

## APPLICATION NOTE

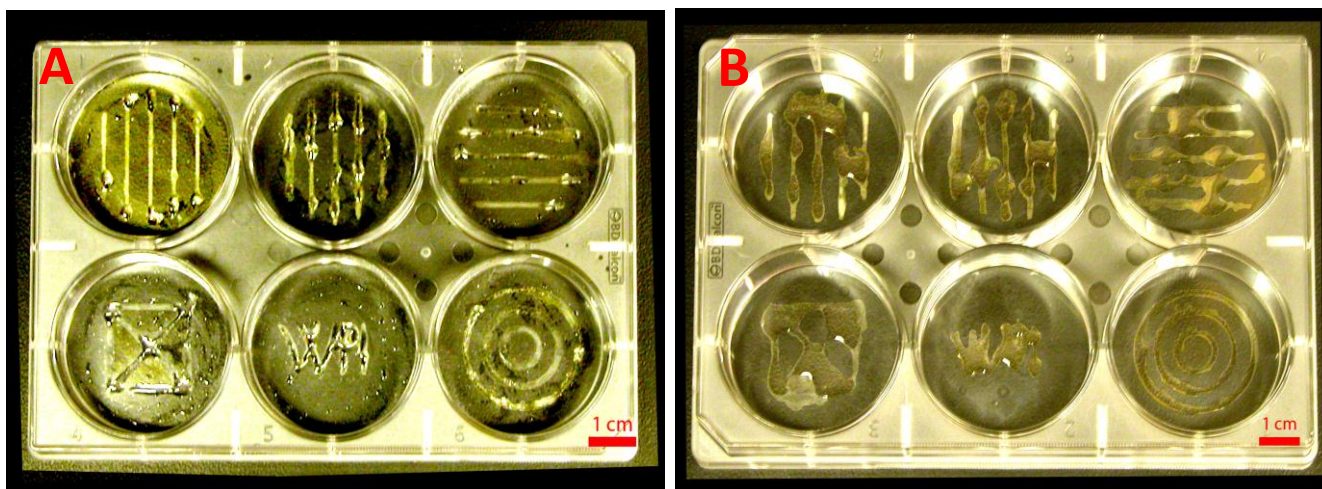
## Bioprinting Human Mesenchymal Stem Cells Suspended in Alginate in Patterns Using the CellJet Without Loss in Cell Viability

### INTRODUCTION

Human Mesenchymal Stem Cells (hMSCs) are multipotent progenitor cells that can differentiate into a variety of tissue types including bone, adipose (fat), cartilage, and muscle<sup>1</sup>. hMSCs hold promise of significantly boosting the regenerative capacity of the recipient tissue after transplantation. There has been scientific interest to develop methods to dispense viable stem cells in desired shapes, patterns or arrays that can be retained over time in culture. Hydrogels such as Alginate that can support cell growth allow this patterning. Alginate is a linear polysaccharide, composed of a repeating disaccharide of  $\beta$ -D-mannuronic acid and  $\alpha$ -L-glucuronic acid, which is soluble in aqueous or buffered salt solutions. Cells added to an alginate solution can be homogeneously distributed in suspension. The polysaccharide can then be cross-linked in the presence of calcium ions to form a polymerized hydrogel that retains its pattern/shape over several days in culture. The porosity of the hydrogel is such that diffusion of large molecules is possible, whereas passive migration of cells is not.<sup>2</sup> As a result, diffusion of nutrient media and oxygen can occur while cells remain in defined patterns. Digilab's **CellJet Cell Printer**, a live-cell dispensing system operating with nanoliter volumes, is designed to handle even the most delicate of cell types while preserving viability. In addition, the CellJet offers the unique ability to dispense viscous liquids and hydrogels such as Alginate on any substrate in specified patterns. The CellJet can thus be a very powerful tool for both basic research such as studying cell biology and translational research such as developing unique modes of cell delivery for cell therapy.

### PROCEDURE

This application note describes a general protocol for dispensing Human Mesenchymal Stem Cells suspended in Alginate to generate simple geometrical patterns in a sterile pre-wet 6-well plate (A) or dry 6-well plate (B).

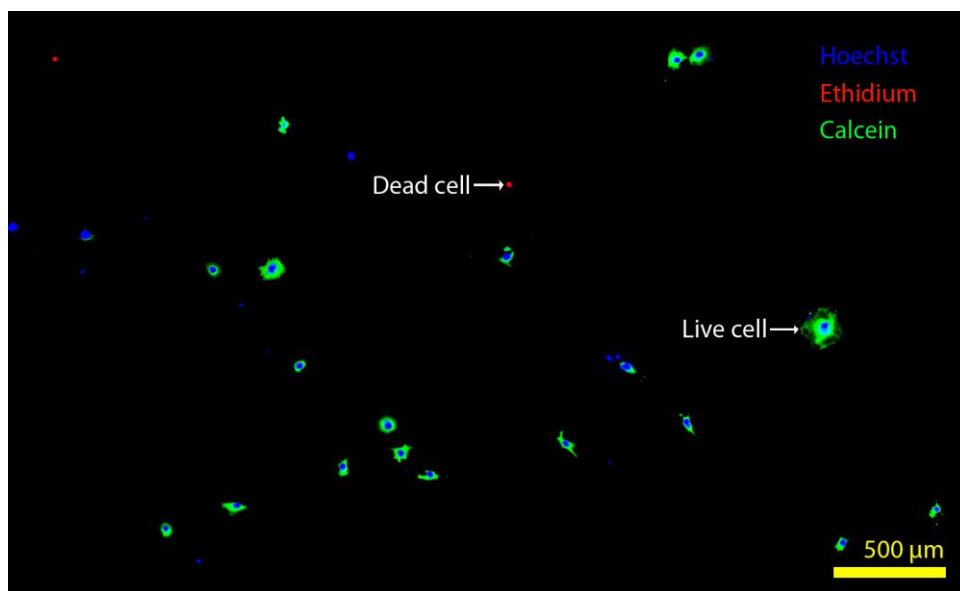


**Figure 1. Bioprinted Human Mesenchymal Stem Cells in 0.5% Sodium Alginate in simple geometrical patterns in sterile pre-wet 6-well plate (A) or dry 6-well plate (B).** 100 nL droplets of hMSC suspension in 0.5% Alginate were dispensed using the CellJet to form continuous patterns in wells of a standard 6-well plate. Pre-wet plate (A) - The bottom of each well was covered with 0.02 M  $\text{CaCl}_2$  and then oven-dried before printing. Dry Plate (B) - No pre-wetting with  $\text{CaCl}_2$  was done prior to printing. The same print program was run for both groups. Top row: First two wells - vertical parallel lines, 3<sup>rd</sup> well - horizontal parallel lines. Bottom row (left to right) - Square with diagonals, "WPI", concentric circles of 4, 8 and 12 mm radii. The sharpness of margins and adherence to programmed pattern is visibly better in patterns bioprinted on pre-wet plates (A) compared to the same printed on dry plates (B). Image taken immediately after all wells were treated with sterile mist of 0.02M  $\text{CaCl}_2$  for 2 minutes.

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Passage 6-8 Human Mesenchymal Stem Cells were suspended in 0.5% (w/v) Sodium Alginate prepared in Mesenchymal Stem Cell Growth Medium (MSCGM) (Lonza), to yield a suspension with a cell concentration of  $1 \times 10^5$  cells/mL. Using the CellJet, the cell suspension in hydrogel was dispensed as 100nL droplets into sterile 6-well plates that were either pre-wet with 0.02 M Calcium Chloride solution or not. The pre-wet plates were oven dried before printing. A combination of drop-by-drop and line-dispense modes were used to generate simple geometrical patterns. The same print program was run in both groups. Immediately after the print run, all plates were treated for 2 minutes with a mist of sterile 0.02 M Calcium Chloride solution to cross-link the Alginate. After bioprinting, 2 mL of MSCGM was added gently from the side of each well, taking care not to disturb the pattern.

The CellJet was programmed to bioprint the following geometrical patterns in each 6-well plate (Figure 1): Top row - vertical parallel lines (5 mm apart) in the first two wells, horizontal parallel lines 5 mm apart in the 3<sup>rd</sup> well. Bottom row (left to right) – A square with 10 mm sides with diagonals, the letters “WPI”, 3 concentric circles of radii 4, 8 and 12 mm. After 24 hours of incubation at 37°C, cells in all wells were stained with a Live-Dead stain (Calcein-AM, Ethidium Homodimer – Invitrogen) and Hoechst stain (Invitrogen) as per manufacturer recommended protocol. Cells were visualized with an inverted microscope in the fluorescent mode using red, green and blue filters. Quality of printed patterns and viability of hMSCs in both pre-wet plates and dry plates were compared.



**Figure 2. Staining for Viability:** Human Mesenchymal Stem cells in all wells were stained with Live-Dead stain (to determine cell viability) and Hoechst stain (to stain the nuclei). Blue (Hoechst dye) = Nuclei of live cells; Red = Nuclei of dead cells (Ethidium); Green = Cytoplasm of live cells (Calcein). Mean viability at 24 hrs in the pre-wet plates group was found to be  $94.90 \pm 2.5\%$  while that in the dry plates group was found to be  $93.47 \pm 3.3\%$ . Statistically, the difference was not significant.

Quality of bioprinted patterns in terms of adherence to programmed/desired print pattern and delineation of edges was visibly better in the patterns printed in 6-well plates pre-wet with 0.02 M  $\text{CaCl}_2$ . Viability of cells was found to be  $94.90 \pm 2.5\%$  in the pre-wet plates group ( $n = 5$ ), while that in the dry plates group ( $n = 5$ ) was found to be  $93.47 \pm 3.3\%$ . An unpaired Students' T-test revealed that the difference was not significant.

This work displays the ability of the CellJet not only to handle cells suspended in viscous media such as 0.5% Alginate (viscosity  $\approx 60$  centipoise) while preserving high cell viability but also to dispense in specified geometrical patterns. It is left to the resourcefulness of the researcher to apply this versatile method for various ends – developing customized 3D cell culture environments, impregnating scaffolds with one or more cell-types and growth factors, generating cell-laden constructs (scaffolds or tissue constructs) de novo, laying down cells on bio-chips, generating customized live-cell arrays, etc.

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## GENERAL PROTOCOL:

1. Write the print program on the AxSys system of the CellJet to generate desired print patterns in wells of a 6-well plate. Test the program with both water and 0.5% Sodium Alginate until satisfactory printing results are achieved. BE SURE TO SAVE THE PROGRAM for later retrieval.
2. Disinfect the humidifier with 10% bleach solution or equivalent, followed by 3 washes of Phosphate Buffered Saline (PBS) in preparation for use with sterile 0.02 M CaCl<sub>2</sub>. The humidifier is used to form a mist of CaCl<sub>2</sub> for cross-linking Alginate after printing.
3. Prepare 0.75 % w/v solution of cell culture grade Sodium Alginate (Sigma-Aldrich) in Mesenchymal Stem Cell Growth Medium (or other cell growth medium according to cell type). Usually, overnight stirring is required to allow the salt to dissolve completely.
4. Prepare 0.02 M solution of Calcium Chloride (Sigma Aldrich) in de-ionized water and bring the pH to 7.
5. Sterilize both the solutions by autoclaving.
6. Prepare sterile 6-well plates for bioprinting: Add 2 mL of 0.02 M sterile Calcium Chloride to each well to cover the bottom with a thin layer. Cover each plate with a sterile, fresh HEPA filter and place the plates in an oven at 60°C for 4 hours to allow all water to evaporate. Inside a laminar flow hood, remove the HEPA filter covers, and replace the lids on all plates.
7. Culture cells in tissue culture flasks until they reach approximately 80-90% confluence and prepare a suspension of cells in MSCGM. Passage 6-8 Human Mesenchymal Stem Cells at 80-90% confluence were used for this work.
8. After cell counting, calculate the volume of cell suspension and 0.75 % Sodium Alginate needed to yield a cell suspension with a concentration of 1x10<sup>5</sup> cells/mL of 0.5% Alginate.
9. Just before the actual printing process, disinfect the CellJet system by cycling 70% Ethanol or equivalent through the entire tubing system, followed by washing with sterile Phosphate Buffered Saline. Also disinfect the deck, wash, waste and vacuum stations by spraying with alcohol followed by dry wiping.
10. Mount the prepared 6-well-plates onto the deck of the CellJet cell printer. Fill the source well with the prepared suspension of human Mesenchymal Stem Cells in 0.5% Alginate.
11. Run the program to bioprint the hMSCs suspended in 0.5% Alginate in specified patterns.
12. Treat all wells of the plate with a sterile mist of CaCl<sub>2</sub> for at least 2 minutes to cross-link Alginate.
13. Fill all wells with 2 mL of MSCGM by pipetting gently along the side of the well, taking care not to disturb the pattern.
14. Incubate at 37°C, under 5% CO<sub>2</sub> for 24 hours. Change cell media every alternate day, or as frequently as required.
15. Analyze printed cells as desired. Viability and patterning precision were examined in this study.

## REFERENCES

1. Pittenger M. F. *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* 284, 143–147. 1999.
2. Kavalkovich K.W. *et al.* Chondrogenic differentiation potential of human mesenchymal stem cells within an Alginate layer culture system. *In vitro Cellular & Developmental Biology – Animal*, 38(8): 457-466. 2002.

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