Supporting Information

Biomimetic drug delivery platforms based on mesenchymal stem cells impregnated with light-responsive submicron sized carriers

Albert R. Muslimov[a,b], Alexander S. Timin[a,c,d,*], Valery R. Bichaykina[e], Oleksii O. Peltek[e], Timofey E. Karpov[d], Aliaksei Dubavik[f], Alexandre Nominé[e,g], Jaafar Ghanbaja[g], Gleb B. Sukhorukov[c,h,i*], Mikhail V. Zyuzin[e,*]

[a]*First I. P. Pavlov State Medical University of St. Petersburg, Lev Tolstoy str., 6/8, Saint-Petersburg, Russian Federation*

[b]*Nanobiotechnology Laboratory, St. Petersburg Academic University, 194021 Saint Petersburg, Russia*

[c]*Research School of Chemical and Biomedical Engineering, National Research Tomsk Polytechnic University, Lenin Avenue 30, 634050 Tomsk, Russia*

[d]*Peter the Great St. Petersburg Polytechnic University, Polytechnicheskaya, 29, 195251 St. Petersburg, Russian Federation*

[e]*Faculty of Physics and Engineering, ITMO University, Lomonosova 9, 191002, St. Petersburg, Russia*

[f]*Department of Optical Physics and Modern Natural Science, ITMO University, 197101 St. Petersburg, Russia*

[g]*Jean Lamour, University of Lorraine/CNRS, Nancy, France*

[h] *Skolkovo Institute of Science and Technology, Moscow, 143025, Russia*

[i]*School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom*

***Corresponding authors:**

1. Prof Gleb B. Sukhorukov. g.sukhorukov@qmul.ac.uk

   Address: Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom

2. Dr Alexander S. Timin. a_timin@mail.ru , timin@tpu.ru.

   Address: National Research Tomsk Polytechnic University, Lenin Avenue 30, 634050 Tomsk, Russia

3. Dr Mikhail V. Zyuzin. mikhail.zyuzin@metalab.ifmo.ru

   Address: Faculty of Physics and Engineering, ITMO University, Lomonosova 9, 191002, St. Petersburg, Russia

Table of Contents

1. Materials

For Au nanorods synthesis: All chemicals were obtained from commercial suppliers and used without further purification. Hexadecyltrimethylammonium bromide (CTAB, ≥ 98.0%), hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$, ≥ 99.9%), L-ascorbic acid (reagent grade), silver nitrate ($AgNO_3$, 99.9999%), sodium borohydride ($NaBH_4$, 99.99%) were purchased from Sigma Aldrich. Oleic acid was obtained from Fisher Scientific. Sodium hydroxide from Solvay (France). Amine-poly(ethyleneglycol)-thiol (NH_2-PEG-SH, MW 1.000) from Laysan Bio, Inc. (China). Hydrochloric acid (HCl, 37 wt. % in water) was purchased from Vekton (Russia). Ultrapure water obtained from a Hydrolab HLP Smart system was used in all experiments. All glassware was cleaned using freshly prepared aqua regia ($HCl:HNO_3$, ratio 3:1 v/v) followed by rinsing with deionized water.

For capsule synthesis: Poly(allylamine hydrochloride) (PAH, M_W = 17,000), Poly(sodium 4-styrenesulfonate) (PSS, M_W = 70,000), tetraethyl orthosilicate (TEOS, M_W = 208.33, 99.9%), bovine serum albumin conjugated with tetramethylrhodamine (TRITC-BSA, M_W=66,463), calcium chloride dehydrate ($CaCl_2$), ethylene glycol (EG), anhydrous sodium carbonate (Na_2CO_3), and ethylenediaminetetraacetic acid trisodium salt (EDTA) were obtained from Sigma-Aldrich and used without further purification. Dextran conjugated with Alexa Fluor 647 (AF647, M_W =10,000) and phalloidin conjugated Alexa Fluor 488 (AF488) were purchased from Scientific Thermo Fisher. Vincristine sodium sulfate (VCR) was obtained from Teva and used without further purification. Ethanol (C_2H_5OH, 95%) was used in sol-gel synthesis. Purified water with specific resistivity higher than 18.2 MΩ cm^{-1} from a three-stage Milli-Q Plus 185 purification system was used.

For cell culture: Alpha Minimum Essential Medium (Alpha-MEM) and Ultraglutamin I were obtained from Lonza, Switzerland. Dulbecco's Modified Eagle Medium/F-12 (DMEM/F-12) was obtained from Gibco. Fetal bovine serum (FBS) was obtained from HyClone, USA. Penicillin and

streptomycin (P/S) were purchased from Biolot, Russia. Trypsin-EDTA solution, transferrin, sodium selenite, and insulin were obtained from Sigma-Aldrich.

For RNA Extraction and PCR: TriZ (RNA extraction reagent) was obtained from Inogene, Russia. RevertAid First Strand cDNA Synthesis Kit was purchased from LifeTechnologies, USA. SYBR Green was obtained from Invitrogen. Deoxynucleotide (dNTP) solution mix, Taq DNA polymerase, forward and reverse primers were obtained from Syntol, Russia.

For tumors immunocytochemistry: Peroxidase Blocking Reagent, secondary antibodies and streptavidin-peroxidase complex were obtained from DAKO Agilent Technologies, USA. Diaminobenzidine buffer solution and Tris-Buffered saline were obtained from Sigma-Aldrich.

For cell imaging: Calcein acetoxymethyl (Calcein AM) and propidium iodide (PI) were obtained from Sigma-Aldrich.

For cell migration: Stromal cell-derived factor-1 (SDF-1) or chemokine C-X-C motif ligand 12 (CXCL12) was purchased from Sigma Aldrich. Transwell permeable inserts were obtained from Corning. The μ-slide chemotaxis chambers were purchased from Ibidi.

2. Synthesis and characterization of Au nanorods

a. Synthesis of sodium oleate (NaOlAc)

Sodium oleate was obtained by adding sodium hydroxide (1.42g, 35.2mmol) to oleic acid (11.12mL, 35.2mmol) dissolved in 100 mL of 70% ethanol. The reaction mixture was stirred overnight at room temperature. The solvent was removed by rotary evaporation and resulted in the product as a white soap, stored in the fridge.

b. Synthesis of Au nanorods

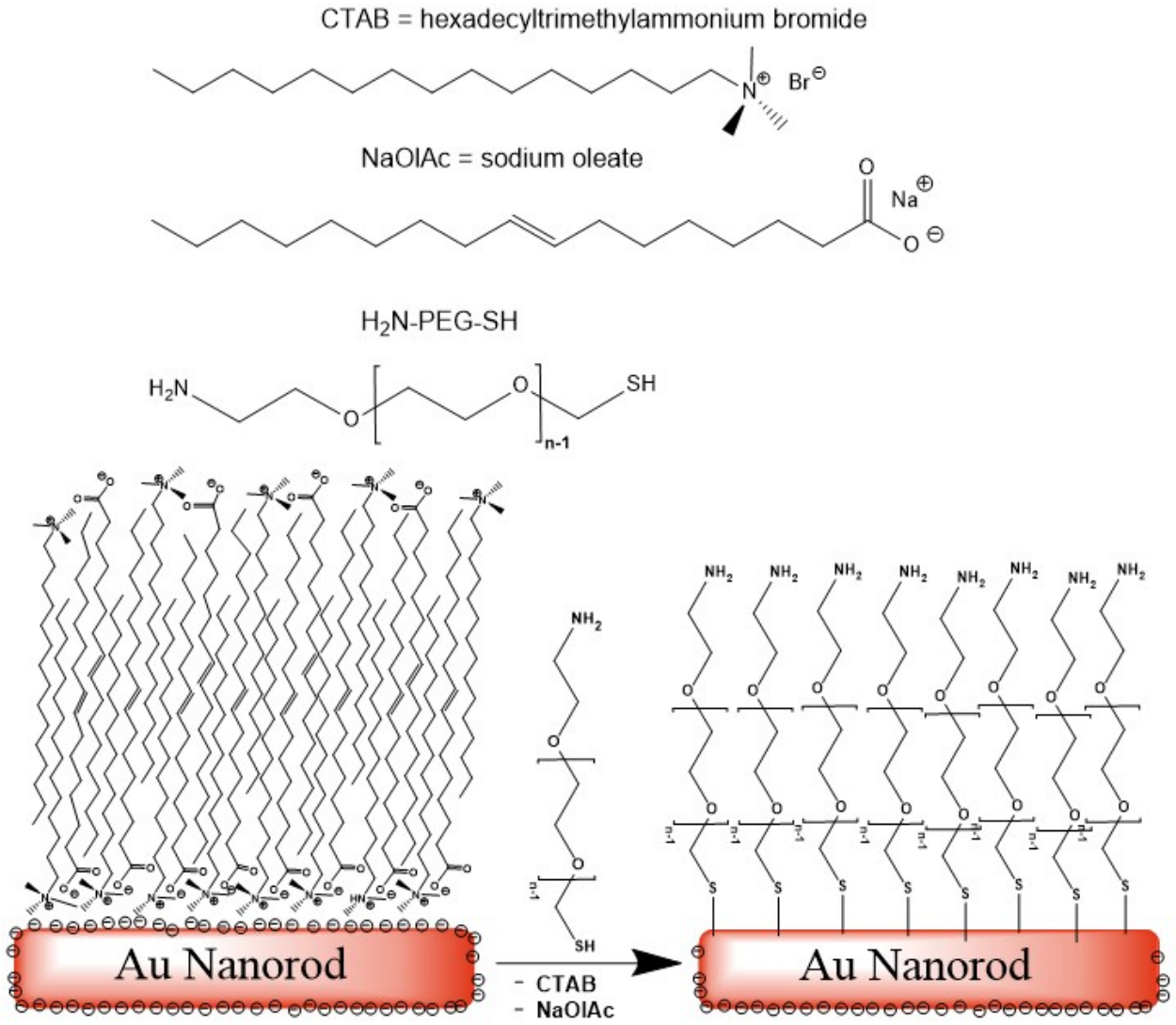
Au nanorods were synthesized using modified protocol.[1] Preparation of the seed solution for gold nanorods growth: The 5 mL of 0.25 mM $HAuCl_4$ was mixed with 2.5 mL of 0.2 M CTAB solution in a 10 mL plastic vial. Then, 0.3 mL of freshly prepared 0.01M $NaBH_4$ was diluted to 0.5 mL with water and was then swiftly injected to the Au(III)-CTAB solution under vigorous stirring at ~1200 rpm. The solution colour changed from yellow to brownish yellow and the stirring was

stopped after 2 min. The seed solution was aged undisturbed at room temperature for 30 min before use.

Preparation of the growth solution: 0.9 g (0.047 M in the final growth solution) of CTAB and 0.1234 g of NaOlAc were dissolved in 25 mL of warm water (~50 °C) in a 100 mL flask. The solution was allowed to cool down to 30 °C and 4.8 mL of 4 mM $AgNO_3$ solution was added. The mixture was kept undisturbed at 30 °C for 15 min after which 25 mL of 1 mM $HAuCl_4$ solution was added. The solution became colorless after 90 min of stirring (700 rpm) and 0.21 mL of HCl (37 wt. % in water) was then introduced to adjust the pH value. After slow stirring at ~400 rpm for another 15 min 0.125 mL of freshly prepared 0.064 M ascorbic acid was added and the solution was vigorously stirred for 30 s. Final step is an injection of 0.01 mL of seed solution into the growth solution. The resultant mixture was stirred for 30 s and left undisturbed at 30°C for 12 h for further nanorods growth.

c. Ligand exchange using H_2N-PEG-SH

15 mL of the as prepared solution was centrifuged for 10 minutes at 7000 rpm. The supernatant was removed from the final precipitated Au nanorods. Then Au nanorods were mixed with 0.5 mL aqueous NH_2-PEG-SH solution (10 mg/mL) and sonicated for 1-2 minutes. The obtained solution was centrifuged again for 10 minutes at 7000 rpm. The addition of aqueous bifunctional PEG to Au NRs and sonication was repeated one more time and stored as is for next experiments (Scheme 1).



Scheme S1. Schematic illustration of ligand exchange using H_2N-PEG-SH

d. Transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX)

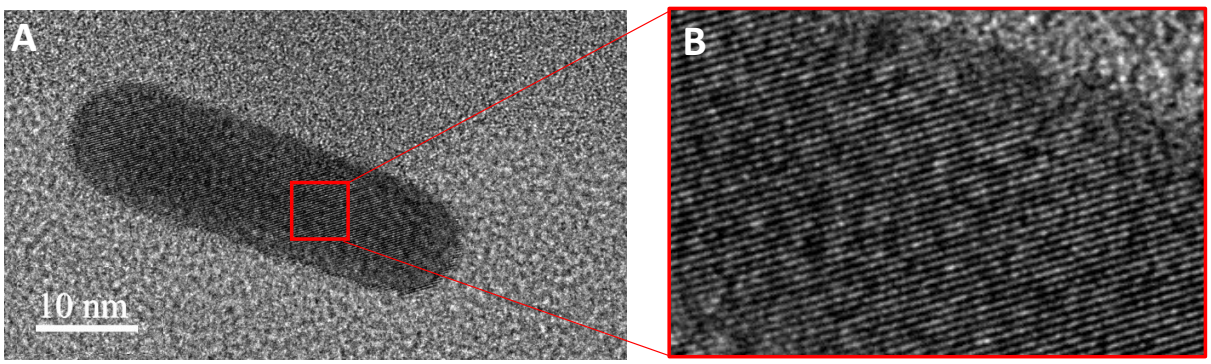
Investigations on the structure and chemical composition of nanoparticles have been carried out with a double corrected JEOL – ARM 200F Cold FEG Transmission Electron Microscope operating at 200 kV equipped with a JEOL Centurio EDS 1sr energy dispersive x-ray spectrometer (EDS). Gatan Digital Micrograph has been used for data processing (Fast Fourrier Transform, line intensity profiles) on TEM images while JEOL Analysis Station software has been used for EDS spectra and mapping.

High-Resolution TEM (Figure S1A,B) shows that the Au NRs are well crystallized and forms a monocrystal. FFT pattern (Figure 2E) and line profile (Figure S1D) converge for interplanar

distances that is are in good agreement with the one of pure gold oriented in the [111] axis (Figure S1C).

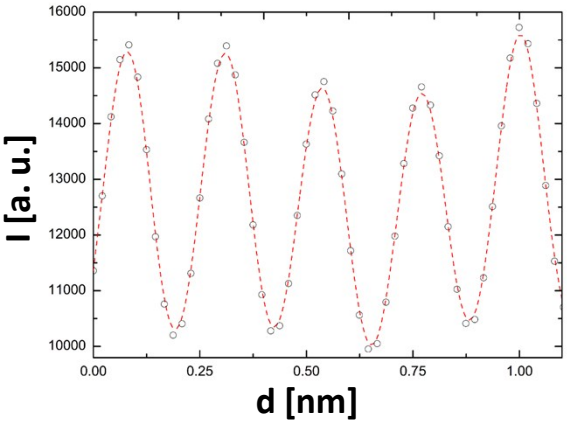
e. UV-Vis spectrometry

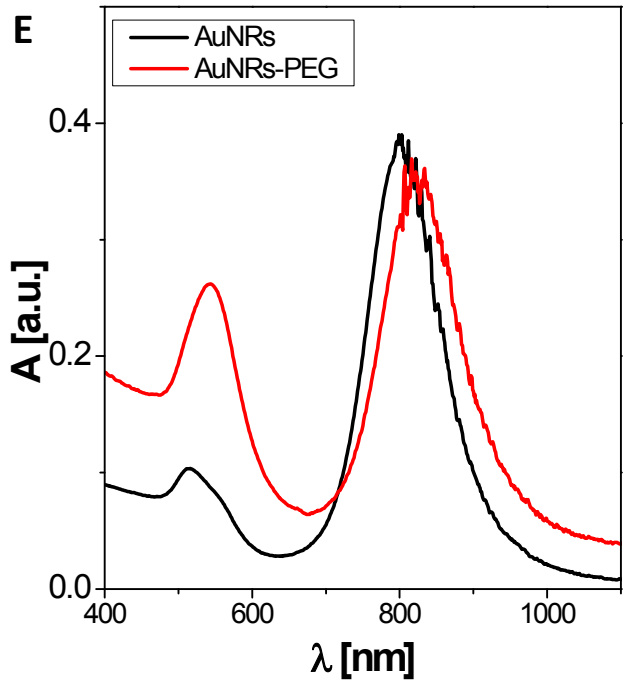
The absorption spectra for the aqueous solutions of the as prepared and after ligand exchange Au NRs were measured in the disposable 10 mm path PMMA cuvettes using spectrophotometer Shimadzu UV-3600 (300-1200 nm interval) (Figure S1E).



C

Measurement	d_{hkl} / nm
Theoretical	0.235
FFT	0.2303
Line profile	0.2304





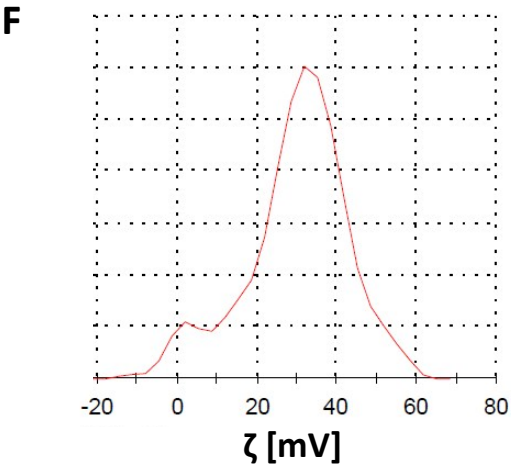


Figure S1. Characterization of Au nanorods. A. HRTEM micrograph of Au NR with B. high magnification micrograph, C. Table of theoretical and experimental interplannar distances, D. Intensity line profile from the interplannar distances. E. Absorption spectra of the for Au nanorods in water before (black) and after (red) ligand exchange using thiolated amino-PEG. F. Measured ζ-potential of PEGylated Au NRs (30 mV).

3. Synthesis and characterization of SubCaps and MicCaps

Submicrometric (**SubCaps**) and micrometric (**MicCaps**) polymer capsules modified with gold nanorods and coated with silica were synthesized as follows:

a. Synthesis of submicrometric capsules modified with Au nanorods coated with silica preloaded with bioactive compounds (SubCaps)

To synthesize submicrometric capsules modified with Au nanorods and coated with silica (**SubCaps**), submicrometric $CaCO_3$ templates were fabricated using co-precipitation reaction by mixing $CaCl_2 \cdot 2H_2O$ and Na_2CO_3 aqueous solutions in an ethylene glycol under magnetic stirring. For this, 2 mL of $CaCl_2 \cdot 2H_2O$ (0.33 M in a 5:1 (v/v) = EG: water solution) were firstly mixed with bioactive molecules to deliver (enlisted in *Table S1*). After this, 386 µL of Na_2CO_3 (0.33 M in a 5:1 (v/v) = EG: water solution) were added to the $CaCl_2$-cargo mixture and left under stirring at 1000 rpm for 30 min at room temperature. Formed submicrometric $CaCO_3$ templates were the mixed 3 times with Milli-Q water by centrifuging (8000 rpm, 3 min) to remove residual salts. To form capsule shell, Layer-by-Layer (LbL) and sol-gel methods were implied. For this, synthetic polyelectrolytes poly(sodium 4-styrenesulfonate) and poly(allylamine hydrochloride) at concentration 10 mg/mL in 0.05 M NaCl were used. Synthesized $CaCO_3$ templates were centrifuged (9000 rpm, 3 min), supernatant was the removed and 1 mL of negatively charged PSS polyelectrolyte solution was added. Dispersions were then sonicated for 3 min and shaken for 10 min. The cores were then washed two times with Milli-Q water. Afterwards, 1 mL of positively charged PAH was added to the cores, followed by 3 min sonication and 10 min shaking. The cores were again washed twice with Milli-Q water. After third PSS monolayer, the 200 µL at concentration 5 mg (Au)/mL of positively charged PEGylated Au nanorods were added to the negatively coated $CaCO_3$ templates, left for 3 min in sonication bath and shaken for 10 min. The cores with attached Au nanorods were then washed 2 times with Milli-Q water. As next, positively charged PAH solution was again added to the cores, followed by 3 min sonication and 10 min shaking. The cores were again washed twice with Milli-Q water. The deposition process was then

repeated 2 times, resulting in (PSS/PAH/PSS/Au NRs/PAH/PSS/PAH) capsule architecture. To reduce the permeability of capsules wall additional silica layer was deposit onto positively charged outer PAH polyelectrolyte layer. To do this, $CaCO_3$ templates were washed twice with Milli-Q water and one time with ethanol. Then, templates were added to the glass beaker containing 15.5 mL of ethanol and 4 mL of Milli-Q water under vigorous magnetic stirring at 700 rpm. Afterwards, 100 µL of TEOS and 100 µL of ammonium hydroxide were added to the particles and were left for 3 hours under magnetic stirring. The silica coated $CaCO_3$ templates were then washed twice with Milli-Q water. To dissolve $CaCO_3$ cores 1 mL of ethylenediaminetetraacetic acid (EDTA, 0.2 M, pH 6) was added to the $CaCO_3$ templates for 10 min. Obtained submicrometric capsules were finally washed twice with Milli-Q water (3500 rpm and 10 min) and dispersed in 1 mL of water.

b. Synthesis of micrometric capsules modified with Au nanorods coated with silica preloaded with bioactive compounds (MicCaps)

To obtain micrometric capsules modified with Au nanorods coated with silica, micrometric $CaCO_3$ templates were fabricated in co-precipitation reaction by mixing $CaCl_2$ and Na_2CO_3 aqueous solutions. For this, 615 µL $CaCl_2$ (0.33 M) was mixed with 2.2 mL of Milli-Q water and 300 µL of cargo (enlisted in Table S1). Then, 615 µL Na_2CO_3 (0.33 M) was added to the mixture under magnetic stirring at 1000 rpm for 30 sec. Formed micrometric sized $CaCO_3$ cores were washed 3 times with Milli-Q water by centrifuging (4000 rpm, 10 s) to remove residual salts. The micrometric capsules loading with bioactive compounds, the formation of the capsules shell and dissolution of $CaCO_3$ templates were performed as described in § 3.a. The concentration of used PAH and PSS polyelectrolyte solutions were 2 mg/mL in 0.5 M NaCl. Finally, micrometric capsules modified with Au nanorods and coated with silica capsules (**MicCaps**) were dispersed in 1 mL of water.

Table S1. Concentrations and molecular weights of loaded cargoes.

Cargo	**Added concentration c [mg/mL]**	**Molecular weight Mw [g/mol]**
Bovine serum albumin conjugated with tetramethylrhodamine (TRITC-BSA)	2	66 463
Dextran conjugated with AlexaFluor 647 (AF647)	2	10 000
Vincristine (VCR)	1	824.97

c. Synthesis of SubCaps and MicCaps postloaded with DAPI

Since the 4',6-diamidino-2-phenylindole (DAPI) used for intracellular nuclei staining possess low molecular weight, **SubCaps** and **MicCaps** DAPI loaded were performed with postloading method. For this, synthesized emply SubCaps and MicCaps (as described in § 3.a. and 3.b.) were mixed with 500 µL of 5 mg/mL DAPI aqueous solution and leaved in shaker for 2 h at 65˚C. Afterwards, **SubCaps** and **MicCaps** were washed 2 times and finally dispersed in 1 mL in Milli-Q water.

d. Scanning electron microscopy (SEM)

Scanning electron microscopy was carried out on a Zeiss SUPRA 40VP with an acceleration voltage of 1 kV. Day before measurements, 35 µL of capsules dispersed in water were dropped onto glass cover slip and let dry overnight.

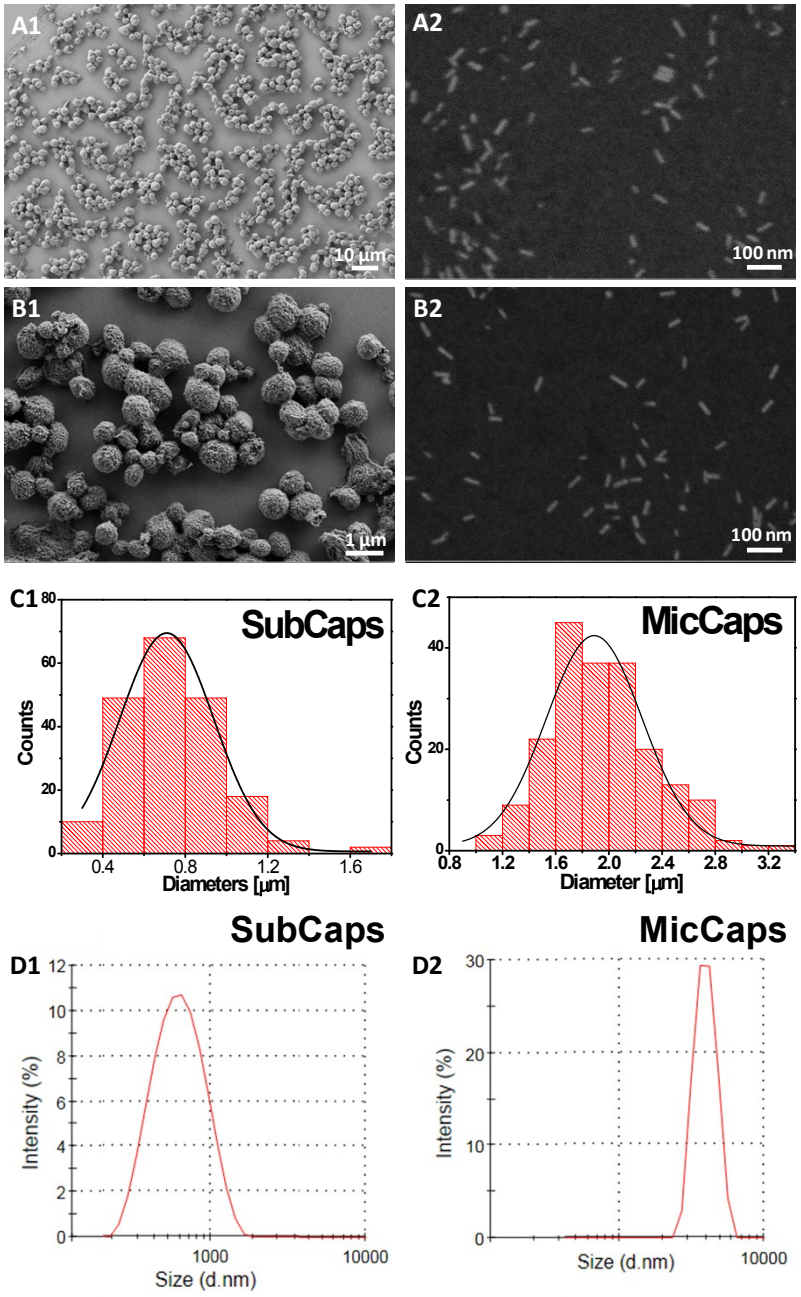


Figure S2. Representative SEM images of capsules. A1. **SubCaps** low magnification micrographs in SE mode, A2. **SubCaps** high magnification micrographs in BSE mode, B1. **MicCaps** low magnification micrographs in SE mode, B2. MicCaps high magnification micrographs in BSE mode. C1. Size distribution of **SubCaps** derived from SEM images. C2. Size distribution of **MicCaps** derived from SEM images. D1. Intensity-weighted distribution of the hydrodynamic diameters of **SubCaps** as determined with DLS in aqueous solution. D2. Intensity-weighted distribution of the hydrodynamic diameters of **MicCaps** as determined with DLS in aqueous solution.

e. Transmission electron microscopy (TEM) and Energy-dispersive X-ray spectroscopy (EDX)

High Angle Annular Dark Field (HAADF) micrograph (Figure S3A,D) shows a strong contrast between the capsule and the NPs that decorates it, which depicts a contrast in average atomic number. The EDS spectra (Figure S3B,E) reveals the presence of silicon, oxygen as well as carbon, sulfur, chloride from the polymer embedding the capsules and a weak signal from gold. However, the line scan shows that the signal of gold is associated with NPs (Figure S3C). The crystallographic investigations based on HRTEM (Figure S1A,B) and the data processing (FFT – Figure 2E) make appear obviously that the capsule is, unlike the Au NRs, amorphous.

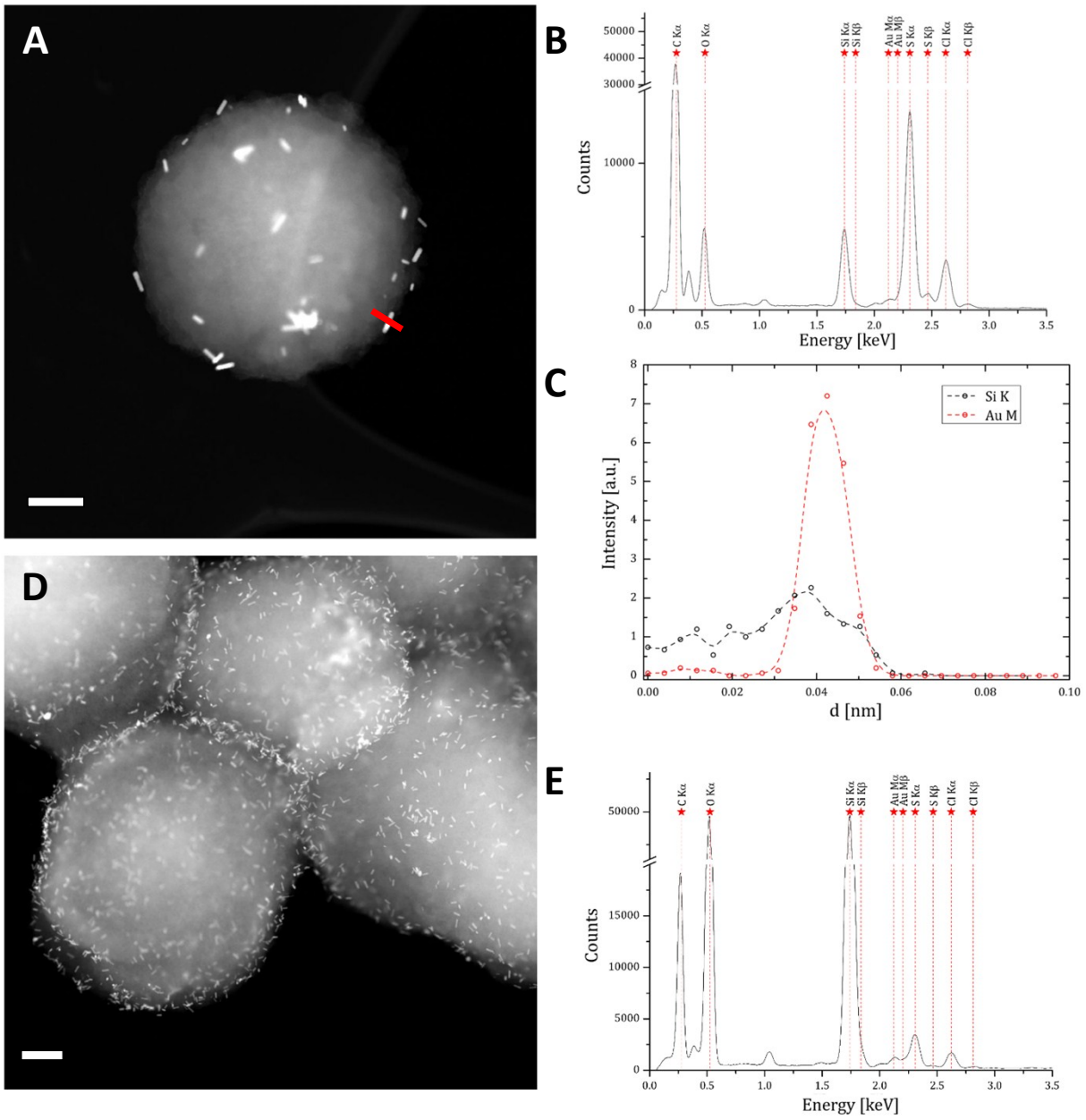


Figure S3: TEM of **SubCaps** and **MicCaps**. A. High Angle Annular Dark Field (HAADF) micrograph of **SubCaps** (scale bar corresponds to 100 nm) with B. EDS spectra of the whole particle and C. Intensity profile of Si and Au along the red arrow. D. High Angle Annular Dark Field (HAADF) micrograph of **MicCaps** (scale bar corresponds to 250 nm) with E. EDS spectra of the whole particle.

f. Confocal laser scanning microscopy (CLSM)

Representative bright field and confocal images were taken with a Carl Zeiss LSM 710 microscope. Objective EC Plan-Neofluar 40x/1.30 Oil DIC, Objective Plan-Apochromat 63x/1.40 Oil DIC and Leica TCS SP8 (Germany).

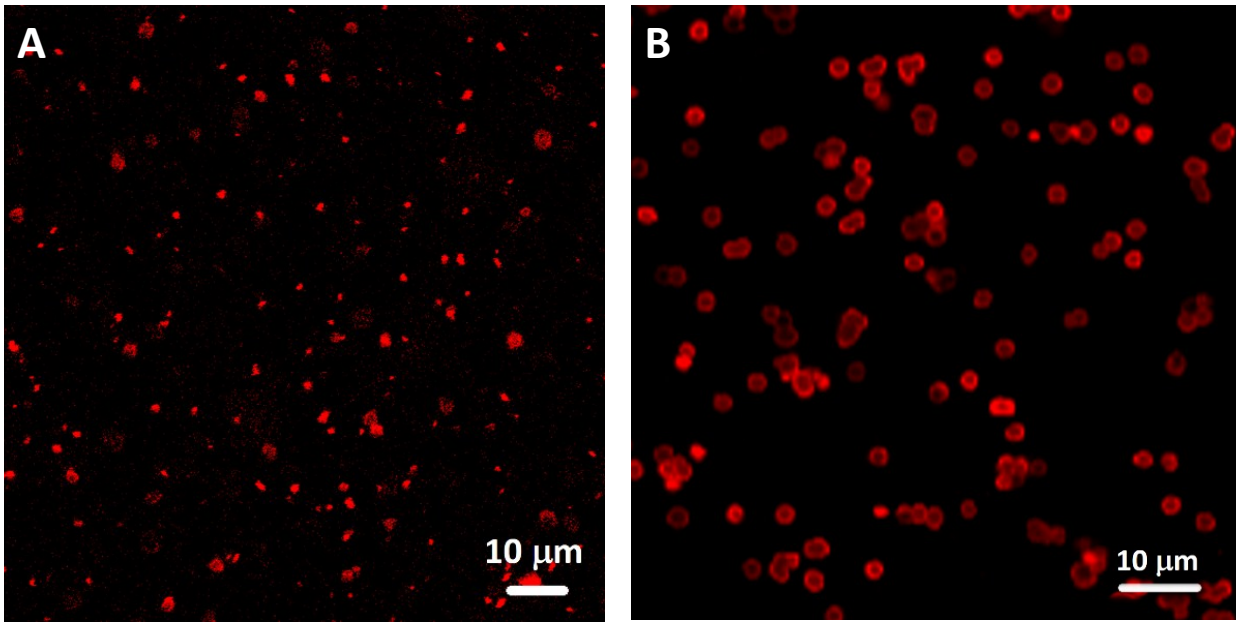


Figure S4. CLSM images of capsules. A. **SubCaps** loaded with BSA-TRITC, B. **MicCaps** loaded with BSA-TRITC.

g. Dynamic light scattering (DLS)

The hydrodynamic diameters of **SubCaps** and **MicCaps**, given as mean values from the intensity distribution of the nanoparticles were measured using a Zetasizer Nano ZS90 (Malvern, UK) equipped with a 4.0 mW He−Ne laser operating at 633 nm and with an avalanche photodiode detector, in the 173° backscattered mode. Before the measurements, the samples were highly diluted in aqueous solution. The measurements were performed at 25 °C.

4. Human mesenchymal stem cells (hMSCs)

Human mesenchymal stem cells (hMSCs) were derived from the bone marrow of healthy donors who gave their informed consent. Cells were isolated using a direct plating procedure. For this, 1 mL of heparinized bone marrow was resuspended in an alpha-MEM medium supplemented with 100 IU/mL penicillin, 0.1 mg/mL streptomycin, 10% of vol. fetal bovine serum, and 2 mM Ultraglutamin I. The hMSCs were cultured under standard conditions (37°C, 5% of CO_2, humidified sterile environment) to >85% confluency. Subsequently, hMSCs were detached with trypsin-EDTA solution (Sigma-Aldrich, UK) and passaged up to the second passage for further experiments.

Confirmation of the cell type was performed using flow cytometry (FACS Aria, BD, USA) with a multicolor antibody panel in accordance with the consensus criteria of the International Society of Cellular Therapy. The analysis shows that cell population expressed low levels (<5%) of hematopoietic markers such as CD45, CD34, CD14, CD20 and high levels (> 95%) of typical hMSC markers, i.e., CD105, CD90, CD73 (Figure S5).

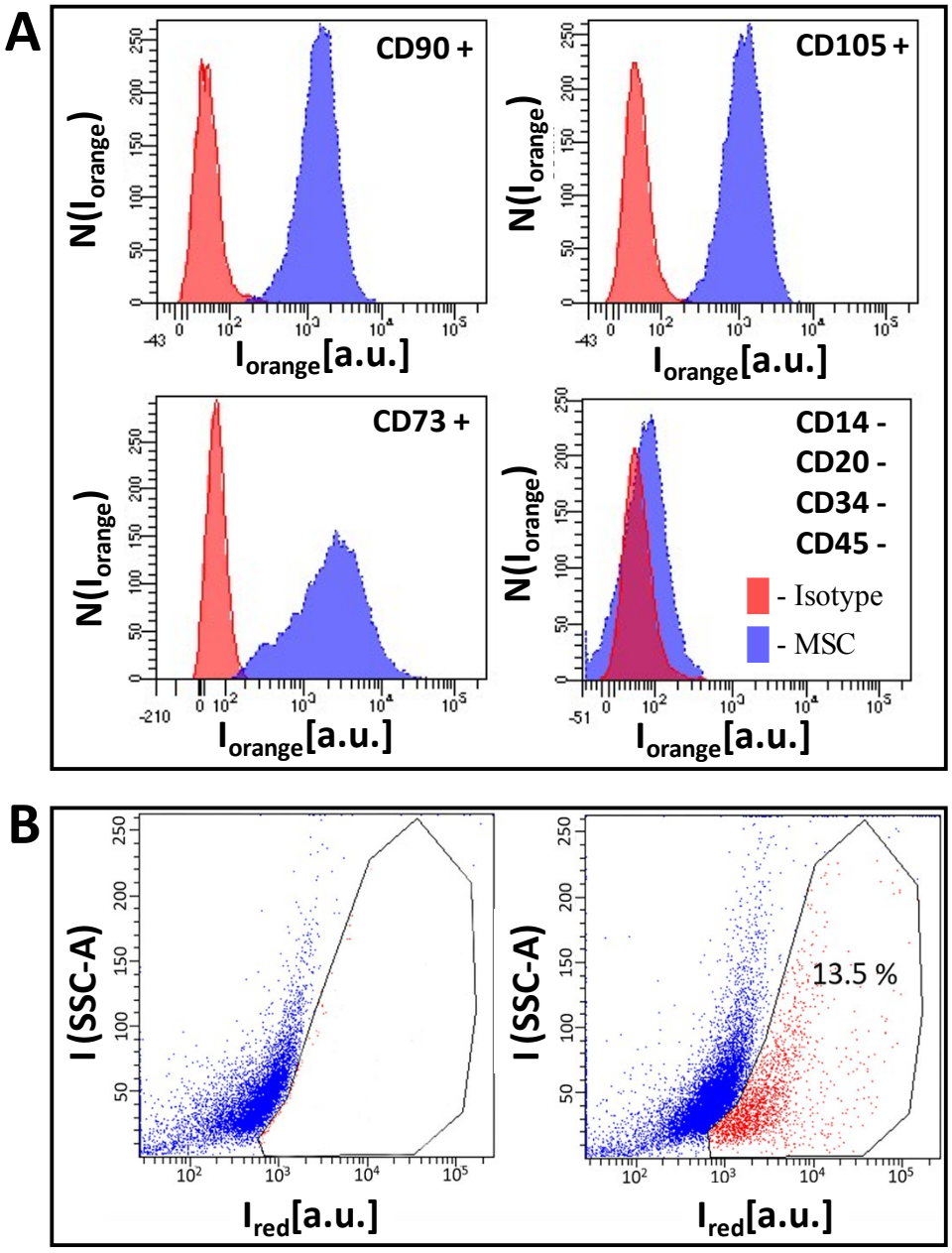


Figure S5. Surface markers of human mesenchymal stem cells (hMSCs). A. Expression of markers in hMSCs was analyzed by flow cytometry. From left to right: CD90, CD105, CD73, and mixture of CD14/CD20/CD34/CD45. Red histograms – cells strained with isotype control; blue histograms – cell isolated with specific antibodies. B. Cell surface expression of CXCR4 was confirmed using flow cytometric analysis. hMSCs were stained with control antibody (isotype) and antibody to CXCR-4 (CXCR-4 PE).

a. Capsules-hMSCs uptake study using CLSM

In order to study the **SubCaps** and **MicCaps** uptake, hMSCs were seeded in 24-well plate with cover glasses (1.9 cm^2 surface area per well, 1 mL of medium per well). After 24 hours capsules loaded with BSA-TRITC were added at the following capsules/cell ratio: 1:1, 3:1, 5:1, 10:1, and left for another 24 hours. Afterwards, cells were fixed and stained with DAPI and Phalloidin conjugated with Alexa Fluor 488. Fluorescence micrographs of cells with the internalized capsules were obtained with a confocal microscope (Leica TCS SP8 (Germany) with Objective EC Plan-Neofluar 40x/1.30 Oil DIC and equipped with a helium-neon laser 543 nm (for BSA-TRITC labeled capsules visualization), an argon laser 488 nm (for visualization of cell membrane labeled with Phalloidin conjugated Alexa Fluor 488), and diode laser emitting at 405 nm (for visualization of nuclei labeled with DAPI). The pinhole was set to 1 Airy unit. All images were obtained and analyzed in Z-stack mode. In order to determine the number of internalized capsules per cell, capsules in at least 50 different cells were counted in each experiment, which was repeated twice. To distinguish capsules inside and outside of the cell, a red fluorescent signal from TRITC was analyzed. If it was surrounded by green fluorescence signal coming from a stained cell membrane, such a capsule was considered internalized. The data of this experiment is represented as a diagram, showing the number of internalized particles per cell (Figure 3B).

b. Capsules-hMSCs association study using flow cytometry

For cell-capsules association assay, the hMSCs were seeded into 24-well plates at an amount of 40 000 cells/well (1.9 cm^2 surface area per well, 1 mL of medium added per well) and incubated for 24 hours at 37 °C, 5% CO_2. Afterward, capsules loaded with BSA-TRITC were added at the following capsules per cell ratio: 1:1, 3:1, 5:1, 10:1. After additional 24 hours of incubation, cells were washed thrice with PBS in order to discard the non-internalized capsuled. Then cells were detached with a trypsin-EDTA solution, which was later neutralized with culture medium supplemented with FBS. The cell suspension was collected into FACS tubes and centrifuged for 5 minutes at 300 x g. Afterward, the supernatant was discarded and the cells associated with

capsules were dispersed in PBS. Subsequently, cells were analysed with flow cytometry (FACSAriaIII, equipped with a helium-neon laser emitting at 543 nm). Side and forward scattering signals were used in order to exclude debris and cell duplets by appropriate gating, which left only the single cells population subject to analysis. In addition to the scattering signal, the red fluorescence originating from associated TRITC-labeled capsules was recorded for all events. Each event in which the red fluorescence signal was stronger than that of the negative control cells, was considered as a cell associated with capsules. The result of the experiment is represented as a percentage of cells associated with capsules plotted against the seeded capsules/cell ratio (Figure S6).

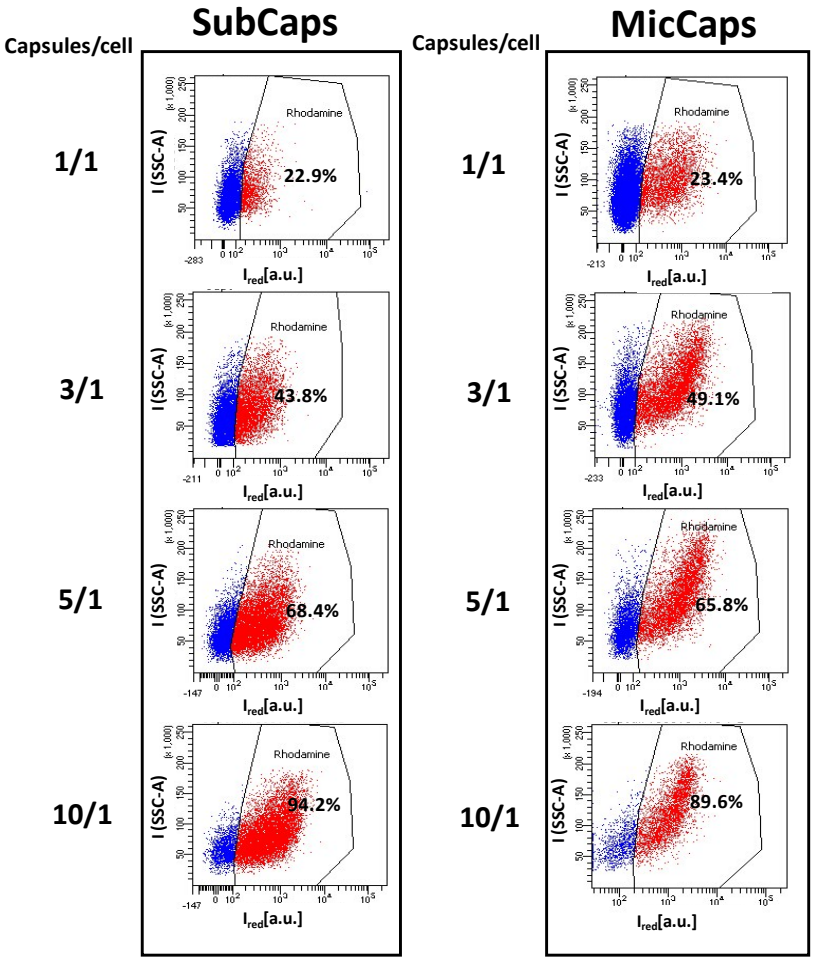


Figure S6. Flow cytometry. The fractions of hMSCs associated with capsules at different cell-to-capsule ratios.

5. Calculation of amount of encapsulated vincristine (VCR)

Absorbance spectra of VCR was used to estimate the amount of encapsulated cargo. For this, after the formation of $CaCO_3$ core-particles with embedded VCR, $CaCO_3$ cores were spun down at 4000 rpm for 10 sec and supernatant was then collected. UV-Vis spectrum of supernatant was measured with fluorometer (Cary Eclipse, Agilent). To calculate the concentration of encapsulated VCR, calibration curve was measured and plotted (known drug concentrations versus absorbance maximum at λ = 298 nm, Figure S7). Thus, knowing the absorbance maximum of encapsulated drugs, the amount of encapsulated cargo was calculated based on the calibration curve.

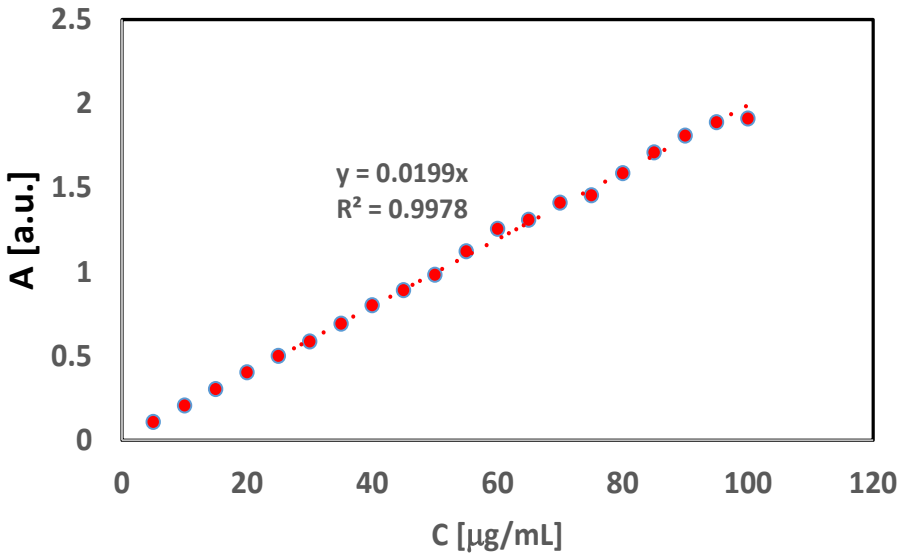


Figure S7. Calibration curve of VCR. Absorbance of VCR at 298 nm measured at known concentrations.

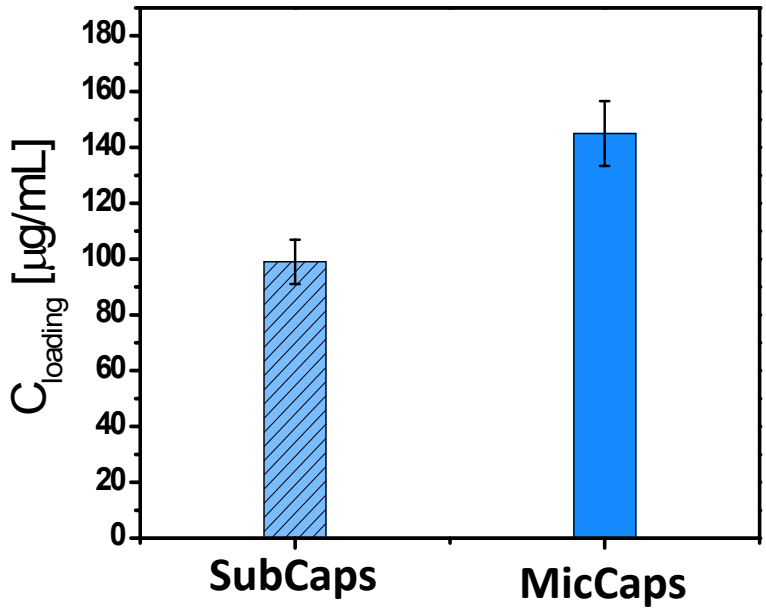


Figure S8. VCR loading. Loading efficiency of VCR into **SubCaps** and **MicCaps**.

6. NIR-laser drug release study

The release of DAPI and VCR was probed with a direct laser writing setup as depicted in Figure S9. A Ti:Sa oscillator (TiF-100, Avesta Project) seeding pulses centered at 820 nm with 50-60 fs pulse duration and 80 MHz repetition rate was expanded and focused on the samples using collecting lens with the focal distance 50 mm. The plate with capsules was mounted on motorized stage for scanning. The same amount of capsules ($2x10^7$) was added to the 48-well plate (V=500 µL, s=1.9 cm^2). After that, NIR-laser was employed to irradiate capsules during different periods of time (5, 6, 8, 10, 14, 28 min) within defined surface area.

The amount of released cargo was measured with UV-Vis spectrometer at $\lambda = 298$ nm. As control, the same amount of capsules was not irradiated with laser. Finally, the percentage of released drugs from capsules was plotted versus time of irradiation.

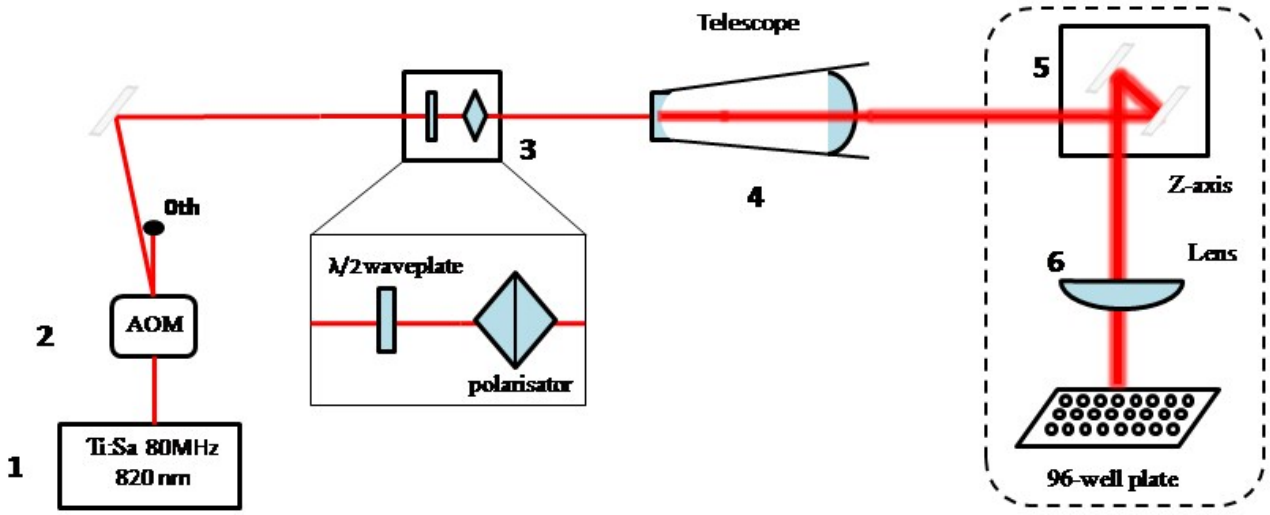


Figure S9. Direct laser writing (DWL) setup used for sample irradiation. 1. Ti:Sa oscillator, 2. Acousto-optic modulator, 3. Attenuator - half-wave plate with polarizer, 4. Beam expander, 5. Galvoscanner, 6. Lens.

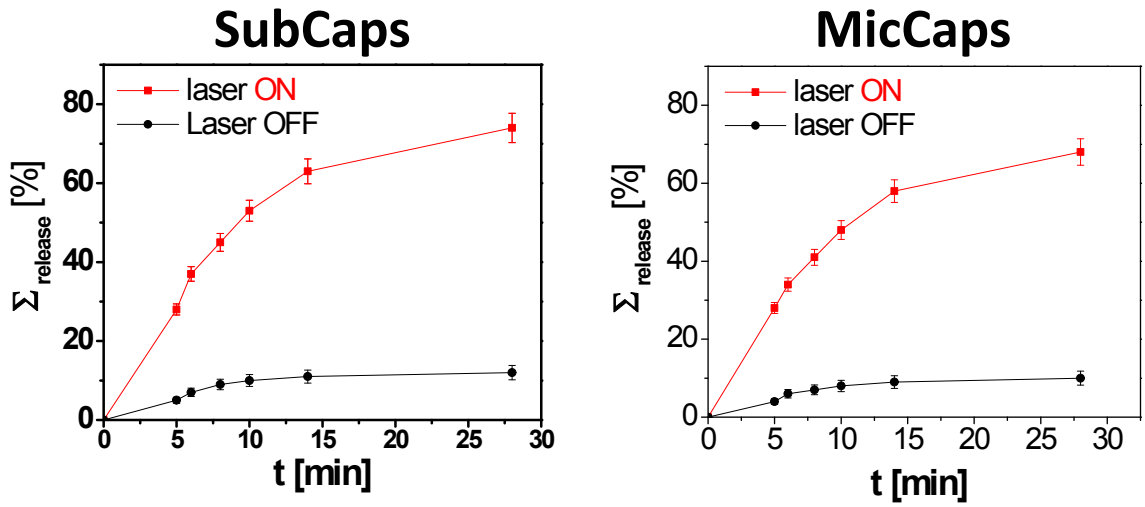


Figure S10. VCR release. Percentage of released antitumor drug VCR from A. **SubCaps** and B. **MicCaps** irradiated during different time periods (red curve) and without irradiation (black curve).

7. Remote activation of bioactive compound (DAPI) inside hMSCs

DAPI was used to visualize remote release of bioactive compounds from **SubCaps** and **MicCaps** inside living hMSCs via nuclei staining. The remote release of DAPI from **SubCaps** and **MicCaps** was performed with setup described in §6. hMSCs were cultivated in Petri dishes (8.8 cm^2) at amount 5000 cells/well. Next day, **SubCaps** and **MicCaps** were added to the cells at amount 10 capsules/cell and cells were leaved in incubator for another 24 h. Then, defined area (1 cm^2) of the Petri dish with attached cells was exposed to laser radiation (820 nm). Under the transmission view of the cells with internalized capsules, the laser beam was focused to the same plane of transmission channel. Following parameters were used to irradiate the cells with internalized capsules: an average laser power = 250 mW, scan speed = 0.1 cm/sec. The average power density of 1.5 mW/μm^2 was used for the capsule irradiation. The hitting of capsules occurred randomly inside scanned area. As controls, cells with **SubCaps** and **MicCaps** without irradiation were used. After laser irradiation, cells were visualized with CLSM after 1 h after irradiation (Leica TCS SP8, Germany, diode laser 405 nm). Control cells were placed in the incubator at 37°C and 5% CO_2 overnight and next day also visualized with CLSM. CLSM images before and after irradiation are presented in the Figure 3.

8. hMSCs viability study

To study toxicity of **SubCaps** and **MicCaps**, LIVE/DEAD assay was used. For this, hMSCs were pre-treated with VCR loaded **SubCaps** and **MicCaps** added at different capsule/cell ratios (1:1, 1:3, 1:5, 1:10) and further seeded in 48-well plates at amount 1.5 x 10^4 cells per well and incubated for 24 h, and 48 h. After that, the cells were detached with 0.25% trypsin-EDTA and stained with Calcein AM (live dye, 0.2 µM) and Propidium iodide (dead dye, 3 µM). After 30 min of staining, the hMSCs were then immediately observed under confocal microscope (Carl Zeiss LSM 710). To visualize live cells (cells stained with Calcein AM), argon laser emitting at 488 nm was used. To visualize dead cells (cell nuclei stained with Propidium iodide), helium neon laser emitting at

543 nm was used. The confocal pinhole was set to 1 airy unit and images were taken with an Objective EC Plan-Neofluar 40x/1.30 Oil DIC. Finally, percentage of living cells were plotted versus added amount of VCR (Figure 4).

9. Spontaneous migration study

Spontaneous migration of hMSCs modified with **SubCaps** and **MicCaps** was studied with lifetime and scratch wound assay.

a. Lifetime study of hMSCs spontaneous migration using optical microscopy

The mobility of hMSCs was studied using the Cell-IQ experimental analytical system based on optical microscopy. For this, hMSCs were seeded into 48-well plates (300 μL of medium added per well, 0.95 cm^2 surface area per well). The next day, capsules were added to the cells at different ratios of 1:1, 5:1, 10:1 capsules per cell. As a control, cells without capsules were used. To assess migration, cells were placed into the Cell-IQ imaging system at 37˚C and 5% CO_2. Different areas of interest (ROI) were selected for further visualization and analysis. Every hour, a snapshot of cells was taken in the study area within 24 hours. Cell-IQ software, after analyzing a series of shots, has formed cell migration paths. The combined trajectories of all cells in the image, their speeds and motion vectors were also determined (Figure 5A).

b. Scratch wound assay

Cells were grown in 48-well plates (300 μL of medium added per well, 0.95 cm^2 surface area per well) until they form a monolayer. Next, the bottom of each well containing hMSC was scratched with a sterile pipette tip. Then the medium (300 μl) was changed in each well, the wells were pre-washed with PBS. Cell migration was estimated using the Cell-IQ imaging system in the same way as in the previous method. The open wound area during time of cell incubation was calculated using the software in Cell-IQ imaging system and plotted (%) open wound area versus time incubation (Figure 5B). Three replicates of two independent experiments were averaged.

10. Directed migration of hMSCs

a. Expression of CXCR4 receptors on hMSCs surface.

The ability of hMSCs to migrate is mostly defined by the availability of surface receptor CXCR4. The Expression of cell surface CXCR4 on hMSCs was detected with phycocerythrin (PE)-conjugated monoclonal anti-CXCR4 (BD Biosicence, USA). Monoclonal antihuman CXCR4-PE (5 μL, 10 μg/mL, 1 h staining) was used to label cells for flow cytometer to determine percentage of CXCR4 positive cells (Figure S5B).

b. Inverted invasion assay (transwell invasion assay)

To measure invasion ability of hMSCs impregnated with capsules towards a gradient of SDF-1, Boyden chamber assay were experiments were carried out. In order to analyze the influence of capsules on cell movement and invasion process commercially available Transwell permeable inserts (Corning) were used (Figure S11). **SubCaps** loaded with VCR were internalized with hMSC through mixing and cultivating under standard conditions for 16 hours at cell-to-capsule ratio of 1:10 in 24-well plate. After that, cells containing VCR loaded **SubCaps** were detached from cultural flask using Triple-E solution (Gibco), counted and placed in complete growth medium to the upper membrane part. SDF-1 was dissolved in complete growth medium and placed to the lower part of the membrane. As controls, (i) hMSCs with empty capsules and (ii) without capsule pretreatment was added to the upper membrane part together with SDF-1 in lower. In case of negative control, (iii) SDF-1 was not added to the lower part of the membrane. Cells were incubated overnight at 37˚C and 5% CO_2. After incubation, lower part of the transwell was gently washed twice with PBS solution and stained with 0.2 μM of Calcein AM (Sigma) for 1 hour. Cell calculation were perform using CLSM (Carl Zeiss LSM 710) by analysis of 10 pictures from each sample with ImageJ software.

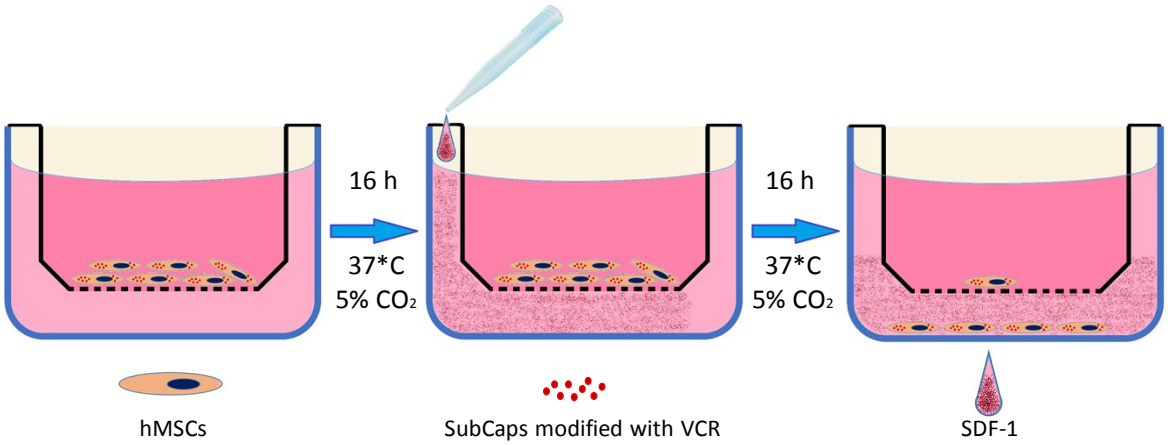


Figure S11. Transwell assay. Schematic illustration of inverted transwell assay procedure.

c. Chemotaxis assay

To study directed migration of hMSCs, commercially available µ-Slide Chemotaxis chambers (Ibidi) were used (Figure S12). Cells were seeded together with **SubCaps** loaded with VCR at amount 10 capsules per cell and were incubated overnight in standard conditions into 24-well plates (40 000 cells/well). Then, cells were washed twice with PBS and detached from cultural flasks with trypsin-EDTA solution. hMSCs were counted with hemocytometer and 6 µL of cell suspension containing 1000 cells was introduced into the middle ports of chamber. Chamber was then placed in incubator into a 10 cm petri dish for 4 hours. After cell attachment, 65 µL of chemoattractant-free medium was introduced into the side port to fill whole chamber. The chemoattractant was added to solution at the concentration 100 ng/mL into the chemoattractant-side. Finally, one side of chamber contained chemoattractant, whereas another was chemoattractant-free. As controls, both sides were chemoattractant-free and contained chemoattractant.

Chambers were then observed under CLSM (Carl Zeiss LSM 710) each 1 hour for 2 days to obtain images of migrating cells. Based on image series, migration of cells was then analyzed with software tool for data analysis from time stack chemotaxis experiments, based on the National Institute of Health's (NIH) ImageJ image processing system.

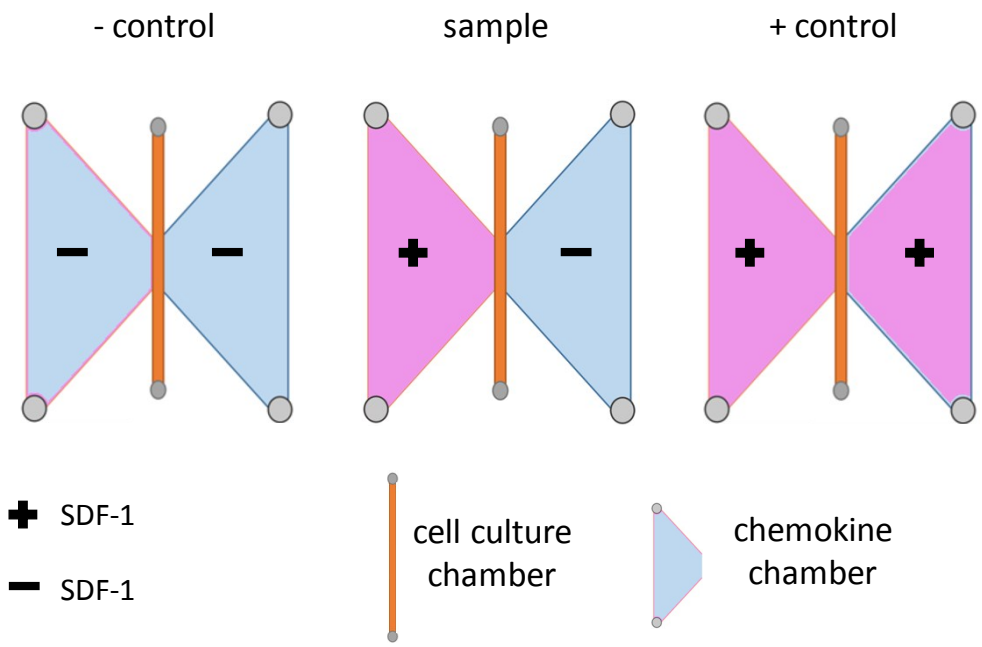


Figure S12. Chemotaxis assay. Design of the μ-Slide Chemotaxis.

11. Proliferation of hMSCs modified with SubCaps

The effect of SubCaps loaded with TRITC-BSA on the cell proliferation was studied at different time periods (24, 48, 72 and 96 h). SubCaps-hMSCs mixture was seeded into 48-well plate and placed at 37˚C and 5% CO_2. After incubation, hMSCs were washed twice with PBS and stained with the Calcein AM (Invitrogen) at concentration 10 μM in PBS for 30 min in order to visualize hMSCs (Carl Zeiss LSM 710, argon laser 488 nm). The obtained images with cell nuclei were examined using the FIJI cell counter java application to count the number of cells. The total number of evaluated images was 10. All measurements were made in triplicate, and error bars corresponded to the standard deviation.

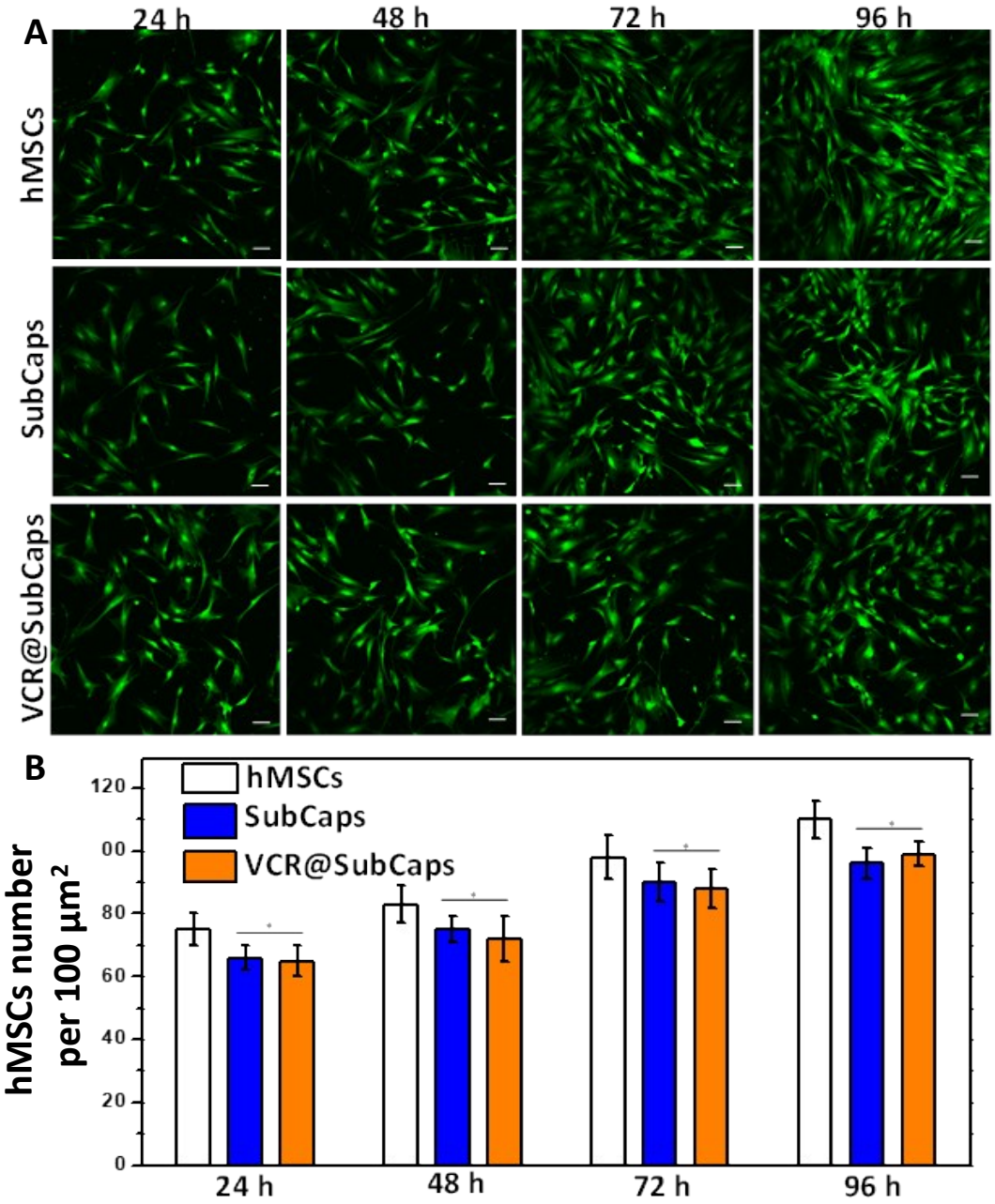


Figure S13. A. CLSM images of hMSCs stained with calcein AM (green) incubated with SubCaps and VCR@SubCaps added at 10 capsules/cell after 24, 48, 72 and 98 h of incubation. B. Number of proliferated hMSCs with and without SubCaps and VCR@SubCaps derived from CLSM images.

12. Experiments with melanoma spheroids

a. Melanoma cells

In order to develop tumor cell cultures in vitro biopsy samples from solid tumor patients (n=11) were used. Cells were cultured (medium: 80% DMEM/F12, 20% FBS, insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), antibiotics (streptomycin with penicillin at a concentration: 100 μg/ml and 100 units/ml, respectively); 37 ° C, 5% CO2, 100% humidity) until reaching 80% confluent monolayer. Then cells were reseeded to the 25 cm2 vials (5 mL medium and $1x10^6$ cells) using 0.25% trypsin solution. Afterward, cell cultures were considered ready for further experiments.

RNA Extraction and reverse transcription

For RNA extraction from harvested primary patient-derived cells (MSCs and tumor cells) TriZ reagent was used in accordance with the manufacturer's instructions. The quantity and purity of RNA were determined using NanoDrop 2000 (Thermo Scientific, USA). For further studies only samples with the 260 nm/280 nm ratio higher than 1.7 were used. The first-strand cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (LifeTechnologies, USA) from 1 μg of total RNA following the product protocol.

CXCL12 expression analysis with quantitative real-time polymerase chain reaction (qPCR).

For real-time qPCR SYBR Green was used as a detection chemical. The reaction conditions were: 10 μl of 2.5x SYBR green PCR master mix containing dNTP mix 2.5mMeach 10xPCR buffer, 5 Units of Taq DNA polymerase and 2.5 mM MgCl2 (Syntol, Russia), 300 nM of forward primer, 300 nM of reverse primer, and 5 μl of cDNA template per each well in a total reaction volume of 25 μl. The qPCR was carried out with the CFX96 thermocycler instrument (Biorad, USA) and the following amplification protocol: 95°C for 10 min; 95°C for 15 s, 60°C for 1 min for 40 cycles. Each sample was analyzed in triplicate. Each primer assay was conducted with No template

controls (NTC). Relative quantification values of CXCL12 and other tumor genes versus TBP and ABL genes expression were measured with following primers:

Table S2. Primers sequences used in qPCR analysis of tumor cells.

Gene	**Primers (forward primer; reverse primer)**
GAGE	5'-AGCTGCTCAGGAGGGAGAGGAT-3' 5'-GGTGACCCTGTTCCTGGCTA-3'
HAGE	5'-GCCACAAGTGCCATGTCAAA-3' 5'-CCTTCAAGTCATCCCACGTT-3'
MAGEA1	5'-GAAGGAACCTGACCCAGGCT-3' 5'-AATCCTGTCCTCTGGGTTGG-3'
NY-ESO-1	5'-TCTGAAGGAGTTCACTGTGT-3' 5'-AGACAGGAGCTGATGGAGAG-3'
PASD1	5'-GTGGGAAATGTTTGCATTCT-3' 5'-AGCTTCATCACTGACTGCCT-3'
SCP1	5'-AAAAGGAACAGAACAAGAAC-3' 5'-TGTGGTAATGGCAGTTAACT-3'
SEMG1	5'-TCCTCATCTTGGAGAAGCAA-3' 5'-TGGGAAAATTCACTTGGTAA-3'
SLLP1	5'-ACTTCGGGCTGGACGGATAC-3' 5'-GCGTTGAAACCGCTTGTGAA-3'
SPANXA1	5'-GAGGAGCGTCCCCTGTGATT-3' 5'-AGCAGGTTGCGGGTCTGAGT-3';
SSX1	5'-GTATATGAAGAGAAACTATAAGG-3' 5'-TATTACACATGAAAGGTGGG-3'
CXCL12	5'-*TGCCAGAGCCAACGTCAAG*-3' *CAGCCGGGCTACAATCTGAA*-3'
TBP	5'-*GAGCTGTGATGTGAAGTTTCC* -3' 5'-*TCTGGGTTTGATCATTCTGTAG*-3'
ABL	5'-GCTGCTCGCTGGAACTCC-3' 5'-GTGATGTAATTGCTGGGGACC-3'

Each sample was analyzed in triplicate. The relative genes expression levels were calculated using the comparative 2-ΔCt method with normalization to the Ct value of TBP and ABL reference gene, results were expressed in percentage and shown in Figure S13.

Tumors immunocytochemistry

To prepare samples for immunocytochemical studies, tumor cells were seeded on 8, 4, and 2-well culture plates with complete medium (80% DMEM/F12, 20% FBS, antibiotics (streptomycin with penicillin at a concentration: 100 μg/ml and 100 units/ml, respectively). When the 80% confluent monolayer was reached, cells were washed with PBS and fixed with absolute acetone (5 minutes).

Afterward, cells were dried and stored at -30°C in an airtight container until cooling. Then samples thawed at room temperature and were left in distilled water for 2 minutes. After washing samples were processed using DAKO® Peroxidase Blocking Reagent before immunocytochemical staining. Thermal unmasking of antigens was performed for 5 minutes at a temperature of 90 ° C in two buffers: EDTA buffer (pH 9.0) and citrate buffer (pH 6.0). Afterward, samples were immediately cooled in distilled water of room temperature to stop the reaction. Then cells were incubated with poly- and monoclonal antibodies in a humid environment for 1 hour at 37°C. After incubation samples were washed with Tris-Buffered saline (TBS) and incubated with secondary antibodies with the streptavidin-peroxidase complex in accordance with product protocol (DAKO Agilent Technologies, USA).

For the detection of the antigen-antibody reaction was used diaminobenzidine buffer solution, which was performed after treatment with alcohol solutions (ethyl 95°, isopropyl) and xylene mixture of isomers and placed under cover glass using Canadian balsam. To exclude stromal elements contamination of tumor cells, anti-fibroblast antibodies were used.

Obtained data are shown in Figure S13.

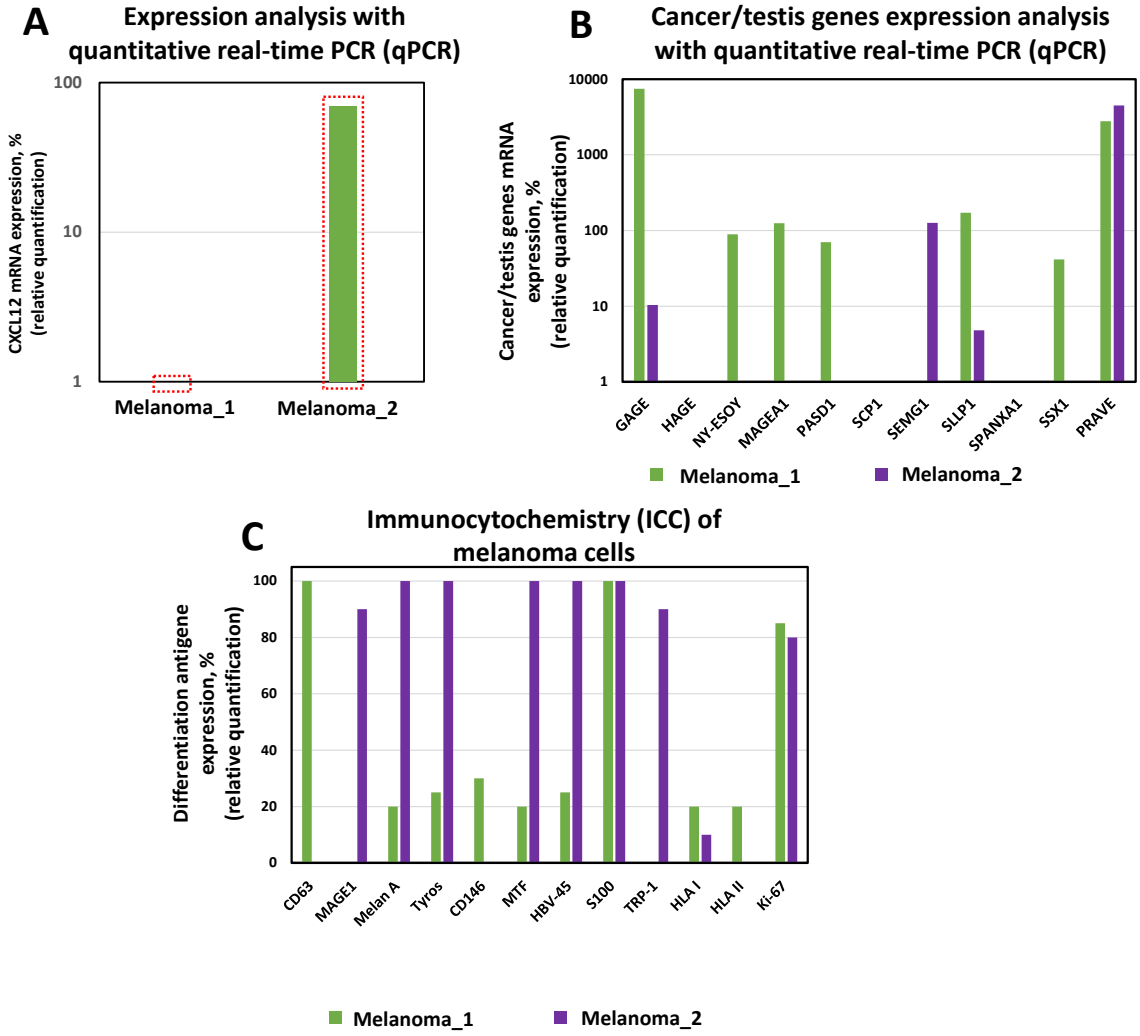


Figure S14. Characterization of melanoma cells. A. CXCL12 mRNA expression analysis with qPCR in various melanoma. Relative quantification values of CXCL12 versus TBP/ABL gene expression are reported. The increased level of CXCL12 is evidenced in melanoma_2 cells. B. Cancer/testis genes expression analysis using qPCR. The increased level of GAGE, NYESO1, MAGEA1, PASD1, SLLP1, SSX1, PRAME is evidenced in melanoma_1 , GAGE, SEMG1, SLLP1, PRAME is evidenced in melanoma_2; B) Immunocytochemistry (ICC) of melanoma cells (melanoma_1 and melanoma_2). Differentiating antigens CD63, MelanA, Tyrosinase, CD146, HBV-45, TRP-1, MITF, S100, gp100, HLA class I, II and proliferated cells markers were included in IHC diagnostic panel.

b. Formation of tumor spheroids

"Hanging drop" technique was used to form tumor spheroids. For this purpose, such a concentration of cells was created so that there were 3000 cells (2400 tumor cells and 600 hMSC) in 15 µl. A 15 µl drop of the formed droplet was placed on the inside of the lid of a 35 mm petri dish, to which 1 ml of PBS was previously added to maintain humidity. Then petri dish. The petri dish was then incubated at 37 ° C and 5% CO^2. The culture medium inside the drop changed every 3 days, and after 6 days the formed tumor spheroids were carefully washed with DMEM.

c. Migration and invasion of hMSCs loaded with AF647 into spheroid 3D

In a 25 cm^2 culture flask, 10^5 hMSK and 10^6 **SubCaps** labeled with AF647 were seeded and left overnight at 37°C, 5% CO_2, in order to further study the migration and invasion of hMSC. Then the cells were washed three times with PBS, stained with TRITS and detached from the plastic using trypsin-EDTA solution. After 5 minutes, culture medium was added to trypsin to inactivate it. Next, the cells internalized with the capsules were poured into a centrifuge tube and centrifuged for 5 minutes at a speed of 300 g. Then the supernatant was poured out, and the hMSCs was re-suspended in 1 ml of new culture medium. Cell counting was performed using a hemocytometer, then 1000 hMSCs and 1 tumor spheroid (stained with 0.2 µM calcein AM) were mixed with 1 mg/ml collagen solution. After that, the mixture of collagen cells was added to a pre-coated thin layer of agarose (1%) with a 35 mm Petri dish and incubated at 37 ° C, 5% CO^2. Migration and cell invasion were observed at different points in time (0, 24, 48 h) using a laser scanning confocal microscope (CLSM, Zeiss 710). The cells pre-stained with Calcein AM were visualized using an argon laser with a wavelength of 488 nm. To visualize hMSCs stained with TRITC, a helium neon laser 543 nm was used. To visualize AF647 loaded capsules, a helium neon laser 633 nm was used. Objective EC Plan-Neofluar 40x/1.30 Oil DIC was used for imaging (Figure S14). Next, we plotted the total number of hMSCs associated with the tumor spheroid.

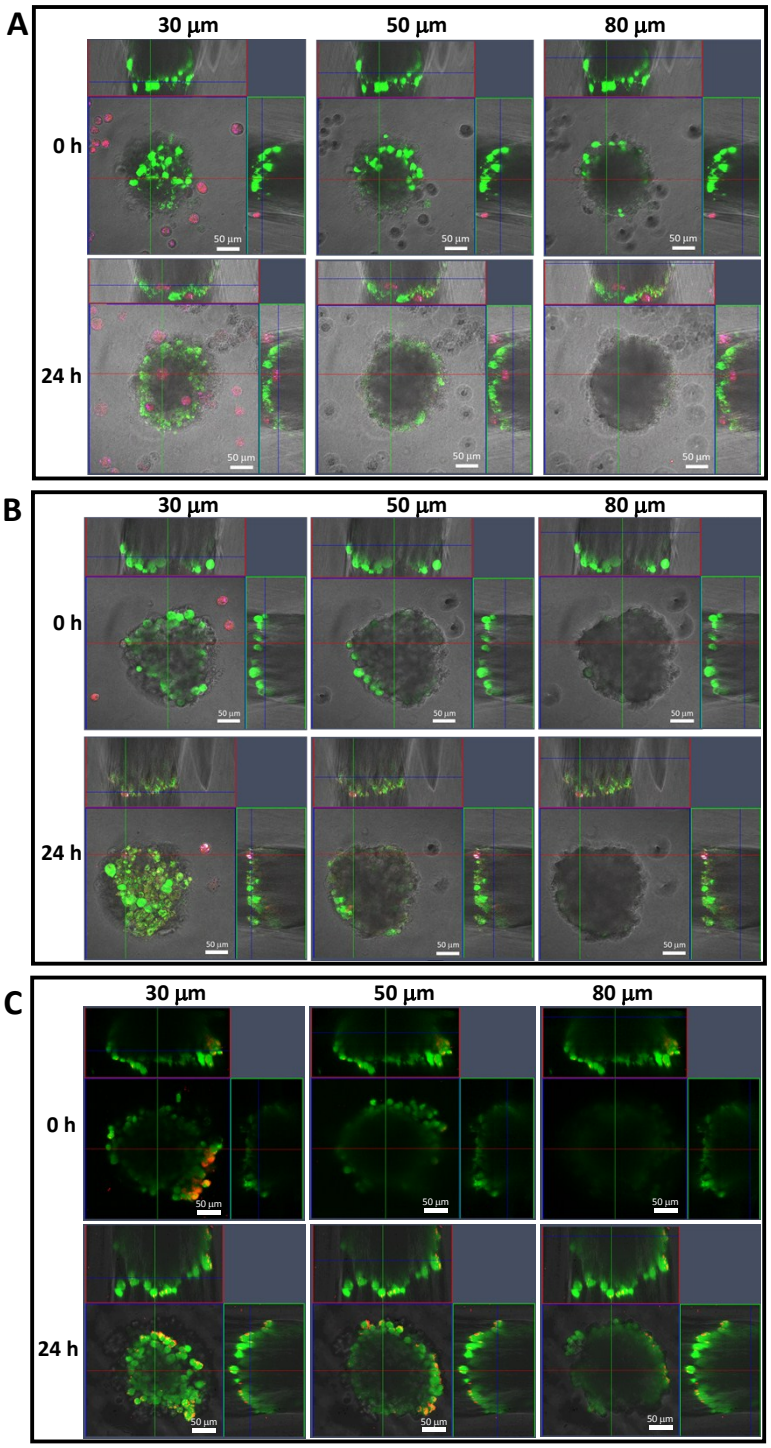


Figure S15. Invasion of hMSCs. Z-stack CLSM images of A. tumor spheroids melanoma_2 incubated with hMSCs modified with SubCaps loaded with AF647, B. tumor spheroids melanoma_1 incubated with hMSCs modified with SubCaps loaded with AF647, C. tumor spheroids melanoma_2 incubated with SubCaps loaded with AF647.

d. 2D viability of tumor cells

In order to study the cytotoxic effect of tumor cells on anticancer drug (vincristine), 2 selected cell lines (melanoma_1 and melanoma_2) were seeded into 48-well plate at an amount of 15 000 cells per well (300 µL of complete medium added per well, 0,95 cm^2 surface area per well) and left overnight. Then vincristine was added to into each well to achieve different concentrations: from 1 ng/mL - 16 ng/mL. After 24 hours of incubation with vincristine LIVE/DEAD assay with CLSM was performed as described in §8 (Figure S15).

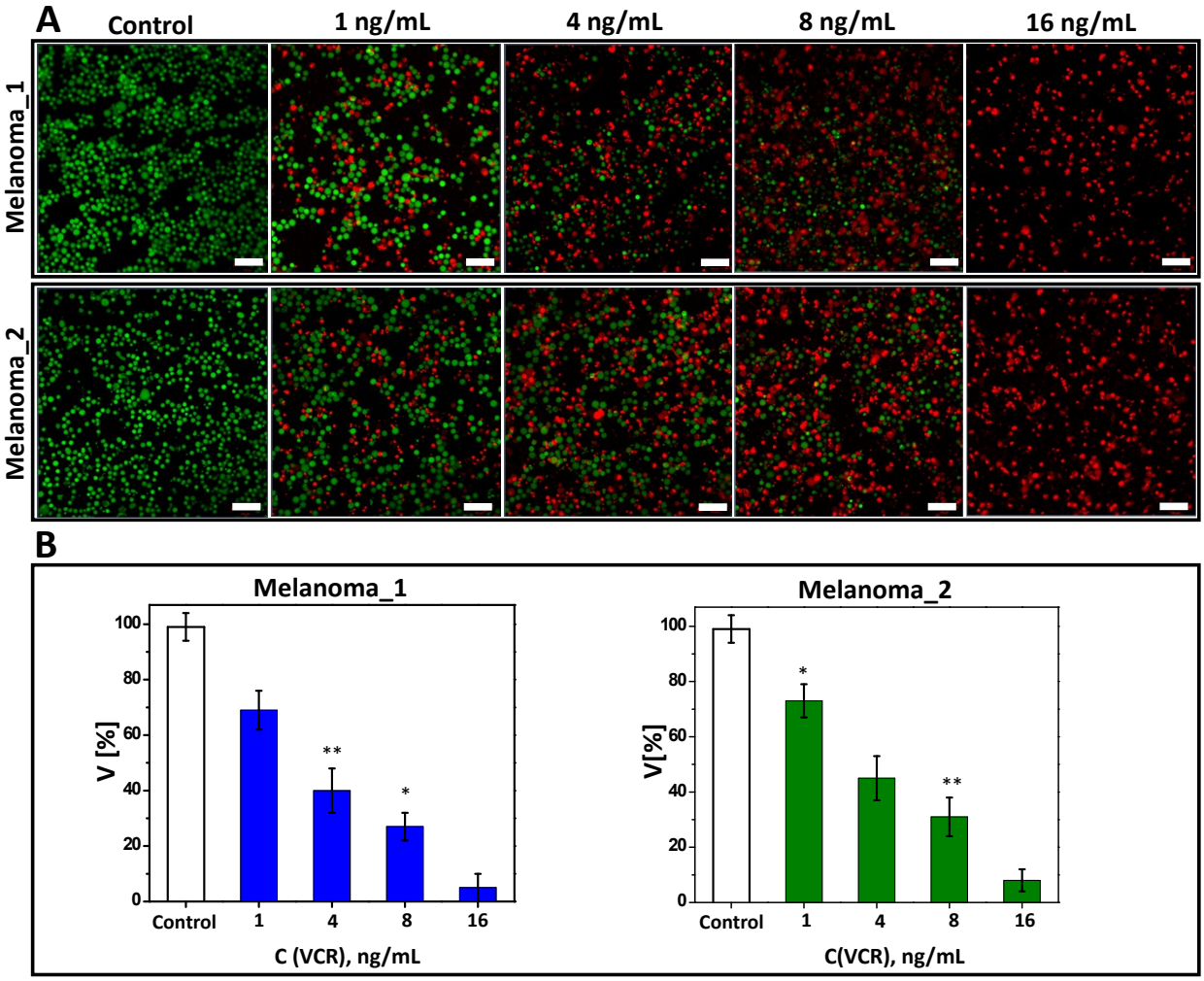


Figure S16. Evaluation of drug resistance of 2D tumor cell cultures against VCR. A. LIVE/DEAD assay of 2D melanoma cultures at different concentration of VCR (Scale bars correspond 100 µm.). B. Viability of melanoma cells (Data are presented as mean ± standard deviation, * represents $p < 0.05$ and ** represents $p < 0.001$).

e. Remote activation of VCR delivered by hMSCs with capsules using NIR-irradiation

To evaluate delivery efficiency of antitumor drug (VCR) using individual capsules (**SubCaps**) and our cell-based system, the LIVE/DEAD assay was performed.

In case of VCR@Caps, the following procedure was performed:

The **SubCaps** loaded with VCR at different amount of capsules (500, 2000, 4000, which corresponds to 2, 5, 10 ng of VCR) were added to the previously formed spheroids (150-250 μm). For this, the medium of drop containing spheroid was replaced with new cell growth medium containing different amounts of capsules.

In case of hMSCs/VCR@Caps, the following procedure was performed:

For this, 10^5 hMSCs and 10^6 **SubCaps** loaded with VCR were seeded into 25 cm^2 culture flask and left overnight at 37 ˚C, 5% CO_2. After that, cells were washed 3 times with PBS and detached with 1.25 mL of trypsin-EDTA. After 5 min, trypsin was inactivated with 2.5 mL of complete culture medium. Cells with internalized capsules were then collected into centrifuge tube and spun down at 300 x g for 5 min. Then, supernatant was discarded and hMSCs were re-suspended in 1 mL of new growth medium. After counting with hemocytometer, different amounts of hMSCs (100, 250, 500 cells, what corresponds to 2, 5, 10 ng/mL of VCR) were added the previously formed spheroids (150-250 μm) of each tumor cell type (melanoma_1 and melanoma_2) as follows. Cell culture medium of drop containing spheroid was replaced with new cell growth medium containing different amounts of hMSCs with VCR@SubCaps.

After 24 h of incubation, all samples were transferred into 96-well plate and irradiated with NIR-laser as described in §6.

Then, the cell viability of tumor cells was evaluated with LIVE/DEAD assay. For this, spheroids were stained with Calcein AM (live dye) and Propidium iodide (dead dye) by adding 0.2 μM of Calcein AM and 3 μM of Propidium iodide in cell medium of 96-well plated and incubated for 30 min at 37˚C . Note that hMSCs are resistant against several anticancer drugs,[2] therefore, it is assumed that VCR was toxic mostly to tumor cells. Spheroids were then immediately observed

under confocal microscope (Carl Zeiss LSM 710). To visualize live cells (cells stained with Calcein AM), argon laser emitting at 488 nm was used. To visualize dead cells (cell nuclei stained with Propidium iodide), helium neon laser emitting at 543 nm was used. The confocal pinhole was set to 1 airy unit and images were taken with an Objective EC Plan-Neofluar 40x/1.30 Oil DIC. Spheroids were scanned in 3 Z-planes with step 1.10 µm. Images were then analyzed with FIJI open source image analysis software. Finally, percentage of living cells were plotted versus added amount of VCR.

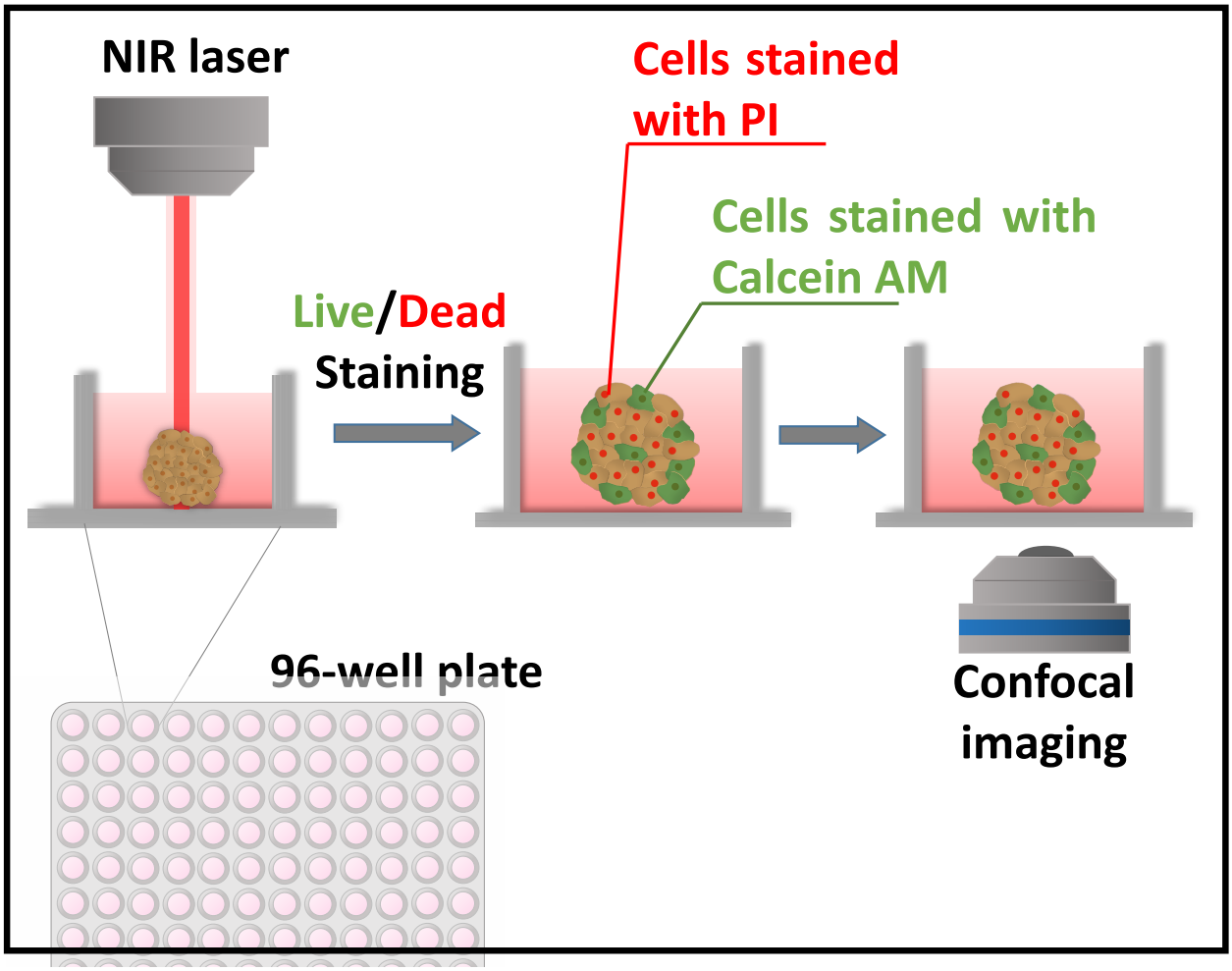


Figure 17. Schematic illustration of laser setup. Experimental steps for the remote controlled release of antitumor drug VCR using NIR-laser irradiation on 3D melanoma spheroid model.

References

[1] X. Ye, C. Zheng, J. Chen, Y. Gao, C. B. Murray, *Nano Lett* 2013, **13**, 765–771.

[2] B. C. Bellagamba, B. R. R. de Abreu, I. Grivicich, C. F. Markarian, E. Chem, M. Camassola, N. B. Nardi, R. R. Dihl, *Genet Mol Biol* 2016, **39**, 129–134.