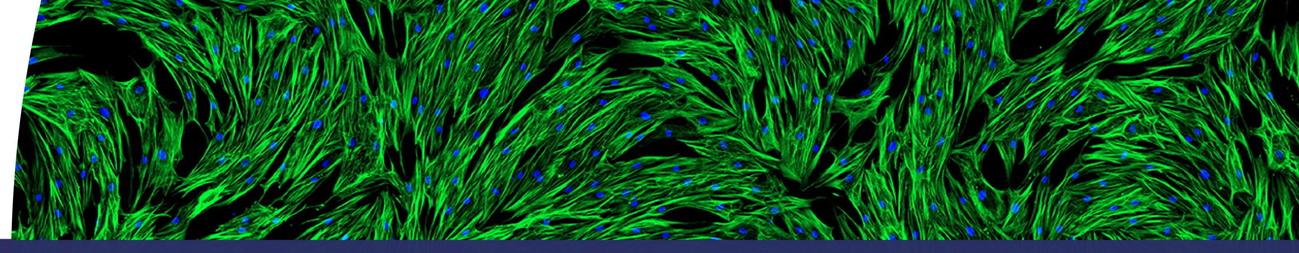

Bioengineering breast cancer dormancy in the bone marrow niche

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INTRODUCTION AND BACKGROUND

Breast cancer dormancy is a phenomenon that takes place in the bone marrow niche following breast cancer cell (BCC) dissemination and migration from the primary tumour. Disseminated BCCs can exist as small dormant micrometastases in the bone marrow that evade chemotherapy from drugs that target actively-proliferating cells, such as doxorubicin (DOX), for many years until environmental cues trigger fatal recurrence. Within this niche, **BCCs communicate with resident mesenchymal stem cells (MSCs)** and haematopoietic stem cells (HSCs); our previous work has shown that **secreted metabolite cargo in MSC extracellular vesicles is essential for slowing BCC growth**[1].

Our overall collaborative project goal is to **model BCC dormancy and study BCC recurrence** in a bioengineered bone marrow niche hydrogel model. We aim to use (i) **metabolite-loaded lipid nanoparticles (LNPs)[2-5] to drive dormancy**, and (ii) hydrogel drug-loaded nanosensors within our hydrogels, alongside Brillouin microscopy, which is non-invasive mechanobiology microscopy, to detect and combat recurrent BCCs. This model will then be integrated on-chip to serve as a platform for studying recurrence in response to environmental factors like infection, to allow high-throughput diagnostic and therapeutic screening (**Figure 1**).

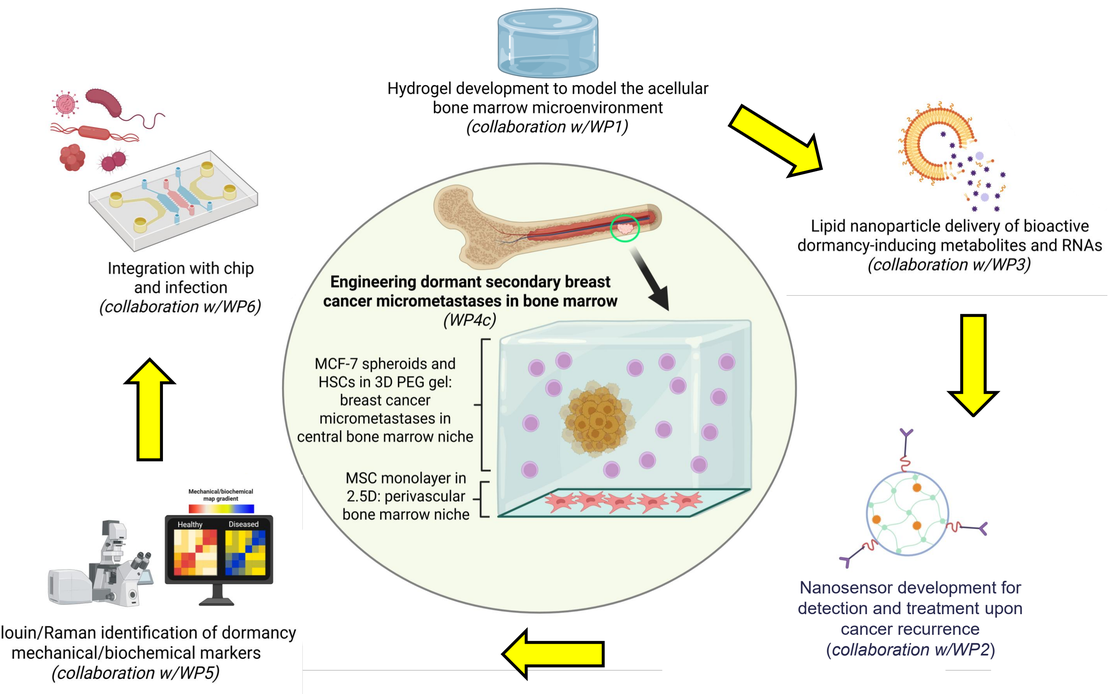


Figure 1. Overview of breast cancer dormancy model development and collaborative interactions with other work packages (WPs) within our EPSRC programme grant StemNiche; this data focuses on the delivery of dormancy-inducing metabolites.

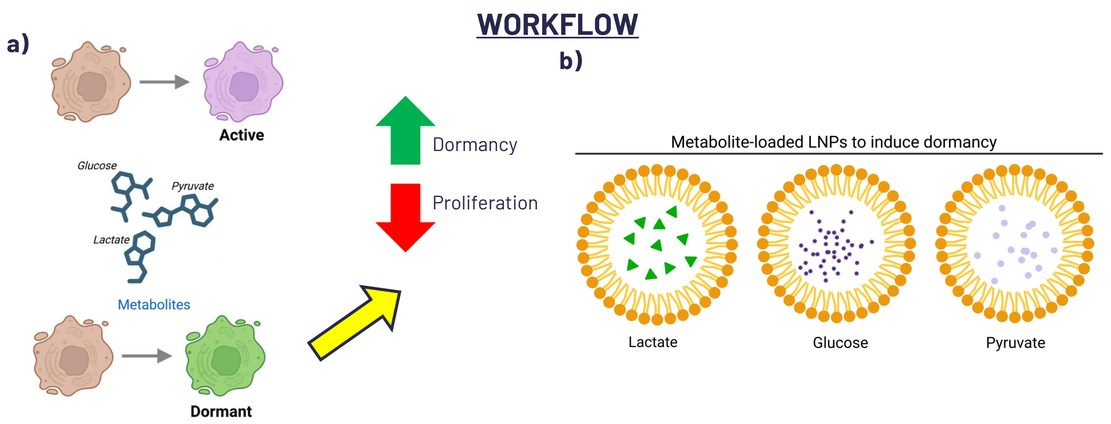


Figure 2. Workflow of developing a breast cancer dormancy model in the bone marrow niche: (a) Using bioactive metabolites (glucose, lactate, pyruvate) to induce BCC dormancy. **(b)** Development of LNP technologies for delivery of metabolites

METHODS

- **Breast cancer cell model** : MCF7, an oestrogen receptor-positive (ER+) breast cancer cell line.
- **Treatments** : MCF-7 cells were seeded at a density of 20k cells/cm^2 and treated for up to 48 h with metabolite cocktail (50 mM glucose, 40 mM l-lactate and 8 mM sodium pyruvate); 1 µM rotenone, an inhibitor of mitochondrial ATP production, was used as a control.
- **Readouts** : MCF7 cell proliferation (Ki-67, EdU), cell cycle arrest (p27) and dormancy induction (COUP-TFI).
- **LNP metabolite delivery** : LNPs were delivered to MCF-7 cells at a concentration of 50k/cell for uptake imaging or 300k/cell for metabolite delivery

RESULTS SUMMARY

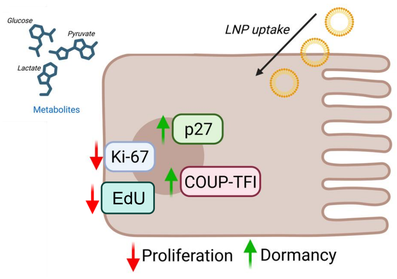
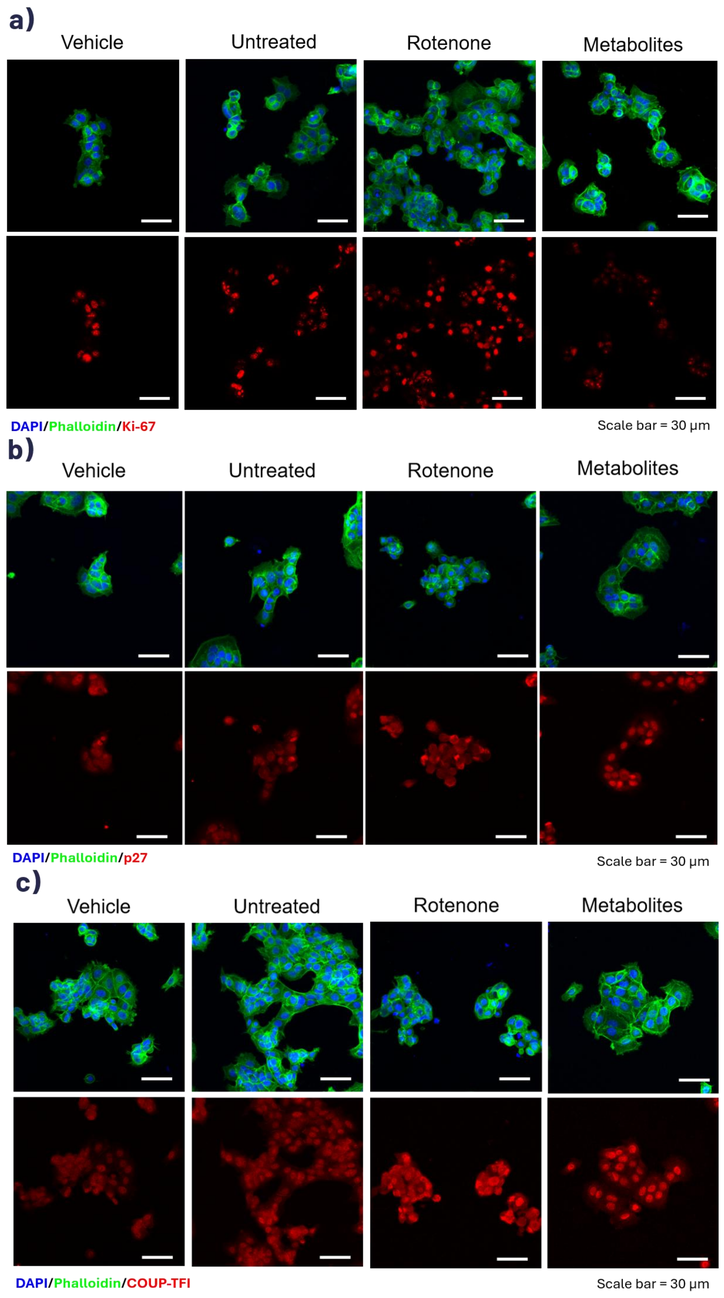


Figure 3. Schematic representation of MCF-7 LNP uptake and metabolite-induced dormancy with suppression of proliferation markers

- Dormancy-associated metabolites induce a dormancy phenotype in BCC cells by regulating proliferation, cell cycle arrest, and dormancy marker expression.
- LNPs are appropriately sized to mimic extracellular vesicles (EVs) and effectively encapsulate dormancy-associated metabolites.
- LNPs are efficiently taken up by BCC cells.
- **LNP delivery of bioactive metabolites induces a dormancy response in BCC cells.**

RESULTS 1 : Metabolites induce BCC dormancy



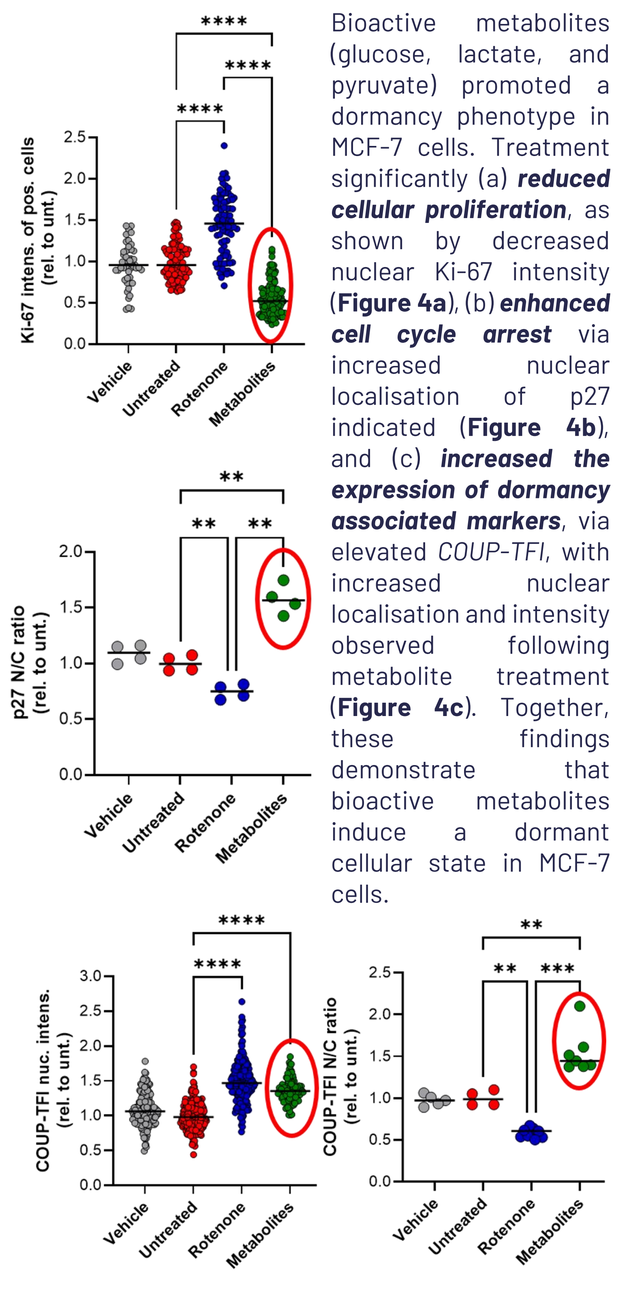
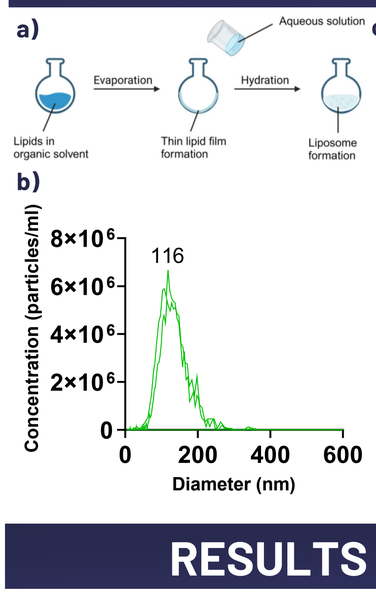


Figure 4. Metabolites induce BCC dormancy: Representative fluorescent microscopy images of MCF-7 cells after 48 hours treatment with bioactive metabolites (left) and quantification of relative intensities (n=83-124) and nuclear: cytoplasmic ratios (n=4-9) (right) for the following markers: Ki-67 **(a),** p27 **(b),** COUP-TFI **(c).** **=p≤0.01, ***=p≤0.001, ****=p≤0.0001 as determined by appropriate parametric/non-parametric one-way ANOVA tests following outlier removal, equal SD determination and normality tests.

Bioactive metabolites (glucose, lactate, and pyruvate) promoted a dormancy phenotype in MCF-7 cells. Treatment significantly (a) ***reduced cellular proliferation***, as shown by decreased nuclear Ki-67 intensity (**Figure 4a**), (b) ***enhanced cell cycle arrest*** via increased nuclear localisation of p27 indicated (**Figure 4b**), and (c) ***increased the expression of dormancy associated markers***, via elevated *COUP-TFI*, with increased nuclear localisation and intensity observed following metabolite treatment (**Figure 4c**). Together, these findings demonstrate that bioactive metabolites induce a dormant cellular state in MCF-7 cells.

RESULTS 2 : EV-mimetic LNPs encapsulate metabolites



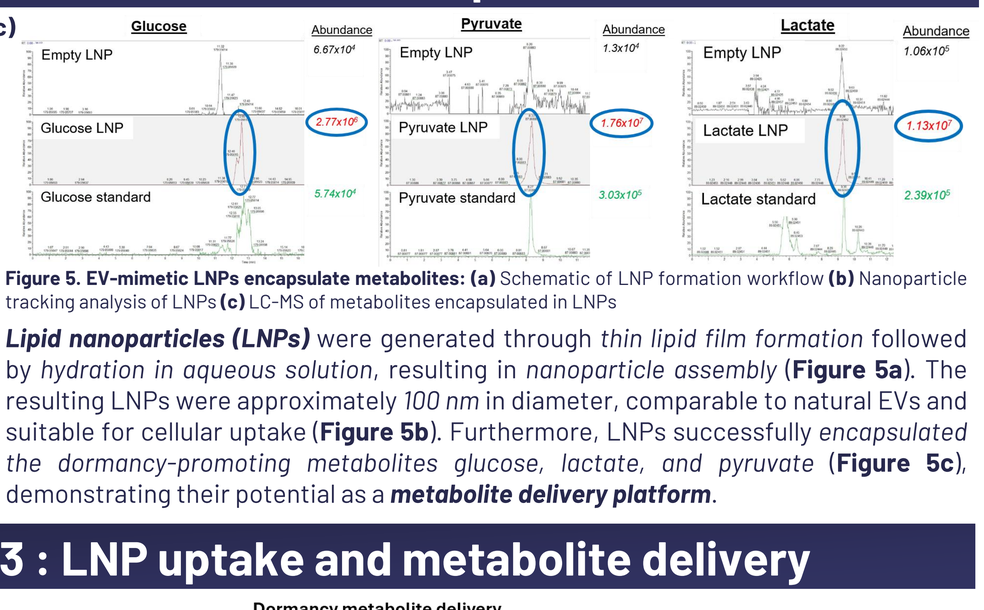


Figure 5. EV-mimetic LNPs encapsulate metabolites: (a) Schematic of LNP formation workflow **(b)** Nanoparticle tracking analysis of LNPs **(c)** LC-MS of metabolites encapsulated in LNPs

Lipid nanoparticles (LNPs) were generated through *thin lipid film formation* followed by *hydration in aqueous solution*, resulting in *nanoparticle assembly* (**Figure 5a**). The resulting LNPs were approximately *100 nm* in diameter, comparable to natural EVs and suitable for cellular uptake (**Figure 5b**). Furthermore, LNPs successfully *encapsulated the dormancy-promoting metabolites glucose, lactate, and pyruvate* (**Figure 5c**), demonstrating their potential as a ***metabolite delivery platform***.

RESULTS 3 : LNP uptake and metabolite delivery

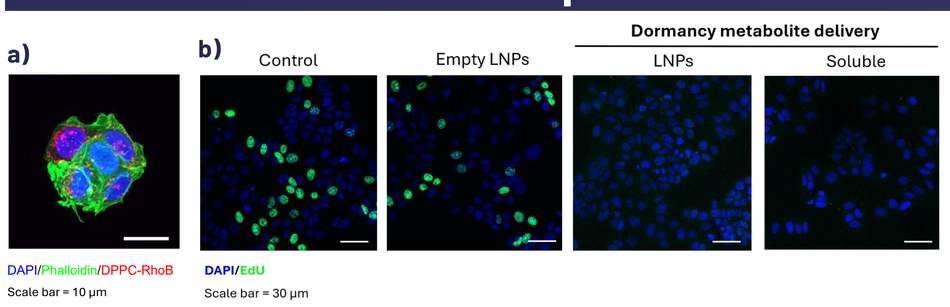


Figure 6. LNP uptake and metabolite delivery: Representative fluorescent microscopy images of MCF-7 cells following DPPC LNP uptake after 4 hrs treatment **(a)** and EdU staining after 48 hrs treatment with dormancy metabolites delivered in LNP or soluble form **(b).**

LNPs were readily *taken up* by MCF-7 cells over 6 hours, with *peak uptake* observed at *4 hours* (**Figure 6a**). Following internalisation, *delivery of dormancy-promoting metabolites reduced expression of the S-phase proliferation marker EdU* (**Figure 6b**), demonstrating that *metabolite-loaded LNPs elicit a biological dormancy response* in MCF-7 cells.

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