Electronic supplementary information (ESI) for

A Microfluidic Origami Chip for Synthesis of Functionalized Polymeric Nanoparticles

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Device design and fabrication

The device design consists of a microchannel (300 μm × 300 μm cross-section and 6 cm long) with three inlets and one outlet. The three inlets have the same channel width of 100 μm, and the angle between two adjacent inlet channels is 20 °. The microfluidic device was fabricated using standard soft lithography with SU8-2150 master mold on a silicon substrate. SU-8 2150 photoresist (MicroChem Corp) was first spin-coated on a 4 inch silicon wafer at 500 rpm for 10 sec followed by 2000 rpm for 30 sec to obtain the desired thickness (300 μm). The coated wafer was soft-baked for 1 hr at 95 °C and then exposed to ultraviolet light with exposure energy of 400 mJ/cm^2 through a photomask containing the pattern of microfluidic channels. After exposure, the wafer was baked again at 95 °C for 25 min and soaked in SU-8 developer (MicroChem Corp) to dissolve the unexposed photoresist. The patterned SU-8 master mold was then hard-baked at 150 °C for 15 min to anneal microcracks in SU-8.

Degassed PDMS (mixed in a 10:1 ratio of PDMS base with curing agent, Sylgard 184, Dow Corning Inc.) was spin-coated over the mold at 500 rpm for 30 sec to achieve a thickness of 500 μm, and baked at 80 °C for 1 hr in an oven. The PDMS with embedded channels was subsequently diced by a razor blade and removed from the master mold. Three inlets and one outlet were punched through the PDMS with a needle with flattened tip. The PDMS slab was then bonded with another layer of flat PDMS of 500 μm after oxygen plasma treatment. Three plastic tubes were inserted through the inlet ports and secured by the liquid PDMS on the top of device. The assembled device was finally placed into an oven at 70 °C for 30 min to cure the liquid PDMS and seal the intersection seam between PDMS and tube. To create a 3D curved geometry, the inlet part of microchannels was first fastened with tape, and the middle part was lifted up or folded to form an arc or a double spiral.

Finally, the outlet part of microchannels was anchored with another tape (Fig. S1).

Materials

Poly(D, L-lactide-*co*-glycolide) (PLGA, lactide:glycolide = 75:25) was purchased from SurModics. Dimethylformamide (DMF), trifluoroethanol (TFE) and doxorubicin (DOX) were purchased from Sigma. 200 mg PLGA and 1.2 mg DOX were dissolved in 5 mL DMF and 3.8 mL TFE to prepare 2 % PLGA-DOX organic solution. Water was deionized with a Millipore Milli-Q water purification system, and used as sheath fluid during synthesis experiments.

Two human carcinoma cell lines, MCF-7 and HeLa were grown to confluence in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a 5% CO_2, 37 °C incubator (Thermo Scientific). Medium was changed every 2 – 3 days.

Experimental set-up

In the experiment of synthesizing PLGA-DOX nanoparticles, the microfluidic origami chip was first primed with deionized water containing 0.1% w/v Tween 20 (AMRESCO Inc.). 2 % PLGA-DOX organic solution was pumped into the microchannel through the middle inlet, while water was pumped into the channel through two side inlets. The flow rates of solutions were precisely controlled by adjusting the parameters of three independent syringe pumps (Longer Precision Pump Co., Ltd.), which were connected to the microchannel through three inlet plastic tubes. After each experiment, we used DMF to wash microchannels.

Dynamic light scattering

Size distribution of PLGA-DOX nanoparticles was analyzed three times for each sample using dynamic light scattering (DLS, Zetasizer 3000HS, Malvern Instruments Ltd.). The sample of 1 mL nanoparticle suspension was in a DLS cuvette, and the measurement was performed with detection optics arranged at 173 °. After each measurement, we used deionized water to rinse the cuvette three times. The size distribution of PLGA-DOX nanoparticles synthesized by 2D flat microchannels closely fit an exponentially increasing curve with the increased flow rate of organic solution (Fig. S2). The polydispersity index (PDI) of PLGA-DOX nanoparticles was much smaller than 0.4, suggesting that nanoparticles were essentially monodisperse (Fig. S3).

Electron microscopy

We used scanning electron microscopy (SEM, Hitachi S4800) and transmission electron microscopy (TEM,FEI Tecnai T20) to characterize PLGA-DOX nanoparticles. Nanoparticle samples were sputter-coated with gold before observation with SEM. Nanoparticle suspensions were deposited onto carbon-coated copper grids before observation with TEM which was operated at an acceleration voltage of 200 kV.

UV-vis spectra

DOX solutions at different concentrations from 0.2 to 20 μg/mL were measured with UV2450 spectrophotometer (Shimadzu). The UV-Vis spectra were acquired by the multifunctional microplate reader (TECAN, Infinite®200 PRO). The characteristic absorption of DOX solution was around 480 nm (Fig. S5(a)). The absorbance at 480 nm at varying DOX concentrations was fit linearly to get the

characterization curve (Fig. S5(b)).

Cellular uptake

For both human carcinoma cell lines (MCF-7 and HeLa), cells were grown in culture dishes until confluence before co-incubation with PLGA-DOX nanoparticles. The nanoparticle suspension or free DOX at DOX concentration of 50 μg/mL was added into high-glucose DMEM supplemented with FBS and penicillin/streptomycin. The final DOX concentration was 10 μg/mL, existing inside PLGA-DOX nanoparticles or in the presence of free DOX. The prepared medium containing nanoparticles or free drug was used to culture cells at 37 °C, 5% CO_2 for 4 hr. After incubation, cells were washed twice with phosphate buffered saline (PBS), and visualized using a Zeiss LSM 710 confocal microscope system (Carl Zeiss). Samples were excited with 488 nm (green) laser line to localize PLGA-DOX nanoparticles or DOX inside cells. After obtaining scanned slices, we used Zen 2009 Zeiss software suite (Carl Zeiss) to automatically overlay bright-field and fluorescent images.

Cell viability

Similar to the experiments of cellular uptake, the nanoparticle suspension or free DOX at 50 μg/mL was added to different amounts of DMEM to obtain a final DOX concentration of 2 – 20 μg/mL. MCF-7 or HeLa with cell density of 1×10^6 cells/mL was incubated with medium containing different amounts of DOX for defined time intervals. For each time point (12 hr, 24 hr, and 48 hr), cell morphology was observed by a Leica DMI 6000 microscope (Leica Microsystems). Meanwhile, cell viability was tested using cell counting kit-8 (CCK-8, Beyotime). This commercial kit could produce soluble purple formazan in the presence of viable cells, and its absorbance increased linearly

as cells proliferated.

Reynolds number and mixing time in 2D microchannels

The microchannel used for nanoparticle synthesis was 300 μm wide, 300 μm high and 6 cm long. The flow rate of organic solution in the middle stream was 0.3125 mL/hr to 2.5 mL/hr, controlled by the syringe pump. Two side water streams had the same flow rates, each of which was 20 mL/hr. To describe flow characteristics in closed microchannels, the Reynolds number (Re) was given by:

$$\mathrm{Re} = \rho U D_h / \mu$$

where U is the maximum velocity within microchannel, ρ and μ are the density and the dynamic viscosity of the fluid, respectively, D_h is the hydrodynamic diameter of microchannel (four times the channel cross section divided by the perimeter). For ρ = 1000 kg/m^3, μ = 0.001 Pa·s, D_h = 300 μm, the calculated Re numbers were listed in Table S1.

The mixing time governed by diffusion ($\tau_{mix,diff}$) inside 2D microchannels was calculated by:

$$\tau_{mix,diff} \sim \frac{w_f^{\,2}}{4D} \approx \frac{w^2}{9D} \frac{1}{(1+1/R)^2}$$

where w_f is the characteristic length, and D is the diffusion coefficient (10^{-9} m^2/s).

$$w_f \approx \frac{2w}{3(1+1/R)}$$

where w is the channel width (300 μm), and R is the flow ratio in the range of 7.75×10^{-3} – 0.059. The estimated mixing time was from 0.59 ms to 30.86 ms with the increased flow ratio, which was also listed in Table S1. We should note the mixing time obtained here was for 2D flat channels. To avoid

clogging inside microchannels, we designed a wide microchannel and applied a low flow ratio. As a result, the calculated mixing time was higher than that from ref. 7 and 13 in the main text, in which the channel width was 20 μm and 50 μm, respectively. For a complex microfluidic system such as 3D curved microchannels, we used a numerical simulation to quantitatively examine the fluid transport inside.

Estimated time of formation of PLGA-DOX nanoparticles

$$C_{PLGA} \approx \frac{concentration}{molecular\ weight} = \frac{2mg/mL}{100000g/mol} \times 6.02 \times 10^{23} mol^{-1} = 1.204 \times 10^{22} m^{-3}$$

$$m_{PLGA} \approx \frac{\pi}{d} a^3 \rho_{PLGA}$$

where a is the diameter of PLGA-DOX nanoparticles, and ρ is the density of PLGA. For a = 100 nm (or 234 nm), and ρ =0.5 g/cm^3, the calculated mass is 2.62×10^{-16} g (or 3.35×10^{-16} g).

$$n_{PLGA} \approx \frac{m_{PLGA}}{molecular\ weight} \times 6.02 \times 10^{23} mol^{-1} = \frac{4\pi}{3} l^3 C_{PLGA}$$

For m_{PLGA} = 2.62×10^{-16} g (or 3.35×10^{-16} g), the estimated diffusion length scale (l) is 315 nm (or 737 nm).

$$\tau_{formation} \approx \frac{l^2}{D_{PLGA}}$$

The diffusion coefficient of PLGA is 10^{-11} m^2/s. For nanoparticles of 100 nm (or 234 nm), the estimated time of particle formation is 9.92 ms (or 54.3 ms).

Numerical simulation

Numerical simulation was used to investigate the mixing process in the complex microchannels. The flow field and species transportation in these microchannels were solved by commercial CFD

software Fluent 6.4 (Ansys Inc.). Hexahedral grids were generated using Gambit (Fluent 6.4, Ansys Inc.) for three different types of microchannels, namely straight, double spiral, and arched microchannels. The simulated mixing time was listed in Table S2.

Table S1. The flow ratio, Re number, and mixing time by diffusion in 2D flat microchannels

Flow rate (organic) (mL/hr)	Flow rate (water) (mL/hr)	Flow ratio	Renolds number	Mixing time $\tau_{mix,diff}$ (ms)
0.3125	40	0.0078	55.99	0.59
0.625	40	0.0154	56.42	2.30
1.25	40	0.0303	57.29	8.65
1.875	40	0.0448	58.16	18.37
2.5	40	0.0588	59.03	30.86

Table S2. The simulated mixing time inside the microfluidic origami chip with different geometries

Geometry	Flow rate (organic) (mL/hr)	Mixing time τ_{mix} (ms)
2D flat channels	1.25	8.27
2D flat channels	2.5	29
3D arc channels	1.25	5.9
3D arc channels	2.5	16
3D double spiral channels	1.25	5.51
3D double spiral channels	2.5	14.5

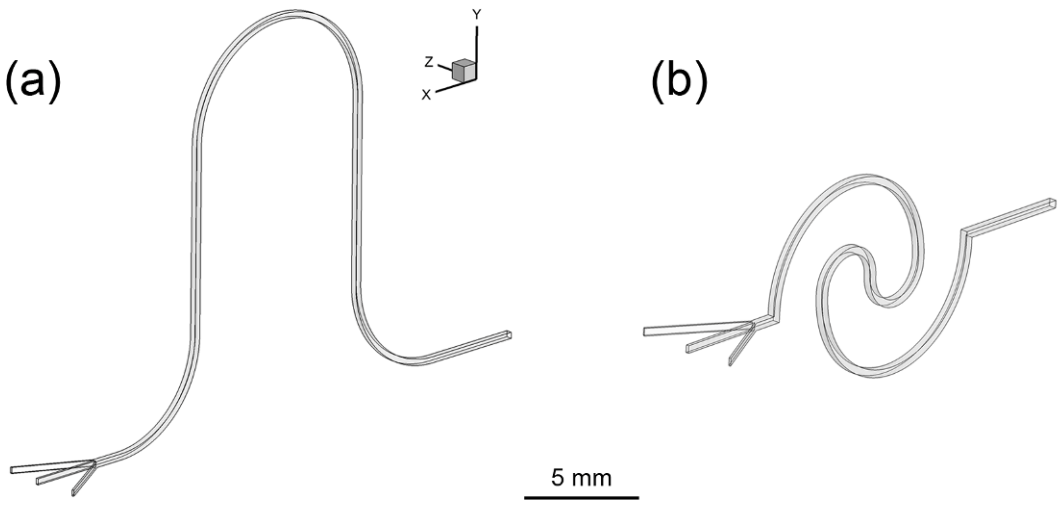

Fig. S1 (a) The origami chip forms a 3D arc geometry by manual folding. (b) The origami chip forms a 3D double spiral geometry by folding. The origami chip is drawn to scale.

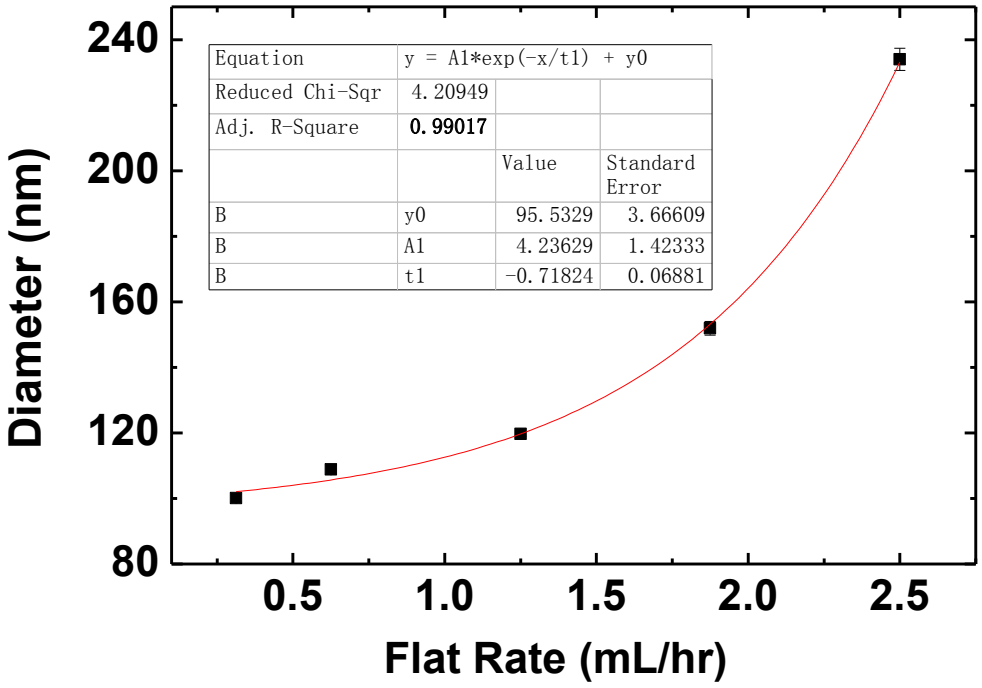

Fig. S2 Size distribution of PLGA-DOX nanoparticles synthesized by 2D flat microchannels. The size of nanoparticles is increased from 100 ± 1.18 nm to 234 ± 3.38 nm in an approximately exponential trend with increasing flow rate of organic stream.

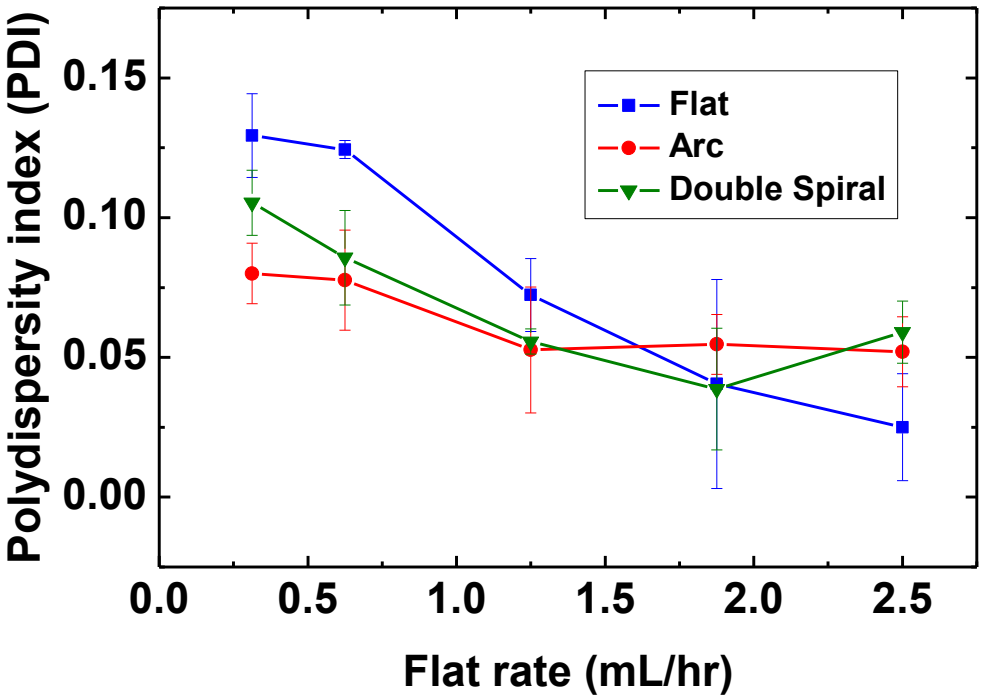

Fig. S3 Polydispersity index (PDI) in dynamic light scattering measurements. The PDI is less than 0.13, indicating a monodisperse dispersion of PLGA-DOX nanoparticles.

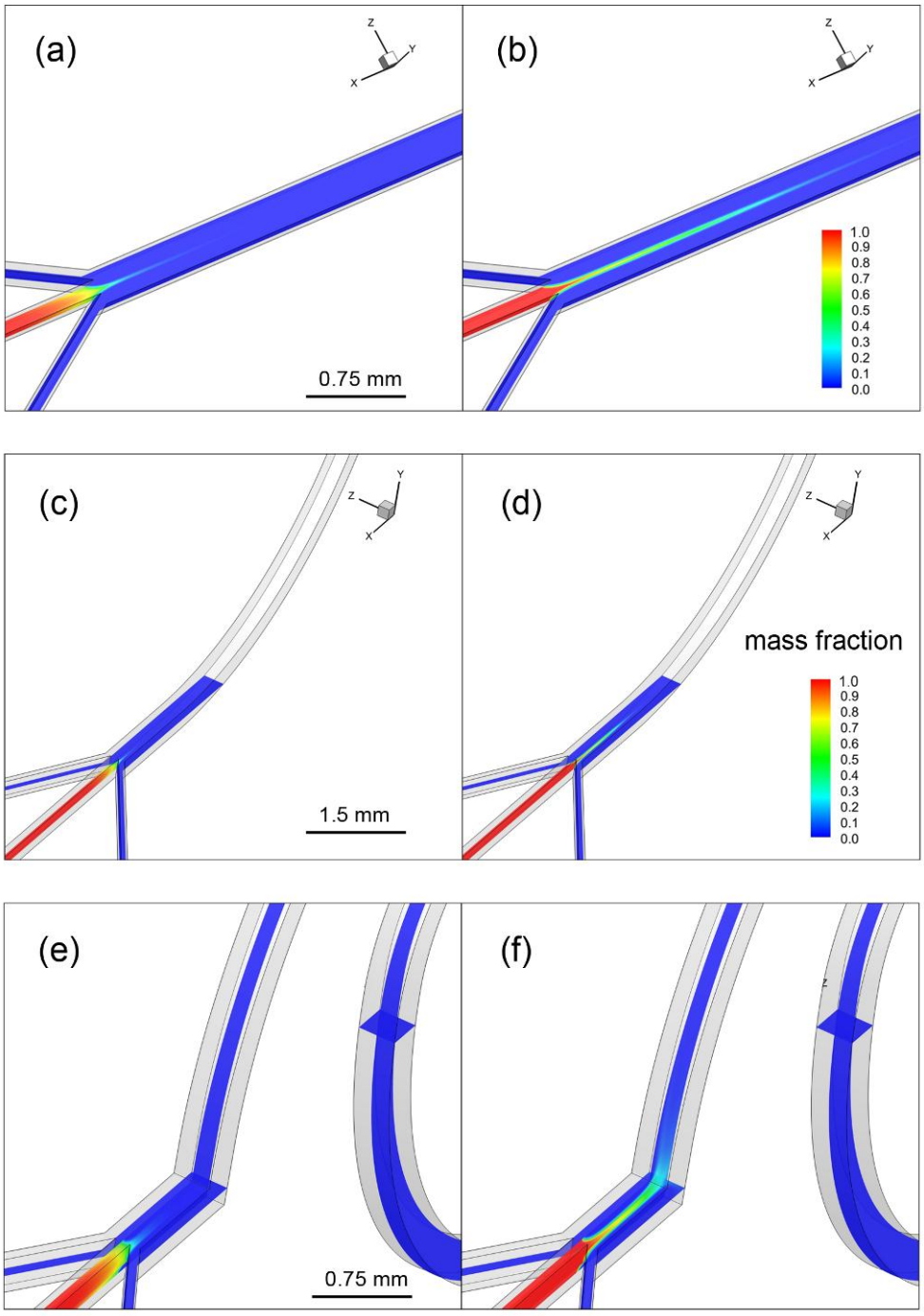

Fig. S4 Prediction of rapid mixing in (a) 2D flat microchannels at 1.25 mL/hr for organic stream. (b) 2D flat microchannels at 2.5 mL/hr for organic stream. (c) 3D arc microchannels at 1.25 mL/hr for organic stream. (d) 3D arc microchannels at 2.5 mL/hr for organic stream. (e) 3D double spiral microchannels at 1.25 mL/hr for organic stream. (f) 3D double spiral microchannels at 2.5 mL/hr for organic stream.

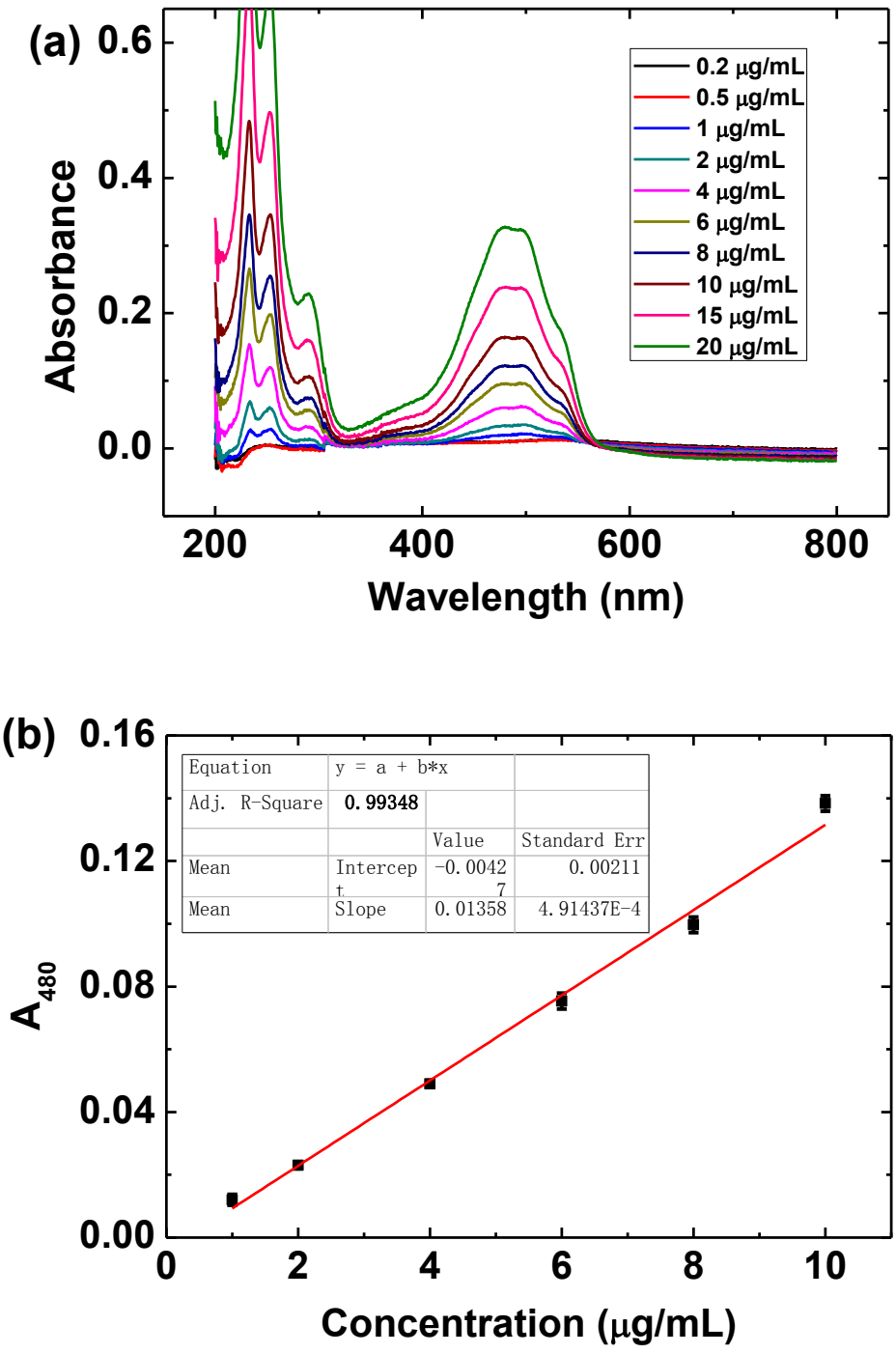

Fig. S5 (a) UV-vis spectra of DOX at different concentrations. (b) Linear fit of absorbance of DOX at 480 nm at concentrations from 1to 10 μg/mL.

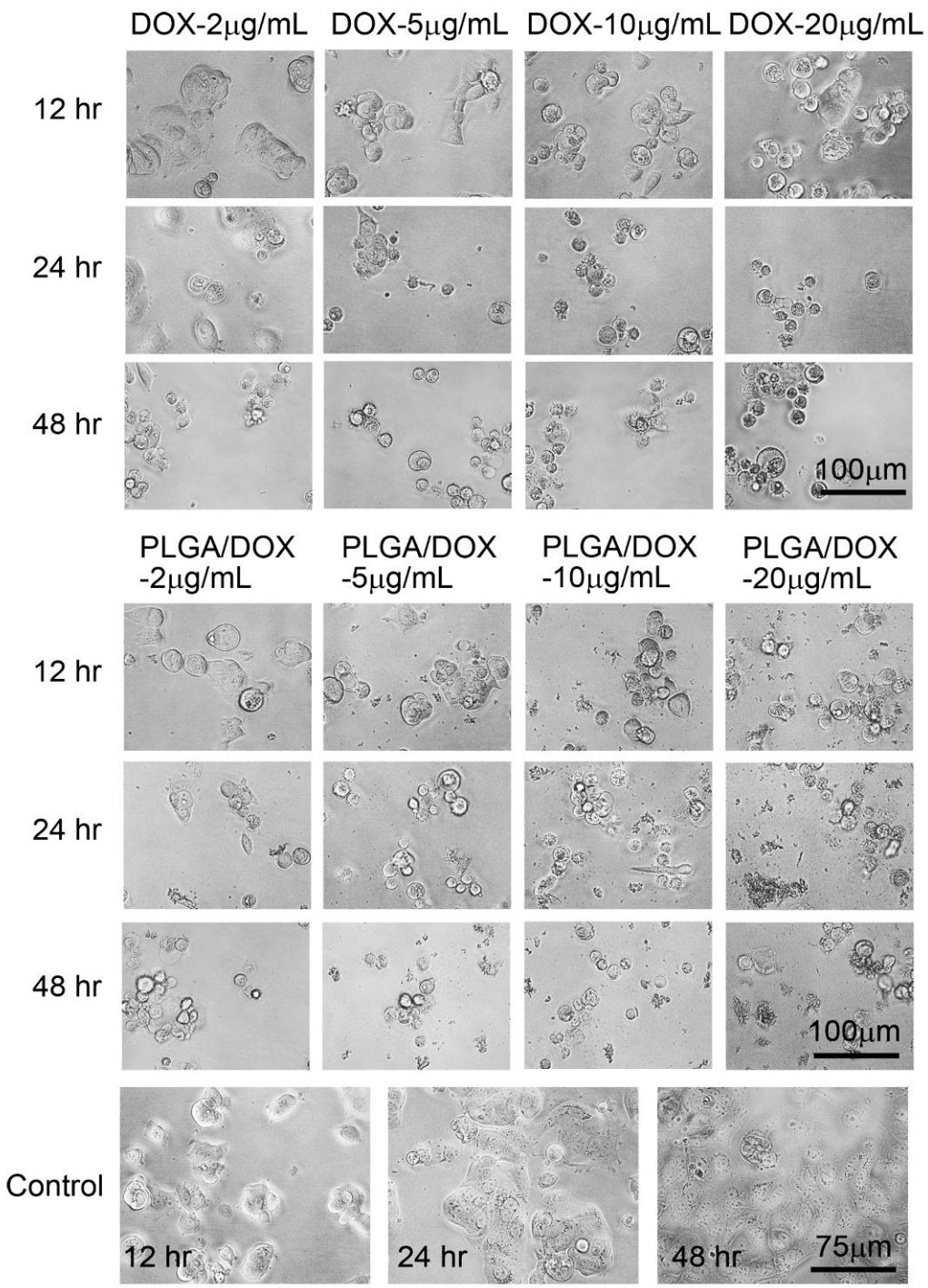

Fig. S6 Microscopic images showing changes in MCF-7 cell morphology as a function of incubation time. MCF-7 cells are treated with PLGA-DOX nanoparticles at DOX concentration of 2 – 20 μg/mL and free DOX at equal concentration.

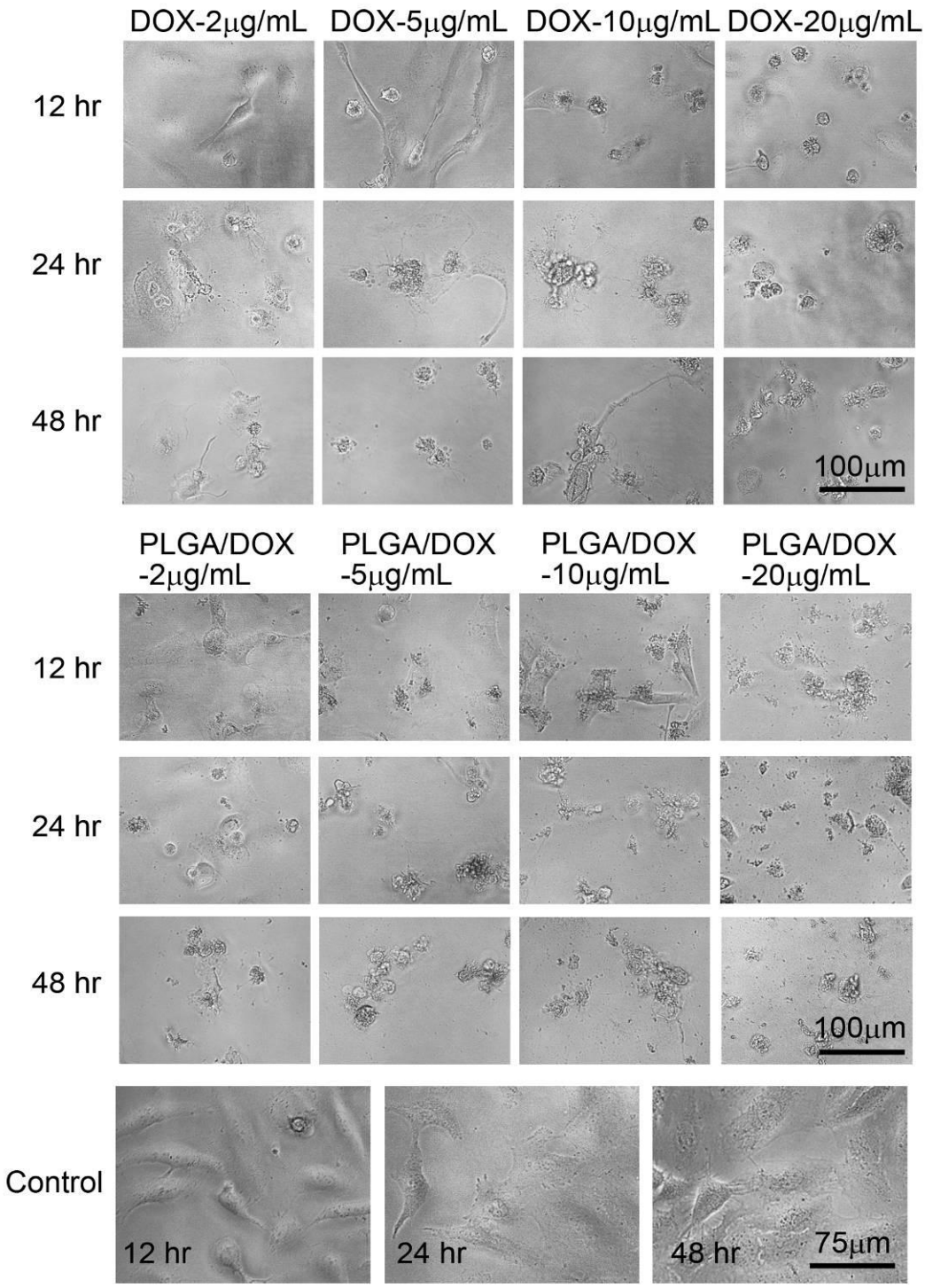

Fig. S7 Microscopic images showing changes in Hela cell morphology as a function of incubation time. HeLa cells are treated with PLGA-DOX nanoparticles at DOX concentration of 2 – 20 μg/mL and free DOX at equal concentration.