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Laboratory Automation and Biomanufacturing Engineering – Automated Cell Application

IMMEDIATE DROP ON DEMAND TECHNOLOGY (I-DOT)

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Introduction

The handling and dosing of cells is the most critical step in the microfabrication of 3D-cell-based assay systems for screening and toxicity testing applications. The use of robotic workstations for liquid handling has increased enormously over the last decades, in order to meet the requirements for high accuracy and high throughput of current bioanalytics. The immediate drop on demand technology (I-DOT) overcomes the challenge of dispensing cells in a small volume precisely and with low shear stress. I-DOT provides a fast and flexible non-contact liquid handling system enabling dispensing cells and liquid without the risk of cross-contamination to a precise volume even in nanoliter range.

Methods

Liquid is dispensed from a special source plate with a nozzle (Ø100 µm) at the bottom. The small diameter keeps the liquid due to capillary forces inside the well and prevents leaking under normal pressure. To release a defined amount of liquid a short compressed air pulse is given through a quick release valve into the well, exceeding the capillary pressure in the nozzle (figure 1). Droplets with a defined volume can be spotted directly onto SBS-plates or other cell culture devices.

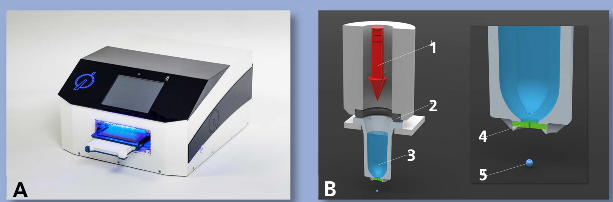

Fig.1: Setup of I-DOT. A: Design of the I-DOT, source and target slides ejected. B: Scheme of the operating principle of the immediate drop on demand technology 1) compressed air pulse, 2) seal ring, 3) source well filled with liquid, 4) nozzle with orifice, 5) droplet. ©Fraunhofer IPA.

In this study MCF-7, human skin fibroblasts (phSD) and human mesenchymal stem cells (hMSCs) were tested for viability, adherence capability, apoptosis induction, and proliferative and differentiation capacity after printing. As a control, cells were dispensed using commercially available pipette systems.

Results

The viabilities of all cell types after the printing process were comparable to the control, all cells had a viability > 80 % (figure 2-4).

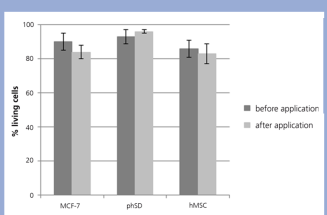

Fig. 2: viability of MCF-7, fibroblasts and hMSCs before and after printing.

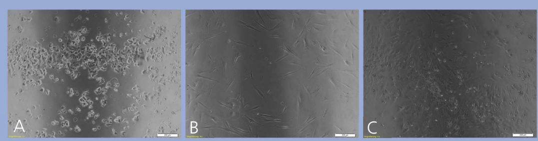

Fig.3: Light microscopic shots of MCF-7 (A), fibroblasts (B) and hMSCs (C) after printing.

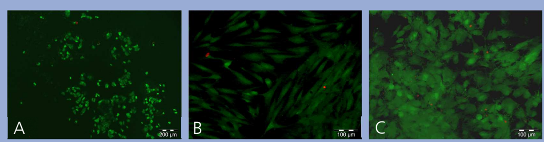

Fig. 4: Live/dead staining of MCF-7 (A), fibroblasts (B) and hMSCs (C) after printing. Living cells are stained with green-fluorescent calcein-AM, dead cells are stained with red-fluorescent ethidium homodimer-1.

Printed as well as manual pipetted cells showed similar proliferation rates, with the strongest rate for MCF-7, followed by primary skin cells and hMSCs. After printing no apoptotic induction was detected as confirmed by TUNEL staining (figure 5).

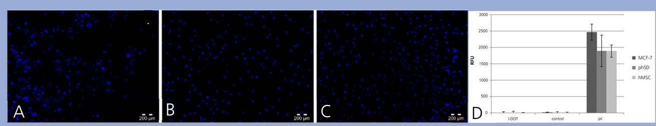

Fig. 5: TUNEL staining of MCF-7 (A), fibroblasts (B) and hMSCs (C) after printing. Apoptic cells are stained with TMR-red, DAPI staining of cell nucleus. D: Quantification of TMR-red staining.

Furthermore, the I-DOT application did not affect hMSCs in undergoing adipogenic and osteogenic differentiation, as demonstrated histologically (figure 6).

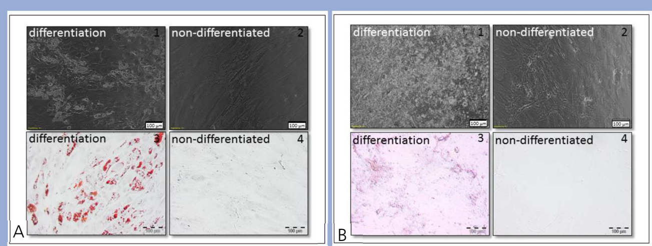

Fig.6: Differentiation of hMSCs after printing. A: adipogenic differentiation (oilred O staining); B: osteogenic differentiation (alizarin red staining)

Conclusion

I-DOT enables to apply accurate cells without detrimental fluorochrome tagging and offer promising potential for single cell and non-invasive stem cell application as a platform for 3D-tissue culture build-up or biomarker screening.