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ARTICLE TYPE

Light-responsive nanocomposite sponges for on demand chemical release with high spatial and dosage control

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We present a biocompatible device for on demand chemical release in the form of a light-activatable sponge-like nanocomposite scaffold, which assures an excellent control over the principal parameters of the chemical release and dosage in order to sustain effective therapeutic action. The sponge consists of a porous biopolymer scaffold containing a dispersion of gold nanorods, which acts as an absorber of the incoming laser light, and of thermosensitive micelles, which serve as a reservoir for the drug molecules to be released. The photothermal response of the nanoparticles contained inside the sponge triggers a contraction in proximal micelles, thus promoting the expulsion of the drug that in turn is released from the sponge to the external environment. The peculiar physiochemical and structural properties of the nanocomposite sponges impart a number of interesting features to the proposed drug release system, including the possibility of spatially-confining the therapeutic treatment as well as of precisely controlling the amount of released drug as a function of duration and power of the excitation light.

1. Introduction

Stimuli-responsive or “smart” materials capable of undergoing conformational and chemical changes upon receiving external signals are attracting great attention, thanks to their potential applications in different key areas such as biotechnology, pharmacology and material science.¹ The release of therapeutic agents from implantable devices and medical dressings can obtain important advantages from this emerging technology. Early activation systems for drug release include direct heating of the implant while in recent examples an electromagnetic stimulus, such as a light beam or an oscillating magnetic field, has been proposed to control the heating process.^{2,3} However, these systems still suffer from some limitations, including large-scale changes in shape and size or a complete melting necessary for activating the release mechanism, which can ultimately reduce the spatial control over the therapeutic response. Most importantly, a remotely-triggered or “active” implant for chemical release (in addition to a control over delivering in time and space) should also provide precise dosage of the amount of species released in order to sustain effective therapeutic actions. Lastly, a reliable setting of the temperature levels reached at the implant site is of particular relevance in the case of a thermal stimulus, in order to prevent damages to adjacent tissues. These aspects, which have been marginally faced so far, may jeopardise the therapeutic performances and ultimately prevent a successful translation of this technology to patients.

Here we introduce a thermosensitive implantable device in the form of a light-activatable sponge-like nanocomposite scaffold that has the potential of overtaking the limitations of previously-

reported strategies. The sponges proposed exploit the concurrence of two principal components: a) gold nanoparticles, which respond efficiently to an external light stimulus,⁴ thus producing a controlled temperature enhancement, and b) amphiphilic micellar drug-carriers, which undergo a modification of their chemo-structural properties as a result of a heating event,⁵ thus allowing the release of a drug as a function of main irradiation parameters.

2. Experimental

2.1 Preparation of nanocomposite sponges

Low-molecular chitosan (Sigma-Aldrich) was dissolved to a final 3.5% (w/v) in a 0.2 nM acidic dispersion of gold nanorods (NRs) ((40 ± 6) nm × (10 ± 2) nm), 3.9 ± 0.5 aspect ratio, prepared as previously described.⁶ Poly(ε-caprolactone)-b-poly(oxyethylene)-b-poly(ε-caprolactone) (PCL-PEO-PCL) copolymer was synthesised as described in Supplementary Information. Fifty microliters of an ethanol solution of the copolymer (30 wt%) were then rapidly injected into 150 μL of the NRs-chitosan composite and mixed until complete homogenization. For the release measurements, the PCL-PEO-PCL alcoholic solution also included 1 mg mL⁻¹ of either Rhodamine 6G (R6G), acridine orange (AO) or Doxorubicin (Dox) (Sigma Aldrich). To obtain the sponges, 70 μL of the solution were poured into 0.5 cm² polystyrene circular moulds. After 180 min of evaporation of the solvent under controlled conditions (25 °C, N₂ flux), insolubilization of the sponge was obtained by treatment with NaOH 1M followed by abundant rinsing with ultrapure water.

Samples were stored in ultrapure water at 4°C until further investigation.

2.2 Characterization of nanocomposite sponges

Optical properties were characterized by a Jasco V-560 spectrophotometer. The ultrastructural characteristics of freeze-dried samples were studied using a Fei Quanta 200 environmental SEM, operated at 25 kV under standard high-vacuum conditions. A backscattered electrons detector was used to detect the NRs distribution. For the TEM analysis, fixed wet samples were infiltrated in epoxy resin. Contrasted sections (80 nm) were inspected under a Philips CM-12 microscope running at 80 kV. Dynamic Light Scattering (DLS) and Z-potential measurements were performed on a ZetaPlus BIC90 particle size analyser (Brookhaven Instruments Corporation); micelles (1 mg mL⁻¹), obtained by injecting a stock alcoholic solution of copolymers into ultrapure water under stirring, were filtered (1.30 µm) and analysed at fixed temperatures. DSC measurements were performed using a Mettler Toledo DSC1 apparatus with hermetically-sealed aluminium pans at 5 °C min⁻¹ rate and under a nitrogen flow. Fluorescence measurements were performed using a LS50B spectrofluorimeter (Perkin Elmer) equipped with a Haacke thermostat. DPD simulations were carried out under Materials Studio (v5.0) package according to a previous report⁷ and Supplementary Information. The water structuring within micelles (20 wt%) was studied by deconvolution of the OH stretching band of FTIR spectra (Shimadzu 8300 spectrometer) acquired at different temperatures, according with Ref. 8.

2.3 Drug release experiments

The in-solution release was evaluated in PBS (800 µL, pH 7.4) at different temperature values (produced by a Thermo Blok 780, Asal). The buffer solution surrounding the sample was collected and replaced with fresh buffer after each period of treatment. Similar experiments were conducted on samples exposed to varying irradiation times and laser intensities. Human HeLa cells were cultured in phenol-red-free DMEM supplemented with 10% fetal bovine serum (FBS), 1 mM glutamine, 100 U mL⁻¹ penicillin and 100 µg mL⁻¹ streptomycin in 5% CO₂ at 37°C. Laser experiments were performed using a bench setup consisting of an AlGaAs diode laser peaked at 810 nm (Mod. WELD 800, El.En S.p.A.) coupled with a 600-µm-core optical fiber and a precision stage showing 6-mm or 2-mm size holes (Fig. S11). HeLa cell viability after 4 h from laser treatment was evaluated by Cellstain double staining kit (Sigma Aldrich, Fig. S9). Fluorescence images were acquired using a Leica DMI3000B microscope. Dox intracellular fluorescence was measured in similar samples by averaging the maximum peak intensity of 50 cells per sample.

3. Results and Discussion

Nanocomposite sponges were fabricated by including gold nanoparticles and amphiphilic micelles within a porous chitosan-based matrix,⁹ with the latter having the main role of providing a bioaffine cross-brace structure for the above-mentioned particulate. We engineered these components in order to obtain final 0.8-cm-diameter × 300-µm-thickness scaffolds that feature

easy-handling and flexibility characteristics, biocompatibility, and stability in aqueous environment (Figs. 1, S1,S2).

Here we have focused on NRs as convenient light-transducers, thanks to their unique absorbance properties within the so-called therapeutic window of the visible spectrum (i.e. the 700 ÷ 1200 nm range), where light has its maximum penetration into tissues, which is highly preferable with a view to biomedical applications inside the body.^{10,11} A dispersion of NRs corresponding to a final 0.2 nM concentration was obtained inside a 3.5% w/v chitosan hydrogel (Figs. 1a,b), which conferred a strong ~810 nm optical absorption band (Fig. S2). The hydrogel bulk displayed a porous interconnected network with a ~300 nm pore size (Fig. S3), which accounted for a good balance between easy crossing of drug molecules and acceptable resistance against mechanical stress on the scale of foremost biological tissues (Fig. S4).¹² The porosity of the bulk translated into a ~24 nm surfaces roughness (Figs. S3c), which appeared suitable for cell attachment and growing (Fig. S5). The sponges were further engineered to contain a dispersion of self-assembled micelles obtained from triblock copolymers of PCL-PEO-PCL with the twofold role of efficient storage of drug molecules and of sustaining thermodependent processes.¹³ Typical micellar sizes of ~500 nm were observed (Figs. 1b,S6) along with a tendency to form aggregates at either high concentrations or after introduction into the constrained porous network of the sponges (Fig. 1a), which is mainly ascribed to moderate Z-potential values (-21 ± 1 mV at 25 °C). These micelles show a phase transition centred around 40 °C, which is preserved once they are introduced into the porous matrix (Fig. 1c). This transition correlates well with the observation of an enhanced chemical release from the sponges when these are subjected to a temperature increase above the physiological value (Fig. 1d). Typical fluorescence spectra against temperature of the sponges loaded with AO (used as probe of the local micellar arrangement¹⁴) revealed characteristic discontinuities in intensity and position of the maximum emission peak corresponding to 40 °C (Fig. 1e), in accordance with DSC results. Here the fluorescence emission signal showed a profile reversing the expected trend, which is a progressive decrease due to the increased probability of nonradiative decay of the excited state of the probe while raising the temperature. The rapid enhancement of emission intensity is interpreted as a fast decrease in the AO self-quenching characterizing the transfer of the probe from a packed state of the micellar interior to a free state of the external aqueous environment. A contemporary shift in the emission maximum can be ascribed to a change in polarity and/or in the ionization state of the probe molecules, which experience an expulsion from the hydrophobic micellar interior to the polar surrounding solution.¹⁵ Further insights into the release mechanism were provided by the observation of an abrupt and reversible volume contraction of the micelles in response to an alternate switching between 25 °C and 50 °C (Fig. 1f). The contraction was accompanied by an increase in the Z-potential values, which suggests a reorganization of both the charge distribution and the water molecules on the micellar surface. To investigate the molecular basis of this contraction we carried out a Dissipative Particle Dynamics (DPD) simulation. The results evidenced a modulation in the interaction parameters calculated on single PCL and PEO blocks as a function of the temperature

(Table S1). In particular, a concurrence of events including the opposite solubility trends of PEO and PCL blocks in water, together with the improved PEO affinity for PCL at higher temperatures, should induce a contraction of the PEO shell toward the PCL core (Figs. 1g and S7)¹⁶ and, in turn, a reduction in the micellar size. This deformation can trigger a water flow crossing at the micellar border, which is expected to enhance the release of drug molecules as previously discussed.³ An interdigitation of the PEO shell into the PCL core was further supported by FTIR analysis (Fig. 1h). This showed a sharp decrease in the larger water networks contained inside the micellar bulk at the expense of smaller water connections as the temperature increased above 40 °C, which is in line with a reduction in the intramolecular spaces (Fig. S8).⁸ These results describe a release mechanism initiated by the photothermal response of NRs contained inside the sponges, which triggers a contraction in proximal micelles, thus promoting the expulsion of drug molecules that in turn are released from the sponge to the external environment (Fig. 1i).

We proved the possibility of regulating the temperature generated inside the sponge upon laser illumination (continuous wave emission from a diode laser) through a variation in the laser intensity and with a linear relationship within the temperature range of interest for the activation of the release mechanism (i.e. ~ 40–50 °C) (Figs. 2a,b). This possibility is a consequence of the reliable photoresponse of gold nanoparticles, which keep optical properties unaltered even after repeated irradiation cycles.¹⁰ Immediate outcomes include the prevention of superheating effects that may induce irreversible thermal damages to health tissue. In addition, a reliable control over the temperature generated by an external light stimulus is pivotal in performing a precise dosing of drug escaping from the sponge. We found that predetermined drug amounts can be released by directly acting on laser emission parameters, such as irradiation time and/or laser intensity, which can control the activation kinetics of the micelles contained inside the supporting chitosan scaffold (Figs. 2c,d). This inspires the concept of customized pharmacological treatments and fully exploits the potential of stimuli-responsive implants (Fig. 2e).

After a preliminary parameterisation, we demonstrated the feasibility of nanocomposite sponges to carry out a controlled chemotreatment of cancer cells. Sponges loaded with doxorubicin (Dox) were placed in contact with a HeLa cells monolayer. By varying the irradiation conditions, we succeeded in inducing different yields of cell death, as was revealed by the calcein (CA)/propidium iodide (PI) assay (Figs. 3a–d). As expected, larger activation times and laser intensities were shown to provide more effective chemotherapy, in accordance with the copious PI staining (red) at the expense of CA-positive viable cells (green). These results are plotted in Fig. 3e, which shows the relationship between cell viability and internalized Dox, expressed as intracellular Dox fluorescence of cell samples treated under different laser conditions. We note that the low supraphysiological temperatures (40–45 °C) reached upon laser-activation of nanocomposite sponges are considered ineffective in inducing any irreversible thermal damages to cells¹⁷ (Fig. S9). This circumscribes the cytotoxic effects we observed in the antitumor Dox activity. However, the latter is known to be

enhanced by the synergic assistance of a hyperthermic effect, which stimulates drug uptake by temporarily increasing the plasmatic membrane fluidity.¹⁸ This insight was confirmed by the prevalence of PI-stained cells adjacent to the irradiated portion of sponge, as can be observed at lower microscope magnification for the optimal conditions 0.46 W cm⁻², t_{irr} = 5 min (Fig. 3f). Therefore, a spatial control over drug release has been proven to occur, which we ascribe to a thermally-induced hyperpermeability effect experienced by cells that lie in intimate contact with the irradiated sponge.

Conclusions

In conclusion we have introduced a novel hybrid nanocomposite material, which can be employed as an effective and reliable laser-activatable tool for drug release. Nanocomposite sponges demonstrated the possibility of a control over the time of delivery and the amount of released drug. In addition, the presence of separate thermosensitive microphases (micelles) inside a thermally-inert chitosan scaffold (Fig. 1c) makes it possible to keep the overall structure of the implant unaltered during irradiation (Fig. S10). When coupled with a focused laser beam, this feature may ultimately assist in performing a spatially-confined release. Thanks to previously-demonstrated properties of laser-activated NRs-doped chitosan films, including their adhesion to biological tissues,⁹ the proposed material may become a multifunctional platform for minimally invasive therapies based on laser application.

Acknowledgements

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Notes and references

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- [†] Electronic Supplementary Information (ESI) available: synthesis of PCL-PEO-PCL copolymer; MTT assay, absorption profiles, SEM and AFM analyses, stress-strain curves, cell adhesion experiments for the sponges; DPD simulations, AFM and FTIR analyses of the PCL-PEO-PCL micelles; laser experimental setup. See DOI: 10.1039/b000000x/
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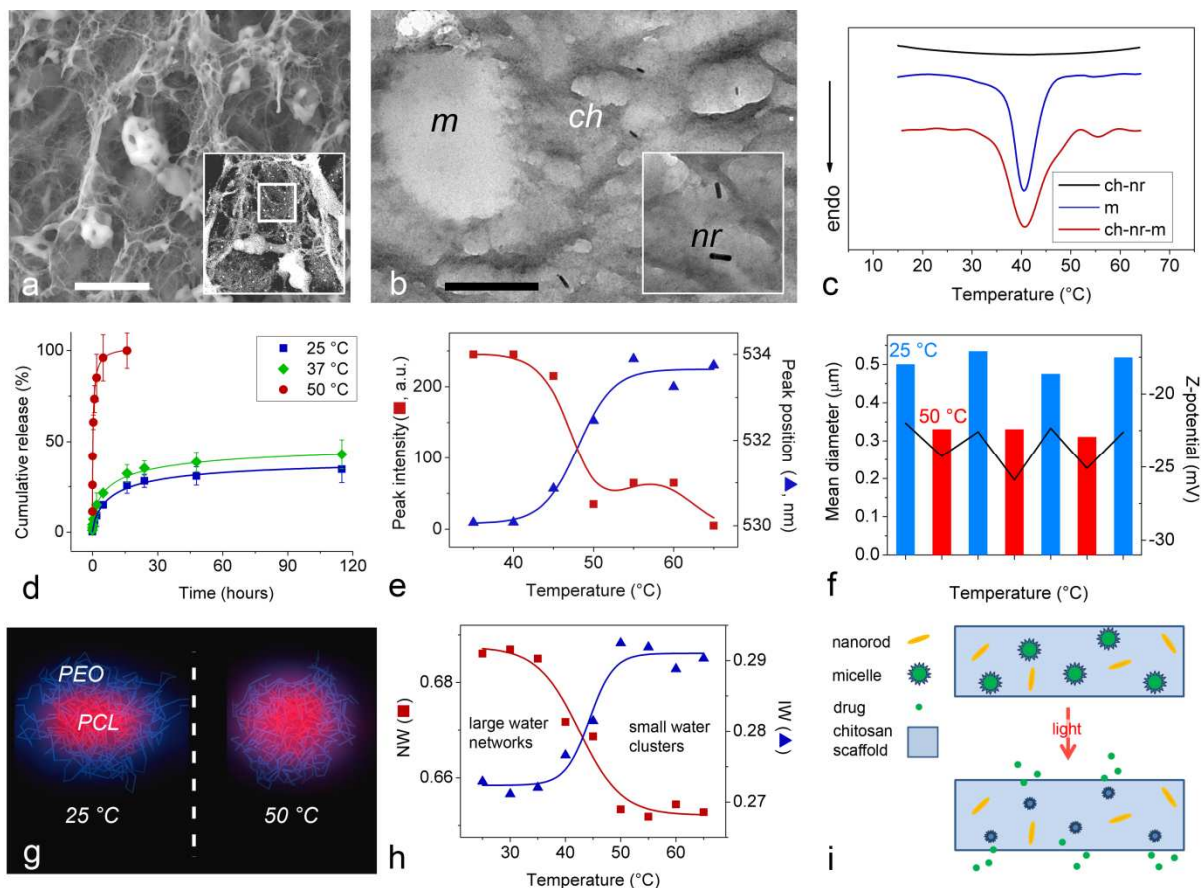


Fig. 1. (a) SEM image of a sponge showing its internal porous architecture containing isolated or clustered PCL-PEO-PCL micelles (bar = 10 μm). Inset: selected area with magnified NRs intercalated within the chitosan backbone (square). (b) TEM micrograph of a cross-sectioned sample, further magnified in the inset. (ch = chitosan matrix, m = micelle, nr = nanorod; bar: 500 nm). (c) DSC curve (2nd run) of: chitosan-NRs scaffolds (black), a 20 wt% micelle solution (blue) and a nanocomposite sponge (red). (d) In vitro cumulative release of R6G from nanocomposite sponges at different temperatures; error bars were based on the SD of triplicate samples. (e) Variation with temperature of fluorescence peak intensity and position of AO contained inside the sponges. (f) Micellar size contraction (blue and red) and Z-potential variation (black) by switching temperature between 25 $^{\circ}\text{C}$ and 50 $^{\circ}\text{C}$. (g) DPD simulation of the PCL (red) and PEO (blue) blocks densities at 25 $^{\circ}\text{C}$ and 50 $^{\circ}\text{C}$. (h) Thermal behaviour of large (NW) and intermediate (IW) water connections contained inside PCL-PEO-PCL micelles as measured by FTIR analysis. (i) Cartoon of the release mechanism of nanocomposite sponges.

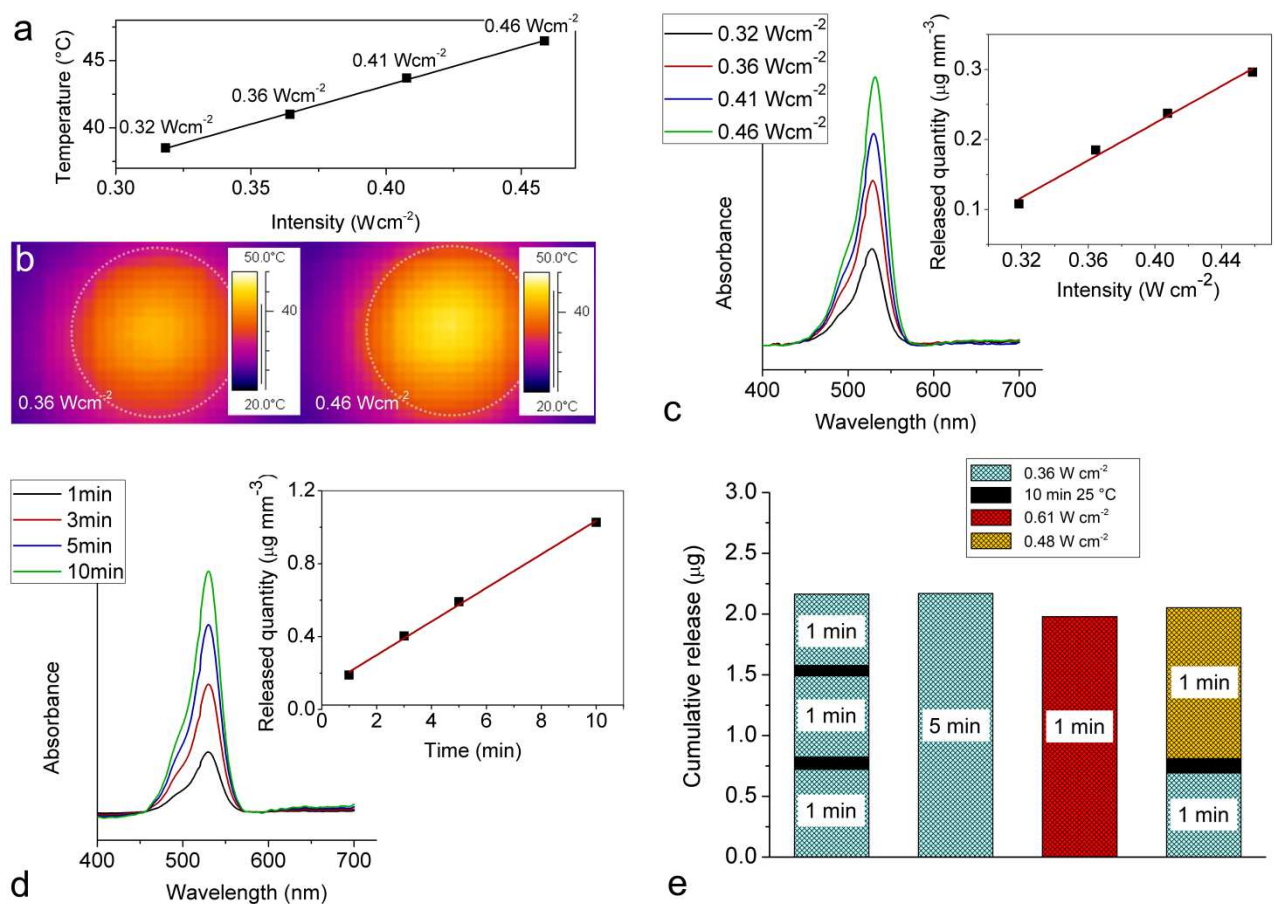


Fig. 2. (a) Linear trend between the intensity of the incident laser beam and the peak temperature reached during 1 min of laser treatment on nanocomposite sponges. (b) Exemplary maps of temperatures registered with a thermocamera and reached after 1 min of irradiation at 0.36 W cm^{-2} (left) and 0.46 W cm^{-2} (right). Absorbance profiles of R6G released from the sponges as a function of (c) laser intensity (at fixed irradiation time = 1 min) and (d) of time (at a fixed laser intensity = 0.36 W cm^{-2}). Insets: linear increase in the released quantity of R6G as a function of intensity (c) and time (d). (e) Similar released amounts of R6G obtained by irradiating the sponges with different combinations of irradiation time and intensity. Repeated treatments on the same sponge are plotted in the same rectangle separated by 10 min equilibrium intervals at 25°C .

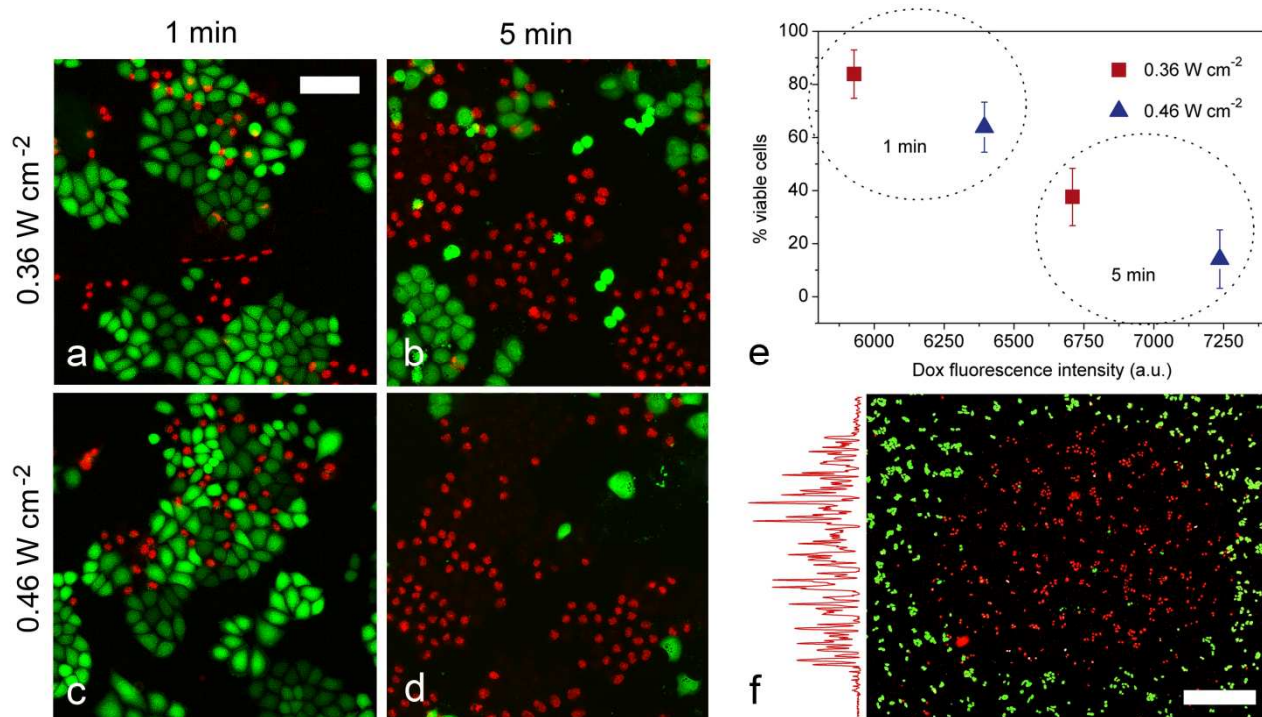
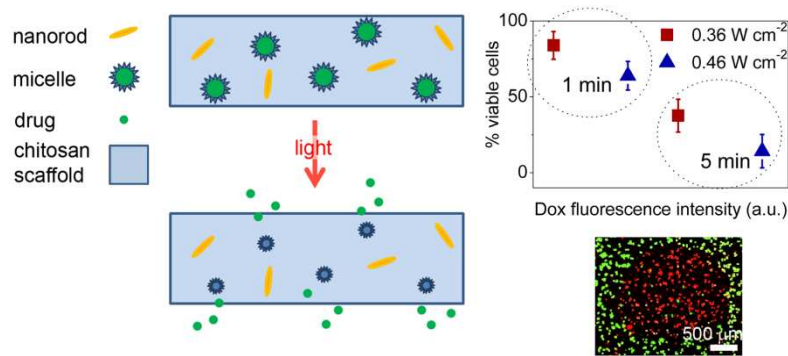


Fig. 3. Fluorescence images showing viable (in green, CA stain) and dead (in red, PI stain) HeLa cells treated with Dox released from overlying sponges that are subjected to different irradiation conditions (a,b = 0.36 W cm^{-2} ; c,d = 0.46 W cm^{-2} ; a,c = 1 min; b,d = 5 min; bar = $100 \mu\text{m}$). (e) Decrease in cell viability caused by Dox treatment at different laser intensities and irradiation times (expressed as % calcein-positive cells averaged over 4 samples $\pm$ SD) as a function of internalized Dox (evaluated as intracellular Dox fluorescence, $n = 4$). (f) Spatial control over Dox release by irradiating a 2-mm area of a sponge (0.46 W cm^{-2} , 5 min) placed in contact with HeLa cells: Dox-induced cell death is confined to those cells in close proximity with the irradiated portion of the sponge (bar = $500 \mu\text{m}$) (the confinement of the PI stain is emphasized by the profile on the left).

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Laser activation of nanocomposite sponges promotes the
expulsion of the drug to the external environment, providing a
precise control over the amount of released drug and a spatial-
confinement of the therapeutic treatment.



[Supplementary Information]

Light-responsive nanocomposite sponges for on demand chemical release with high spatial and dosage control

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Synthesis of the PCL-PEO-PCL triblock copolymer. PCL-PEO-PCL copolymer was synthesized according to literature procedures with a few modifications [C.B. Liu, C.Y. Gong, M.J. Huang, J.W. Wang, Y.F. Pan, Y.D. Zhang, G.Z. Li, M.L. Gou, K. Wang, M.J. Tu, Y.Q. Wei, Z.Y. Qian *J Biomed Mater Res B Appl Biomater* 84 165 (2008)]. A two-necked round-bottom flask was charged, under nitrogen atmosphere, with 15 g (131 mmol) of ϵ -CL, 15 g of PEG 2000 and 0.15 g (0.37 mmol) of $\text{Sn}(\text{Oct})_2$. The resulting mixture was heated under stirring at 130 °C for 6 h and subsequently degassed under vacuum for 30 min before cooling to room temperature. The resulting semisolid material was dissolved in the minimum amount possible of dichloromethane and then precipitated with an excess of cold petroleum ether. The solid sample was finally filtered, washed on a Buchner funnel and dried under vacuum at room temperature for 24 h. The copolymer was re-dissolved in EtOH and dialyzed at room temperature using a dialysis membrane (cut-off 3000 Da) for two days. This dialyzed solution was evaporated under vacuum to afford a translucent off-white solid material. FT-IR (KBr): 3428 (-OH st.), 1720 (C=O st.), 1241 (C-O-C st.) cm^{-1} . $^1\text{H-NMR}$ (400 MHz, CDCl_3 , 293 K): δ = 1.36-1.42 (m), 1.62-1.67 (m), 2.30 (t), 3.64 (br s), 3.69 (t), 4.06 (t), 4.22 (m). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3 , 293 K): OH-C⁽¹⁾H₂-(C⁽²⁾H₂)₂-C⁽³⁾H₂-C⁽⁴⁾H₂-C⁽⁵⁾O-[OC⁽⁶⁾H₂-C⁽⁷⁾H₂-C⁽⁸⁾H₂-C⁽³⁾H₂-C⁽⁴⁾H₂-C⁽⁵⁾O-]_xOC⁽⁹⁾H₂C⁽¹⁰⁾H₂-[OC⁽¹¹⁾H₂C⁽¹¹⁾H₂]_y: δ 24.5 (C; 3), 25.5 (C; 8), 28.3 (C; 7), 32.3 (C; 2), 34.1 (C; 4), 62.5 (C; 1), 63.4 (C; 9), 64.1 (C; 6), 69.1 (C; 10), 70.5 (C; 11), 173.5 (C; 5).

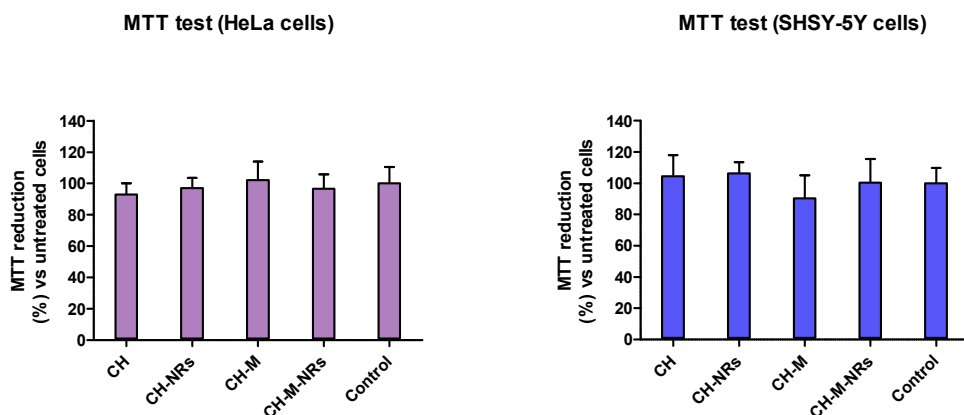


Fig. S1. MTT reduction inhibition assay [T. Mosmann *J Immunol Methods* 65 55 (1983)] for cell viability showed no cytotoxicity of blank nanocomposite sponges on human cervical cancer cells (HeLa) and on human neuroblastoma cells (SHSY-5Y). Chitosan (CH) scaffolds alone and containing gold nanorods (NRs), PCL-PEO-PCL micelles (M) or both were placed in the cell culture media at a corresponding concentration of 1 mg mL^{-1} and then added to the cells. Cell viability was evaluated after 24 hour application of the sponges on cells previously plated for 24 hours. Cell viability was expressed as % of MTT reduction in treated cells with respect to untreated cells (taken as 100%). The values shown are means $\pm$ SD of three independent experiments carried out in triplicate.

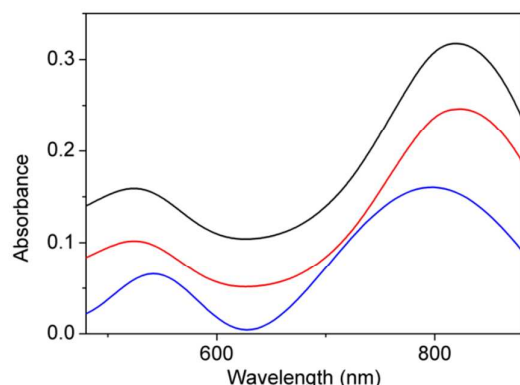


Fig. S2. Comparison between the absorption profiles of a nanocomposite sponge (red) containing a mixture of 3.5 % w/v chitosan and 0.2 nM NRs as described in the Experimental Section of the main text, and the initial colloidal NRs dispersion (black) prepared as described in previous papers [F. Ratto, P. Matteini, F. Rossi, R. Pini *J Nanopart Res* 12 2029 (2009); P. Matteini, F. Ratto, F. Rossi, S. Centi, L. Dei, R. Pini *Adv Mater* 22 4313 (2010)] and used for the preparation of the sponges used in the experiments. The sponges were stored in aqueous solution at 4°C where they retained unmodified optical characteristics for at least 30 days. The blue profile corresponds to an absorption spectrum of a sponge fabricated by using too large amounts of NRs (0.8 nM), showing a characteristic plasmon-coupling behaviour, in accordance with previous results [P. Matteini, F. Ratto, F. Rossi, S. Centi, L. Dei, R. Pini *Adv Mater* 22 4313 (2010)].

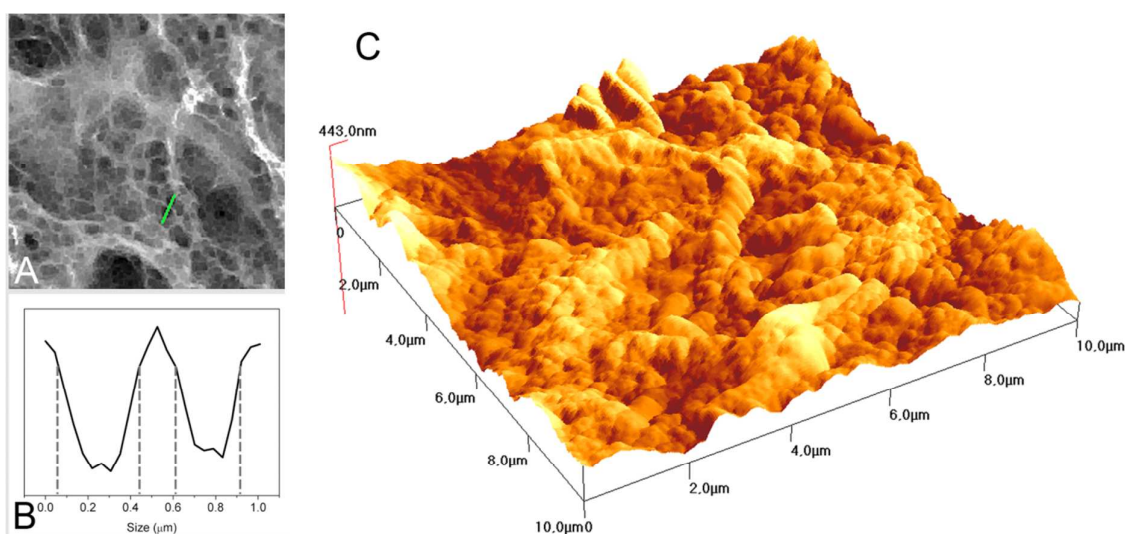


Fig. S3. (a) SEM micrograph ($7 \times 7 \mu\text{m}^2$) evidencing the porous architecture of a cross-sectioned sponge. The presence of a finely interconnected porous substructure confers both biomimetic properties [A. Di Martino, M. Sittinger, M.V. Risbud *Biomaterials* 26 2983 (2005)] and potential for granting a comfortable passage of the released species. (b) Example of a quantification of the porosity obtained under the ImageJ platform by considering the line profile along the green line displayed in (a). The analysis was conducted on 100 randomly distributed pores. Average pore size of ~ 300 nm were detected in accordance with TEM observations (Figure 1b). Images were acquired on a Fei Quanta 200 environmental SEM, operated at 25 kV under standard high-vacuum conditions. (c) 3D AFM topography of a sponge surface ($10 \times 10 \mu\text{m}^2$) obtained by operating in contact mode with a Quesant Q-Scope 250 instrument and silicon cantilevers (CSC17/Ti-Pt/15, Schafer) of tip radius ≤ 10 nm. Samples were freeze-dried before measurements in order to preserve their morphology. RMS roughness was calculated by WsXM v3.1 software (Nanotec electronica S.L.): the average RMS roughness value of 10 images ($10 \times 10 \mu\text{m}^2$) was reported in the main text.

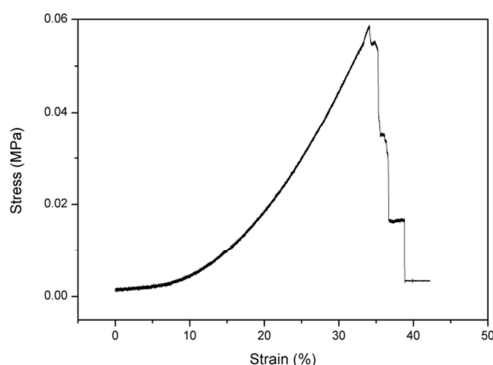


Fig. S4. Representative stress-strain curves of a nanocomposite sponge. Wet sponges were resized to $(6 \times 6) \text{ mm}^2$ samples and secured with tensile grips to a tensiometer (Asper s.r.l.). An increasing load was applied at an elongation rate of $10 \mu\text{m s}^{-1}$. Measurement were carried out in water bath at 25°C . Young's modulus $E = (\sigma \varepsilon^{-1})$, where σ is the stress and ε is the strain of deformation, was taken as a measurement of sample stiffness. Average E values of $0.15 \pm 0.02 \text{ MPa}$ were typically measured, which are comparable to those of native tissues [I. Levental, P.C. Georges, P.A. Janmey Soft Matter 3 299 (2007)] and can be considered appropriate for safe storage of drugs for long times inside the body without the risk of detrimental fractures.

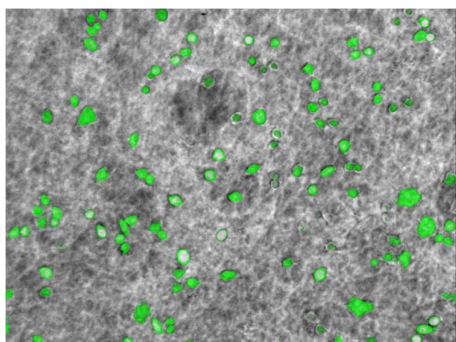


Fig. S5. HeLa cells adhesion to the sponge surface after 24 h from seeding. The green fluorescence produced by calcein stain indicates the presence of vital cells ($1.8 \times 1.3 \mu\text{m}^2$). This image is obtained by merging the green fluorescence channel with the bright field acquisition. Cells were cultured in accordance with the protocol reported in the Experimental Section for laser experiments but using petri dishes containing a sponge laid at the bottom.

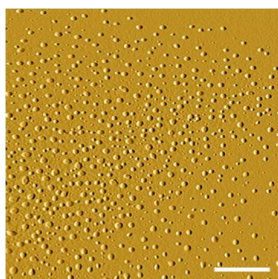


Fig. S6. AFM topography of micelles (we used a 1 mgmL^{-1} solution) deposited on a mica substrate carried out on a Quesant Q-Scope 250 instrument operated in tapping mode at room temperature (Bar = $2 \mu\text{m}$).

Table S1. Repulsion parameters a_{ij} of the PCL-PEO-PCL/water system calculated at 25°C and at 50°C . a_{ij} describe the repulsion interactions between i and j pairs or components of the system. For a

bead density of 3, as used in this work, the repulsion parameter between like particles was $a_{ii} = 25$ according to Groot and Madden [R.D. Groot, T.J. Madden, J. Chem. Phys. 108 8713 (1998)]. Flory-Huggins parameters used to obtain a_{ij} parameters were simulated using the Material Studio Blends module.

T=25°C	water	PEO	PCL
water	25.00		
PEO	25.90	25.00	
PCL	62.20	41.03	25.00
T=50°C	water	PEO	PCL
water	25.00		
PEO	27.99	25.00	
PCL	56.68	37.25	25.00

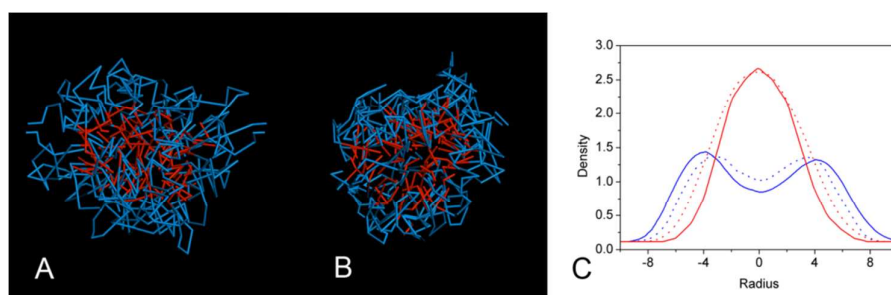


Fig. S7. Simulated cross-sections of the PCL-PEO-PCL micelles at 25 °C (a) and 50 °C (b) (the solvent was omitted for clarity; PCL block is red, PEO block is blue) by using the repulsion a_{ij} parameters reported in Table S1. (b) Simulated density profiles of PCL-PEO-PCL micelles at 25 °C (solid lines) and 50 °C (dashed lines). (The ABA system (PCL-PEO-PCL) was modelled as a 2+9+2 spring-bead chain. The solvent (water) was modelled as single DBD bead. Simulations were performed in a cubic cell of size $10 \times 10 \times 10 r_c^3$ with periodic boundary conditions and by running 100,000 DPD steps. For convenience the cut-off radius r_c , the particle mass m , and $k_B T$ were all taken as unity. Adjacent particles in the polymer chain interacted via a linear spring with a harmonic spring constant of 4.0. The volume fraction of the copolymer was set to 0.1. The repulsion parameters a_{ij} of PCL and PEO blocks as a function of temperature were calculated from the Flory-Huggins parameters χ_{ij} according to the expression: $a_{ij} = 25 + 3.50\chi_{ij}$). The repulsion between the PCL blocks and water at 25 °C decreases at 50 °C (Table S1), which can be ascribed to the breaking of the physical crosslinks connecting the PCL strands together [S.J. Bae, J.M. Suh, Y.S. Sohn, Y.H. Bae, S.W. Kim, B. Jeong *Macromolecules* 38 5260 (2005)] and to the consequent increased solvation of the PCL strands. Contemporary, the PEO shell shrinks as the temperature increases in accordance with the inverse solubility–temperature relationship previously reported [F.E. Bailey Jr., R.W. Callard *J. Appl. Polym. Sci.*, 1 56 (1959); and Y. Zhao, H.J. Liang, S.G. Wang and C. Wu *J. Phys. Chem B* 105 848 (2001)]. On the base of these observations and previous studies [J.W. Lee, F. Hua, D.S. Lee *J. Control. Release* 73 315 (2001)], at low temperatures (e.g. 25 °C) and water solution the PCL blocks form domains separated from PEO blocks due to hydrophobic interactions. As temperature rises (e.g. at 50 °C), PEO blocks become more hydrophobic and shrink due to dehydration. At the same time the hydrophobic interactions within the PCL blocks are loosened. This process is responsible for domain breaking and interdigitation between PCL and PEO blocks which at a larger scale is hypothesized to translate in a reduced micellar size as corroborated by the DLS measurements (Figure 1f).

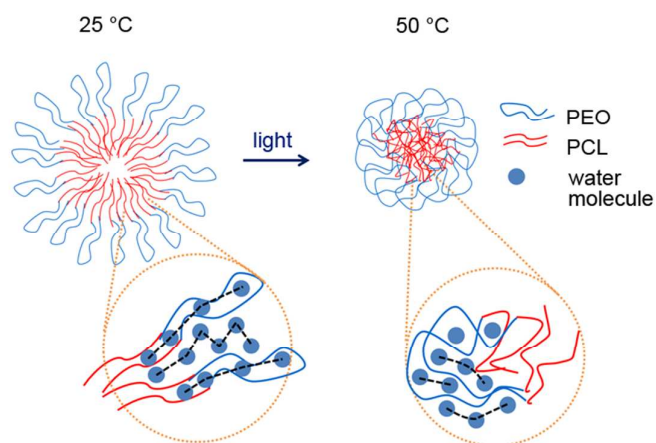


Fig. S8. Proposed scheme of water molecules arrangement inside a PCL-PEO-PCL micelle at 25 °C and 50 °C on the base of FTIR analysis (Figure 1h). Large water networks contained inside micelles at ambient temperature are replaced by short water connections as the micelle shrinks upon rising temperature (water connections are represented with a dashed line).

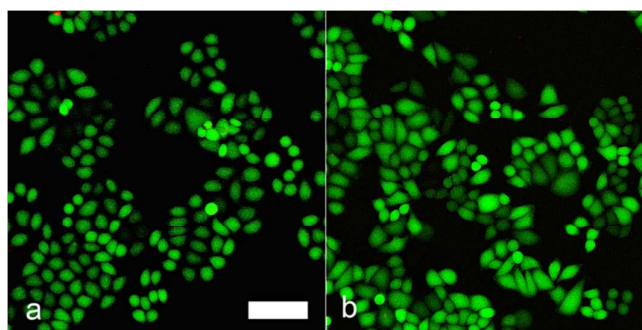


Fig. S9. Control experiments: blank (without Dox) sponges were placed in contact with HeLa cells monolayers and then subjected to different laser irradiation conditions in accordance with Figure 3 (a = 0.36 W cm⁻²; b = 0.46 W cm⁻²; t_{irr} = 5 min; bar = 100 μm). Viable cells are stained by calcein, showing green fluorescence; dead cells are red fluorescent due to propidium iodide stain. The percentage of viable cells in (a) and (b), as determined from the expression:

$$\% \text{ viable cells} = \frac{n^{\circ} \text{ viable cells}}{(n^{\circ} \text{ viable cells} + n^{\circ} \text{ dead cells})} \times 100,$$

is close to 100 %.

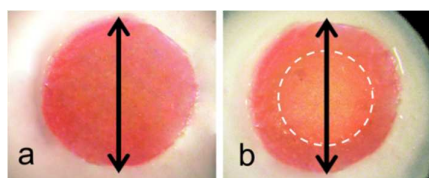


Fig. S10. A nanocomposite sponge loaded with Dox, before irradiation (a), and after 30 s from laser irradiation (0.46 W cm⁻², 5 min). The size, as indicated by arrows is not altered by the laser treatment (the irradiated portion of the sponge is indicated with a dashed circle).

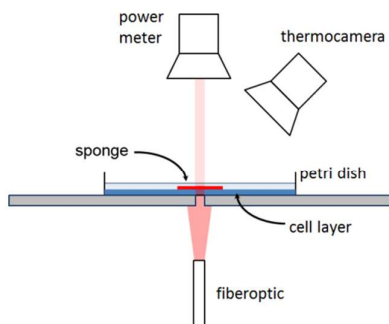


Fig. S11. Schematic overview of the experimental setup used in the irradiation experiments.

Laser experiments were performed with a bench setup consisting of an AlGaAs diode laser peaked at 810 nm (Mod. WELD 800, El.En S.p.A.) coupled with a 600- μm -core optical fiber and a precision stage showing 6-mm or 2-mm size central holes. In a preferred configuration, a sponge was laid in a 35 mm-size cultured petri dish, which was positioned over the centre of the stage. The laser treatment was provided by keeping the fiberoptic tip 10-20 mm below the stage in order to enhance the uniformity of the laser spot. The temperature was measured by an infrared thermocamera (Thermovision A20 FLIR Systems Inc.) and the transmitted laser power was monitored with a power meter (Ophir, Model PD2A). Maximum temperature values reached within the irradiated areas were collected during the laser treatment.