Selective adsorbtion of low density lipoprotein from blood using porous silicon

Praveen Kumar S*, Ramesh R, Aravind T

Department of Electronics and Communication Engineering, Saveetha Engineering College, Thandalam, Chennai 602105, Tamilnadu, India

*Corresponding author

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Abstract

This work presents the isolation of Low Density Lipoprotein (LDL) from blood. The blood molecules with size less than 1µm are filtered in the size based crossflow filter technique. Filtered molecules are passed through the Porous Silicon (PSi). The immobilized Anti-Apolipoprotein B (AAB) on the surface of Porous Silicon is used to adsorb the LDL. Isolation of LDL based on Porous Silicon eliminates the calculation of the other lipoproteins and Triglycerides. Filter with AAB coated PSi is designed and simulated in COMSOL Multiphysics software. Copyright © 2018 VBRI Press.

Keywords: Low Density Lipoprotein, Crossflow filter, Size based separation, Porous Silicon

Introduction

The major lipids present in the blood plasma are Cholesterol, Fatty acids, Triglycerides and Phospholipids. Cholesterol synthesized from the liver in association with the proteins is transported through the plasma that forms Lipoprotein. The Lipoproteins are classified as Very Low Density Lipoprotein (VLDL), Intermediate Density Liporprotein (IDL), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) based on their size. The function of LDL is to provide cells with cholesterol which is needed for steroid hormone synthesis and membrane formation. The size of the LDL is globular in shape with the average diameter of about 22nm to 27nm**[1]**. This LDL is referred to as Bad Cholesterol because when they invade the damaged endothelium cell on blood vessels, they get accumulated and causes Atherosclerosis. So the LDL quantity should be maintained. As per National Cholesterol Education Program (NCEP), the normal level of LDL should be <120 mg/dL (<3.34 mmol/L). However the detection of LDL level in blood remains a challenging task.

In convention techniques, the LDL is detected using Friedwald equation by subtracting the High Density Lipoprotein (HDL) and Triglycerides from the total Cholesterol **[2]**. But this method does not predict the accurate level of LDL. Langmuir – Blodgett films of Polyaniline is used for detection of LDL by immobilizing the Anti-Apolipoprotein B (AAB) on the film which can adsorb Apolipoprotein B **[3]**. It is found to detect LDL upto 0.39µm (130 mg/dl) with sensitivity of 11.25Kohm.µm^{-1}. The Nickel Oxide (NiO) thin film can also be used to detect LDL by immobilizing AAB [4] with sensitivity of 12Kohm.µm-1. NiO thin film based LDL detector can be used for 15 times without any degradation in the performance. Biosensors can also be used to sense LDL **[1]**.

Amount of harmful cells in the blood can be estimated by removing the cell selectively. Size based crossflow filter is the emerging technology because of its high accuracy. The Circulating Tumor Cells (CTC) from the blood is filtered by allowing the blood sample to flow through the microfluidic device in which the filter made of polyethylene glycol diacrylate (PEGDA) **[10]**. The shape of the filter is conical in order to ensure that no blood molecules get clog when passing through the filter. The CTCs also can be extracted by using the Polydimethylsiloxane (PDMS) microfiltration membrane (PMM). The thickness of PMM is 60um and the hole size is about 6.9-10.8µm and the center to center distance is 25µm **[11]**.

In this work, the PSi is used as the platform to detect the LDL. Formation of porous membrane on the silicon wafer is first made possible by Uhlir **[6]**. He found the porous formation accidentally when trying to polish the surface of the silicon. AAB cannot be directly bonded on the PSi layer. To bond the AAB, the PSi need NH_2 ion group [3 & 4]. To form NH_2 group on the outer shell, PSi layer should be oxidized and Amino Silanized **[5]**. Pore size and porosity is the major concern in the PSi. Pores in the silicon wafer can be formed by electrochemical etching process. The pore size and porosity depends upon the type of wafer and its resistivity, Electrolyte (Concentration of HF (Hydroflouric acid) and Ethanol), applying current and etching time **[7]**. By applying 30mA/cm^2 current with 15% of HF in electrolyte form pore size of 50nm to 75nm with porosity of 62% **[8]**. The double tank electrochemical cell can be used to create pores in sapphire wafer (double side polished) and this needs back side illumination. It is found that uniform

pores is formed in P-type wafer when compared to N-type wafer and also hole diameter and length also increases in P-type wafer **[9]**.

In this paper, the cross-flow filter with membrane pores of 1µm size is designed to separate the molecules with size less than 1µm from the whole blood. Barrier is developed in the microfluidic channel to ensure the blood flows close to filtering membrane layer. AAB, which is the antigen of LDL, is coated on PSi layer and placed below the membrane layer in order to adsorb LDL from the filtered molecules. Microfluidic device for filtering LDL molecules is virtually fabricated. This microfluidic device with different barrier length is designed, simulated and analyzed its velocity profile for better filtering efficiency.

Design concept

Microfilter structure

Size based crossflow microfiber is the emerging and useful technology in the field of medical science. Pores are created based on the size of molecules to be filtered. The size of LDL is 22-27nm. But the blood has many other components with size in the range of 1-21nm. Hence it is not possible to filter LDL using size based filtration technique. The blood with nano sized molecules are filtered first and then the LDL is selectively extracted by using AAB coated PSi layer. This increases the efficiency. The structure of the filter with AAB coated PSi layer is shown in **Fig. 1**.

The **Fig. 1** shows the structure of filter with three chambers. The chamber 1 is the microfluidic channel in which the blood flows from inlet to outlet. The chamber 1 also has barrier to make the blood flow closer to the membrane layer for effectively filtering of components. The chamber 2 is the filtering chamber. The molecules with size less than 1um are passed though the center membrane layer. The substrate of the filter is made of Porous Silicon which is coated with AAB in chamber 3. The filtered blood interacts with AAB coated PSi which leads to the adsorption of LDL. The remaining nanosized molecules such as glucose, aminoacids, etc., are removed away through the outlet. Then the LDL adsorbed PSi is taken away and processed for calculation of LDL.

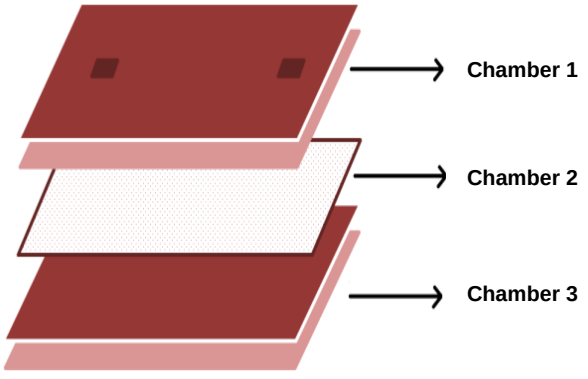

Fig. 1. Structure of membrane filter.

Porous silicon formation

The porous silicon can be used to adsorb the LDL molecule selectively. The pores in the silicon wafer are formed by using the Eletrochemical cell. Single sided p-type wafer with boron doped in <100> orientation can be used as the substrate because the hole concentration in p-type wafer is more and the holes formation is uniform. The electrochemical etching is carried out in Teflon cell. The electrolyte is prepared from 48% aqueous HF solution and ethanol in the ratio of 3:1 and it is poured into the teflon cell. The bottom of the wafer has metallic contact which acts as anode. The platinum mesh is used as the cathode to provide power supply and this mesh is immersed in the electrolyte solution. 30mA/cm^2 current is applied through platinum mesh for 15 mins. This can form pore size of approximately 50-75nm **[5,7]**.

Immobilization of AAB on PSi

To immobilize the AAB, PSi needs NH_2 ion group. So the PSi needs to be oxidized. This increases the hydrophilic nature of PSi, because the water based buffer solution cannot infiltrate through the pores. Then the oxidized PSi layer is Silanized. Further AAB is prepared using 0.4M N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC) and 0.1M N-hydroxy-succinimide (NHS) solution. The prepared AAB is poured thus the AAB is bonded on the surface of PSi.

PSi is washed thoroughly with Phosphate Buffer Saline (PBS) to remove any unbounded or loosely bounded particles. This process is schematically shown in **Fig. 2**. The prepared PSi is kept at 4^{0}C when not in use **[3,5]**.

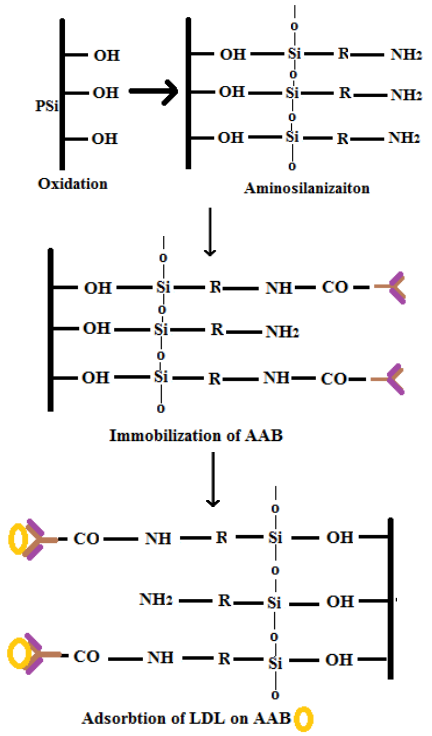

Fig. 2. Immobilization of AAB on PSi

Material selection

As the filter is used for blood cell separation, the material should be biologically compatible. Many bio-compatible materials are available to fabricate the device. Among all, PDMS is the most commonly used because of its high bio-compatibility, more economical and readily available. So PDMS layer is used as the filter layer. The bottom of the filter has AAB coated PSi layer. The porous silicon layer increases the surface to volume ratio. So large number of LDL molecules can get adsorbed. The remaining chambers are made from glass. PDMS, PSi and glass are bio-medically more compatible. The properties of PDMS and Silicon are given in below **Table 1**.

Table 1. Properties of PDMS and Silicon.

Properties	PDMS	Silicon
Density	0.97 Kg/m^3	2329 Kg/m^3
Dynamic Viscosity	1.45e3 Pa.s	0.8 Pa.s
Relative Permittivity	3.5	11.7
Electrical Conductivity	4e13	1
Young's modulus	0.61e6 Pa	170e9 Pa
Poisson ration	0.5	0.28
Thermal Conductivity	0.15 W/mK	130 W/mK

Virtual Fabrication

Preparation of membrane layer (PDMS)

The silicon wafer (p-type, <100> orientation and 475µm) is used to create the mold for PDMS using photolithography. The silicon wafer is spin coated with positive photoresis material. The glass mask is used to transfer the pattern on the silicon wafer by UV rays. The silicon wafer is etched with deep reactive ion etching (DRIE) and this result in master mold.

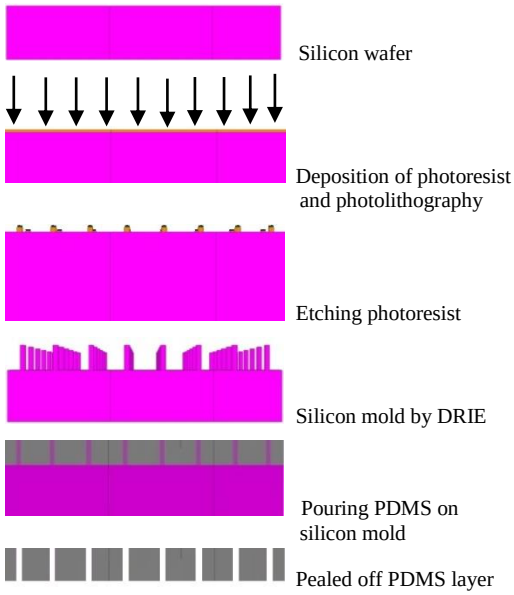

Fig. 3. Fabrication of PDMS membrane layer.

The silicon master was silanized with anti-sticking agent in desiccators overnight. Then the PDMS is prepared by mixing the PDMS prepolymer and the curing agent in the ratio of 10:1 by weight. Prepared PDMS is poured on the master mold and cured at 75ºc for 2hrs. The cured PDMS layer is pealed off from the master mold and forms the membrane layer. This PDMS layer acts as a filter for filtering the molecules less than 1µm.

Microfluidic device

The silicon wafer is adhesively bonded with glass using BCB (Benzocyclobutene). Initially, the chrome is deposited by sputtering on the glass to protect the glass during wet etching. Positive photoresist material is spin coated on the top of chrome which allows the pattern to transfer on the chrome during photolithography. Glass is anisotrophically etched by wet etching technique using HF (Hydroflouric) acid and then the chrome is etched by electrochemical etching. The glass is plasma treated with O_2 (30W, 160mTorr, 30s). Plasma treatment of PDMS layer is also done. The plasma treated glass chamber and PDMS are tightly bonded. PDMS layer is covered with corning glass and packed together to form a microfluidic device.

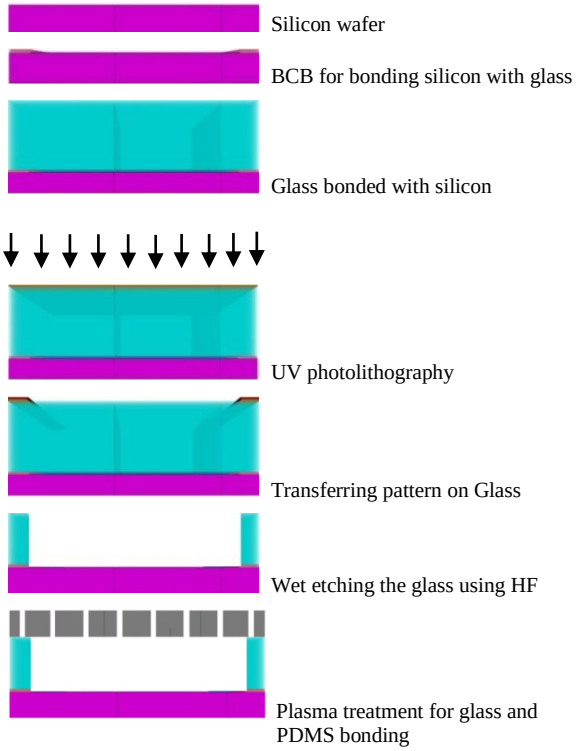

Fig. 4. Fabrication of microfluidic device.

Results and discussions

The laminar flow module is used to find the velocity (flow rate) of fluid through the filter. Dimension of inlet and outlet1 is 250µm wide so that all major molecules of blood can enter into the filter. The chamber 1 and chamber 3 is 3mm long and 450µm deep. The outlet2 is

100µm wide. The middle filter layer is 50µm thick and the bottom porous silicon is 50µm thick. The pores in the filter layer is 1µm wide through which the molecules with size <1µm will get pass to outlet 2. The filter structure is drawn in 2D module. The bottom substrate has Porous Silicon layer with AAB coated. The inlet velocity is set to 0.0015 m/s (0.2ml/min).

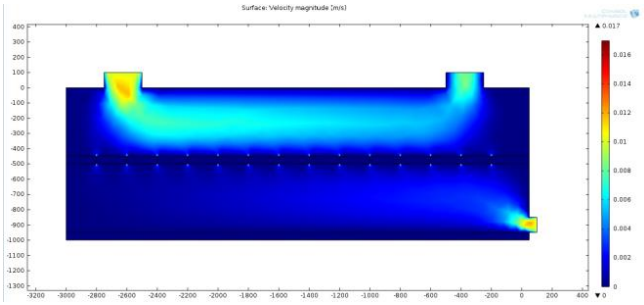

Fig. 4. Velocity profile of filter.

Fig. 4 shows the vel ocity profile of the blood through the cross flow filter. From the result, it is clear that the blood flow is not closer to the membrane filter. As it is not closer, the cell separation is not efficient because some molecules will get escape to outlet1. In order to have efficient filtration, the barrier is built in chamber 1. The barrier makes the blood to flow closer to the filtering membrane. Thus the efficiency is increased. The location of barrier decides the efficiency of particle separation. The structure with barrier closer and away from the membrane is designed and shown in **Fig. 5 and 6**.

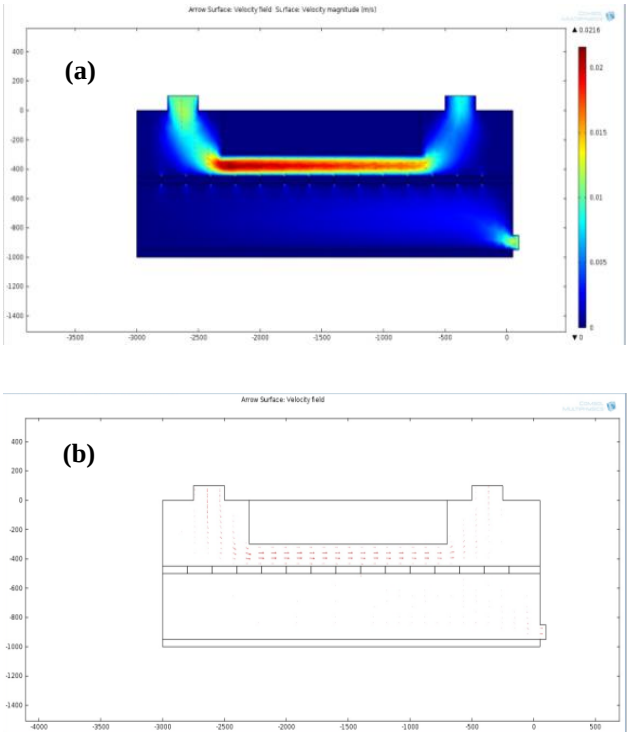

Fig. 5. Filter with barrier closer to membrane (a) Velocity profile (b) Blood flow line diagram.

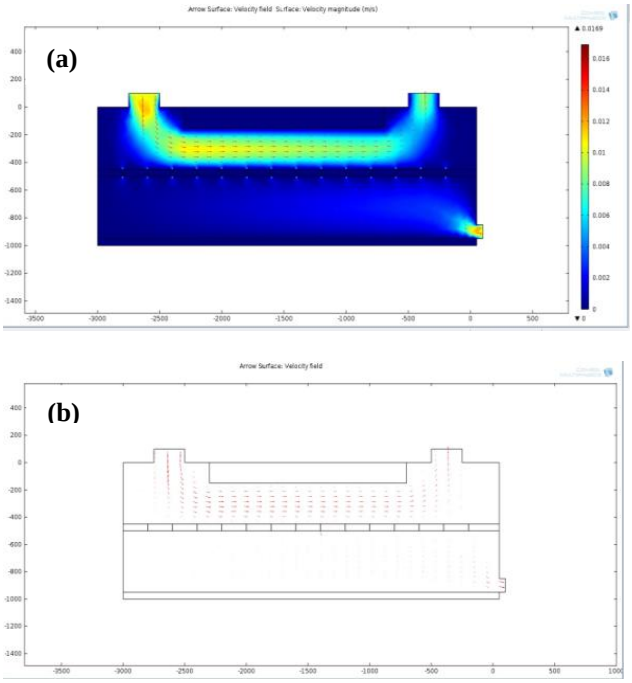

Fig. 6. Filter with barrier away from membrane (a) Velocity profile (b) Blood flow line diagram.

The **Fig. 5 and 6** shows the velocity flow and blood movement in the filter with barrier closer and away from the membrane layer. When the barrier is closer to the porous membrane layer, velocity flow is high. So the particles to be filtered move faster. So particles filtering efficiency will be low.

When the barrier is not closer to the membrane layer, the blood flows with reduced velocity. Since the particle movement is slow, more number of desired size particles can be filtered. The blood movement in the chamber 3 is more in this structure and this is shown in **Fig. 6(b)**.

The velocity at the channel, maximum velocity attained and velocity at the outlet for various filter structures is noted from the simulation result and graphically shown below in **Fig. 7 and 8**. **Fig. 7** shows the velocity magnitude in filter l without barrier and **Fig. 8** represents the velocity magnitude in filter with barrier 150µm and 250µm away from the membrane layer.

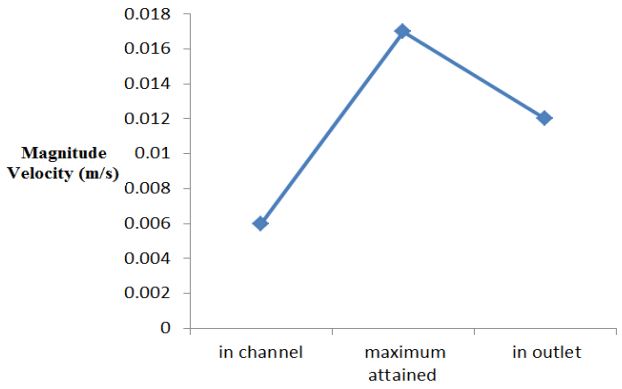

Fig. 7. Velocity magnitude in filter without barrier.

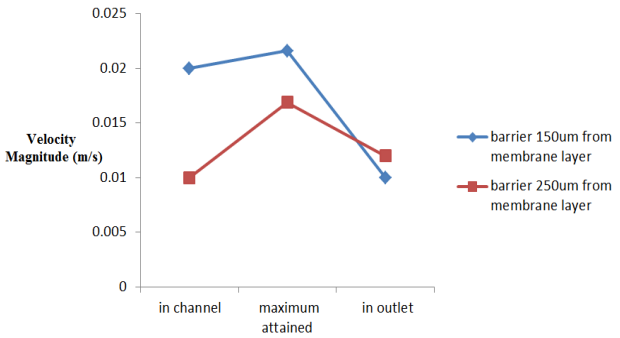

Fig. 8. Velocity magnitude in filter (a) 150µm gap (b) 250µm gap.

The filtered molecules flow towards the outlet 2 through the chamber with PSi. So the LDL molecules of size 22-27nm enters the pores in the membrane filter. When going towards the outlet 2, it gets adsorbed on the AAB coated PSi layer. Then this PSi layer analyzed for the detection of LDL molecule adsorbed.

Particle Trajectory module is used to demonstrate the particle filtration through the membrane layer and the adsorbtion and it is shown in **Fig. 9**. The molecules with size less than 1µm is filtered through the membrane layer and the filtered molecules are allowed to flow upon the porous membrane where the AAB is coated. The LDL molecules gets adsorbed on this porous layer and the residue molecules are left away through the output 2. The adsorbed molecules if processed further to calculate the quantity of LDL.

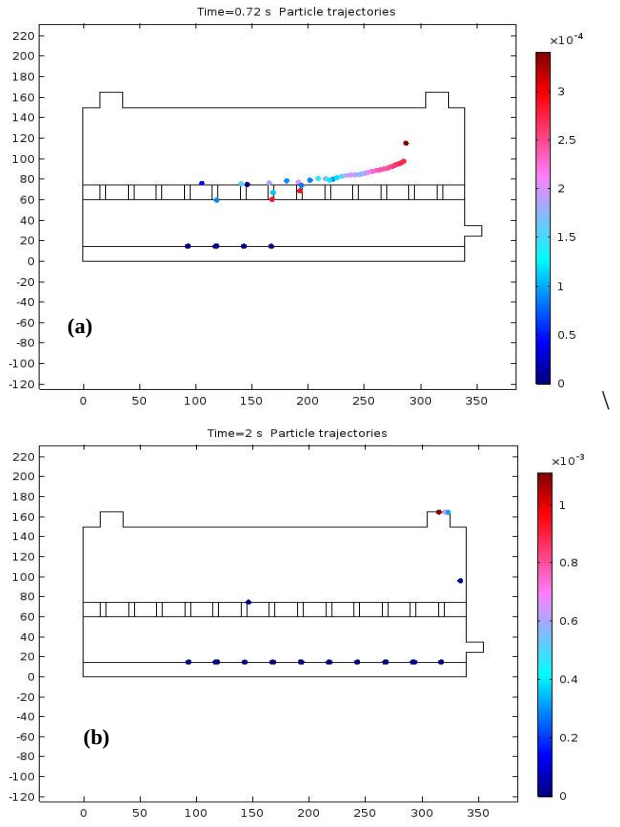

Fig. 9. Particle Trajectory module (a) Filtration of molecules (b) Adsorbtion of LDL on porous layer.

In order to increase the filtering efficiency, the flow in the channel should be laminar. Because in the turbulent flow, the flow pattern of the fluid is unpredictable. Whereas, in laminar flow, the flow pattern of the molecules can be predicted. So we should predict the flow pattern. In microfluidics, to have laminar flow, Reynolds number should be less than 100 else flow will be turbulent. Reynolds number is given as

$$R_e = \frac{\rho v L}{\mu} \quad (1)$$

where ρ is density of fluid, v is the velocity of fluid through the channel, L is the length of channel and μ is the absolute viscosity of fluid.

As the device deals with the blood molecules, density of blood is considered which is 1025kg/m^3. The inlet velocity of fluid is 0.0015 m/s (which is 0.2mL/min). The length of the channel is 150um. The absolute viscosity of fluid (blood) is 3.5 x 10^{-3} Pa.s. By using the above values, Reynolds number calculated is 53.98. The calculated Reynolds number value is less than 100. This indicates that the flow will be laminar. As the flow is laminar, the filtering will be more efficient.

In microfluidics, the flow through the microchannel will has some loss. This loss can be due to friction or this loss can be due to inlet to outlet pressure changes. When the blood flows through the microchannel, as the blood touches the wall of the microchannel, there exists friction. Due to this friction, loss occurs. The friction for the fully developed laminar flow is

$$f = \frac{24}{R_e}(1 - 1.3553\,a + 1.9467\,a^2 - 1.7012\,a^3 + 0.9564a^4 - 0.2537\,a^5) \quad (2)$$

Where R_e is Reynolds number, a is aspect ratio. Aspect ratio depends upon the width and depth of the channel and can be calculated as $a = \frac{\text{depth}}{\text{width}}$. For microchannel with length 3000µm and depth 450µm, the aspect ratio calculated is 0.15. With aspect ratio and Reynolds number, friction factor calculated is 0.018385. The loss due to the pressure changes is directly proportional to the friction factor, density of fluid, velocity and inversely proportional to hydrodynamic diameter. The hydrodynamic diameter is given as $D_h = \frac{2wd}{w+d}$ Where w and d is the width and depth of the channel. Hydrodynamic diameter is calculated to be 782.60µm. The pressure loss for rectangular microchannel can be estimated as

$$\Delta P = 2f\frac{L}{D_h}\rho U^2 \quad (3)$$

where L is the length of the microchannel, P is the density of fluid, U is the velocity of fluid and D_h is the hydrodynamic diameter. With the calculated value, the pressure drop calculated is 3.18 Pa. The loss coefficient due to pressure drop can be calculated using formula,

$$K_a = \frac{2.\Delta P}{\rho.U^2} \quad (4)$$

With the pressure drop (8.279 Pa), density of fluid (blood density is 1025kg/m3) and velocity (0.0015m/s), the loss coefficient due to pressure drop is 2.765. The calculation done above gives the approximate loss value.

Conclusion

In this work, the crossflow filter with PSi layer is designed and simulated. The molecules with size less than 1µm are filtered through the membrane layer which is made of PDMS. LDL is selectively adsorbed from the filtered blood by allowing the blood to flow through the PSi. The crossflow filter is mathematically analyzed for Reynolds number and loss due to friction and pressure drop. The Reynolds number of 53.98 is calculated for the filter and the loss due to pressure drop computed is approximately 2.765.Virtual fabrication of the microfluidic device is done and the filtering device with PSi layer can be fabricated to selectively adsorb the LDL in future.

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Supporting information

Supporting informations are available from VBRI Press.

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