

APPLICATION NOTE

Bioprinting Human Mesenchymal Stem Cells using the CellJet without loss in cell viability

INTRODUCTION

Adult Stem cells are characterized by both the ability to undergo numerous cycles of cell division while still maintaining an undifferentiated state and the ability to differentiate into diverse cell types present in their tissue of origin¹. Human Mesenchymal Stem Cells (hMSCs) are multipotent progenitor cells that can differentiate into a variety of tissue types including bone, adipose (fat), cartilage, and muscle². hMSCs hold promise in helping cure diseases of these tissues either by aiding repair or significantly boosting regeneration capacity in the tissue where they are transplanted. Clinical use of autologous hMSCs for therapy is facilitated by the ready availability of these cells from the bone marrow in adults and the Wharton's jelly of the umbilical cord in neonates, among other sources. Research and translation to therapy can be accelerated greatly by the ability to dispense viable stem cells in desired patterns or arrays on various substrates in an automated controlled manner which is precise, reproducible, and fast. Recently, Digilab has developed the **CellJet Cell Printer**, a live-cell dispensing system operating with nanoliter volumes designed to handle efficiently even the most delicate of cells while preserving viability.

PROCEDURE

This Application note describes a simple protocol for dispensing nanodroplets of Human Mesenchymal Stem Cells into wells of 96-well plates pre-filled with growth medium, so as to preserve cell viability. Passage 6-8 Human Mesenchymal Stem Cells at >90% confluence were washed, trypsinized, and resuspended in fresh growth medium to make final cell concentration 1×10^6 cells/mL. The cell suspension was either dispensed using the CellJet or manually pipetted into sterile 96-well plates, with each well pre-filled with 200 μ L of sterile Mesenchymal Stem Cell Growth Medium (MSCGM) (Lonza). Cells were incubated at 37°C for 24 hours and stained with a Live-Dead stain (Calcein-AM that fluoresces green in the cytoplasm of a live cell, Ethidium Homodimer that binds to DNA of a dead cells) (Invitrogen), as per manufacturer recommended protocol. Cells were visualized by using an inverted microscope at 4x magnification in the fluorescent mode using Red and Green filters. Manual tiling was performed to cover the entire well for all wells. Images were preprocessed for analysis using Adobe Photoshop CS5. All Images were analyzed using CellProfiler (<http://www.cellprofiler.org>) (Broad Institute, Cambridge, MA). Viability of hMSCs in the two groups (Bioprinted using the CellJet and manually pipetted) were compared for statistically significant difference using unpaired students T-test.

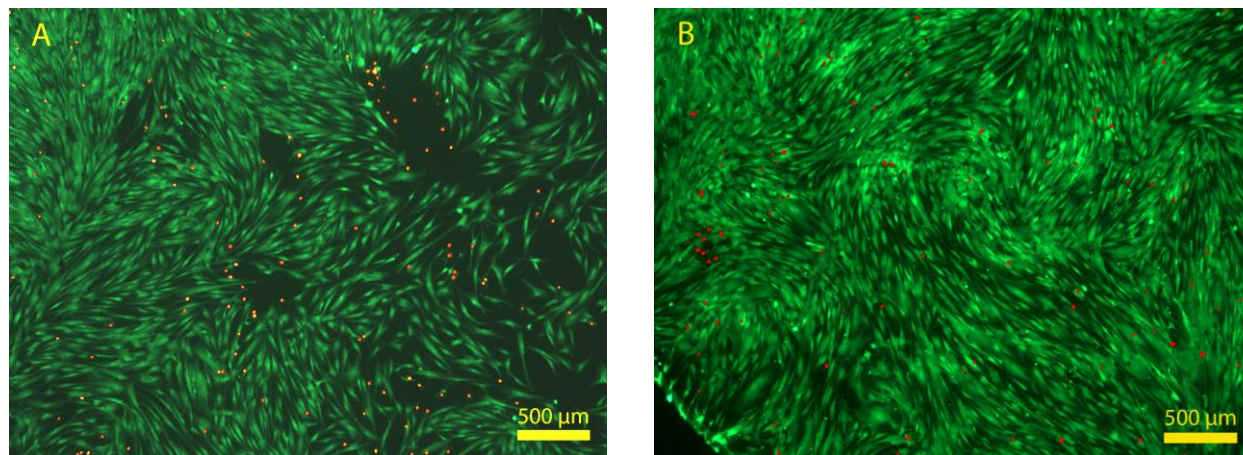


Figure 1. Bioprinted human Mesenchymal Stem Cells. 20 μ L of hMSC-cell-suspension was (A) dispensed using the CellJet or (B) manually pipetted in 96-plate wells, each containing 180 μ L of MSCGM. Cells were incubated for 24 hours at 37°C and stained with Live-Dead stain (Invitrogen). Green = Live cells. Red = Dead cells.

APPLICATION NOTE

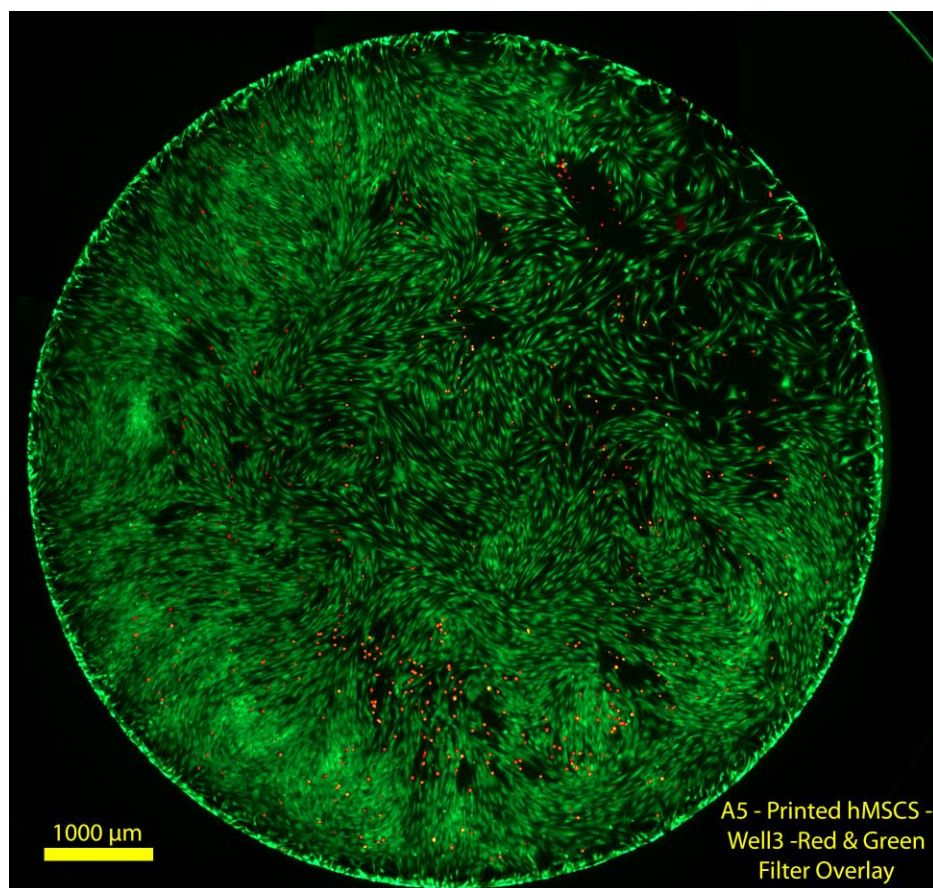


Figure 2. Bioprinted Human Mesenchymal Stem Cells in a 96-plate well. 20 μL of hMSC-cell-suspension was dispensed using the CellJet in 96-plate wells, each containing 180 μL of MSCGM. Cells were incubated for 24 hours at 37°C, and stained with Live-Dead stain (Invitrogen). Manual Tiling was done while microscopic imaging to cover the entire well at 4x magnification. Green = Live cells. Red = Dead cells. Percentage of viable cells was found to be 92%.

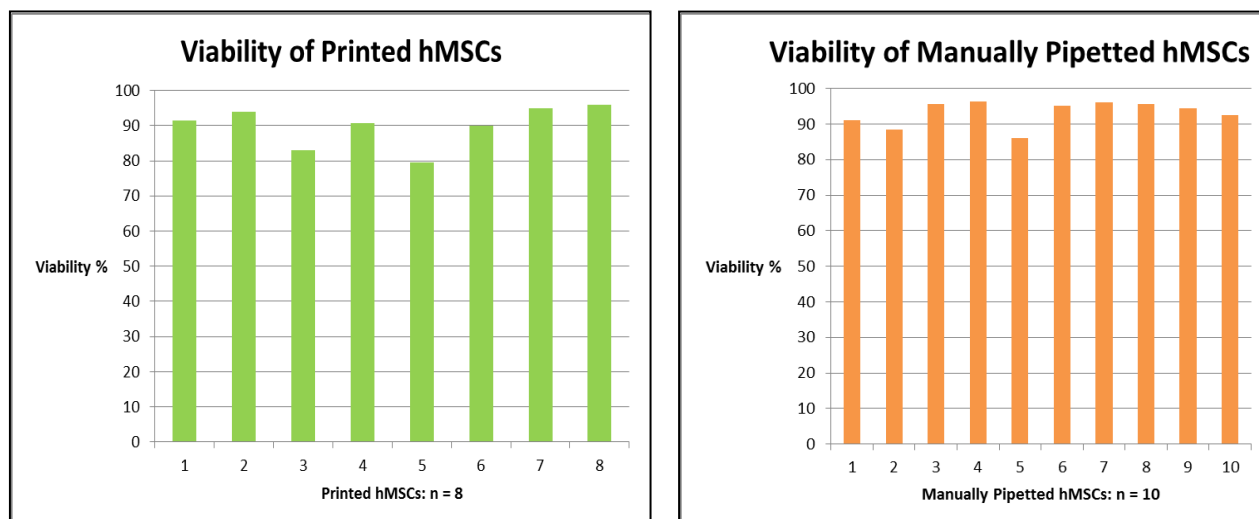


Figure 3. Viability of Bioprinted hMSCs compared to manually pipetted hMSCs. Mean viability of Bioprinted hMSCs was found to be 90% ($\pm 6\%$) and that of manually pipetted hMSCs was 93% ($\pm 4\%$). Viability in both groups was compared using unpaired Student's T-test and the difference was not statistically significant. ($p = 0.196$).

APPLICATION NOTE

Viability of Human Mesenchymal Stem Cells dispensed using the CellJet, which uses Digilab's patented synQUAD dispensing technology, was found comparable to cells dispensed manually by pipetting. Mean viability of Bioprinted hMSCs was found to be 90% and that of manually pipetted hMSCs was 93%; the difference was statistically not significant.

Consistently high viability has been achieved with various cell types with the CellJet using droplet sizes between 100 nL to 4 μ L. Using droplets smaller than 100 nL should not be attempted without prevention of evaporation by taking steps such as performing cell dispensing inside a humidity chamber or surrounding small droplets with water or growth medium. In general, dispensing this type of cells into wells pre-filled with growth medium is the best way of cell printing to ensure highest cell viability.

GENERAL PROTOCOL:

1. Culture cells in tissue culture flasks until they reach approximately 80-90% confluence.
2. Aspirate the conditioned medium, carefully wash the attached cells with sterile Phosphate Buffered Saline (PBS) (or equivalent wash solution); aspirate PBS.
3. Add trypsin solution (0.25% w/v, 1 ml/75 mm²) by gently spreading over the surface and incubate at 37°C until the cells assume round configuration (check periodically under microscope), rock the flask gently to assist detachment. Add fresh growth medium to the flask in 1:2 ratio by volume to neutralize the action of trypsin. (E.g. if 1 mL of Trypsin was added, add at least 2 mL of growth medium).
4. Resuspend the cells in the fresh growth medium, collect the cells by low-speed centrifugation, aspirate the supernatant medium, and resuspend the cells in 1 ml of fresh growth medium. Place the cells on ice. Take an aliquot of the cell suspension for cell counting.
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 planning to seed the cells. Otherwise, prepare the plate or slide for dispensing cells on a dry surface. Please refer to the CellJet manual for details.
6. Dilute the cell suspension with growth medium to reach optimal concentration in the range of (1-2) x 10⁶ cells/mL and load into the CellJet system. Up to 250 μ L of cell suspension can be used 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. Dispensing smaller volumes (as low as 50 nL) without significant loss in viability is possible if dispensing cells into growth medium. Use valve open times at least 1.2msec.
8. Cover the plate with a lid, place into CO₂ 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 being performed.

REFERENCES

1. <http://stemcells.nih.gov/info/basics/basics1.asp>
2. Pittenger, M. F. et al. Multilineage potential of adult human mesenchymal stem cells. Science 284, 143–147 (1999)

WORLDWIDE OFFICE
84 October Hill Road
Holliston, MA 01746
United States
Phone: 508 893 3130
Fax: 508 893 8011

EUROPEAN OFFICE
18 Blackstone Road
Huntingdon, Cambridgeshire
PE29 6EF United Kingdom
Phone: [+44] 1480 426 700
Fax: [+44] 1480 426 767

ASIAN PACIFIC OFFICE
6th Fl. Yokohama World Porters
2-2-1 Shinkou, Nakaku
Yokohama, Japan 231-0001
Phone/Fax: 045 651 6252