Application of CellJet Technology for Precise "Printing" of Adherent Mammalian Cells with no Loss in Viability.

INTRODUCTION

Several different emerging technologies are highly dependent on precise, reliable and fast distribution of viable cells in minute volumes. Among these technologies are cell and tissue engineering, live cells microarray assays, high content analysis - just to name a few. Recently, Digilab has developed CellJet, a dispensing system operating with nanoliter volumes designed for operation with live eukaryotic cell lines.

Here we describe a simple protocol for dispensing nanodroplets of adherent mammalian cells either into liquid nutrient medium, or onto dry surfaces with minimal, or no losses in their viability. For comparative purposes, distribution of the particular cells was done by using either a CellJet technology, a peristaltic dispenser or manually. For fresh cell suspension preparation, mammalian cells at the middle of their logarithmic phase of growth attached to the surface of a Petri dish, were washed with PBS, trypsinized according to the recommended procedure, resuspended in a fresh growth medium and dispensed through either CellJet, or peristaltic system into wells filled with (Figures 1), or without (Figures 2-4) growth medium. In the latter case, the wells were filled with appropriate growth medium after 1-6 hrs allowance for attachment of the cells to the plastic surface. Cells (live, or stained with Trypan Blue, or Propidium Iodide) were visualized by using MIAS-2 microscopy at 2-10x magnification in either brightfield, or fluorescent mode and eaZYX image analyzing software. We found that trypsinized adherent cells could be dispensed directly on plastic without loss of viability (Figures 2, 4) when dispensed at 0.1 mm from the surface. Growth capacity of cells dispensed with CellJet was comparable to the one dispensed either manually, or with peristaltic dispenser (Figure 3).

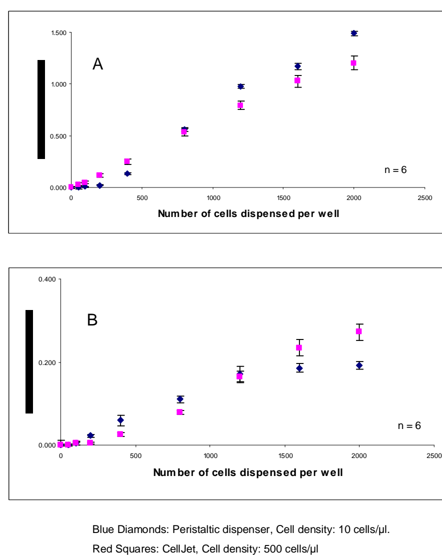


Figure 1. Comparison of viability of HeLa (A), or primary endothelial (B) cells dispensed with either CellJet printer or peristaltic dispenser. Different volumes of the cell suspension (500,000 cells/ml), ranging from 100 nl to 4 µl, were dispensed with CellJet. Alternatively, the cell suspension was diluted 50-fold and 5 µl to 200 µl were dispensed with the peristaltic dispenser (PD) Four (A), or nine (B) days after seeding, MTT viability assay was performed according to standard procedure. (Best fit linear regression: A: $R2=0.9964$ for CellJet, and $R2=0.9791$ for PD; B: $R2=0.9722$ for CellJet, and $R2=0.9435$ for PD).

Figure 2. Growth of HeLa cells after "printing". HeLa cells were dispensed using CellJet technology into dry flat bottom wells of 96-well plates and allowed to adhere for 5 hrs after which the wells were filled with growth medium and incubated for the specified number of days at 37 ºC, 5% CO_2 and humidity at 95%. Live cells images were captured at 5x magnification.

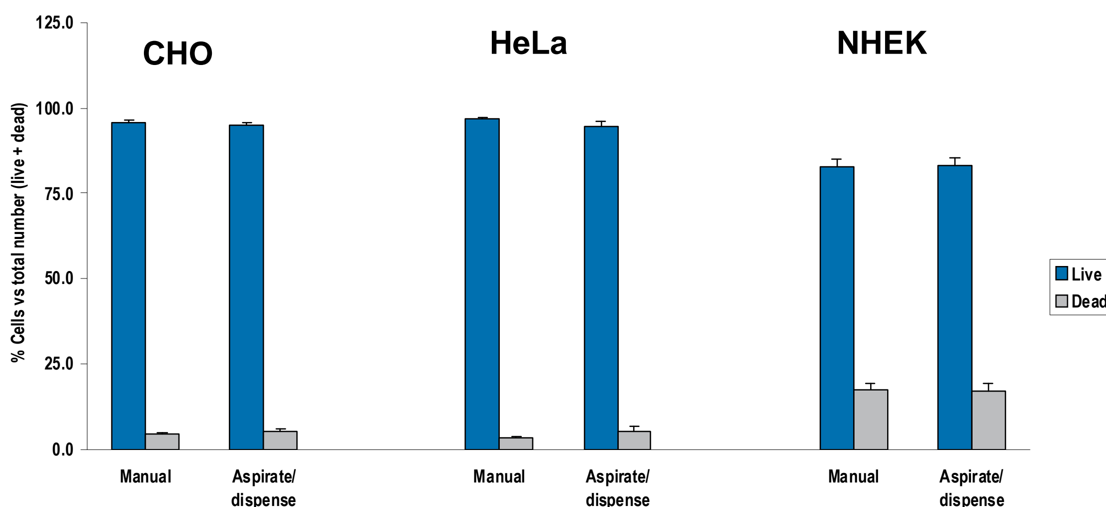


Figure 3. Distribution of adherent cells. With CellJet technology. Trypsinized cells were aspirated & dispensed (not through the valve), drop by drop and from the top of the plate. The cell density was ~ 500,000 cells/ml and V = 4 µl. The number of dead cells was assayed with PI and image analysis using MIAS-2 reader. And eaZYX image analyser software For manual dispensing (pipetting) the cell suspension was diluted 50x and V = 200 µl. Graphs represents average percentages and CV, n = 12 for pipetting and 60 for CellJet dispensing. About 2000 cells were counted per well.

Adherent cells can be dispensed in small volumes resulting in growing micro-confluent cultures. Cells dispensed in volumes of less than 1-2 µl grew fine when dispensed into liquid medium but did not grow efficiently after adherence when dispensed on plastic. Cells dispensed by "on-the-fly" procedure on plastic at 0.1 mm from the surface with speeds of 10 or 20µl/sec appeared more spread, than at 5µl/sec (Figure 4). To summarize, adherent cells can be dispensed in small (nano- and micro-liter) volumes resulting in growing micro-confluent cultures.

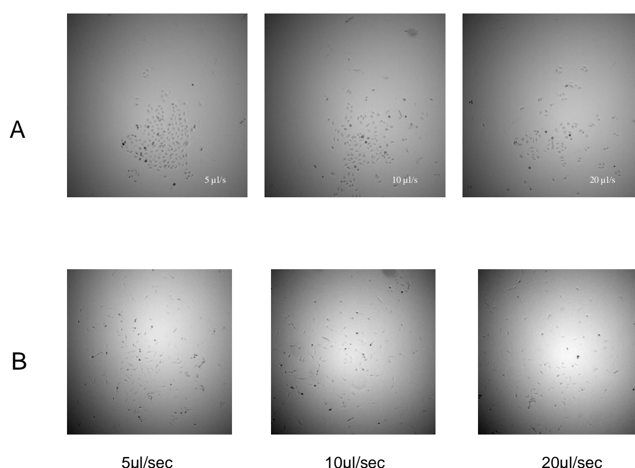
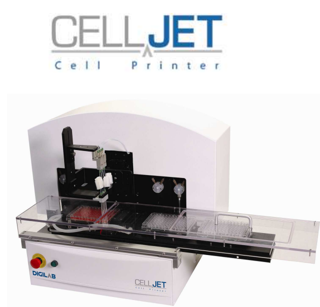


Figure 4. Effect of speed of "on-the-fly printing" on HeLa (A), or primary endothelial (B) cells viability. A 2µl aliquote of the cell suspension (10^5 cells/ml) was dispensed by CellJet technology at different speeds as indicated. After five (A), or nine (B) hrs adherence, growth medium was added and cells were incubated for 1 day at 37ºC, 5% CO_2 and 95% humidity before live cells images were captured at 5x magnification.

PROCEDURE

1. Grow cells in Petri dishes until the middle of logarithmic phase of growth.
2. Aspirate the conditioned medium, carefully wash the attached cells with ice-cold PBS; aspirate PBS.
3. Add 0.5 ml/10 mm dish of corresponding trypsin containing solution and incubate at RT until the cells assume round configuration, aspirate the trypsin solution.
4. Resuspend the cells in 2-5 ml of fresh growth medium, collect the cells by low speed centrifugation, aspirate the medium and resuspend the cells in 1 ml of fresh growth medium. Place the cells on ice. Take an aliquote of the cell suspension for cell counting using either hemocytometer, or cell counter.
5. Prepare the plate where the cells are going to be dispensed. Fill up the wells with minimal volume of the fresh growth medium (just to cover the surface) if you are planning to seed the cells. Otherwise, prepare the plate, or the slide for dispensing the cells on a dry surface, we recommend following particular instructions from the CellJet manual.
6. Dilute the cell suspension with growth medium to reach optimal concentration in the range of $(50\text{-}500)\text{x}10^3$ cells/ml and load the CellJet system. You can use up to 250µl of cell suspension at a time with the coiled tubing.
7. Dispense the cells using CellJet cell printer into the wells, or onto the plastic surface. Using at least 100nl of the suspension is recommended to achieve higher cell viability. You may dispense smaller volumes (as low as 20nl) if you are dispensing cells into growth medium without significant lose in viability. If the "on-the-fly" method is chosen for dispensing with CellJet, use volumes not smaller than 1µl per drop and speeds larger than 10µl/sec. Reference to the CellJet manual to avoid common mistakes.
8. Cover the plate with a lid, place into CO_2 incubator for at least 10 min to allow the cells to attach to the surface. If printing onto a slide was performed, place the slide in a humidity chamber for at least 10 min.
9. Add more growth medium if necessary and incubate as specified for the assay you are performing.

CELLJET Cell Printer



CellJet Cell Printer

MIAS-2

MIAS-2 Imager

eaZYX

eaZYX Image Analyzer

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