Smooth Muscle Cells: Use of **CellJet** Technology for Precise Dispensing with no Loss in Viability.

INTRODUCTION

Tissue engineering is a methodology directed to improve or replace biological functions of a part, or a whole organ. Once a wild fantasy belonging in science-fiction books, it recently became one of the hot areas of research at the intersection of cell biology, engineering, and medicine. Tissue and – even more - artificial organ engineering depends largely on development of a fast and reliable technology of building functional blood vessels. Vascular replacement surgery strongly depends on creating replacement vessels by means of tissue engineering of small-diameter blood vessels. Because all tubular structures, including blood vessels, are predominantly built of smooth muscle cells, there is a demand for smooth muscle tissue growth as well as precise, reliable and fast distribution of viable smooth muscle cells in minute volumes. Recently, Digilab has developed CellJet, a dispensing system operating with nanoliter volumes designed for operation with live eukaryotic cell lines, including smooth muscle cells.

Here we describe a simple protocol for dispensing nanodroplets of mammalian smooth muscle 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 or manually.

For fresh cell suspension preparation, mammalian cells at the middle of their logarithmic phase of growth attached to the surface of a tissue culture flask, were washed with PBS, trypsinized according to the recommended procedure, re-suspended in a fresh growth medium and dispensed through either CellJet, or manually into wells filled with growth medium, or without it. In the latter case, the wells were filled with appropriate growth medium after 10-60 min allowance for attachment of the cells to the plastic surface. Cells were visualized by using microscopy at 1-20x magnification in either brightfield, or fluorescent mode (after staining with calcein AM for live cells, or ethidium homodimer for dead cells). We found that muscle cells in volumes lower than 100 nl could be dispensed with CellJet directly on plastic without significant loss of viability (Fig. 1) when dispensed at no further than 5 mm from the surface and surrounded by several 2 µl droplets of growth medium. Growth capacity of cells dispensed with CellJet or manually was comparable.

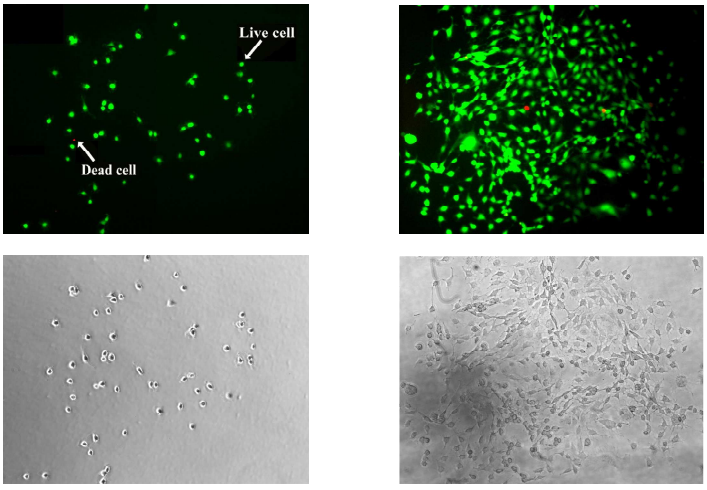

Figure 1. Human Muscle Primary Cells printed with CellJet technology into a 24-well plate. Initial dispense deposited four 2µL growth medium drops around the well center. Secondary dispense: one drop of cell suspension ((A) 50nL, or (B) 100nL) was dispensed at 20µL/sec and dispense height 5mm in the center of the well. After 10 min incubation, 0.3mL of fresh medium was added to each well and the cells were placed into CO_2 incubator for 4 hrs. Staining procedures: Calcein AM to visualize live cells (green fluorescent), Ethidium Homodimer to visualize dead cells (red fluorescent). Phase contrast image at 10x magnification. Average cell viability was calculated to be >95%.

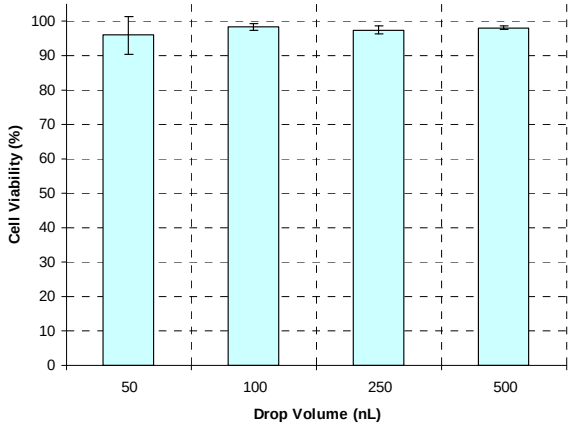

Figure 2. Smooth muscle cell viability two days after their dispensing at different droplet volume as measured by live/dead cell staining. Plotted are average values ± standard deviation from 20 independent measurements.

PROCEDURE

1. Grow up primary cells in Petri dishes, or tissue flasks for 2-4 generations.
2. Aspirate the conditioned medium, carefully wash the attached cells with ice-cold PBS; aspirate PBS.
3. Add trypsin containing solution (0.5 ml/80 mm^2) and incubate at room temperature until the cells assume uniform 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 to 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. If you are planning to dispense cells in droplets smaller than 100 nl, consider increasing local humidity around the droplet.
6. Dilute the cell suspension with growth medium to reach optimal concentration in the range of (200-1000)x10^3 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) without significant lose in viability if you are dispensing cells into growth medium. If the "on-the-fly" method is chosen for dispensing with CellJet, use volumes not smaller than 1µl per drop and speeds in the range of (10-20)µl/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.

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