Conceptual design and 3D modeling of a microfluidic device for liver cells investigation

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Abstract: *The cell engineering is one of the most developing fields during the last decade needing specially fabricated polymer microfluidic devices and systems. One of the main functionalities of the microfluidic devices is to mimic the in-vivo environment where the cells and tissue live. The various types in-vitro microfluidic devices and systems could replace the experiments with animals in the biomedical investigations. The aim of this publication is 3D modeling and simulation of a microfluidic device for liver cells investigation. Suitable materials could be used with main properties related with fully transparency and bio-compatibility of the selected polymers. A new technology for development of the microfluidic device will be proposed, incorporating a thin layer of liver cells for investigation of their behavior during treatment with different substances. The conceptual work principle of the developed bio-chip will be presented. The future investigations, related to the fabrication of a real physical prototype and research experiments will be mentioned briefly in conclusions.*
Keywords*: CELL ENGINEERING, POLYMER MICROFLUIDIC DEVICES, 3D CAD MODELING, LIVER CELL INVESTIGATIONS*

1. Introduction

The liver is one of the most complicated organs in the human body. The main "building blocks" of the liver are the lobules. Each liver lobule consists of several hexagonal microscopic structures where the liver cells are radially located around the central vein. In the periphery of each lobule, up to 6 portal triads are located at the apex of the hexagons. The triads consists from a portal vein, hepatic artery and bile duct. There are vascular sinusoids connecting the central vein of each lobule with the portal vein and hepatic artery. The sinusoids are surrounded by the main liver cells - hepatocytes (HPs). The liver sinusoid's wall is mainly composed by three cell types (liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs), and hepatic stellate cells (HSCs)). The LSECs are perforated by small fenestrae therefore the gap between the liver sinusoid and the HPs (Disse space) is easily filled up with blood. The direct exposure of HPs to the blood stream increases the surface for transportation between HPs and blood flow. This process facilitates various liver functions as resorption and release of nutrients, and detoxication [1, 2].

Microfluidics handles and analyzes fluids at the submillimeter scales. Microfluidic technology enables the implementation of advanced platforms (such as micro-total-analysis systems [3], lab-on-a-chip [4], lab-on-a-disc [5], organ-on-a-chip (OOC) [6], or body-on-a-chip (BOC) [7]) for research in life sciences. Some of the significant advantages of microfluidic devices, particularly for biological research, are direct screening, better control of the fluid flow, low consumption of reagents, mimicking the *in vivo* cellular microenvironment and low sample requirement [8].

In the last few years, the organ-on-a-chip became one of the most important and emerging technologies emulating human organs and especially the human liver. Organ-on-a-chip systems are microfluidic devices, used to culture live cells in microfluidic chambers with continuous flow conditions mimicking the physiological function of tissues and organs *in-vivo*. The microfluidic devices from the type organ-on-a-chip are used mainly for evaluating the safety and efficacy of different drugs *in-vitro*. Because of the size of the microfluidic devices they could be used for modeling and simulation of extremely fine and complex main components of the tissues (liver sinusoids), rather than creating a complete liver tissues. Liver chips have been used in different format for better cultivation of HPs [9]. Various geometries and dimensions of the cell and tissue culture chamber have been developed where oxygen and nutrition gradients were fine tuned. The creation of a successful liver microfluidic device is related with a lot of important parameters that could be taken into account. (medium flow, mass transfer of nutrients and metabolites, purification, sufficient supply of oxygen, mechanical forces as shear stress) [9].

A conceptual design and 3D modeling of a liver microfluidic device have been proposed in this publication. In fact, a microfluidic device with two microchannels have been modeled, mimicking the liver sinusoid and Disse space (the most important part of the liver which is in direct contact with the HPs). A 3D model of the liver chip has been created using a suitable CAD software. New geometry of the liver chip has been presented for better culturing and investigation of HPs. Appropriate pressures driving the laminar flow into the microchanels have been applied and as a result, the wall shear stress have been obtained. A suitable fabrication technology has been described regarding the concept design of the liver chip. The working principle of the new microfluidic device has been presented comparing the advantages and disadvantages with the developed already organ-on-a-chip liver devices.

2. Conceptual Design

The direct contact of the hepatocytes with the blood, circulating in the liver, is realized through a Disse space. A huge amount of blood penetrates via the fenestrea of the LSECs, in the liver sinusoids. The microfluidic device, presented in the current publication, represents an *in-vitro* 3D model of Disse space and the liver vascular sinusoid. The liver chip consists from one microfluidic channel, separated in two identical parallel microchannels with a polymer perforated membrane (the diameter size of the microholes is up to 400 nm). The dimensions of each one of the microchannels are 0.1 (height) / 1 (width) / 15 (length) mm. The thickness of the polymer membrane is 0.01 mm and the diameter size of the microholes is up to 0.4 μm. The HPCs under investigation could be cultured at the bottom side of the lower microchannel.

The KCs and LSECs (from liver sinusoids) could be cultured over the polymer perforated membrane. The HSCs will be cultured on the lower surface of the membrane. Various chemical substances and/or drugs could be injected into the microfluidic device - a laminar fluid flow. The region where the HPCs will be cultured is in the middle of the channel as a circular shape. On this way more liver cells could be investigated at once (fig. 1). The feeding of the microfluidic liver chip with four types of cells is realized by the protocol from [1].

3. 3D Modeling and Simulation of a liver sinusoid microfluidic device

Two main types (type 1 and type 2) [1, 10] of microfluidic devices for liver cells investigation are analysed. A standard organ-on-a-chip device consists from 2 microfluidic channels separated by a perforated membrane. The geometry of the microchannels and the membrane, as well as the fabrication materials are extremely important during the design of the liver microfluidic devices. Both commercially available liver on-a-chips from [1, 10] are modeled using Solid Works (fig. 1, fig. 2).

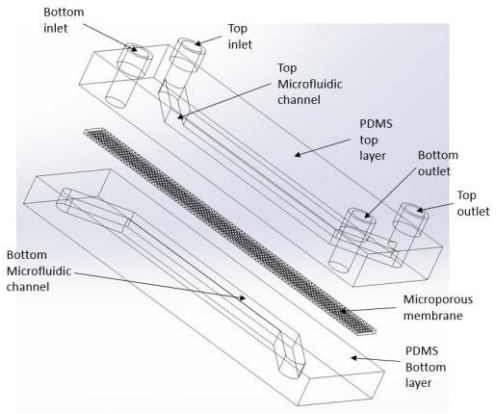

Fig. 1 *3D modeling of the liver-on-a-chip device (type 1). The device consists from 3 main parts – 2 parts with identical straight micro-channels and a polymer perforated (microporous) membrane.*

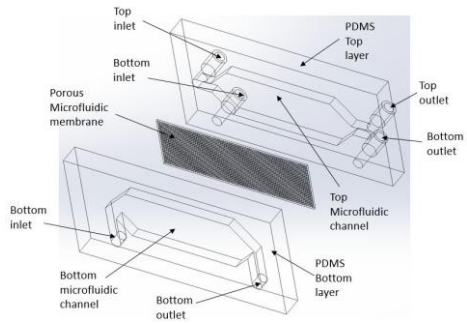

Fig. 2 *3D modeling of the liver-on-a-chip device (type2). The device consists from 3 main parts – 2 parts with micro-channels extended in the middle (complex shape) and a polymer perforated (microporous) membrane.*

Regarding models' features, it is proposed a new design of a microfluidic device for HPCs analysis. It consists from one channel separated in two identical parallel microchannels. The height, length and width of each one of the microchannels are 0.1 mm, 15 mm and 1 mm, respectively. In the middle, the shape of the microchannels is a circle with a diameter size equal to 5 mm. Both microchannels are separated from a perforated membrane with a thickness from 0.01 mm. The micro-holes of the membrane are 400 nm in diameter (fig. 3).

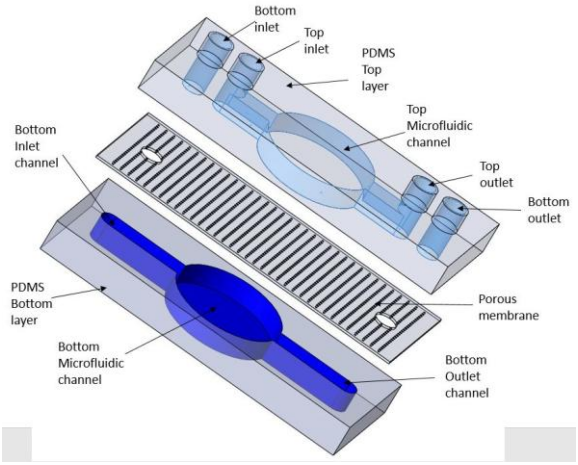

Fig. 3 *3D modeling of a liver-on-a-chip (new design). The device consists from 3 main parts – 2 parts with identical micro-channels (extended in the middle as a circle shape) and a polymer perforated membrane.*

All three microfluidic structures, before analyzed, are simulated with a computational fluid dynamics (CFD) software.

The STL files, created with SolidWorks have been imported in Flow 3D (CFD software). A suitable mesh has been created for simulation. The fluid used for CFD is water at 37ºC. A Newtonian fluid has been defined, calculating the Reynolds number using this equation:

$$Re = \frac{\rho u D_H}{\mu} \quad (1)$$

where

ρ - density fo the fluid (kg/m^3)

u - mean velocity of the fluid (m/s)

μ - dynamic viscosity of the fluid (Pa.s = N.s/m^2=kg/(m.s))

D_H - hydraulic diameter of the pipe (In our case the cross-section of the microchannel is a rectange. Therefore, $D_H = \frac{4A}{P}$, where - A - cross-sectional area of the microchannel (m^2); P - wetted perimeter of the microchannel (m)

For all simulated microfluidic devices, the velocity (input parameter) is equal to 0.24 mm/s. The main goal is to obtain the shear stress into the microchannel, simulating the liver sinusoid (upper channel).

The shearing is a deformation process that may impact the structure of the LSECs situated at the top surface of the perforated membrane. For a Newtonian fluid (laminar flow), the shear stress (τ) is directly proportional to the shear rate (γ) which in turn is proportional to the fluid velocity gradient (dv/dy) [11]. The LSECs which are located close to the lower plate (membrane) are exposed to the maximum shear stress (fig. 4).

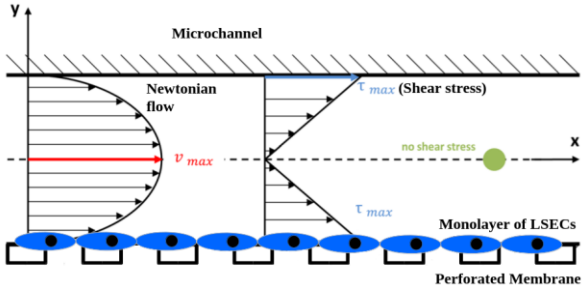

Fig. 4 *Illustration of velocity profile and shear stress for a Newtonian liquid in a channel between two plates (plate and a perforated membrane – simulated as a solid wall). The shear stress of all 3 types of microfluidic devices are identical.*

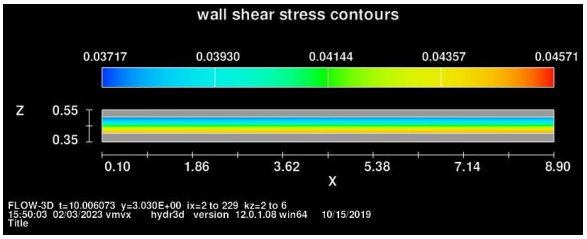

Fig. 5 *Simulation results the microfluidic device – type 1; The wall shear stress (straight microchannel) is up to 0.022 dyn. The pressure along the microchannel is approximately 1014 mbar.*

The shear stress of all 3 types of microfluidic devices are identical. The obtained results could be seen on figures 5, 6, 7, respectively. Therefore all of them could be used successfully for investigation of HPCs situated in the proximity of the liver sinusoid.

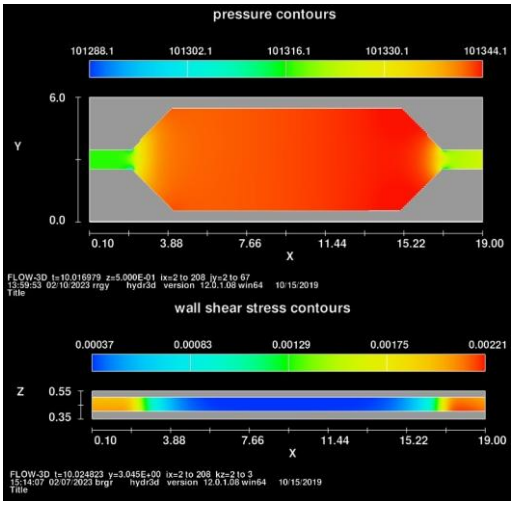

Fig. 6 *Simulation results of the microfluidic device – type 2; The wall shear stress is up to 0.028 dyn. The pressure along the microchannel is approximately 1013 mbar. The pressure in the wide part of the microfluidic device is 0.01 dyn.*

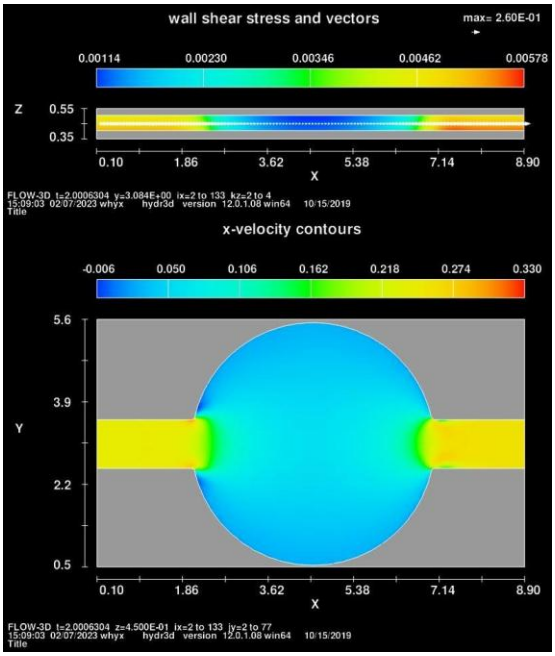

Fig. 7 *Simulation results of the new microfluidic structure – type 3; The pressure in the wide part (circle) of the microfluidic device is up to 0.01 dyn. The wall shear stress of the narrow part (straight channel) is up to 0.057 dyn. The pressure is approximately 1014 mbar.*

Regarding the simulation results, a micropump with a pressure range from 0 to 2000 mbar could be applied for control of the Newtonian fluid into the future prototype of the organ-on-a-chip liver sinusoid device. Companies as Elveflow have this type of micropumps available on the market. Our optical system BioFlux 200 also could be used for creation of such laminar flows with the same pressures.

4. Fabrication Technology of a Liver Sinusoid on a Chip Microfluidic Device

The proposed microfluidic device mimicking the liver sinusoidal structure will consist from 2 main blocks with identical microchannel geometry. The height of the microchannel will be 0.1 mm. The width of the straight part is 1 mm. The region in the shape of a circle will be with a diameter size from 5 mm. The length of the microchannel is 9 mm.

The geometry of the microfluidic device will be fabricated by a xurography technique [12], performed at low cost in a straightforward manner.

The microchannel will be drawn using a CAD software. The molds will be cut by a cutting plotter from a vinyl paper. The mold will be transferred to any polymer / glass substrate (petri dish) using an adhesive. The PDMS prepolymer will be prepared by mixing a commercial prepolymer and a curing agen (10:1 ratio) and poured onto master mold in the petri dish and cured in an oven at 80ºC for 20 minutes. Using a blade, the microchannels will be cut off and the inlet and outlet holes will be made using a suitable micro puncher. Both identical parts of the microfluidic device will be aligned with a mask aligner. The perforated (the diameter size of the holes is 400 nm) polymer membrane (commercially available) will be incorporated between both PDMS parts. The whole microfluidic structure will be sealed finally.

5. Conclusions

In this paper, a new design of a microfluidic device mimicking the liver sinusoidal structure has been proposed. 3D models of the proposed microfluidic device and two types of the most widely used liver on-a-chip devices have been prepared for CFD simulation. The results obtained, helped to estimate the wall shear stress generated over the polymer membrane where the LSECs are cultured. Regarding the used pressure, suitable micropumps could be selected for creation of an appropriate Newtonian flow. As a future activity, a fabrication technology based on a soft lithography has been proposed. Regarding the perforated polymer membrane, we have plans to develop innovative membrane using two photon polymerization technique and our high-technological equipment for 3D micro/nano-printing (Photonic Professional GT2 – Nanoscribe).

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