# Microfluidic Lab-on-Chip System Development for Cell Culture Cultivation

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**Abstract:**

Lab-on-chip systems are microfluidic devices that can be used as powerful tools for research in fields of molecular biology and bioengineering. Novel chip systems such as miniaturized microfluidic human tissue and organ models are powerful platforms to *ex vivo* study functional units of any important biological and physiological parameters of their *in vivo* counterparts. Also, development of microfluidic system with mathematical modeling described fluid dynamics in a chip can be used in real-time monitoring of the transport, efficacy, and cytotoxicity of potential drugs on the same platform.

We have developed a Chip system that is suitable for cultivation of cells or primitive tissues. The sandwich chip design is composed of acrylic plates - material that is transparent for easy optical following of cells growing. It is manufactured by milling processes performed on in-house developed 3D CNC router– 3D CNC milling machine and by the FDM 3D printing process on Creality 3D CR-10max printer. The main components are: i) bottom plate with cylindrical main chamber for cells growing and two supplying channels; ii) top plate with other half of the channel as well as two holes for tubing fittings.

Connection of Chip to a peristaltic pump gives a microfluidic system physiologically relevant microenvironment for cell growth. Fluidic control mimics conditions in real *in vivo* systems and leads to successful cell proliferation.

Organ-on-a-chip concept is relatively new technology, and it will take a greater impact on research in the upcoming years. In combination with computational modeling, organ-on-a-chip device can bring faster, safer, and more useful findings in organ(s) behavior.

In near future, organ-on-chip systems promise to be powerful platforms for *ex vivo* studies of functional units of some organs, for specific biological processes, for examination of tumor with its microenvironment. Also, these small devices can be used for personalized/precision medicine and drug screening for direct treatment decision-making. The chip model that we developed can be used for successful growth of cells or smaller tissues in conditions as suitable as in a living system.

**Keywords:** lab-on-chip, microfluidics, tissue engineering

## 1 Introduction

Lab-on-chip systems, which have recently emerged as powerful tools in research, are microfluidic devices that aim to recreate relevant physiological features [1]. 'Engineered tissue' culture on a chip is a promising technology with relative simplicity of sample acquisition and availability [2]. Device mathematical modeling describes fluid dynamics chip that can be used in real-time monitoring of the transport, efficacy and cytotoxicity of potential drugs on the same platform [1,2]. The most common microfluidic devices are fabricated in the elastomer poly(dimethylsiloxane) (PDMS) and operated using variable-control syringe pumps [3]. Integration of fine fluidic control in platform with cell-laden chamber compartments inside developed a new approach for tissue mimicry [4]. Organ-on-a-chip studies have shown the importance of producing physiologically relevant microenvironments with respect to static flow well-plate systems [5].

Recent observations constitute a proof of concept that miniaturized microfluidic human tissue and organ models are powerful platforms to study *ex vivo* functional units of some organs (e.g., liver), specific biological processes (e.g., angiogenesis), tumor with its microenvironment and for drug preclinical testing [6,7]. Future work on treatment application on these devices will offer such opportunities, minimizing sample volumes and maximizing control over the manipulation of particles suspended in microfluidic system [2,5]. Organ-on-chip systems promise to be powerful enablers of personalized/precision medicine and drug screening in order to direct treatment decision-making [1,2]. Animal models can satisfy requirement and provide an *in vivo* setting to study treatment, but they have their own complications, they are expensive, ethically questionable and fail to provide a precise recapitulation of results to human physiology [1,7]. *Ex vivo* assays, such as Organ-on-a-chip, that predict therapy response may fill these gaps [8].

Novel chip systems can be tailored to reconstruct important biological and physiological parameters of their *in vivo* counterparts. Furthermore, multiple organ-on-a-chip models can be connected in a similar manner in which they are arranged *in vivo*. So, they can provide analyzes of multiorgan interactions making a body-on-a-chip system comparable to an entire human body [9,10].

In this paper we presented a chip produced for growing cells in conditions similar to physiological ones. With connection to a peristaltic pump, a controlled flow was obtained, which enables successful growth and proliferation of cells in the system.

## 2 Methodology

The chip is sandwich designed, composed of two acrylic plates 5 mm thick. Selected material is transparent for microscopic following of cell growth. The channels are designed and milled on the inner side of both panels. The SolidWorks 2020 [11] CAD software package was used to design and build the 3D model of the chip.

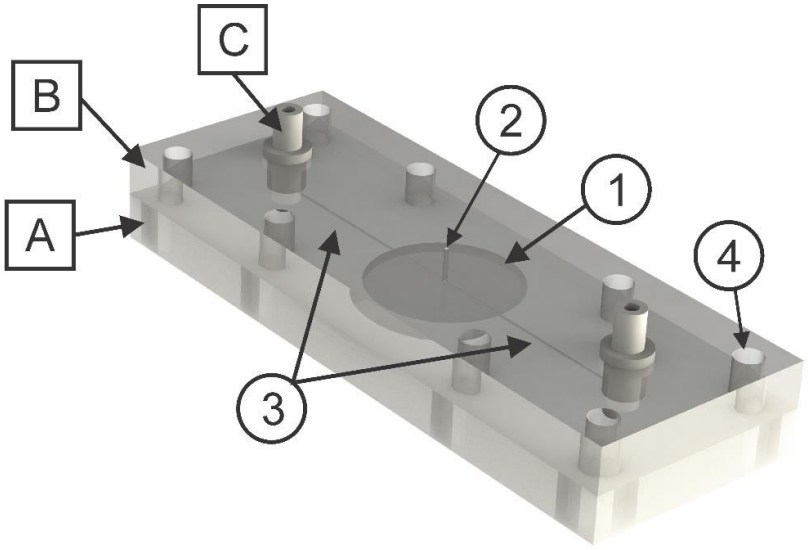


**Fig. 1.** Chip: A - bottom part; B - top part; C - tube fitting; 1 - main chamber; 2 - air pillar, for removing bubbles; 3 - supply channels

The main components are: bottom plate (A - Fig. 1), top plate (B – Fig. 1), which were cut on laser. Also, the plates are drilled holes (4 – Fig. 1), on laser, for easy bolting them. The cylindrical main chamber for cells cultivation (1 – Fig. 1) as well as two supplying channels (3 – Fig. 1) with half of the cylindrical cross-section are milled in this plate. The other half of the channel is milled in the top plate (B – Fig. 1) as well as two holes for tubing fittings. At the end of each supplying channels tubing fittings (C –Fig.1) are attached and the purposes are to provide an easy connection between the chip and the rest of the experimental equipment (e.g. peristaltic pump). Those fittings are manufactured by the FDM 3D printing process.

To achieve computational model that will include simulation of the fluid flow through the microcapillaries, it can be used the Navier-Stokes equation and continuity equation (Fig.2) [12]. To observe fluid flow through in the chip we will use convection-diffusion equation and additional reaction-diffusion partial differential equation, PDE [13]. The model will be solved with finite element method, FEM, in our in house built solver PAK. Also, the numerical model can be additionally extended to include multi-scale simulations, up to the nano-scale level, by applying the previously developed numerical model that was successfully applied to model the motion of circulating tumor cells in microfluidic chips [14].

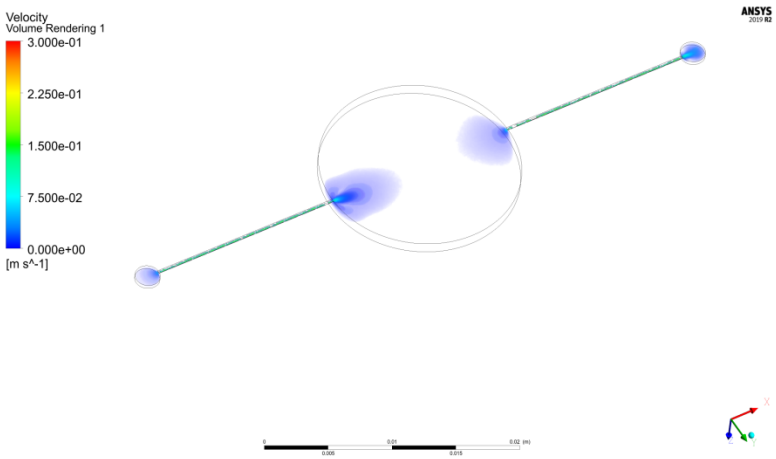


**Fig. 2.** Fluid velocity — PAK solver, input fluid velocity 0,03 ml/s.

Cell line HeLa (Human cervix epithelioid carcinoma) used for seeding in Chip was obtained from ATCC –American Type Culture Collection, Manassas, VA, USA. The cells were propagated and maintained under optimum conditions and standard protocols in DMEM medium (Dulbecco's Modified Eagle Medium, Gibco, USA) supplemented with 10% fetal bovine serum (Gibco Invitrogen, USA) and antibiotics 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma Aldrich). Experiments were performed in incubator in a humidified atmosphere of 5% $CO_2$ at 37°C. Cells were seeded at a concentration of 200,000 cells per ml and were in static conditions for 24 h while adhering to the base (both in the chip and in the 6-well). The seeded cells in the 6-well served as a control group for comparison with the group under dynamic conditions, which represents a microfluidic chip system. After 24 h incubation, the cells in the chip were switched on medium flow, and their proliferation was observed for 24 and 48 hours, respectively.

## 3 Results

We have developed a microfluidic Lab-on-chip system that is suitable for cultivation of cells or primitive tissues. Fig. 2 shows computer designed model of our Chip, and on Fig. 3 there is a real model after the production process.

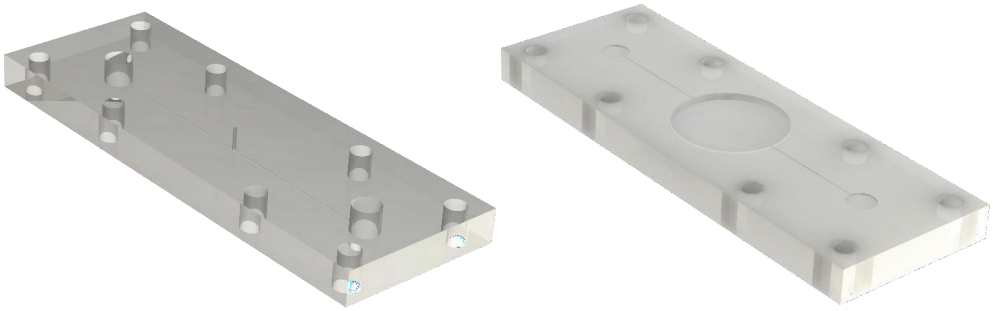

**Fig. 2.** Computer designed model

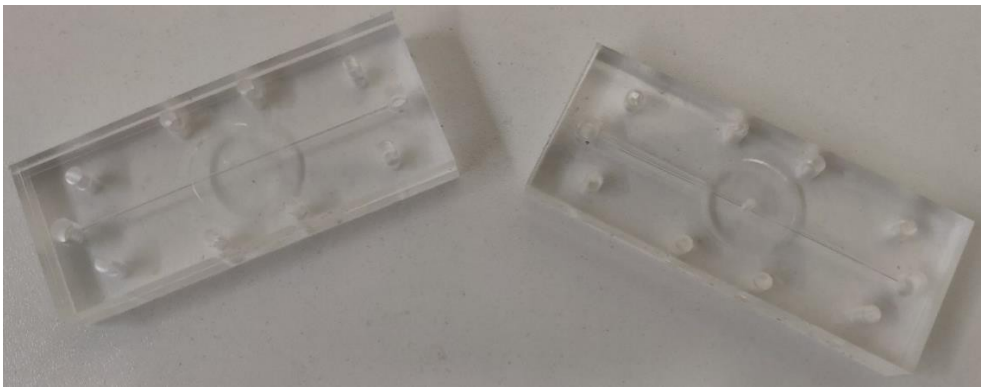

**Fig. 3.** Real model

Dimensions of main components (bottom and top plates) are 30 mm by 70 mm and 5 mm thick. The cylindrical main chamber for cells cultivation has a diameter of 16mm and a depth of 0.8 mm. Two supplying channels are with half of the cylindrical cross-section with diameter 0.25 mm.

The high-speed laser cutting and engraving machine LASERCUT 1209 was used to cut the chip according to the given dimensions (Fig. 4). LASERCUT 1209 is a system in which the working or active substance ($CO_2$) is in a gaseous state. The power of the tube is 220 W. The working surfaces of the laser are 1200 mm x 900 mm. The maximum tool travel along the Z axis is 250 mm, and the positioning accuracy is 0.1 mm. LaserGrav V8.98 software package was used to prepare for cutting. The parameters used for cutting chip with the dimensions 70 mm x 30 mm and for making holes are: cutting speed 5 mm/s, max power (%) 50 and corner power (%) 40.

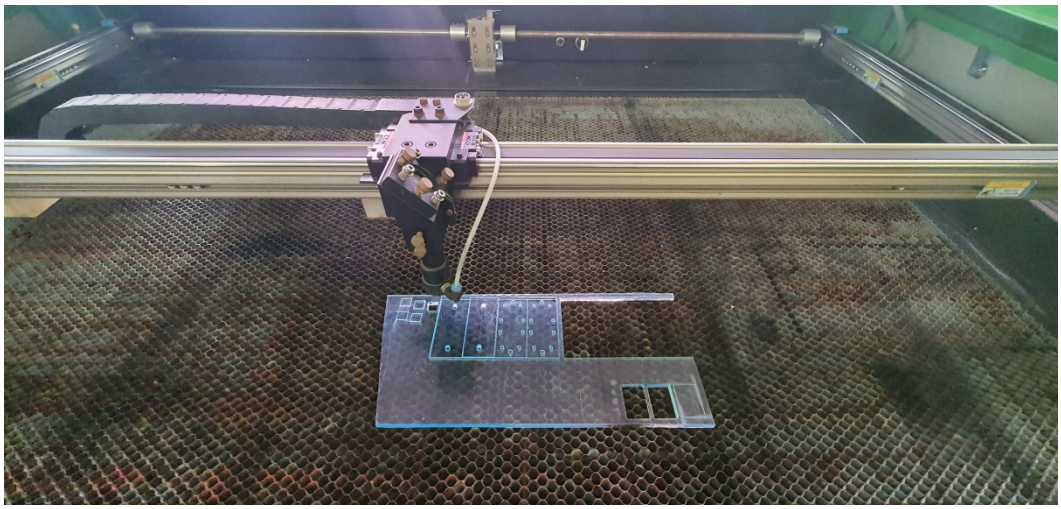

**Fig. 4.**Laser cutting

Milling processes are performed on in-house developed 3D CNC router – 3D CNC milling machine. This machine is designed for very precise work with an installed 2.2 KW water cooling milling head. This head provides max. 24000 RPM. For the axial precise (with error of 0.01 mm per 100 mm) and robust operation of the machine, a hybrid system was used. A highly precise encoder was added to each stepper motor, and hybrid step drivers with feedback coupling were used to control them. In this way, stable operation is ensured and the problem of skipping steps that occurs in machines with classic stepper motors is overcome. For milling larger chambers and openings on plates, a milling machine with 2 blades and a diameter of 2 mm was used, while for small supply channels, a flute with a ball end and diameter of 0.25 mm was used. With a cutting depth of 0.25 mm is ensured a semicylindrical cross-section profile of supplying channels (Fig.5).

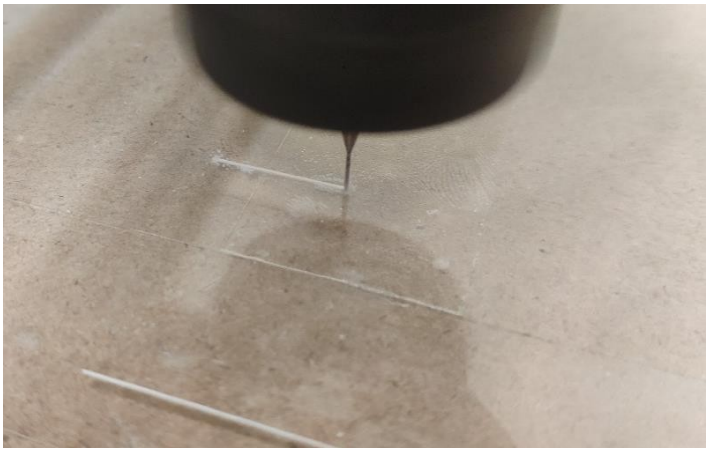

**Fig. 5.** Milling process, ball end flute 0.25 mm

Tubing fittings that are manufactured by the FDM 3D printing process are from PLA material (Fig. 6). Creality 3D CR-10max printer was used, with a high-quality printing setup (layer thickness 0.1 mm).


**Fig. 6.**3D printing of tubing fittings

When the Chip is connected to a peristaltic pump, we get a microfluidic system with a physiologically relevant microenvironment for cells or tissues (Fig.7). Peristaltic pump provides fine fluidic control that mimics conditions in real *in vivo* systems, and thus leads to successful cell proliferation.

**Fig. 7.** Chip connected to a peristaltic pump with flow

After seeding the cells in the Chip and in the presence of controlled flow of 0.03 ml/min medium, the cells are successfully cultivated. After 24 and 48 hours of flow-controlled Chip experiment in incubator, the cells in the chip survived and divided undisturbed.

Compared to the static model such as seeding cells in 6 wells (oftenly used *in vitro* studies), cells

from the chip are cultivated in conditions similar to physiological. We concluded that cells on the Chip were grown more successfully in comparison to the static conditions (6-well). Fig.8a shows the cells from the static 6-well, while Fig.8b shows the cells after growing under dynamic conditions in the Chip system.

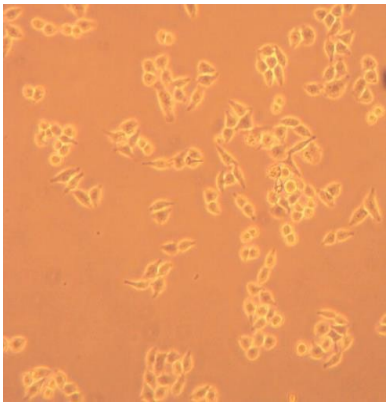

**Fig.8a.** Cells seeded in the 6-well

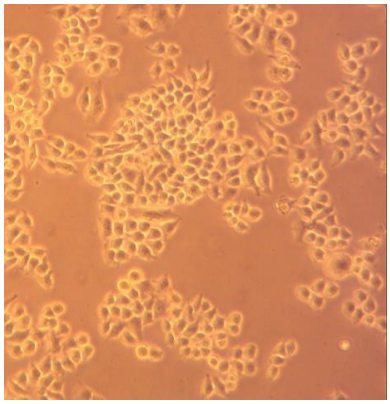

**Fig.8b.** Cells in the Chip

## 4 Discussion

Lab-on-Chip is a system that is primarily intended to replace *in vivo* experiments due to its simplification, shortening of testing time, and ethical issues regarding the use of a large number of animals. Our chip model can be used for successful growth of cells or smaller tissues in conditions as suitable as in a living system.

By further system developing we aim to create a Lab-on-chip microfluidic device that can be used for various purposes. More improved Chip systems will be manufactured by inserting multiple channels for different fluids, by inserting more chambers where several types of cells will be seeded, with deeper central chamber that will create the conditions for the cultivation of 3D cells seeded in hydrogels or various tissues.

## 5 Conclusion

We have developed functional lab-on-chip system and were able to cultivate cells in such conditions that successfully mimics growing cells as *in vivo* systems. With further work we will improve microfluidic devices in order to use them in several directions for research in the field of molecular biology and bioengineering.

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**Conflict of interest declaration:**

The authors declare that they have no conflict of interest.

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