## A 3D bioprintable hydrogel with tuneable stiffness for exploring cells encapsulated in matrices of differing stiffnesses.

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In vitro cell models have undergone a shift from 2D models to 3D models that better reflect the native microenvironment. The advances and new materials available to the field have opened an opportunity to transition this research to 3D bioprinting to provide high-throughput capabilities. Recently, our team and collaborators have developed a bespoke drop-on-demand printer and demonstrated its capability to produce large quantities of reproducible structures with high fidelity.<sup>1-3</sup> Drop-on-demand printing offers several advantages such as exact control over material volume and precise deposition in defined locations. Here, we developed and printed a bioink system with tuneable stiffness to expand the range of bioinks available for drop-on-demand printing by utilizing a 4-armed polyethylene glycol with maleimide functionalized arms. The complementary crosslinker comprised of a matrix metalloprotease degradable peptide and a 4-armedthiolated polymer in various ratios which was adjusted to control the stiffness of the network. Furthermore, the modularity of the system allows for the easy addition of biological motifs. By printing 60 structures every 10 minutes, the highthroughput capability was validated by assessing the variability and size distribution of printed structures. Also, the application of this system in drop-on-demand printing is validated in this work using MCF-7 cells which were monitored for viability, proliferation, and migration. This work explores the versatility of this bioink in a high-throughput capacity and demonstrates its capability as a platform for studying cell behavior in a range of environments.

## References

1. Utama, R. H.; Atapattu, L.; O'Mahony, A. P.; Fife, C. M.; Baek, J.; Allard, T.; O'Mahony, K. J.; Ribeiro, J. C. C.; Gaus, K.; Kavallaris, M.; Gooding, J. J., A 3D Bioprinter Specifically Designed for the High-Throughput Production of Matrix-Embedded Multicellular Spheroids. *iScience* **2020**, *23* (10), 101621.

2. Utama, R. H.; Tan, V. T. G.; Tjandra, K. C.; Sexton, A.; Nguyen, D. H. T.; O'Mahony, A. P.; Du, E. Y.; Tian, P.; Ribeiro, J. C. C.; Kavallaris, M.; Gooding, J. J., A Covalently Crosslinked Ink for Multimaterials Dropon-Demand 3D Bioprinting of 3D Cell Cultures. *Macromol Biosci* **2021**, *21* (9), e2100125.

3. Jung, M.; Skhinas, J. N.; Du, E. Y.; Tolentino, M. A. K.; Utama, R. H.; Engel, M.; Volkerling, A.; Sexton, A.; O'Mahony, A. P.; Ribeiro, J. C. C.; Gooding, J. J.; Kavallaris, M., A high-throughput 3D bioprinted cancer cell migration and invasion model with versatile and broad biological applicability. **2021**. (submitted)