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Operative Unit "Microbiology and Clinical Microbiology"
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303-307 Des Voeux Road Central
Sheung Wang, Hong Kong (China)

Chieti, 19 February 2020

Ref.: Evaluation of the antibacterial activity of photocatalytic painting materials – final report.

Dear Sirs,
please find enclosed the final report on the results concerning the evaluation of the antibacterial activity of BLG6-5% photocatalytic painting materials.

Best regards.

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TEST REPORT

Material: Photocatalytic painting materials

Product: Ready-to-use photocatalytic painting materials

Samples: BLG6-5% and painting material without photocatalytic agent (control)

Test information:

- Treated specimen: photocatalytic painting material applied, as a 1 mm-thickness film, onto stainless steel 50x50 mm coupon;
- Not-treated specimens: painting material without photocatalytic agent applied, as a 1 mm-thickness film, onto stainless steel 50x50 mm coupon;
- Method: film adhesion (*Fine ceramics – Test method for antibacterial activity of semiconducting photocatalytic materials (ISO 27447:2019)*);
- Cover film: Pyrex7740, 40x40 mm square – 0.10 mm thickness;
- Light source, irradiation intensity, and time exposure: LED (at 1000 lux) or UVA (at 0.25 mW/cm²), exposure for 4, 8 and 24 hours;
- Test bacterial strains: *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 27853;
- Volume of test inoculum: 100 µl;
- Each sample was assayed in triplicate, on two different occasions.

Sampling: made by Customer

Date of sampling: 5 December 2019

Sample preparation: made at “G. d’Annunzio” University of Chieti-Pescara

Analysis start/end dates: 7 January 2020 – 5 February 2020

Table 1. Antibacterial activity of BLG6-5% formula against *E. coli* following LED irradiation for 4, 8 and 24 hours.

BLG6-5%		4h	8h	24h
No. of bacteria inoculated [cells/coupon]	860.000			
A - average number of viable bacteria of non-treated specimens, just after inoculation (t=0h) [cells/coupon]	700.000			
B_L - average number of viable bacteria of non-treated specimens, after irradiation [cells/coupon]		670.000	2.000.000	180.000
C_L - average number of viable bacteria of photocatalytic treated specimens, after irradiation [cells/coupon]		15	10	0
R_L - photocatalyst antibacterial activity, after irradiation		4.6	5.3	6.3
Antibacterial activity R%		>99.99	>99.99	>99.99
B_D - average number of viable bacteria of non-treated specimens, after being kept in a dark place [cells/coupon]		730.000	920.000	16.000.000
C_D - average number of viable bacteria of photocatalytic treated specimens, after being kept in a dark place [cells/coupon]		5	0	0
ΔR - photocatalyst antibacterial activity value with irradiation		0	0	0

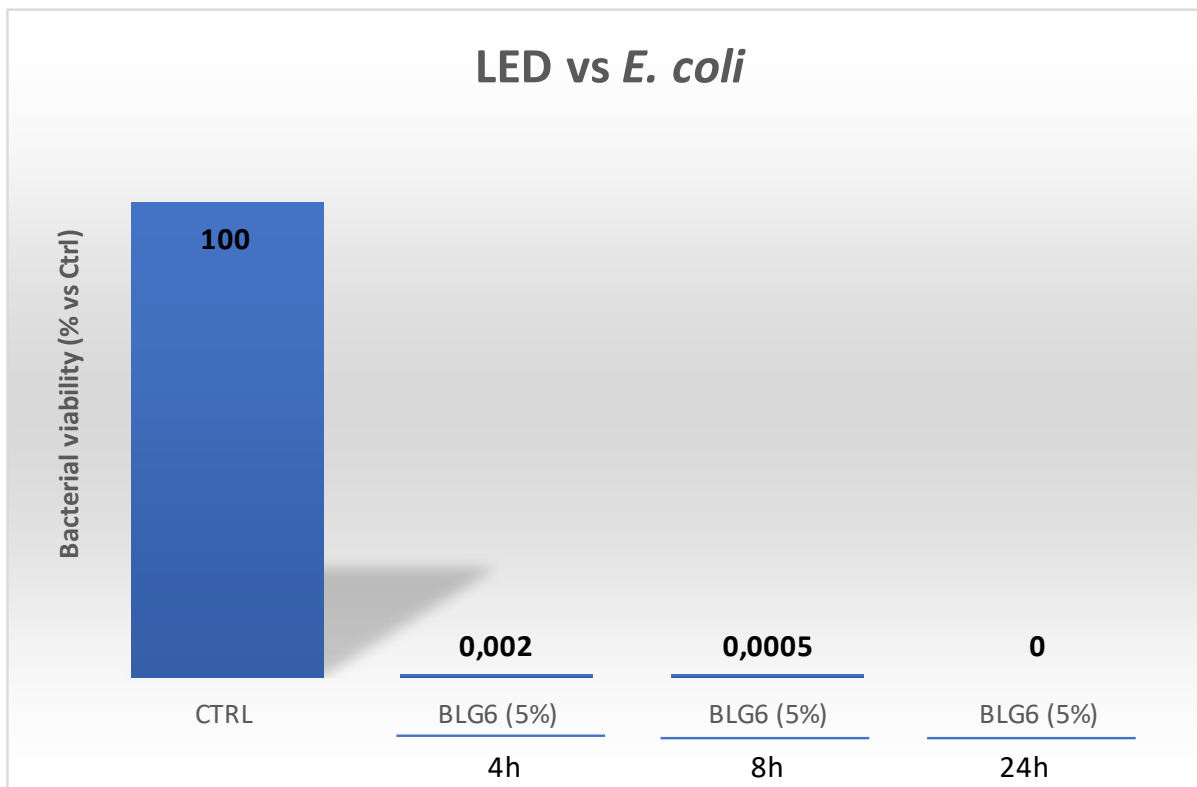


Figure 1. Antibacterial activity of BLG6-5% formula against *E. coli* following LED irradiation for 4, 8 and 24 hours.

Table 2. Antibacterial activity of BLG6-5% formula against *P. aeruginosa* following LED irradiation for 4, 8 and 24 hours.

BLG6-5%		4h	8h	24h
No. of bacteria inoculated [cells/coupon]	780.000			
A - average number of viable bacteria of non-treated specimens, just after inoculation (t=0h) [cells/coupon]	770.000			
B_L - average number of viable bacteria of non-treated specimens, after irradiation [cells/coupon]		1.100.000	4.300.00	51.000.000
C_L - average number of viable bacteria of photocatalytic treated specimens, after irradiation [cells/coupon]		0	0	33
R_L - photocatalyst antibacterial activity, after irradiation		7.0	7.6	6.2
Antibacterial activity R%		>99.99	>99.99	>99.99
B_D - average number of viable bacteria of non-treated specimens, after being kept in a dark place [cells/coupon]		910.000	5.200.000	46.000.000
C_D - average number of viable bacteria of photocatalytic treated specimens, after being kept in a dark place [cells/coupon]		0	5	0
ΔR - photocatalyst antibacterial activity value with irradiation		0	0	0.1

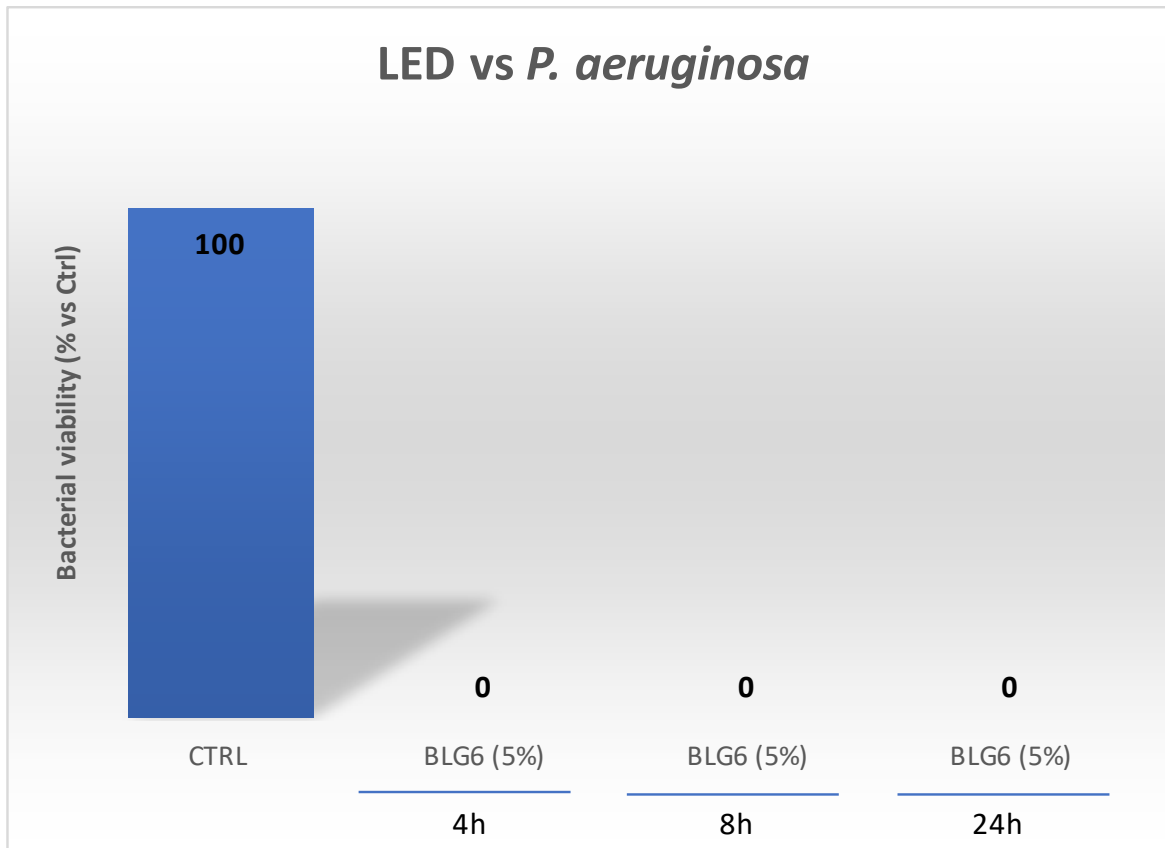


Figure 2. Antibacterial activity of BLG6-5% formula against *P. aeruginosa* following LED irradiation for 4, 8 and 24 hours.

Table 3. Antibacterial activity of BLG6-5% formula against *S. aureus* following LED irradiation for 4, 8 and 24 hours.

BLG6-5%		4h	8h	24h
No. of bacteria inoculated [cells/coupon]	800.000			
A - average number of viable bacteria of non-treated specimens, just after inoculation (t=0h) [cells/coupon]	790.000			
B_L - average number of viable bacteria of non-treated specimens, after irradiation [cells/coupon]		820.000	820.000	600.000
C_L - average number of viable bacteria of photocatalytic treated specimens, after irradiation [cells/coupon]		7	0	0
R_L - photocatalyst antibacterial activity, after irradiation		5.0	6.9	6.8
Antibacterial activity R%		>99.99	100	100
B_D - average number of viable bacteria of non-treated specimens, after being kept in a dark place [cells/coupon]		710.000	680.000	610.000
C_D - average number of viable bacteria of photocatalytic treated specimens, after being kept in a dark place [cells/coupon]		0	0	0
ΔR - photocatalyst antibacterial activity value with irradiation		0	0	0

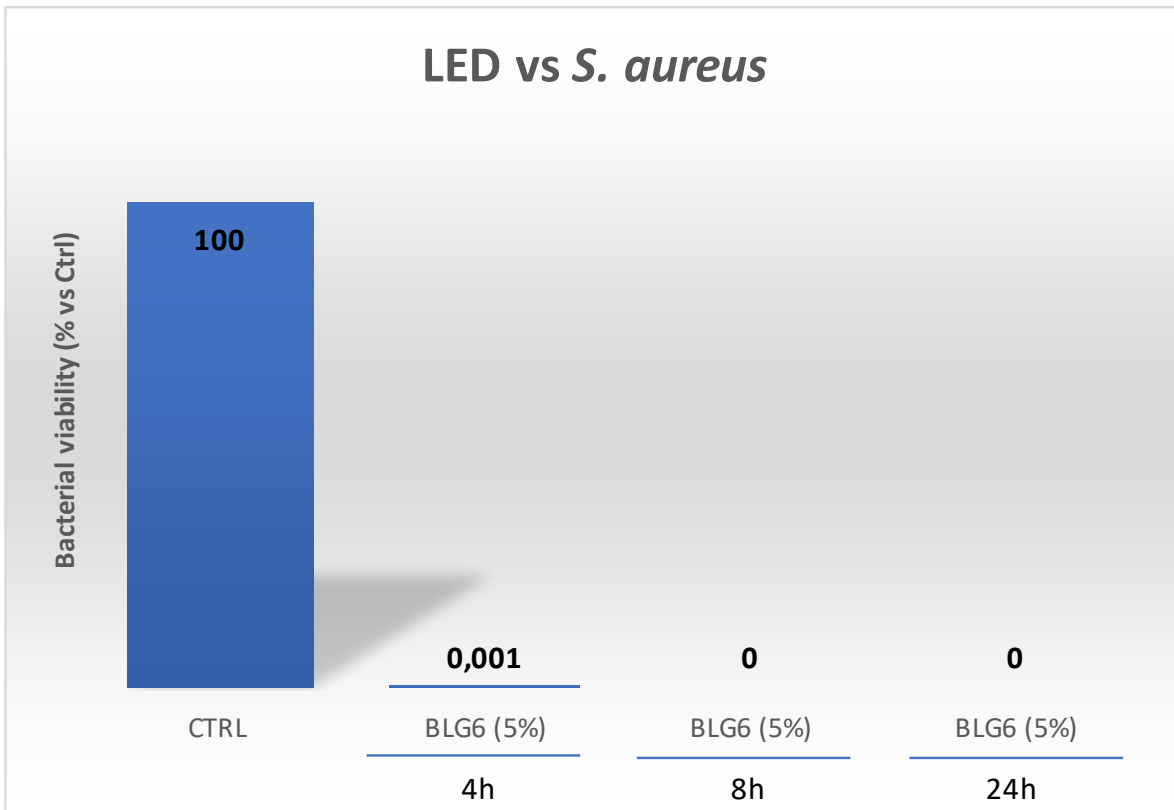


Figure 3. Antibacterial activity of BLG6-5% formula against *S. aureus* following LED irradiation for 4, 8 and 24 hours.

EXPOSURE TO LED - SUMMARY

BLG6 5% under LED	4h	8h	24h
<i>S. aureus</i>	✓	✓	✓
<i>E. coli</i>	✓	✓	✓
<i>P. aeruginosa</i>	✓	✓	✓

✓ Bacterial killing reached $\geq 99.99\%$, although the effect was NOT DUE to photocatalysis

Table 4. Antibacterial activity of BLG6-5% formula against *E. coli* following UVA irradiation for 4, 8 and 24 hours.

BLG6-5%		4h	8h	24h
No. of bacteria inoculated [cells/coupon]	880.000			
A - average number of viable bacteria of non-treated specimens, just after inoculation (t=0h) [cells/coupon]	870.000			
B_L - average number of viable bacteria of non-treated specimens, after irradiation [cells/coupon]		750.000	510.000	1.000.000
C_L - average number of viable bacteria of photocatalytic treated specimens, after irradiation [cells/coupon]		0	13	14
R_L - photocatalyst antibacterial activity, after irradiation		6.9	4.6	4.9
Antibacterial activity R%		100	>99.99	>99.99
B_D - average number of viable bacteria of non-treated specimens, after being kept in a dark place [cells/coupon]		700.000	2.300.000	12.000.000
C_D - average number of viable bacteria of photocatalytic treated specimens, after being kept in a dark place [cells/coupon]		5	0	0
ΔR - photocatalyst antibacterial activity value with irradiation		0	0	0

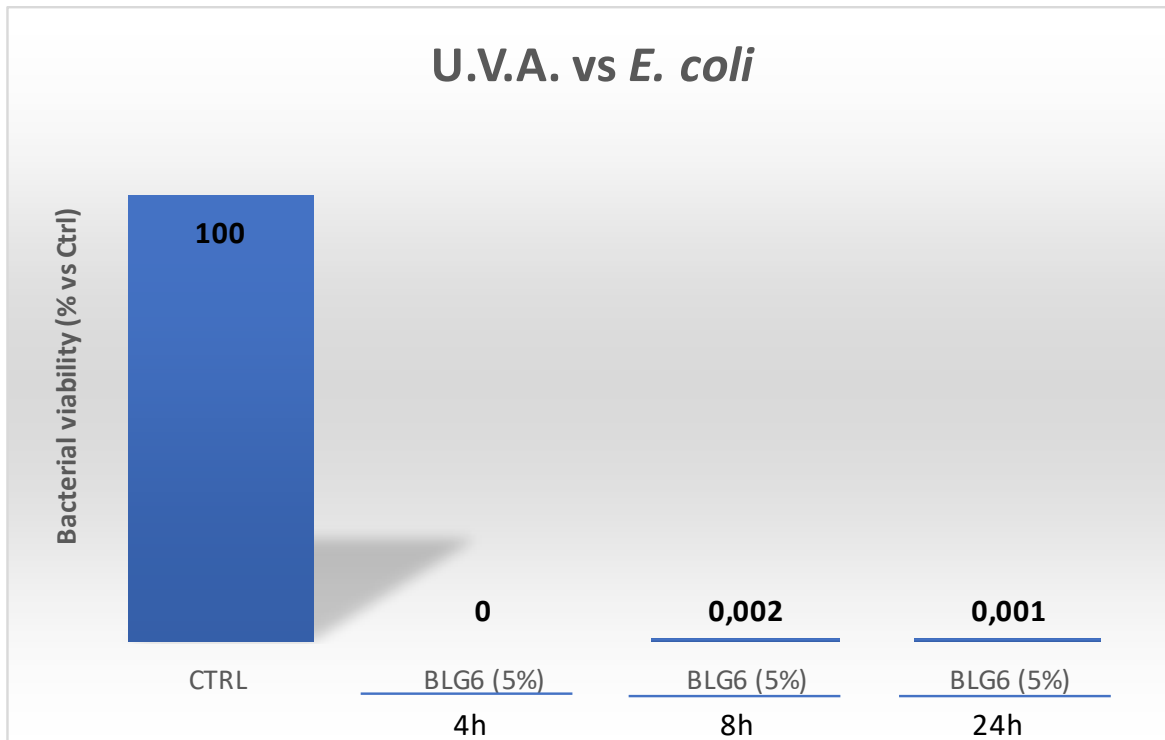


Figure 4. Antibacterial activity of BLG6-5% formula against *E. coli* following UVA irradiation for 4, 8 and 24 hours.

Table 5. Antibacterial activity of BLG6-5% formula against *P. aeruginosa* following UVA irradiation for 4, 8 and 24 hours.

BLG6-5%		4h	8h	24h
No. of bacteria inoculated [cells/coupon]	880.000			
A - average number of viable bacteria of non-treated specimens, just after inoculation (t=0h) [cells/coupon]	880.000			
B_L - average number of viable bacteria of non-treated specimens, after irradiation [cells/coupon]		720.000	2.500.000	57.000.000
C_L - average number of viable bacteria of photocatalytic treated specimens, after irradiation [cells/coupon]		0	5	0
R_L - photocatalyst antibacterial activity, after irradiation		6.9	5.7	8.8
Antibacterial activity R%		100	>99.99	100
B_D - average number of viable bacteria of non-treated specimens, after being kept in a dark place [cells/coupon]		910.000	5.200.000	46.000.000
C_D - average number of viable bacteria of photocatalytic treated specimens, after being kept in a dark place [cells/coupon]		0	5	0
ΔR - photocatalyst antibacterial activity value with irradiation		0	0	0.1

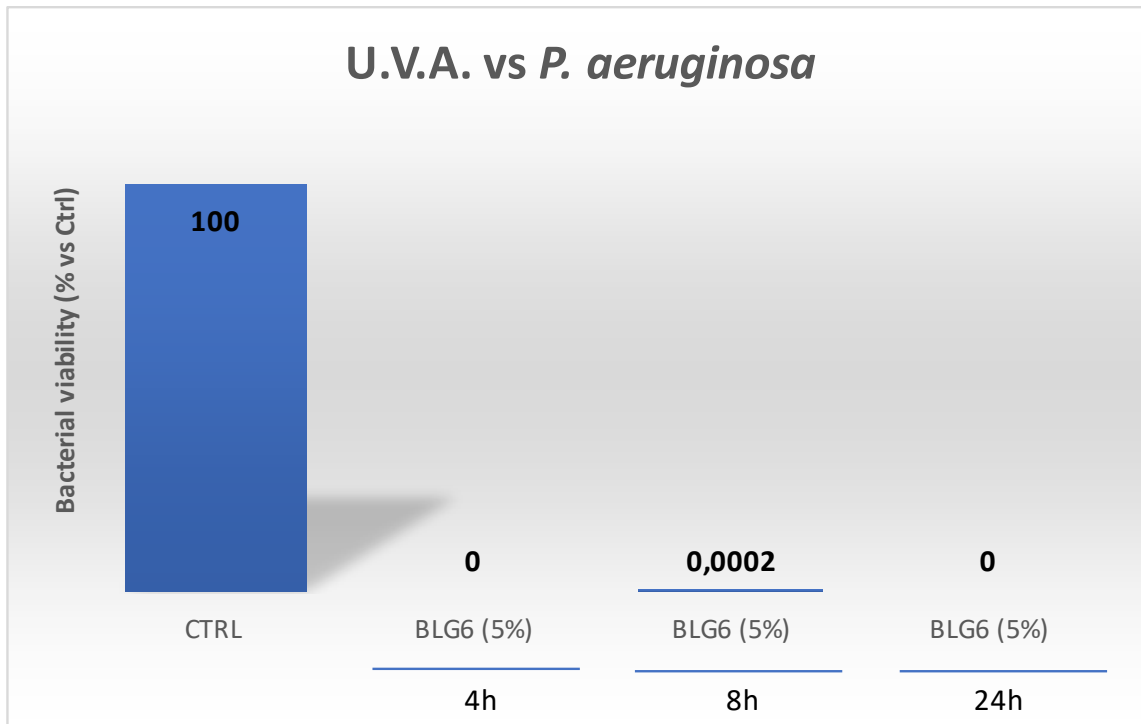


Figure 5. Antibacterial activity of BLG6-5% formula against *P. aeruginosa* following UVA irradiation for 4, 8 and 24 hours.

Table 6. Antibacterial activity of BLG6-5% formula against *S. aureus* following UVA irradiation for 4, 8 and 24 hours.

BLG6-5%		4h	8h	24h
No. of bacteria inoculated [cells/coupon]	660.000			
A - average number of viable bacteria of non-treated specimens, just after inoculation (t=0h) [cells/coupon]	650.000			
B_L - average number of viable bacteria of non-treated specimens, after irradiation [cells/coupon]		530.000	296.000	610.000
C_L - average number of viable bacteria of photocatalytic treated specimens, after irradiation [cells/coupon]		65	5	0
R_L - photocatalyst antibacterial activity, after irradiation		3.9	4.8	6.8
Antibacterial activity R%		99.9	>99.99	100
B_D - average number of viable bacteria of non-treated specimens, after being kept in a dark place [cells/coupon]		810.000	690.000	340.000
C_D - average number of viable bacteria of photocatalytic treated specimens, after being kept in a dark place [cells/coupon]		0	5	0
ΔR - photocatalyst antibacterial activity value with irradiation		0	0	0.3

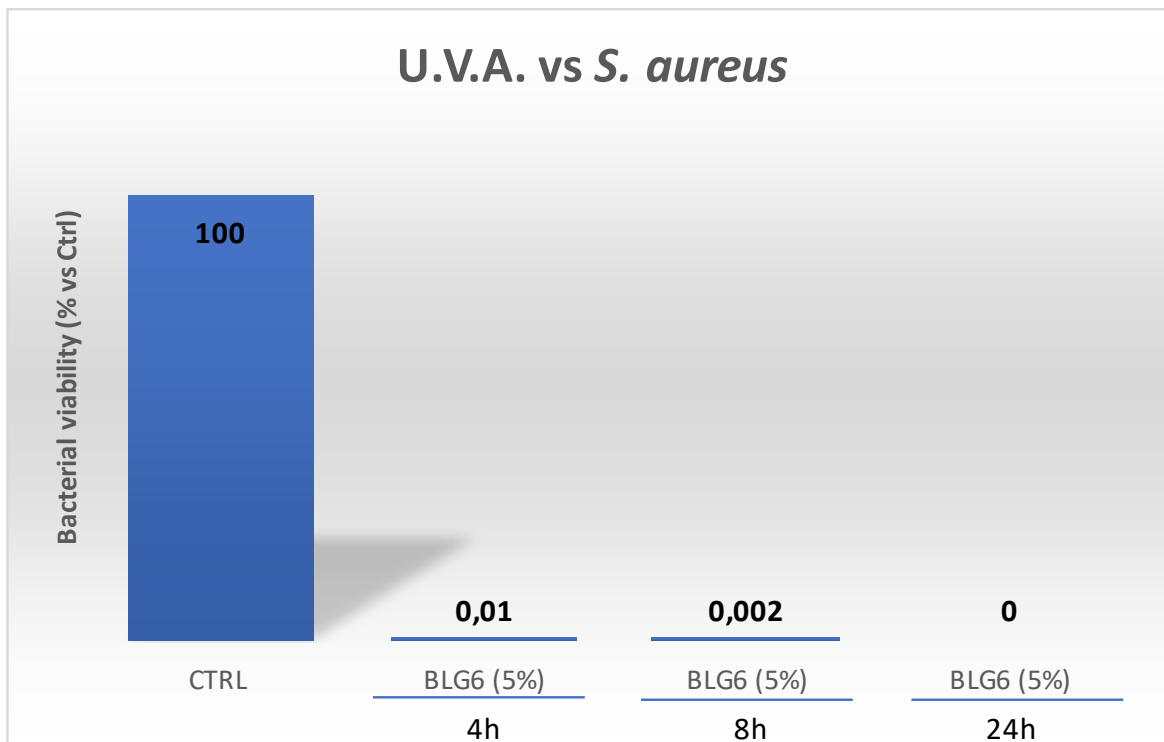


Figure 6. Antibacterial activity of BLG6-5% formula against *S. aureus* following UVA irradiation for 4, 8 and 24 hours.

EXPOSURE TO UVA - SUMMARY

BLG6 5% under UVA	4h	8h	24h
<i>S. aureus</i>	✓	✓	✓
<i>E. coli</i>	✓	✓	✓
<i>P. aeruginosa</i>	✓	✓	✓

✓ Bacterial killing reached $\geq 99.99\%$, although the effect was NOT DUE to photocatalysis

CONCLUSIONS

- BLG6-5% formula:
 - ALWAYS reached the desired endpoint (killing $\geq 99.99\%$) regardless of exposure time, irradiation source, and tested bacterial species;
 - however, as suggested by ΔR values, this activity WAS NOT DUE to the photocatalytic effect but rather to other formula components.