Developing Microfluidic Devices for Assisted Reproductive Technologies

Darien Nguyen

2019 CNF REU Intern

Intern Affiliation: Chemistry, Winthrop University

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CNF REU Principal Investigator: Alireza Abbaspourrad, Food Science and Technology, Cornell University

CNF REU Mentor: Amir Mokhtare, Food Science and Technology, Cornell University

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Contact: nguyend4@winthrop.edu, alireza@cornell.edu, am2964@cornell.edu

Website: http://cnf.cornell.edu/education/reu/2019

Primary CNF Tools Used: ABM contact aligner, Class II resist spinners (SU-8), plasma cleaner/PDMS casting room

Abstract:

The gaining popularity of Assisted Reproductive Technologies (ART) such as *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) calls for the introduction of more affordable and less tedious processes rather than the typical manual operations. In order for ICSI to occur, the Cumulus Oocyte Complexes (COCs) retrieved from the ovaries must be processed in order to remove the tightly-packed cumulus cells surrounding them. As of yet, this tedious and unstandardized process is being done manually by skilled embryologists, which result in variability and unavailability. The focus of this project is to develop microfluidic devices to denude the COCs for ICSI in order to reduce the tyranny of manual operations and push towards automated reproducible operations. However, many microfluidic devices are fabricated through conventional PDMS microfluidic processes which can be potentially harmful and result in loss of the precious oocytes. Herein, we report the fabrication and testing of a non-PDMS and reversibly-bonded microfluidic device using artificial eggs similar to COCs.

Summary of Research:

In order to reduce the tyranny of the current tedious, manual processes in Assisted Reproductive Technologies (ART), microfluidic devices emerge as a plausible solution. Microfluidic devices have been recently used in a few parts of the ART procedure such as sperm selection, insemination, etc. However, microfluidics has rarely been applied to improve the processing of oocytes for ICSI, which calls for the removal of the tightly-packed cumulus cells surrounding the oocyte. This research aims at developing microfluidic devices to denude cumulus cells from oocytes to not only reduce manual operations but also to increase the standardization within the ART process.

First of all, we used AutoCAD 2019 to prepare a variety of designs for the denudation of the COCs. They range from a simple single inlet/outlet port in which jagged walls intend to denude the cumulus cells off the COCs to more complex designs intending to vacuum the cumulus cells off the COCs. These designs were constructed for bovine COCs, which have a size of 400-500 μm and an oocyte size of about 150-200 μm.

We employed photolithography fabrication methods for making PDMS microfluidic devices. SU-8 molds were developed on a silicon wafer and coated with parylene to act as a mold release. PDMS was then casted on the wafer and slowly peeled from the wafer to ensure channels remain intact. The PDMS channels were then punched for the inlet and outlet ports and bonded to glass slides using a plasma oxygen bonding method.

In order to test these PDMS microfluidic devices, we developed an automated system consisting of a microcontroller and a set of solenoids that are connected to the computer and controlled through a GUI (Figure 1). Different programs were coded for the microcontroller for each desired design. Porous, partially-crosslinked polymeric beads, dispersed in water, were used to simulate COCs and were successfully run through the channels in the PDMS microfluidic devices. However, many challenges were revealed along with this individual success.

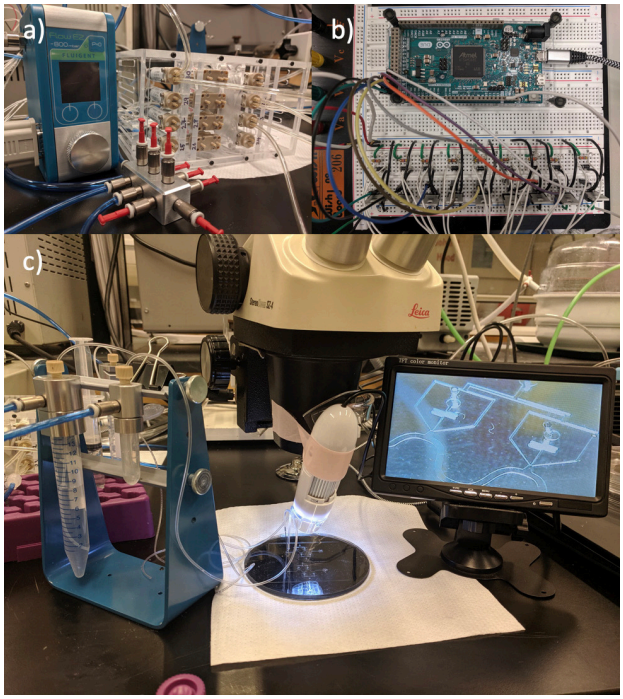

Figure 1: Equipment set-up for testing microfluidic devices. Figure 1a shows the magnetic valves and also the Fluigent pump. Figure 1b shows the Arduino microcontroller controlling the magnetic valves. Figure 1c shows the observation deck for the microfluidic devices.

The particles tended to stick together when traveling from the transferring tube to the microfluidic device, causing them to clog the channels. Therefore, designs were modified to a two-inlet port design where one inlet was used to only flow the media, while the other flows the particles. Although this reduced the number of clog incidents, it did not fully resolve the problem. A new microfluidic device (Figure 2) was fabricated on acrylic using the CNC Milling Machine, in order to not only prevent COCs from sticking together, but also expose them to hyaluronidase, a chemical used in oocyte processing to break down the cumulus cells.

PDMS itself is known to be deformable, allow absorption, and permit leaching, which could be harmful to cells; therefore, it potentially has a very low chance to be applied in the real and large scale. In addition, the conventional PDMS microfluidic device relies on permanent bonding to glass, which could result in damages or wasting an egg if the process were to go wrong. A new design requiring two layers of SU-8 was proposed that would apply a vacuum in order to seal the channels to a substrate that could be reversed if needed. The masks were also reverse polarized so that the channels would be etched into SU-8. The SU-8 channels were enclosed using a flat slab of PDMS and a vacuum was applied to fully enclose the channels (Figure 3).

Finally, the polymeric beads did not accurately simulate eggs due to their hydrophilic properties and the lack of an outer layer that could be removed to simulate the cumulus cells. A microfluidic device was created in order to create artificial eggs from hydrogel (Figure 4). A solid particle, acting as the oocyte, would be encapsulated in loosely-bonded hydrogel, acting as the cumulus cells.

Results, Conclusions, and Future Work:

A working, reversibly-bonded, non-PDMS microfluidic device was fabricated in order to denude COCs. Artificial eggs were produced from hydrogel through another designed microfluidic device. In the future, the non-PDMS microfluidic device would be tested using actual bovine COCs to determine the effectiveness of the denudation and physical stress towards the oocyte. This would be compared to the current physical processes of denudation and be determined to be advantageous or not. In addition, further development on making the microfluidic device more automated would be in action.

Acknowledgements:

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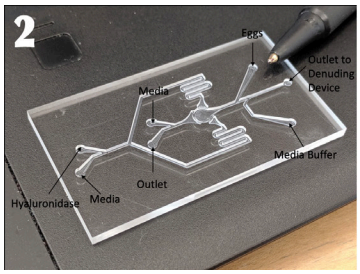
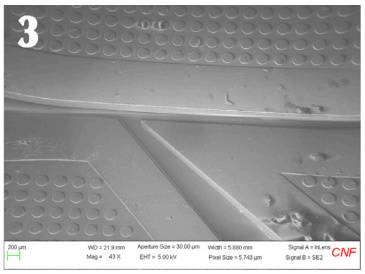
References:

[1] L. Weng, G. Y. Lee, J. Liu, R. Kapur, T. Toth, and M. Toner, Lab Chip, 2018, 18, 3892.





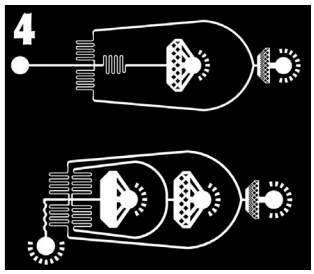


Figure 2, left: *Acrylic microfluidic device aimed at exposing and separating the COCs.* ***Figure 3, middle:*** *SEM image of the SU-8 reversible vacuum design.* ***Figure 4, right:*** *Mask designs for microfluidic hydrogel particle maker.*