Extracellular Vesicle-Mediated Drug Delivery and Uptake in GFP-Tagged MDA-MB-231 Spheroids: A 3D Fluorescence-Based Theranostic Model

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1. Introduction

Extracellular vesicles (EVs) have emerged as critical mediators of intercellular communication in cancer, carrying proteins, nucleic acids, and lipids that influence tumour progression and therapeutic response. Traditional two-dimensional (2D) cultures fail to capture the spatial and biochemical complexity of tumours, whereas three-dimensional (3D) spheroid models more accurately reproduce in vivo conditions, including gradients of oxygen, nutrients, and extracellular matrix interactions.

Fluorescence-based tracking provides a powerful approach to study EV uptake, distribution, and penetration in these 3D systems. Such visualisations are essential for developing strategies to engineer EVs as targeted drug delivery vehicles. Importantly, integrating fluorescence analysis with positron emission tomography (PET)–compatible probes can bridge preclinical discoveries with clinical imaging, enabling real-time monitoring of EV behaviour in vivo.

This study applies fluorescence-guided imaging to examine the dynamics of EV interaction with breast cancer spheroids, aiming to generate insights that inform the development of PET-compatible theranostic platforms.

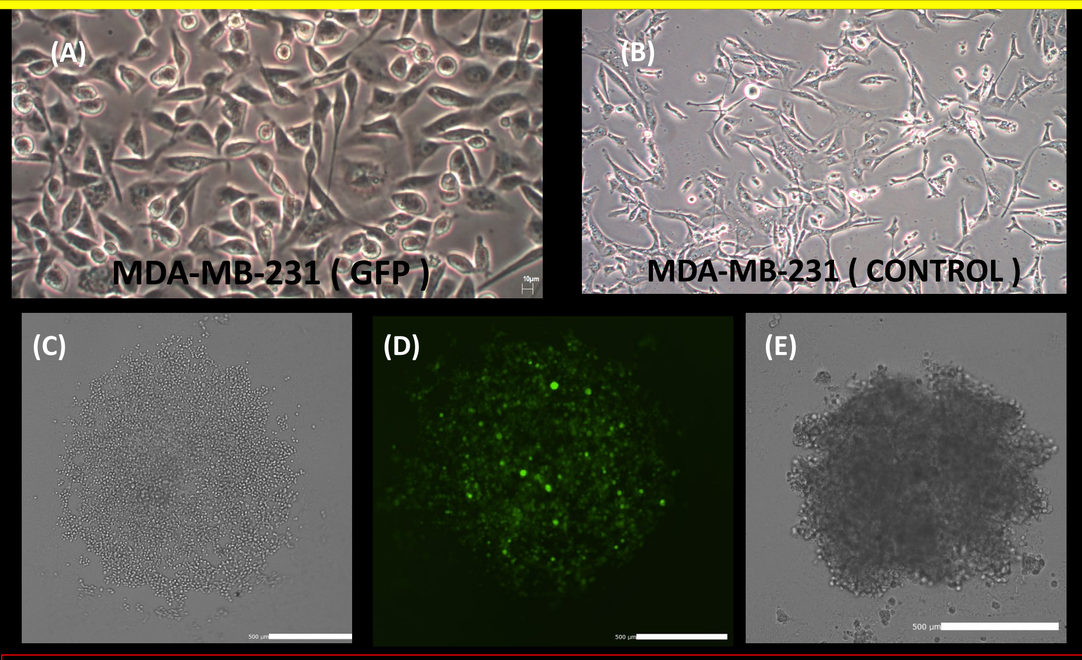
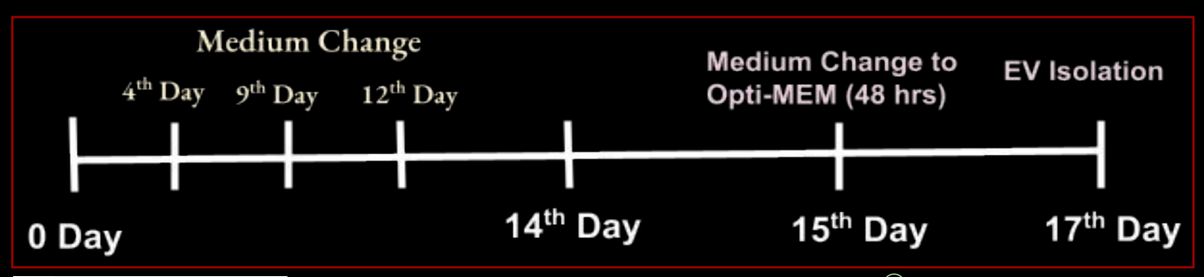
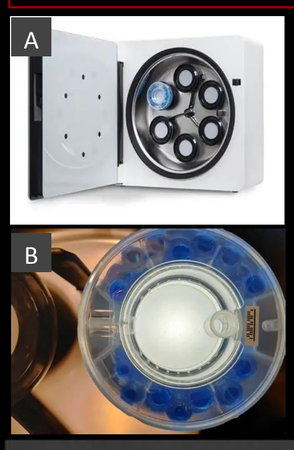


Fig. 1: Representative images of MDA-MB-231 GFP (A) and Control cells (B) and spheroids (C , D and E). GFP-expressing spheroids form more loosely associated, cluster-like structures (C). The GFP fluorescence signal highlights the distribution of GFP-positive cells (D) within the spheroid architecture, while Control spheroids (E) display a compact, well-organized morphology. Scale: 500 µm

2. Methodology

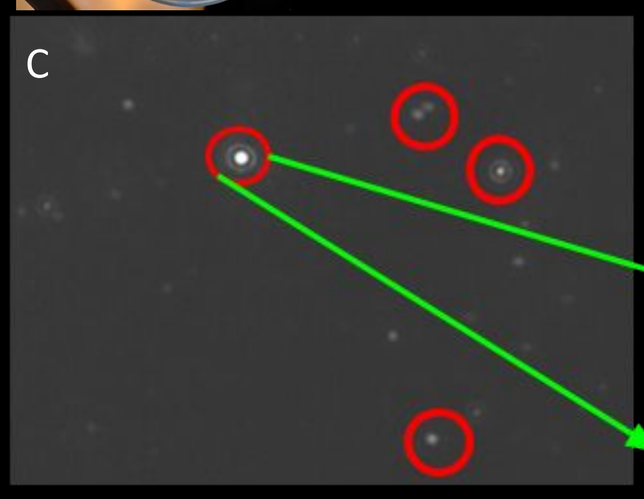
a) 3D cell culture (Spheroids)



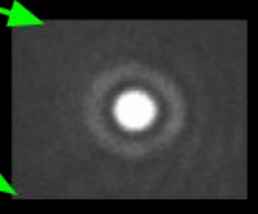


3D culture devices (Celvivo®)

MDA-MB-231 cells were expanded in 2D, then cultured in CelVivo® (A) clinostat bioreactors (B) to form scaffold-free spheroids. Control spheroids developed compact structures, while GFP-expressing spheroids formed loose clusters. Mature spheroids after 14 days isolated EV's from GFP- expressing spheroids, followed by NTA, after attaining desired concentration, they were stained with PKH26 and incubated with controls for 24 hrs followed by imaging to evaluate EV secretion, uptake, and distribution over time.



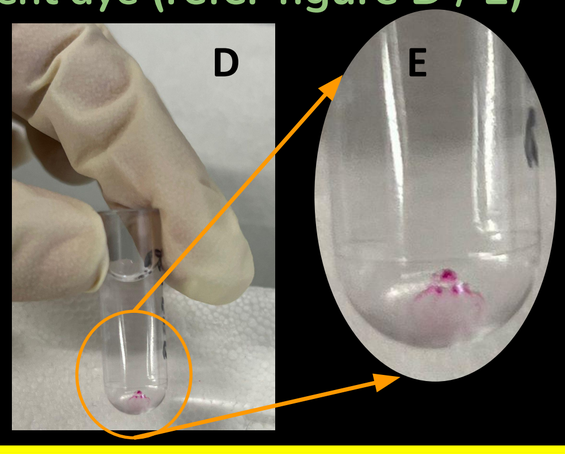
MDA MB 231 - GFP EVs visualisation through nanoparticle tracking analysis (refer figure C)



A single GFP-EV picturization

Staining with PKH26 red fluorescent dye (refer figure D / E)

PKH26 is a lipophilic fluorescent dye widely used to label extracellular vesicles (EVs) for uptake and tracking studies. In this study, EVs were stained using PKH26 at a final concentration of approximately 1 µg/mL for 24 hrs (refer Figure D and E). This working concentration enabled the reliable visualisation of EVs without compromising particle integrity or introducing excessive free-dye artefacts.



3. Results

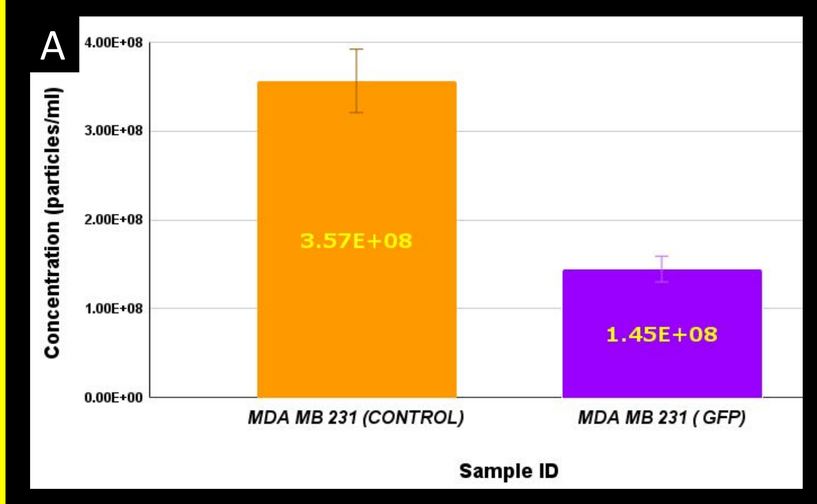
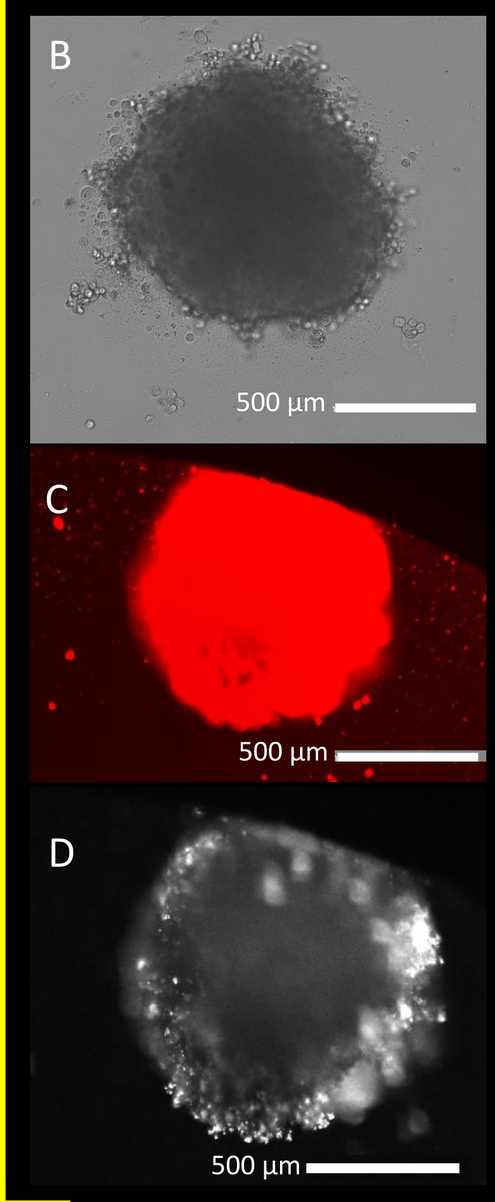


Fig. 3.A: The bar chart demonstrates reduced EV yield from GFP-expressing spheroids relative to controls, suggesting that GFP expression and altered spheroid morphology may impact EV secretion dynamics.



EV Labeling

- PKH26 at 1 µg/mL → strong, stable fluorescence
- Size distribution preserved; minimal aggregation

NTA Analysis

- Particle concentration: 10^7–10^8 particles/mL
- Control spheroids: 3.57×10^8 particles/mL
- GFP spheroids: 1.45×10^8 particles/mL
- Vesicle integrity maintained

Spheroid Uptake

- Fig. B: Control spheroids → compact, solid structure
- Fig. C: PKH26-labeled EV uptake → localized penetration
- Fig. D: Masked imaging (3500 µs, red channel)
  clear EV spatial distribution
- GFP spheroids → loose, cluster-like morphology; heterogeneous uptake

Impact

- Robust EV tracking in 3D tumor models
- Supports fluorescence-guided monitoring
- Foundation for PET-compatible theranostics

4. Conclusion

This study demonstrates that fluorescence-based imaging in 3D spheroid models enables precise visualization of EV uptake and distribution, capturing the complex tumor microenvironment more effectively than 2D systems. The results highlight distinct EV secretion and uptake dynamics in GFP-expressing versus control spheroids, underscoring the influence of tumor heterogeneity. Importantly, these findings establish a foundation for integrating fluorescence guided assays with PET-compatible probes, paving the way for translational theranostic platforms. Such dual modality approaches hold significant promise for advancing EV-based drug delivery, enabling real-time, non-invasive monitoring from bench to bedside.

5. References

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