Photodynamic Toluidine Blue-Gold Nanoconjugates as a Novel Therapeutic for *Staphylococcal* Biofilms

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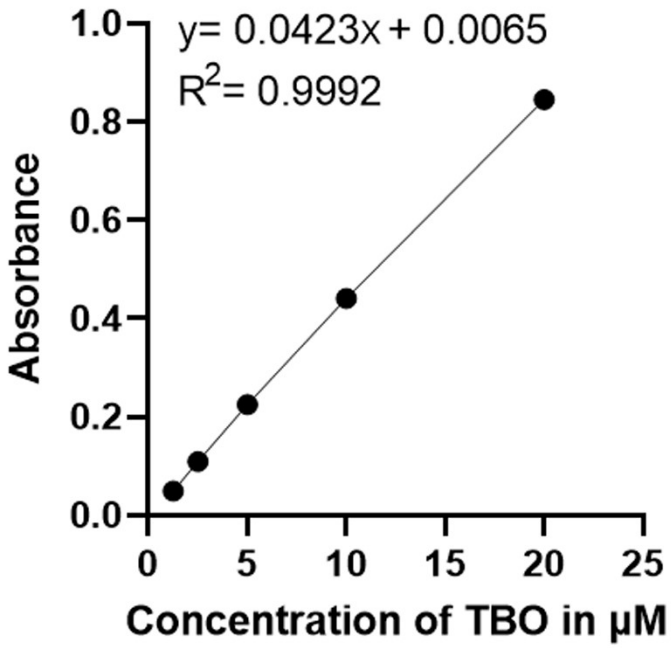


Figure S 1. Calibration curve of Toluidine Blue O (TBO) solution at different concentrations. The curve was used to indirectly calculate the quantity of electrostatically conjugated TBO onto PEG-GNPs@TBO and PEG-GNSs@TBO nanoconjugates.

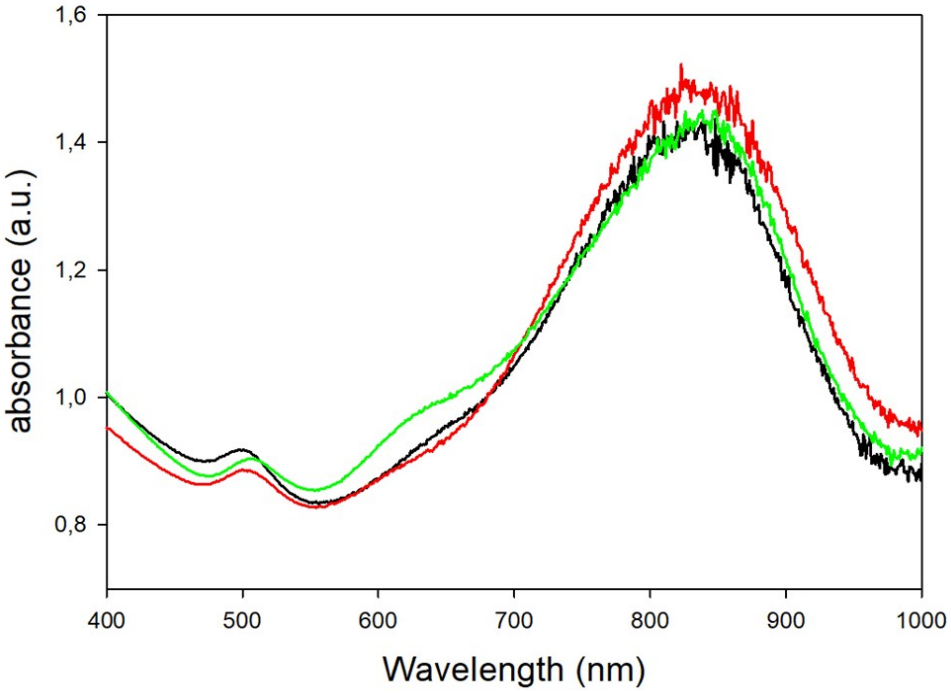
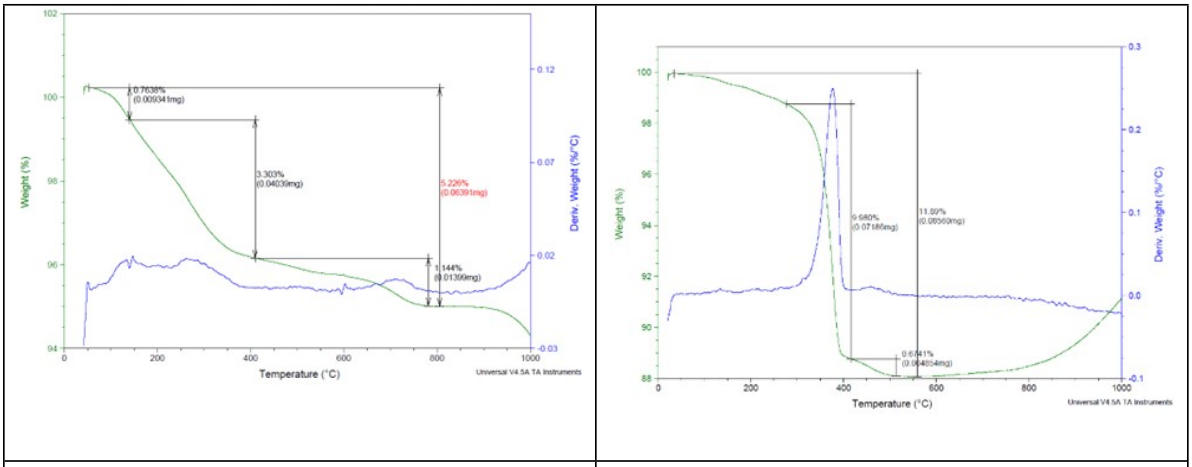
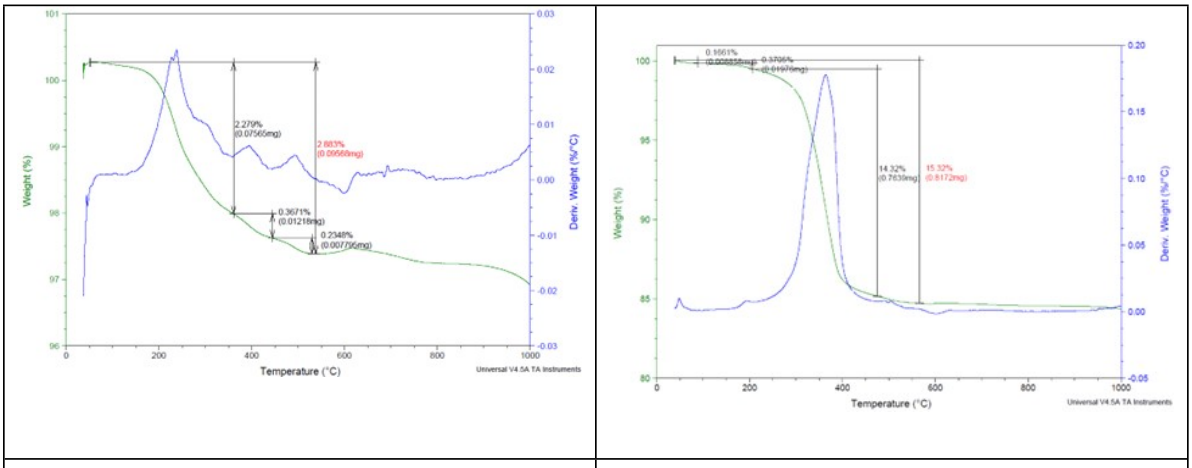


Figure S 2. Absorption spectra of three different GNS batches (before pegylation). The similar profiles indicate the good reproducibility of the synthetic approach.



A B

Figure S 3A, B. TGA measurements on as-prepared GNP (A) and on PEG-GNP (B). The green lines are the % of weight vs T (°C) profiles, and the blue profile are their derivate. In Figure S3A, the first weight loss (0.7638 %, at T < 200 °C) is typically attributed to water desorption, while the second and third weight loss are attributed to citrate decomposition, for a total of 5.226-0.7638 = 4.46%); in Figure S3B, the weight loss (T < 200 °C) is water, and the remaining weight loss is PEG-COOH (mw 5000), 9.980 + 0.6741 = 10.65%.



C D

Figure S 3C, D. TGA measurements on as-prepared GNS (C) and on PEG-GNS (D). The green lines a the % of weight vs T (°C) profiles, and the blue profile are their derivate. In Figure S3C, the total weight loss, including adsorbed water, is just 2.88 %; in Figure S3D, the weight loss at T < 200 °C is water, and the remaining weight loss is PEG-COOH (mw 5000) 14.32%.

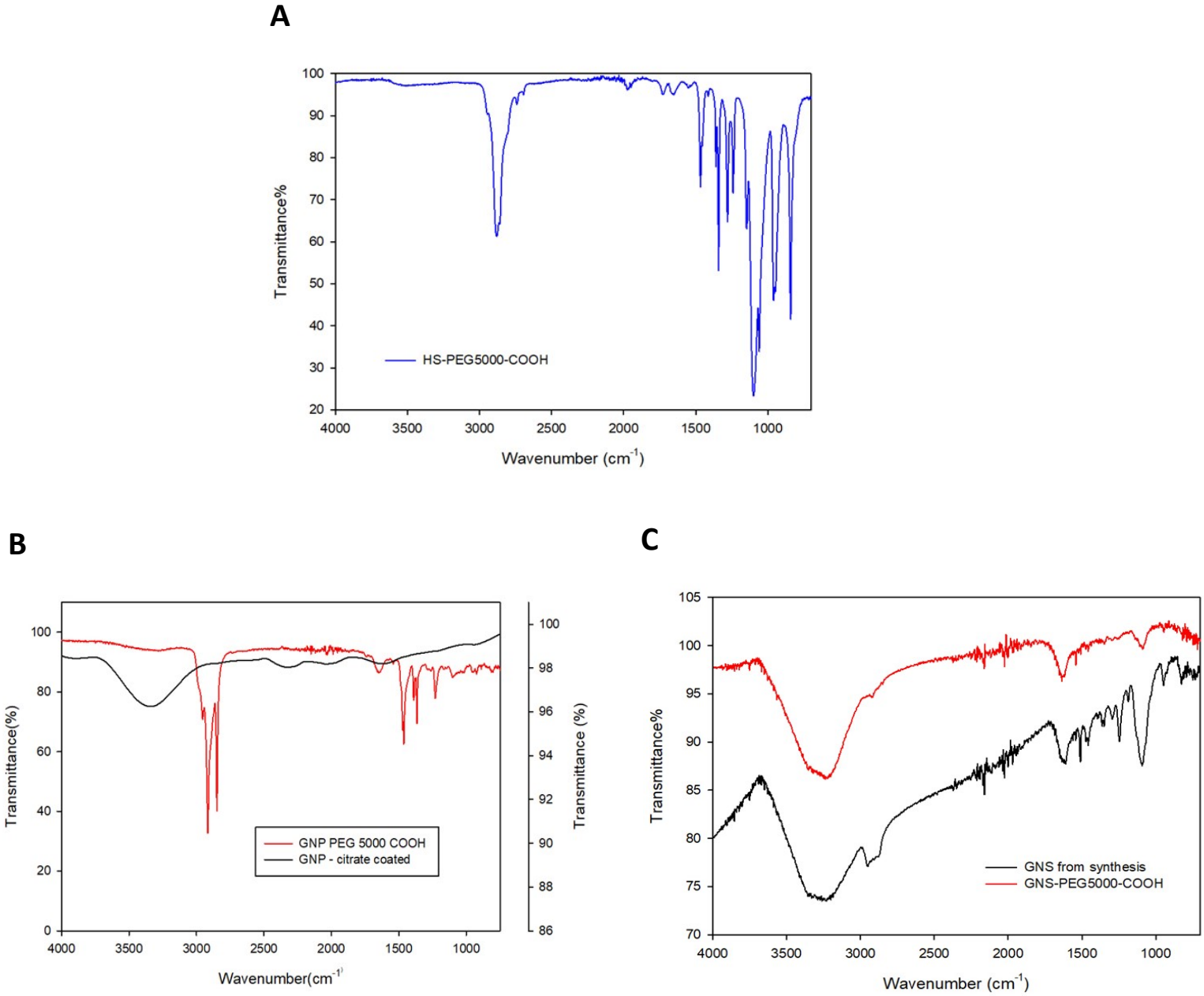


Figure S 4A. FTIR spectrum of HS-PEG-COOH MW 5000. ***S 4B.*** FTIR spectra of citrated-coated GNPs (black spectrum right displaced vertical axis) and of PEG-GNPs (red spectrum, left vertical axis). ***S 4C***. FTIR spectra of Triton X-100 coated GNSs (black spectrum) and of PEG-GNSs (red spectrum).

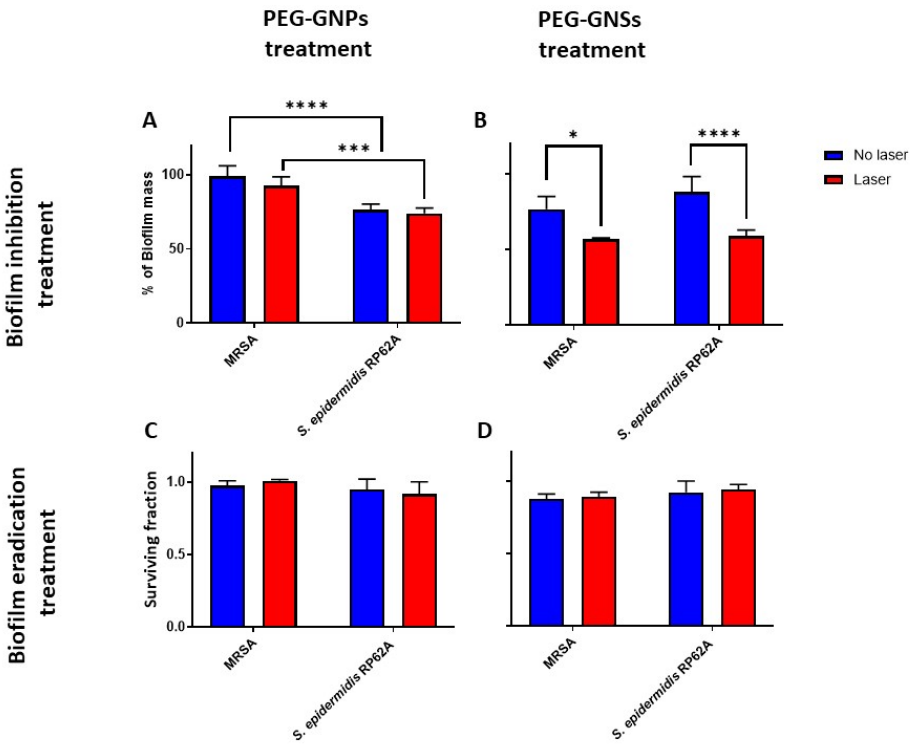


Figure S 5A) aPDI effects of PEG-GNPs@ or 5B) PEG-GNSs on Staphylococcal biofilm formations. *To evaluate the aPDI effects exerted by TBO-unconjugated PEG-GNPs [A] or PEG-GNSs [B] on biofilm formations by MRSA and S. epidermidis RP62A planktonic cultures, CV assay was performed. Percentage of biofilm mass formation was calculated as indicated in Materials and methods in paragraph* ***(2.2.6.1). 5C, D) aPDI effects of PEG-GNPs or PEG-GNSs on the bacterial viability of preformed Staphylococcal biofilms.*** *To evaluate the aPDI effects on the bacterial viability of 24-hour preformed biofilms of MRSA and S. epidermidis RP62A upon treatments with PEG-GNPs [C], PEG-GNS [D], an MTT assay was performed. Surviving fraction was calculated as indicated in Materials and methods in paragraph (**2.2.6.1**). Data were expressed as mean ± standard deviations (n = 3). Test groups were compared using one-way variance analysis (ANOVA), followed by Bonferroni post hoc, for multiple comparisons where: * p < 0.05, ***p < 0.001, ****p < 0.0001*

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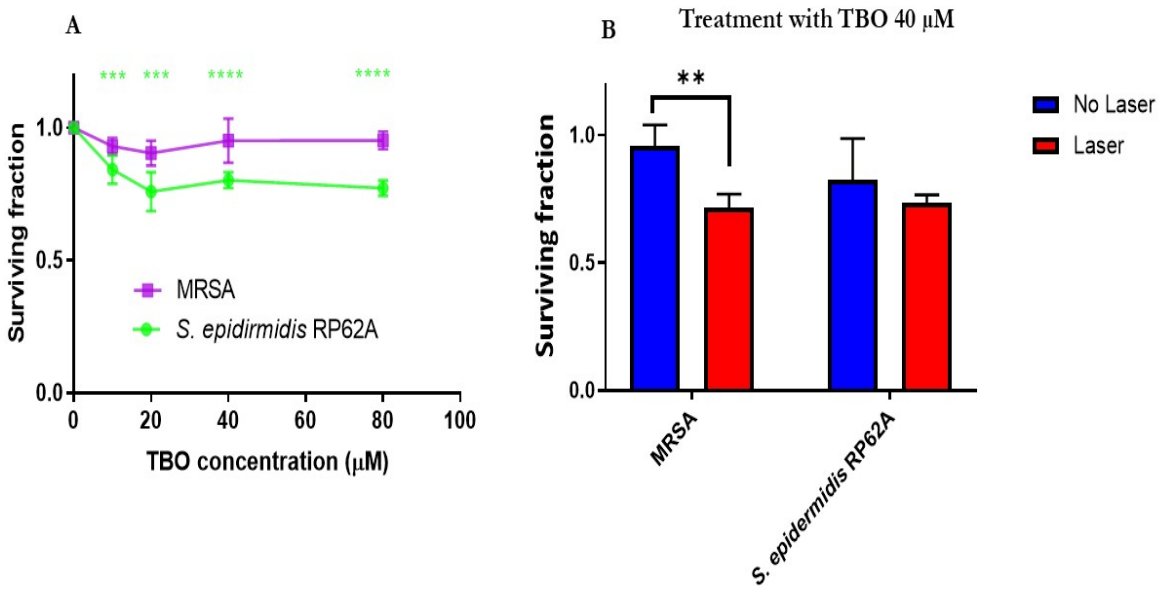

Figure S 6A) Dose-dependent effects of TBO solutions on the bacterial viability of preformed biofilms. *To evaluate the intrinsic dose-dependent effect of different TBO concentrations on the bacterial cell viability of 24-hour preformed biofilms of MRSA and S. epidermidis RP62A, an MTT assay was performed. TBO solutions at different concentrations were incubated with preformed biofilms for 24 hours at 37° C prior to conducting the MTT assay. The UV absorbance of solubilized formazan salts at 570 nm was recorded as an indication of bacterial cells viability. 6**B) aPDI light-dose effects of TBO (40μM) solution on the bacterial viability of preformed biofilms.*** *To evaluate the aPDI effects of TBO (40 μM) on the bacterial cell viability of 24-hour preformed biofilms of MRSA and S. epidermidis RP62A, an MTT assay was performed. 200 μL of 40 μM TBO solution were added to the preformed biofilms, and the samples were incubated for 1 hour at 37°C in dark conditions. Next the samples were exposed to laser irradiation (~ 0.2 W/cm^2 at 638 nm for 15 min) prior to conducting MTT assay. In both experiments surviving fraction was calculated as indicated in Materials and methods in paragraph **(2.2.6.1)**. Data were expressed as mean ± standard deviations (n = 3). Test groups were compared using one-way variance analysis (ANOVA), followed by Bonferroni post hoc, for multiple comparisons where: **p < 0.01, ***p < 0.001, ****p < 0.0001.*

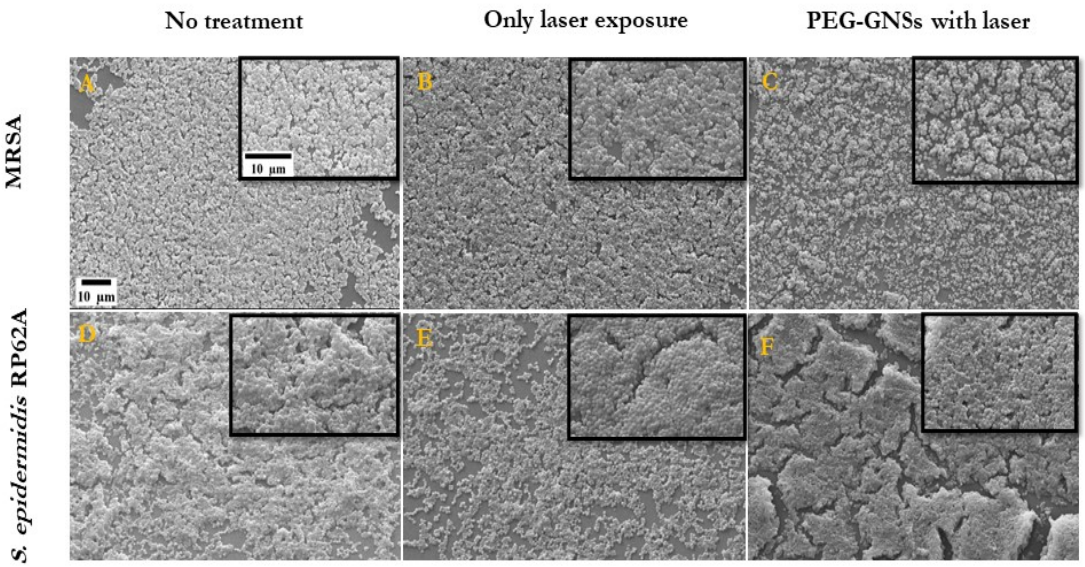

Figure S 7 SEM images (Biofilm inhibition treatment). *SEM images of biofilm formations of MRSA* ***[A, B, C]*** *and S. epidermidis RP62A* ***[D, E, F]*** *planktonic cultures upon exposure to different treatments as indicated. SEM images [within the black frames] represent specific sections from same corresponding images at a larger magnification. Scale bar: 10 µm.*

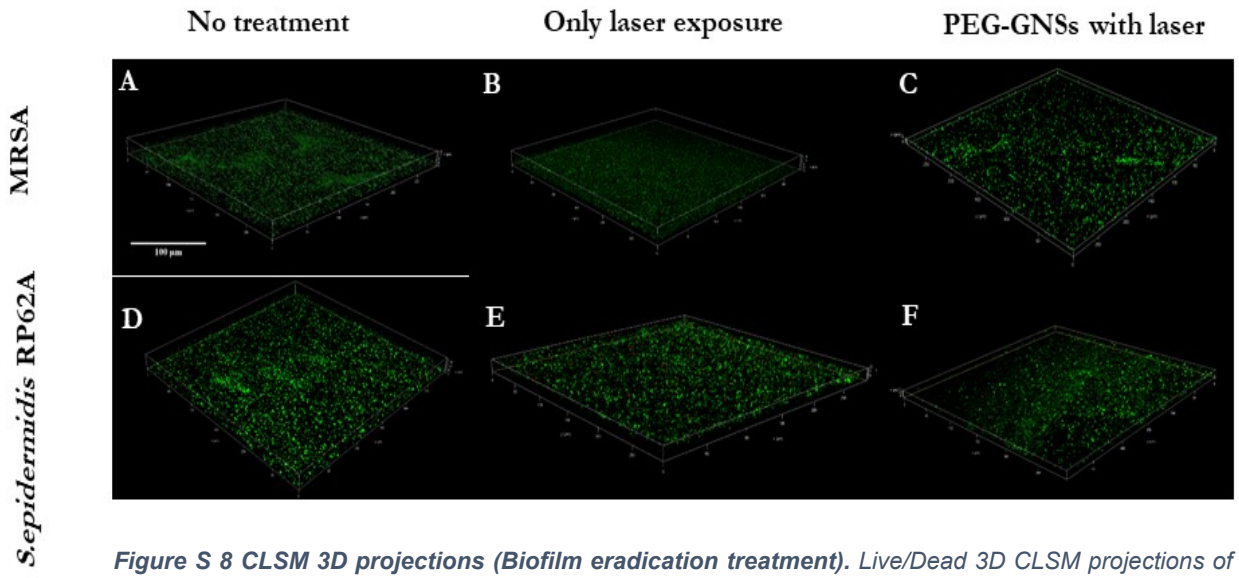

Figure S 8 CLSM 3D projections (Biofilm eradication treatment). *Live/Dead 3D CLSM projections of MRSA [**A, B, C**] and S. epidermidis RP62A [**D, E, F**] preformed biofilms upon exposure to different treatments as indicated. Live cells were stained in green by SYTO 9 and dead cells stained in red by Propidium iodide PI. Scale bar: 100 µm.*

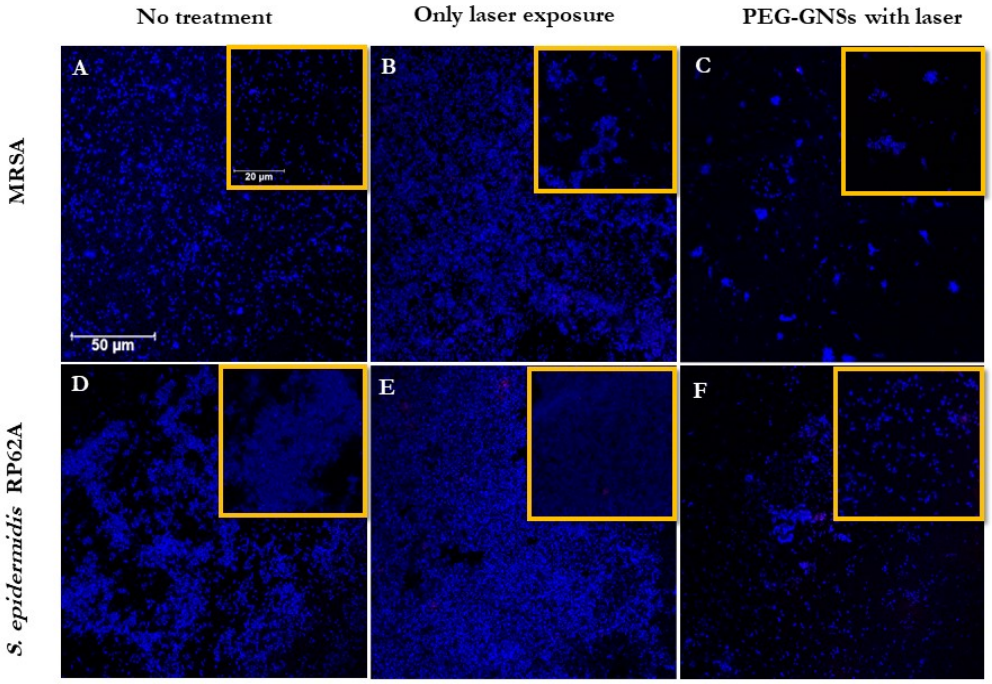

Figure S 9 CLSM images for ROS detection. *CellROX® Deep Red/Hoechst 33342 stained CLSM images for the ROS detection on MRSA [**A, B, C**] and S. epidermidis RP62A [**D, E, F**] preformed biofilms upon exposure to different treatments as indicated. Scale bar: 50 µm. CLSM images [within yellow frames] represent a selected section from the same corresponding images at a larger magnification.*

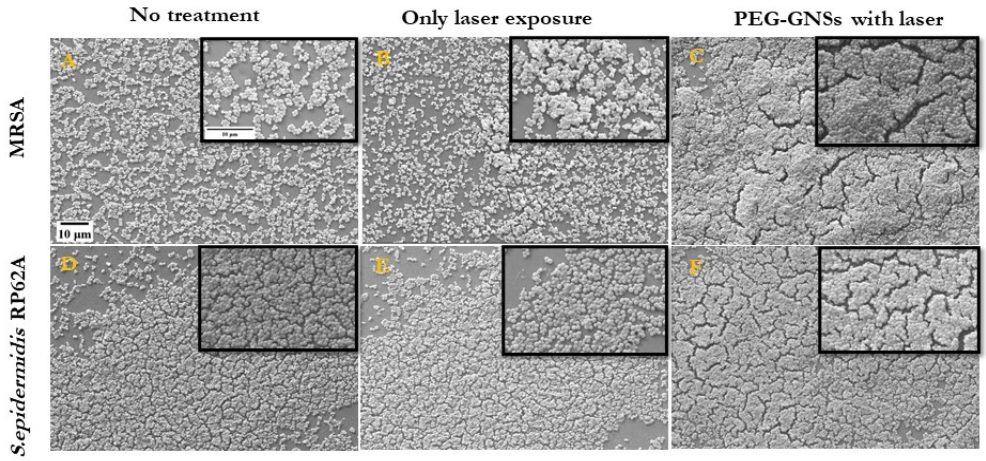

Figure S 10 SEM images (Biofilm eradication treatment). *SEM images of MRSA [**A, B, C**] and S. epidermidis RP62A [**D, E, F**] preformed biofilms upon exposure to different treatments as indicated. SEM images [within black frames] represent specific sections from same corresponding images at a larger magnification.*