Confinement induces drug resistance in breast cancer

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Introduction: During breast cancer progression, confinement imposed by the interface of the mammary gland lumen and its surrounding extracellular matrix is thought to be a key driver of cancer heterogeneity and drug resistance[1,2]. Herein, drop-on-demand printing[3] is exploited to meet the challenge of mimicking this complex interface within an *in vitro* setting to explore the role for confinement in driving breast cancer heterogeneity and drug resistance.

Materials and Methods: Alginate (1.5-3 %w/v) and $CaCl_2$ (2-4 %w/v) were co-printed through drop-on-demand printing (Rastrum, Inventia) to generate bioinert cup-shaped hydrogels with tailorable stiffness as determined through compression testing (MCR301, Anton Paar). MCF7 cells grow to conform to the cup cavities (≤10 days) to generate confined spheroids with well-defined shape (Fig. 1A). Unconfined spheroids were prepared using ultra-low attachment plates. Cell survival (CellTiter-Glo®) was measured in response to cytotoxic drugs (doxorubicin, 0-120 µM). Drug resistance markers (CD44, CD133) were probed through immunofluorescence staining and imaged using confocal microscopy (LSM800, Zeiss).

Results and Discussion: Drop-on-demand printing allowed fabrication of cups with circular cavities that enabled the growth of confined spheroids with similar dimensions as unconfined spheroids (0.49±0.04 versus 0.56±0.06 mm^2 area and 0.47±0.04 $*10^6$ versus 0.48±0.06 $*10^6$ cells). Confinement could thus be studied through a direct comparison between confined and unconfined spheroids, wherein confined spheroids demonstrated higher drug resistance than unconfined spheroids (IC_{50}: 16.3±1.9 and 7.7±2.3 µM, Fig. 1B). Concomitantly, confinement drove the emergence of drug resistant ($CD44^{high}$, $CD133^{high}$) populations at the confined spheroid's edges (Fig. 1C). It was hypothesized that interfacial stress caused by cup's physical confinement caused emergence of these drug-resistant populations. To verify this hypothesis, cup stiffness was varied to tune the interfacial stress of confinement (5.5±1.1, 9.9±4.9 and 18.5±3.9 kPa), which resulted in an increase in expression of drug resistance molecular markers with a concurrent increase in IC_{50} values (2.4±1.3, 3.8±1.7 and 10.5±2.5 µM), supporting a "*interfacial stress—stemness—drug resistance*" relationship.

Conclusions: The direct comparison of confined and unconfined spheroids, uniquely enabled by the drop-on-demand printing platform, revealed the importance of confinement and the extracellular matrix in breast cancer models, for the study and treatment of cancer heterogeneity and drug resistance.

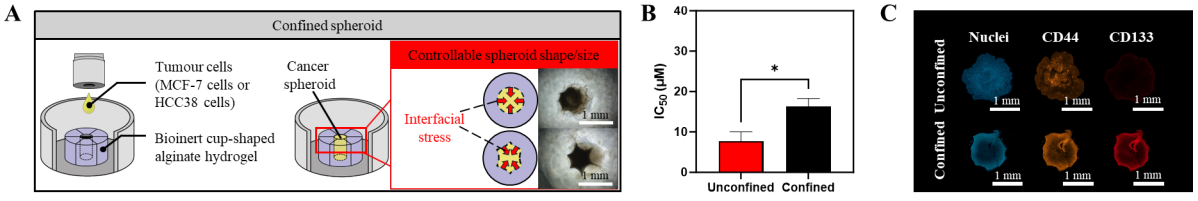

Figure 1: Drop-on-demand printing (A) enabling a direct comparison between confined and unconfined spheroids to reveal (B) interfacial stress-driven emergence of drug resistance in breast cancer (C).

References:

[1] Kalli, M.; Stylianopoulos, T. *Frontiers in Oncology* **2018**, *8*, 55. [2] Lee J.; Kilian, A.A.; et al. *Nature Materials* **2016**, *15*, 856. [3] Utama, R.H.; Gooding, J.J..; et al. *iScience* **2020**, *23*, 101621.