Overcoming Autofluorescence Barriers: A Novel NIR Method for Studying Electric Field-Driven Nanoparticle Penetration in Peritoneal Models

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Intraperitoneal drug delivery holds potential for treating peritoneal metastases, yet limited tissue permeability constrains drug penetration. To augment nanoparticle (NP) penetration, we examined the impact of electromotive drug administration (EMDA) in different peritoneal tissues. Traditional fluorescent NP face challenges due to tissue autofluorescence (AF), impeding their visualization. Our study employs near-infrared NP and Fluorescence Lifetime Imaging Microscopy (FLIM) for accurate quantification, offering enhanced penetration and discrimination from the AF. Freshly excised peritoneal tissue fragments (1cm) from porcine, rat, and human donors, with approval from the ethics committee, were rinsed with PBS for hydration. Tissues were exposed to a suspension of cationic amine-modified polymeric beads (Merck) in isotonic saline within a custom-designed device for temperature control to study the effect of EMDA (pulsed Direct current electrical stimulation, 30 mA, 30 mins) in comparison of Passive Diffusion (no stimulation). After exposure, tissues were sectioned and evaluated under microscopy. Confocal microscopy initially employed for NP localization was hampered by significant spectral overlap between conventional fluorescent beads (100 nm, Ex/Em: 481/644 nm) and peritoneal tissue autofluorescence (Ex/Em: 475/680 nm). To overcome this limitation, subsequent samples as positive control utilized cationic RL-100 nanoparticles (30-75 nm, prepared according to literature method (1)) doped with far-red emitting aza-BODIPY dye compared with tissue without NPs. Samples were treated in the same manner and analyzed under FLIM microscopy, using HC PL Apo 10x/0.4 air objective lens, excited at 475nm, and emission collected at 639-698 nm (HyD R detector), showed differentiated RL-100 NPs from AF based on their unique lifetime and spectral characteristics (2). Phasor FLIM analysis of passive diffusion sample performed by LAS X software revealed distinct patterns of NP localized on tissue edges with an average lifetime. Interestingly, the NP lifetime was shorter than the free NP lifetime (fig.1A green ROI on a phasor plot), which probably reflects the impact of tissue environment on NP fluorescence. Heterogeneity was observed in AF phasor plots, with no analogous positions detected in the NIR channel. Results highlight the utility of nIR-NP and FLIM co-application for reliable detection and differentiating NP from AF in peritoneal tissue models. Observed differences in NP lifetime suggest potential avenues for further EMDA investigation into interaction dynamics between NP and peritoneal tissues.

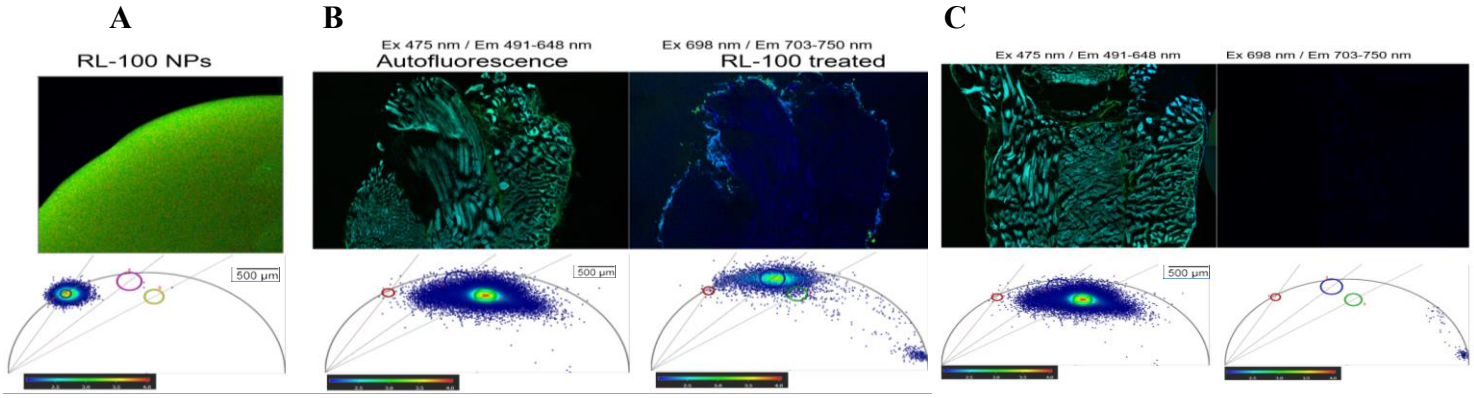

Figure 1: FLIM Microscopy images of **a.** RL-100 NP solution with phasor plot, **b.** RL-100 NP uptake separated in different channels. **c.** Untreated tissues with autofluorescence BG. In the Phasor plot, circle 1 shows the localization of free RL-100 particles; circle 2 shows the localization of RL-100 treated with tissues; circle 3 shows the localization of autofluorescence of the treated rat tissues.

References: 1. Borisov SM, Mayr T, Mistlberger G, Waich K, Koren K, Chojnacki P, Klimant I. Precipitation as a simple and versatile method for preparation of optical nanochemosensors. Talanta. 2009 Oct 15;79(5):1322-30.
2. Debruyne AC, Okkelman IA, Heymans N, Nobis M, Borisov SM, Dmitriev RI. Live microscopy of multicellular spheroids with multi-modal near-infrared nanoparticles reveals differences in oxygenation gradients. bioRxiv. 2023:2023-12.