Monolayers of gold nanostars (GNS) are grafted on mercaptopropyltrimethoxysilane-coated glass slides. In the formed monolayers the local surface plasmon resonance of GNS can be tuned in the 700–1100 nm range. Upon laser excitation of the nearIR LSPR an efficient photothermal response is observed, inducing local hyperthermia and efficient killing of *Staphylococcus aureus* biofilms.

Biofilms are sessile microbial communities, usually constituted by the Gram+ *Staphylococcus aureus* and *Staphylococcus epidermidis*, embedded in a self-produced extracellular polymer matrix. They may develop on the surface of prostheses, catheters and implants, leading to severe infections in an impressive number of hospitalized patients. Conventional antibiotics do not eradicate biofilms from the surfaces where they have formed and often surgical removal of the implant is the only affordable solution. It has been proposed that coating medical devices with Ag nanoparticles prevents biofilm formation but with controversial results, as Ag nanoparticles are effective towards Gram+ bacterial strains but much less towards Gram-. We propose here a new approach based on gold nanostars (GNS). Gold is not intrinsically antibacterial but GNS possess two or more localized surface plasmon resonances (LSPR) that undergo thermal relaxation upon irradiation. Moreover, at least one LSPR falls in the near-IR range (NIR, 750–1100 nm) where tissues and blood are semi-transparent. Following some examples of glass surfaces coated with gold nanoparticle monolayers, we have now prepared bulk glass materials coated with a GNS monolayer, that proved to be very efficient in photothermal biofilm laser-treatment against *S. aureus* biofilms, suggesting the possibility of fabricating medical devices with the same coating: once internalized, they would not need to be removed if a biofilm is formed on their surface but may be treated in situ, i.e. through tissues.

GNS are prepared in water according to a seed-growth method developed by us using laurylsulfobetaine (LSB) as the directing and coating agent. The obtained colloidal solutions are mixtures containing mainly asymmetric branched gold nanoparticles (70–80%) and a lower percentage of symmetric 6-branched nanostars, see Fig. 1B for a

![Fig. 1](image-url)
representative TEM image. Their absorption spectrum in solution is dominated by the LSPR of the main component, whose maximum can be positioned in the 750–1150 nm range as a function of the reaction conditions. Adhesion of a monolayer of GNS on glass is obtained by formation of first a monolayer of MPTS on the surface, according to an established procedure. The obtained glass|MPTS surfaces are then dipped into a colloidal solution of GNS (Au = 0.06–0.07 g L$^{-1}$), typically for 18 hours, yielding glass|MPTS|GNS surfaces, with the formation of thiolate–gold bonds (all experimental details in ESI† Sections S1 and S2).

The weak nature of the LSB–Au interaction favours the facile LSB surface displacement by the MPTS thiol groups and efficient GNS adhesion. The slides are sonicated in water 3 times for 3 min before use and dried with a N$_2$ flux. They can be kept in air at room temperature, under light exposure, with no degradation after 3 months (as checked with UV-Vis spectra). Control experiments on plain glass gave no coating. GNS-bearing slides are intensely colored but transparent [photographs in ESI† Fig. S3A–E] and absorption spectra are directly recorded on dry slides using a common UV-Vis spectrophotometer. The position of the LSPR maximum can be placed at the desired wavelength with a large interval (550–1100 nm), see e.g. Fig. 1C, depending on the LSPR of the chosen coating solution. The surface is densely populated by GNS, as shown by Scanning Electron Microscope (SEM) images, Fig. 1A (ESI† Fig. S4A and B for larger images). The grafted GNS maintain the shape they have in the coating colloidal solution. A LSPR blue shift of 52 nm (average on 70 preparations, $\sigma = 10$ nm) is observed on passing from the colloidal solution in water to the monolayer on glass in air (ESI† Fig. S3F), due to change in the local refractive index ($n$). Glass|MPTS|GNS surfaces prepared on the internal wall of an optical glass cuvette, then filled with different solvents (ESI† Fig. S5), show a linear trend for $\lambda_{\text{max}}$ vs. $n$ with a 300 nm/n unit variation, and when water is the solvent $\lambda_{\text{max}}$ reaches a similar value as in colloidal solution. Glass|MPTS|GNS slides prepared with dipping times shorter than 18 hours show a less dense coating than seen in Fig. 1A, which corresponds to a less intense UV-Vis-NIR absorption (ESI† Fig. S4C for SEM images). This prompted us to study the kinetics of coating. Using colloidal solutions with $\lambda_{\text{max}} = 850$ nm ($\lambda_{\text{max}}$ on glass = 808 nm), glass|MPTS slides are taken off at different times along a period of 140 hours. Absorption spectra are recorded on each slide and the total Au content determined by fully oxidizing the GNS monolayer in a small measured volume of aqua regia (3.0 mL). This is finally analysed using standard techniques for diluted cations in solution (ICP-OES, inductively coupled plasma optical emission spectroscopy). Spectra are normalized by setting the absorbance to zero at 480 nm (Fig. 1D) and a sharp ascending-plateau trend is obtained by plotting Abs at $\lambda_{\text{max}}$ vs. time (Fig. 1E, coloured circles). The trend parallels the quantity of Au per cm$^2$ found by oxidation/ICP-OES, Fig. 1E (half-filled squares). Full coating is reached in <18 hours, confirming that the routine time for preparation is suitable. Many slides were prepared with 18 hours coating time in GNS solutions from different syntheses, and the total Au per cm$^2$ calculated, with variations from preparation to preparation, albeit in a limited range (1.5–3.5 $\mu$g cm$^{-2}$). By normalizing the absorption spectra at 480 nm and determination of the total gold quantity on the slide by ICP-OES, a linear plot for Abs at LSPR maximum vs. Au per cm$^2$ is obtained (ESI† Fig. S6A).

We used this as a calibration plot in all further preparations, in order to quickly calculate the Au per cm$^2$ surface concentration on each freshly prepared slide. For the sake of uniformity, in all the following experiments slides were used with Au content only in the 2.0–3.0 $\mu$g cm$^{-2}$ range. The efficient photothermophysical properties of the GNS used in this work have been already demonstrated in their colloidal solutions.† Now we use dry glass|MPTS|GNS slides with LSPR maximum at ~800 nm (Fig. 2A). The slides were irradiated with continuous laser sources at 750, 800, 850, 900 and 950 nm, with irradiance of 0.8 W cm$^{-2}$ (ESI† Fig. 2A). The trend smoothly follows the LSPR band profile (red triangles, Fig. 2A). When using the 800 nm source, the power was also varied, showing that $\Delta T$ linearly increases from 2 to 20 °C when irradiance increases from 0.08 to 0.8 W cm$^{-2}$ (Fig. 2B, inset). Fig. 2B shows the $\Delta T$ vs. time trends at each irradiance (laser at 800 nm), showing steep ascending-plateau profiles with a rapidly reached maximum equilibrium temperature (<20 s). It is worth mentioning that a significant increase in T is observed using a laser beam intensity (0.08 W cm$^{-2}$) four times lower than the maximum permitted exposure for skin, as established by the American National Standards Institute (ANSI) Laser Safety Standards.3a,b

For the antibiofilm studies a series of 32 round glass slides (diameter 1.0 cm) were prepared, with LSPR centred at 802 $\sigma = 12$ nm (ESI† Fig. S7) and a gold surface concentration of 3.0 ($\sigma = 0.4$) $\mu$g cm$^{-2}$. The microorganism used is a methicillin-resistant S. aureus LP strain, which was shown to be an efficient biofilm producer.1c For biofilm growth an overnight culture of S. aureus LP was diluted at 1:50 in tryptone soy broth (TSB) containing 0.25% glucose, and aliquots (0.500 mL) of the diluted bacterial suspension were inoculated into 24-well microplates containing either plain glass slides or glass|MPTS|GNS slides, and incubated for 16 hours at 37 °C. Some of the wells were laser-treated with a AlGaAs laser diode with emitting light at 808 nm, irradiance of 0.090 W cm$^{-2}$ (also taking into account the $\approx 24$% loss introduced by the plate bottom), and a waist with a diameter matching that of the glass slides (ESI† details and experimental set up). The exposure time to laser was 5, 10 and 30 minutes.
Photothermal therapy is a potentially powerful tool against biofilms.\textsuperscript{10} In this communication we demonstrate that bulk glass surfaces bearing controlled microgram quantities of gold (up to 3.0 µg cm\textsuperscript{-2}) as firmly grafted, stable monolayers of nanostars can be prepared. Laser irradiation of the GNS monolayer at their NIR LSPR results in efficient photothermal conversion. Using irradiance values significantly lower than the maximum permissible exposure of skin, with a laser source at 808 nm we observed that the photothermal action of the GNS monolayer efficiently induces cell death in \textit{S. aureus} biofilms by hyperthermia. This coating approach may be in principle also applied on steel, ITO, silica, \textit{i.e.} any surface that is prone to MPTS functionalization. Using a laser source inside the biological “transparent” NIR window, our findings could lead to useful coatings for internalized medical devices and implants: biofilms that eventually form on their surface may be laser-treated \textit{in situ}, avoiding surgical removal.

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Notes and references


