UNIVERSAL DRUG DELIVERY PLATFORM FOR ANTICANCER THERANOSTICS BASED ON DUMBBELL-LIKE Fe_3O_4-Au NANOPARTICLES

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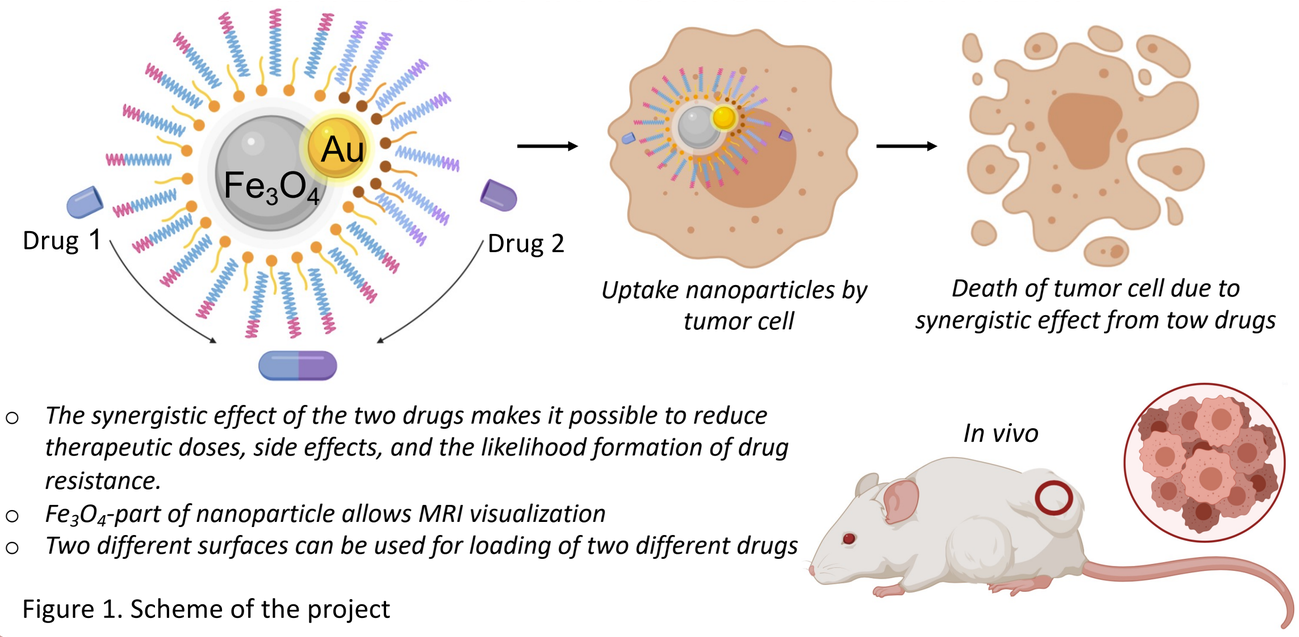
INTRODUCTION

Anticancer therapy is a significant challenge today. The use of nanocarriers as a promising method can influence the pharmacokinetics and biodistribution of drugs, as well as reduce side effects. Combinations of drugs such as doxorubicin and paclitaxel in certain ratios have been shown to exhibit a synergistic effect, while using drugs simultaneously can reduce the development of resistance and the total administered dose [1,2]. To establish the effect produced by a combination of two drugs, *in vitro* studies were carried out and the combination index CI were determined for combinations doxorubicin with cisplatin and doxorubicin with paclitaxel. The synergistic effect corresponds to CI <0.9 [3]. However, delivering combinations of drugs to tumor cells *in vivo* at a given molar ratio is difficult due to differences in the chemical structure and properties of anticancer drugs (hydrophobicity and charge). In this work, magnetic dumbbell-like Fe_3O_4-Au nanoparticles (MNPs) are proposed. Firstly, due to their magnetic properties, MNPs can be used for magneto-resonance imaging which allows to track biodistribution of MNPs. Secondly, the presence of two chemical surfaces (Fe_3O_4 and Au) allows us to modify MNPs with different molecules in order to load two different types of drugs at given ratios.

1. Khafaji, M., Zamani, M., Vossoughi, M., & Iraji Zad, A. (2019). Doxorubicin/Cisplatin-Loaded Superparamagnetic Nanoparticles As A Stimuli-Responsive Co-Delivery System For Chemo-Photothermal Therapy. International journal of nanomedicine, 14, 8769–8786.
2. Jiang, Y., Zhou, Y., Zhang, C. Y., & Fang, T. (2020). Co-Delivery of Paclitaxel and Doxorubicin by pH-Responsive Prodrug Micelles for Cancer Therapy. International journal of nanomedicine, 15, 3319–3331.
3. Tardi, P., Johnstone, S., Harasym, N., Xie, S., Harasym, T., Zisman, N., Harvie, P., Bermudes, D., & Mayer, L. (2009). In vivo maintenance of synergistic cytarabine:daunorubicin ratios greatly enhances therapeutic efficacy. Leukemia research, 33(1), 129–139.

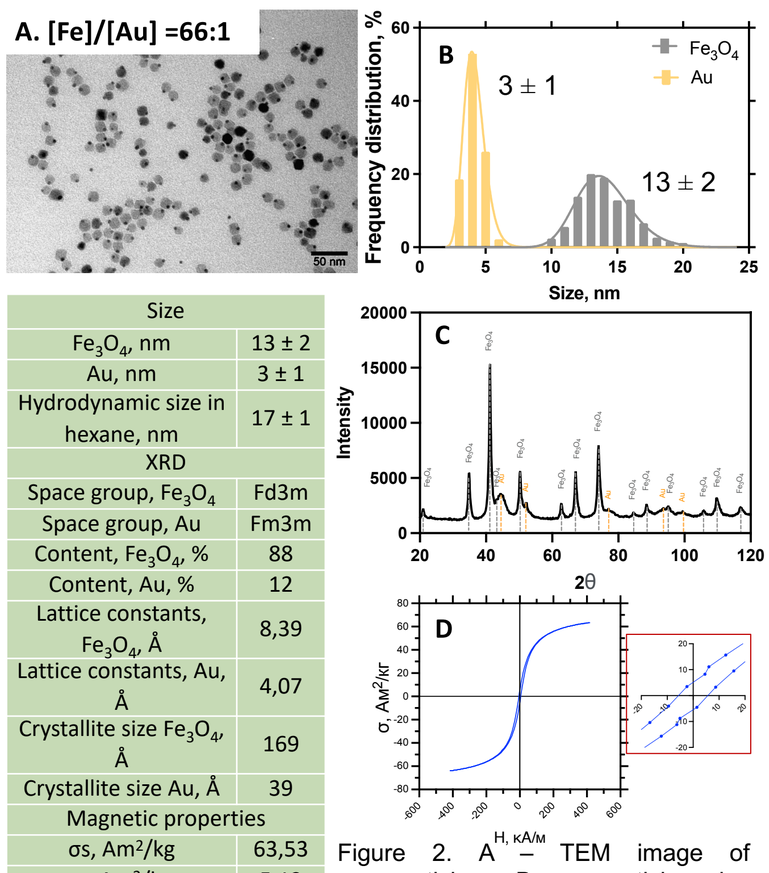
STRATEGY OF ANTICANCER TREATMENT



- *The synergistic effect of the two drugs makes it possible to reduce therapeutic doses, side effects, and the likelihood formation of drug resistance.*
- *Fe_3O_4-part of nanoparticle allows MRI visualization*
- *Two different surfaces can be used for loading of two different drugs*

Figure 1. Scheme of the project

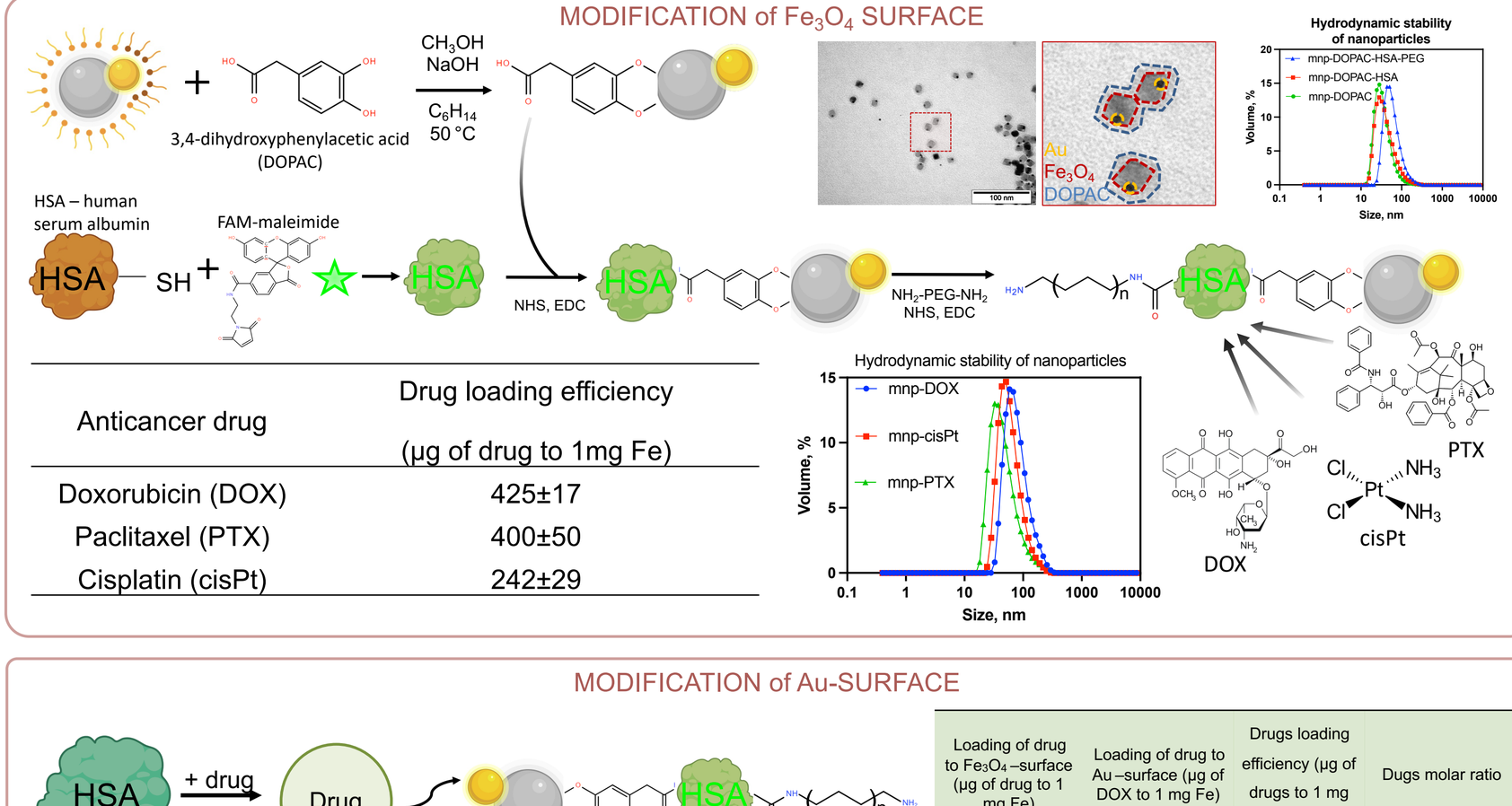
PROPERTIES OF SYNTHESIZED NANOPATICLES



Size	
Fe_3O_4, nm	13 ± 2
Au, nm	3 ± 1
Hydrodynamic size in hexane, nm	17 ± 1
XRD	
Space group, Fe_3O_4	Fd3m
Space group, Au	Fm3m
Content, Fe_3O_4, %	88
Content, Au, %	12
Lattice constants, Fe_3O_4, Å	8,39
Lattice constants, Au, Å	4,07
Crystallite size Fe_3O_4, Å	169
Crystallite size Au, Å	39
Magnetic properties	
σs, Am²/kg	63,53
$σ_r$, Am²/kg	5,12
$σ_r/σ_s$	0,081
H_c, kA/m	5,7
H_{max}, kA/m	418

Figure 2. A – TEM image of nanoparticles; B – particle size distribution by frequency percent of total particle; C – XRD patterns of nanoparticles; D – magnetic hysteresis loop

MODIFICATION of Fe_3O_4 SURFACE

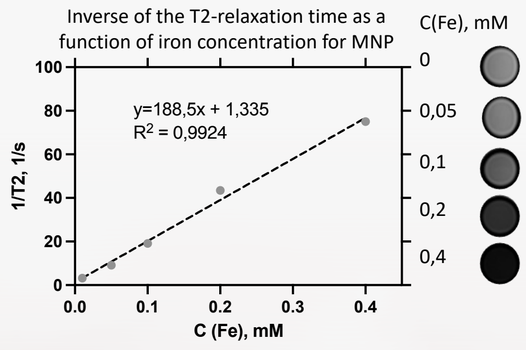


Anticancer drug	Drug loading efficiency (μg of drug to 1mg Fe)
Doxorubicin (DOX)	425±17
Paclitaxel (PTX)	400±50
Cisplatin (cisPt)	242±29

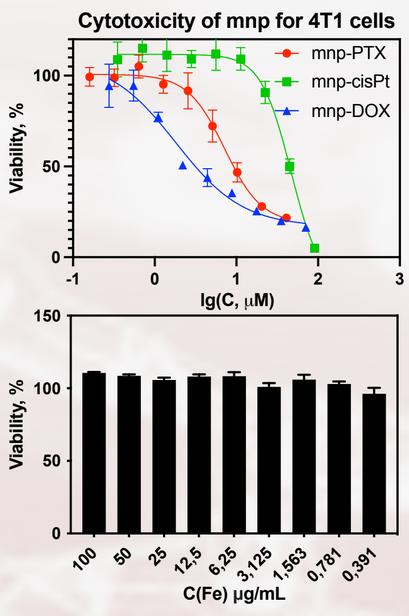
MODIFICATION of Au-SURFACE

Loading of drug to Fe_3O_4 –surface (μg of drug to 1 mg Fe)	Loading of drug to Au –surface (μg of DOX to 1 mg Fe)	Drugs loading efficiency (μg of drugs to 1 mg Fe)	Dugs molar ratio
400±50	370±15	770	DOX:PTX=1,3:1
242±29	608±115	850	DOX:cisPt=1,3:1

In vitro STUDIES



Cell line	IC50 mnp-DOX-cisPt	CI
4T1	7,4±0,8	0,42
CT26	55,7±23,8	0,84
Cell line	IC50 mnp-DOX-PTX	CI
4T1	1,67±0,56	0,22
CT26	3,9±0,7	0,17



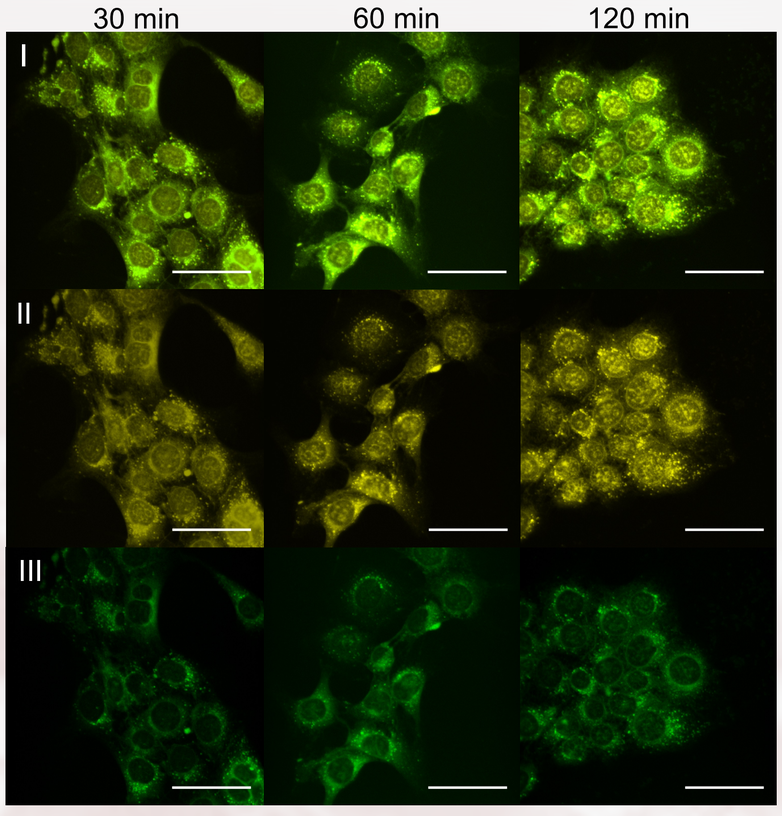
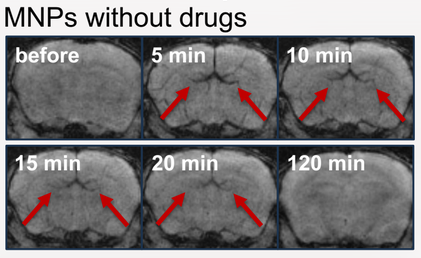


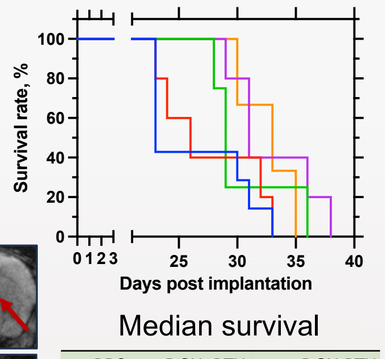
Figure 3. I—mnp-DOX internalization process in 4T1 cells. I—merge, II—Dox fluorescence, III—FAM (fluorescein amidite) fluorescence, corresponding to MNPs, laser scanning confocal microscopy, scale bar 50 um.

In vivo STUDIES

For therapy study BALB/c mice were subcutaneously implanted with CT26 cells.

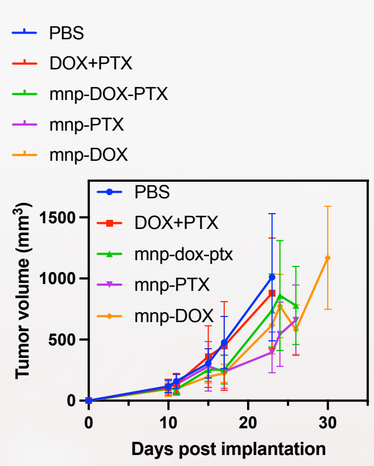
Mouse brain MR-images before and after injection of MNPs without drugs





Median survival

PBS	DOX+PTX	mnp-DOX-PTX
23	26	
mnp-PTX	mnp-DOX	29
31	33	



CONCLUSIONS

The goal of this research was to develop a delivery system for a combination of antitumor agents with a synergistic effect based on Fe_3O_4-Au magnetic nanoparticles (MNPs) for cancer therapy. At the moment, this work is in progress and requires additional research.

- As a result of this work, we have synthesized dumbbell-shaped nanoparticles with sizes of 13 ± 2 nm for Fe_3O_4 and 3 ± 1 nm for Au. We have also studied their phase composition and magnetic properties.
- Sequential functionalization of the surface of Fe_3O_4 nanoparticles in the composition of Fe_3O_4-Au nanoparticles with molecules such as DOPAC, human serum albumin (HSA), fluorescein maleimide-bound, and NH_2-PEG-COOH has led to the formation of nanoparticles that can load 425 ± 17 μg of doxorubicin (DOX) per mg (in terms of iron), 400 ± 50 μg of paclitaxel (PTX), and 242 ± 29 μg of cisplatin (cisPt).The resulting Fe_3O_4-Au nanoparticles loaded with combinations of anticancer drugs retain the synergistic effect of the drugs.
- The combination index (CI) for doxorubicin (DOX) and cisplatin (cisPt) in free form for 4T1 and CT26 cell lines was equal to 0.25 and 0.14, whereas the CI for the mnp-DOX-cisPt combination increased up to 0.42 and 0.84 for 4T1 and CT26 cell lines, respectively. The CI of free DOX and paclitaxel (PTX) was 0.71 and 0.79, whereas for the mnp-DOX-PTX combination we observed a decrease of CI up to 0.22 and 0.17 for 4T1 and CT26 cell lines, respectively.
- The Fe_3O_4 -Au MNPs-loaded drug combinations increased the median survival time by 6-10 days compared to the control group, and significantly reduced tumor volume compared to the control. The group receiving the free DOX-PTX combination had a median survival time of only 3 days greater than the control, and did not affect tumor volume growth rate.