“Upconverting and magnetic nanoparticles for cancer therapy”

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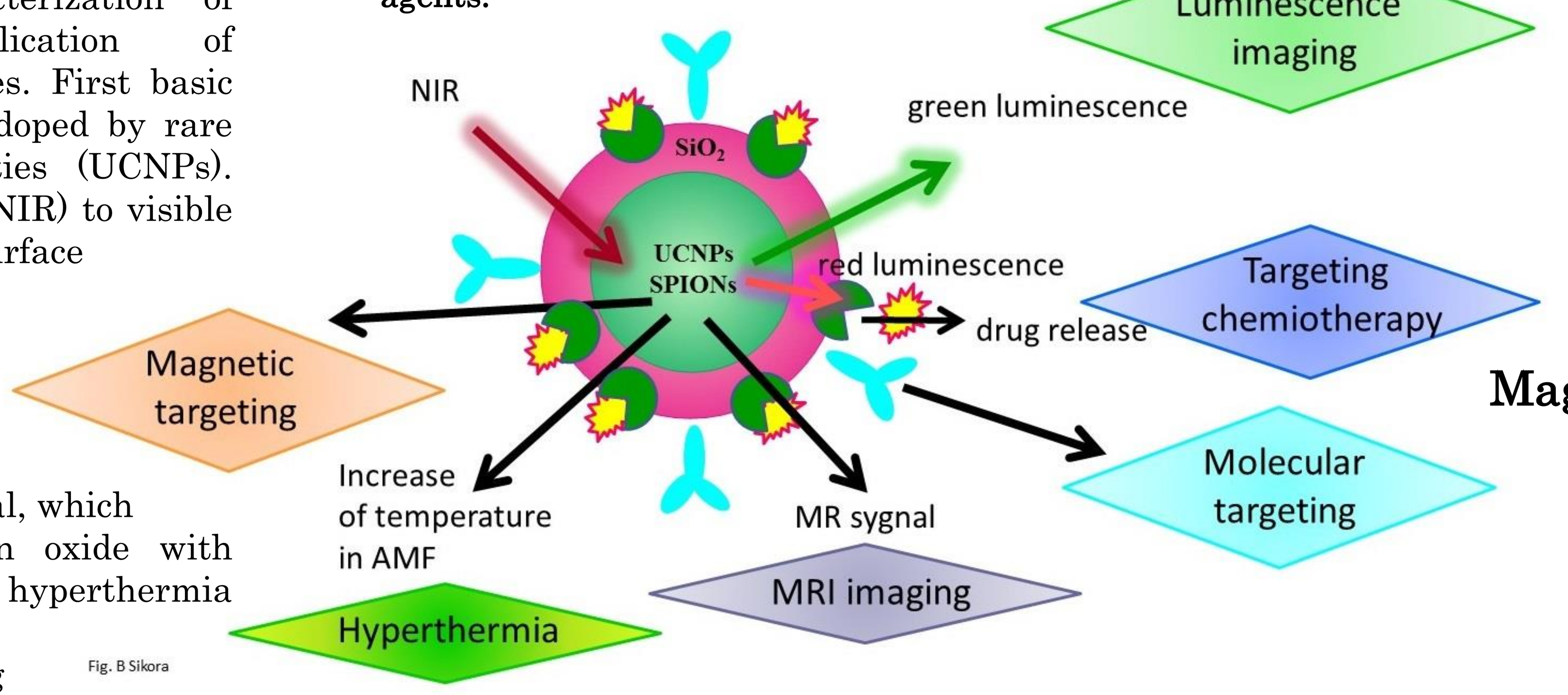
Introduction

In our studies we optimize process of synthesis, characterization of physical properties and potentially biological application of nanoconstructs based on optical and magnetic properties. First basic material is yttrium fluoride nanoparticles – $NaYF_4$ – doped by rare earth ions: Yb/Er and Yb/Tm, with upconverting properties (UCNPs). The nanoparticles are capable to convert near-infrared (NIR) to visible (VIS) and ultra-violet (UV) light. We functionalized the surface of UCNPs by silicon oxide and by photosensitizer (PS) for photodynamic therapy (PDT). In system, $NaYF_4$:20%Yb,2%Er@SiO_2-PS, NIR is absorbed by upconverting core than energy is transferred to PS which can produce reactive oxygen species in aqueous environment and damage cancer cells. Second nanomaterial, which we want to connect with upconverting part, is iron oxide with superparamagnetic properties (SPION). In magnetic hyperthermia therapy, under exposure to alternating magnetic field, the nanoparticles start to increase the temperature (destroying the cells). The same nanoparticles can be used as a contrast agents in magnetic resonance imaging. Combination of both presented materials (UCNPs and SPIONs) in one SiO_2 shell will allow to create multifunctional nanosystem with two independed anti-cancer therapies.

The model of multifunctional opto-magnetic nanoparticle as a anticancer agents.

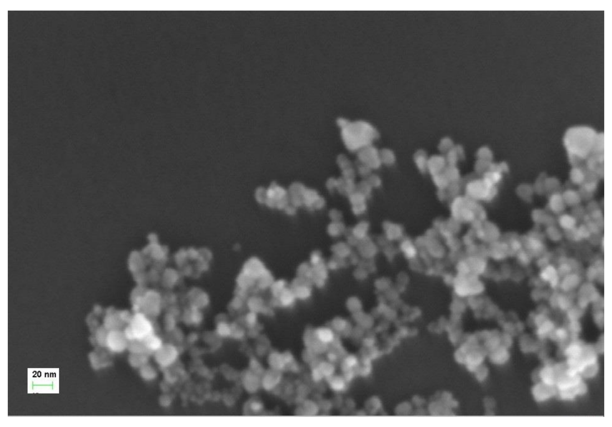


drug

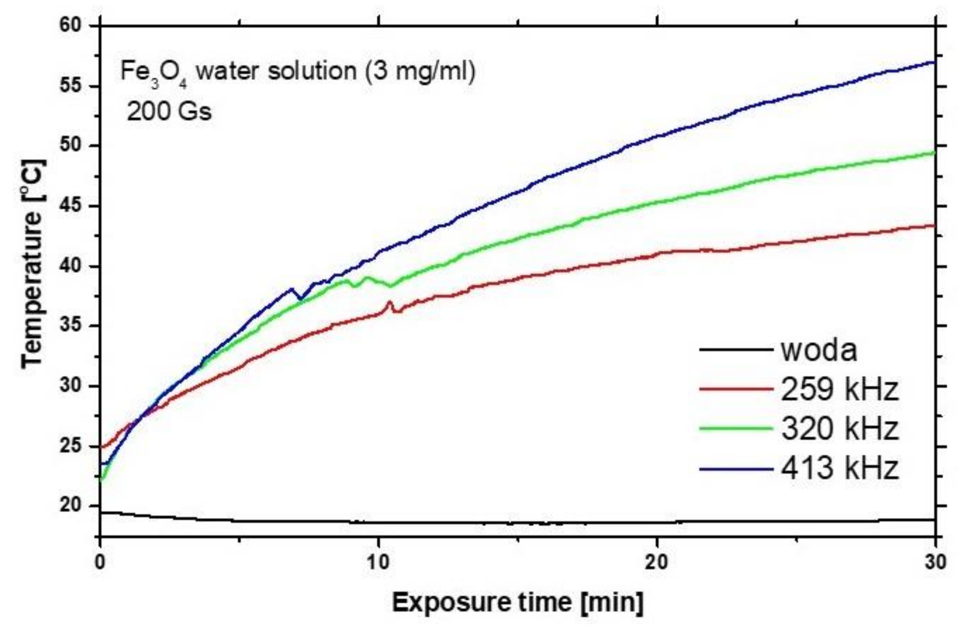
Antibody

NIR – near infrared (excitation light for UCNPs)
UCNPs - upconversion nanophosphors $NaYF_4$
SPIONs - superparamagnetic iron oxide nanoparticles Fe_3O_4
PDT – photodynamic therapy
MR – magnetic Resonance
AMF - alternating magnetic field

SEM image of superparamagnetic Fe_3O_4

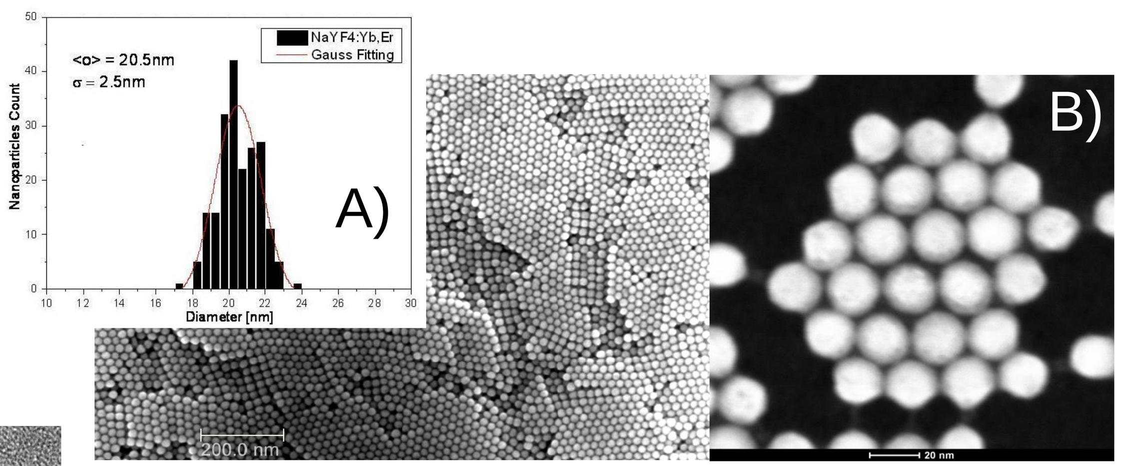


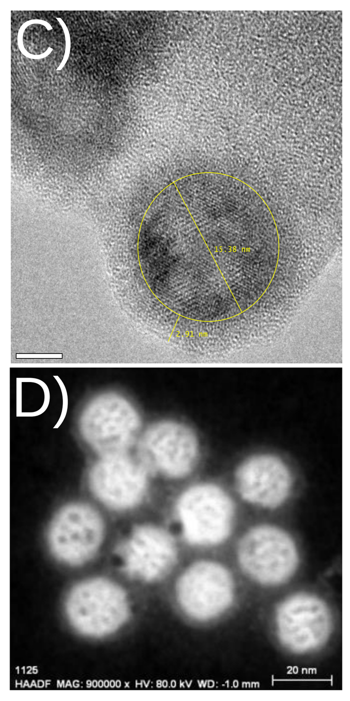
Magnetic hyperthermia measuremnt of Fe_3O_4 NPs

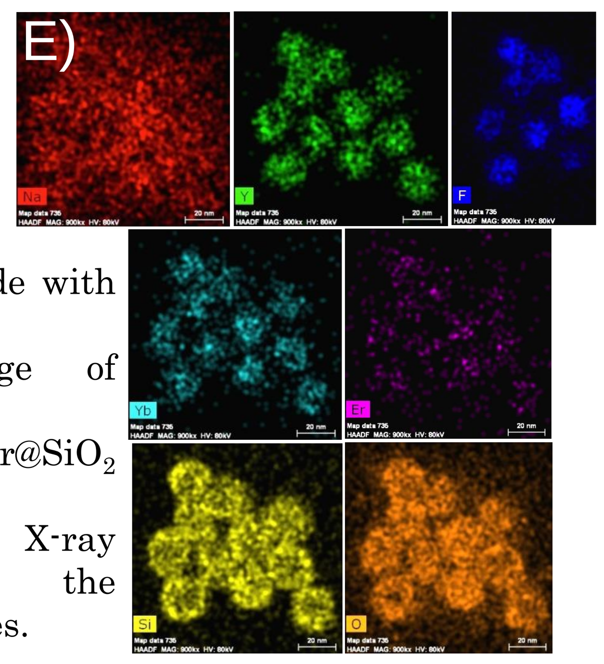


Temperature measurement results of water solution Fe_3O_4 nanoparticles under exposure by magnetic field (with different frequency) during 30 minutes.

Scanning and Transmission Electron Microscopy of β-$NaYF_4$:20Yb,2%Er and β-$NaYF_4$: 20%Yb,2%Er@SiO_2 nanoparticles

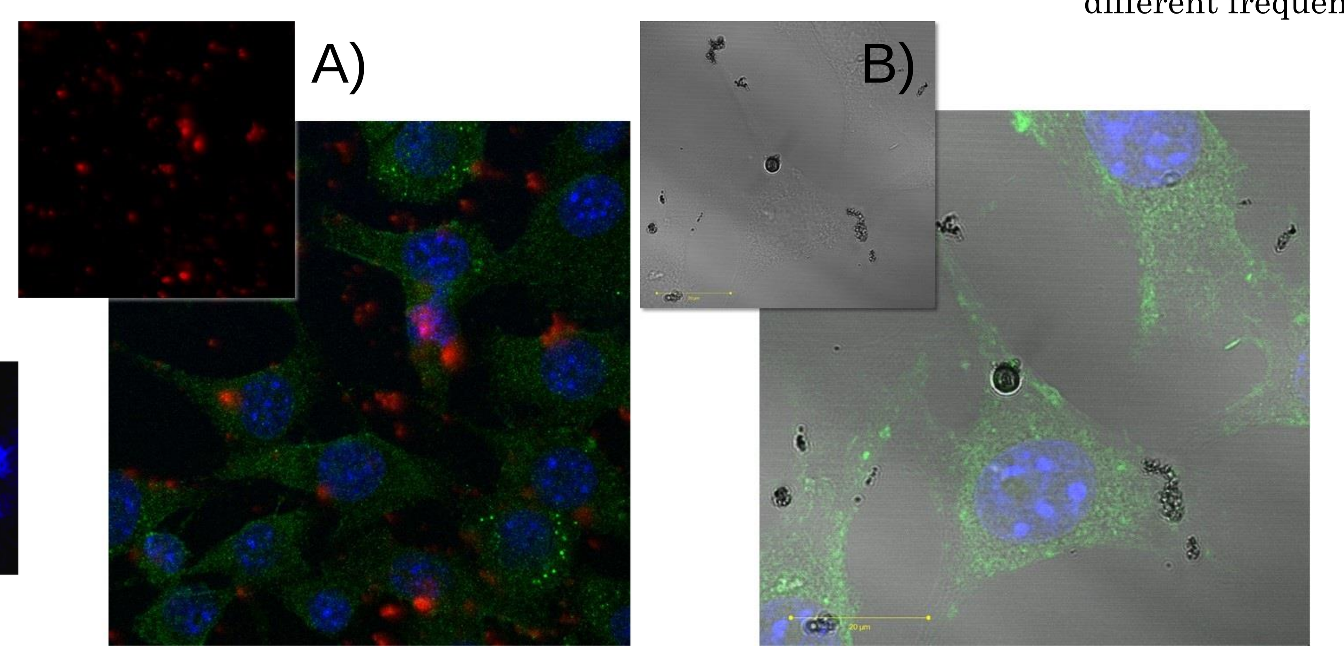






A: The SEM image of β-$NaYF_4$: 20%Yb,2%Er nanoparticles (insert shows histogram of nanoparticles size)
B: The TEM image of β-$NaYF_4$:20%Yb,2%Er nanoparticles. The size of nanoparticles was near 20 nm (image made in dark-field mode with used HAADF detector).
C: High resolution TEM image of $NaYF_4$:Yb^{3+}-Er^{3+}@SiO_2 nanocapsules
D: TEM image of $NaYF_4$:20%Yb,2%Er@SiO_2 nanocapsules.
E: EDX (energy dispersive X-ray spectroscopy) mapping of the $NaYF_4$:20%Yb,2%Er@SiO_2 nanocapsules.

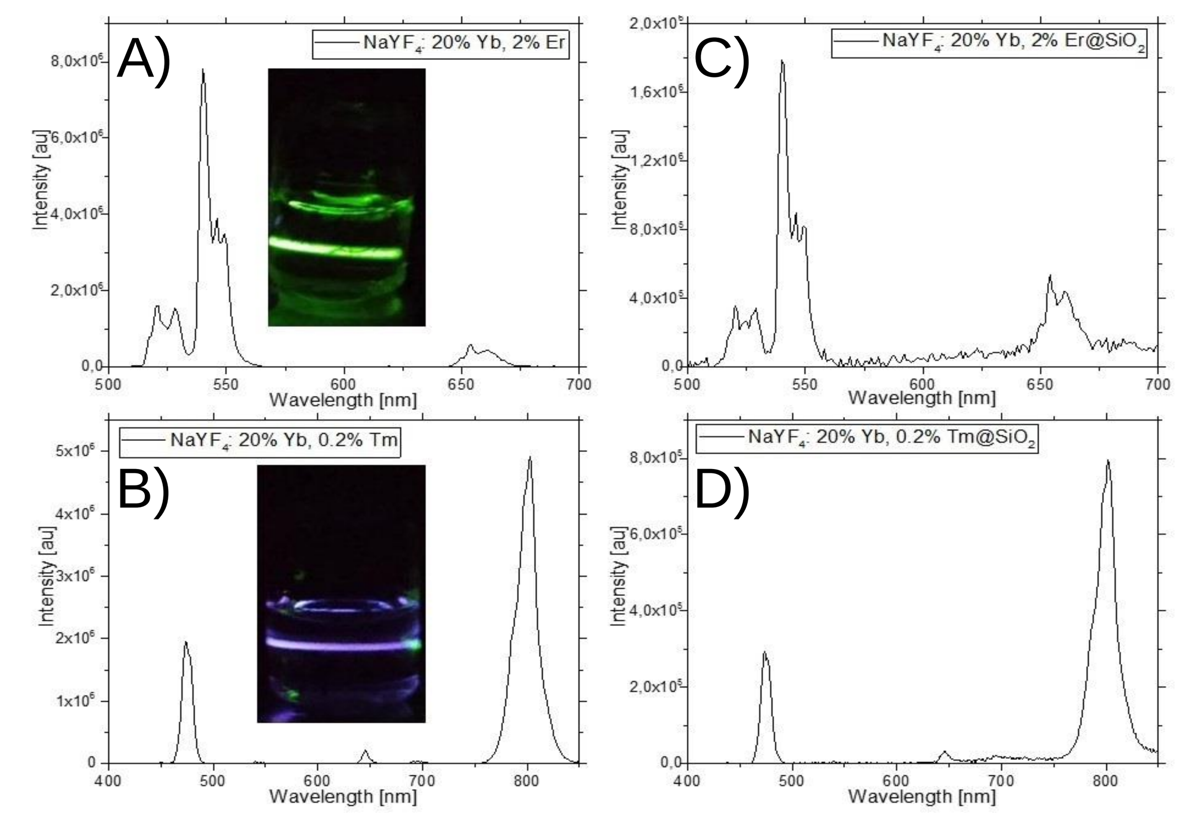
Nanoparticles inside breast cancer cells (4T1)



Clusters of UCNPs are shown in red (A; excitation by femtosecond laser, power density: 2.42 W/μm², wavelength: 980 nm; detection 500-730 nm). The SPIONs are shown on the image B in visible light transmission mode; inserts show only channels of NPs.

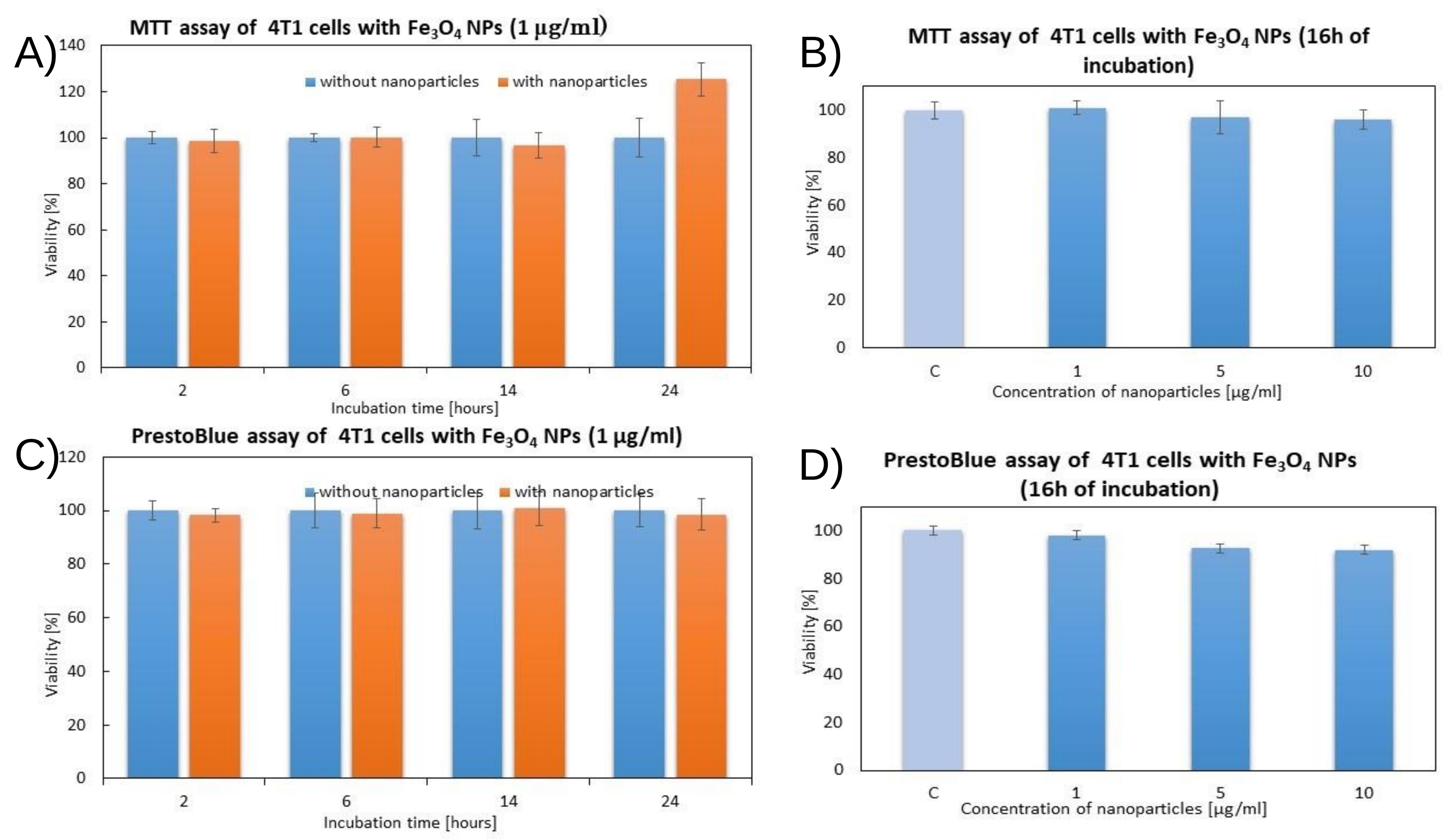
Confocal image of 4T1 cells, A: after 6 hours incubation with $NaYF_4$:20%Yb,2%Er@SiO_2–SPCD nanoparticles, B: after 3 hours of incubation with Fe_3O_4@PVMMA nanoparticles. In both cases early endosomes were stained with primary anti-EEA1 rabbit antibody and secondary goat anti-rabbit antibody conjugated with AlexaFluor488 - highlighted in green (excitation by CW laser with power density: (A) 0.043 and (B) 0.027 W/μm², wavelength: 488 nm, detection 495-572 nm). The blue colour indicates nuclei stained with Hoechst33258 (excitation by femtosecond laser, power density: (A) 0.46 and (B) 0.46 W/μm², wavelength: 705 nm; detection 425-475 nm).

Luminescence of β-$NaYF_4$:20%Yb,2%Er, β-$NaYF_4$:20%Yb,0.2%Tm, β-$NaYF_4$: 20%Yb,2%Er@SiO_2 and β-$NaYF_4$:20%Yb,0.2%Tm@SiO_2 nanoparticles



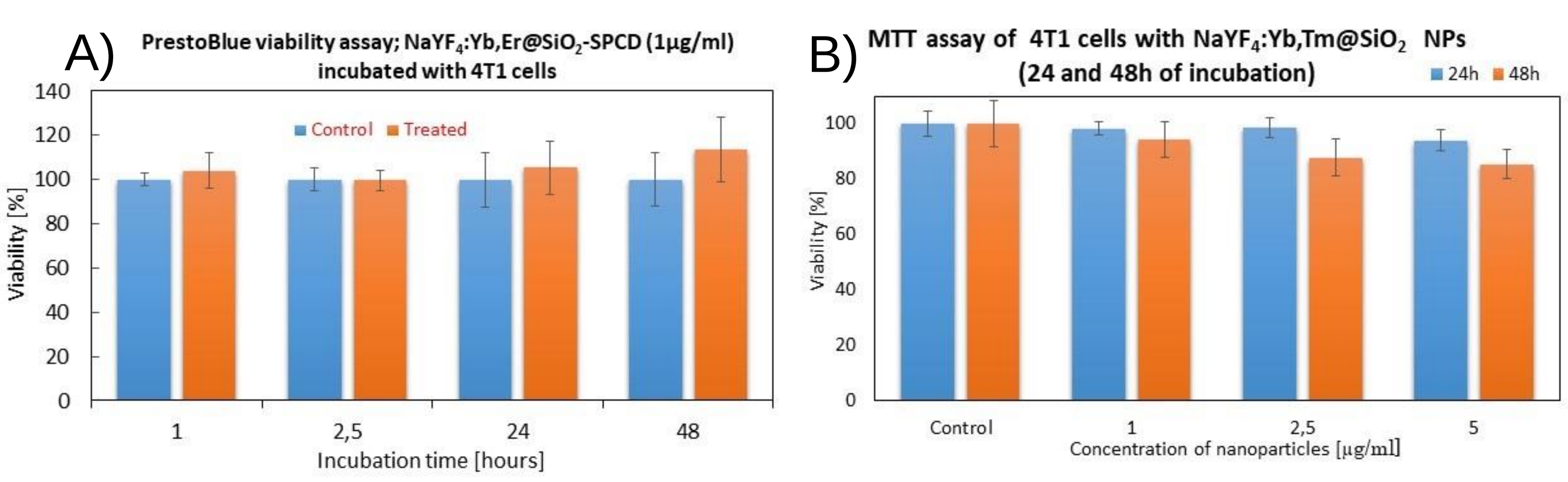
Luminescence of the (A) $NaYF_4$:20%Yb,2%Er, (B)$NaYF_4$:20%Yb,0.2%Tm, (C)$NaYF_4$:20%Yb,2%Er@SiO_2 (D)$NaYF_4$:20%Yb,0.2%Tm@SiO_2 nanoparticles in cyclohexane (without SiO_2 shell) and water (coated nanoparticles) solution (1mg/ml). Excitation wavelength was CW - 980 nm, power density – 12.14 W/cm². Insert shows digital images of nanoparticles solutions.

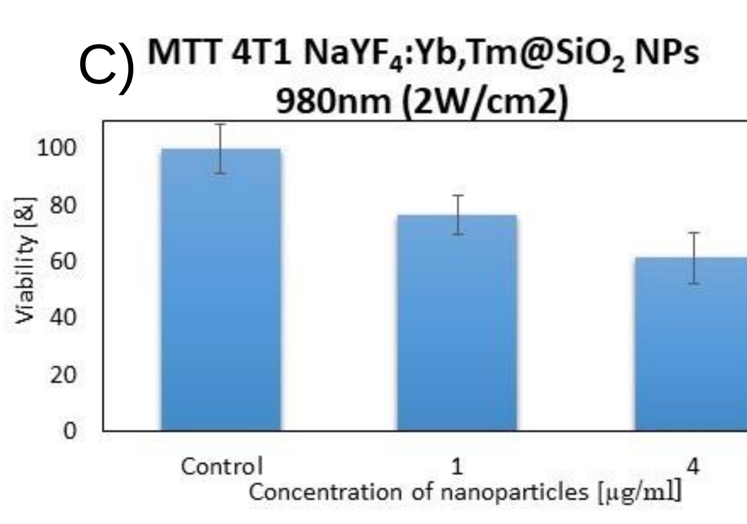
Cytotoxicity of Fe3O4 nanoparticles



Cytotoxicity of Fe_3O_4 nanoparticles at 4T1 cells line. Cells incubated in different periods of time (A and C) and different concentrations of nanoparticles (B and D). The viability of cells after treatment specified using two independent commercial cytotoxicity assay: MTT (A and B) and PrestoBlue (C and D).

Cytotoxicity of $NaYF_4$:20%Yb,2%Er@SiO_2–SPCD and $NaYF_4$: 20%Yb,0.2%Tm@SiO_2. Pilot experiment of photodynamic therapy with living cells





Toxicity of $NaYF_4$:20%Yb,2%Er@SiO_2–SPCD (A) and $NaYF_4$:20%Yb,0.2%Tm@SiO_2 (B) made on 4T1 (breast cancer) cells incubated with nanoparticles in the dark.

C) The results of 4T1 cells viability, incubated with $NaYF_4$:20%Yb,0.2%Tm@SiO_2. Cells were irradiated by 980nm NIR laser for 10 minutes (1 minute of irradiation and 1 minute break – total irradiation time 5 minutes) with 2W/cm² power density.

Conclusions

1. **Yttrium sodium fluoride nanoparticles doped by rear-earth ions were synthetized with narrow size distribution (~20nm) and wide luminescence emission range (green-red and blue-red) depend of using doped ions.**
2. **The main advantage of using UCNPs is excitation light wavelength – near-infrered. The NIR light can be used for imaging of biological tissues without biological background (autofluorescence) and relatively low level of cytotoxicity.**
3. **Therapeutic potential of thulium doped nanoparticles proved with experiment on living cancer cells.**
4. **Upconverting nanoparticles were functionalized by SiO_2 shell and photosensitizer (SPCD).**
5. **The $NaYF_4$ doped by rare earth ions nanoparticles have relatively low cytotoxicity level without excitation by NIR, checked by two kinds of viability assays. The Fe_3O_4 nanoparticles have love cytotoxicity without exposure by magnetic field (without hyperthermia).**
6. **Functionalized $NaYF_4$:Yb,Er@SiO_2-SPCD and Fe_3O_4 coated by polymer (PVMMA) are capable to penetrate inside living cells.**

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