between biofilm and sedimented bacteria - however, since the MTPs were continuously shaken and liquid in the wells was pipetted out, we assume that absorption (and scattering) was mainly caused by biofilms
- the microscope images confirm the existence of biofilms

⇒ **main results:**

- 1) blue and violet irradiation can reduce biofilm formation
- 2) blue light had stronger impact in these biofilm experiments, which is contrary to results for planktonic bacteria
- 3) for *B. subtilis* biofilm formation was even totally prevented, therefore, this might also be possible for other bacteria if the irradiation intensity is high enough

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