School of Nanomedicine

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GOLD NANOPARTICLES AND ENDOTHELIAL PROGENITOR CELLS: A WIN-WIN ALLIANCE FOR TARGETING TUMORS

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INTRODUCTION: Plasmonic photothermal therapy utilizes biologically inert gold nanorods (AuNRs) that convert light into heat capable of eliminating cancerous tissue. This approach has lower morbidity than surgical resection and can potentially synergize with other treatment modalities including chemotherapy and immunotherapy. In this work, we propose alternative NIR-sensitive, tumor tropic cellular vectors, called Endothelial Colony Forming Cells (ECFCs), enriched with chitosan-coated AuNRs. ECFCs display a great capability to intake AuNRs without losing viability and exerting an in vitro antitumor activity per se.

EXPERIMENTAL: Melanoma cells (M6) and ECFCs were exposed over night to 100 µM AuNRs before evaluating intracellular uptake both with conventional optical microscope, TEM and photoacoustic imaging (PA). We also evaluated the behavior of AuNRs-ECFC in 3D-culture, performing M6 spheroids then plating AuNRs-ECFCs. We then sought to determine AuNRs- ECFCs' antitumor activity in vivo using a human melanoma xenograft rat model. AuNRs- ECFCs injected in caudal vein retain their ability to migrate to tumor sites in vivo 1 day after injection and stay in the tumor mass for more than 1 week.

RESULTS AND DISCUSSION: The PA signal provided from ECFC loaded with AuNRs exhibited a stronger enhancement compared to AuNRs-M6, without detectable spectral shift. As expected, ECFCs loaded with AuNRs, thanks to their ability to enter the spheroid, exert their antitumor activity by reducing the volume of the sphere, compared to control spheroids plated with unloaded ECFCs. Besides, the PA signal provided from AuNR–ECFCs inside spheroids exhibited a strong enhancement. Histological analyses of explanted tumor mass demonstrate that gold is still retained after 1 week from injection and organs including liver, spleen, kidney, and lung did not show any morphological alteration compared to control rats treated with unloaded ECFCs.

CONCLUSIONS: We demonstrated in vitro that AuNRs-loaded ECFCs are able to generate higher photoacoustic signals than AuNRs loaded in M6 cells. 3D cultures confirm the cytostatic effect of AuNRs-ECFC on tumor. In vivo, we show, via immunohistochemical analysis, a great tumor-homing efficiency of AuNRs–ECFCs after a bolus intravenous administration and their permanence inside the tumor masses 1 week after administration.

Intracellular uptake of AuNRs

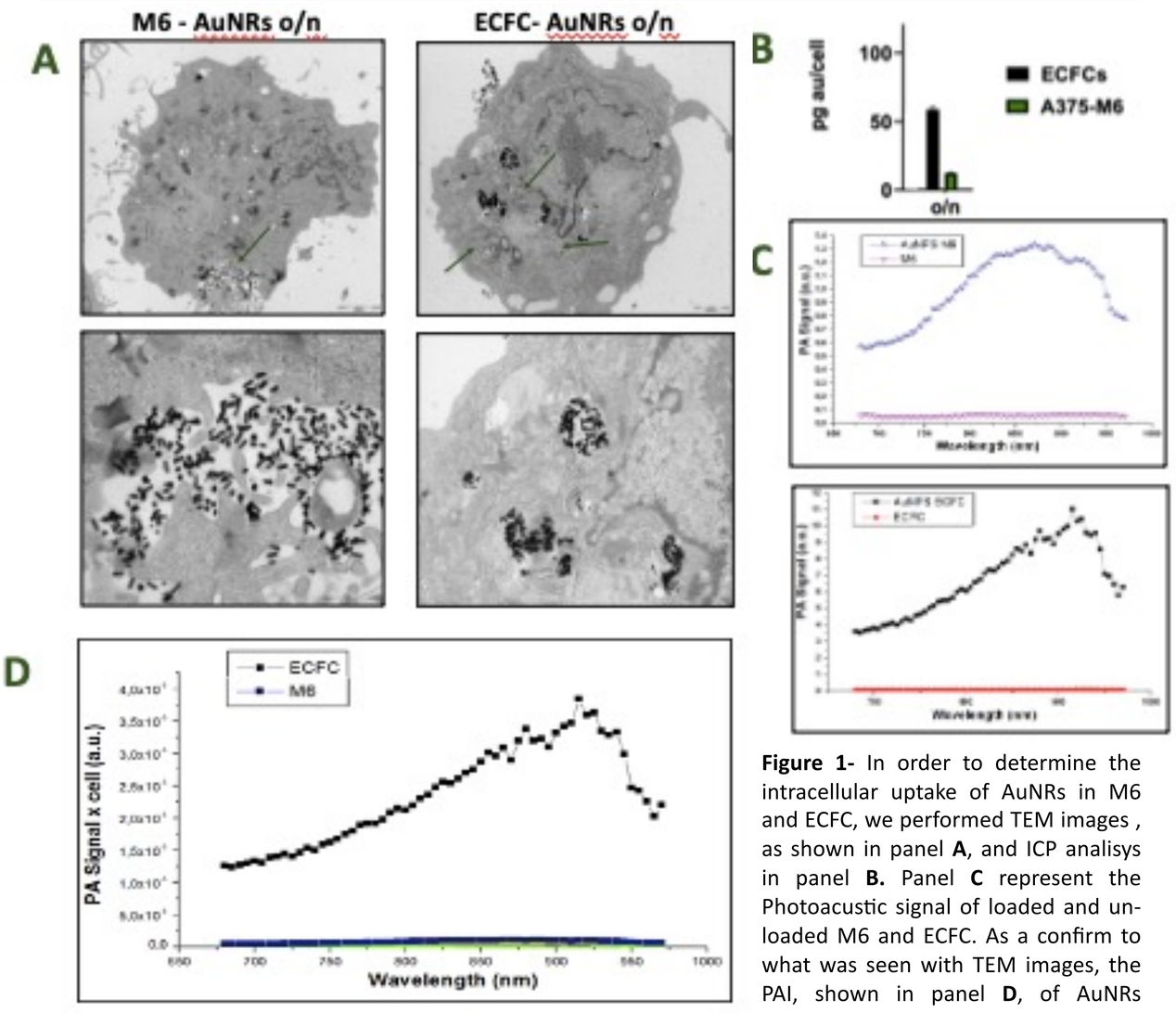
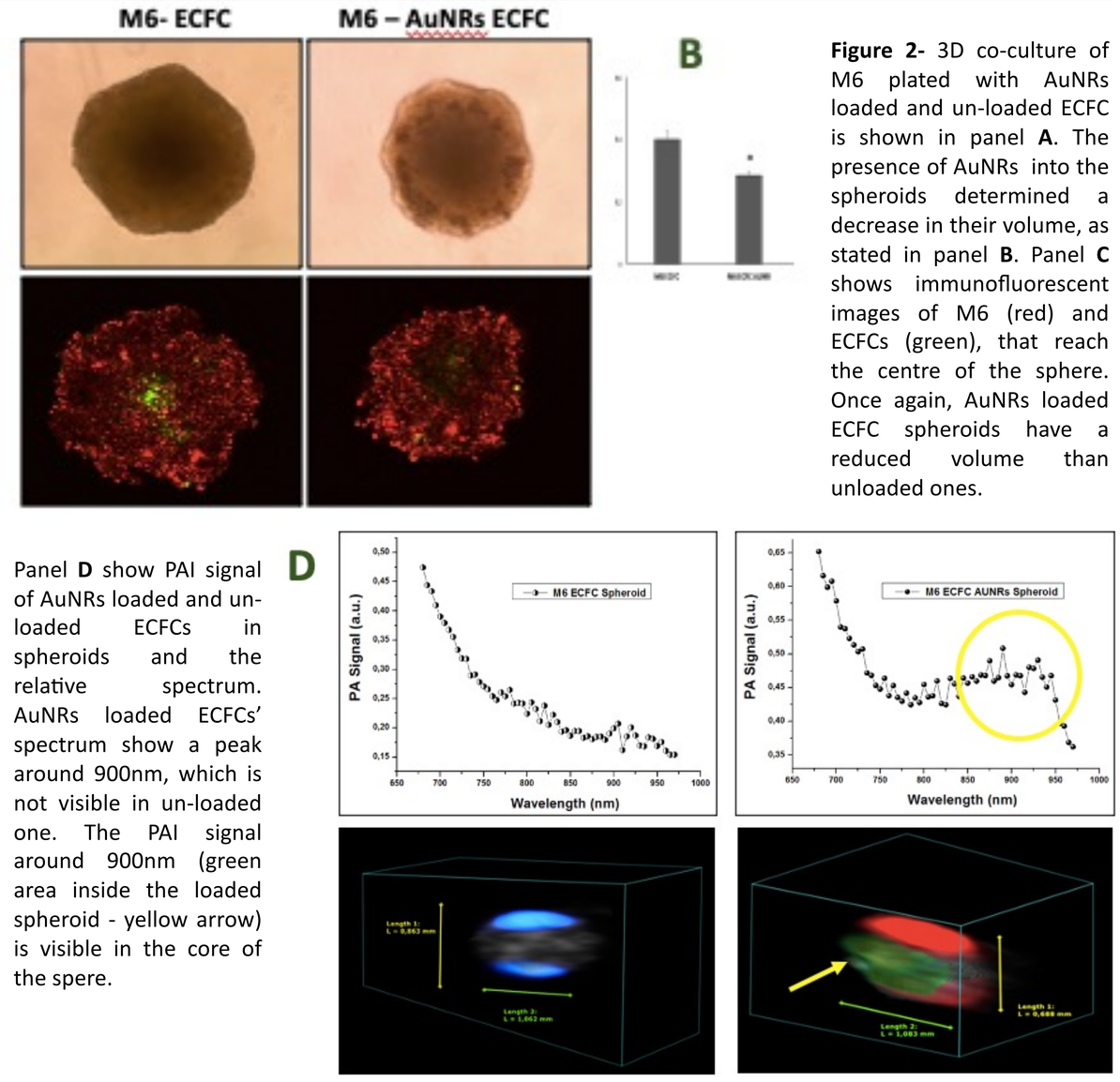


Figure 1- In order to determine the intracellular uptake of AuNRs in M6 and ECFC, we performed TEM images , as shown in panel **A**, and ICP analisys in panel **B.** Panel **C** represent the Photoacustic signal of loaded and un-loaded M6 and ECFC. As a confirm to what was seen with TEM images, the PAI, shown in panel **D**, of AuNRs loaded ECFC resulted way higher than the one of loaded M6.

Effects on AuNRs on 3D co-colture



Panel **D** show PAI signal of AuNRs loaded and un-loaded ECFCs in spheroids and the relative spectrum. AuNRs loaded ECFCs' spectrum show a peak around 900nm, which is not visible in un-loaded one. The PAI signal around 900nm (green area inside the loaded spheroid - yellow arrow) is visible in the core of the spere.

Figure 2- 3D co-culture of M6 plated with AuNRs loaded and un-loaded ECFC is shown in panel **A**. The presence of AuNRs into the spheroids determined a decrease in their volume, as stated in panel **B**. Panel **C** shows immunofluorescent images of M6 (red) and ECFCs (green), that reach the centre of the sphere. Once again, AuNRs loaded ECFC spheroids have a reduced volume than unloaded ones.

In vivo uptake of AuNRs

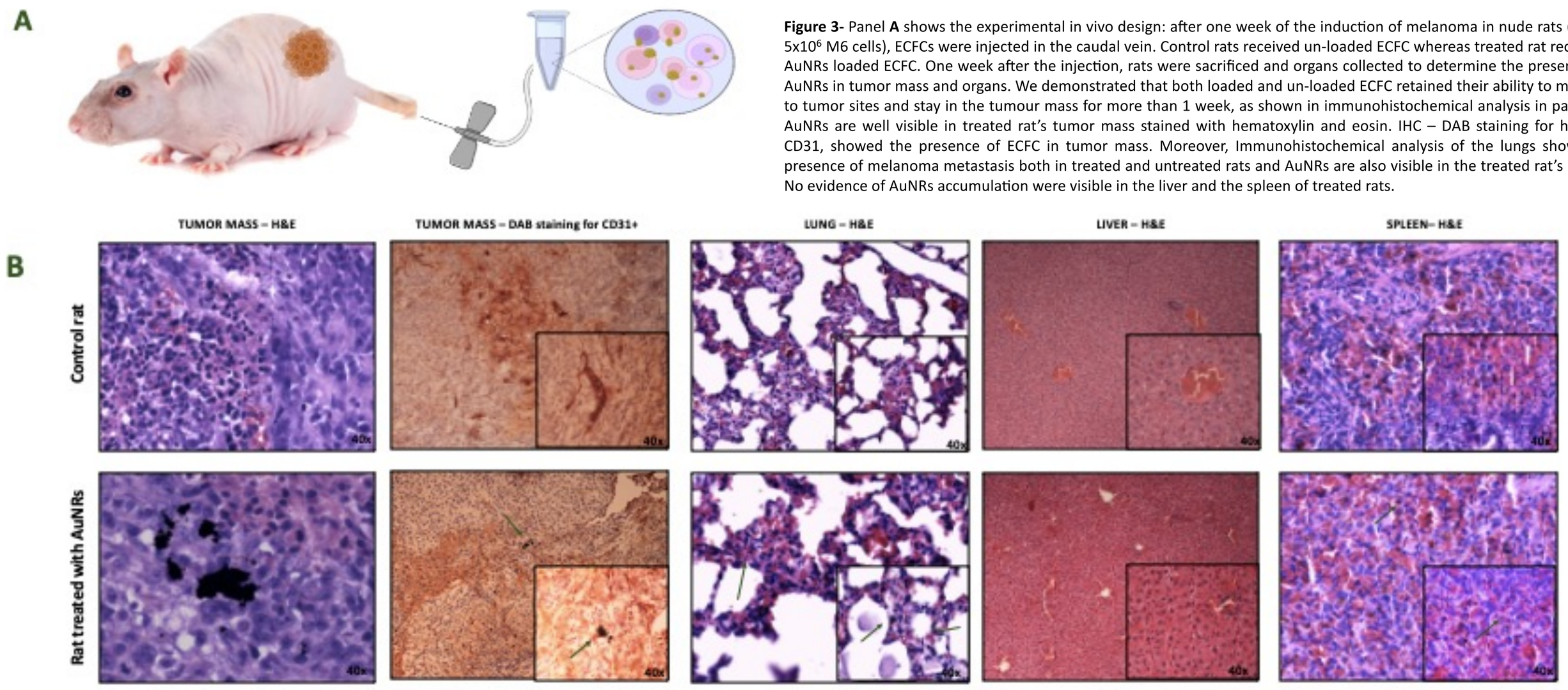


Figure 3- Panel **A** shows the experimental in vivo design: after one week of the induction of melanoma in nude rats (using $5x10^6$ M6 cells), ECFCs were injected in the caudal vein. Control rats received un-loaded ECFC whereas treated rat received AuNRs loaded ECFC. One week after the injection, rats were sacrificed and organs collected to determine the presence of AuNRs in tumor mass and organs. We demonstrated that both loaded and un-loaded ECFC retained their ability to migrate to tumor sites and stay in the tumour mass for more than 1 week, as shown in immunohistochemical analysis in panel **B**. AuNRs are well visible in treated rat's tumor mass stained with hematoxylin and eosin. IHC – DAB staining for human CD31, showed the presence of ECFC in tumor mass. Moreover, Immunohistochemical analysis of the lungs show the presence of melanoma metastasis both in treated and untreated rats and AuNRs are also visible in the treated rat's lungs. No evidence of AuNRs accumulation were visible in the liver and the spleen of treated rats.

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