HUMAN UTERINE CERVIX-ON-A-CHIP:
ESTABLISHING THE FIRST 3D/DYNAMIC IN VITRO MODEL TO STUDY THE DEVELOPMENT OF CERVICAL CARCINOMA AND HUMAN PAPILOMA VIRUS MECHANISM OF ACTION

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OBJECTIVE

To develop a microfluidic «uterine cervix-on-a-chip» platform that let the cultured cells to take characteristic positions similar to those observed in native human uterine cervix. This platform can also help to study the unknown transformation zone of cervix, which is the target zone of Human Papilloma virus (HPV).

METHODS

A microfluidic device prepared by demolding cured polidimetilsiloxano (PDMS). On the chip, we carried out cell culture and co-culture of ectocervical epithelial cells (cells type: Ect1/E6E7) and endocervical epithelial cells (cells type: End1/E6E7) cell lines. Endocervix epithelial cell has been marked by green fluorescent protein (GFP) to provide a convenient way to measure transduction efficiency into endocervix cells via fluorescence and to differentiate them from ectocervix epithelial cells. Numerous experiments have been conducted to check the functionality of the chip, such as live/dead assay, prestoBlue cell viability, 2D migration of cells (scratch tests) and 3D migration of cells by the use of 3D printers.

RESULTS

We have found that both cell lines can grow from both sides of the chip to reach each other in order to get a transformation zone and prepare the squamo-columnar junction. This multilayer-cell junction contains both types of epithelial cells and can mimic conveniently the transformation zone of cervix.

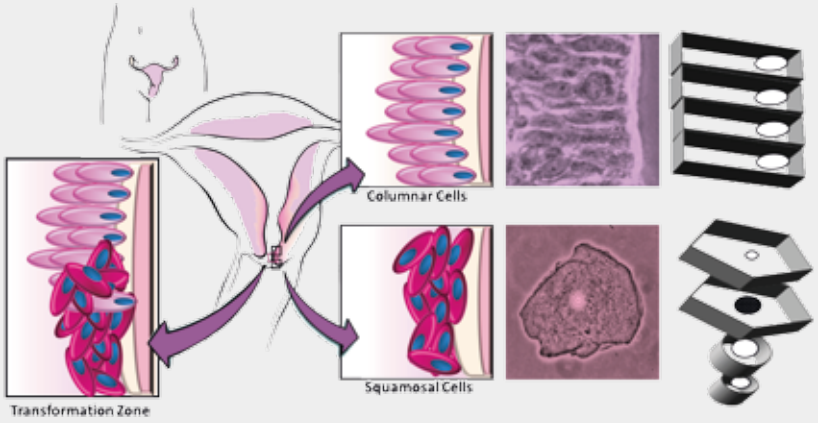

Figure 1. Formation of the epithelial cells at the transformation zone of the cervix

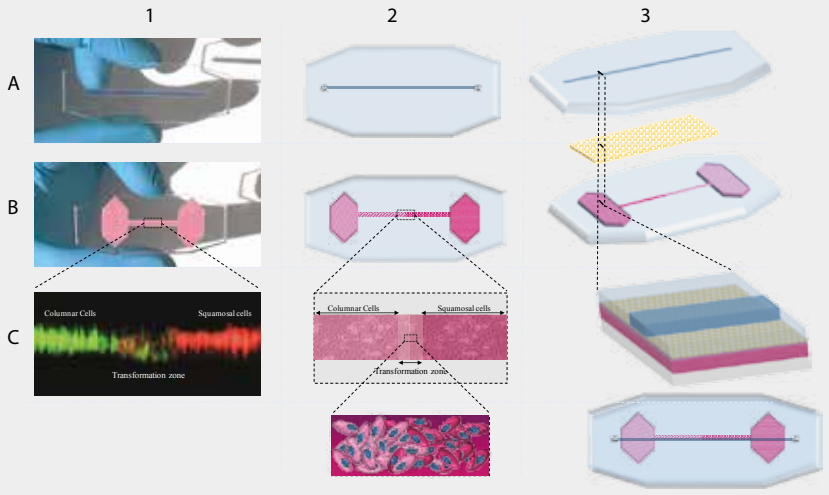

Figure 2. Illustration showing the distribution of epithelia of the ectocervix (dark pink) and endocervix cells (pale pink). (A) Upper layer of the chip (B) Lower layer of the chip (3) Two PDMS layers are aligned and irreversibly bonded to form two sets of parallel microchannels separated by a 22-Qm-thick polycarbonate membranes, containing an array of through-holes with an effective diameter of 22-Qm. Scale bar, 200 Qm. (C) Long-term microfluidic co-culture produces a tissue-tissue interface consisting of a single layer of the Columnar epithelium (stained with Cell Tracker Green) closely next to a monolayer of the Squamous epithelium (stained with Cell Tracker Red) (C1), both of which express intercellular junctional structures stained with antibodies to VE-cadherin. Scale bar, 50 Qm.

CONCLUSIONS

Uterine cervix-on-a-chip may provide a powerful alternative in vitro model for studies on uterine physiology, real-time, high-resolution imaging, and analysis of biological responses in the cervix, as well as drug development. This established uterine cervix-on-a-chip is simple, effective, and easy to operate. It is expected to have important applications in personalized treatment of HPV infection lesions and cervical cancer and to play a potential role in other clinical treatments and tissue engineering.

Khorsandi D, Gaslain Y. and Emsellem C. are Procare Health Iberia S.L. employees . Combalía J, Cortés J. and Palacios S. are consultants of Procare Health Iberia S.L. Rest of authors declare no conflict of interest.

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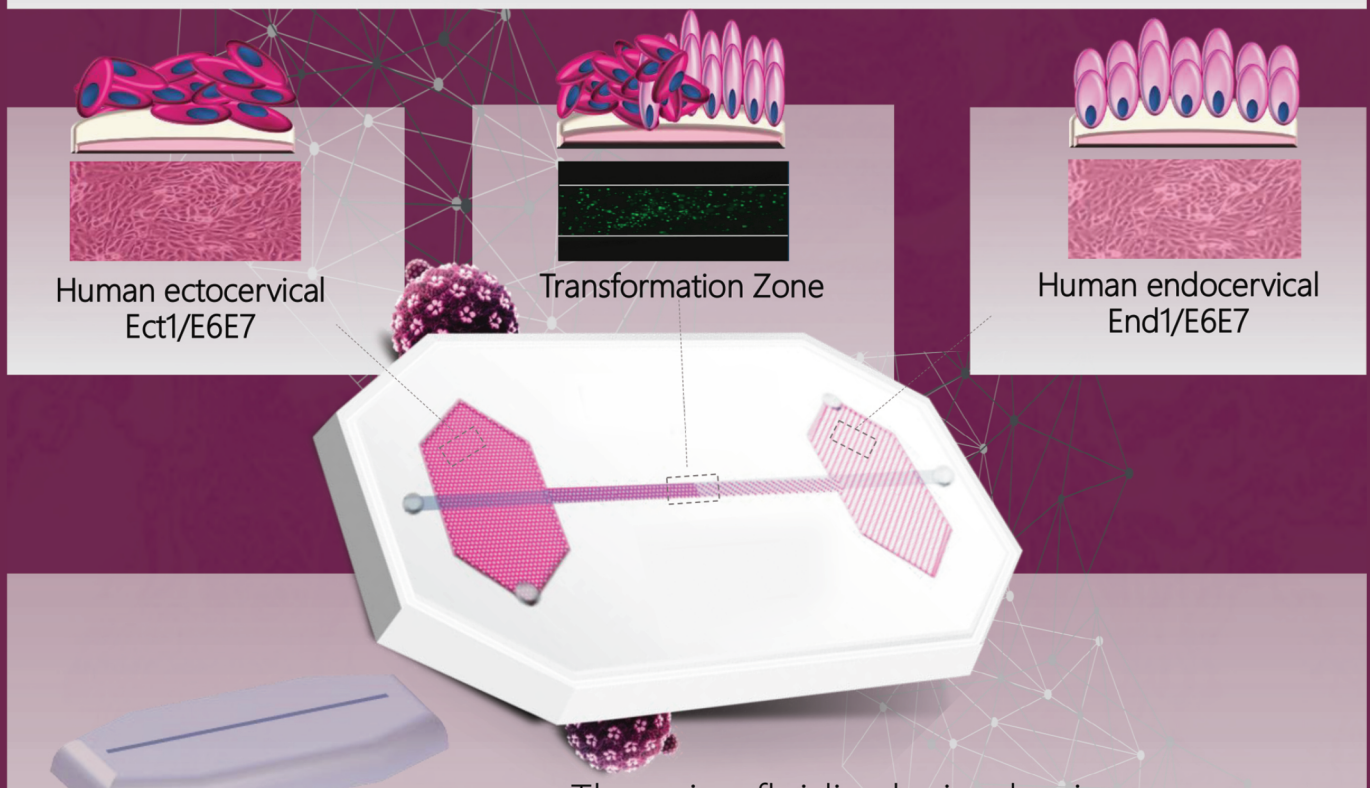
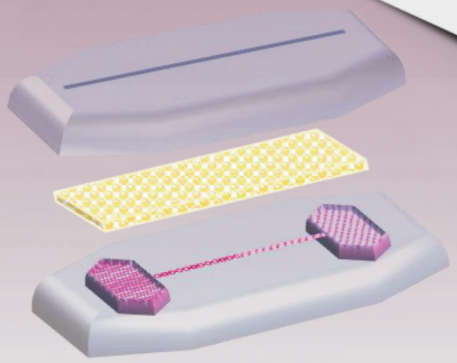
The cost of drug discovery is steadily increasing owing to the limited predictability of two-dimensional (2D) cell culture and animal models. The objective of this study was to develop a microfluidic 'uterine cervix-on-a-chip', a dynamic 3D platform that let the cultured cells mimic the native human uterine cervix histomorphology *in vitro* aiming to study the transformation zone of cervix during Human Papilloma virus (HPV) infection and cervical cancer development.





- The microfluidic device having an upper and a lower channel was prepared by demolding cured poly dimethyl siloxane (PDMS) . A 0.4 µm porous polycarbonate membrane divided these two layers.

Conclusion

Uterine cervix-on-a-chip may provide the first *in vitro* model for studies on cervix physiology, real-time and high-resolution imaging, and analysis of biological responses in the cervix, as well as drug development. This technology can allow both types of epithelial cells to grow on from both sides of the chip to reach each other and make the Squamo-columnar junction, which is the target zone of HPV.

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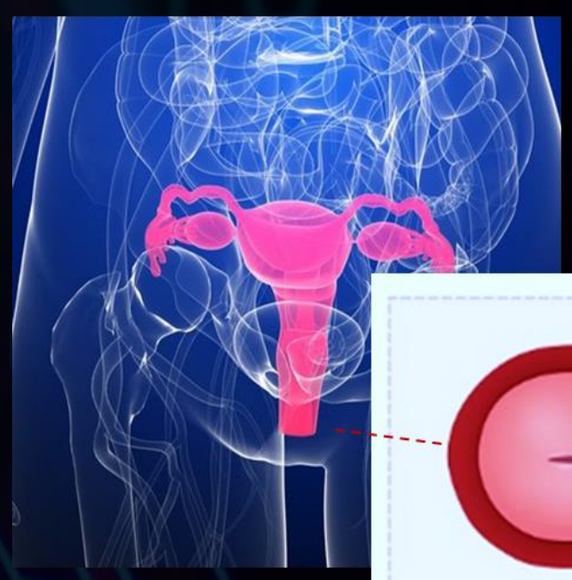
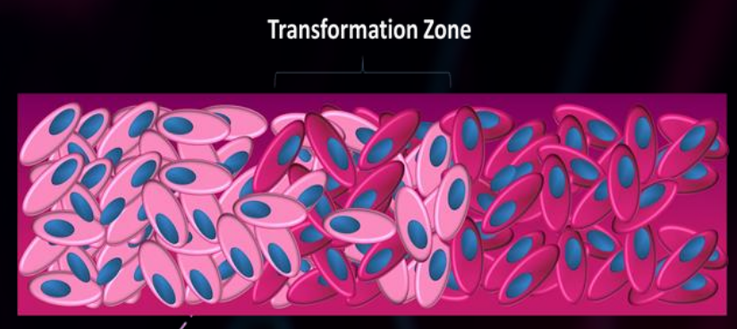
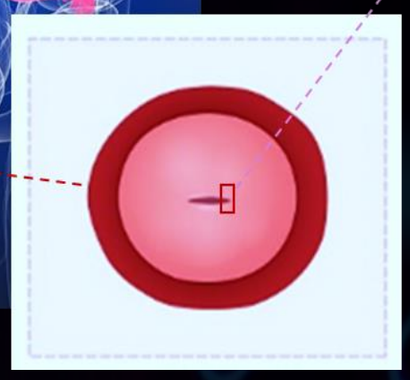
INTRODUCTION

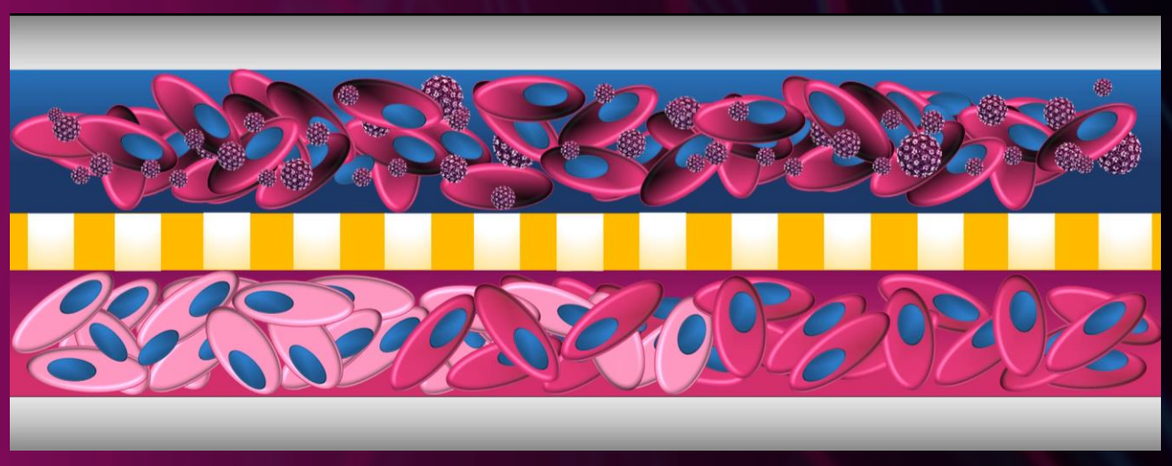
The cost of drug discovery is steadily increasing owing to the limited predictability of two-dimensional (2D) cell culture and animal models. The objective of this study was to develop a microfluidic 'uterine cervix-on-a-chip', a dynamic 3D platform that let the cultured cells mimic the native human uterine cervix histomorphology *in vitro* aiming to study the transformation zone of cervix during Human Papilloma virus (HPV) infection and cervical cancer development.





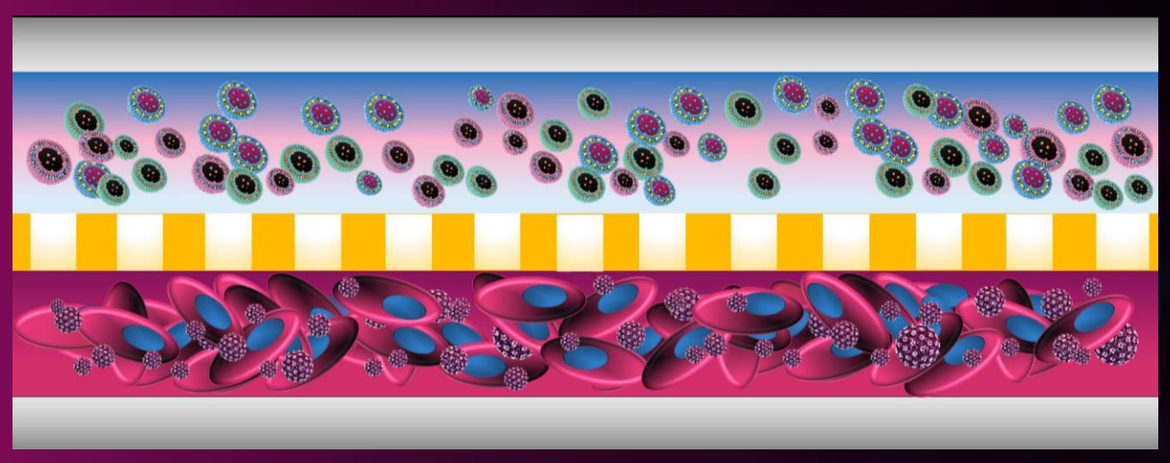


1. Culturing healthy epithelial cells in lower chamber of the chip.



2. Adding infected cell via upper chamber of the chip to mimic the infection and let the viruses go over the porous membrane.

3. By changing the upper layer, the infected model is ready to test new drugs and treatments.



4. By adding the new treatment/ drug compound to the system through upper chamber, we can detect the treatment's mechanism of action on the infected viruses real time.

CONCLUSION

Uterine cervix-on-a-chip may provide the first *in vitro* model for studies on cervix physiology, real-time and high-resolution imaging, and analysis of biological responses in the cervix, as well as drug development. This technology can allow both types of epithelial cells to grow on from both sides of the chip to reach each other and make the Squamo-columnar junction, which is the target zone of HPV.

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BACKGROUND AND OBJECTIVES

Predicting the effects of drugs before human clinical trials is at the heart of drug screening and discovery processes. The cost of drug discovery is steadily increasing owing to the limited predictability of two-dimensional (2D) cell culture and animal models. The conjunction of microfabrication and tissue engineering led to organ-on-a-chip technologies, which offer an alternative to conventional preclinical models for drug screening. The objective of this study was to develop a microfluidic 'uterine cervix-on-a-chip' a dynamic 3D platform that let the cultured cells mimic the native human uterine cervix histomorphology *in vitro*. This platform would be able to be used as an *in vitro* model to study the transformation zone of cervix during Human Papilloma virus (HPV) infection and cervical cancer development.

METHODS

A microfluidic device prepared by demolding cured polidimetilsiloxano (PDMS). On the chip, we carried out cell culture and co-culture of ectocervical epithelial cells (Ect1/E6E7) and endocervical epithelial cells (End1/E6E7) cell lines. Endocervix epithelial cell has been marked by AAV-GFP control viruses to provide a convenient way to measure transduction efficiency into endocervix cells via fluorescence and to differentiate them from ectocervix epithelial cells (Figure 1). Numbers of experiments have been designed to check the functionality of the chip, such as live/dead assay, prestoBlue cell viability, 2D migration of cells (scratch tests) (Figure 2) and 3D migration of cells by the use of 3D printers (Figure 3).

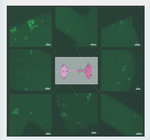

Figure 1- Growth of cells in different parts of the chip after 7 days

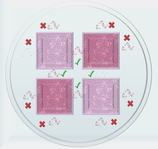

Figure 2- 2D cell migration (scratch test)

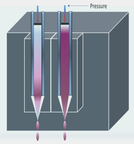

Figure 3- 3D cell culture and migration of cells

RESULTS

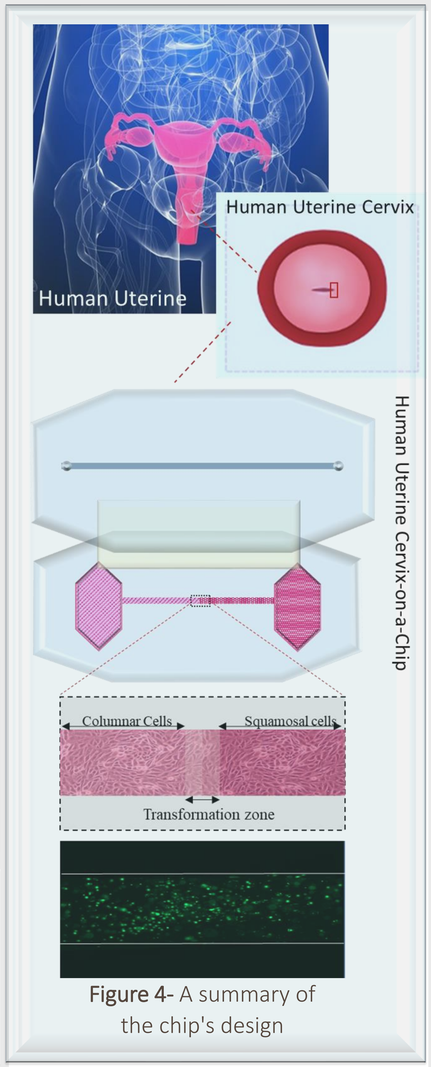

Figure 4- A summary of the chip's design

As a preliminary result, we have made a non-toxic, functional, In vitro model for human uterine cervix that allows both types of epithelial cells to grow from both sides of the chip to reach each other in order to make the squamo-columnar junction. This multilayer-cell junction can mimic the transformation zone of the cervix* (Figure 4C).

*This is an ongoing project and this presentation is just reporting the results of its first phase.

FUTURE PLAN

The next phase of the project is planed to mimic the infection via upper chamber and test the new treatments on the infected cells in order to check the cytokines changes and immune response of the cells and HPV mechanism of action (Figure 4D).

CONCLUSION

Uterine cervix-on-a-chip may provide a powerful alternative in vitro model for studies on cervix physiology, real-time, high-resolution imaging, and analysis of biological responses in the cervix, as well as drug development. This established uterine cervix-on-a-chip is simple, effective, and easy to operate. It is expected to have important applications in personalized treatment of HPV infection lesions and cervical cancer and to play a potential role in other clinical treatments and tissue engineering.

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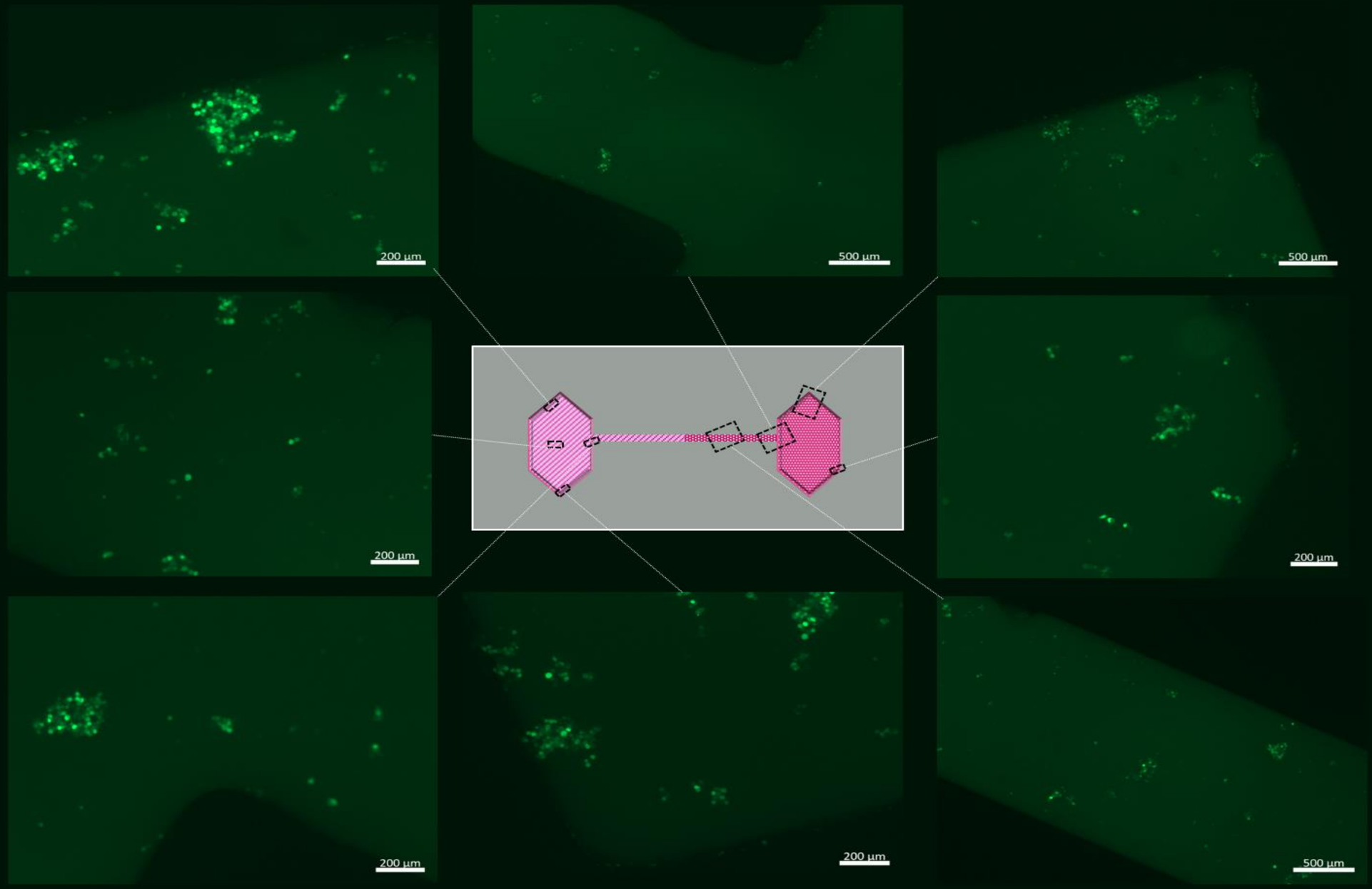
New Directions



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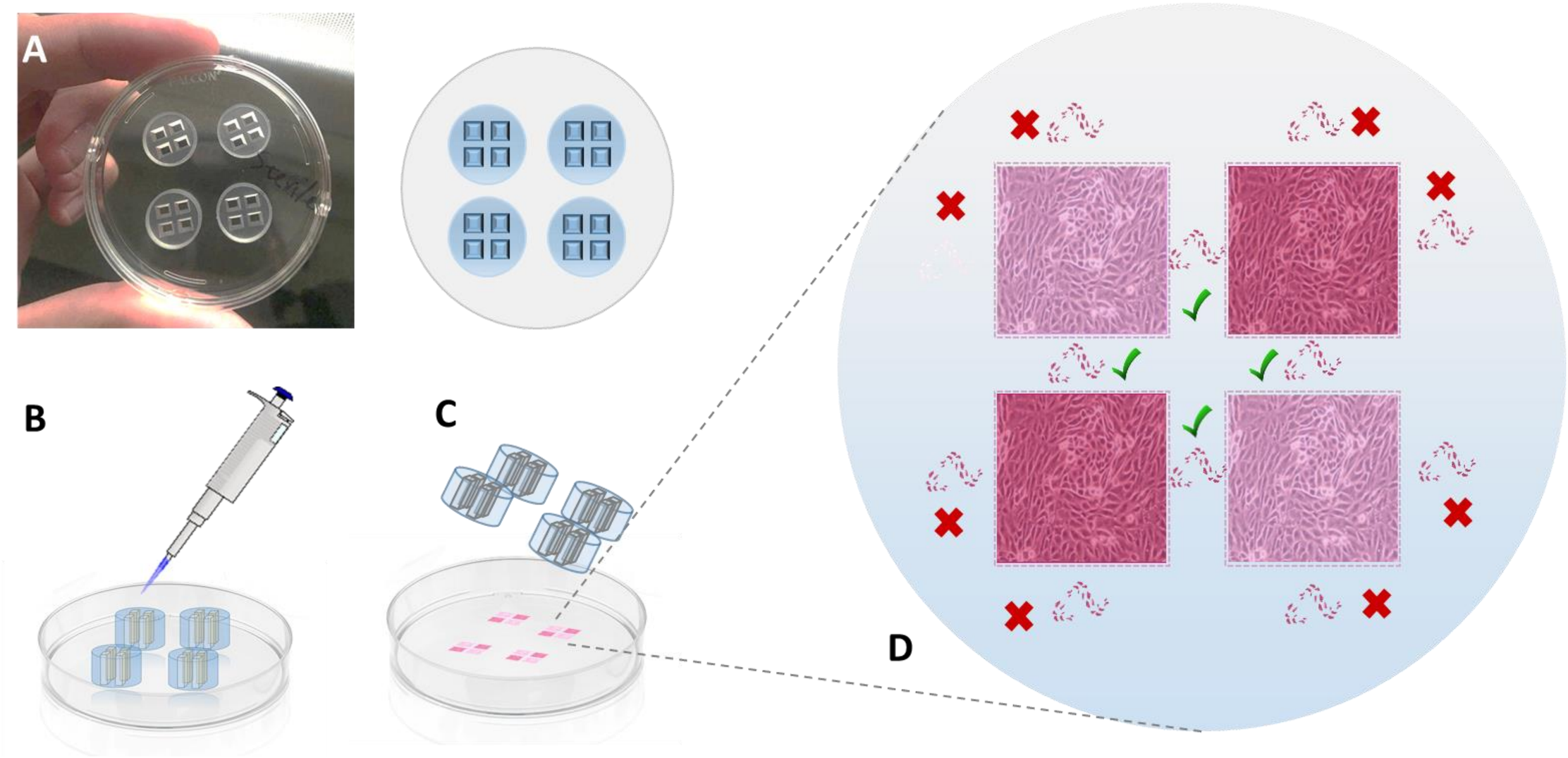

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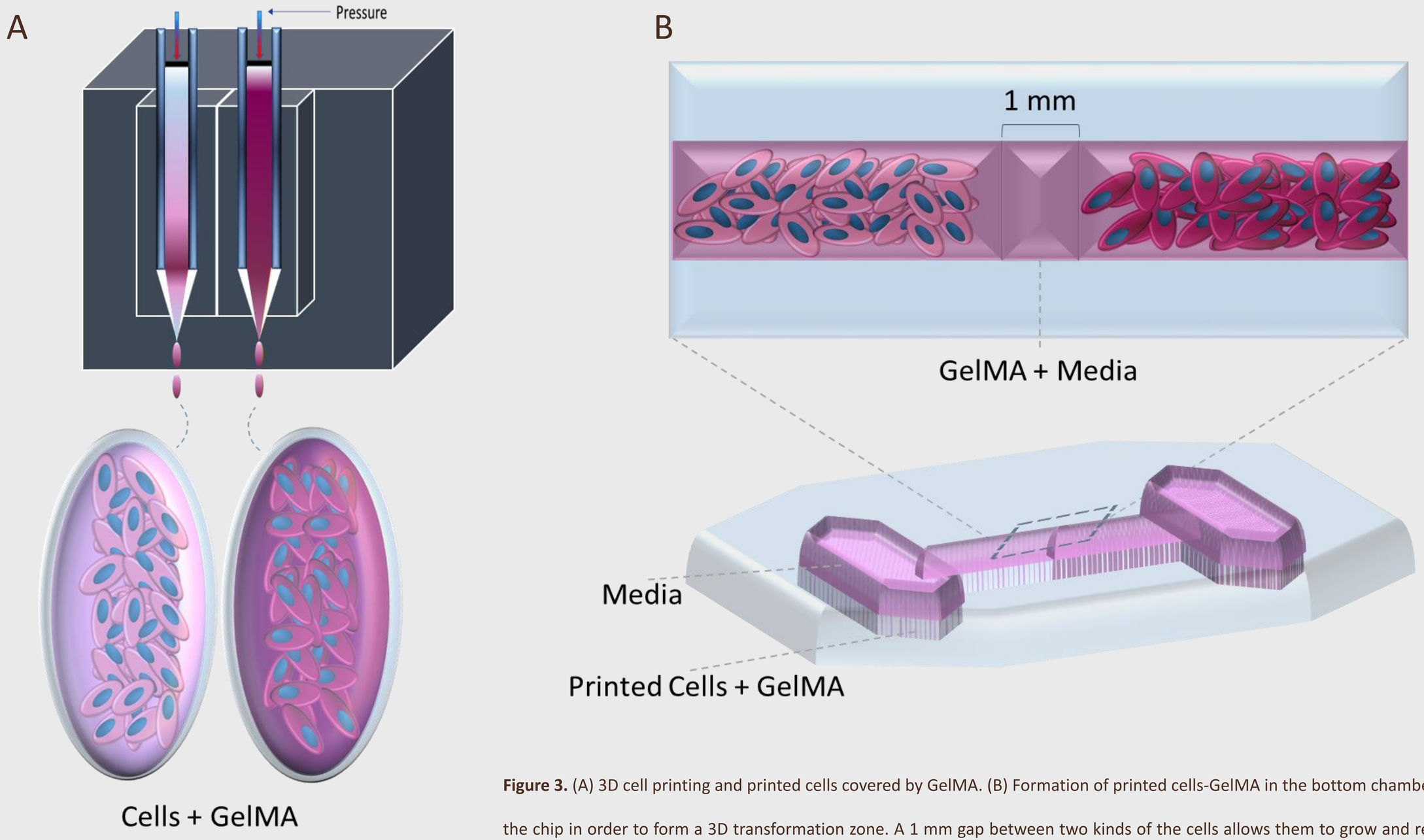


Figure 3. (A) 3D cell printing and printed cells covered by GelMA. (B) Formation of printed cells-GelMA in the bottom chamber of the chip in order to form a 3D transformation zone. A 1 mm gap between two kinds of the cells allows them to grow and reach each other to make the transformation zone.

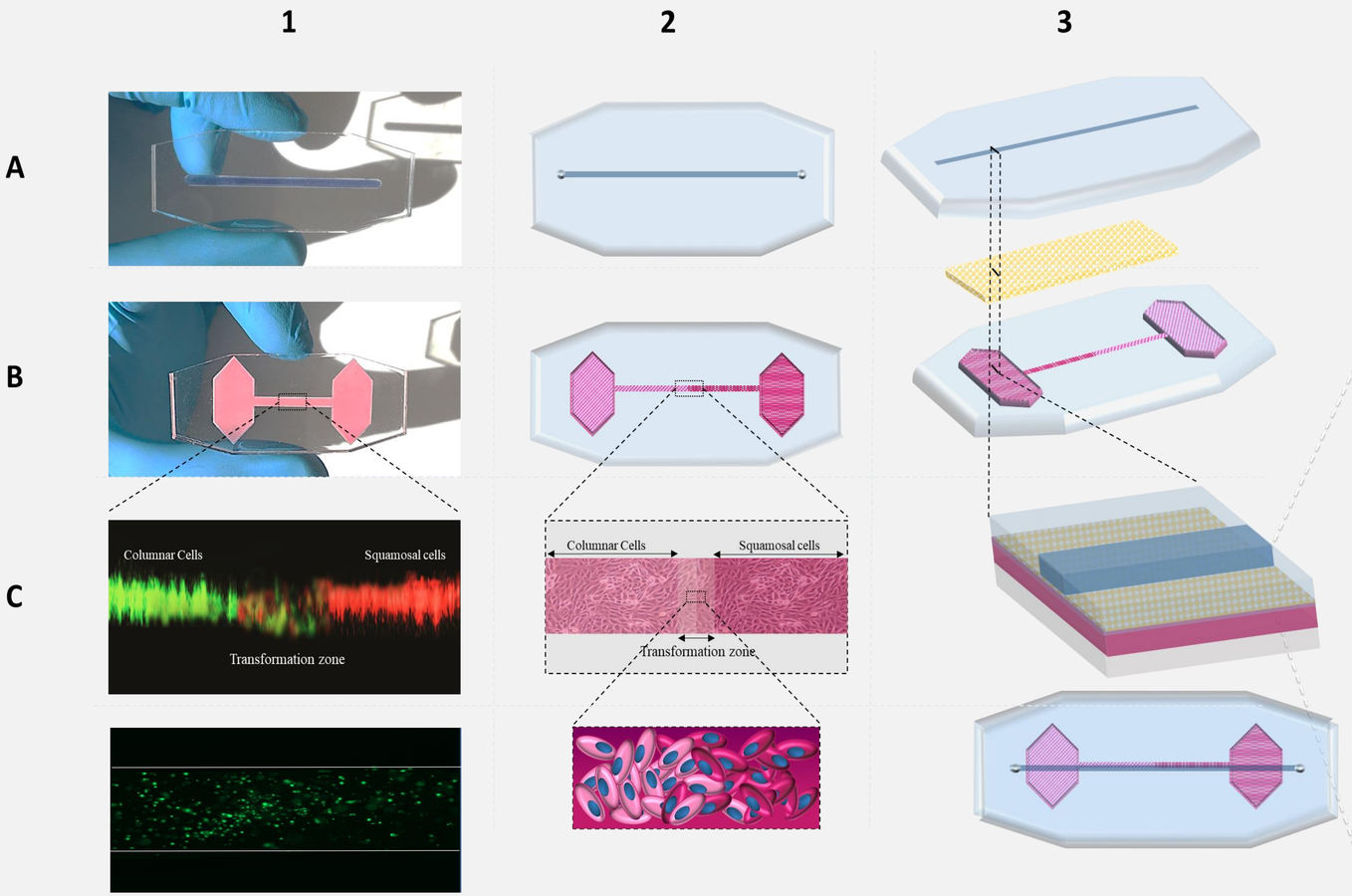
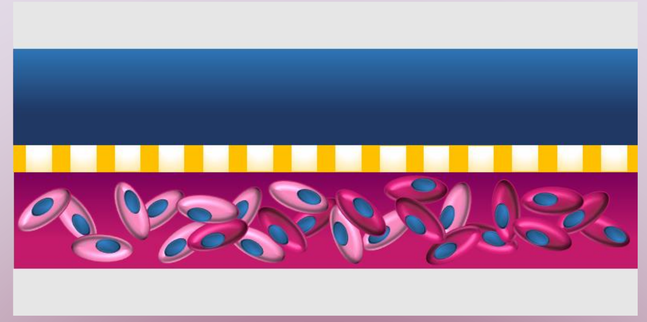


Figure 4. Illustration showing the distribution of epithelia of the ectocervix (dark pink) and endocervix cells (pale pink). (A) Upper layer of the chip (B) Lower layer of the chip (3) Two PDMS layers are aligned and irreversibly bonded to form two sets of parallel microchannels separated by a 22-µm-thick polycarbonate membranes, containing an array of through-holes with an effective diameter of 22-µm. Scale bar, 200 µm. (C) Long-term microfluidic co-culture produces a tissue-tissue interface consisting of a single layer of the Columnar epithelium (stained with Cell Tracker Green) closely next to a monolayer of the Squamous epithelium (stained with Cell Tracker Red) (C1), both of which express intercellular junctional structures stained with antibodies to VE-cadherin. Scale bar, 50 µm. (D) Future plan and the mechanism of mimicking the infection and testing new treatments.

D



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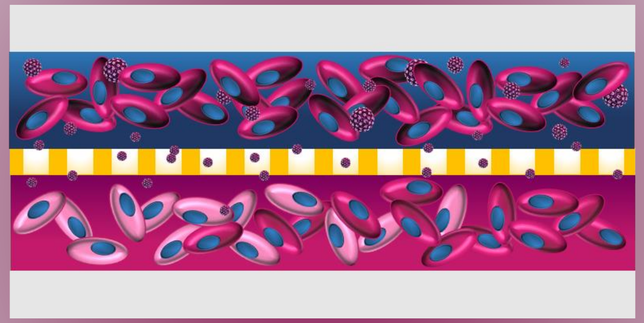

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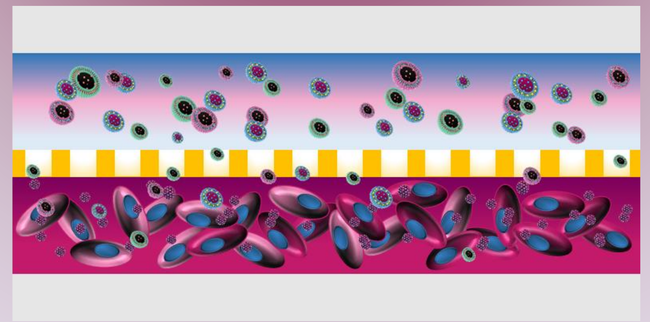

3. By changing the upper layer, the infected model is ready to test new drugs and treatments.