Layer-by-Layer Printing of Hep3B Cells in Hydrogels for Cancer Cell Migration Assays

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Abstract

Introduction

- Rapid assessment of the invasion potential of various cancers in three-dimensional (3D) cell culture via layer-by-layer printing of cells encapsulated in hydrogels has been studied.
- Microarray bioprinting technology on a microwell chip has been explored to create 3D cancer-like tissue structures and study cancer cell migration.
- Alginate, a negatively charged biopolymer, forms a hydrogel via ionic crosslinking. Oxy-methacrylated alginate (OMA) is polymerized via near-ultraviolet light in the presence of a photoinitiator.

Goal

- To demonstrate rapid creation of cancer tissue-like structure via microarray 3D-bioprinting and develop a high-throughput, 3D cancer cell migration assay.

Methods

- Layer-by-layer cell culture conditions were optimized in OMA by varying exposure time, photoinitiator concentration, and OMA concentration
- Cells were cultures for three days to test viability.
- 3D cancer cell migration was demonstrated by printing two layers of hydrogels into the microwells: the bottom layer with a mixture of alginate and matrigel, and the top layer with Hep3B cells in alginate .
- Printed cells were cultured for 14 days to investigate cell migration in 3D.

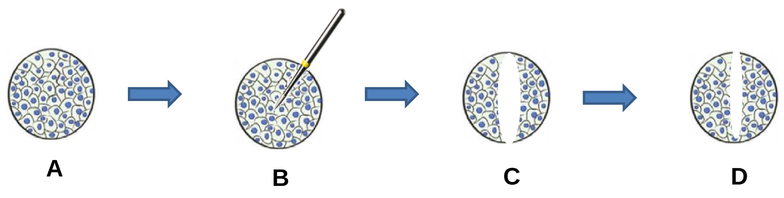
Conclusions

- Migration in OMA was moderately successful.

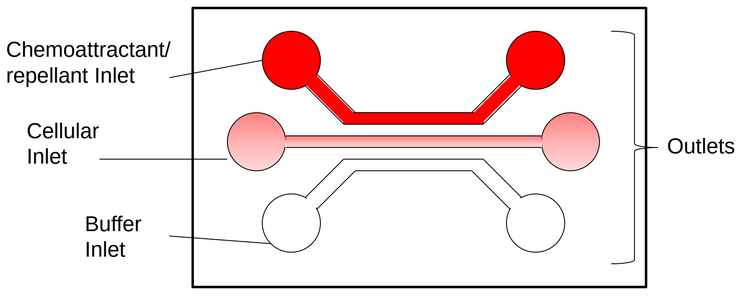
Future direction

- Determine bidirectional migration potential and extend time of migration assays.
- Use growth factors to induce migration in OMA-45

Introduction: Traditional Cell Migration Assays

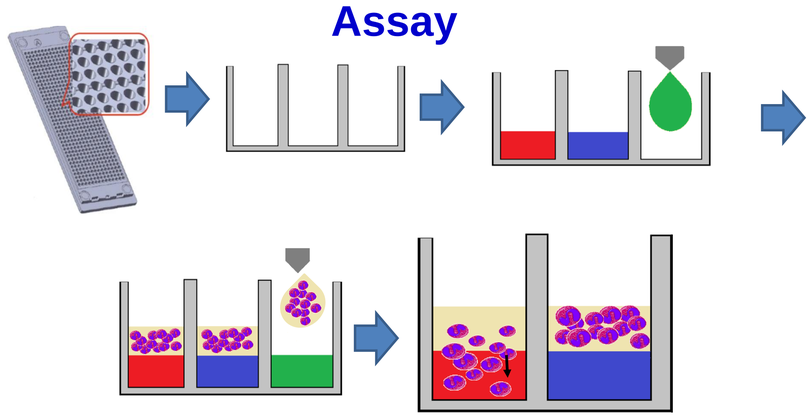


Wound Healing Assay: (A) Cells are cultured in well plates. (B) After the monolayer is obtained, a wound is created in the monolayer of cells. (C) Size of the wound being measured with time (D) Cells closing the wound.



Chemotaxis Assay on a Microfluidic Device: Cells respond to the presence of a chemical by migrating towards or away from it. https://biofluidics.bee.cornell.edu/device.html

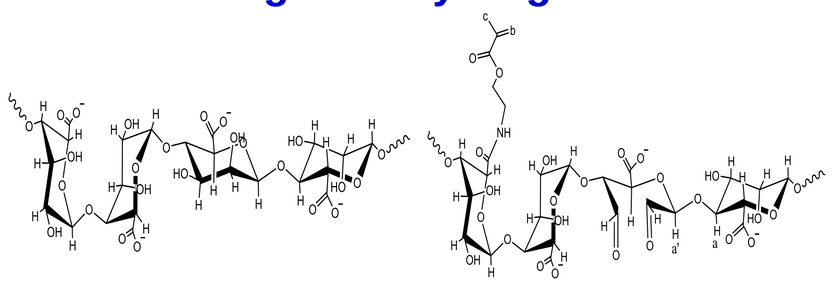
Our Cancer Cell Migration Assay



3D cell migration assays

As an alternative to 2D cell migration assays, 3D tumor spheroids in which cells encapsulated in alginate are printed in layers on microwell chip platforms can lead to more *in-vivo* like cell migration studies and more predictive behavior of cancer cells.

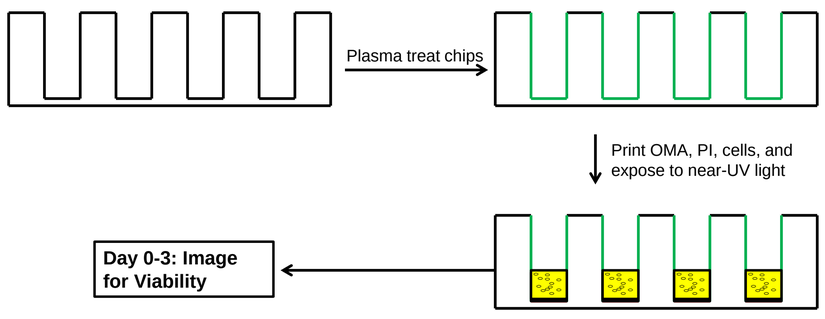
Alginate Hydrogel



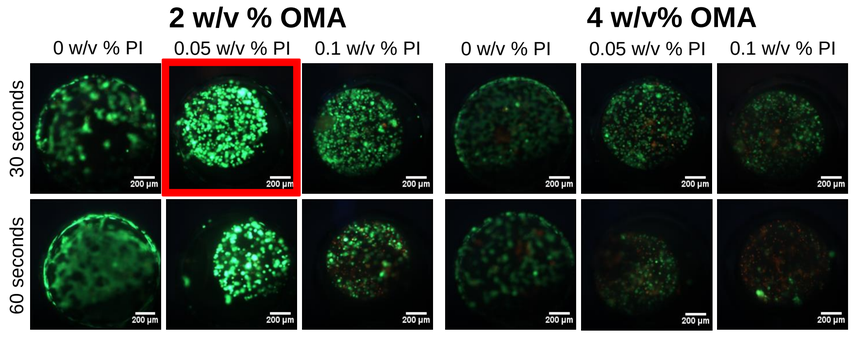
Calcium- crosslinked alginate | **Oxy-methacrylated alginate (OMA)**

- Alginate normally forms a hydrogel in the presence of divalent cations (e.g., Ca^{2+}). OMA forms a hydrogel with photoinitiator (PI) generating free radicals under UV that initiate the polymerization process. **Reference: Jeon, et al., *Biomaterials* 33, 3503-3514 (2012)**

Optimizing conditions for cells cultured in OMA

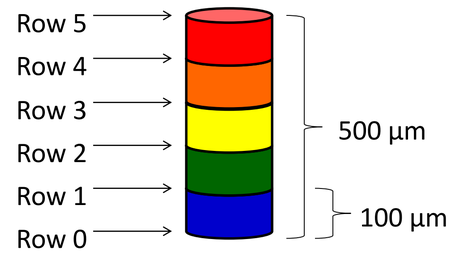


Microwells were printed with one droplet of 640 nL of Hep3B cells at a seeding density of 4 x 10^6 cells/mL while encapsulated in a mixture of 45% methacrylated OMA and Irgacure2959, our photoinitiator (PI). Alginate and PI concentration were varied during the initial print. Right after printing, microwells were placed under near-ultraviolet light (365 nm) exposure for 2 and 4 minutes. After three days of culturing, images were obtained using a multiband filter of the chip scanner.



Images of Hep3B cells encapsulated in 45% OMA exposed at ~4 mW/cm^2. Images were obtained at 300 µm above the bottom of the microwell.

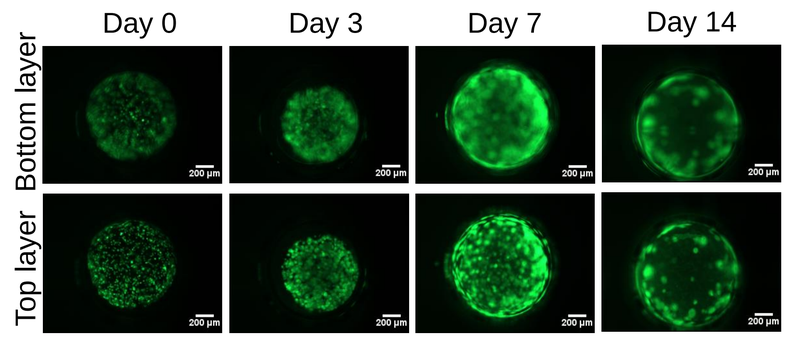
Imaging for Cell Migration Assays



Cells were imaged at various z-positions after staining with fluorescent dyes.

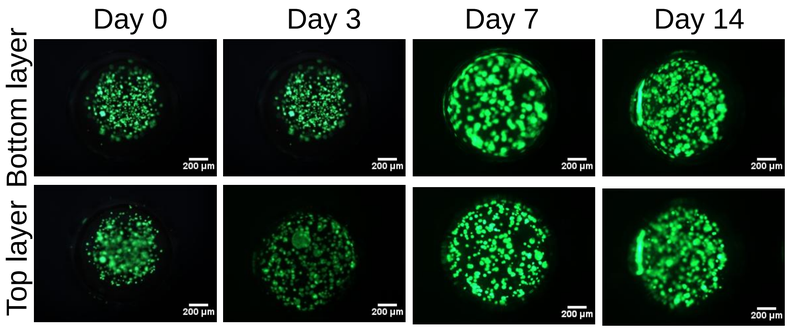
Migration of cells towards Matrigel

Calcium- Crosslinked Alginate



For calcium-crosslinked alginate, microwells were printed with 320 nL of $CaCl_2$, followed by drying and printing the first layer of 320 nL of 0.75 w/v % alginate and Matrigel (0 to 2 mg/mL) mixture. After 15 minutes of incubation, a second layer of 320 nL of Hep3B cells (6 x 10^6 cells/mL) encapsulated in 0.75% alginate was printed. Cell layers were cultured for 14 days to investigate for cell migration in 3D. Images above indicate migrate in response to a 1.5 mg/mL Matrigel bottom layer.

OMA



For OMA, 320 nL of 2 w/v % alginate, 0.05 w/v % PI and Matrigel (0 to 2 mg/mL) mixture were printed in the first layer. Microwells were exposed to 2min 365 nm, then incubated at 37^{o}C. After 15 minutes of incubation, a second layer of 320 nL of Hep3B cells (4 x 10^6 cells/mL) encapsulated in 2 w/v % OMA was printed, along with 0.05 w/v % PI. Cell layers were cultured for 14 days to investigate for cell migration in 3D. Images above indicate migrate in response to a 1.5 mg/mL Matrigel bottom layer.

Conclusions

- **Viable cells are possible with OMA-45.**
- **No Hep3B cell migration was observed within 1 week, but some was observed up to 2 weeks out.**
- **Migration needs to be conducted out for longer periods of time (e.g., 4 weeks).**
- **Matrigel may be leaching from the bottom layer**
- **Better chemoattractants (e.g., growth factors) are necessary to simulate cancer cell migration in 3D.**

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