Live Cells Dispensing with CellJet Printer.

INTRODUCTION

Figure 1. ***Digilab's Live Cell Printer CellJet***
(http://www.digilabglobal.com/products-celljet)
(Catalogue numbers CJ100001-1 – CJ100004-1)

Three dimensional (3D) printing has opened a revolutionary era in biology and medicine; many experts now predict that 3D-bioprinting will emerge as the leading manufacturing paradigm of the 21st century. Yet, automatic delivering of live mammalian cells without their damaging is one of the greatest challenges in bio-printing. To address the challenge, Digilab has recently developed Live Cell Printer CellJet (Fig. 1), an automatic dispensing system operating with nanoliter volumes specifically designed to print live cells and hydrogels in any 2D and 3D configuration on any surface, micro-titer well dish, or culture dish with minimal loss in cell viability. This system suits perfectly for operation with live eukaryotic cell lines and was successfully used with a number of mammalian cell lines in combination with major types of hydrogels (bio-inks).

EXPERIMENTAL

CellJet is a unique dispensing platform based on operating one, or many (up to 16) precise hydraulically driven systems connected with dispensing tips through solenoid valves. This opens possibility to simultaneously dispense up to 16 different cell types. The system is designed the way that cells in the liquid never contact any metal parts inside the valves or pumps, thus significantly enhancing overall viability of the dispensed cells. Experimental data confirmed that viability for most types of mammalian cells dispensed through CellJet system, compared to viability of the same type of cells with the same hydrogel seeded manually, exceeds 95% (Fig. 2). The list of cell lines and primary cells tested so far for viability with CellJet consists of Human Embryonic Stem cells (HuES7, OxF2, NCL-1); Human Myeloid cell line U937 (ATCC CRL-1593.2); Human Mesenchymal Stem cells (hMSC); Human Muscle Stem cells; Human Fibroblasts; Human Hepatocytes; Human Cardiomyocytes; Human Epidermal Keratinocyte cell line NHEK; human Adenocarcinoma line HeLa (ATCC CCL-2); Mouse Smooth Muscle cells; Mouse Fibroblasts; Murine Embryonic Stem Cells (unique, genetically engineered); Murine Hybridomas (at least two kinds: "robust" and "fragile"); Chinese Hamster Ovary cell line CHO-K1 (ATCC CCL-61); Yeast cells; Bacterial cells (such as *E.coli* etc.) [1, 2]

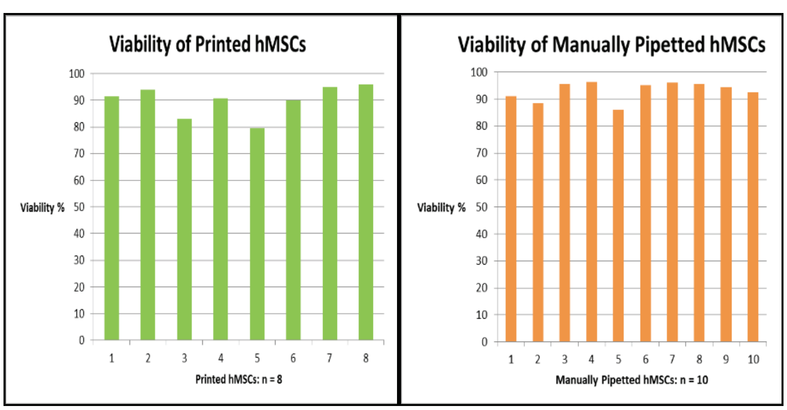

Figure 2. ***Viability of Bioprinted hMSCs compared to manually pipetted hMSCs into 96-well plate.*** 20 µL of hMSC-cell-suspension was dispensed using the CellJet in 96-plate wells, each containing 180 µL of MSCGM. Cells were incubated for 24 hours at 37°C, and stained with Live-Dead stain (Invitrogen). Manual Tiling was done while microscopic imaging to cover the entire well at 4x magnification.

CellJet instrument provides researchers with rare opportunity to utilize the great mechanical precision of the system (positioning specs: dispensing increments 1.3 µm. repeatability <± 10 µm) along with the ease in optimization of many parameters of liquid dispensing, which are critical for cell's viability (like drop volume, drop delivery speed, delivery type (drop-by-drop, or on-the-fly), distance of the delivering tip from the surface etc); its ceramic tips deliver clean, sharp drops. This is the reason why CellJet can correctly dispense even extremely viscous liquids at nanoliter levels with dispense accuracy below 10%. Because most of hydrogels are very viscous, such ability of CellJet instrument opens the door to creation of very fine and precise bio-constructions. Fig.3 & 4 demonstrate some examples of printing fine structures by CellJet using alginate as external matrix. The list of Extra-cellular matrices that have been printed using CellJet includes also: Collagen; Matrigel (Corning); Agarose; some Peptide-based self-assembling matrices (like PuraMatrix [3] and octapeptides [4]); PEG; Dextrans – all at different concentrations.

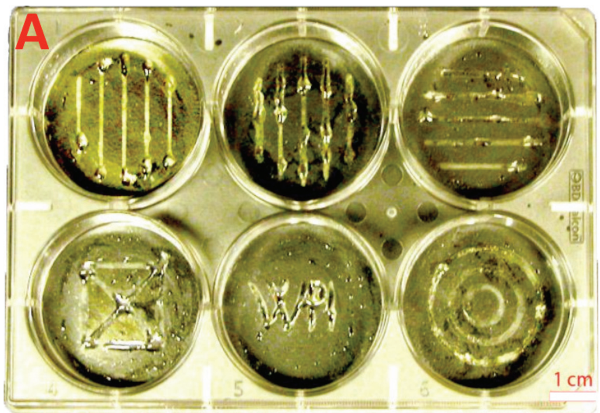

Figure 3. *Programmed patterns printed by the cell printer inside wells of a 6-well plate using 1% Sodium Alginate.* Top row: left to right: 2 wells with vertical parallel lines, 3rd well with horizontal parallel lines. Bottom row: left to right: square with diagonals, the letters WPI, 3 concentric circles.

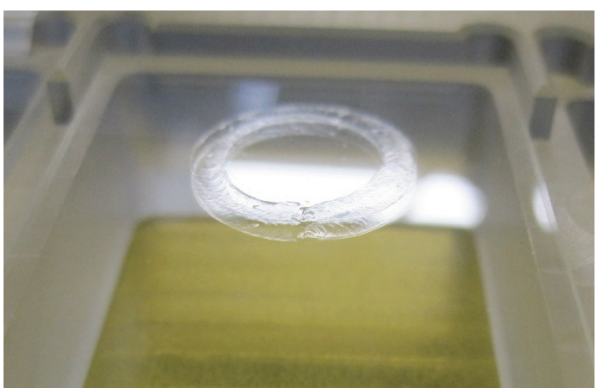

Figure 4. *Printing a 10 mm diameter ring of 1% Sodium Alginate on Glass slide.* Printing circular structures and adding layers of the same opens the possibility of forming tubes using biological material.

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