Перспективы нано и микроносителей как средств доставки противоопухолевых препаратов и генной терапии

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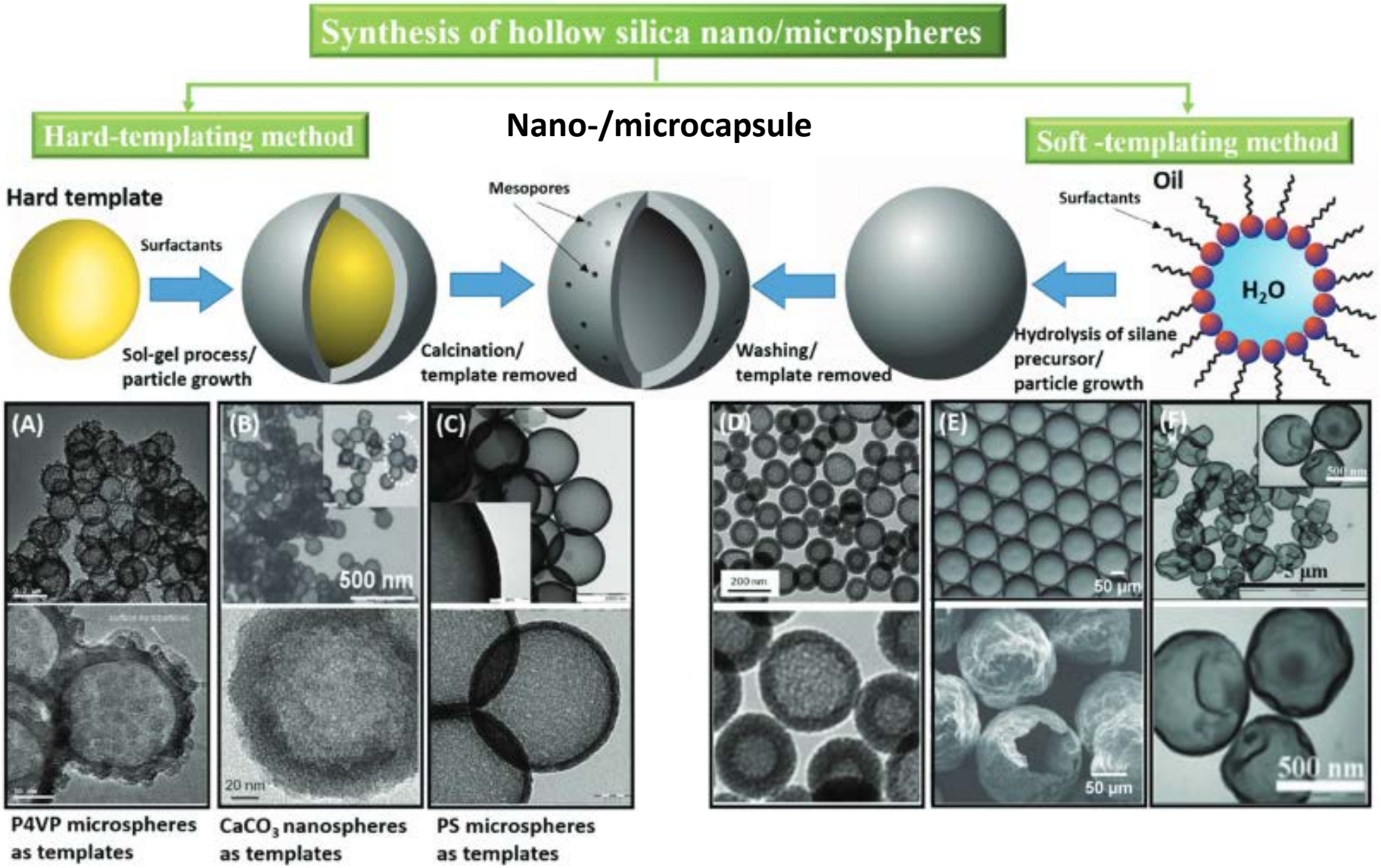
Санкт-Петербург
19-20 апреля 2018 г

Design of new drug delivery systems based on polyelectrolyte, hybrid microcapsules and nanoparticles



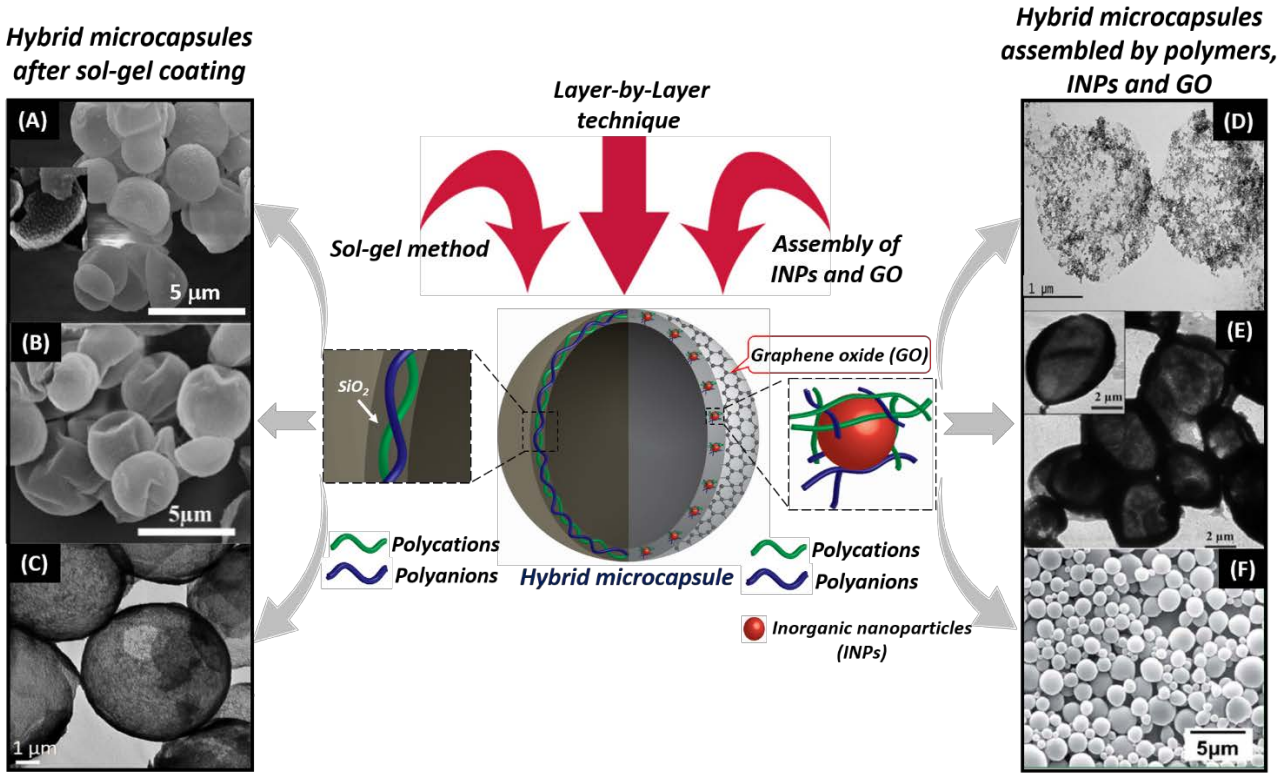
Methods of preparation of drug carriers:

- layer-by-layer technique
- Sol-gel chemistry
- Nanoparticle immobilization
- Antibody functionalization

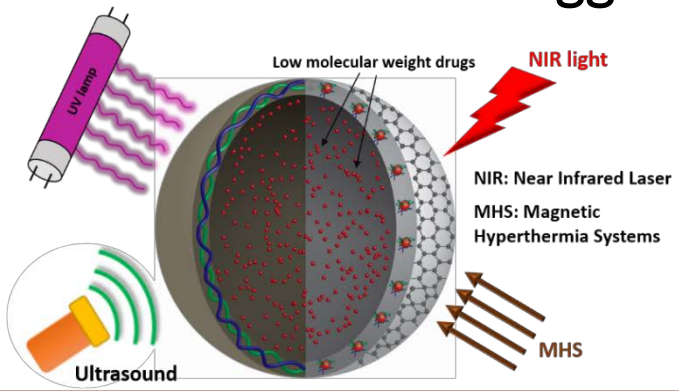
A. S. Timin, Hui Gao, D. A. Voronin, D. A. Gorin and G. B. Sukhorukov, **Adv. Mater. Inter**. 2017

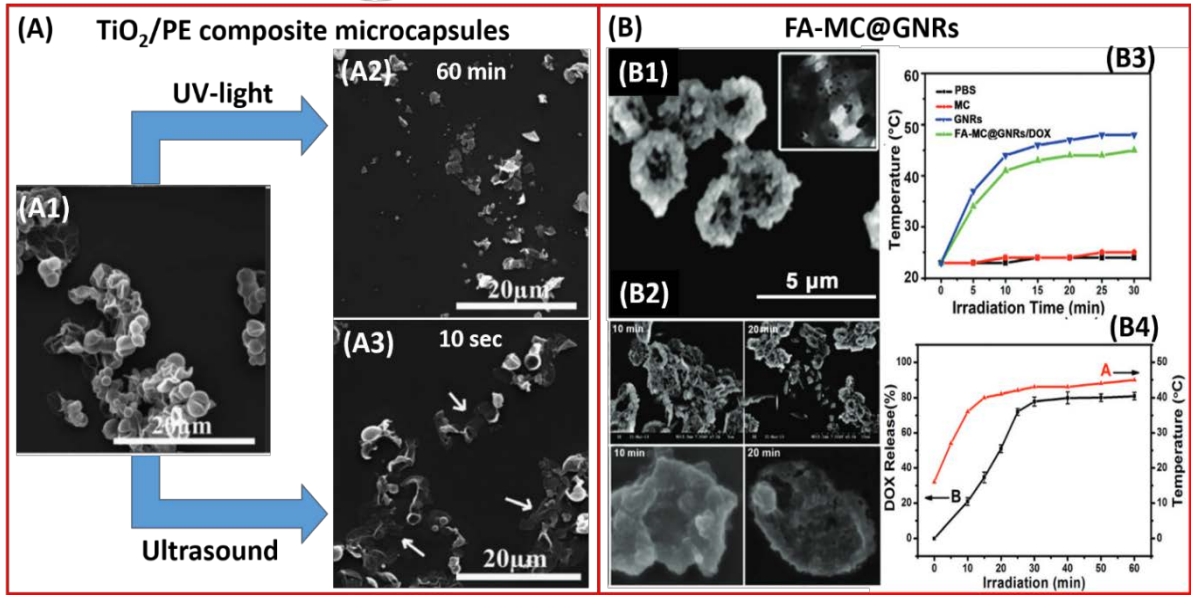
Design of new drug delivery systems based on polyelectrolyte, hybrid microcapsules and nanoparticles

Different ways of functionalization



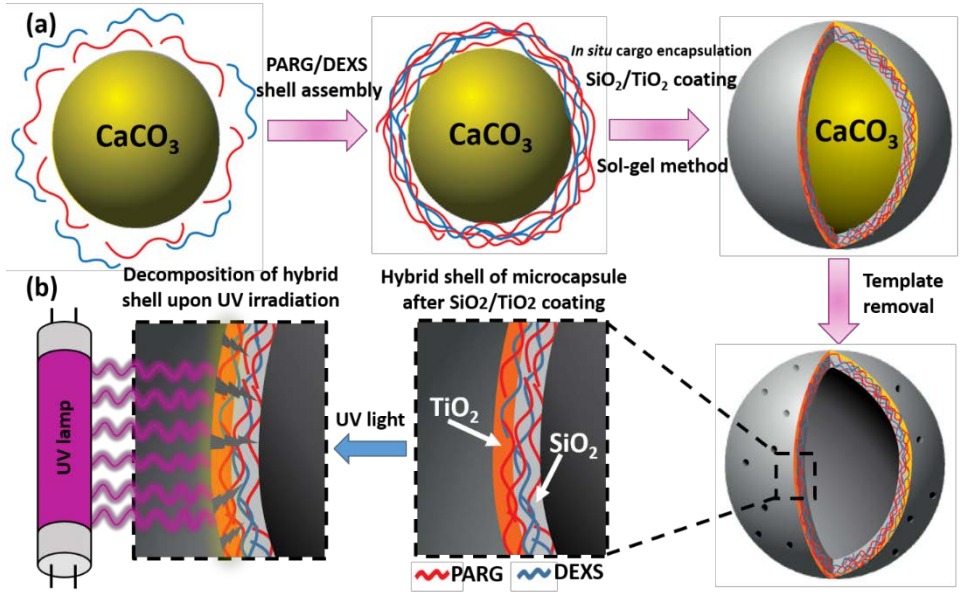
Different mechanisms of triggering



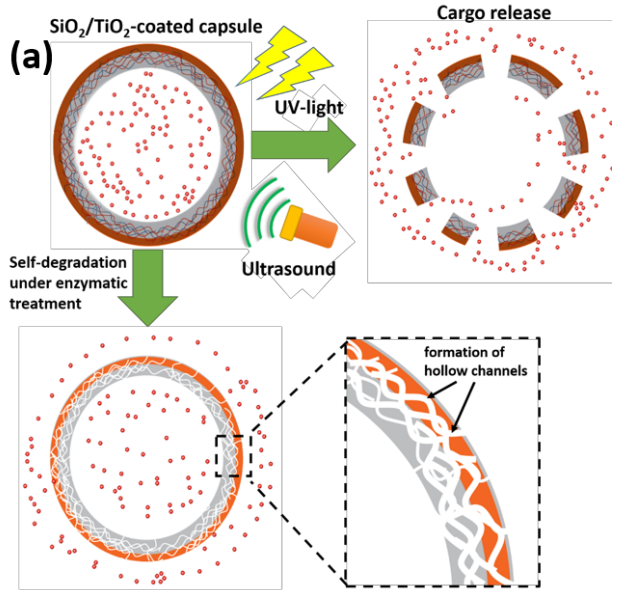


A. S. Timin, G. B. Sukhorukov, **Multi-layer microcapsules: fresh insights and new applications Expert Opinion on Drug Delivery**. — 2017. — Vol. 14, iss. 5.

Schematic illustration of preparation of triple-responsive capsules

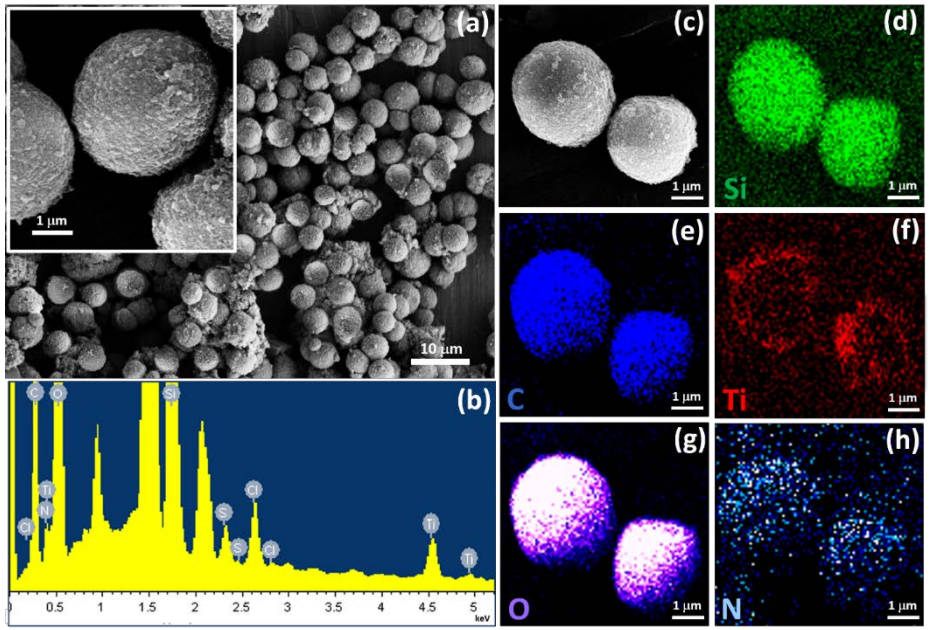


Capsule destruction

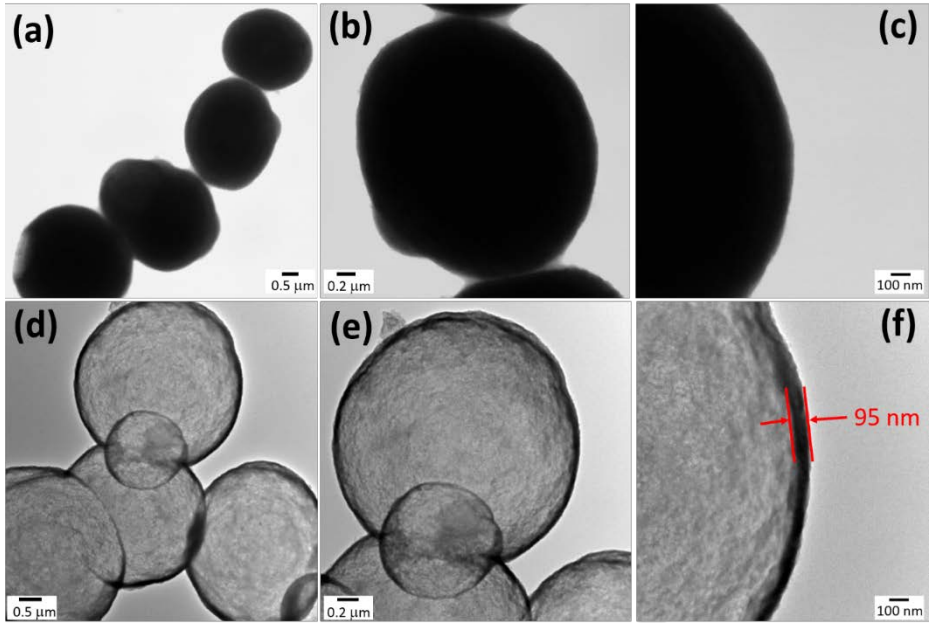


Timin A.S., Lepik K.V., Muslimov A.R., Sergeev V.S., Vilesov A.D., Sukhorukov G.B., Afanasyev B.V. **Materials Chemistry B**. – 2016. – V. 4. – P. 7270 – 7282.

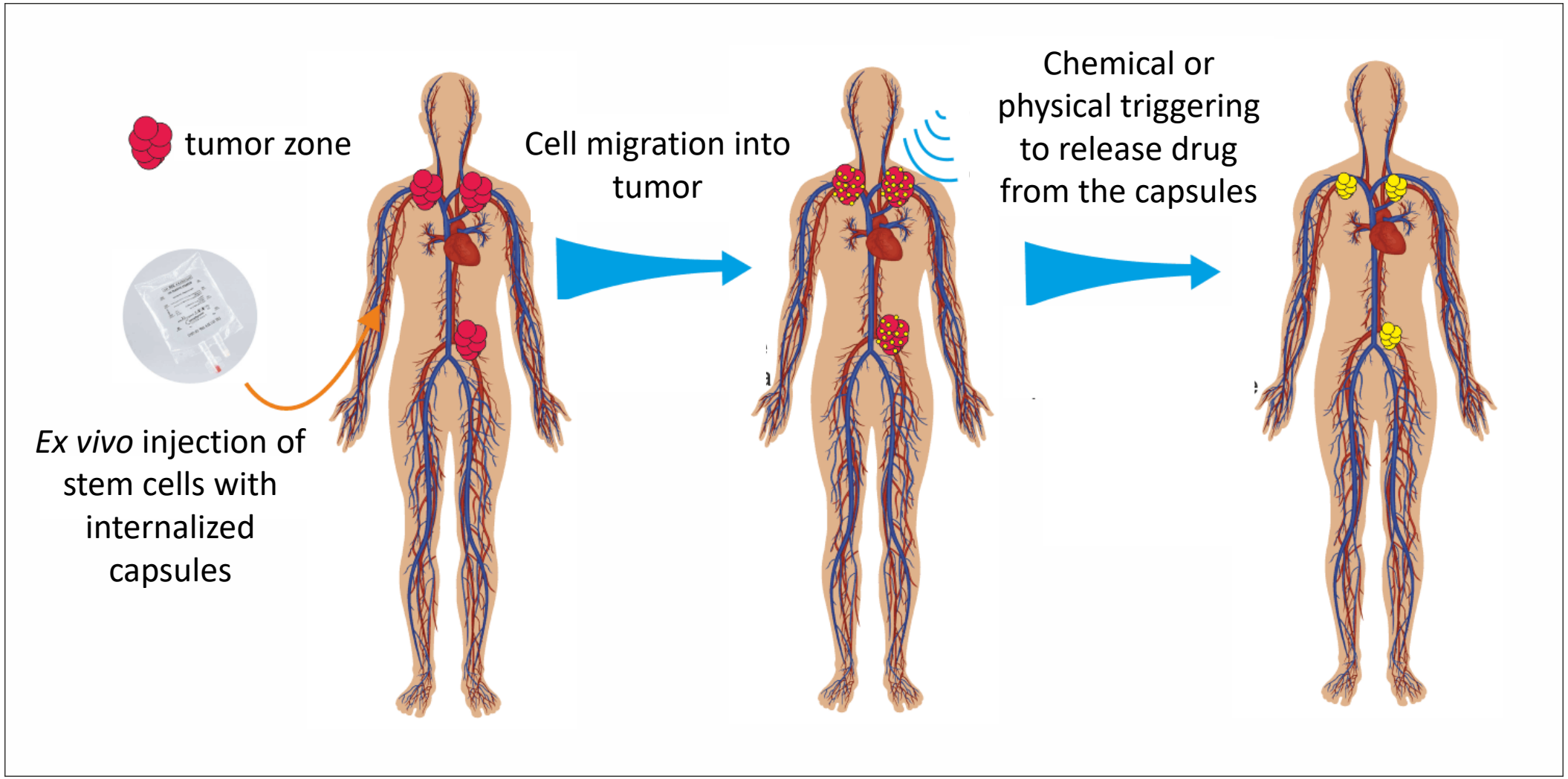
SEM images of capsules



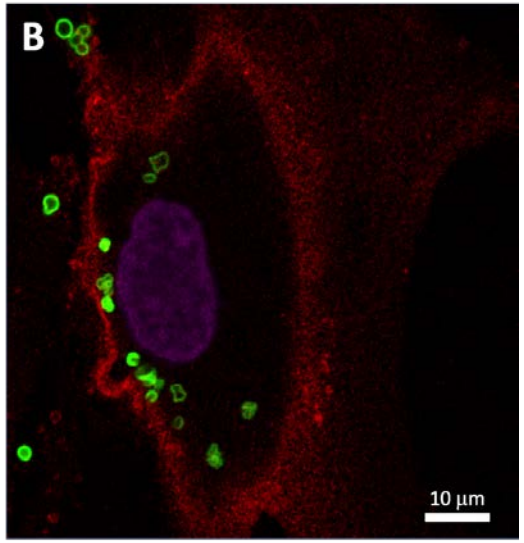
TEM images of capsules



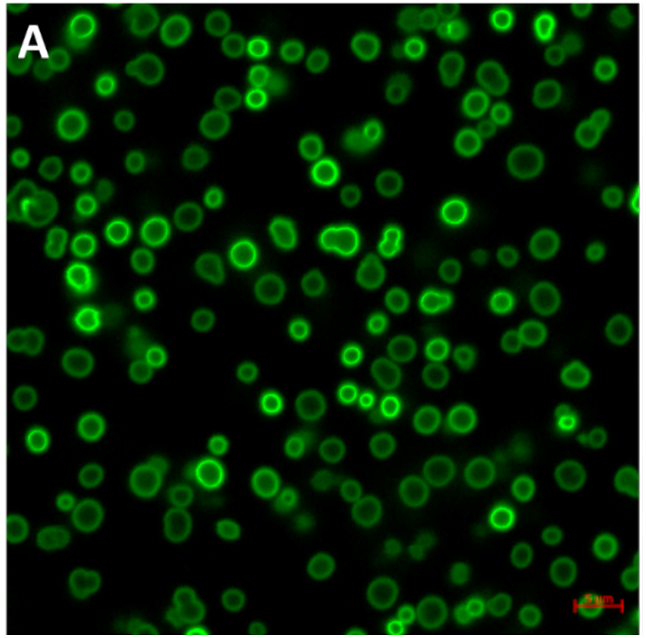
Functionalization of stem cells by multifunctional drug carriers for study of homing effect and migration potential

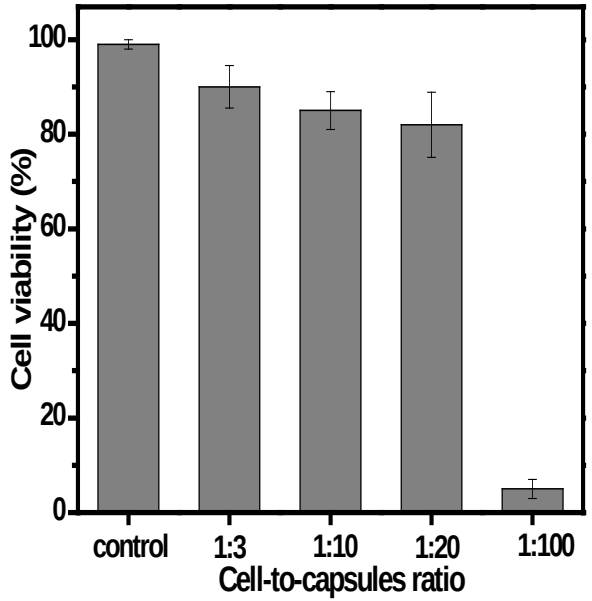


Capsule uptake by stem cells

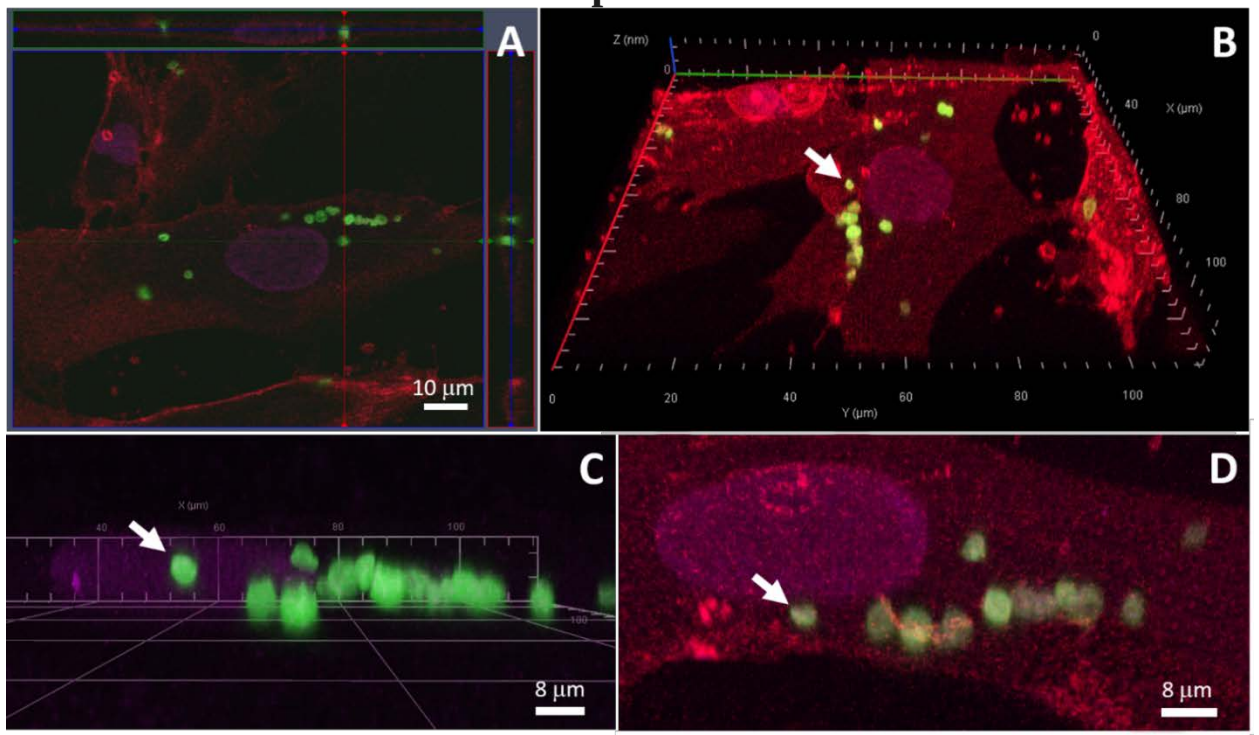


Magnetic capsules



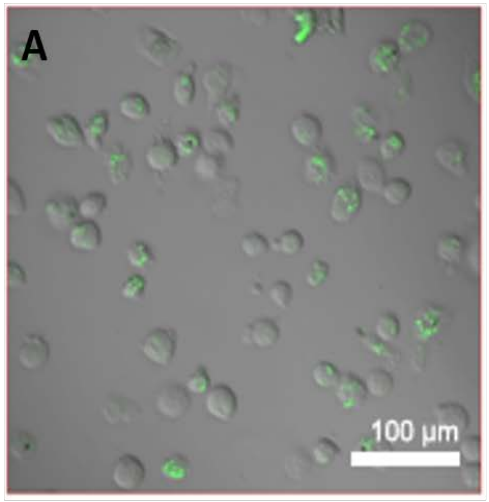


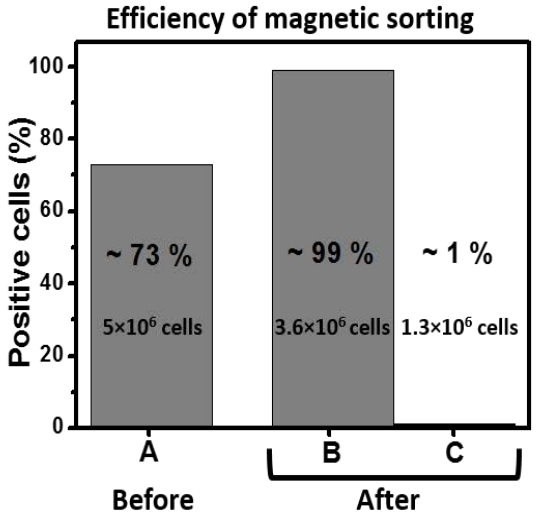
Z-stack imaging of stem cells containing magnetic capsules

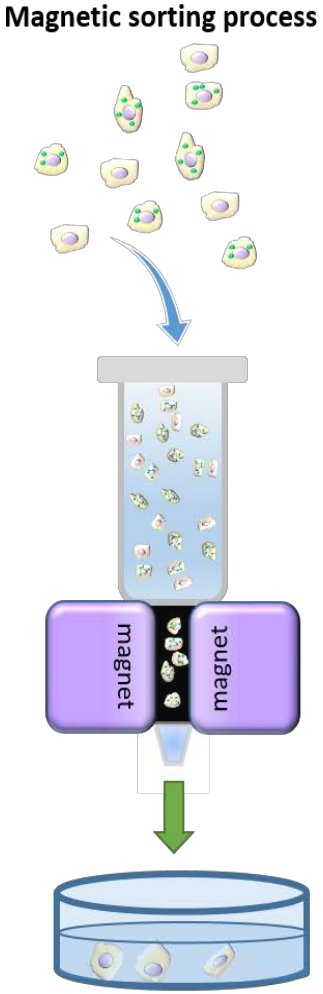


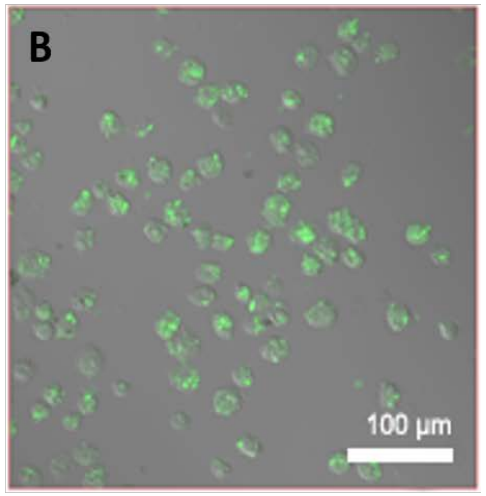
Lepik K.V.; Muslimov A.R.; Timin A.S.; Sergeev V.S.; Sukhorukov G.B. et al. *Advanced Healthcare Materials. 2016, 5(24), 3182–3190.*

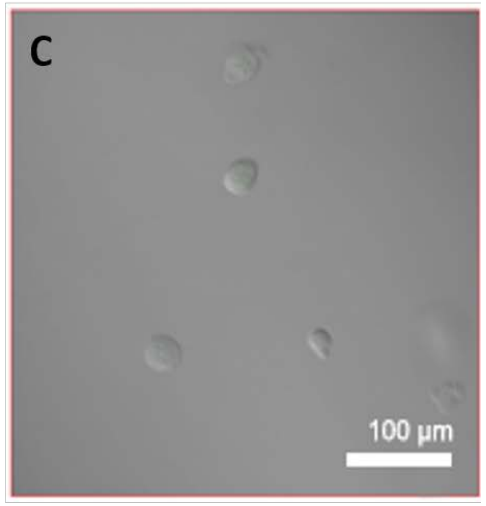
Magnetic separation of stem cells



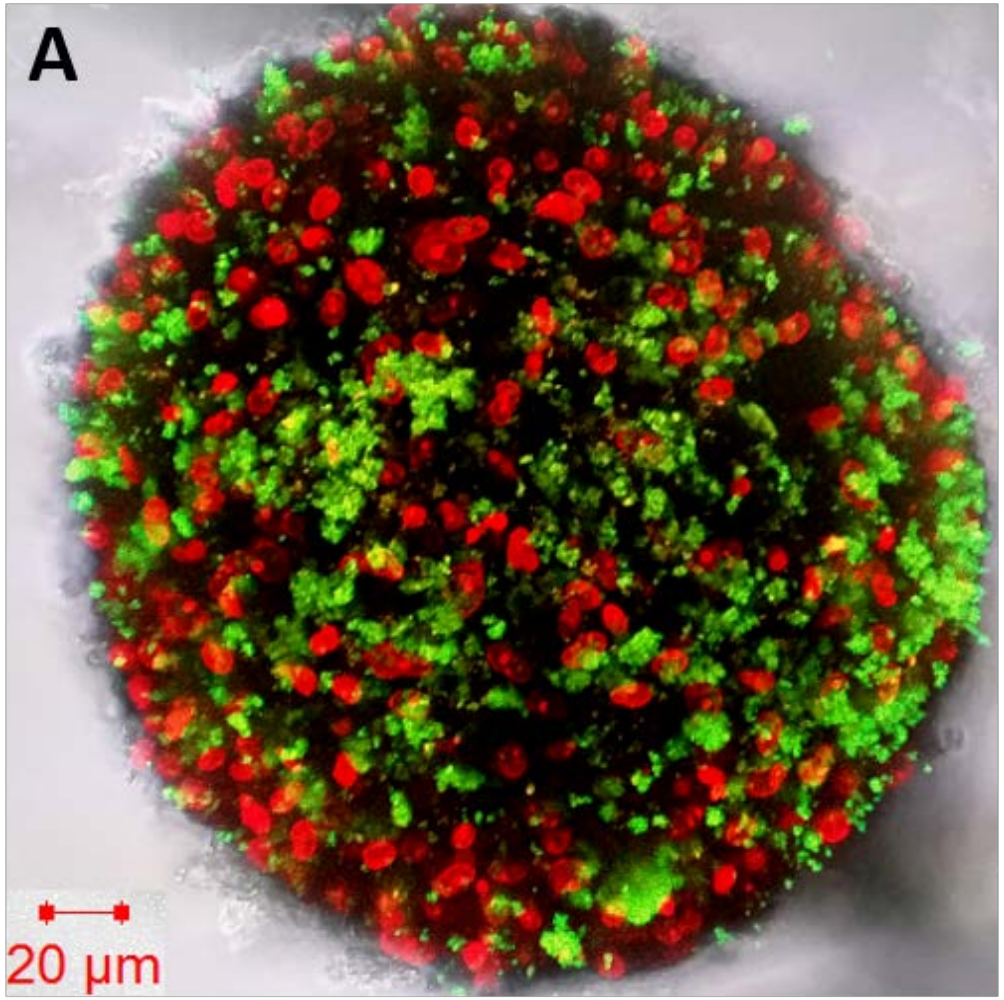








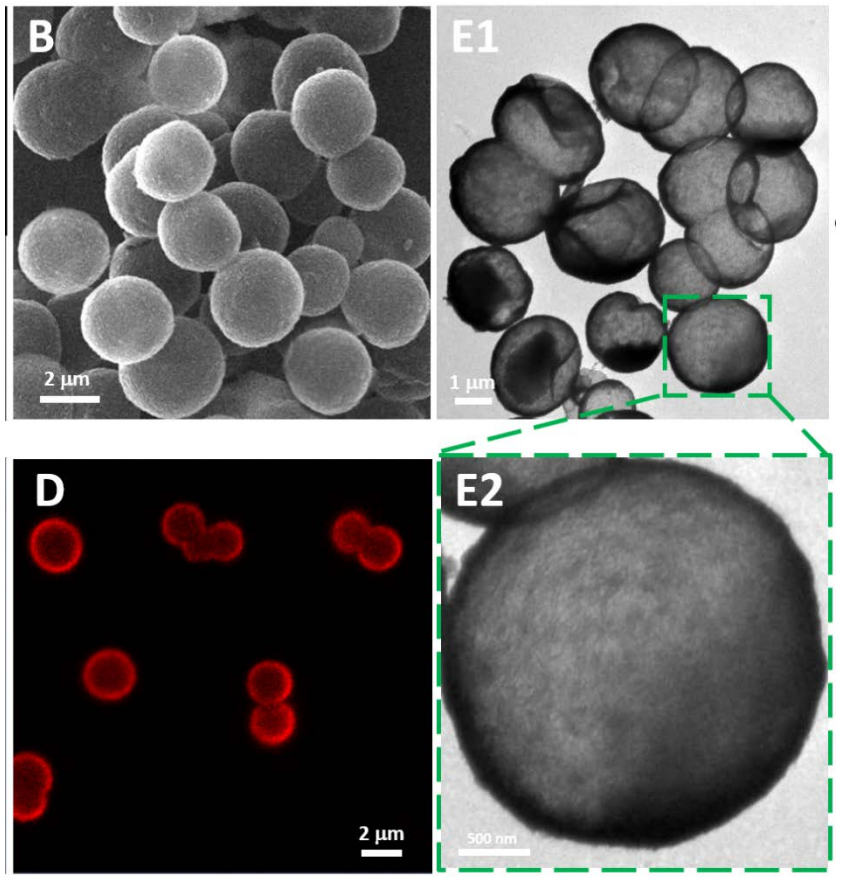
Magnetic cell spheroid with capsules



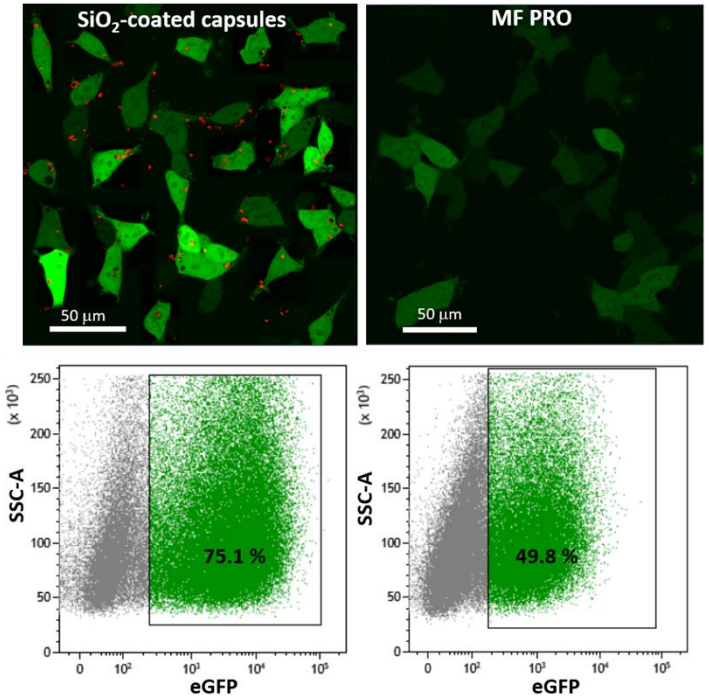
Lepik K.V.; Muslimov A.R.; Timin A.S.; Sergeev V.S.; Sukhorukov G.B. et al. *Advanced Healthcare Materials. 2016, 5(24), 3182–3190*.

Non-viral gene delivery/CRISPR-Cas9

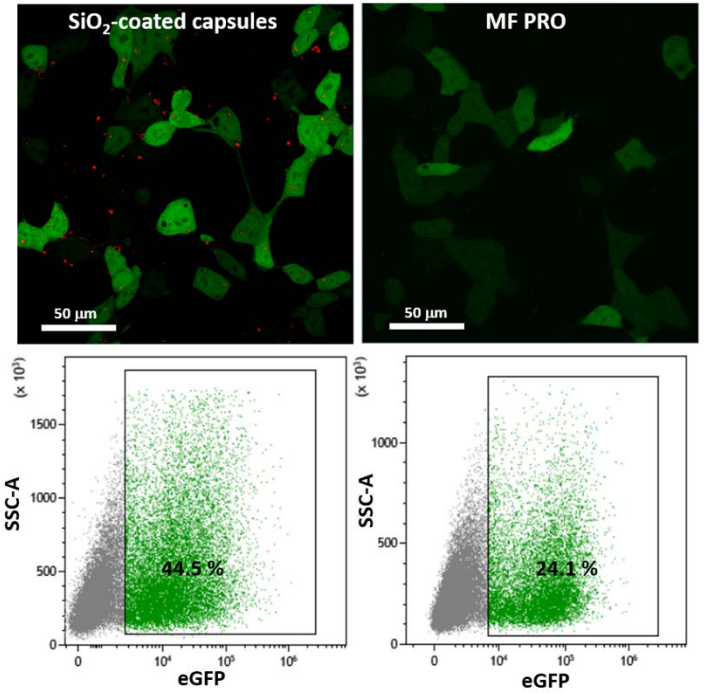
SEM, TEM and CLSM images of hybrid microcapsules



Transfection of EGFP-mRNA

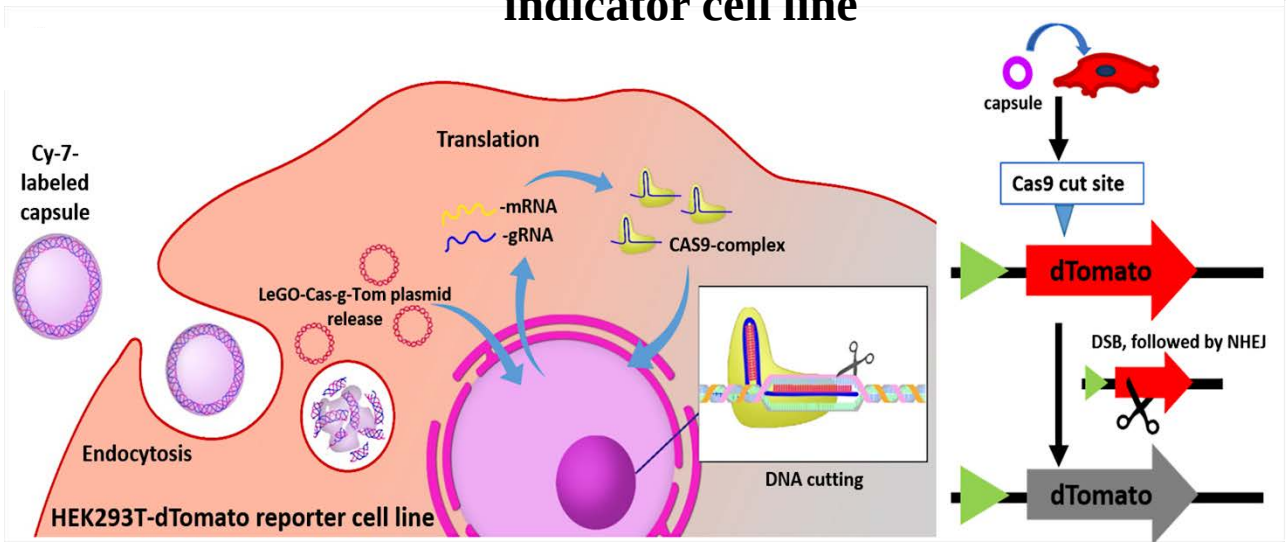


Transfection of EGFP-pDNA

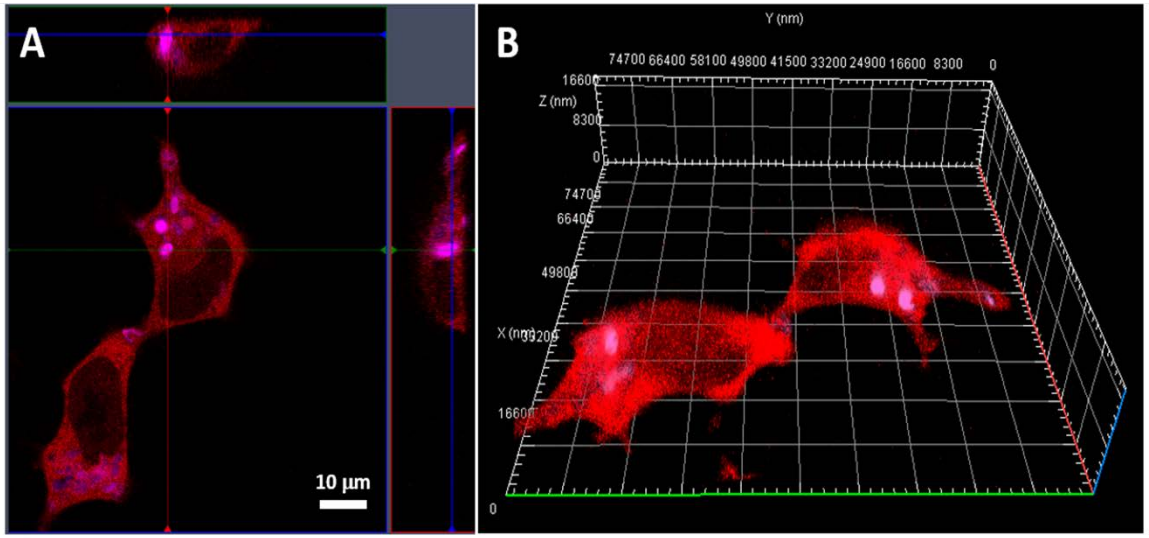


Timin A.S. et al., Efficient gene editing via non-viral delivery of CRISPR-Cas9 system using polymeric and hybrid microcarriers/Nanomedicine: NBM, 2017 DOI: http://dx.doi.org/10.1016/j.nano.2017.09.001

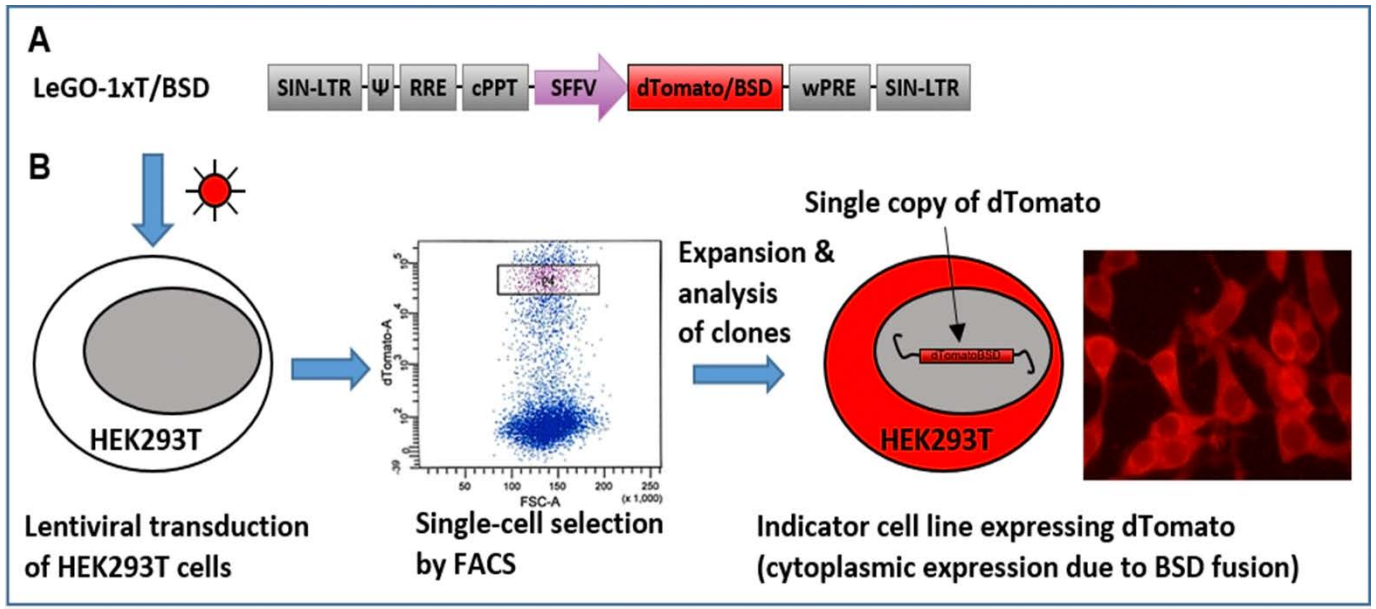
Principle of dTomato knockout in the HEK293T-based indicator cell line



Capsules Internalization in HEK293T-dTomato cells



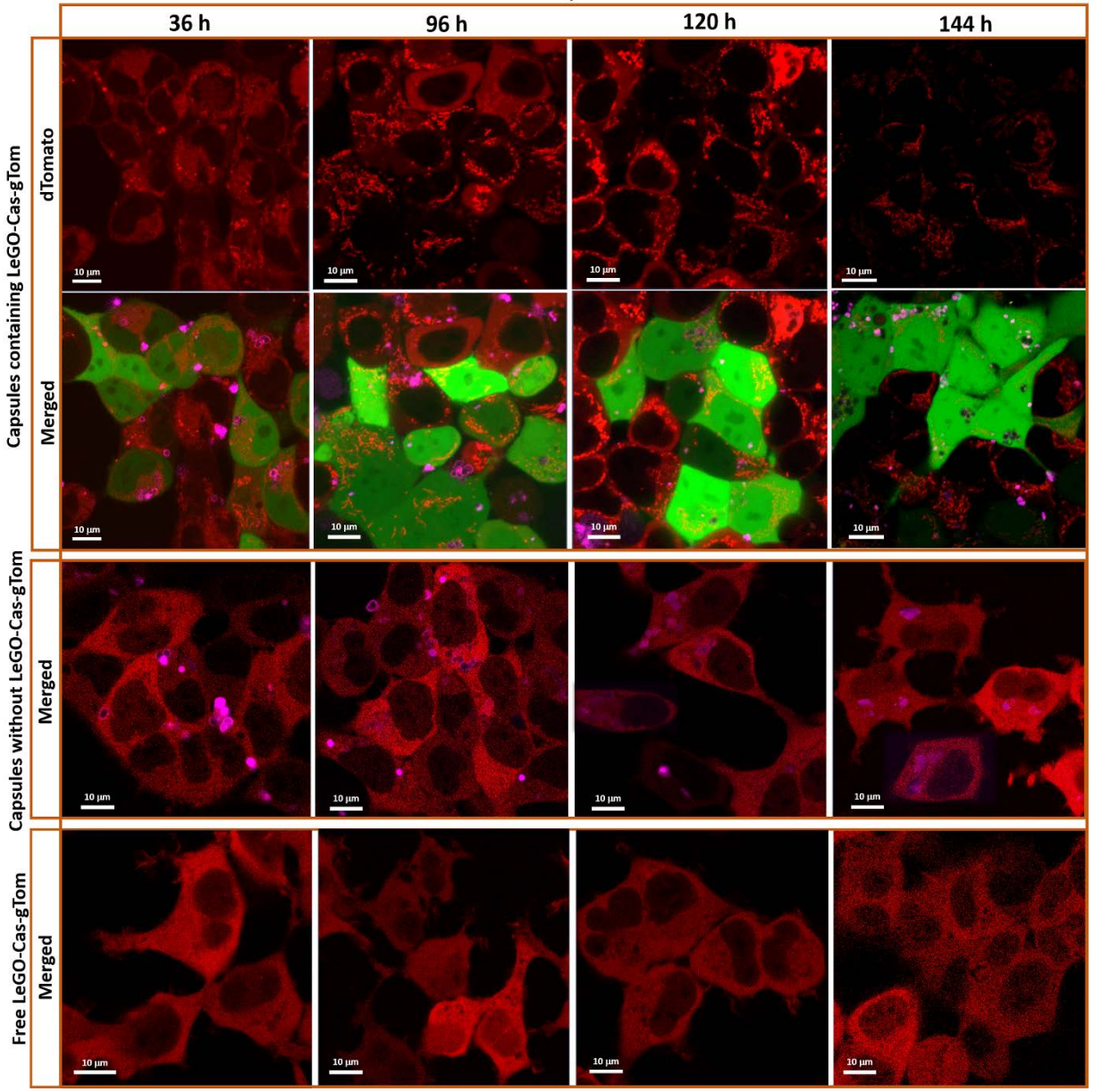
Preparation of indicator cell line expressing dTomato



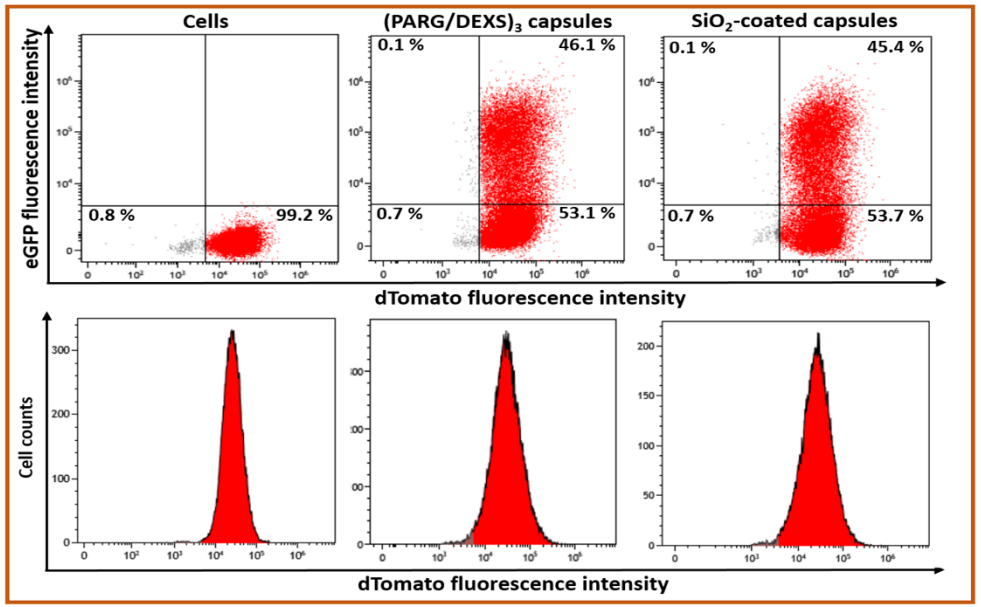
Prof. Boris Fehse

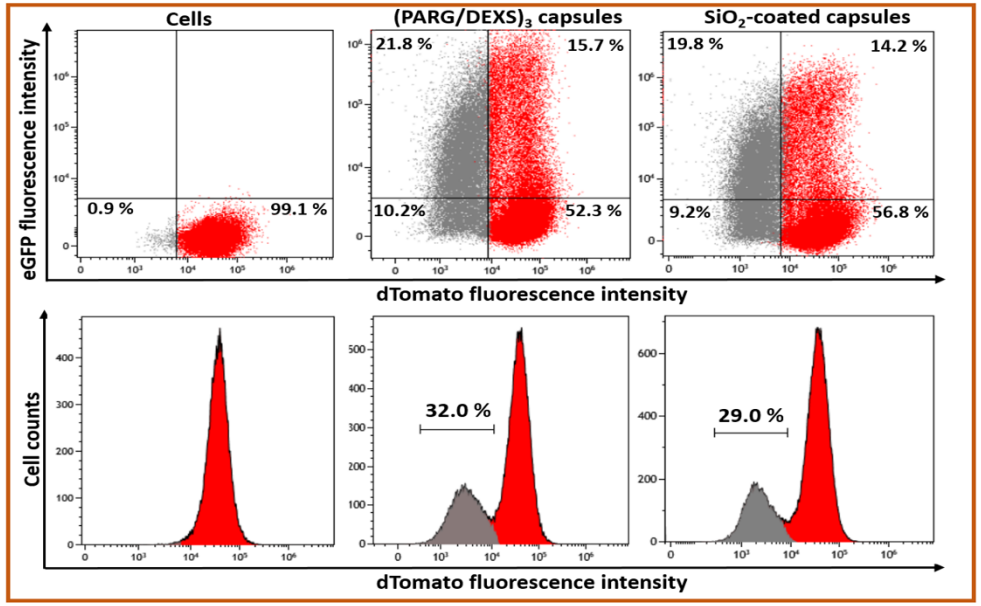
Head of scientific laboratory, Centre for oncology, Department of Stem Cell transplantation, University of Hamburg

Successful transfection of HEK293T with capsules containing Cy-7/LeGO-Cas-gTom plasmid results in efficient editing of the dTomato gene

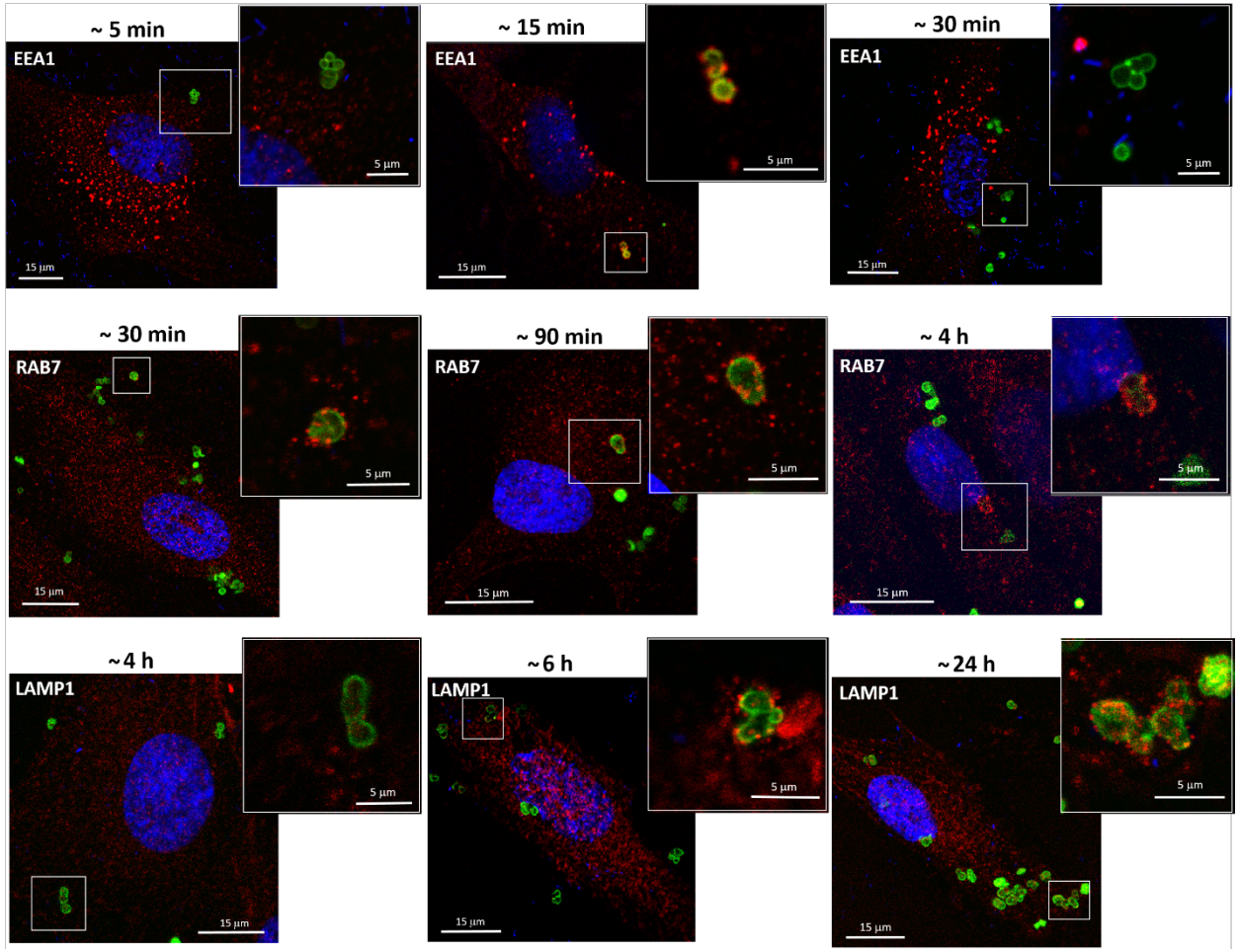


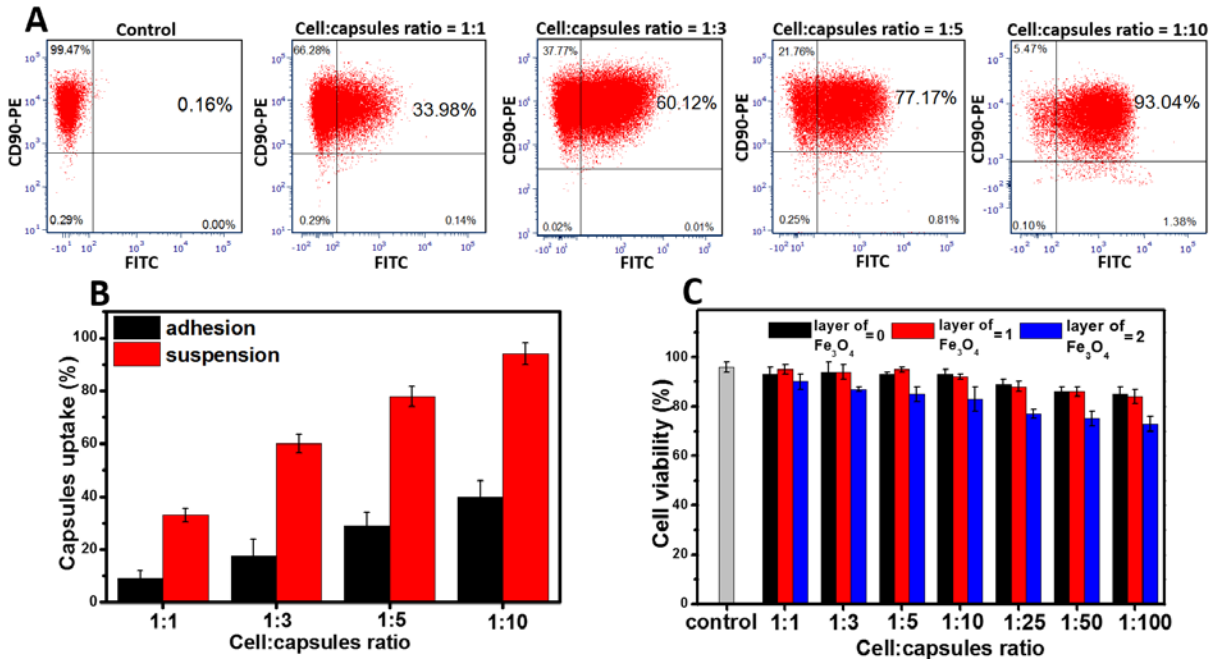
High efficiency CRISPR-Cas9-mediated dTomato knockout after transfection with both $(PARG/DESX)_3$ and SiO_2-coated capsules



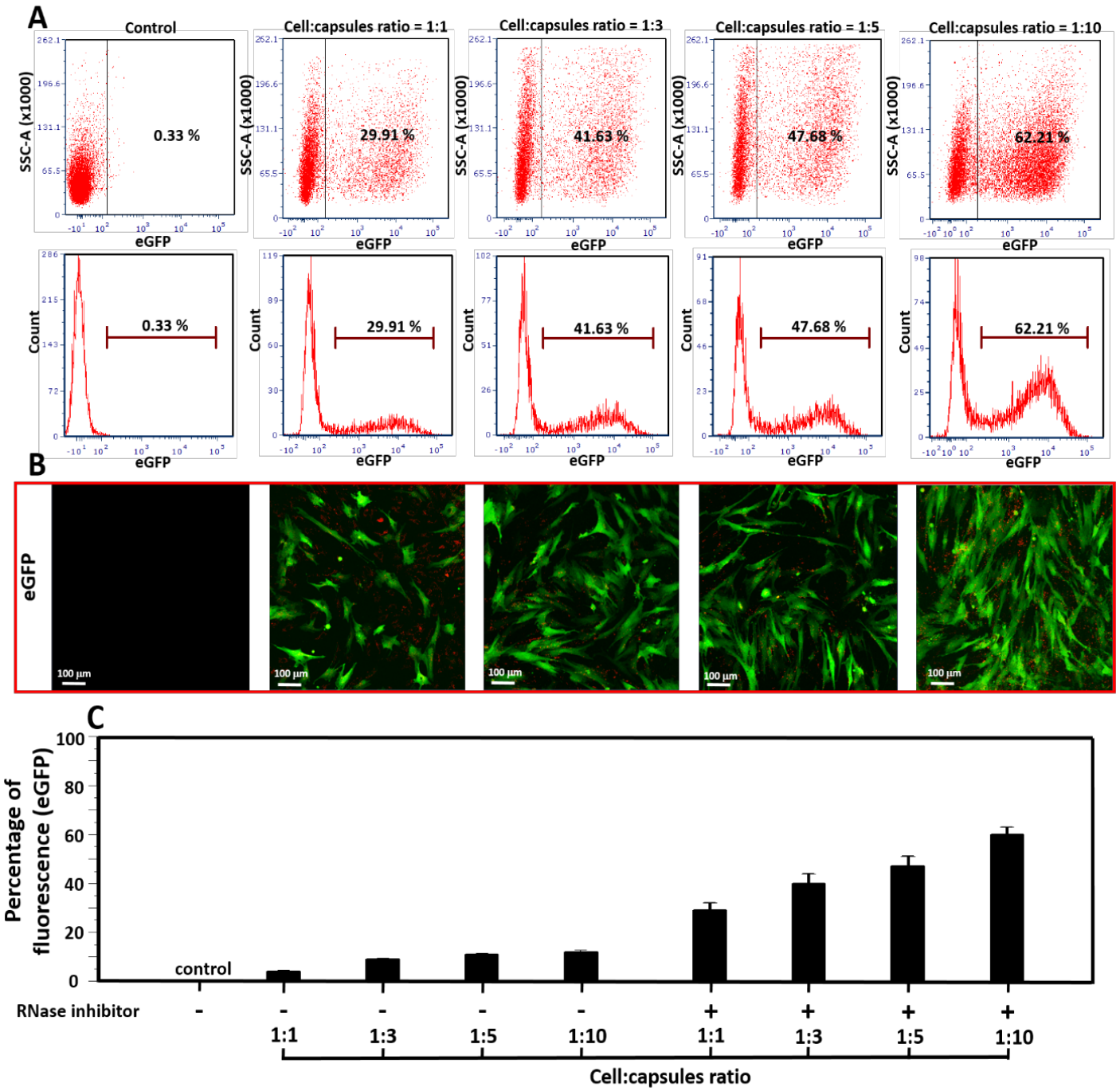


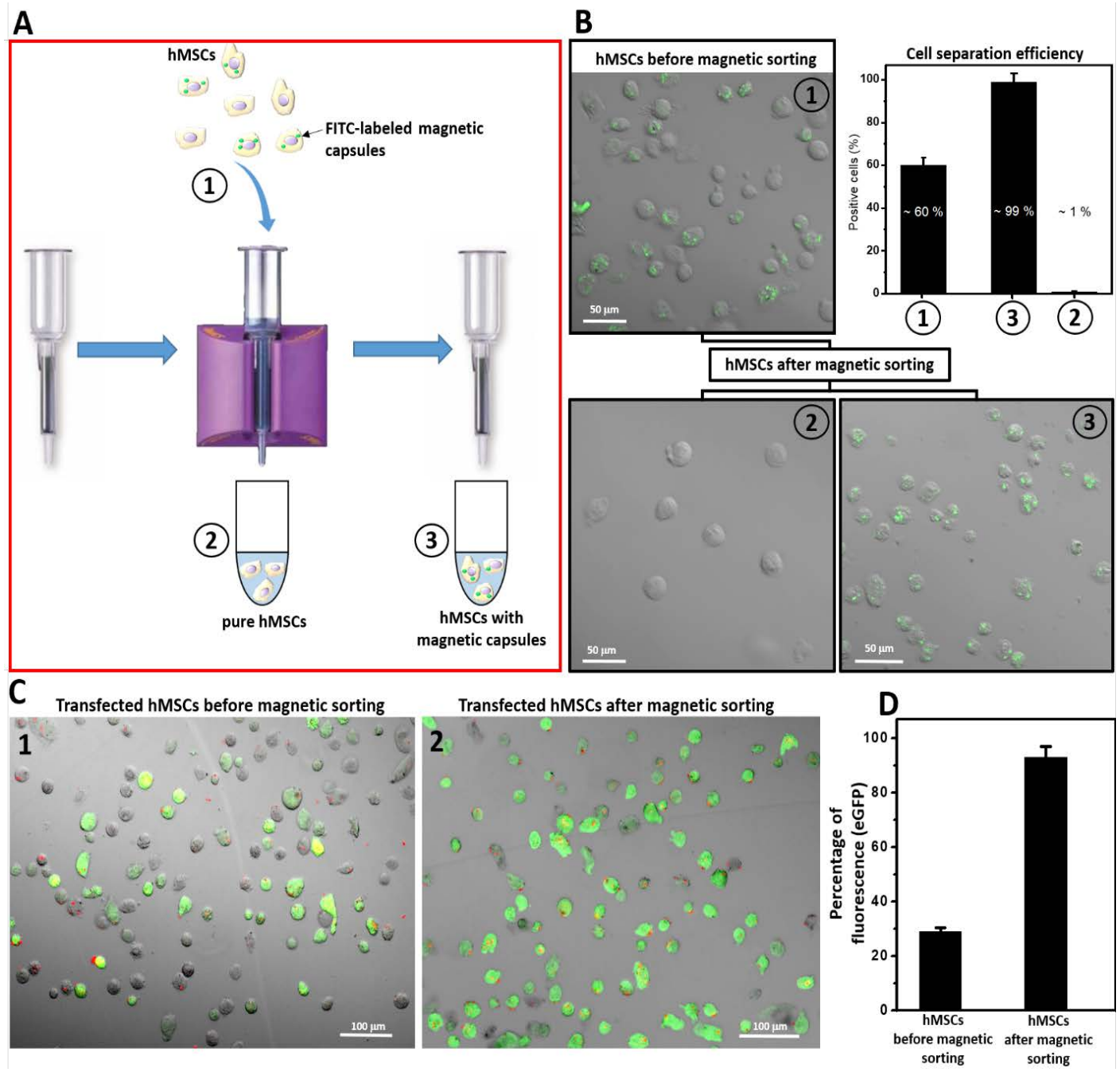
Microcapsules for mRNA delivery into MSCs





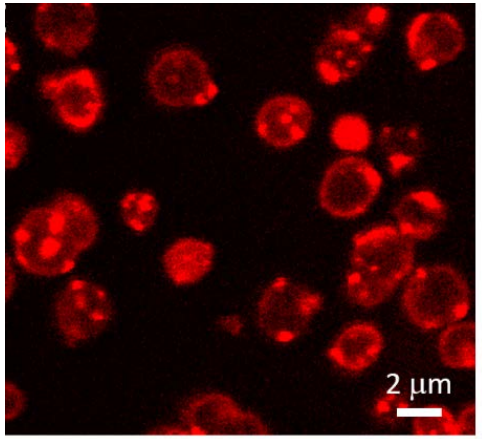
Muslimov A.R., **Timin A.S.**, Petrova A., Epifanovskaya O., Shakirova A., Lepik K., Gorshkov A., Il'inskaja E., Vasin A., Afanasyev B., Boris Fehse, and Sukhorukov G.B., Mesenchymal stem cells engineering: microcapsules assisted gene transfection and magnetic cell separation/ ***ACS Biomater. Sci. Eng***. – 2017. – DOI: 10.1021/acsbiomaterials.7b00482

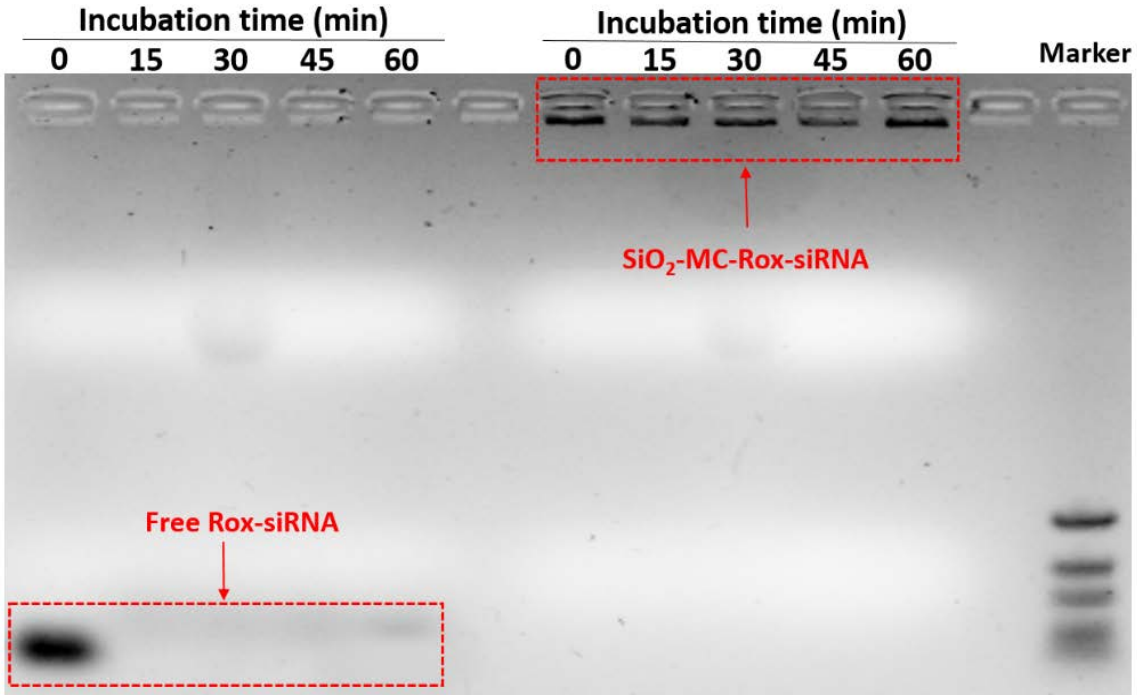

A
Control
Cell:capsules ratio = 1:1
Cell:capsules ratio = 1:3
Cell:capsules ratio = 1:5
Cell:capsules ratio = 1:10
SSC-A (x1000)
eGFP
Count
0.33 %
29.91 %
41.63 %
47.68 %
62.21 %
B
eGFP
100 µm
C
Percentage of fluorescence (eGFP)
control
RNase inhibitor
Cell:capsules ratio
1:1
1:3
1:5
1:10

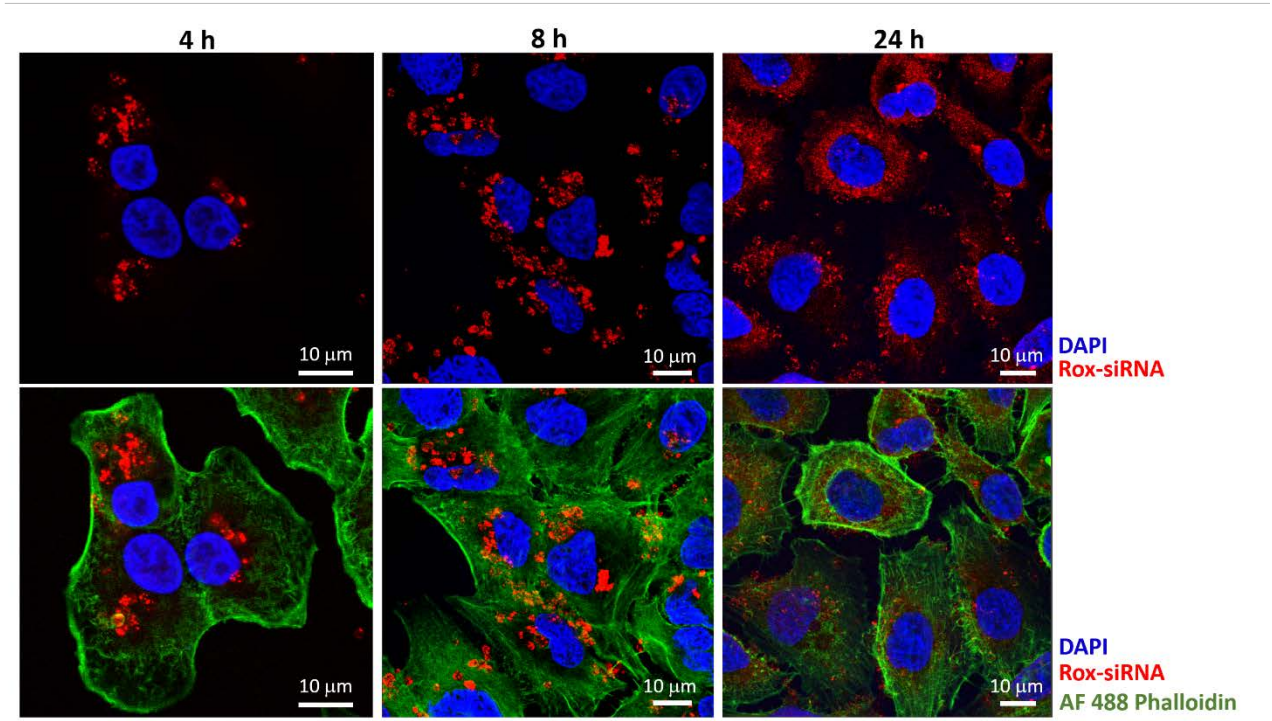

A
hMSCs
FITC-labeled magnetic capsules
1
2
3
pure hMSCs
hMSCs with magnetic capsules
B
hMSCs before magnetic sorting
1
50 µm
Cell separation efficiency
Positive cells (%)
100
80
60
40
20
0
~ 60 %
~ 99 %
~ 1 %
1
3
2
hMSCs after magnetic sorting
2
50 µm
3
50 µm
C
Transfected hMSCs before magnetic sorting
1
100 µm
Transfected hMSCs after magnetic sorting
2
100 µm
D
Percentage of fluorescence (eGFP)
100
80
60
40
20
0
hMSCs before magnetic sorting
hMSCs after magnetic sorting

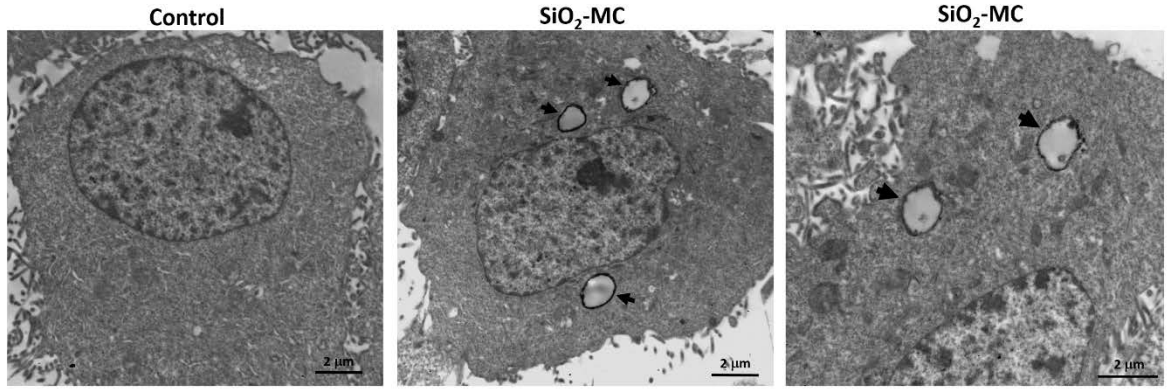
Non-viral delivery of antiviral siRNA against influenza A virus

SiO_2-microcapsules (SiO_2-MC/Rox-siRNA)

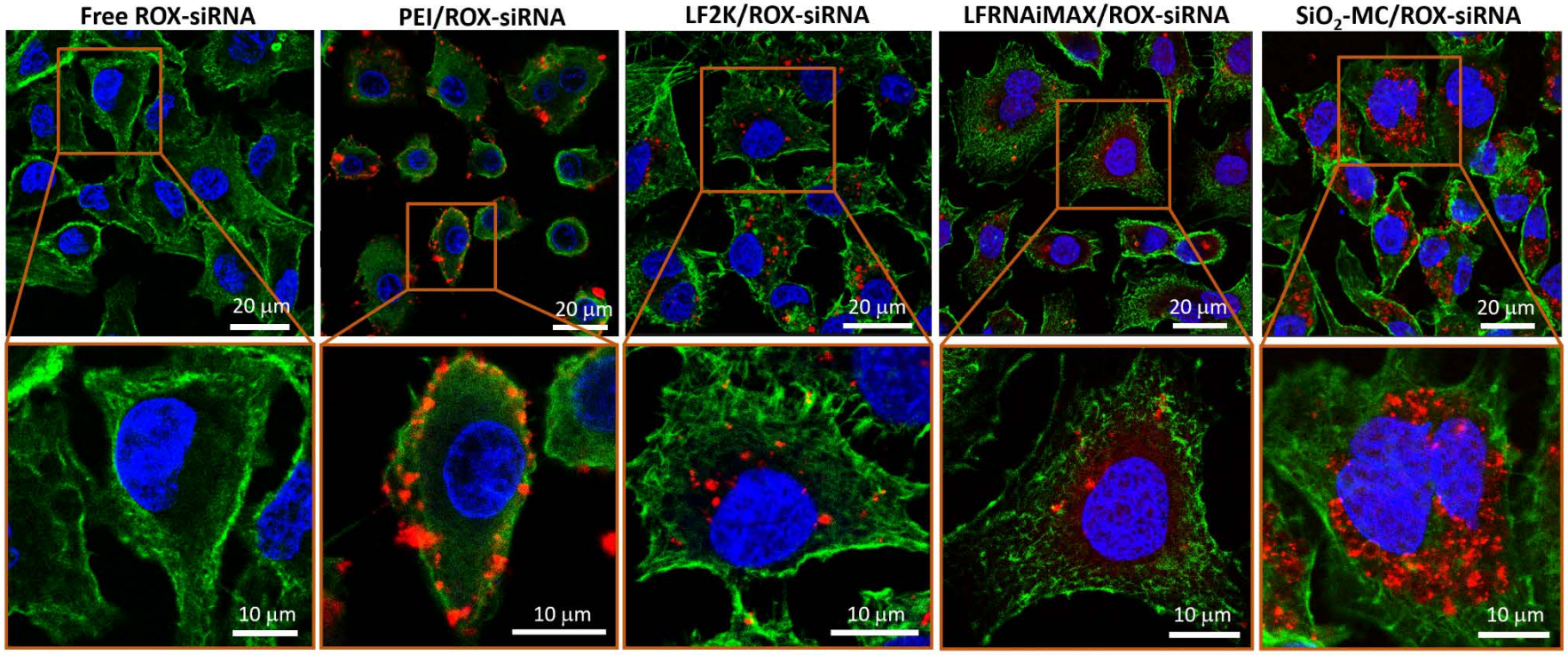








CLSM images of intracellular delivery of ROX-siRNA using different non-viral vectors



Abbreviations:

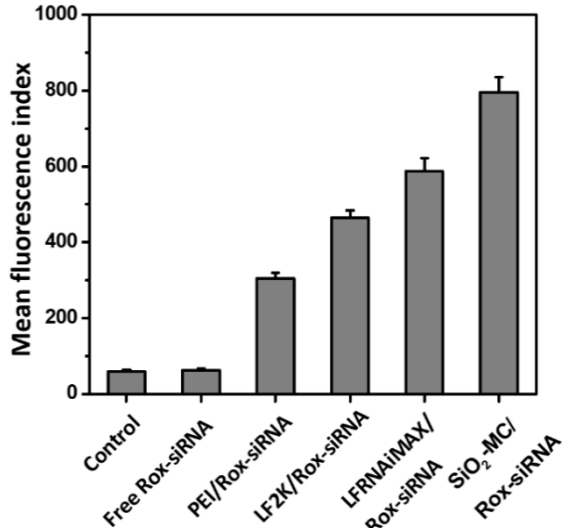
PEI – polyethylenimine

LF2K – Lipofectamine 2000

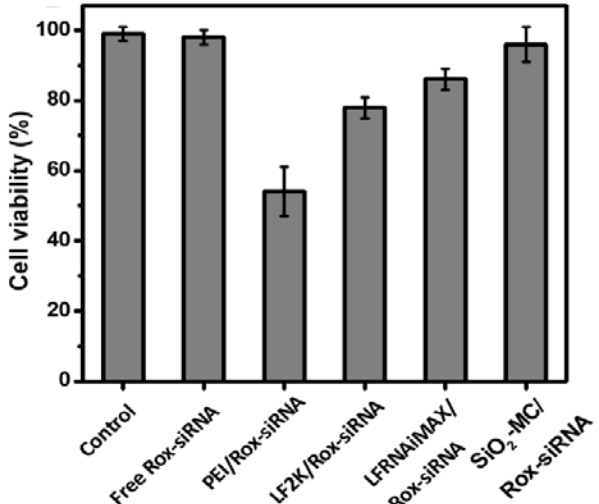
LFRNAiMAX – Lipofectamine RNAiMAX

SiO_2-MC – SiO_2-microcapsules

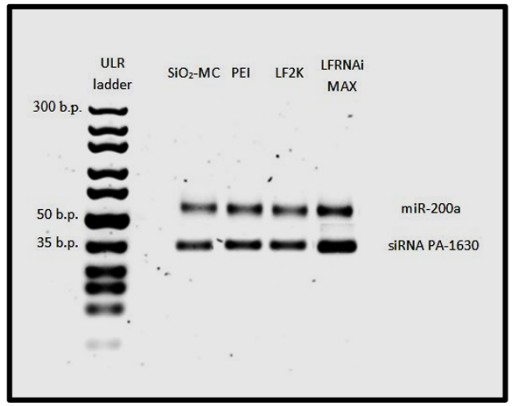
Flow cytometry



MTT assay

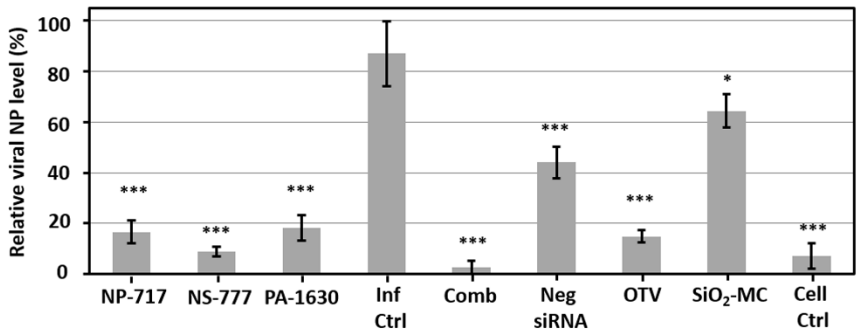


Gel-phoresis of PCR products after siRNA transfection

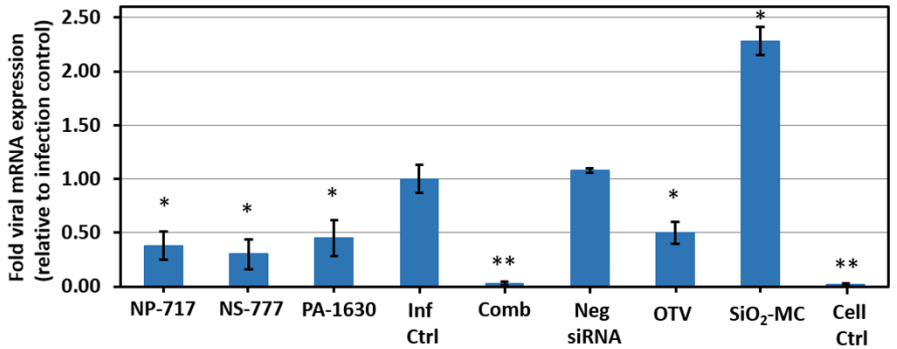


In vitro antiviral effect of siRNA-loaded microcapsules

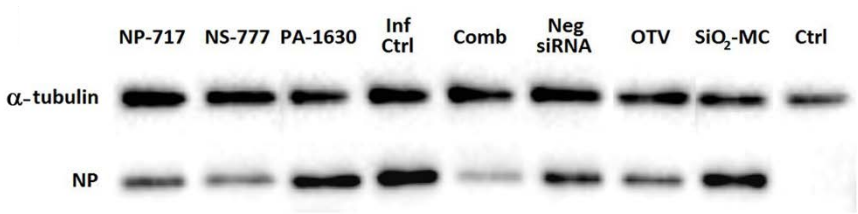
Relative viral NP level in infected A549 cells measured by ELISA



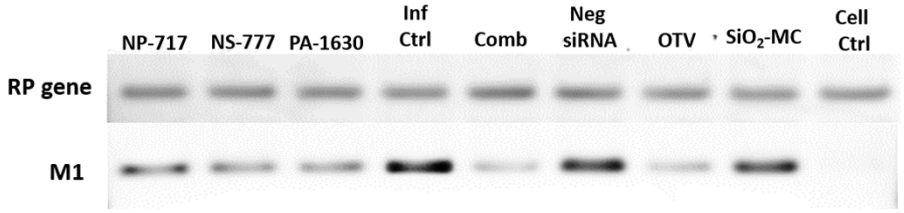
Relative IAV M1 mRNA expression of culture lysates determined by RT-qPCR



Relative viral NP level in infected A549 cells measured by western blot analysis normalized to alpha-tubulin



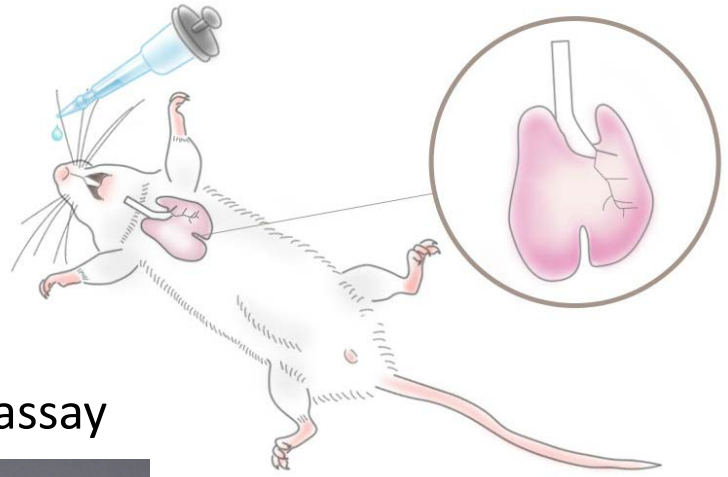
Gel electrophoresis of PA-1630 siRNA PCR products extracted from cells



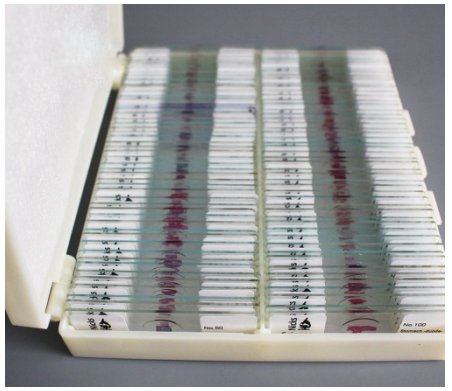
A. Petrova, A.S. Timin, et al. *Antiviral research* (submitted)

In vivo study

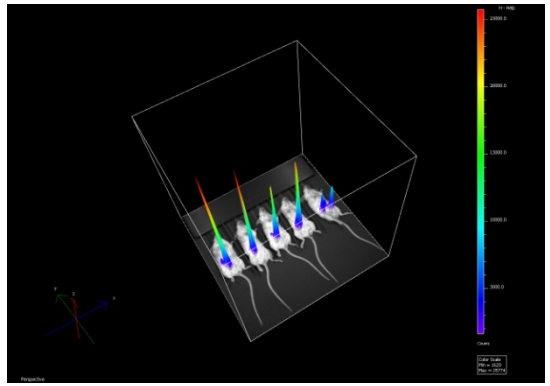
Capsules administration



Histological assay



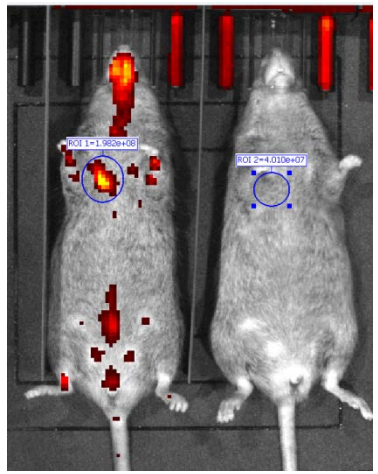
IVIS assay



Virology *in vivo* assay

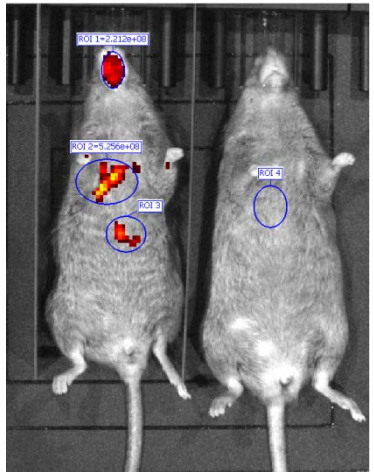
In vivo study

Microcapsules

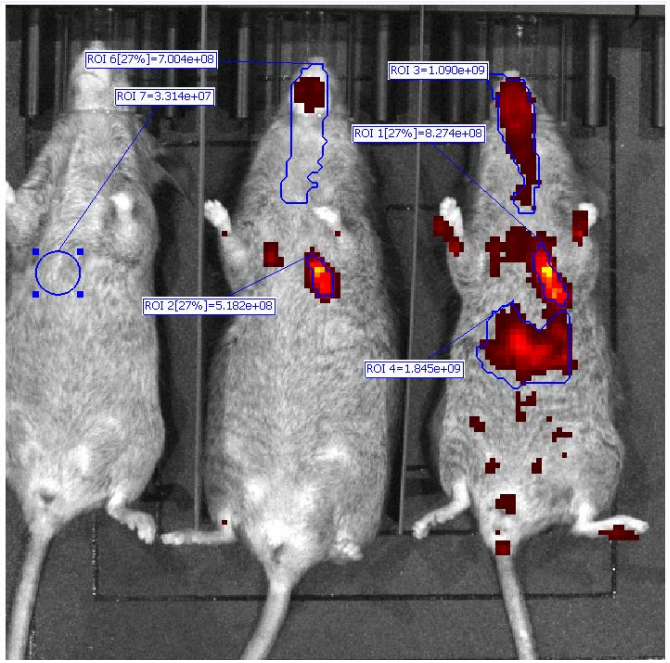


ROI = 1,982e+08

Submicron capsules

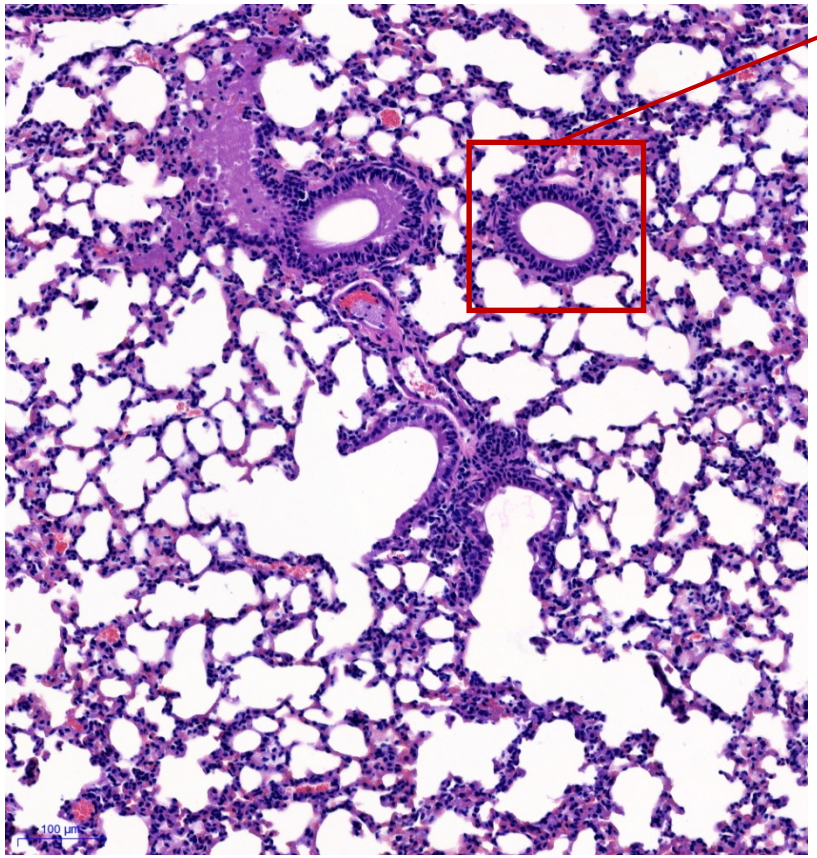


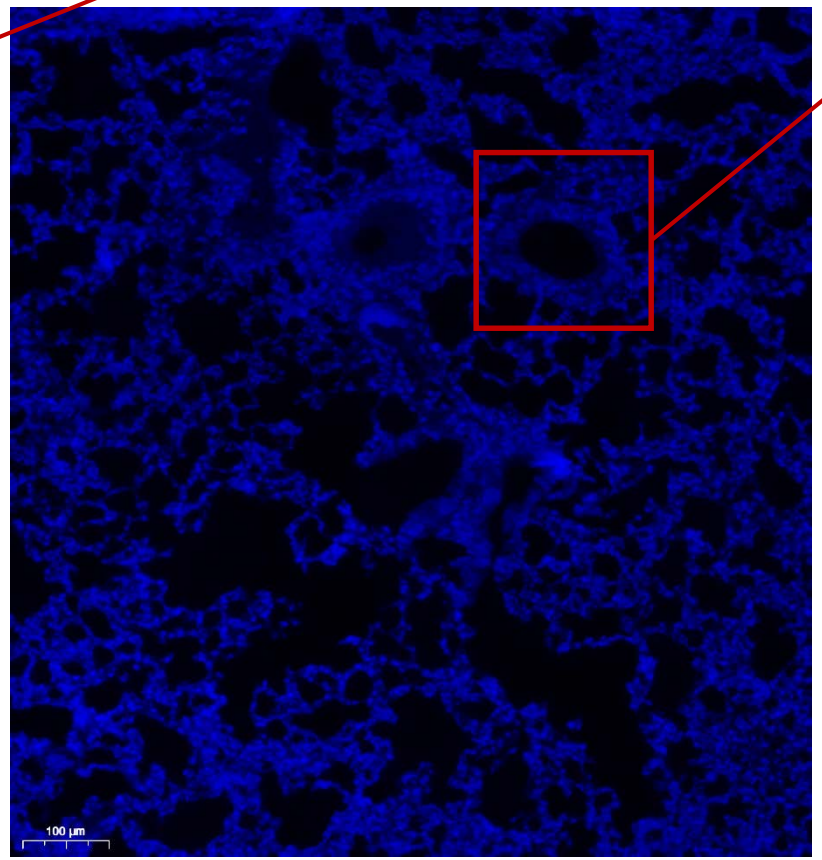
ROI = 5,256e+08

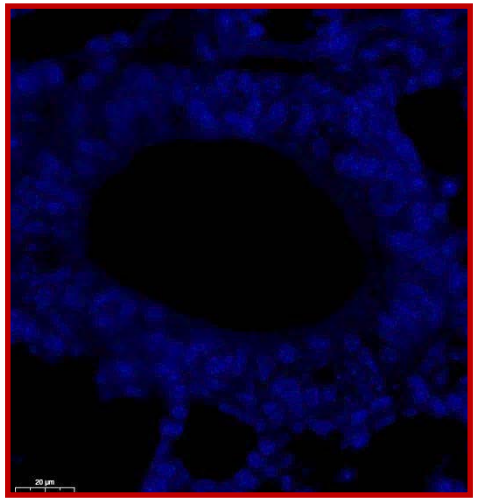


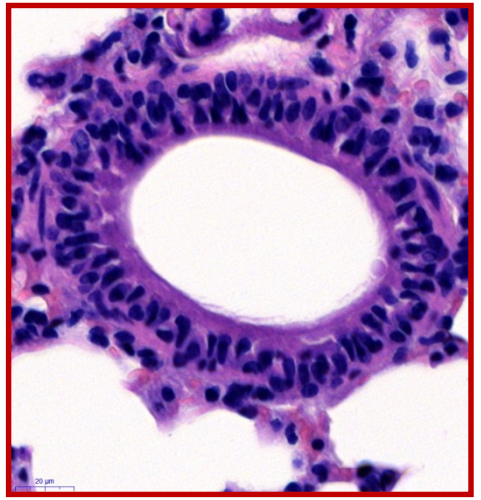
Control/Microcapsules/Submicron capsules

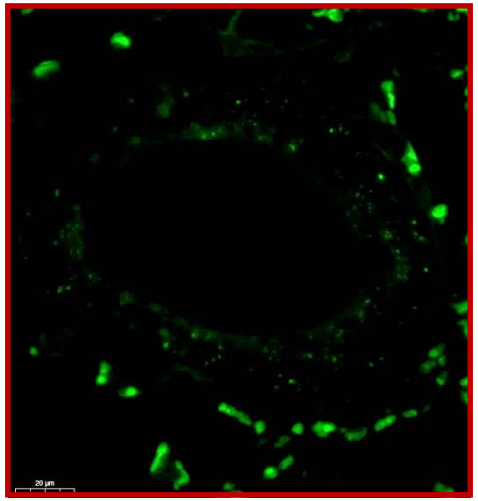
Histological data











Blue – DAPI (cell nuclei)
Green – FITC (capsules)

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