# The interplay of breast cancer size and shape in drug resistance

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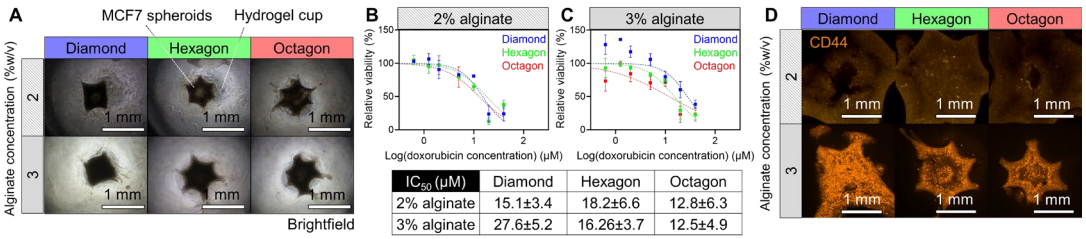
**Introduction:** The biophysical forces exerted onto pre-invasive breast cancer due to the stiffening of the surrounding breast extracellular matrix (ECM) are thought to induce drug resistance[1]. Concomitantly, studies have suggested that curvature of the cancer-ECM interface affect the biophysical forces perceived by cells, suggesting a potential interplay between ECM stiffness and cancer shape[2]. Herein, drop-on-demand printing[3] is exploited to meet the unmet challenge of controlling ECM stiffness and cancer shape independently, enabling a reductionist study into the potential interplay between these variables in driving drug resistance.
**Materials and Methods:** Alginate (1-3 %w/v) and $CaCl_2$ (4 %w/v) were co-printed (Rastrum, Inventia) to generate cup-shaped hydrogel cell culture models in which MCF7 cells were dispensed to form cancer spheroids (≤5 days) within the confines of the cup. The cup meanwhile acted as a physical mimic of the breast ECM. Hydrogel stiffness was determined through compression testing (MCF301, Anton Paar). Cell survival (CellTiter-Glo®) was measured in response to cytotoxic drug doxorubicin (0-80 μM). Drug resistance markers (CD44, CD133) were stained and imaged using confocal microscopy (LSM800, Zeiss).
**Results and Discussion:** Spatial control over droplet deposition allowed tailoring of the cup model shape at the spheroid interface. MCF7 cells conformed to the surrounding cavity, thus facilitating control over spheroid curvature through modulating cavity shape (Fig. 1A). Meanwhile, cup model stiffness was independently adjustable in an alginate concentration-dependent manner (1.7±1.2-18.5±3.9 kPa). The model cavities were designed to keep the spheroid size constant, regardless of spheroid curvature and cup stiffness, enabling systematic study into the effect of spheroid shape and ECM stiffness on drug resistance. While spheroid curvature did not affect drug sensitivity in low stiffness (9 kPa) environments, curvature-dependent drug sensitivity was observed when the cancer was grown within high stiffness (19 kPa) surroundings (Fig. 1B-C). Exclusively in this high stiffness environment, there was also a dependency on the spatial emergence of a drug-resistant phenotype ($CD44^{high}$, $CD133^{high}$, Fig. 1D), supporting a "*stiffness-curvature-drug resistance*" interplay wherein drug resistance emerges as a consequence of the shape/stiffness-defined biophysical force regime.
**Conclusions:** Our model uniquely identified that ECM stiffness and cancer shape interplay to affect breast cancer drug resistance. Understanding the molecular mechanisms driving this interplay will be instrumental for the study and treatment of breast cancer.




| $IC_{50}$ (μM) | Diamond | Hexagon | Octagon |
|---|---|---|---|
| 2% alginate | 15.1±3.4 | 18.2±6.6 | 12.8±6.3 |
| 3% alginate | 27.6±5.2 | 16.26±3.7 | 12.5±4.9 |

**Figure 1:** Drop-on-demand printing enabling independent control over cancer spheroid shape and ECM stiffness (A). These factors interplay to affect drug sensitivity (B-C) and drug resistance (D).

**References:** [1] Taubenberger, A.V; Guck. J.; et al. *Adv Biosyst* **2019**, e1900128. [2] Lee, J.;Kilian, K.K.; et al. *Nat Mater* **2016**, *15*, 856-62. [3] Utama, R.H.; Gooding, J.J.; et al. *iScience* **2020**, *23*, 101621.