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[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 $Sn(Oct)_2$. 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}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). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$, 293 K; $OH\text{-}C^{(1)}H_2\text{-}(C^{(2)}H_2)_2\text{-}C^{(3)}H_2\text{-}C^{(4)}H_2\text{-}C^{(5)}O\text{-}[OC^{(6)}H_2\text{-}C^{(7)}H_2\text{-}C^{(8)}H_2\text{-}C^{(3)}H_2\text{-}C^{(4)}H_2\text{-}C^{(5)}O\text{-}]_xOC^{(9)}H_2C^{(10)}H_2\text{-}[OC^{(11)}H_2C^{(11)}H_2\text{-}]_y$): 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**).

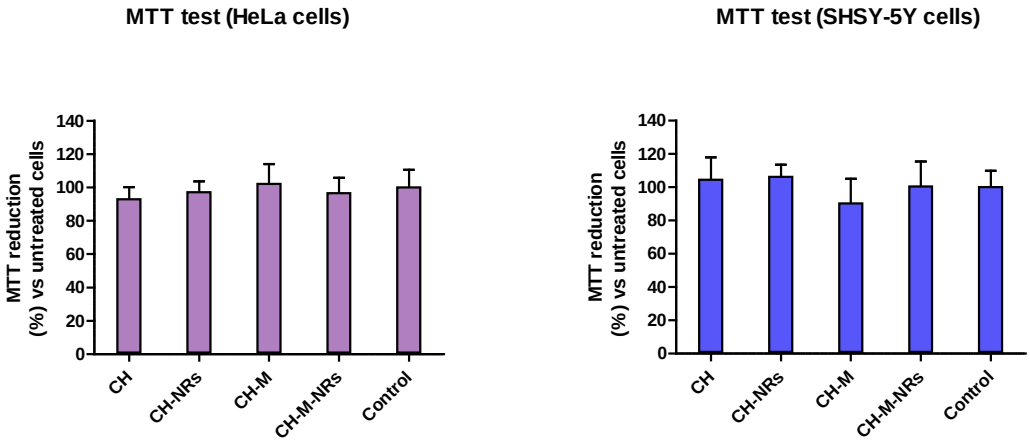

Fig. S1. MTT reduction inhibition assay [T. Mosmann J Immunol Methods 65 55 (1983)] for cell viability showed no cytotoxicity of blank nanocomposite sponges on human cervical cancer cells (HeLa) and on human neuroblastoma cells (SHSY-5Y). Chitosan (CH) scaffolds alone and containing gold nanorods (NRs), PCL-PEO-PCL micelles (M) or both were placed in the cell culture media at a corresponding concentration of 1 $mgmL^{-1}$ 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 ± SD of three independent experiments carried out in triplicate.

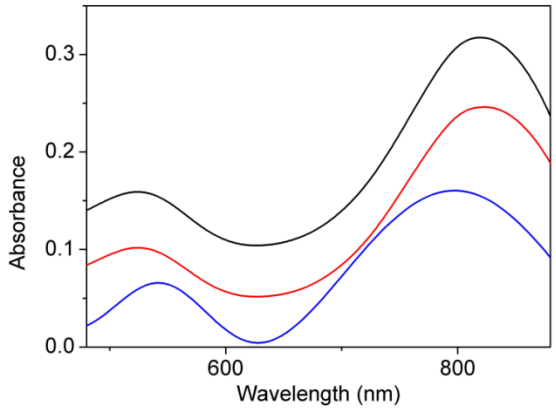

Fig. S2. Comparison between the absorption profiles of a nanocomposite sponge (red) containing a mixture of 3.5 % w/v chitosan and 0.2 nM NRs as described in the Experimental Section of the main text, and the initial colloidal NRs dispersion (black) prepared as described in previous papers [F. Ratto, 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)].

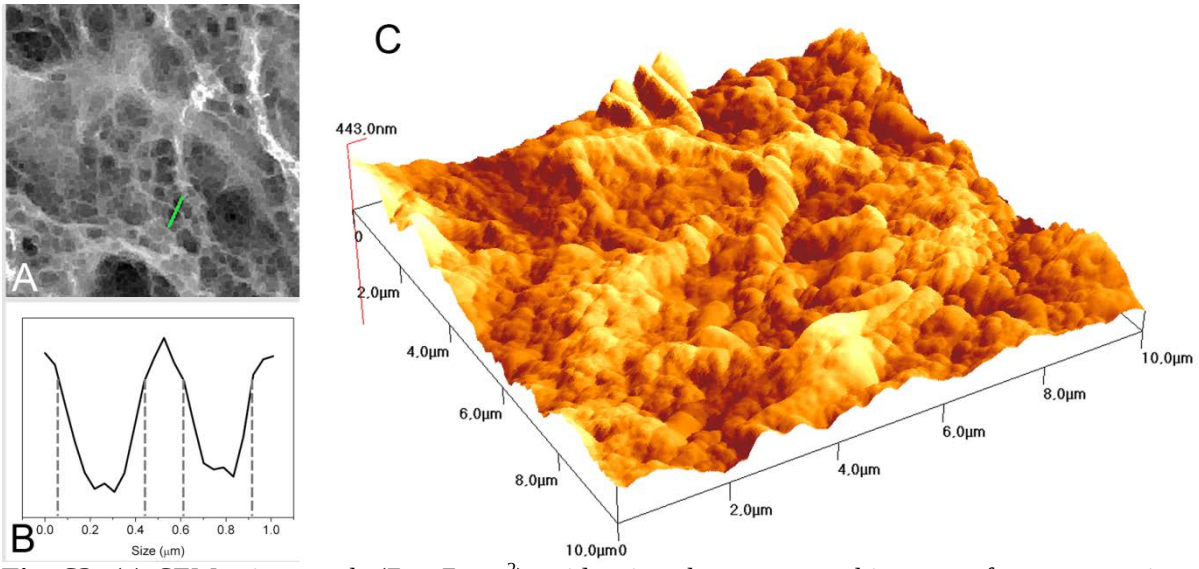

Fig. S3. (a) SEM micrograph (7 × 7 μm^2) evidencing the porous architecture of a cross-sectioned sponge. The presence of a finely interconnected porous substructure confers both biomimetic properties [A. Di Martino, M. Sittinger, M.V. Risbud Biomaterials 26 2983 (2005)] and potential for granting a comfortable 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 × 10 μm^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 × 10 μm^2) was reported in the main text.

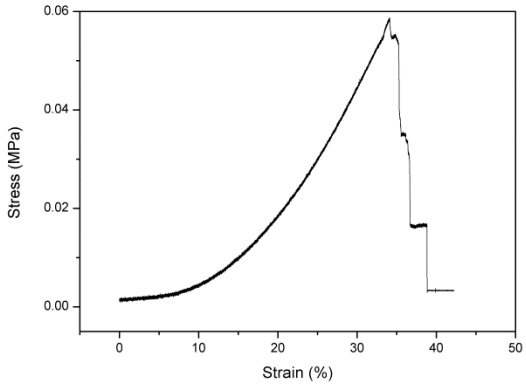

Fig. S4. Representative stress-strain curves of a nanocomposite sponge. Wet sponges were resized to (6 × 6) mm^2 samples and secured with tensile grips to a tensiometer (Asper s.r.l.). An increasing load was applied at an elongation rate of 10 μm s^{-1}. Measurement were carried out in water bath at 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 ± 0.02 MPa were typically measured, which are comparable to those of relevant soft mammalian tissues such as carotid artery, thyroid and lung among others [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.

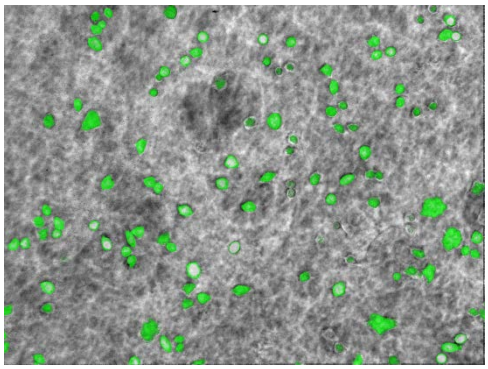

Fig. S5. HeLa cells adhesion to the sponge surface after 24 h from seeding. The green fluorescence produced by calcein stain indicates the presence of vital cells (1.8 × 1.3 μm^2).This image is obtained by merging the green fluorescence channel with the bright field acquisition. Cells were cultured in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

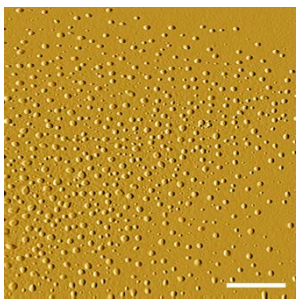

Fig. S6. AFM topography of micelles (we used a 1 $mgmL^{-1}$ solution) deposited on a mica substrate carried out on a Quesant Q-Scope 250 instrument operated in tapping mode at room temperature (Bar = 2 μm).

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

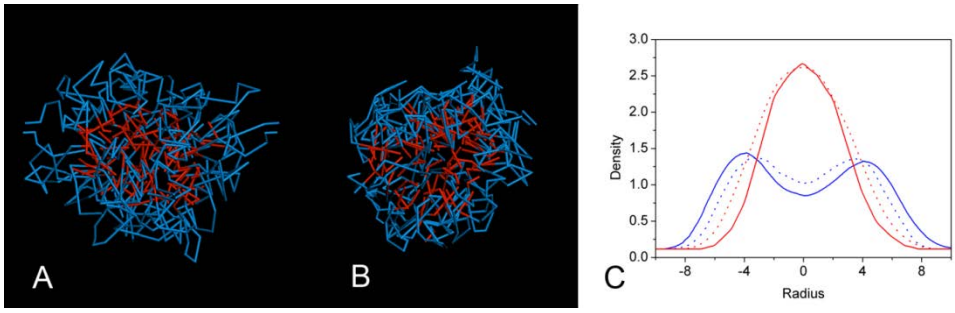

Fig. S7. Simulated cross-sections of the PCL-PEO-PCL micelles at 25 °C (a) and 50 °C (b) (the solvent was omitted for clarity; PCL block is red, PEO block is blue) by using the repulsion a_{ij} parameters reported in Table S1. (b) Simulated density profiles of PCL-PEO-PCL micelles at 25 °C (solid lines) 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 $10\times10\times10r_c^3$ 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_BT 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).

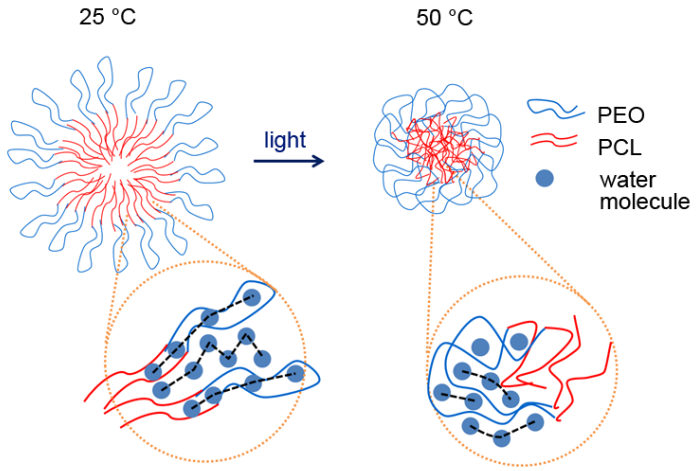

Fig. S8. Proposed scheme of water molecules arrangement inside a PCL-PEO-PCL micelle at 25 °C and 50 °C on the base of FTIR analysis (Figure 1h). Large water networks contained inside micelles at ambient temperature are replaced by short water connections as the micelle shrinks upon rising temperature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

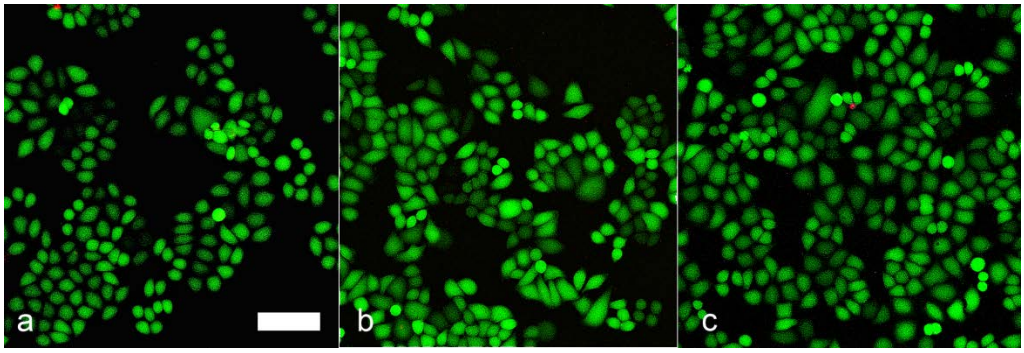

Fig. S9. Control experiments: (a,b) blank (without Dox) sponges were placed in contact with HeLa cells monolayers and then subjected to different laser irradiation conditions in accordance with Figure 3 (a = 0.36 W cm^{-2}; b = 0.46 W cm^{-2}; t_{irr} = 5 min; bar = 100 μm). (c) Dox-loaded sponge kept in contact with HeLa cells for 5 min and not exposed to laser light. Cell viability was checked after 4 h from the treatment in accordance with S.H. Kim et al. [S.H. Kim and J.H. Kim Cancer Research 32 323 (1972)]. 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), (b), and (c) as determined from the expression:

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

is close to 100 %.

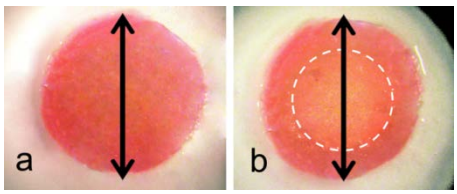

Fig. S10. A nanocomposite sponge loaded with Dox, before irradiation (a), and after 30 s from laser irradiation (0.46 W cm^{-2}, 5 min). The size, as indicated by arrows is not altered by the laser treatment (the irradiated portion of the sponge is indicated with a dashed circle).

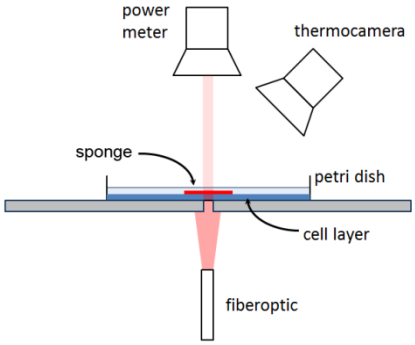

Fig. S11. Schematic overview of the experimental setup used in the irradiation experiments.
Laser experiments were performed with a bench setup consisting of an AlGaAs diode laser peaked at 810 nm (Mod. WELD 800, El.En S.p.A.) coupled with a 600-µm-core optical fiber and a precision stage showing 6-mm 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.