# The effect of 3D bioprinted well-defined tumour microenvironments to the drug response of tumour spheroids

*Lin Zhu* [1,2], *M. A. Kristine Tolentino*[1,2], *Panthipa Suwannakot*[1,2], *Kang Lin*[1,2], *Kristopher A. Kilian*[1,2,5], *Maria Kavallaris*[1,3], *Richard D. Tilley*[1,4], *J. Justin Gooding*[1,2]*

[1]School of Chemistry
, The University of New South Wales, Sydney, NSW, Australia
[3]Children's Cancer Institute, Lowy Cancer Research Centre, UNSW, Sydney, NSW, Australia, The University of New South Wales, Sydney, NSW, Australia
[2]Australian Centre for Nanomedicine
[4]Mark Wainwright Analytical Centre, The University of New South Wales, Sydney, NSW, Australia
[5]School of Materials Science and Engineering, The University of New South Wales, Sydney, NSW, Australia

*Lin.zhu3@unsw.edu.au* and *justin.gooding@unsw.edu.au*

The tumour microenvironment (TME) plays an important role in cancer progression, invasion, metastasis, and drug resistance[1]. TME consists of tumour cells, non-malignant cells including stromal fibroblasts, endothelial cells, immune cells, and the non-cellular components of extracellular matrix such as collagen, fibronectin, hyaluronan, lamininand, and others[2]. In this research, a high-throughput drop-on-demand 3D bioprinter was used to produce matrix-embedded multicellular tumour spheroids to screen for the anticancer drug doxorubicin. 3D culture conditions more closely resemble the natural environment for cells and so can bridge the gap between 2D and *in vivo* studies. MCF-7 breast cancer cells, HFF1 human skin fibroblast cells, and their combined cultures were bioprinted in 3D polyethylene glycol hydrogels and grown as cell cultures. These hydrogels were then functionalised with the cell adhesion peptide arginylglycylaspartic acid (RGD). Four matrix conditions were investigated: high (2.5 kPa) and low (0.7 kPa) stiffness matrix, and the addition or lack of RGD. Using $IC_{50}$ assay, we found that the co-culture tumour spheroids showed the least effective drug uptake compared to cells cultured separately. The presence of HFF1 in the co-culture could modify the matrix properties, thus affecting the breast tumour spheroids response to the drug treatment. Furthermore, the presence of RGD motif in the extracellular matrix decreased the drug uptake of all cultures.

**References**

[1] Quail, D. F.; Joyce, J. A. *Nature medicine* **2013,** *19* (11), 1423-37.
[2] Boedtkjer, E.; Pedersen, S. F. *Annual review of physiology* **2020,** *82*, 103-126.