Microfluidic device for hydroxyapatite crystals growth process study

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Abstract. Heart problems tend to occupy a leading position among elderly diseases. Organ-on-a-chip is suitable technology for researches of human body mechanisms without animal models. One of the widely spread diseases is valve calcification. To study growth of hydroxyapatite crystals a microfluidic device was created. For simulating the heart valve on microfluidic device COMSOL Multiphysics® was used. The device was fabricated from polydimethylsiloxane using spin-coating method for membrane which acts as a valve and soft lithography for the other parts.

1. Introduction
In recent years in the most developed countries a population aging is observed. This is accompanied by an increase of valvular heart defects number associated with valve calcification. Moreover, calcific lesions of the heart tend to occupy a leading position among elderly diseases. According to [1] among population over 55 years old calcified valvular heart disease was diagnosed in 45.8% of cases [2, 3]. Investigation of calcific deposits growth process, namely hydroxyapatite crystals, is an important task for understanding causes of heart valve calcification.

To solve this problem organ-on-a-chip (ONC) technology can be used. ONC is a new way for advanced researches. In vitro surrogate models, that can reproduce the complex structure and functionality of living human organs, are necessary for understanding diverse biological reactions of human organism to various external stimuli.

There are plenty of systems simulating blood vessels [4], kidneys and intestines [5]. However, there are not so many works where the mechanical model of the organ is observed. During an organ model creation it is not always possible to reduce it proportionally with preserving its functions. Therefore, an allometric reduction is applied [6, 7].

In this work the microfluidic device simulation was obtained for topology design consideration. Suitable membrane thickness was chosen based on simulation results. Microfluidic device (MFD) was made of polydimethylsiloxane (PDMS) using spin-coating method for membrane and soft lithography for bulk parts. Thus, an attempt to create the mechanical model of heart valve via membrane was made.
2. Simulation
To design the MFD topology COMSOL Multiphysics® was used. For that purpose simulation requires solving the fluid-structure interaction problem. The calculation was performed in real time in order to trace the dynamics of the valve membrane movement with a time-varying fluid flow. To reduce the calculation time 2D model was used (figure 1, 2).

The simulation was carried out in order to determine the device channel sizes and the thickness of the membrane. It is necessary that the membrane should not touch the channel walls when fluid flows through them. As a result membrane thickness from 10 um to 100 um showed higher stability. Membranes of 30 um and 50 um thickness, located between channels, could be used for better device sealing. It is possible to use both thicknesses but 50 um is easier to manipulate during assembly.

For creating the heart rate via membrane oscillation peristaltic pump was used. Selection of peristaltic pump frequency resulted from allometric scaling of the heart rate which corresponds to normal human heart rate around 60 beats per minute. In accordance with the geometrical size of the device, much less than human heart of a natural size, a heart rate of 124 beats per minute was selected. It was also important to make sufficiently wide edges around the channels for better device sealing (figure 3). To simulate the heart rate the sine modulus function was used with the appropriate coefficients applied to the input stream with parabolic velocity profile.

![Figure 1] Maximum deflection of 50um PDMS membrane. Arrows show the velocity of fluid flow. Max flow range 0.01 m/s. 1 – input channel, 2 – membrane, 3 – output channel.

![Figure 2] Maximum deflection of 30um PDMS membrane. Arrows show the velocity of fluid flow. Max flow range 0.002 m/s. 1 – input channel, 2 – membrane, 3 – output channel.

3. Membrane and chip fabrication
The MFD consists of three parts: two bulk parts with channels and membrane located between them and clamped at one end. Whole device was fabricated from PDMS using spin-coating method for membrane and soft lithography for the other parts. To produce all the parts was used PDMS (Sylgard 184, Dow Corning) consisting of silicon elastomer base and curing agent, which were mixed in ratio 10:1.

For membrane fabrication 100 ul of uncured PDMS mixture was placed at the centre of acrylic glass plate. Spin-coating method consists of two steps. First the 100 ul drop was spun on speed 300 r. p. m. during 20s for spreading it on the plate surface and after the speed was ramped up to 3000 r. p. m. for 15s for removing PDMS excess. Afterward the plate was cured at 80°C for 1.5 hour. The membrane with thickness 30 um PDMS membrane is showed on figure 4. There is an important point here, it is necessary to make all the manipulations with PDMS no later than 15 minutes after mixing, otherwise it will not spread well on the plate. Changing the speed and time of rotation it is possible to manipulate the thickness of membrane.
The other parts of the device are made of PDMS mixed in the same way using the soft lithography method. Mixture of PDMS poured onto a structured mold then cured at 80°C for 2 hours. The membrane of the desired size was cut by mechanical method with a sharp scalpel and after the membrane was placed on one part of the device so as to cover the channel. The membrane is strongly electrified in air so all manipulations with it were carried out in distilled water. After placing the membrane, MFD parts with membrane were pulled out of the water and dried. For assembling and sealing MFD was treated with O₂ plasma for 1.5 minutes.

4. Results
As a result of the work the suitable membrane thickness and channel sizes of MFD were chosen according to Comsol simulation for heart valve modelling. The PDMS membrane was obtained by spin-coating method in two steps. The minimum thickness of obtained membrane was determined as 30 um. Behaviour of 30 um and 50 um membrane thicknesses were simulated in a periodic flow. MFD was made of PDMS using soft lithography for bulk parts with further assembling with 50 um membrane via sealing.

Using a peristaltic pump in a variable flow that causes membrane oscillations in the device it is possible to get different modes of the microfluidic device operation. The simulation provides data on the flow rate that can be created for the mode of membrane oscillations in which the membrane, after deflection, has time to return to its original position. The porous structure of PDMS allows to create a lot of the crystallization centres so if we pump a sufficiently concentrated solution through the channels, at a temperature of 37 degrees (human body temperature), it can be observed calcific deposits growth process on the membrane as in real human heart. Trial experiments were carried out with distilled water. It showed that MFD is sealed and membrane oscillates according to obtained simulation. In the future, it is planned to conduct experiments with concentrated solution of hydroxyapatite crystals.

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