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

INTRODUCTION

Stem cells are characterized by the ability to renew themselves and differentiate into a diverse range of specialized cell types. For a practical purpose, a stem cell is a cell that has the potential to regenerate tissue over a lifetime. There is a strong public belief that stem cell therapy has the potential to dramatically change the treatment of many human diseases. Stem cell therapy technologically is highly dependent on precise, reliable and fast distribution of viable stem 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 stem cells.

Here we describe a simple protocol for dispensing nanodroplets of mammalian muscle stem cells either into liquid nutrient medium, or onto dry surfaces with minimal, or no losses in their viability. Distribution of the stem cells done by using a CellJet technology was found comparable to manual dispense.

For fresh cell suspension preparation, stem cells at the middle of their logarithmic phase of growth in a tissue culture flask, were washed, trypsinized, resuspended in a fresh growth medium to make final cell concentration $1x10^6$ cells/ml (if not stated otherwise) and dispensed through CellJet into wells. Cells were visualized by using microscopy at 2-20x magnification in either brightfield, or fluorescent mode. We found that stem cells could be dispensed directly onto plastic without loss of viability when dispensed at not further than 5mm from the surface to keep the droplet closer to spherical form and minimize its spreading over a surface area. Growth capacity of cells dispensed with CellJet was comparable to manual method. Although we got good results using droplets as small as 50 nl (Fig.1-2A), using droplets smaller than 100 nl should not be attempted without taking steps toward prevention of liquid evaporation from the droplets. Such steps may involve performing cell dispensing inside a humidity chamber, or by surrounding it with droplets of water, or growth medium. In general, dispensing this type of cells into wells pre-filled with growth medium is the safest and fastest way of cell printing.

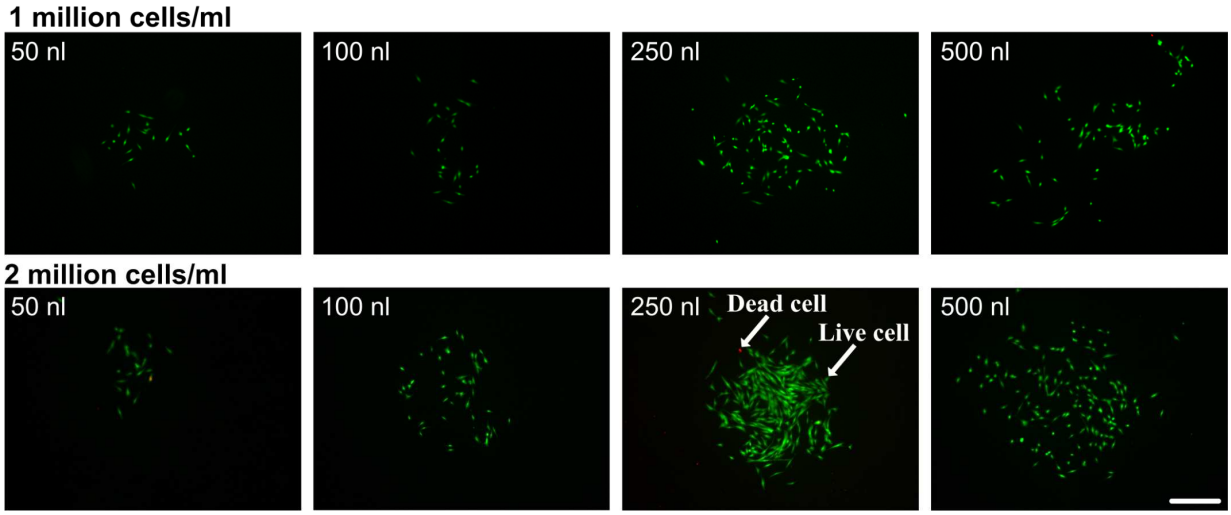

Figure 1. Human Muscle Stem Cells printed with CellJet technology into a 24 well plate. Initial dispense deposited four media 2 µl drops around well center. Secondary dispense: cells at two different concentrations in the volume as stated were dispensed as one drop at 20 µl/sec and low dispense height in well center. Cells were seeded for 10 min after which 300 µl of fresh media was added to each well and cells were incubated in CO_2 incubator for 24 hours. Staining Assays: Calcein AM for live cell staining, Ethidium Homodimer for dead cell staining.

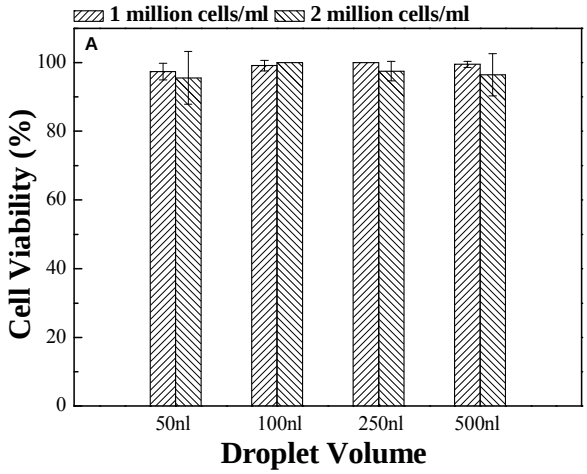

Figure 2. Smooth muscle cell viability two days after their dispensing at different initial cell concentrations and droplet volume as measured by live/dead cell staining. Plotted are average values ± standard deviation from 20 independent measurements.

PROCEDURE

1. Grow cells in tissue culture flasks until the cells reached about 70% confluence state.
2. Aspirate the conditioned medium, carefully wash the attached cells with ice-cold PBS; aspirate PBS.
3. Add trypsin solution (0.5 ml/80 mm^2), gently spread it over the surface and incubate until the cells assume round configuration (check periodically under microscope), aspirate the trypsin solution.
4. Resuspend the cells in 3-7 ml of fresh growth medium, collect the cells by low-speed centrifugation, aspirate the medium, 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 (1-2)x10^6 cells/ml and use it to load the CellJet system. You can use up to 250 µl 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 100 nl of the suspension is recommended to achieve higher cell viability. You may dispense smaller volumes (as low as 50 nl) without significant lose in viability if you are dispensing cells into growth medium. Use valve open times at least 1.2msec.
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.