

Supplementary Material for Manuscript: Effective biofilm eradication on orthopedic implants with methylene blue based antimicrobial photodynamic therapy *in vitro*

Table S1: Photodynamic inactivation of planktonic *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Cutibacterium acnes* using different concentrations of methylene blue (MB) as average log₁₀ reduction of two or three independent biological replicates. The stars indicate 100% killing, i.e., no regrowth. MB only controls were performed with the determined minimum bactericidal concentration without regrowth (MBC_{100%}).

Log10 reduction

Methylene blue				
concentration (µg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>S. epidermidis</i>	<i>C. acnes</i>
0.25	1.57	1.42	2.49	2.30
0.50	2.06	3.17	4.48	3.98**
1.00	4.11	6.19**	6.07**	5.94*
2.50	5.67	6.19*	6.21*	6.26*
5.00	5.91*	6.19*	6.07*	5.94*
10.00	5.98*	6.19*	6.07*	5.94*
MB only	2.39	2.03	2.20	1.23
Light only	0.15	0.03	0.00	0.24

*100% killing, i.e. no regrowth

**Minimum bactericidal concentration with 100% killing and no regrowth (=MBC_{100%})

Methylene blue only was tested with the concentration of the MBC_{100%} (5 µg/ml for *S. aureus*, 1 µg/ml for *E. coli* and *S. epidermidis*, 0.5 µg/ml for *C. acnes*)

Table S2: Photodynamic inactivation of early (2-day-old) and mature (6-day-old) *Staphylococcus aureus* biofilms formed on polyethylene (PE), titanium alloy (TAV), cobalt-chromium-molybdenum (CCM) and polymethyl methacrylate (PMMA) based bone cement discs. The average log₁₀ reductions of two or three independent biological replicates using different methylene blue (MB) concentrations are presented. The stars indicate 100% killing, i.e., no regrowth. MB only controls were performed with the determined minimum bactericidal concentration without regrowth (MBC_{100%}).

Log10 reduction

Methylene blue concentration (µg/ml)	PE		TAV		CCM		PMMA Cement	
	2d	6d	2d	6d	2d	6d	2d	6d
1.00	1.70	1.12	1.50	1.61	1.20	1.12	1.02	1.65
10.00	2.80	3.96	2.82	3.66	2.03	3.05	1.98	3.79
50.00	4.73	5.54	4.03	5.04	3.83	3.89	2.46	4.97
100.00	5.62*	5.87*	4.41*	5.68*	5.34*	4.56*	3.46*	5.62*
MB only	3.34	2.73	1.54	3.37	2.70	3.18	2.35	3.39
Light only	0.00	0.05	0.31	0.37	0.06	0.22	0.07	0.31

Table S3: Photodynamic inactivation of mature biofilms of methicillin resistant *Staphylococcus aureus* (6d), *Escherichia coli* (6d), *Staphylococcus epidermidis* (6d) and *Cutibacterium acnes* (8d) formed on polyethylene (PE) discs. The average log₁₀ reductions of two or three independent biological replicates using different methylene blue (MB) concentrations are presented. The stars indicate 100% killing, i.e., no regrowth. MB only controls were performed with the determined minimum bactericidal concentration without regrowth (MBC_{100%}).

Log10 reduction

Methylene blue				
concentration (µg/ml)	MRSA	<i>E. coli</i>	<i>S. epidermidis</i>	<i>C. acnes</i>
1.00	1.66	0.48	1.28	1.46
10.00	5.13	1.37	2.14	2.93
50.00	4.04	2.73	3.94*	3.09**
100.00	6.91*	5.28*	5.44*	3.75*
MB only	3.06	3.64	2.68	1.96
Light only	0.00	0.00	0.11	0.00

MB, Methylene blue

6d biofilm for MRSA, *E. coli* and *S. epidermidis*, 8d biofilm for *C. acnes*

*100% killing, i.e. no regrowth

**minimum bactericidal concentration with 100% killing and no regrowth (=MBC_{100%})

Methylene blue only was tested with the concentration of the MBC_{100%}

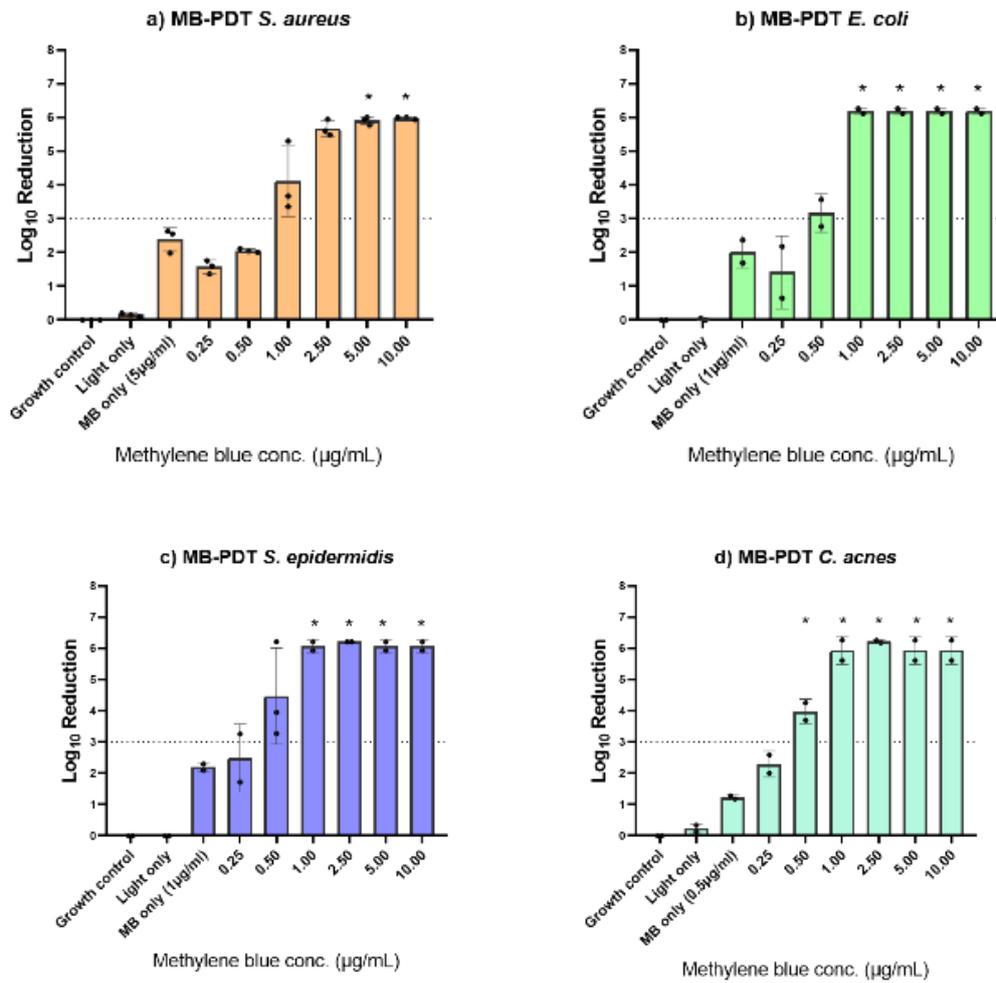


Figure S1. Photodynamic inactivation of planktonic *Staphylococcus aureus* (a), *Escherichia coli* (b), *Staphylococcus epidermidis* (c) and *Cutibacterium acnes* (d) using different concentrations of methylene blue (MB). The bars show the average log₁₀ reduction with the standard deviation from two or three independent biological replicates. The dotted line signals a bactericidal effect (3 log₁₀ reductions). The stars above the bars indicate 100% killing, i.e. no regrowth. MB only controls were performed with the determined minimum bactericidal concentration without regrowth (MBC_{100%}).

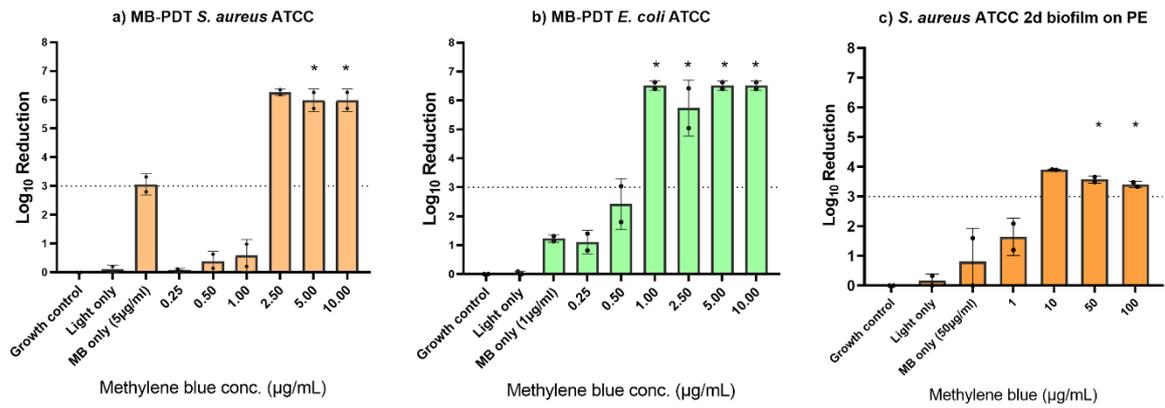


Figure S2: Photodynamic inactivation of planktonic ATCC *Staphylococcus aureus* (a), planktonic ATCC *Escherichia coli* (b) and 2-day-old ATCC *Staphylococcus aureus* biofilm formed on polyethylene (PE) (c) using different concentrations of methylene blue (MB). The bars show the average log₁₀ reduction with the standard deviation from two independent biological replicates. The dotted line signals a bactericidal effect (3 log₁₀ reductions). The stars above the bars indicate 100% killing, i.e. no regrowth. MB only controls were performed with the determined minimum bactericidal concentration without regrowth (MBC_{100%}).

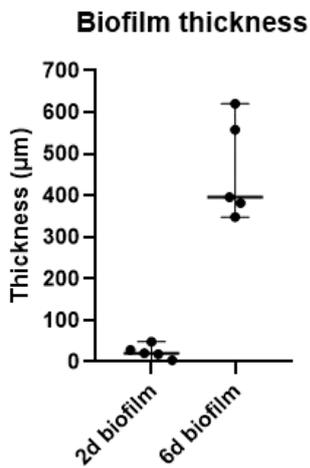


Figure S3: Biofilm thickness of 2-day-old and 6-day-old *Staphylococcus aureus* biofilm.

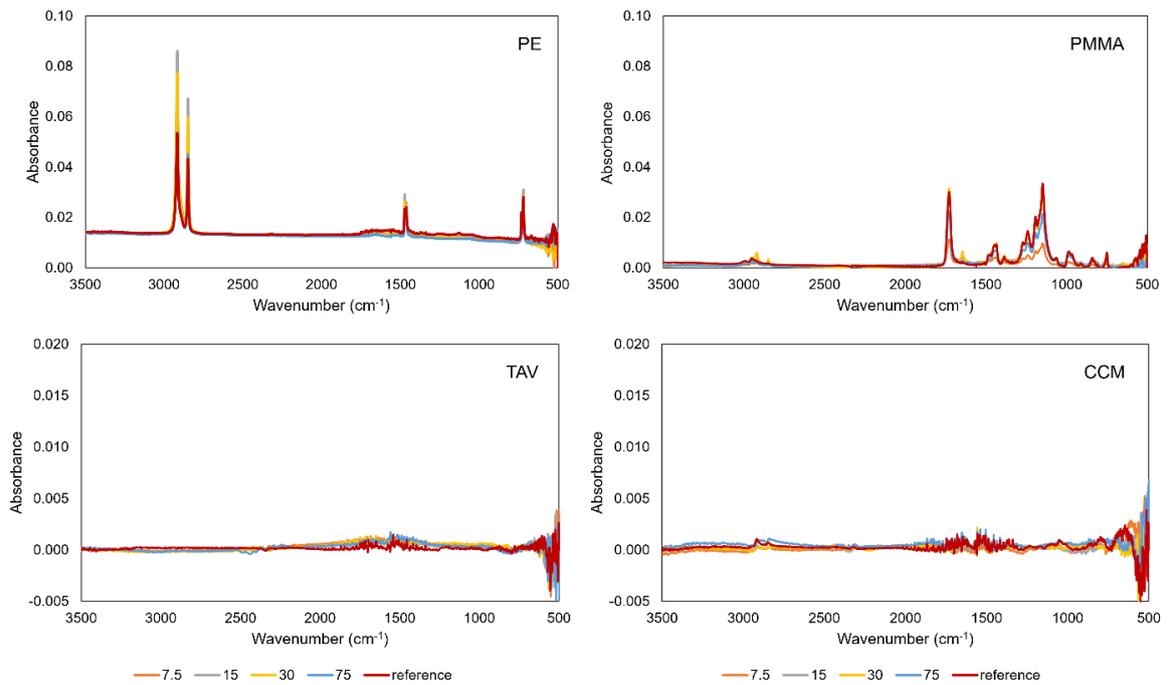


Figure S4: Attenuated total reflection infrared spectroscopy spectra of four different albumin-coated implant materials (PE, polyethylene; PMMA, polymethyl methacrylat; TAV, titanium alloy; CCM, cobalt-chromium-molybdenum) treated with methylene blue photodynamic therapy (MB-PDT) using light doses ranging from 7.5 to 75 J/cm². No differences between the reference and the treated samples are visible. Adsorption of albumin was only measured on the PMMA cement materials.

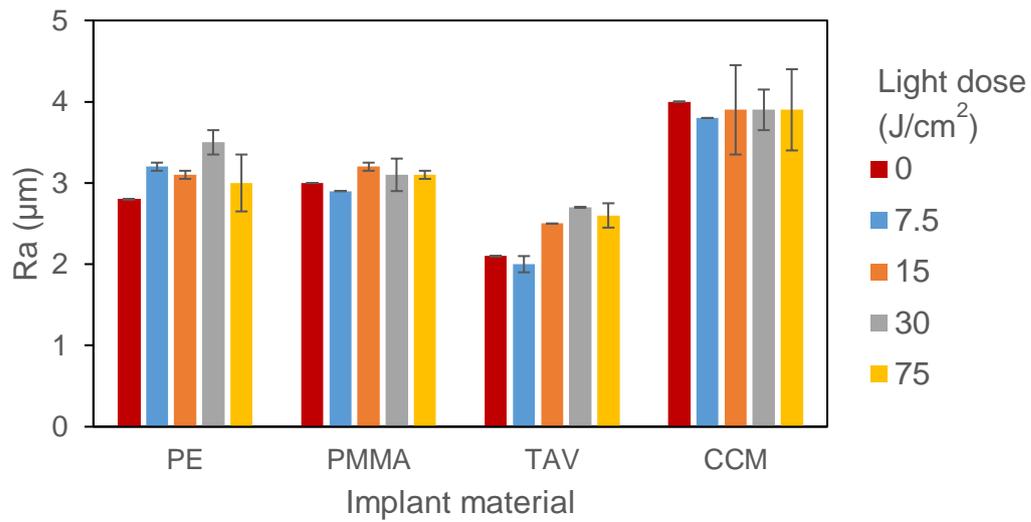


Figure S5: Roughness average (Ra) values of four different albumin-coated implant materials (PE, polyethylene; PMMA, polymethyl methacrylat; TAV, titanium alloy; CCM, Cobalt-chromium-molybdenum) treated with methylene blue photodynamic therapy (MB-PDT) using light doses ranging from 7.5 to 75 J/cm² compared to the untreated (0 J/cm²) reference materials.

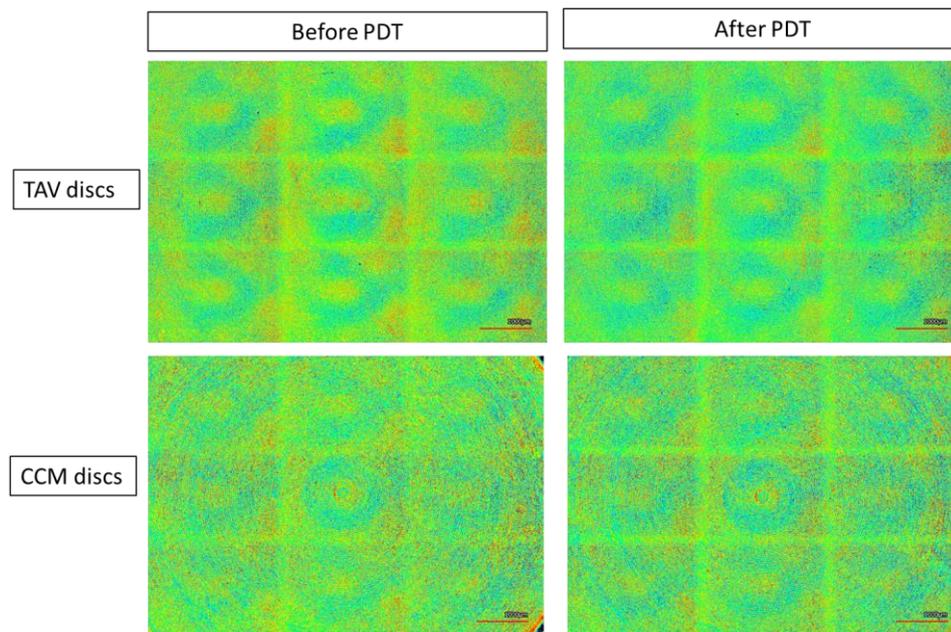


Figure S6: Example confocal laser scanning microscopy pictures of implant discs (top: TAV, titanium alloy; bottom CCM, cobalt-chromium-molybdenum) before PDT (left) and after PDT (right). Scale bar = 1000μm. No cracks or damages are visually seen after photodynamic therapy.