

3D Design and Prototyping of a Microfluidic Device for Blood Cells' Investigations

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Abstract: The present study offers an important technological approach for the development of a disposable microfluidic channel using 3D nanoprinter - Photonic Professional GT2 (Nanoscribe, Germany). This publication aims to present 3D modelling, simulation, and prototype of a 3D nanoprinted microfluidic device for the investigation of blood cells. The design of 3D model of a microchannel is realized by the 3D CAD analysis software – SOLIDWORKS. A suitable laminar flow is generated by using computational fluid dynamics (CFD) software. As a result, the critical points of the pressure, velocity and wall shear stress into the microfluidic channel are obtained. An actual physical prototype of the proposed microfluidic device is developed, using a highly innovative technology of 3D nanoprinting by two-photon polymerization. Experimental studies with dilute erythrocyte suspensions are conducted to test the functionality of the developed real-world prototype of nano 3D printed microchannel.

Keywords: MICROFLUIDICS, MICROCHANNELS, 3D MODELING, 3D NANOPRINTING, TWO PHOTON POLYMERIZATION, RED BLOOD CELLS

1. Introduction

Microfluidics is the science and technology of systems that process or manipulate small (10^{-9} to 10^{-18} l) quantities of fluids using channels from tens to hundreds of micrometers in size [1, 2]. The first applications of microfluidic technologies are for the analysis, separation and detection with high resolution and sensitivity of various substances in blood rheology –hemorheology, and chemical analysis [2, 3]. In recent years, microfluidics has had wide application from fluid physics to the food industry, cell biology, biomedicine, pharmacy, etc. [4, 5, 6].

The microfluidic device (also referred to as lab-on-a-chip or micro total analysis systems – μ TAS) is one or some microfluidic channels with inlet/outlet openings to perform respective micro-functions and biomedical analysis [5]. It allows for single unit operation (e.g., mixing, separation, droplet generation, particle manipulation, heating, and detection) or incorporates multiple unit operations [3, 7].

One of the long-term, goals in the field of microfluidics is to create integrated and portable clinical diagnostic devices for home usage, eliminating time-consuming laboratory analysis procedures [8]. Through the application of microfluidic technologies, it is expected to improve the efficiency in the detection of biomolecules and the monitoring of individualized diagnostic and therapeutic studies *in vitro* and *in vivo* [8].

Hemorheological alterations in the majority of different diseases are connected with blood rheology disturbances, such as the increase of blood and plasma viscosity, cell aggregation enhancement, and reduction of the red blood cells (RBCs) deformability. Thus, the visualizations and measurements of blood cells' deformability, flowing in microfluidic devices, can provide vital information to diagnose early symptoms of blood diseases and consequently to be used as a fast clinical tool for the early detection of biomarkers. For instance, RBCs rigidity is correlated with myocardial infarction, diabetes mellitus, and hypertension, among other blood diseases [8, 9, 10].

Various microfluidic devices are used, in order to understand better the cells' behavior in blood vessels. The analysis of rheological properties of blood cells is of a great interest among the scientist of the biomedical field, since it is strongly dependent on the interactions and mechanical cells' proprieties [8, 9, 10].

The study of the rheological properties of blood and mimicking the RBC's flow at *in vitro* conditions is essential for the design, performance, and optimization of the microfluidic devices. Valuable progress in personalized medicine for new treatments and diagnosis approaches can be achieved by combining the unique features of hemorheology and microfluidic technology for single-cell analysis [8, 9, 10].

Microfluidic devices can be categorized into active and passive types, according to the fluid flow control principle [6]. The passive microfluidic devices (microchannels, microvalves, and microfilters), use physical properties, such as surface tension, capillary action, and gravity, to manipulate the fluid flow. These devices can automate simple operations such as fluid transport, separation, and mixing.

Active microfluidic devices use an external power source to manage the fluid flow and manipulate samples. These devices can be controlled precisely in dynamic mode by using electric, magnetic, thermal, or acoustic fields. They can automate complex operations such as sample manipulation, cell sorting, and drug screening with high throughput, but this process would require more complex manufacturing and control techniques. Usually, these devices are more expensive from the passive one [6].

Hybrid microfluidic devices combine both methods to achieve better functionalities. The hybrid microfluidic devices offer a versatile platform for different applications, including biomedical research, clinical diagnostics, and new drug discovery.

Microfluidic technologies have many useful characteristics such as: small size of microfluidic devices and usage of few very small amounts of samples and reagents (at microliters size), low price, short analysis time, small footprints for analytics devices, etc. These features offer fundamentally new possibilities for controlling the concentrations of molecules and cells in space and time [1, 3].

In general, two types of techniques can be used to manufacture microfluidic devices: subtractive and additive [11, 12, 13]. In subtractive techniques, the material is removed from a substrate to create microchannels and other features, whereas in additive techniques, the material is added layer by layer to build a three-dimensional structure [14]. Common subtractive techniques include hot embossing and laser ablation, while general additive techniques include 3D printing, soft lithography, and inkjet printing [15]. These techniques offer various advantages, including low-cost, rapid prototyping, and the ability to manufacture microfluidic devices with complex geometries [16, 17].

3D printers are a synonym for unique rapid prototyping devices. The affordability and speed of these printers allow their implementation in the field of mass production [17]. 3D printing, also known as additive manufacturing, has become one of the world's best tools for small-scale manufacturing [18].

Among the various 3D printing approaches, two-photon polymerization (2PP) enables the highest spatial resolution, with characteristics smaller than the Abbe diffraction [17, 18] limit that are produced due to nonlinear light-induced effects in the photosensitive material.

Micro 3D printing from 2PP finds many applications, in micro-robotics, biosensing, biomedical research, optical data storage, micro-optics, antireflective surfaces, photonic metamaterials, photonic crystals or mechanical metamaterials [18].

Nano-3D printing, is a hybrid process that combines digital light projection 3D printing with nanoscale-relief patterning. Nano-3DP enables a variety of novel applications with higher-resolution surface details than currently available [19].

All of the above said, the authors of these study dedicate their scientific efforts in the field of the microfluidics combined with 3D printing of special technical devices. Hence, the present study aims to create a design, simulation, and prototype of a 3D nanoprinted microfluidic device mimicking a postcapillary vessel for blood cells' investigations.

2. Materials and Methods

The design of 3D model of a microchannel is done by 3D CAD analysis software – SOLIDWORKS. A file of type STL (mesh format file) is generated on the basis of SOLIDWORKS software environment. For studying fluid flow dynamics, this file is very important during the transfer of complex 3D geometries into the computational fluid dynamics (CFD) software - Flow 3D.

A highly innovative two-photon polymerization (2PP) technology is used for 3D printing of the designed microfluidic device. The 3D nanoprinter - Photonic Professional GT2 (Nanoscribe, Germany) is applied for two-photon polymerization to produce a filigree structure from a 3D shape via high-precision 3D printing. 2PP 3D printing is a direct laser writing (DLW) technique in which a solid structure is written into a liquid resin voxel by voxel by scanning a femtosecond-pulsed tightly focused laser beam.

The 3D model of the microchannel, designed in SOLIDWORKS (.stl file) is imported into the specialized design Photonic Professional GT2 (PPGT2, Nanoscribe) software (Describe), which is used to select the recipe (printed material, substrate and resin type), mode of operation, dimensions of the printing layers.

In Describe a special job file is generated that is used as an input to the nanoprinting process. A glass substrate is fixed on a special holder. A drop of a photoresist suitable for working with glass substrates is put on the substrate. The holder is placed in the 3D printing machine (PPGT2 Nanoscribe). PPGT2 3D printer is controlled by an easy-to-use graphical user interface of the specialized software (NanoWrite) from Nanoscribe. NanoWrite is launched, which calibrates the overall system, loads the work file from Describe, and performs the nanoprinting operation.

After turning on the 3D printing process, the holder with the polymer microstructure is removed from the 3D printer. The glass substrate with the microchannel is washed exclusively with isopropanol solution and the geometry of the printed microchannel is analyzed under a microscope.

The manufactured microchannel is tested for patency by filling it with a diluted erythrocyte suspension. The flow of erythrocytes is created with a syringe pump and observed using a light microscope Primostar 3 Zeiss with integrated digital camera.

3. Results

Postcapillary blood vessel sizes are less than 1 mm in diameter. Multiple microfluidic channels with different heights and widths and different printing modes are designed and prototyped in order to mimic these post-capture venules.

In this study, a microfluidic device is presented that is most suitable for monitoring blood cells throughout the channel volume, and in close proximity to the channel walls. This is a necessary condition when covering the channel with endothelial cells or

adhesion molecules when creating *in vitro* experimental models of blood vessels.

The designed microfluidic device is elaborated with the following dimensions: height/width /length - 0.075 x 0.35 x 7 mm. A 3D model of the microchannel is created (Fig. 1) by using SOLIDWORKS software environment. The thickness of the wall of the microchannel is 0.05 mm. The height of the bottom of the channel is 0.02 mm.

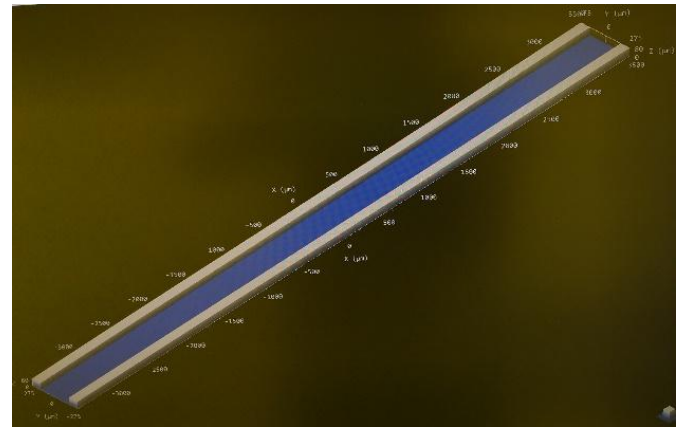


Fig. 1 3D model of the microchannel, generated in SOLIDWORKS.

The selected 3D design of microchannel should be tested for the suitable input fluid flow parameters, which will create the laminar flow that is required when studying dilute blood cell suspensions.

For this purpose, the .stl file created in the SOLIDWORKS is imported in Flow 3D (CFD software). A suitable mesh for simulation of the fluid flow in the microchannel is generated (mesh cell size of 0.025 x 0.025 mm). The flow through the microchannel is tested with a fluid - water, at a temperature of 20°C. The boundary conditions of the model are defined by setting a fluid flow rate of 0.026 mm³/s, which is obtained at a Reynolds number <1 (a necessary condition for laminar flow). The pressure contours (Fig. 2), x-velocity contours (Fig. 3) and wall shear stress contours (Fig. 4) are obtained as a result.

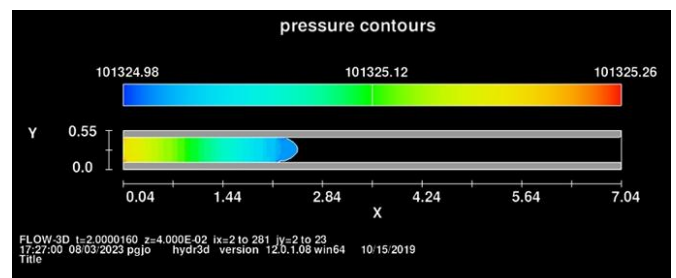


Fig. 2 Simulation results of the pressure (g/mm²) in the microfluidic channel.

From the simulation results, the pressure along the microchannel is approximately 101325 g/mm² (Fig. 2). In the central region of the flow, the fluid velocity increases to 1.41 mm/s, while at the channel walls it decreases to 0.02 mm/s (Fig. 3).

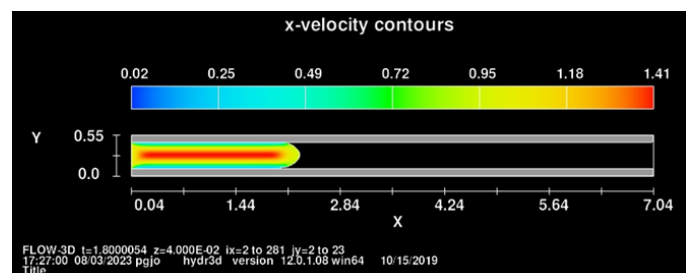


Fig. 3 Simulation results of the velocity (mm/s) in the microfluidic channel.

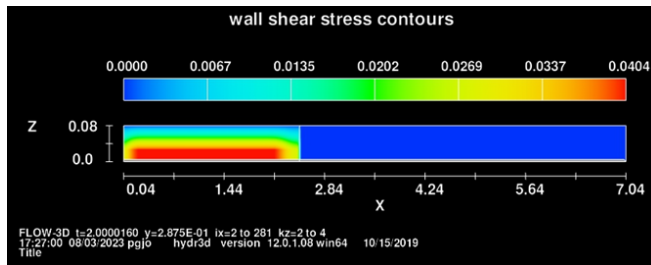


Fig. 4 Simulation results of the wall shear stress (g/mm/s^2) in the microfluidic channel.

The wall shear stress on the bottom of the microchannel has maximum value of 0.04 g/mm/s^2 (Fig. 4).

After simulating the fluid flow in the channel with the chosen geometry, the microfluidic device is constructed by nanoprinting with PPGT2 3D printer. The 3D model of the microchannel is imported in Describe using the .stl file. A special job file is generated in Describe, containing the whole information, regarding the recipe (printed material, substrate and resin type), mode of operation, dimensions of the printing layers. In this case, the recipe - IP-S 25x ITO Shell (3D MF) is used, which is suitable for printing on a glass substrate with an optical magnification of x25.

The Shell and Scaffold mode of operation is selected in order to reduce the printing time. The size of each layer is $1 \mu\text{m}$. A glass substrate is fixed on a special holder. A drop of a photoresist IP-S is put on the substrate. The holder is placed in the 3D printing machine and NanoWrite is launched for calibration of the whole system. The nanoprinting procedure is launched and the entire process can be monitored in real time using a special digital camera (part of PPGT2 3D printer) (Fig. 5). The Shell and Scaffold mode polymerizes the photoresist only along the skeleton of the selected network. In this way, the printing time is reduced, but the microstructure becomes more fragile.

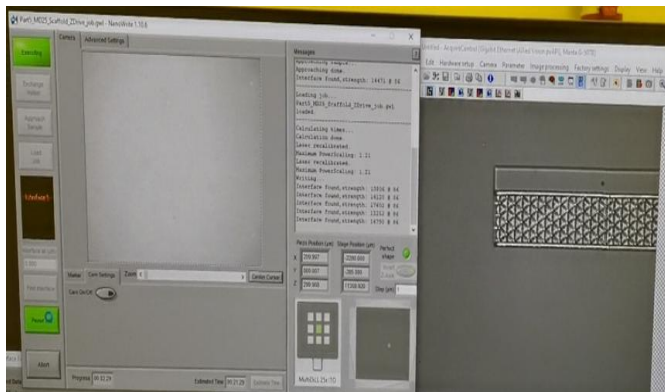


Fig. 5 Screenshot of the graphical user interface of the specialized software (NanoWrite) from Nanoscribe for control of PPGT2 3D nanoprinter (on the left) and visualization (part of the microchannel) of the nanoprinting procedure in real time (on the right) by a digital camera from Nanoscribe.

The holder with the polymer microstructure is removed from the 3D nanoprinter, after the end of the 3D printing process, when the 3D process is finished.

The glass substrate with the microchannel is carefully washed with a solution of isopropanol. The geometry and the size of the developed microchannel is studied under a microscope (Fig. 6).

Some defects of the geometry (blue circles – Fig.6) during the 3D nanoprinting procedure are clearly visible. The width of the microchannel is almost 0.37 mm measured by microscope Primostar 3 Zeiss. Future scientific work is related to the

optimization of the recipe and decreasing the number of defects in the microstructure.

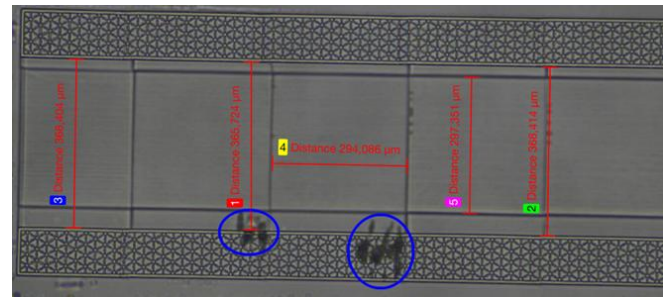


Fig. 6 Image from microscope Primostar 3 Zeiss, of the 3D nanoprinted microchannel.

The constructed microchannel is tested by filling it with a diluted erythrocyte suspension. The flow of erythrocytes is created with a syringe pump and observed using an optical microscope Primostar 3 Zeiss with integrated digital camera (Fig. 7). Experimental results with diluted erythrocyte suspensions show that the channel is passable and the flow through it is laminar.



Fig. 7 Image of the developed microchannel filled with a diluted erythrocyte suspension.

4. Discussion

3D printing has changed the field of microfluidics significantly and also created innovative microfluidic devices that are usually impossible to be designed employing other approaches. Three-dimensional printing allows science and technology to be transformed by producing custom-made low-cost equipment requiring specialized equipment [20].

So far, the 3D-printed microfluidic-based miniaturized devices have various biomedical applications. The group of scientists Yeong et al. fabricated porous scaffolds for cardiac tissue engineering by selective laser 3D printing technique [21]. Wu et al. fabricated 3D biomimetic microvascular networks embedded in the hydrogel matrix by multidirectional printing [22]. Towards the development of a rapid, sensitive and specific influenza virus detection system, Krejcova et al. have produced a 3D-printed bead-based microfluidic chip [23]. Among the innovators, Gowers et al are the first who developed a 3D-printed wearable microfluidic device integrated with clinical microdialysis probes and needle biosensors for continuous monitoring of human tissue metabolite levels [24]. Kadimisetty and others. have developed a novel, low-cost gravity flow microfluidic immunosensor for the detection of three cancer biomarker proteins [25]. Oh and others developed and elaborated a 3D-printed blood viscosity assay [26]. Chen et al. designed a 3D printed microfluidic device in order to isolate circulating tumor cells from human blood samples [27].

Visualization and measurements of blood cells flowing in microfluidic devices are of a great importance in providing not only essential information about the rheological properties of blood cells, but it is also vital information for diagnosing early symptoms of

diseases during clinical research. Microfluidic devices combined with flow imaging techniques can be used to assess the cardiac risk profile of a particular patient and help medical staff to decide on the appropriate treatment and therefore to increase the chances of patient's survival. Analysis of single cells, particularly red blood cells, provides unique and specific information on patient stratification, for example overall outcome of clinical care and treatment of diabetes, hypertension, among other blood diseases [8, 9, 10].

The present study offers an important technological approach for the rapid production of low-cost microfluidic channel with the help of which the rheological properties of blood cells can be studied in various diseases. By innovative two-photon polymerization (2PP) technology 3D nanoprinting microfluidic device was suggested and constructed. Our proposed 3D microchannel design and prototype can be embedded in a holder with micro-insert, connected to a micropump and microscope, forming an active microfluidic system. The nano 3D printed channel can be used to evaluate hemorheological properties - erythrocyte aggregation and deformability. The channel walls can be coated with suitable cells or adhesion molecules to create *in vitro* experimental models imitating *in vivo* conditions in blood vessels—for the purposes of laboratory and clinical practice.

5. Conclusion

The microfluidic device is developed for the study of the rheological properties of the blood. Every stage of 3D modeling and simulation of the prototype is described. The simulation results of the pressure, velocity, and wall shear stress measured in the microchannel are of a great importance for laminar flow generation. Innovative technology such as two-photon polymerization is used in the 3D nanoprinting process with extremely precise PPGT2 Nanoscribe equipment. Experimental studies with dilute erythrocyte suspensions are conducted to test the functionality of the developed real-world prototype of nano 3D printed microchannel.

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