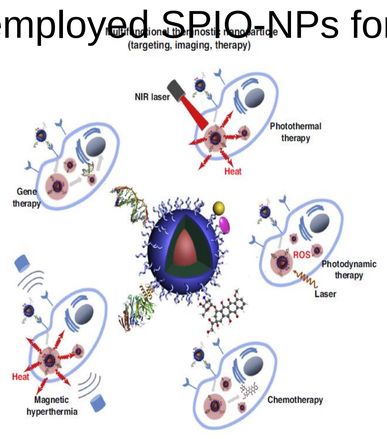
Multifunctional iron oxide nanoparticles for targeting metastatic breast cancer cells

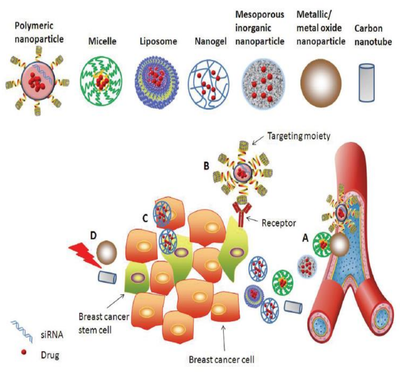
Catalano Enrico[1], Geisler J [2], Kristensen VN [1]
[1] Department of Clinical Molecular Biology (EpiGen), Akershus University Hospital, University of Oslo (UiO), Oslo, Norway. [2] Department of Oncology, Akershus University Hospital, 1478 Lørenskog, Norway;

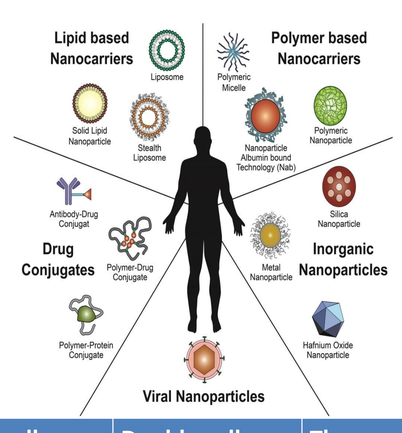
Introduction

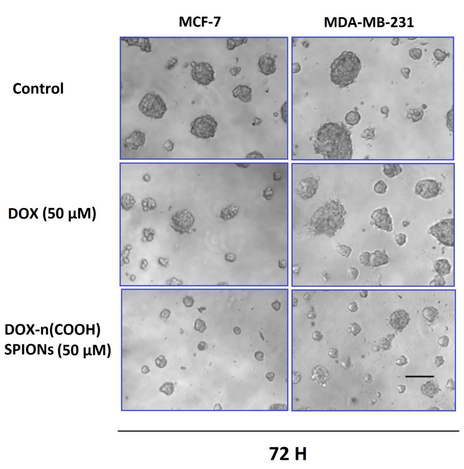
Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide, accountings for 25% of all cancer cases and 15% of all cancer deaths among females. Nanocarriers can be effective anticancer drug delivery systems for preventing and treating breast cancer.

The ability of SPIONs to be functionalized and concurrently respond to a magnetic field has made them a useful tool for theranostics – the fusion of therapeutic and diagnostic technologies that targets to individualize medicine. Fe_3O_4 are superior to other metal oxide nanoparticles for their biocompatibility and stability and are, by far, the most commonly employed SPIO-NPs for biomedical applications.



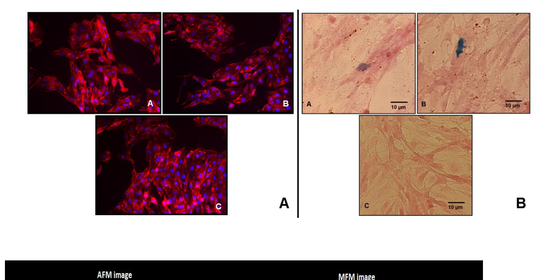


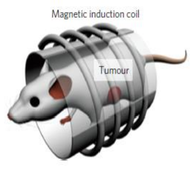




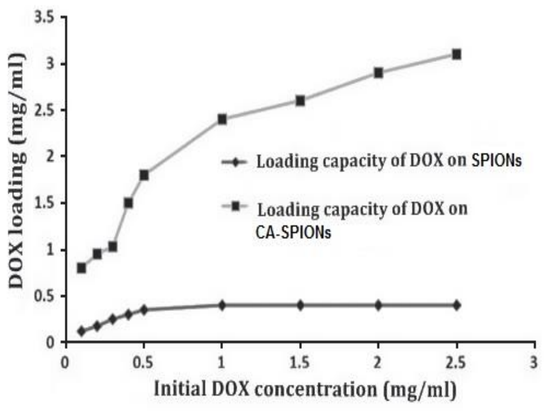
Invasion capacity of matrigel-embedded 3D cultures of MCF-7 and MDA-MB-231 cells is reduced with DOX-SPIONs. Cells were grown in a semi-solid matrigel matrix. Then, 3D cultures were exposed to the indicated doses of the drugs. After 72 h was taken pictures and quantified the spheres size. Scale bar= 100 µm.

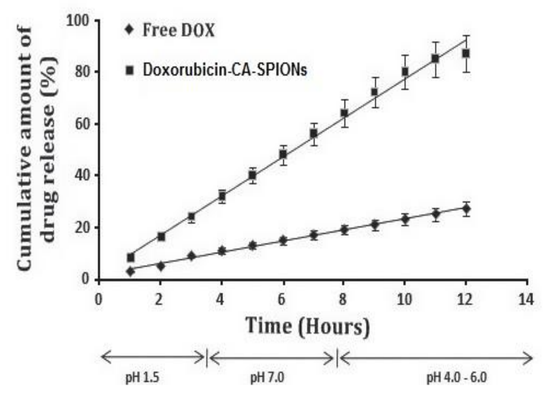
Hyperthermia selectivity treatment on breast cancer cells	Single cell colture	Double cell colture	Three cell coltures
For hyperthermia treatment, cells were exposed to alternating magnetic fields (530 kHz and 18.30 ± 1.59 kA/m).	**MCF-10A**	**MCF-7 + MCF10A**	**MDA-MB-231 + MCF-7 + MCF10A**
	MDA-MB-231	**MDA-MB-231 + MCF10A**	
	MCF-7		

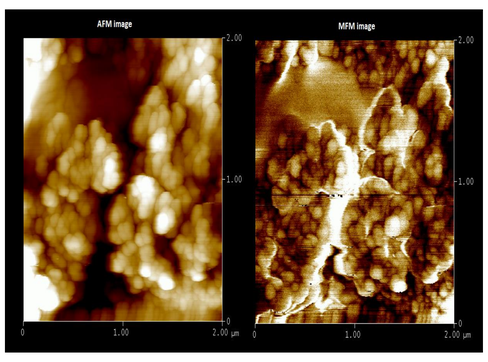




Loading efficiency of doxorubicin on SPIONs

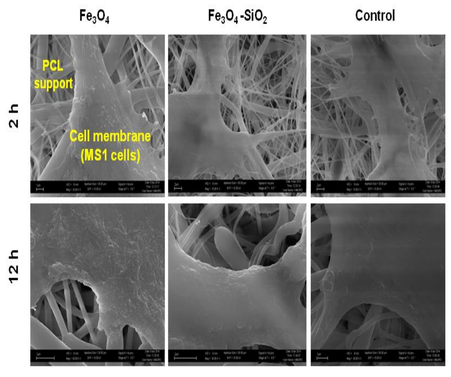






Cytocompatibility in dynamic conditions (Fe_3O_4 and Fe_3O_4-SiO_2 NPs)

Cells were analyzed with FESEM equipped to EDS probe, in order to evaluate the presence, if any, of Fe_3O_4 and Fe_3O_4-SiO_2 nanoparticles deposits.



Using dynamic culture conditions, the cells morphology appeared typically elongated.

Materials and methods

In vitro evaluation on breast cancer cells

The therapeutic effect of DOX-CA-SPIONs was evaluated by cell viability assay on the following cell lines: MCF-7 (Human breast cancer cells ER+/PR+), Triple negative MDA-MB-231 breast cancer cells, MCF-10A (Mammary epithelial cells). In this way we tried to verify the selectivity of magnetic hyperthermia treatment induced by SPIONs on killing breast cancer cells respect to normal mammary epithelial cells.

Conjugation of DOX to CA-SPIONs

For drug conjugation to modified CA-SPIONs, the concentration (50 µg/ml) of carboxylic acid-coated SPIONs was first sonicated with 0.5, 1, 5 and 50 µM concentrations of DOX solution for 0.5 h and then stirred overnight at room temperature in the dark. All the samples were centrifuged at 18 000 x g for 1 h. The DOX concentration of all the samples was measured using a standard DOX concentration curve, generated with a UV-Vis spectrophotometer at the wavelength of 488 nm.

References.
[1] Sun, C.; Lee, J. S. H.; Zhang, M. Adv. Drug Delivery Rev. 2008, 60, 1252.
[2] Z. Li et al., Mat Sci Eng C 2010, 30: 990-996.

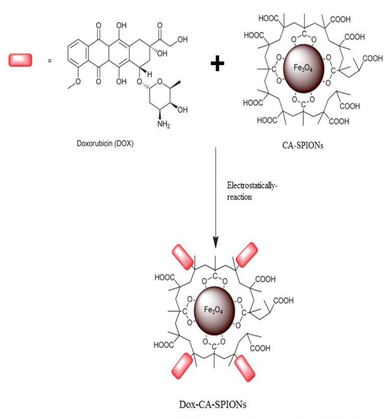
Results

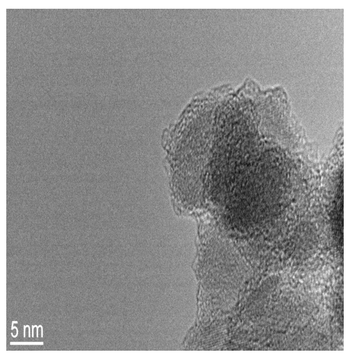
Targeting chemotherapy effect

Conjugation of doxorubicin to CA-SPIONs showed to increase the killing effect on breast cancer cells without side effects on normal mammary epithelial cells (MCF-10A).

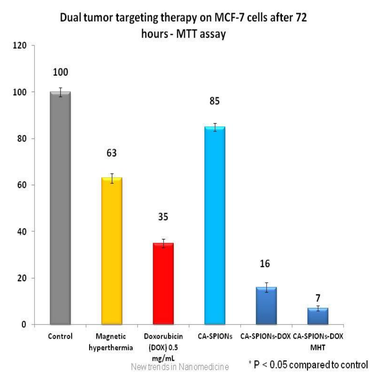
Physicochemical characterization, Dynamic light scattering analysis and Zeta Potential

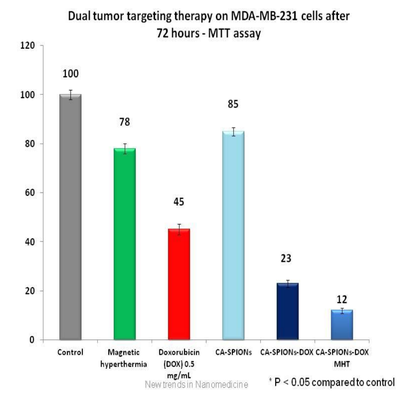
Conjugation CA-SPIONs/DOX	Mean diameter	Zeta Potential
50 µg / 0 µM	92.0 nm	- 45 mV
50 µg / 0.5 µM	137.2 nm	- 37.4 mV
50 µg / 1 µM	109.9 nm	- 49.9 mV
50 µg / 5 µM	154.4 nm	- 34.1 mV
50 µg / 50 µM	254.7 nm	- 41.7 mV



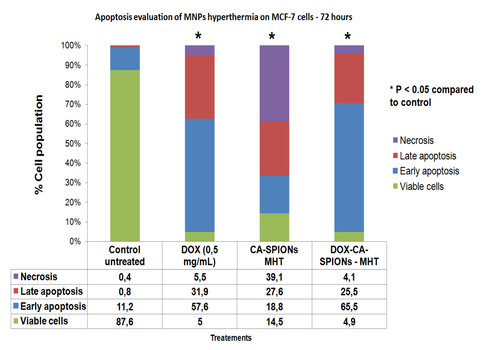


Cell viability evaluation

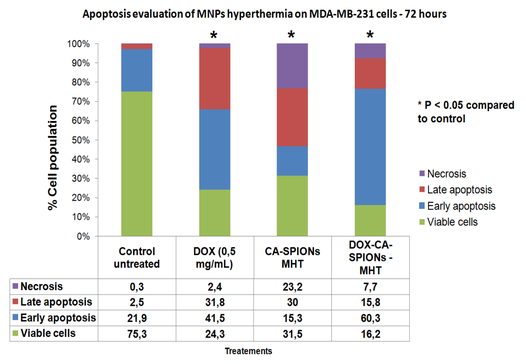




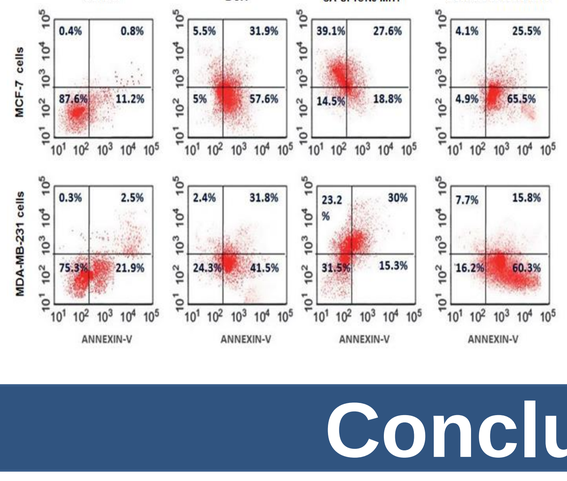
Apoptosis evaluation

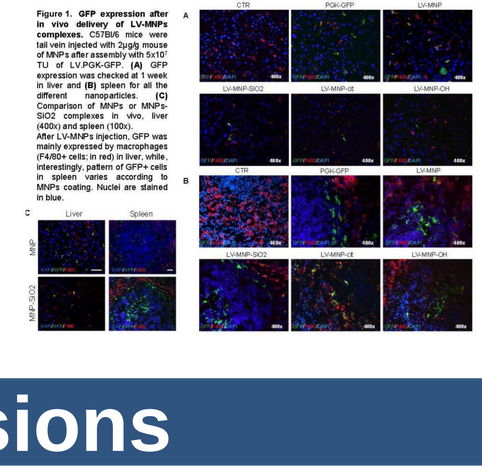


	Control untreated	DOX (0,5 mg/mL)	CA-SPIONs MHT	DOX-CA-SPIONs - MHT
Necrosis	0,4	5,5	39,1	4,1
Late apoptosis	0,8	31,9	27,6	25,5
Early apoptosis	11,2	57,6	18,8	65,5
Viable cells	87,6	5	14,5	4,9



	Control untreated	DOX (0,5 mg/mL)	CA-SPIONs MHT	DOX-CA-SPIONs - MHT
Necrosis	0,3	2,4	23,2	7,7
Late apoptosis	2,5	31,8	30	15,8
Early apoptosis	21,9	41,5	15,3	60,3
Viable cells	75,3	24,3	31,5	16,2





Conclusions

The results demonstrated the potential of the DOX-CA SPIONs to achieve dual tumor targeting by magnetic field-guided in breast cancer cells, and exploit the incredible possibilities to kill in a target way the cancer cells. This project is an upgrade in the breast cancer treatment in the framework of personalized nanomedicine. MNPs are the most optimal choice for use as anticancer agents due to their unusual beneficial properties, most notably enhanced drug availability for prolonging the drug effects in tumor tissues. In conclusion, this study indicates that SPIONs are promising therapeutic agents for magnetic hyperthermia of breast cancer cells and offers a dual magnetotherapeutic approach with iron oxide nanoparticles as the sole heat mediators. We demonstrated that iron oxide nanoparticles can be remotely activated with an alternating magnetic field, achieving a very efficient heat conversion. These results are promising for applications in biomedicine, but further *in vivo* and *in vitro* investigations of the efficacy and safety of these magnetic nanoparticles are necessary to optimize their use in various applications like targeted drug delivery for anticancer therapy.

Confocal microscopy images of doxorubicin-loaded iron-oxide nanoparticles showed a very targeted and localized harmful cellular effect of DOXCA-SPIONs in the intracellular environment of breast cancer cells that induce higher apoptotic effect. Nanotechnology also has the potential to generate unique and highly effective theraputic agents and revolutionize the cancer treatment of patients to kill cancer cells without affecting the nearby healthy tissue.

Acknowledgments: The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7-PEOPLE-2013-COFUND) under grant agreement n° 609020 - Scientia Fellows.