

APPLICATION NOTE

Application of CellJet Technology for Precise “Printing” of Mammalian Cell Lines Grown in Suspension.

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 microdroplets of mammalian cells grown in suspension 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 were collected by low-speed centrifugation, resuspended in a fresh growth medium and dispensed through either CellJet, or peristaltic system into wells filled with (Figures 1-3), or without (Figures 4-5) growth medium. In the latter case, the wells were filled with appropriate growth medium after 1-6 hrs incubation of the cells on the plastic surface. Cells (live, or stained with Trypan Blue, or Propidium Iodide) were visualized by using eaZYX image analyzing software with MIAS-2 microscopy at 2-10x magnification in either brightfield, or fluorescent mode. We found that free-flowing cells could be dispensed directly onto plastic without loss of viability (Figure 5) when dispensed at 0.1mm from the surface in volumes not less than 4 μ l and growth medium was added within one hour after the dispensing. Growth capacity of cells dispensed with CellJet was in general comparable to the one dispensed either manually, or with peristaltic dispenser (Figures 1-2) with exception of some fragile cells where it was slightly lower with CellJet technology (Figure 2).

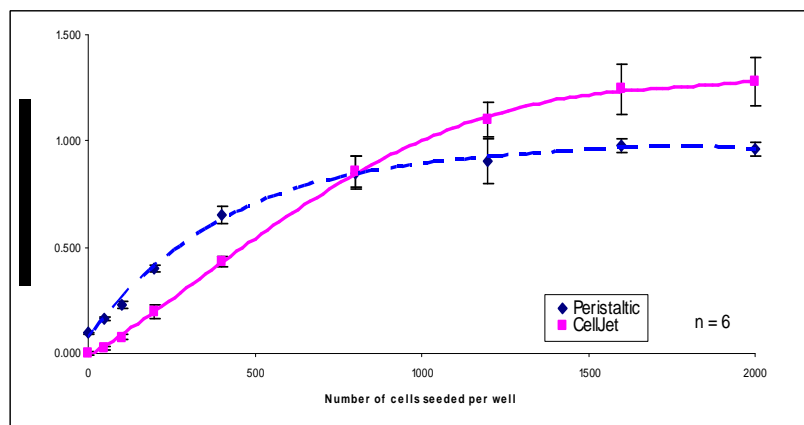


Figure 1. Viability of U-937 cells dispensed with the CellJet printer. Different volumes of a U-937 cell suspension (500,000 cells/ml), ranging from 100 nl to 4 μ l, were dispensed with CellJet Cell Printer into wells pre-filled with RPMI medium. Alternatively, the cell suspension was diluted 50-fold with RPMI and 5 μ l to 200 μ l were dispensed into RPMI pre-filled wells with peristaltic dispenser. Four days after seeding MTT viability assay was performed according to standard procedure. (Best fit linear regression with $R^2=0.9821$ for CellJet, and $R^2=0.9630$ for peristaltic dispenser).

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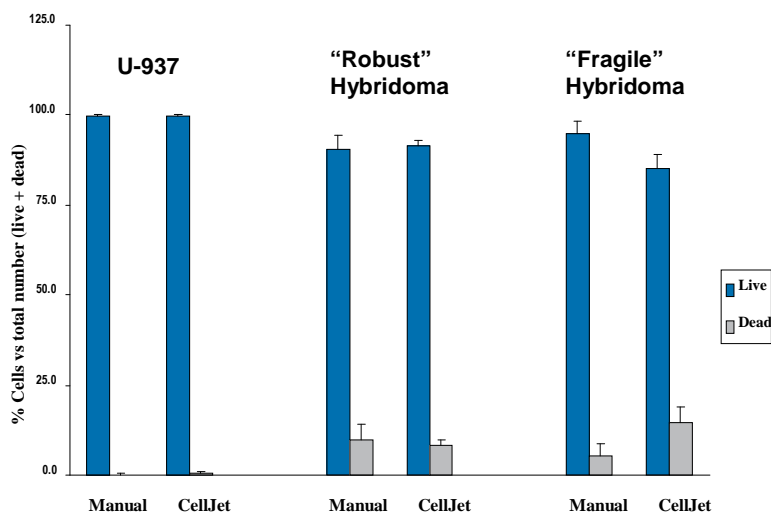


Figure 2. Dispersion of free flowing cells with CellJet technology. Suspension cells, U-937 and two hybridoma's cell lines (Hyb) were dispensed drop by drop through the valve of CellJet cell printer. The cell density was ~ 500,000 cells/ml and $V = 25 \mu\text{l}$ both for manual dispensing (pipetting) and CellJet dispensing. After addition of Trypan blue, the number of viable cells was assayed using a Neubauer counting chamber. Graph represents average percentage and CV, $n = 4$. At least 100 cells were counted per data point.

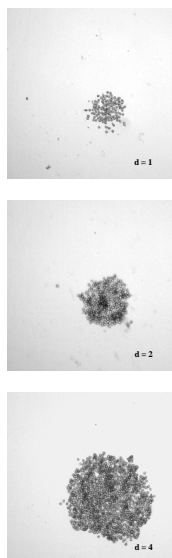


Figure 3. Growth of U-937 cells after CellJet "printing" into growth medium. After dispensing cells in U-bottom plates, growth medium was added. Images at 2.5x magnification were captured on consecutive days after seeding as indicated.

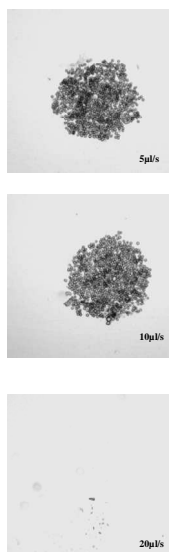


Figure 4. Effect of speed of "on-the-fly printing" by using CellJet technology on U-937 cells viability. Cells were "printed on the fly" in U-bottom plates at different dispensing speeds as indicated. After 4 days incubation images were captured at 2.5x magnification.

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Free flowing cells can be dispensed in small volumes resulting in growing cultures. Cells dispensed in volumes of less than 3 μ l grew fine when dispensed into liquid medium but did not grow efficiently when dispensed onto plastic. Cells dispensed by “on-the-fly” procedure on plastic at 0.1 mm from the surface with speeds of 5 or 10 μ l/sec appeared viable, while those dispensed at 20 μ l/sec were not (Figure 4). Addition of growth medium 4 and more hours after cell dispense also resulted in drastic loss of cell viability. To summarize, free living cells can be dispensed in small volumes using CellJet technology, resulting in growing cultures.

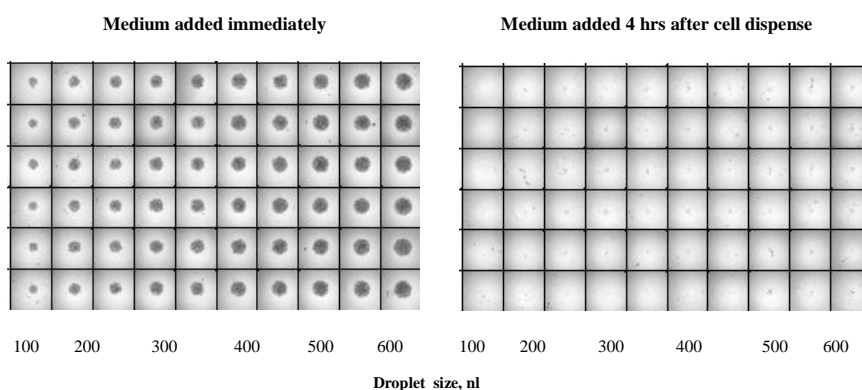


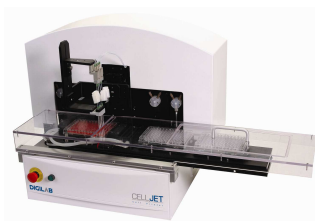
Figure 5. Survival of U-937 cells dispensed by CellJet technology into wells of a U plate as a function of droplet's size and time of growth medium addition. Growth medium was added either immediately (left panel), or four hours (right panel) after cell dispense. The picture similar to the left panel was obtained when the medium was added up to 1hr after the cell dispense (not shown). CellJet parameters: height: top of plate; dispensing speed: default; cell density: 500,000 cells/ml. MIAS-2/esXYZ scanning: was performed 3 days after seeding

PROCEDURE

1. Grow cells in flasks until the middle of logarithmic phase of growth.
2. Collect the cell suspension into a sterile centrifuge tube/bottle and collect the cells by low-speed centrifugation; aspirate the conditioned medium.
3. Resuspend the cells in 1-5 ml of fresh growth medium. Place the cells on ice. Take an aliquot of the cell suspension for cell counting using either hemocytometer, or cell counter.
4. 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.
5. Dilute the cell suspension with growth medium to reach optimal concentration in the range of (50-500) $\times 10^3$ cells/ml and load the CellJet system. You can use up to 250 μ l of cell suspension at a time with the coiled tubing.

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6. Dispense the cells using CellJet cell printer into the wells, or onto the plastic surface. Using at least 3µl of the cell suspension is recommended to achieve higher cell viability if dispensing onto dry plastic surface. You may dispense smaller volumes (as low as 100nl) if you are dispensing cells into growth medium without significant lose in viability. If ‘on-the-fly” mode was used, make sure that growth medium was added to the wells/spots containing the cells within one hour after cell dispense. Reference to the CellJet manual to avoid common mistakes
7. If printing onto a slide was performed, place the slide in a humidity chamber.
8. Add more growth medium if necessary and incubate as specified for the assay you are performing.

CELLJET
Cell Printer

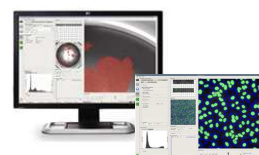
CellJet Cell Printer

MIAS-2



MIAS-2 Imager

eaZYX



eaZYX Image Analyzer

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