# Effect of annealing on the Cytotoxicity of Upconversion Nanoparticles in Different Cell Lines

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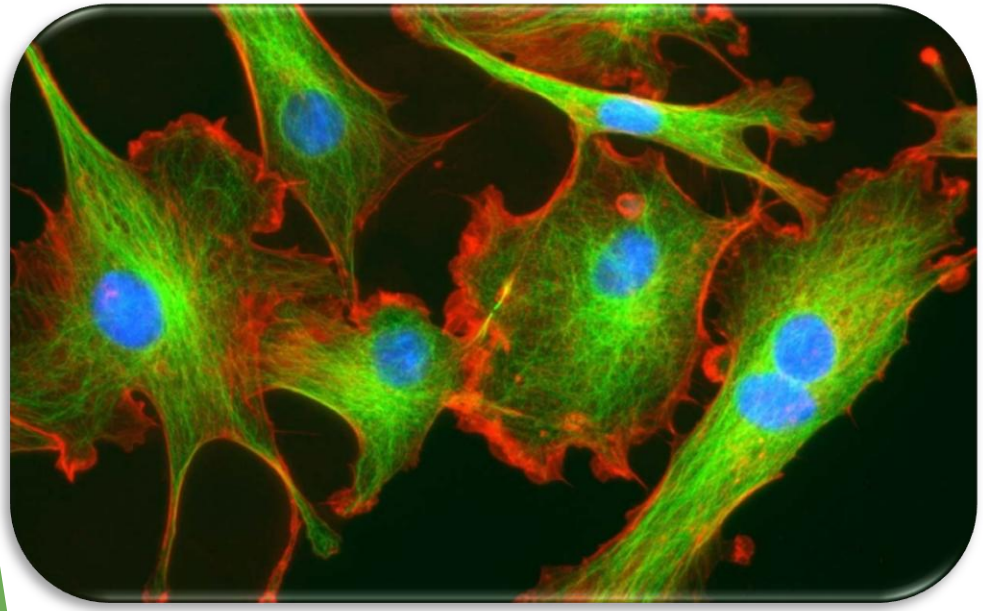
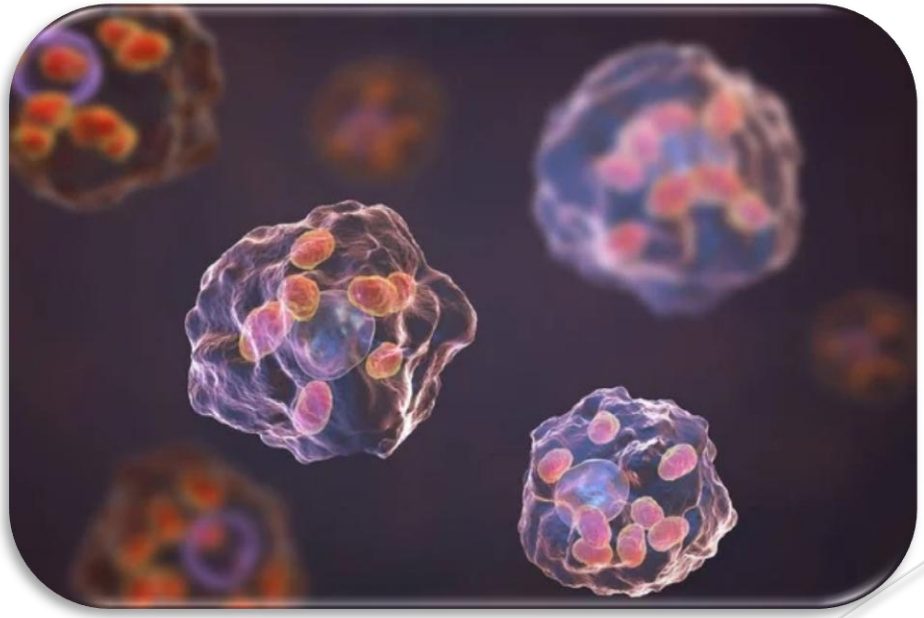
# Aim

We have evaluated the cytotoxicity of UPCNPs $NaYF_4$: $Yb^{3+}$, $Er^{3+}$ unannealed and annealed at 550 $^oC$ on different normal and cancer murine cell lines after 24, 48 and 72 h of incubation.





# Methods and Materials

Theranostic technologies enable the advancement of imaging and therapy in oncology using nanomethods and the development of advanced biomedical products and services for healthcare purposes. With the use of these technologies, theranostics will be able to target the delivery of both active agents and imaging compounds.

Photodynamic therapy (PDT) is a non-surgical method for the treatment of oncological diseases, which is currently intensively used with high efficiency. The experience of clinical application of PDT shows that this method belongs to one of the promising areas in modern clinical oncology. The necessary components of PDT are a photosensitizer (PS) localized in the pathological area and a radiation source of the appropriate wavelength.[1,2] Various classes of substances can be selected as PS, but, as a rule, molecular structures and nanoparticles are considered.[3]

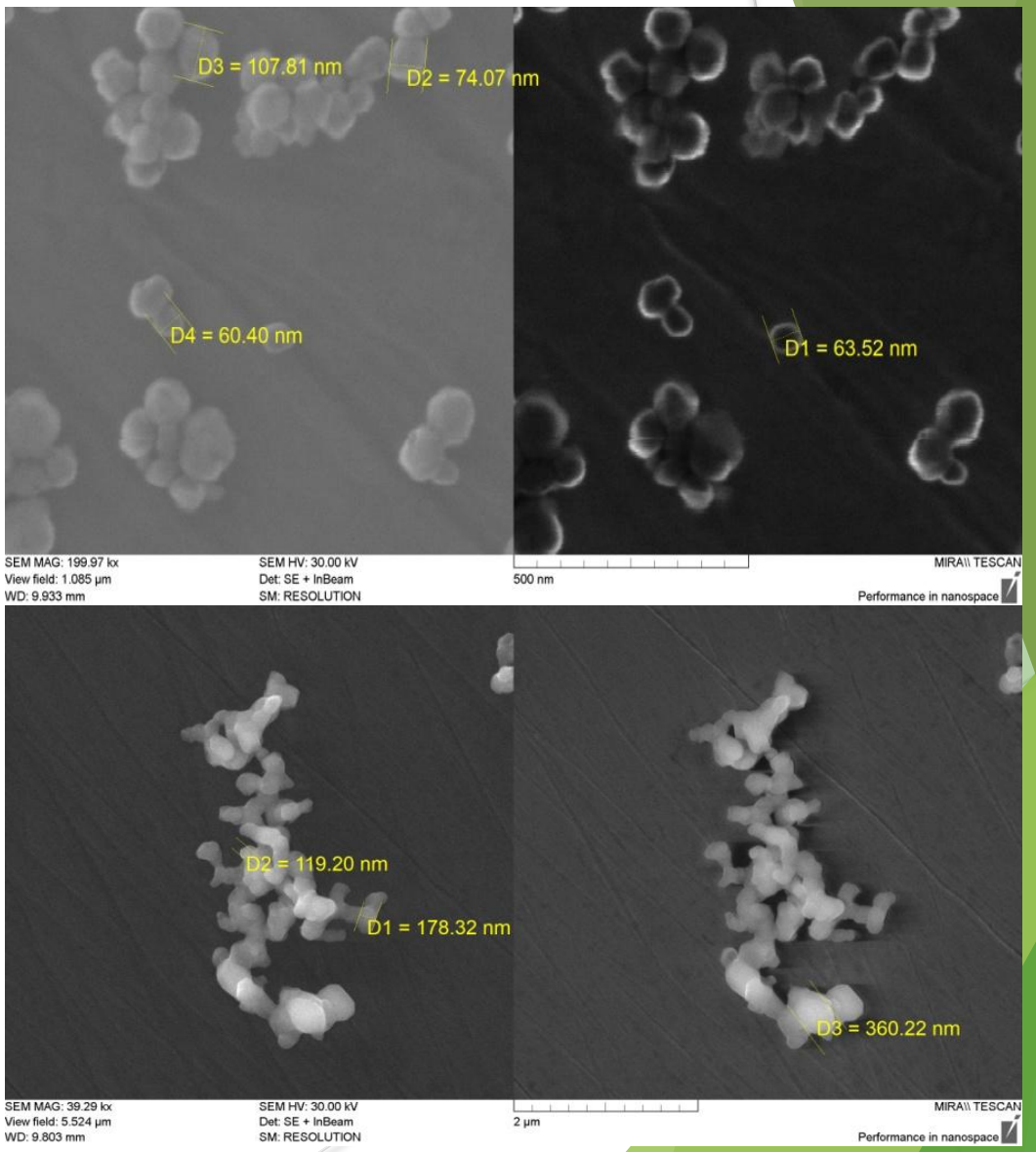
# Methods and Materials

In this regard, it is of interest to combine of upconversion nanoparticles (UCNPs)[4] and phthalocyanine dyes. Dyes are able to further enhance the efficiency of PDT due to nonradiative energy transfer from nanoparticles to phthalocyanine. However, it is necessary for the local concentration of nanoparticles and dye to be maximum in a submicron volume, which is possible to obtain only by encapsulating two components into one carrier.[4] Phthalocyanines conjugate extremely weakly with proteins and very quickly release,[5,6] which naturally will not allow us to create a depot PS. Cyanine dyes, which are also actively used in PDT, effectively bind to proteins through a wide variety of functional groups, and therefore can be an even more effective pair for UCNPs during PDT.[7,8]

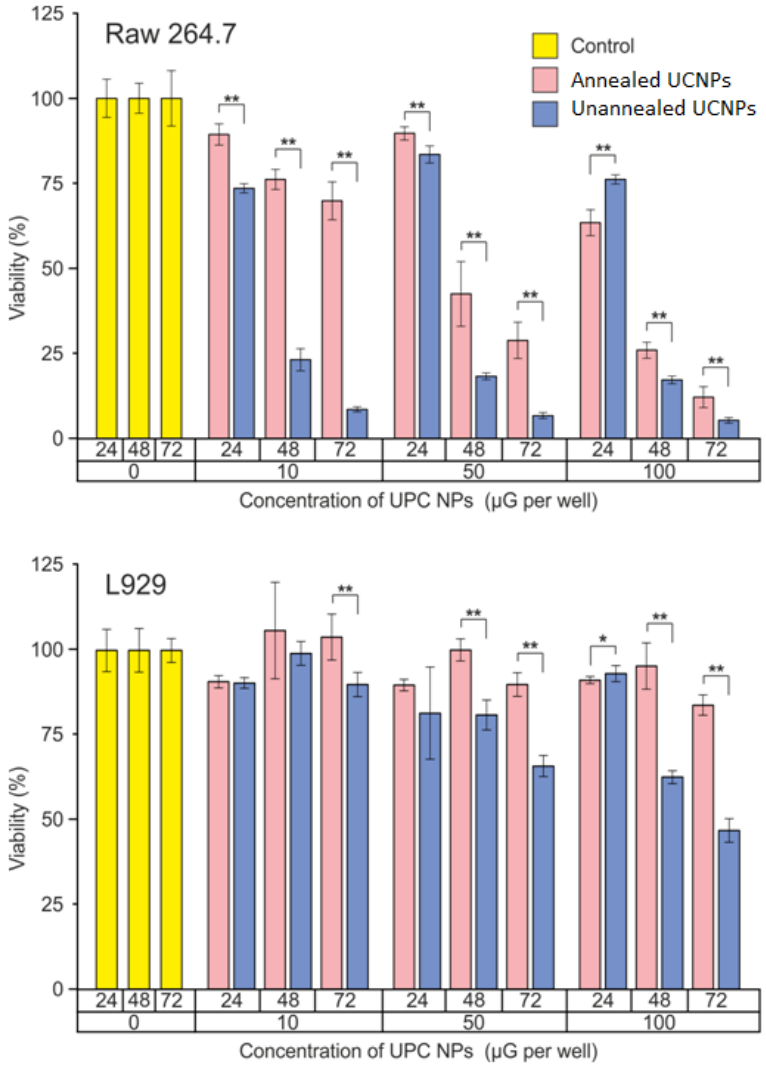
From the point of view of theranostics, an important issue is to increase the efficiency of luminescence of UCNPs. An effective and fairly simple method for increasing the UCNPs intensity is additional heating of the particles—annealing. In this case, as a result of annealing, the luminescence intensity can increase by a factor of 4000.[9] Previously, it was shown that for the UCNPs used in this work, it is typical that the dependence of the luminescence intensity on the annealing temperature takes the maximum value at 550 °C and has local maximum at 400 °C.[10]

# EXPERIMENTAL PROCEDURES

- We used the in-house synthesized UCNPs $NaYF_4$: $Yb^{3+}$, $Er^{3+}$ (fluoride matrix doped with ions of ytterbium and erbium), unannealed and annealed at 550 $^oC$. The UCNPs were synthesized by a hydrothermal method.
- For cells' viability evaluation 10uL of Cell Proliferation Reagent WST-1 (Sigma Aldrich, USA) was added to each well and incubated for 4 h. Cells incubated without particles were used as a positive control.



# RESULTS AND DISCUSSION



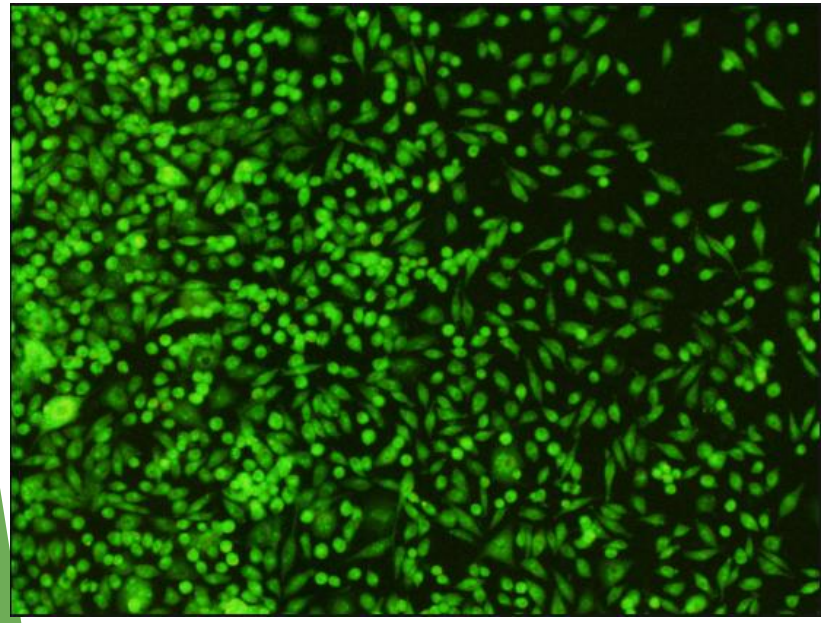

In these experiments we show the absence of toxicity of UCNPs using quantitative (Cell Proliferation Reagent WST-1) method (Fig. 2).

UCNPs provide a dose-dependent and time-dependent cytotoxic effect on all studied cell lines which was most pronounced for the Raw264.7 cell line. It is probably caused by the high phagocytic activity of macrophages. The statistically significant differences in cell viability after 24, 48 and 72 h of incubation of cells with particles were observed just for the macrophage cell line. It is also worth noting that annealed particles are less toxic than unannealed ones.

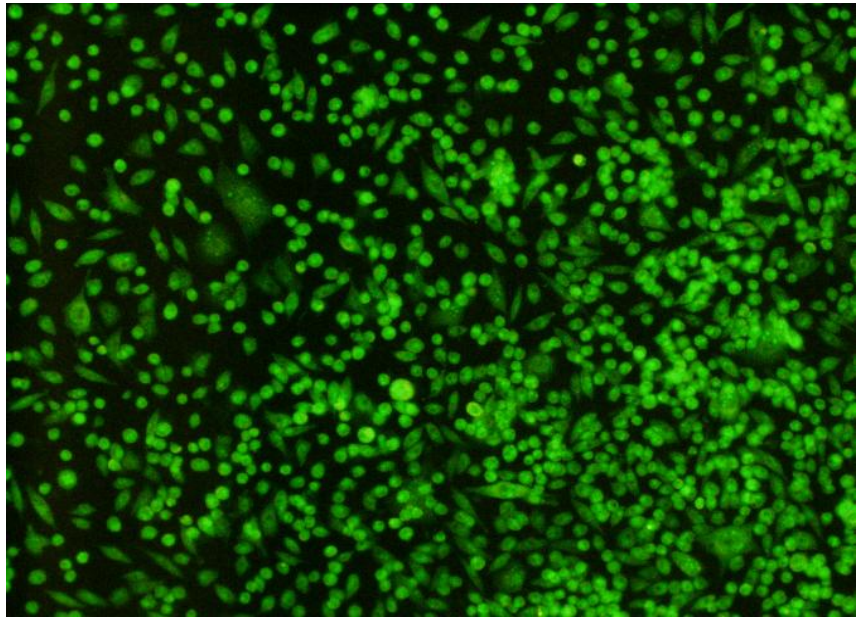
**Thus, according to preliminary data in experiments *in vitro* annealed UCNPs are the most promising particles for the development of cancer treatment methods**

# Cell lines L929

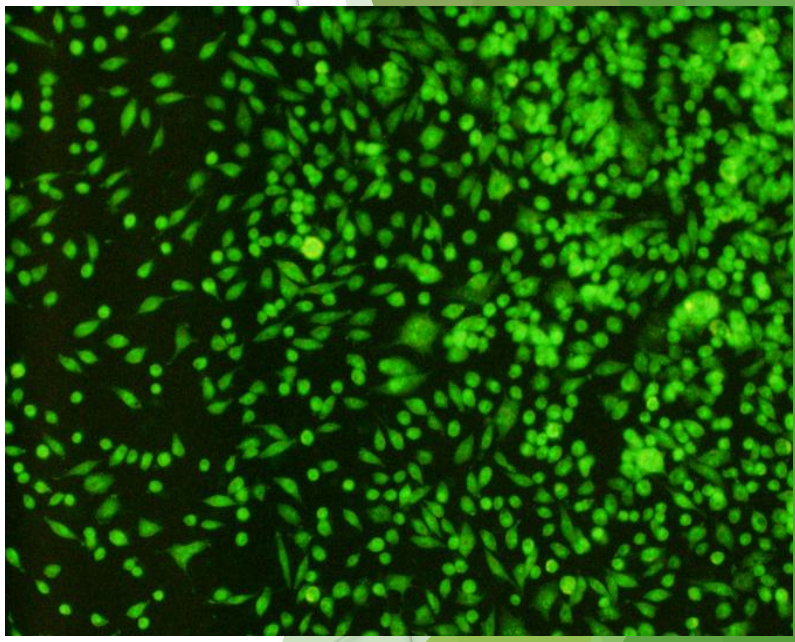
The action of unannealed particles has a concentration dependence, while annealed particles do not have such a dependence, and the greatest toxic properties are exhibited at a concentration of 50 mkg per cell.



Cell control lines L929

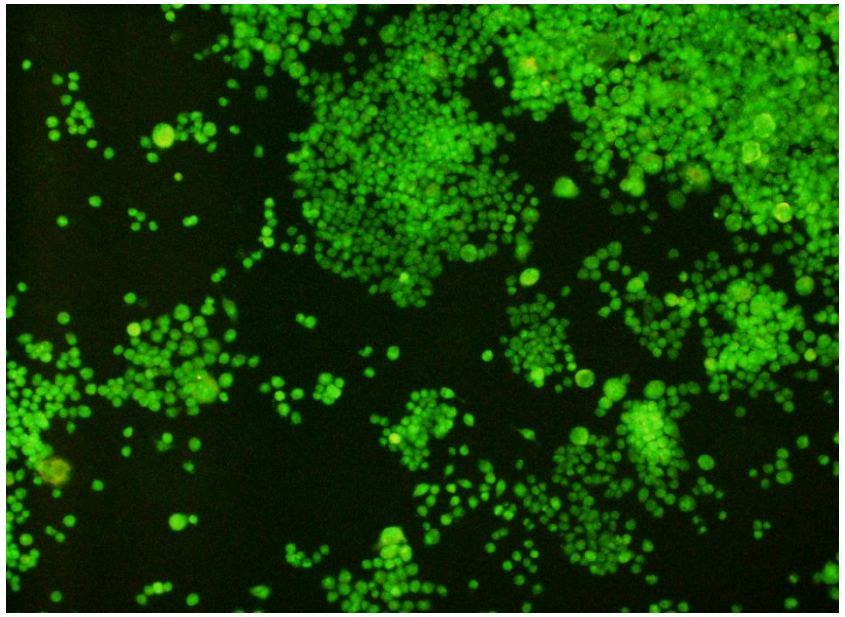


Cell culture of L929 fibroblasts 24 hours after adding a suspension of **unannealed** AKNP at a concentration of 50 mkg per well

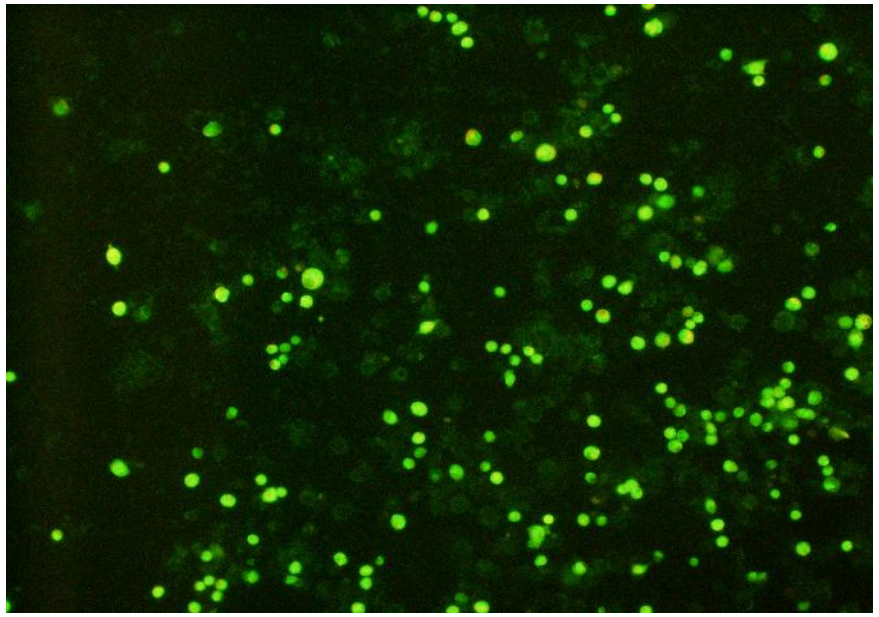


Cell culture of fibroblasts L929 24 hours after adding a suspension of **annealed** AKNP with a concentration of 50 mkg

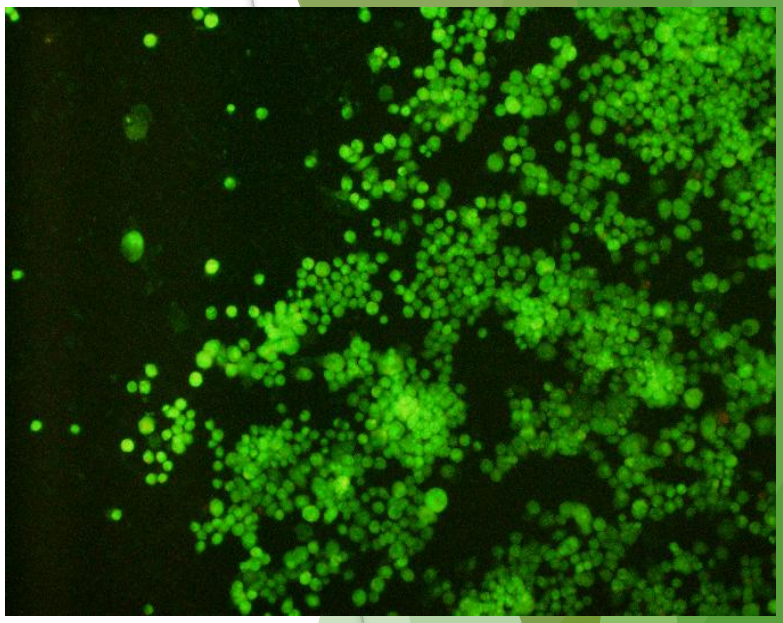
# Raw264.7 cell line



Raw264.7 cell line control. 72 hours



Cell culture of macrophages raw264.7 72 hours after adding a suspension of unannealed AKNP with concentrations 50 mkg per cell



Raw264.7 macrophage cell culture 72 hours after adding a suspension of annealed AKNP at concentrations of 50 mkg per well

# CONCLUSIONS

1. It has been presented the influence of annealing temperature on the cytotoxicity UCNPs on different normal and cancer murine cell lines *in vitro*.
2. The cell viability is scored for cytotoxic effects of UCNPs at dark conditions. UCNPs provide the dose-dependent and time-dependent cytotoxic effect on all studied cell lines which was most pronounced for the Raw264.7 cell line. It is probably caused by the high phagocytic activity of macrophages. The statistically significant differences in cell viability after 24, 48 and 72 h of incubation of cells with particles were observed just for the macrophage cell line. It is also worth noting that annealed particles are less toxic than unannealed ones.
3. **The results obtained can be taken into consideration in the development of complex drugs for photodynamic therapy and the study of ways to increase their efficiency in carrying out the PDT procedure with their use. The drugs should be nano / microcontainers - carriers of acting photodynamic agents, coated with targeted molecules and deliberately destroyed at the delivery site.**

# References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# ACKNOWLEDGMENTS

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