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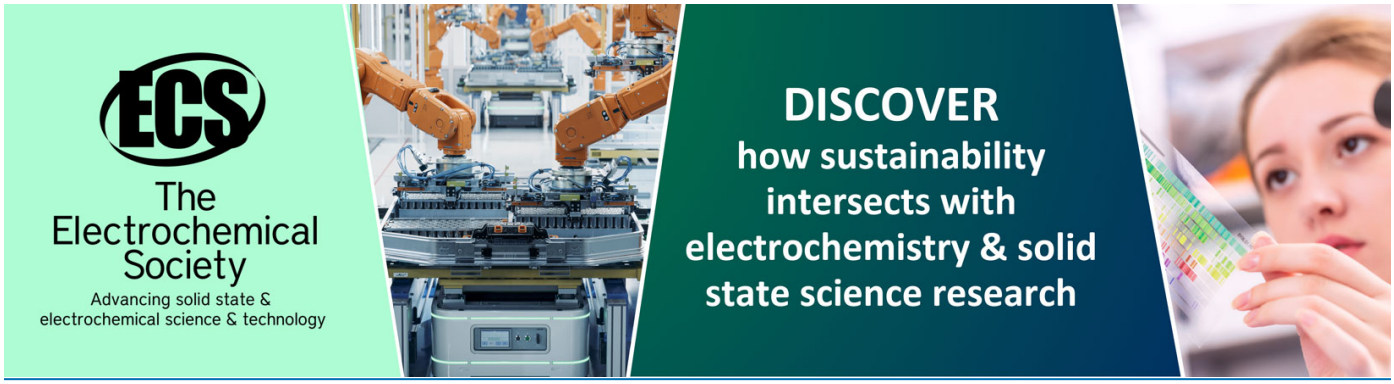

Simulation of the calcium salts formation in micro-sized channels (capillaries) in continuous flow mode

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Abstract. The conception "organ-on-a-chip" is a simulation of a living organism functional unit using a microfluidic device and additional technologies. Such technology can clarify some process in human body, reactions on drugs and help to create the new way of treatment without animal models. Calcific aortic stenosis is characterized as long developing disease that is more often observed among the elderly people. "Organ-on-a-chip" via microfluidic technology can simulate some process of this disease such as the calcium salts deposition in shorter time than it happens in living human body. Moreover it can explain the nature of disease. In this work we investigated the deposition of calcium salts in vessels by imitating the processes in the microfluidic chip and mathematical modelling.

1. Introduction

Calcific aortic stenosis (AS) is the most prevalent heart valve disorder in developed countries [1]. AS is the third-most frequent cardiovascular disease after the coronary artery disease and the systemic arterial hypertension, with a prevalence of 0.4% in the general population and 1.7% in the population >65 years old [2]. Calcific aortic valve disease is characterized by time-dependent wear and tear of the leaflets, lipoprotein deposition, chronic inflammation, and active leaflet calcification. The calcification exists in the vasculature in several forms of calcium phosphate, including calcium hydroxyapatite or different calcium salts [3].

In this work we researched a calcium salt deposition in vessels, imitating this process on microfluidic chip (MFC) by "organ-on-a-chip" concept. "Organ-on-a-chip" is a multi-channel three-dimensional MFC that allows us to simulate organs with a certain scale, thus replacing a living organism with a technical device or a model and reducing the time and cost of research [4]. At the moment, there is a number of organs that have been simulated using MFC: heart, lung, kidney, artery, bone, skin, etc. [5]. In the future, "Organ-on-a-chip" can simplify the study of chronic pathophysiological reactions, computational modeling of liquid-dynamic interactions with metabolites, and the interaction of cells and gases with circulating cells, such as blood, tumor, immune cells and bacteria [6].

Moreover the "organ-on-a-chip" idea can help to imitate not only a whole human organ, but various processes in a living organism, in particular, chemical reactions and mass transfer of substances. This makes it possible to identify the mechanism of salt formation, determine the factors that affect this process and manage it. In the future, this will help explore specific treatment strategies and identify conditions that prevent the salts formation or calcification and the AS development.

2. Materials and methods

Phosphate buffered saline (PBS) and water solution of calcium chloride $CaCl_2$ were used to simulate the calcium salts deposition in vessels.

Calcium phosphates formation was performed in a MFC, its configuration is shown in figure 1. The chip consists of two chambers separated by a thin-layer membrane, in which a cut is made to connect the channels.

The microfluidic chip was made from polydimethylsiloxane (PDMS) by soft lithography. PDMS mixture of 10:1 silicon elastomer and a curing agent (Sylgard 184, Dow Corning) was degassed and poured onto the master mold with subsequent curing at 80°C for 2 h. After complete curing, PDMS replica was peeled off from the mold. Inlet and outlet access holes were made by a biopsy punch [6], the cut in the membrane was made with a thin scalpel. Sealing was achieved by placing the PDMS replica onto a clean glass slide after surface oxidizing in O_2 plasma. Mold was fabricated using standard lithography from SU-8 negative photoresist on a silicon wafer [7].

Registration of the calcium phosphates formation was performed using an optical microscope and a CCD camera with a progressive scan Pike F421b (Germany).

3. Experiment

A number of experiments were carried out on an experimental system (figure 2) consisting of micro-tubes with reagents, a BT100-2J peristaltic pump and a microfluidic chip. The concentration of calcium chloride was varied from 0.2% to 0.8% with a step of 0.2.

Water solution of calcium chloride was continuously loaded into one channel and a buffer into the second by peristaltic pump. Due to pressure difference, the liquid leaked through a cut in the membrane, reagents mixed and an insoluble product – calcium salts – released.

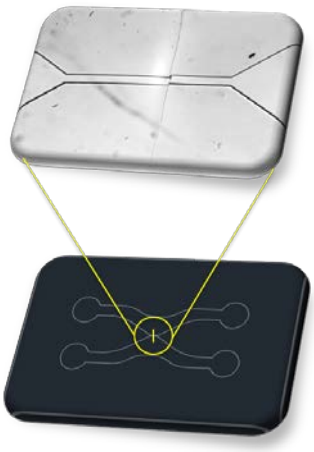


Figure 1. MFC configuration

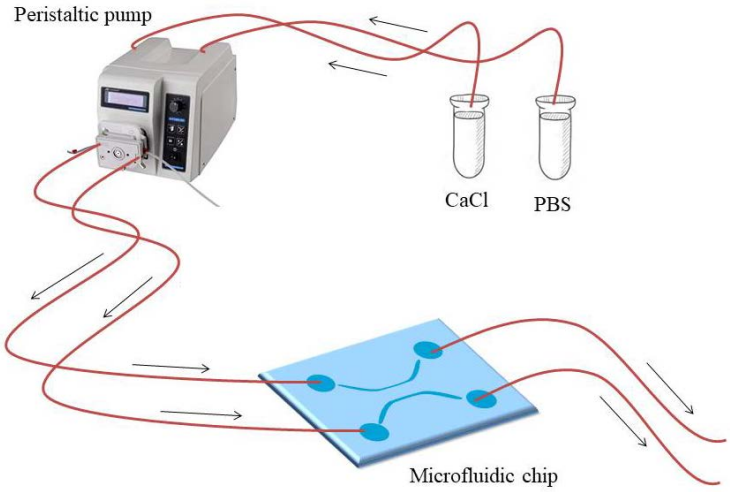
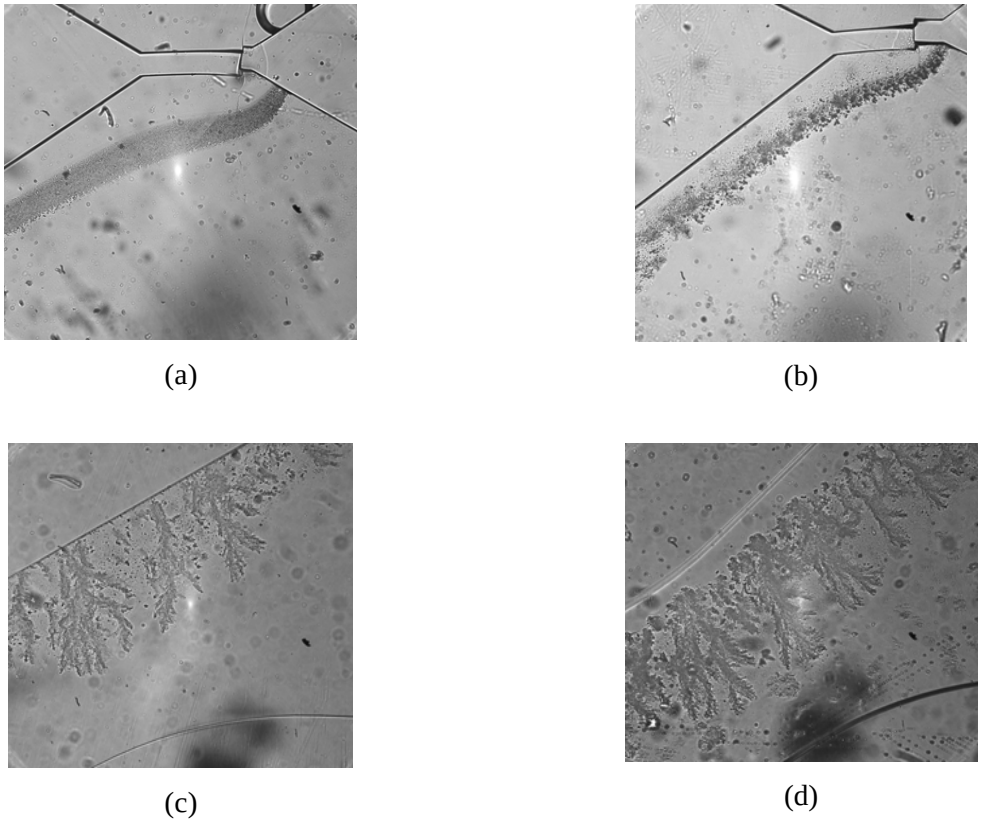


Figure 2. Experimental system

The experiment was carried out for 25 minutes. The $CaCl_2$ solution was supplied at a speed of 20 rpm, which corresponds to a flow rate of 19.5 µl / sec. PBS solution was supplied at the low speed of 10 µl/min for constant updating of the reagent, but in the same time to prevent washing the formed crystals of calcium salts out from channel by the flow. Figure 3 shows images recorded during the formation of calcium phosphates in a microfluidic device at different concentrations of $CaCl_2$.



(a) (b)

(c) (d)

Figure 3. Formation of calcium phosphates in a microfluidic device at different concentrations of $CaCl_2$: (a) 0.2%, (b) 0.4%, (c) 0.6%, (d) 0.8%

4. Mathematical modelling

Mathematical modeling was performed to describe the results of the experiment with the 0,4% $CaCl_2$ concentration. The considered regime is featured by the rates comparability of two processes relevant to the insoluble precipitate formation, namely of the diffusion (deposition) of precipitate on channel walls and of the chemical formation of products during the reagent transfer through the channel.

The goal of mathematical modeling is to explain the shape of the "trace" formed at the bottom of the microchip channel by the reaction product (calcium hydrophosphate $CaHPO_4$).

The studied chemical reaction is

$$CaCl_2 + Na_2(HPO_4) = Ca(HPO_4) + 2NaCl \quad (1)$$

Assumption 1. The second order reaction and its speed are determined by the following formula:

$$\frac{dc}{dt} = kC_1C_2, \quad (2)$$

where k is the reaction rate constant, and $C_{1,\,2}$ is the concentration of reagents.

However, since the reaction components were continuously loaded into the reaction chamber, they can be considered inexhaustible so their concentration remains constant. Then the reaction product concentration should increase linearly according to the law $C(t) = kC_1C_2t$ which corresponds qualitatively to a zero order reaction. Convective transfer of reagents can be described using two specific models with known analytical solutions [8].

Assumption 2. The first model is the submerged jet model, which describes the insertion of $CaCl_2$ through a cut in a microchannel of 90 μm wide and 30 μm deep. According to [8] the radial component of the flow velocity vector:

$$\nu_r = \frac{1}{r}F(\theta) \tag{3}$$

$$F(\theta) = 2\gamma\left(\frac{A^2-1}{(A-\cos(\theta))^2} - 1\right), \tag{4}$$

where θ is the angle from the jet axis, r is the radial displacement, and γ is the kinematic viscosity coefficient.

For a non-Newtonian fluid model and small velocity changes it is possible to use initially the apparent or plastic viscosities. The parameter *A* is the integration constant defined via the radial component of the momentum flow tensor in the jet:

$$\Pi_{rr} = p + \rho V_r V_r, \tag{5}$$

where *p* is the pressure, ρ is the density of the liquid.

For a full truncated jet model, we can obtain the pulse flow *W*:

$$W = 2\pi \int_0^\pi r^2 \Pi_{rr} \cos(\theta)\sin(\theta)\, d\theta \tag{6}$$

$$\Pi_{rr} = \frac{4\nu^2\rho}{r^2}\left(\frac{\left(A^2-1\right)^2}{(A-\cos(\theta))^4} - \frac{A}{A-\cos(\theta)}\right) \tag{7}$$

With the given flow rate of calcium chloride and the channel geometry it is possible to estimate the tensor components of the momentum flow of this reaction component of the initial flow and find the parameter *A* and the velocity profile. The flow rate is very high in this case, so the model is called the strong jet case and *A* is close to 1. For accuracy:

$$A = 1 + \theta_0^2 \tag{8}$$

$$\theta_0^2 = \frac{64\pi\gamma^2\rho}{3W} \tag{9}$$

The jet is a cone with the apex angle of θ_0. According to our conditions, the angle θ_0 is approximately equal to 0,22 radians or 12,5 degrees. Accordingly, *A* is approximately 1,025. The minimum distance from the jet formation point (the channel cut point) to the boundary of the reaction product trace is about 80-90 μm.

The outer boundary of the "trace" can be determined by a decrease in the concentration of incoming calcium chloride (for an untruncated jet according to the law $1/r^2$, for a vertically truncated one $1/r$). It is obvious that a decrease in the reagents concentration leads to the decrease in the reaction rate and the decrease in the concentration of the product.

Assumption 3. The motion of the other reaction component - PBS, and in particular Na_2 (HPO_4), can be described approximately by the flow model that flows around an angular structure with rigid walls and an angle α. The flow area corresponds to the angular range 135 degrees. The speed dependence on the distance traveled r is quite weak (the exponent is equal to π/α^{-1} or 1/3). The boundaries of the "trace" correspond quite well to the streamlines. Discrepancies may be related to the additional longitudinal and transverse diffusion of the reaction product.

Assumption 4. The diffusion coefficient is approximately equal to 7.6.10-10 m^2/s. The equivalent volume of the molecule ball has a radius of about 0.43 nm. Estimation of the diffusion coefficient in the Stokes-Einstein approximation at a temperature of 37°C can give the specified value of the

diffusion coefficient. During the motion along the "track" at a high velocity of about cm/s, the diffusion transfer is from one to several microns.

Assumption 5. To estimate the reaction rate constant we can approximate the corresponding value for the acid-base reaction in a buffer solution by the mechanism

$$A^- + H^+ \rightarrow P\,, \tag{10}$$

where the reaction rate constant is defined as $k = 2{,}05 \cdot 10^{13} \exp\left(\frac{-8681}{T}\right)\ l \cdot m^{-1} \cdot s^{-1}$.

For the considered temperature regime, this corresponds to $k = 14{,}13\ l\ \cdot m^{-1} \cdot s^{-1}$.

The calculation of the amount of reaction product that formed the "trace" can be made based on two options. Firstly, in case where the reaction rate constant is confirmed to be adequate, the final concentration of the reaction product, the number of calcium hydrophosphate particles in the trace zone, and the thickness of the particle layer at the bottom of the chamber can be calculated. Secondly, the calculation can be made on the basis of indirect estimates; at that one needs the amount of both reagents that went into the "trace" zone, the portion of reagents that reacted to form the product, the portion of the product that was not removed from the reaction chamber, but deposited to the bottom of the chamber. The last one requires comparison of the diffusion and convective times of the reagents' motion.

5. Results

From obtained results (Figure 3) we conclude that the observed spatial distribution of the reaction product (calcium phosphates) for small concentrations of $CaCl_2$ qualitatively corresponds to the streamlines when the liquid flows through the membrane cut. At the same time, in cases with higher concentration of $CaCl_2$ we observed the formation of dendritic structures with an increasing characteristic size of self-similar elements.

Mathematical modeling was used to explain the shape of the trace formed at the bottom of the microchip channel by the reaction product - calcium salts. Naturally, the similar mechanism of formation of calcium salts can be observed in a living organism during the AC development. Therefore the developed model can be further applied to describe these processes in capillaries.

Acknowledgments

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