Investigating the effect of new contrast agent in an orthotopic mouse model

Ashish Avasthi[1], Carlos Caro[1], Jose M. Paez-Muñoz[1], Alejandro Domínguez[1], Manuel Pernía Leal[2,*], Maria L. García-Martín[1,3,*]

1. BIONAND, Andalusian Centre for Nanomedicine and Biotechnology, Calle severo ochoa 35,, Universidad de Málaga, 29590 Málaga, Spain
2. Departamento de Química Orgánica y Farmacéutica, Universidad de Sevilla, C/Profesor García González 2, 41012 Seville, Spain
3. Networking Research Center on Bioengineering, Biomaterials and Nanomedicine, CIBER-BBN, 29590 Málaga, Spain

INTRODUCTION

Abstract - Cancer is the second most common cause of death in the world after cardiovascular diseases. There has been a lack of effective diagnosing at an early stage for this malice. Over the years, there have been significant improvements in the diagnostic field but not without issues. In this sense, magnetic resonance imaging (MRI) has arise as a powerful diagnostic tool. In addition, the use of gadolinium (Gd) as the contrast agent lead to better diagnosis, but there are some evidences of health hazards due to use of Gd.

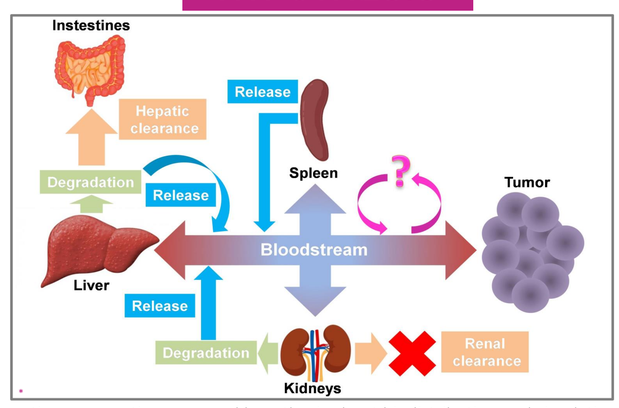
Objective - In this work, we evaluate polyethylene glycol-gallol (PEG-GA) coated iron oxide as an alternative contrast agent for a breast cancer model. These nanoparticles have shown to be of biocompatible nature when interacting with cells. In the orthotopic mouse model, movement of this contrast agent is followed using MRI and its injection-excretion cycle is determined by various methods.

Keywords:

Contrast Agent (CA) - A substance used to increase the contrast of structures or fluids within the body during medical imaging. These agents absorb or alter external energy, which is different from radiopharmaceuticals, which emit radiation themselves.

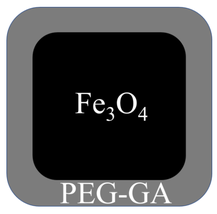
Enhanced Permeation and Retentivity (EPR) – According to literature, molecules of certain sizes tend to accumulate in tumor tissue much more than they do in normal tissues due to leaky vasculature in the tumors.

HYPOTHESIS

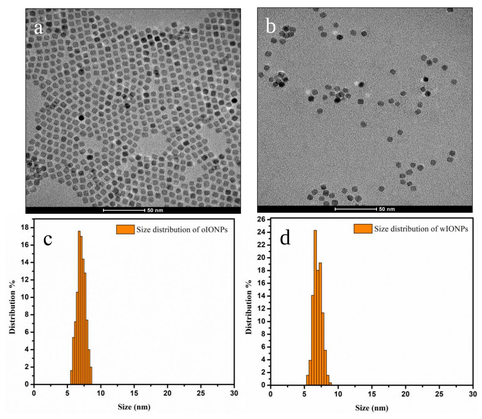


Schematics representing proposed hypothesis about biodistribution and circulation of nps

OBSERVATIONS AND RESULTS



Synthesis of nanoparticles



Iron oxide nanoparticles before (a) and, after (b) ligand exchange; along with their respective (c,d) TEM size distribution

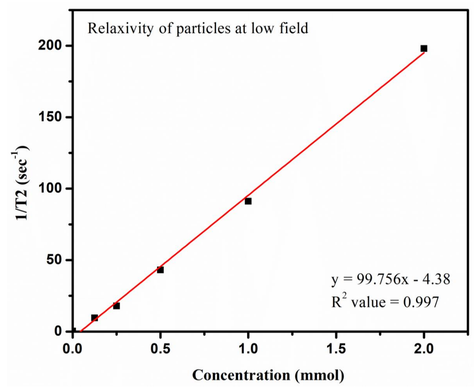
Table 1

TEM SIZE (D_T)	HYDRODYNAMIC DIAMETER IN WATER (D_{HW})	HYDRODYNAMIC DIAMETER IN PBS (D_{HP})	HYDRODYNAMIC DIAMETER IN FBS (D_{HF})
9.33 ± 0.89	24.37 ± 2.15	31.31 ± 2.96	25.96 ± 3.6

Table 2

D_{HP} 0 hr	D_{HP} 24 hrs	D_{HP} 48 hrs	D_{HP} 72 hrs	D_{HP} 168 hrs
31.31 ± 2.96	31.09 ± 4.47	35.99 ± 2.14	34.49 ± 2.866	33.47 ± 3.33

TEM and hydrodynamic diameters of nps in different media (Table 1) as well as their stability in PBS over time (Table 2)



r_2 relaxivity of the nanoparticles at low field

In-vitro evaluation

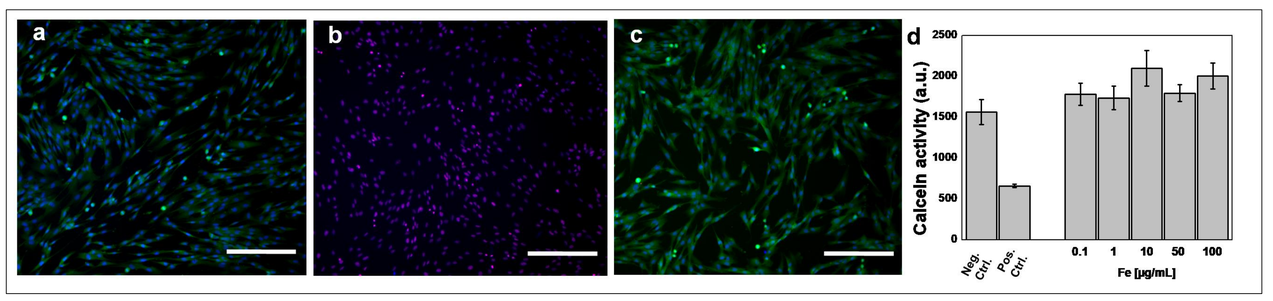


Figure (**left**) (a) Negative control, (b) Positive control, (c) cells exposed to 100 µg/mL of NPs., (d) Calcein intensity

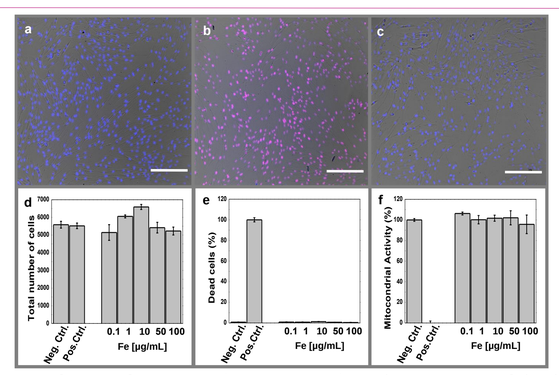
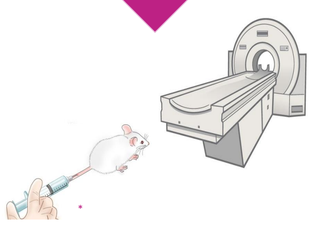
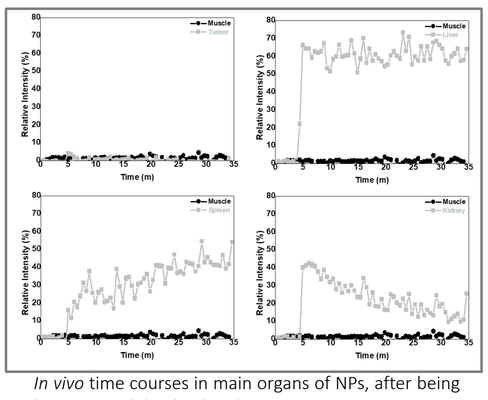


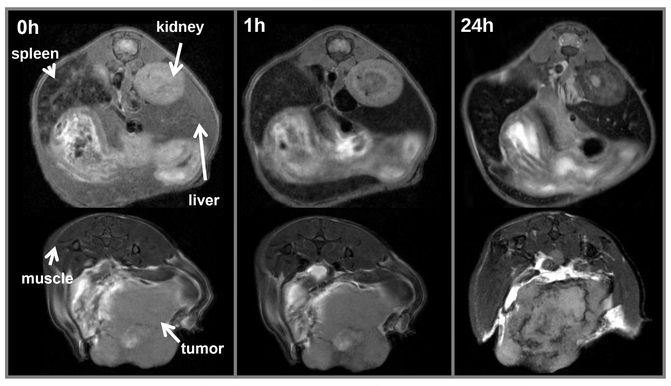
Figure (**right**) a) Negative control, b) Positive control, c) cells exposed to 100 µg/mL of MNPs. d) Total number of cells per well exposed to increasing concentration of MNPs, from 0.1 µg/mL to 100 µg/mL. e) Percentage of dead cells exposed to increasing concentration of MNPs, from 0.1 µg/mL to 100 µg/mL. f) MTT assay of cells exposed to increasing concentration of MNPs, from 0.1 µg/mL to 100 µg/mL.



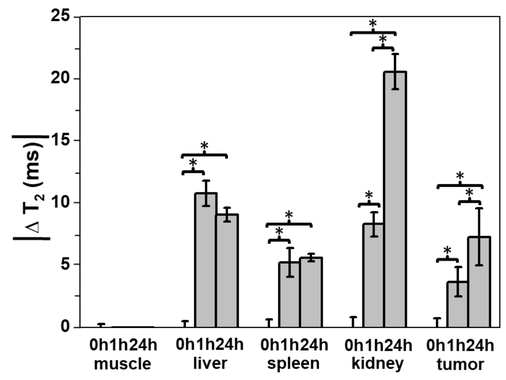
In- vivo evaluation



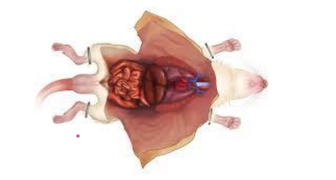
In vivo time courses in main organs of NPs, after being intravenous injection in mice.



Representative T_2-weighted MR images at different experimental time points after intravenous injection of NPs.

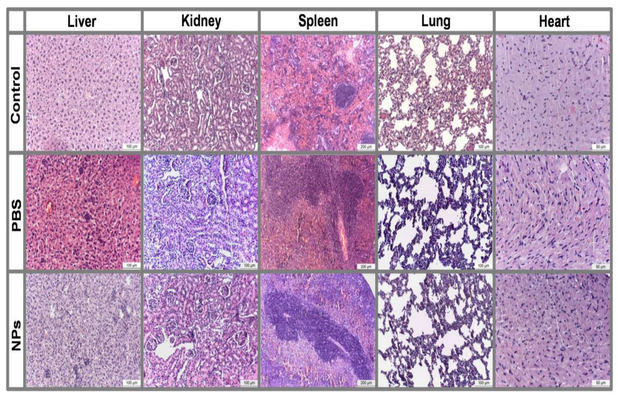


ΔT_2 values of different organs at different time points after intravenously injection of NPs. The average values were obtained by performing three experiments.

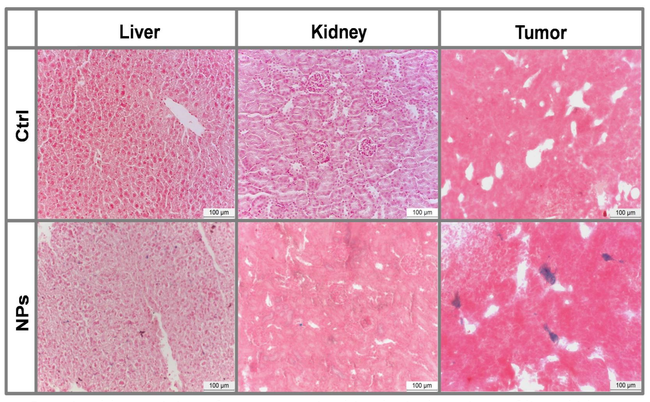


Ex-vivo evaluation

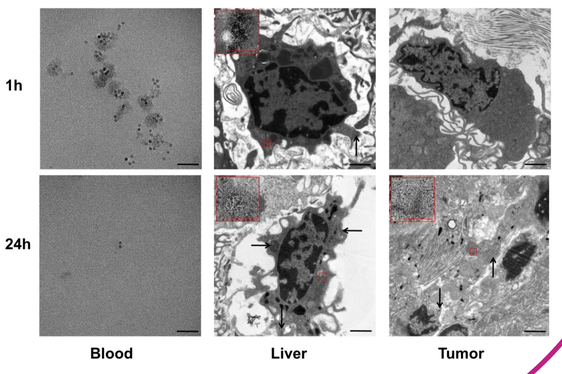
* Image adapted from elsewhere



H&E staining of representative histological sections of main organs: Control mice, without injection (top), 24 hrs post intravenous administration of PBS (centre) and 24 hrs post intravenous administration of NPs.



Prussian blue staining of representative histological sections of liver, kidneys and tumor after 24 hrs of intravenous injection with PBS (top) and after intravenous injection of NPs (bottom).



TEM Of blood, liver tissue and tumor tissue at 1 hr and 24 hrs interval

CONCLUSIONS

1. The synthesized CA is biocompatible and non-toxic.
2. The synthesized PEGylated nanoparticles exhibit long circulations times and it is due to this long circulation times an effective EPR can be seen.
3. Our model revealed metastatic events especially in liver, thus physicochemical properties of these nanoparticles will open new avenues in theranostics of this devastating cancer.

FUTURE PERSPECTIVES

1. This CA has potential to be used as theranostic agent using magnetic hyperthermia and/or drug conjugation or entrapment.
2. This can also be used for molecular targeting by binding this CA to cell specific ligands
3. This contrast agent is currently being investigated to see if it behaves differently when used as active targeting agent.

CONTACT PERSON

Ashish Avasthi
Email -aavasthi@bionand.es

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