An oviduct-on-a-chip microfluidic platform for the investigation of the motility of ciliated cells after cancer treatment

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Introduction

Ovarian cancer is the most lethal type of cancer in women as it is detected, in most cases, at late stages. Chemotherapy to treat ovarian cancer is the most common procedure after surgery. After ovarian cancer treatment, fertility can be affected dramatically in women, for example alkylating-agent-based treatments with Cisplatin has resulted in amenorrhea in 18%–61% of young women and 61%–97% in older women[1]. Ovaries and surrounding organs, such as the fallopian tubes or oviducts can be affected by chemotherapeutic agents. Oviducts are tubular shape organs where fertilization takes place, the oviduct epithelium is mainly composed of secretory and ciliated cells. The ciliated cells are important during the fertilization process creating a sperm reservoir and promoting gamete interaction. Due to the impact of cancer treatments in those organs, new fields such as oncofertility have emerged in order to provide patients with options for the fertility preservation. Here we present a microfluidic platform to evaluate the cell response in the oviduct upon the exposure to chemotherapeutic drugs and, therefore, to investigate the influence on fertility post-treatment.

Methods

Design and fabrication of the microfluidic platform
- Computer aided design (CAD)
- Soft lithography

PDMS substrate

Micropatterned mold

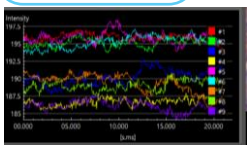
Modification of the polycarbonate (PC) membrane through different methods:
- Plasma activation
- APTES
- Corona discharge

Contact angle measurement and SEM

Cell isolation from bovine oviducts

Seeding of bovine oviduct epithelial cells into the microfluidic platform:
- confluent monolayer
- air-liquid interface

Cilia beating measurement using optical density with an inverted microscope



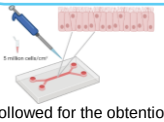


Figure 1.Diagram of the different steps followed for the obtention of the oviduct-on-chip platform.

Results

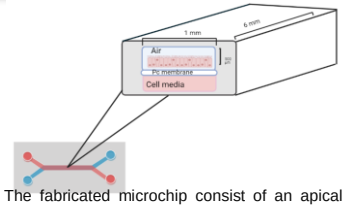
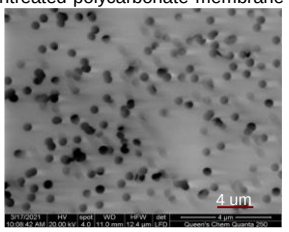


Figure 2. The fabricated microchip consist of an apical and a basolateral compartment separated by a transparent polycarbonate membrane to establish an air-liquid interface

Sample	Contact angle (°)
Unmodified polycarbonate membrane	59.85± 2.3
Plasma activated polycarbonate membrane	43.17 ± 4.9
APTES-modified polycarbonate membrane	93.15± 2.9
Corona discharged polycarbonate membrane	31.53± 2.2

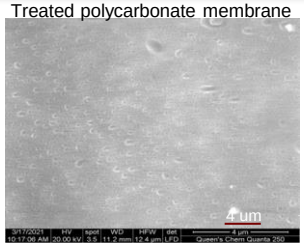
Table 1. The polycarbonate membranes were treated with different methods to promote its bonding with the PDMS layers. The contact angle of water was used to measure the surface free energy of the PC membranes. The static contact angle of PC was shown increase from 59° to 93° with APTES functionalization. Higher decreases were observed with 59° to 43° with plasma and a 59° to 31° with corona discharge

Untreated polycarbonate membrane



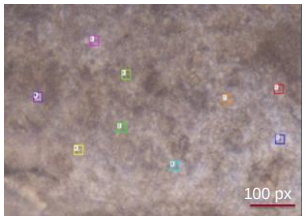
Average pore size= 0.45um±0.061

Treated polycarbonate membrane



Average pore size= 0.27um±0.091

Figure 3. Treatment of the polycarbonate membrane with plasma activation + heating at 100°C induced transparency and decreased the pore size from 0.45 um to 0.27 um.



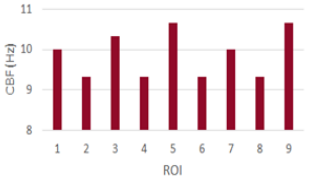


Figure 4. Cilia beating frequencies were measured from regions of interest (ROI) in a confluent cell monolayer inside the chip. The analyzed areas (20x20 pixels) were selected where cilia beating was visible.

Discussion

The designed microchip has allowed cell attachment using a plasma activated polycarbonate membrane without having leakage of cell media. The obtention of a transparent membrane allows for optical access to the cell monolayer compared to the translucent unmodified polycarbonate membrane. The cells have formed a confluent monolayer after one month maintaining the cilia beating (10 Hz average) showing that the platform provides with the suitable conditions to maintain its differentiated state. The obtained oviduct on chip platform may provide insight into the changes of the cilia beating frequency after the exposure to a chemotherapeutic agent and the consequences that this type of treatment can have in the fertility capacity of women.

References

1.Ronn, R., & Holzer, H. E. (2013). Oncofertility in Canada: the impact of cancer on fertility. Current oncology (Toronto, Ont.), 20(4), e338–e344. https://doi.org/10.3747/co.20.1358

Acknowledgments

Natural Sciences and Engineering Research Council of Canada (NSERC) RGPIN-2019-04292, Canada Foundation for Innovation John R. Evans Leaders' Fund Program (No. 319670), Excellence in Research Award (Queen's University) and Early Researcher Award (Government of Ontario). CONACYT scholarship