Embedded breast cancer spheroids as a biomimetic cancer model

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Introduction: Conventional methods to mimic breast cancer *in vitro* rely on non-embedded methods utilising non-adherent culture plastic whereby cancer spheroid formation is driven by cell-cell interactions[1], which may bias the aggregation of a subset of cancer populations within typically heterogeneous cancer cell lines. It is hypothesized that an *in vitro* model in which cancer spheroid are grown within an embedding hydrogel would provide a closer mimic the *in vivo* situation. Herein, the spheroid growth of embedded and non-embedded cell aggregations was compared in four different breast cancer cell lines.

Materials and Methods: Bioinert hydrogel cup structures were fabricated through sequential dispensing of alginate (4 %w/v) and calcium chloride (3 %w/v) in a layer-by-layer fashion.[2] Embedded spheroids were prepared through dispensing of breast cancer cells (MDA-MB-231, HCC38, HCC1806 and SUM159PT cells) within the cup cavities of varying sizes as controlled by printing parameters (300-1000 µm). Non-embedded spheroids were prepared through ultra-low attachment plates. Cavity diameter and spheroid growth were measured and monitored over 7 days of culture through brightfield microscopy with daily Alamar blue assay to determine cell proliferation and viability.

Results and discussion: Non-embedded MDA-MB-231 cells formed spheroids that condensed over time (Fig. 1A). In contrast, MDA-MB-231 cells dispensed within the cups (Fig. 1B) did not form a spheroid but rather migrated out during culture. MDA-MB-231 cells are considered metastatic in nature and natively would migrate rather than form cell-cell contacts, suggesting that the embedded spheroid platform may more faithfully replicate the phenotype of metastatic cell lines. The other three cell lines, pre-metastatic in nature, were able to form non-embedded spheroids with similar appearance (Fig. 1A). When embedded, cells proliferated (Fig. 1C) and formed dense cell-cell aggregations (Fig. 1D) that conformed to the cup cavity, but the size range of formed spheroids was cell line-dependent line (HCC38: < 1000 µm, HCC1806: < 1000 µm, SUM159PT: < 300 µm). The differential size ranges for the embedded spheroids may represent the inherent heterogeneity within these cell lines, making the embedded spheroid model ideal as a pre-metastatic breast cancer model for applications such as drug screening.

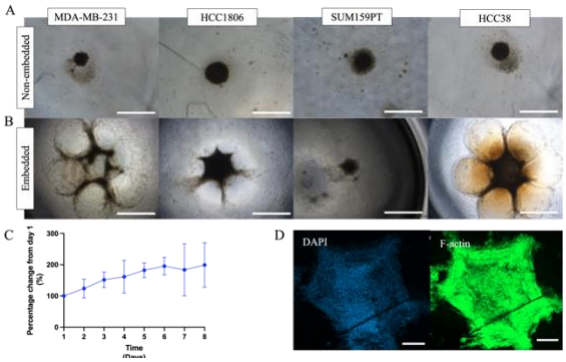

Figure 1: (A) Non-embedded and (B) embedded spheroids. (C) Proliferation and representative F-actin staining of spheroid. Scale bar = 1mm

Conclusions: Embedded spheroids demonstrated vastly different phenotypes to non-embedded spheroids, which may serve to mimic the *in vivo* breast cancer for improving our understanding of breast cancer development and guide drug discovery.

References:

[1] Jubelin, C.; et al. *Cells&Bioscience* **2022,** 12. [2] Utama, R.; et al. *Iscience* **2020**, *23*.