Supplementary information:

Materials and Methods

Materials. Chemical reagents related to nanorods and nanoparticles synthesis were purchased from Sigma-Aldrich. Methoxy-terminated poly (ethylene glycol)(mPEG-thiol, 5kDa) and Amine-terminated PEG (NH_2-PEG-thiol, 10kDa) were purchased from Laysan Bio and Rappe polymere, respectively. Bovine collagen (I) solution of different concentrations 10, 6 and 3mg/ml was purchased from Advanced Biomatrix. RPMI 1640 culture media was purchased from Gibco. µ-slideVI0.4 (Cat#80606) chambers were purchased from ibidi. Other biological reagents were purchased from Sigma-Aldrich and Invitrogen.

Preparation of collagen (I) hydrogels inside µ-channels. µ-slide channels were pre-cooled on ice. To make ~ 7mg/ml collagen hydrogel, 300ul of bovine collagen (I) solution (10mg/ml) was mixed respectively with 50ul phosphate buffer solution (PBS, 10x), 10ul sodium hydroxide (0.1M) and 120ul RPMI (1640) media (containing 10% fetal bovine serum and 1% penicillin streptomycin) and kept cool in ice. Next, 30ul(µ-channel volume) of the above mixture was pipetted into the opening of each µ-channel through one reservoir. The µ-slide was then placed in an incubator, held at 37°C and 5% CO_2, for 4hrs to ensure assembly of intact collagen (I) matrix. Finally, 40ul of PBS (1x) was added to each pair of reservoirs to maintain humidity and avoid drying of hydrogels before use. For the preparation of the other 2 and 4mg/ml collagen hydrogels, initial bovine collagen (I) solutions of 3 and 6mg/ml were used with proportional ratios of PBS (10x), NaOH (0.1M) and RPMI media.

Growth of gold nanorods and their surface-modification with PEG. Gold nanorods with an aspect ratio of 4.1 were synthesized via modified seed-mediated technique. First, a seed solution was prepared by rapid injection of sodium borohydride (1.2ml, 0.01M) into a mixture of $HAuCl_4$ (0.5ml, 0.01) and cetyltrimethyl ammonium bromide (19.5ml, 0.1M) under vigorous stirring. The resulting solution was heated to 60°C for 20min on a stir plate followed by cooling to room temperature. Next, a growth solution was prepared containing $HAuCl_4$ (49.5ml, 0.01M), cetyltrimethyl ammonium bromide (CTAB, 950ml, 0.1M). To this solution, $AgNO_3$ (5ml, 0.01M) and ascorbic acid (7ml, 0.1M) were added subsequently. The yellow color solution became clear. Finally, 20ml of the seed solution was added to the growth solution under moderate stirring and left overnight. The resulting nanorods were centrifuged at 15000 x g for 30 min twice, washed with deionized water (DI, Nanopure™ H_2O) and re-dispersed in DI water. To modify the surface of the gold nanorods with PEG, purified nanorod solution (1nM) was added dropwise in 1:1 volume ratio to mPEG-thiol (1mg/ml, 5k) and allowed to moderately stir at room temperature for 3 hrs. The nanorods were then centrifuged three times at 8000 x g for 20min to remove excess PEG and CTAB.

Synthesis of spherical 50nm and 120nm gold nanoparticles and their surface PEGylation. 50nm and 120nm gold nanoparticles were synthesized via hydroquinone-seeded growth[51]. First, 15nm gold nanoparticles were prepared by reducing gold (III) chloride hydrate (25mM) with sodium citrate tribasic (1mM) under reflux while being vigorously stirred at a volume ratio of 0.125. The as-prepared seed was stored in 0.01% (v/v) Tween 20 solution. Next, 50nm and 120nm gold nanoparticles were prepared by further growth of 15nm gold seeds (100x diluted) in the

presence of gold (III) chloride hydrate (25mM), sodium citrate tribasic (15mM) and hydroquinone (25mM) in a 1:1:1 volume ratio, while the amount of gold colloids was varied to control the particle size. The nanoparticles were then centrifuged twice and re-dispersed in 0.05% (v/v) Tween 20 solution before PEGylation. PEG surface modification in both particle sizes was achieved by incubating nanoparticles with NH_2-PEG-thiol (10kDa) and mPEG-thiol (5kDa) at a molar ratio of 1:4 (1 Amine-PEG/nm^2 and 4 mPEG/nm^2) at 60°C for 1 hr[6]. 50nm and 120nm nanoparticles were then centrifuged three times at 1500 x g and 500 x g for 45min and stored at 4°C before labeling with the fluorescent molecule.

Fluorescent labeling of 50nm and 120nm nanoparticles. AlexaFluor647nm dye was aliquoted as received and stored in a dark box at -20°C. An AlexaFluor647nm aliquot was thawed and dissolved in 50ul sodium bicarbonate (0.1M). Next, 450ul of PEGylated nanoparticles in sodium bicarbonate (0.1M) was incubated with AlexFluor647nm solution at 5x equivalent amount of theoretical Amine-PEG molecules of corresponding nanoparticle surface. Incubation was carried out for 3hrs. 50nm and 120nm nanoparticles were then centrifuged and washed three times at 1500 x g and 500 x g for 45min and stored at 4°C in the dark.

Photo-thermal experiments. A power tunable fiber-coupled continuous wave (CW) laser diode (785nm) was used for exciting gold nanorods. The temperature was monitored using FLIR E60 thermal imager (320x240 pixels) and thermal accuracy of ± 2°C. Photo-thermal temperature profiles of gold nanorods were developed in 96-well plates. 150ul gold nanorods solution were pipetted into a well and the well plate was placed on a pre-heating stage set to 37°C. The sealed well-plate (to avoid evaporation) was stabilized to a final temperature of 33-34°C. Next, a laser beam of 5mm was shun on the well containing gold nanorods and the average instant temperature profile of the well was recorded as a function of time with respect to the control well (which contained distilled water). The same process was applied to all concentrations and laser powers. Emissivity corrections were done using FLIR protocol with respect to black tape body (0.95).
To apply photo-thermal process on collagen (I) μ-channels, 30ul of PEGylated gold nanorods were pipetted into one reservoir of an already prepared collagen (I) channel while the other reservoir was filled with 30ul of blank PBS (1x). The μ-channel was placed on heating stage and stabilized to a temperature of 33-34°C before photo-thermal process. After 1hr, a laser power density of 3W/cm^2 raster radiated an area covering ~ 8 mm of the channel in the vicinity of the reservoir. The fiber radiation scanned this area in a back and forth movement for 6min. The temperature of the entire channel was mapped using thermal imager at 10s intervals.

Reflectance confocal imaging. Collagen (I) hydrogels were prepared in approximately 2, 4 and 7mgl concentrations. The hydrogels were then imaged using a Nikon confocal microscope in reflex mode by scanning a 512x512 pixels ROI at 10x and 20x magnifications under 486.7nm laser. The focal plane was set to top layer of collagen matrix to avoid signal artifacts.

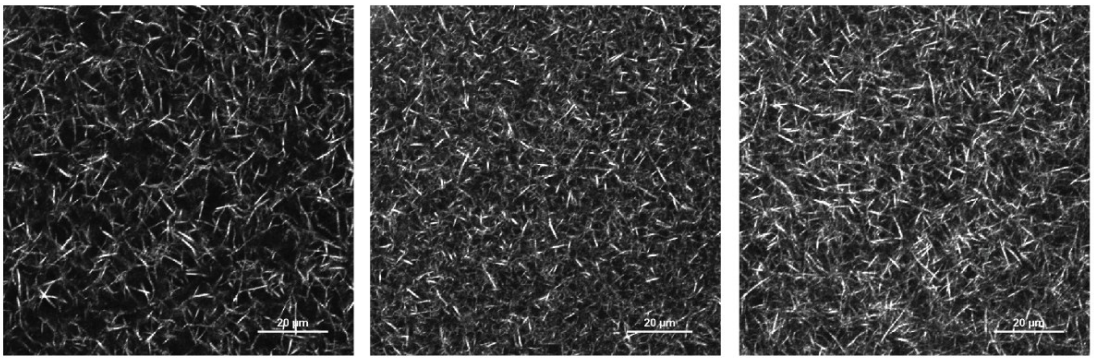
Fluorescent tracking of nanoparticles using confocal microscopy. Collagen (I) hydrogels were prepared in two μ-channels (PTT and control) as previously described. 30ul of PEGylated GNRs were added to one of the reservoirs for both μ-channel while the other reservoirs were filled with the same volume of PBS (1x). After PTT process on one of the μ-channels, both μ-channels were placed on microscope stage for head-to-head imaging on the same spots along both channels. Next,

30ul of fluorescent nanoparticles were added on top of the already added GNRs reservoirs in both μ-channels. Another 30ul of PBS (1x) was added to other reservoirs to counterbalance hydrostatic pressure. Immediately, confocal fluorescent imaging was performed by sequential scanning a 512x512 pixel ROI along the channel at 1mm intervals and 10x magnification under 639.4nm laser for 12hrs. Imaging condition was optimized for laser power and PMT detector to offset background signal and de-saturate nanoparticle signal. Z-focus was kept constant along channel length. The direction of the imaging was from the vicinity of high concentration reservoir towards the other reservoir.

Image analysis and diffusion measurements. Nanoparticles diffusion inside collagen (I) were analyzed from sequential fluorescent images using ImagJ and Matlab. To develop spatio-temporal intensity profiles, mean fluorescence of a fixed ROI (25x25 pixel) was measured along mid-channel axis at different penetration distances for 12hrs and subtracted from background. For intensity-penetration distance profiles, three ROIs were measured for each penetration depth and normalized to reference point intensity (proximity of reservoir) at a fixed time-point for each nanoparticle size. Diffusivity values were calculated by fitting intensity-penetration depth in the mid-channel profiles at fixed time-points to standard 1-D analyte diffusion equation (1).

Figures

Figure S1 | Reflectance confocal images collagen(I) hydrogels at different concentrations: Increase in collagen(I) cocentration lead to higher density of collagen(I) fibers in hydrogel matrice



2mg/ml 4mg/ml 7mg/ml

Figure S2 | Effect of laser power density on GNRs temperature elevation:

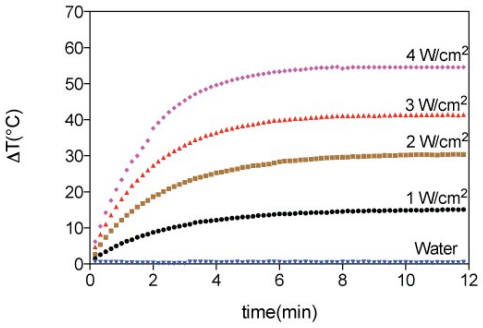


Figure S3 | Representative TEM images of **A**)50nm , **B**)120nm nanoparticles: Both showed monodisperse nanoparticles with relatively spherical shape.