Microfluidic devices to mimic tumor microenvironment

Student(s)

Büşra Nur Ata

Ronaldo Leka

Faculty Member(s)

Assist. Prof. Meltem Elitaş

Supervisor: Hande Karamahmutoğlu

Sabancı Üniversitesi

PURE
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ABSTRACT

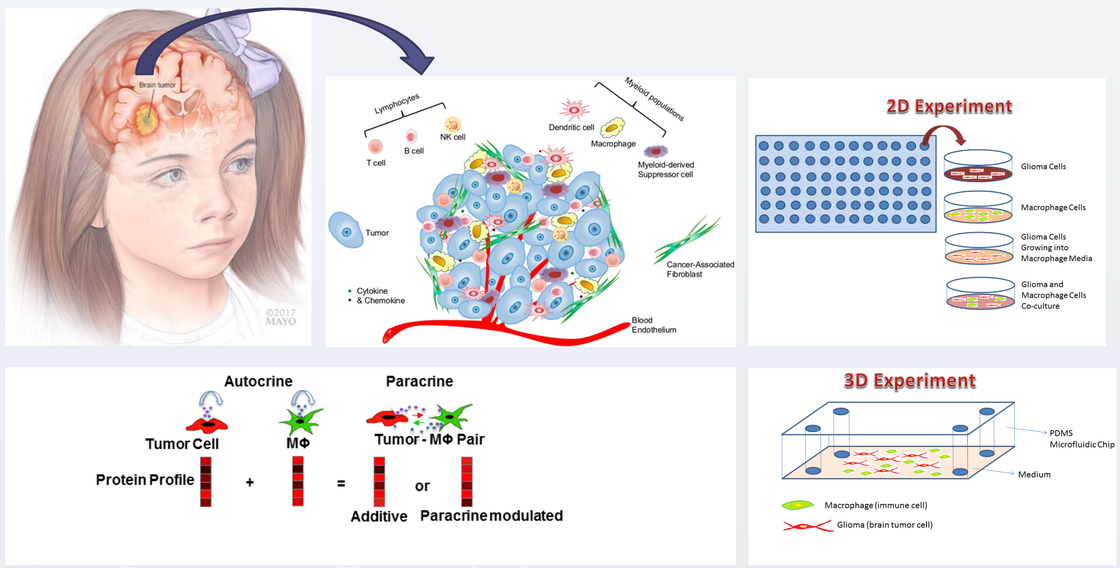

Figure 1. Schematic representation of the project at first glance.

Glioma is one of the most common primary brain tumours among adults. It is incurable, median survival of newly-diagnosed patients is less than 15 months although patients are submitted to combination of surgery, chemotherapy and radiation therapy [1]. Glioma tumor microenvironment is held to responsible for theupatic resistance since tumor microenvironment has a dynamic complex structure involving not only tumor cells but also aberrant neovascularization, stromal and infiltrating immune cells and extracellular matrix[2,3]. Infiltrating tumor-associated macrophage (TAMs) are the dominating immune cell type in malignant glioma by accounting 40% of the tumor mass[4,5]. Studies have been shown that TAMs contribute to tumor progression by playing immunosuppressive role and promoting angiogenesis[2,6].

Molecular mechanisms that enrole TAM differentiation has been studied to develop new cancer drug therapies *in vitro* experiments and mice models[7,8]. However, *in vitro* experiments are insufficient to reflect the physiologically-accurate tumor microenvironment. Additionally, *in vivo* mice models seem the best option to study cancer therapeutic secreenings but it has limitations on monitoring immune cells.

Recently, microfluidic devices have been engineered to mimic tumor microenvironment by allowing control of the dynamic structure of the microenvironment, and co-culturing of different cell types in the same microenvironment[9]. Although cell migration and heterotypic cell culturing have been studied, yet algoritmic analysis of time-lapse cell location of heterotypic cell pairs have not been reported.

OBJECTIVE

Herein, we present a microfluidic device allowing co-culture the glioma and macrophage cells to investigate their interaction at single-cell resolution by microscope imaging, image processing, statistical analysis and machine learning algorithm.

PROJECT DETAILS

Traditional cell culture containers such as plates and flasks provide relatively large surfaces for cell growth compared to the size of the cells. This property leads to poor mimicking of tumor microenvironment and continuous loss of medium through evaporation. In this project, we used microfluidic chips to circumvent these two problems and generate more biologically significant data about the behavior of tumor cells in co-culture with one type of immune cell, the macrophages.

PDMS chip design and fabrication

Microfluidic chips were designed by using the CleWin Layout Editor software and medium flow simulations were performed using COMSOL Multiphysics software. Chip wafers were produced by photolithography and PDMS chips (10% silicone elastomer curing agent) were produced by soft photolithography based on the wafers.

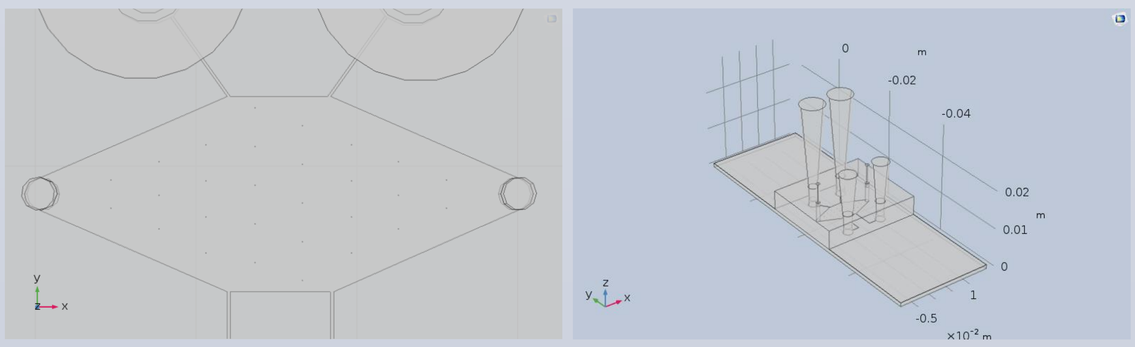

Figure 2. Microfluidic chip design used for simulations in the COMSOL Multiphysics software.

PROJECT DETAILS (continued)

Cell culture

The U87 glioma cell line was maintained using DMEM (10% FBS, 1% penicillin/streptomycin) and the U937 monocyte cell line was maintained using RPMI-1640 medium (10% FBS, 1% penicillin/streptomycin) in T-75 flasks. Medium was changed once or twice a day based on usage.

Macrophage differentiation

Macrophages were obtained from U937 monocytes by incubating $3x10^5$ cells/mL and 50ng/mL PMA in $22cm^2$ dishes for 48 hours. After 2 consecutive medium changes every 24 hours, the macrophages were used in the co-culture experiments.

Co-culture and fluorescence imaging

Cells were seeded in 96-well plates in triplicates at 10^4 cells/well for U87 cells and $2x10^4$ cells/well for macrophage cells in the following configuration: pure cultures of U87 and macrophages, U87 cells incubated in 50% macrophage-used RPMI-1640 medium, and co-culture of U87 and macrophage cells. U87 cells were stained with 1µM CellTracker Red dye, whereas macrophages were stained with 1µM CellTracker Green dye. Images were taken with 10x objective using a Carl Zeiss Axio Observer Z1 inverted microscope. Results are shown in **Figure 3** and **4**.

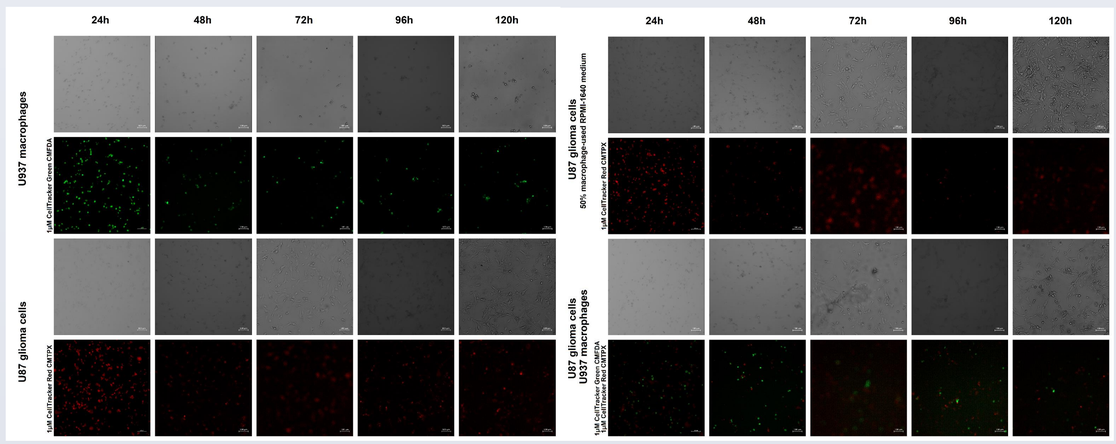

Figure 3. Phase and fluorescence imaging of cells for 5 consecutive days. Images were obtained with a 10x objective using a Carl Zeiss Axio Observer Z1 inverted microscope. Fluorescence images were taken using 20 ms exposure time.

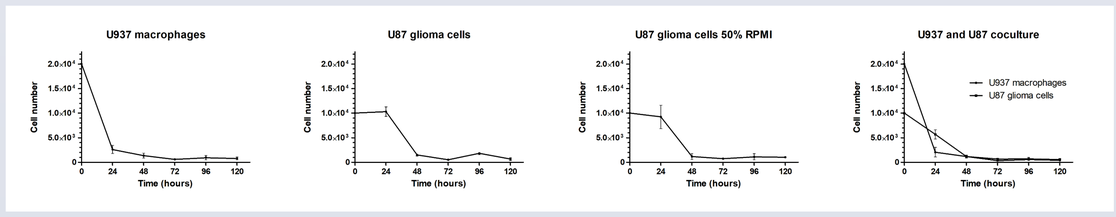

Figure 4. Graphs of cell number of time in hours. The number of cells was counted from fluorescence microscopy images using ImageJ automatic method of counting cells. The total number of cells was estimated from the count as the cells were distributed uniformly in all the wells.

CONCLUSIONS AND FUTURE WORK

As expected, the U87 glioma cells have lower cell survival rate when co-cultured with macrophages. In order to study cell metabolism, cell signaling between the different cell lines, we must:

- perform cell viability assay using CellTiter-Glo®
- perform ELISA using the medium collected from the cells
- use microfluidic chips to mimic the tumor environment more closely to *in vivo* conditions as shown in **Figure 5.**

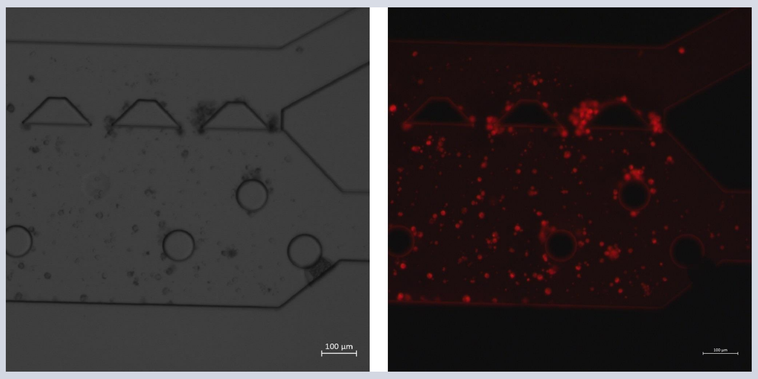

Figure 5. Phase and fluorescence microscopy images of U937 monocytes seeded in a microfluidic chip.

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