

## Appendix A for

Stacking up: A new approach for cell culture studies

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**This PDF file includes:**

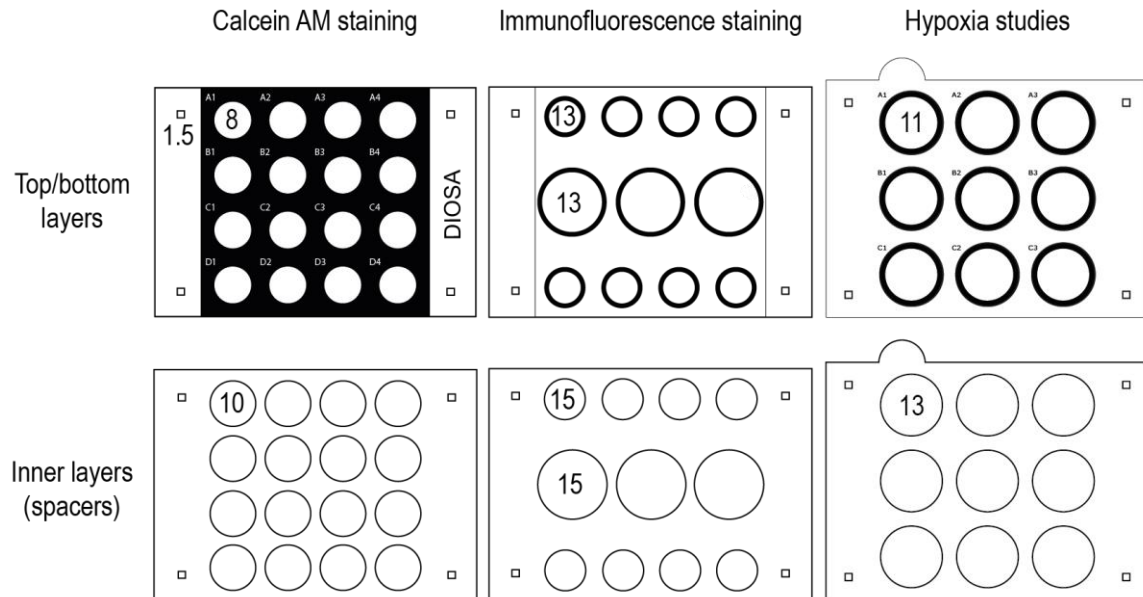
Figures A.1 to A.5  
Caption for Video A

**Other Supplementary Materials for this manuscript includes the following:**

Video A

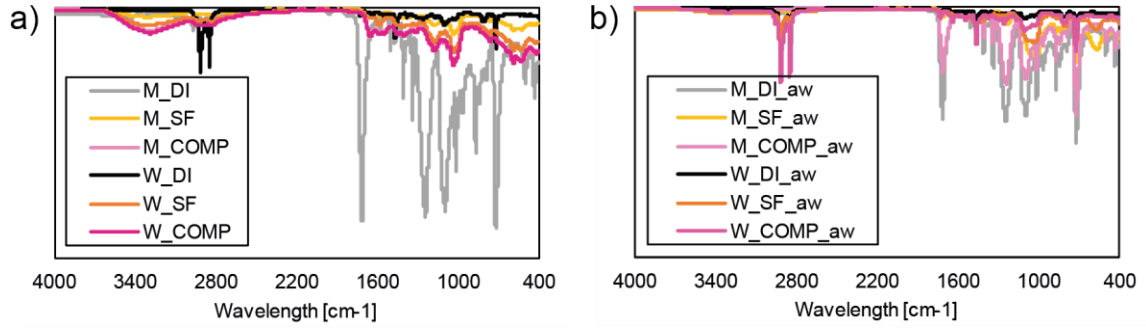
**Figure A.1.**

Layer designs for the printed cell culture platform. It includes the top/bottom layers to be printed with hydrophobic boundaries, and the inner layers to be cut at the desktop cutter. The designs were used for Calcein AM staining, immunofluorescence staining, and hypoxia studies. Numbers inside the wells represent the diameter in millimetres.



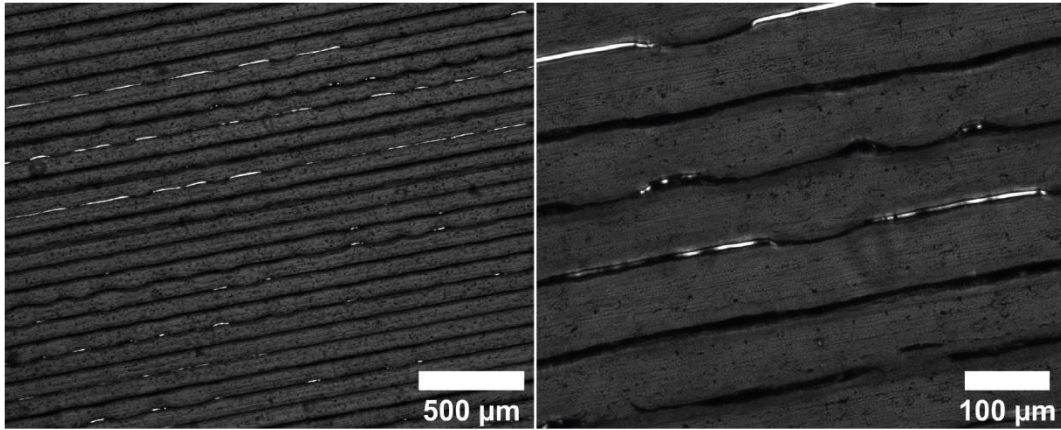
**Figure A.2.**

FTIR spectra for Melinex OD (M) and black wax-printed Melinex OD (W) after contact with deionised water (DI), serum free DMEM (SF), and complete DMEM (COMP) in two conditions: a) no wash after contact, b) wash (aw) after liquid contact



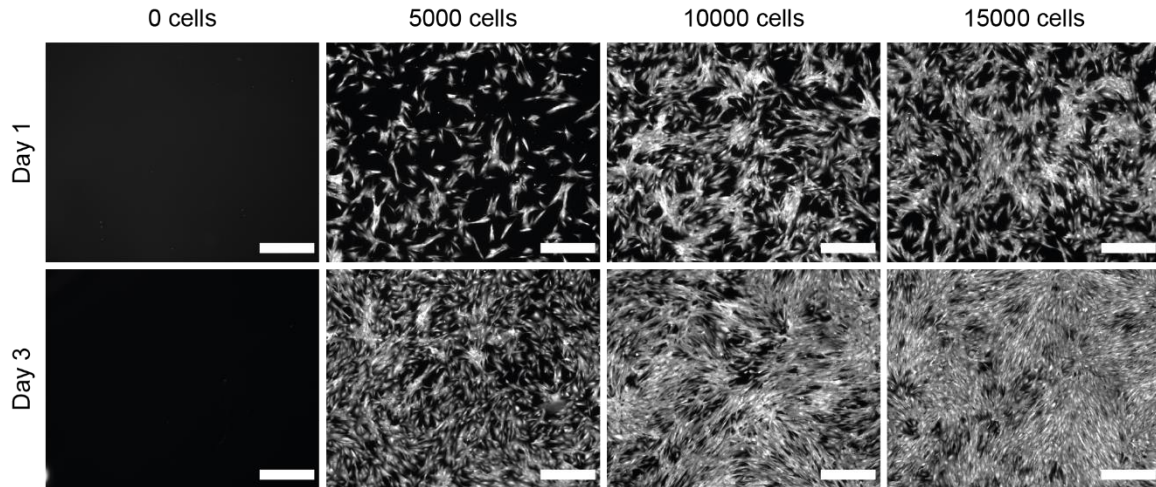
**Figure A.3.**

Microscopy images at different magnifications of black wax ink print on Melinex® OD showing uneven or incomplete fusion after melting.



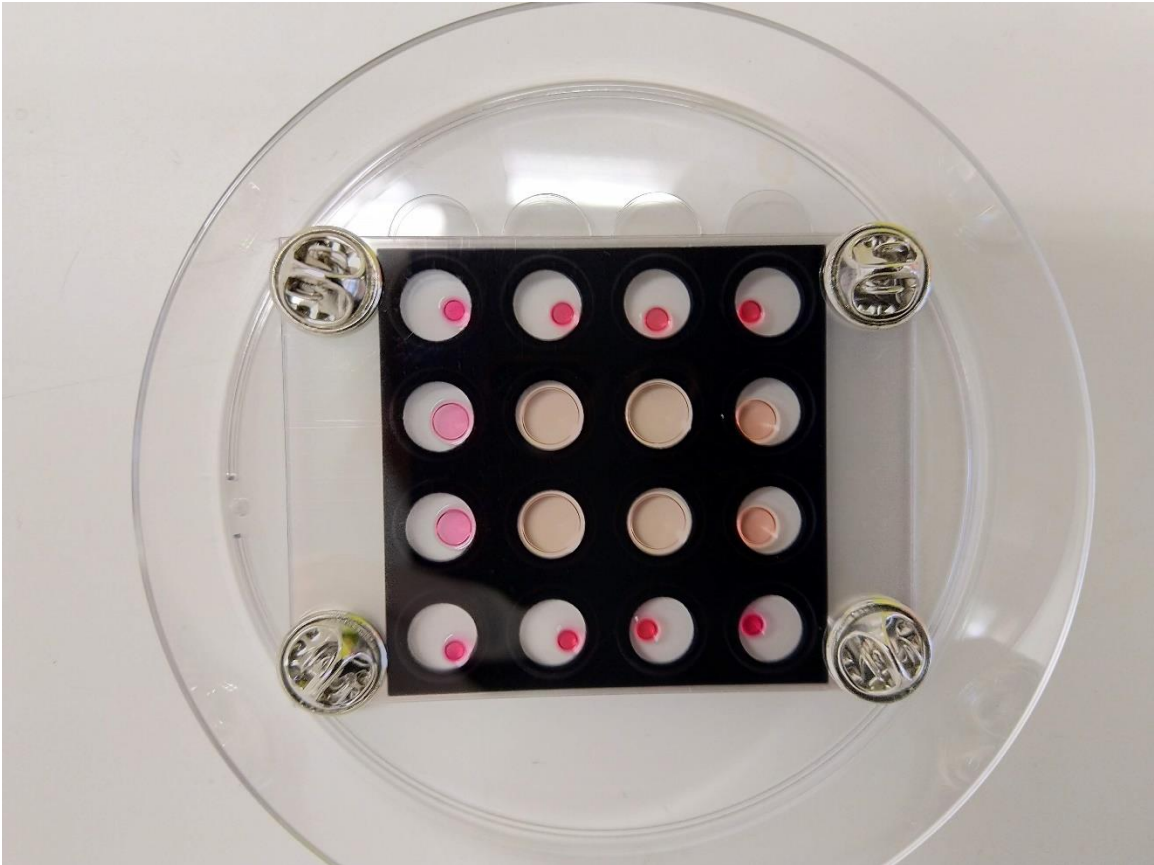
**Figure A.4.**

Fluorescence imaging of HDFs stained with Calcein AM for a cell seeding of 0, 5000, 10000, and 15000 cells at day 1, and day 3 of cell culture. Prior to imaging, cells were treated with 50  $\mu$ M Cisplatin to induce apoptosis. After 10 hours, cells are arrested, and loose the spindle shape morphology characteristic of fibroblasts. Longer treatment times would be needed to reach cell death. Scale bar: 500  $\mu$ m.



**Figure A.5.**

Image of the stacked cell culture platform after four days of cell culture without changing media. The wells closer to the edges of the plastic have dried the most, but kept the column of liquid between the top and the bottom layer. In contrast, the media in the centre wells has not reduced significantly. The different colours in the cell culture media indicate changes in the pH.



**Video A (separate file)**

Demonstration of the stacked cell culture platform.