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Fabrication and Testing of Microfluidic Devices for Blood Cell Separation

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Abstract Blood separation is a strategic preliminary step in preparation to on-chip biological analysis. Two microfluidic devices for on-chip blood separation are presented. Both devices will be integrated to form the separation module of a Lab on Chip for non-invasive prenatal diagnosis. In the first device, a blood plasma separator, the separation of blood cells from plasma is made possible in microchannels by bio-physical effects such as an axial migration effect and Zweifach-Fung bifurcation law. Behaviour of mussel and human blood suspensions were studied alongside the effect of different geometries. The second device aims to separate fetal nucleated red blood cells based on their magnetic susceptibility. Biocompatible materials are used in the manufacturing of both devices.

Keywords: microfluidic devices, blood separation, SU8, microchannel.

1. Introduction

The ability to accurately detect and separate various biological particles from a wide variety of samples including blood, urine, and saliva is an essential process in clinical and research activities. Analysis of a pure cell population from a complex biological system or blood plasma can lead to a greater understanding of disease pathology and help in the development of new therapeutics. Microfluidics permits the analysis of a few microlitres of biological samples, and avoids the painful blood extraction of several dozens of millilitres. Centrifugation and filtration are the two conventional techniques for blood separation in laboratories. Both methods are time-consuming, relatively expensive and might damage cells if not used carefully (Sallam, 1984). In light of the disadvantages outlined above and the benefits that microscale techniques can provide in terms of volume of blood extracted, response time and portability, it is therefore unsurprising that the alternative blood separation on-chip has gained increasing interest over the last few years. Different types of force available at the microscale have been used to separate blood on-chip. Such forces are electrical, magnetic and acoustic (Pamme, 2007; Kersaudy-Kerhoas, 2008). This paper describes the fabrication and testing of two microfluidic devices that are to form the separation module of a lab-on-a-chip for non-invasive prenatal diagnosis (NIPD) as shown in Figure 1. The microsystem envisaged will be a disposable cartridge with integrated modules for separation, preparation and analysis of fetal genetic material from maternal whole blood.

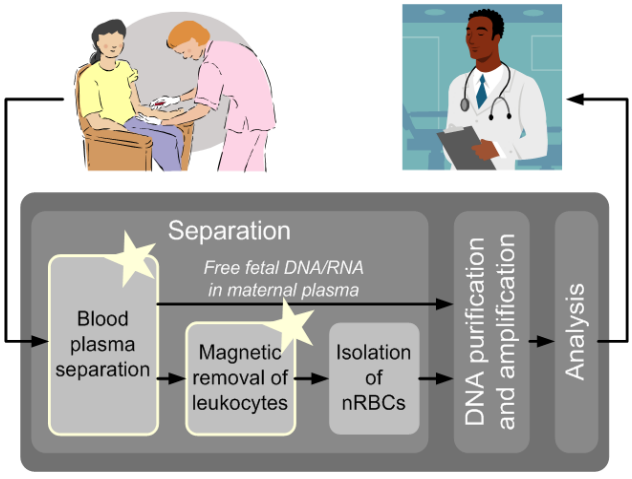

Figure 1: Lab on Chip flow diagram for NIPD. This diagram shows the different steps of a NPID system from the patient to the results. Nucleated red blood cell (nRBC) target fetal cell for analysis. This paper deals with the first two steps in the separation part.

2. Blood Plasma Separator

Firstly plasma is removed from maternal blood to be analysed for cell free fetal nucleic acids and screened for pregnancy indicator proteins. For example low levels of pregnancy associated plasma protein A (PAPP-A) are associated with certain fetal chromosome abnormalities (Cunniff, 2004). Removing the plasma at this stage reduces the levels of blood inhibitors such as immunoglobulin G (IgG) which can affect downstream analysis (Al-Soud *et al*., 2000).

2.1 Theory and Design.

Separation techniques using only hydrodynamic forces, channels geometries and bio-physical effects have lately been developed, based on preliminary work on microcirculation (Fung, 2004).

The design of an optimised blood plasma separator is based on an original in-house device (Kersaudy-Kerhoas, 2008). The use of microchannels ensures laminar flow and the presence of a particle-free layer. Constrictions inducing a high-shear stress zone pulling the particles even more centrally are integrated along the main channel (Faivre, 2006); four bifurcation channels are placed on each side of the main channel after each constriction. Red blood cells exhibit a specific behaviour in bifurcations. Fung demonstrated that cells at a bifurcation have a tendency to travel to the highest flow rate daughter channel, providing that the flow rate ratio is at least 2.5:1 and the dimensions of the cells are comparable to the channel diameter. This effect is sometimes referred to as the Zweifach-Fung bifurcation law. Its origin is found in the asymmetrical distribution of pressure and shear forces on the cell at the bifurcation, pulling it to the channel with the highest flow.

The overall aim of the system is to reach optimum purity efficiency, E_p, defined as:

$$E_p = 1 - \left(\frac{c_p}{c_f} \right) \quad (1)$$

where c_p and c_f are the number of events per mL in the plasma collection outlet and in the feed inlet, respectively.

As shown in Figure 2, the external connection consists of only one inlet to introduce the diluted blood and three outlets. One of these outlets collects the cells and the other two collect the plasma.

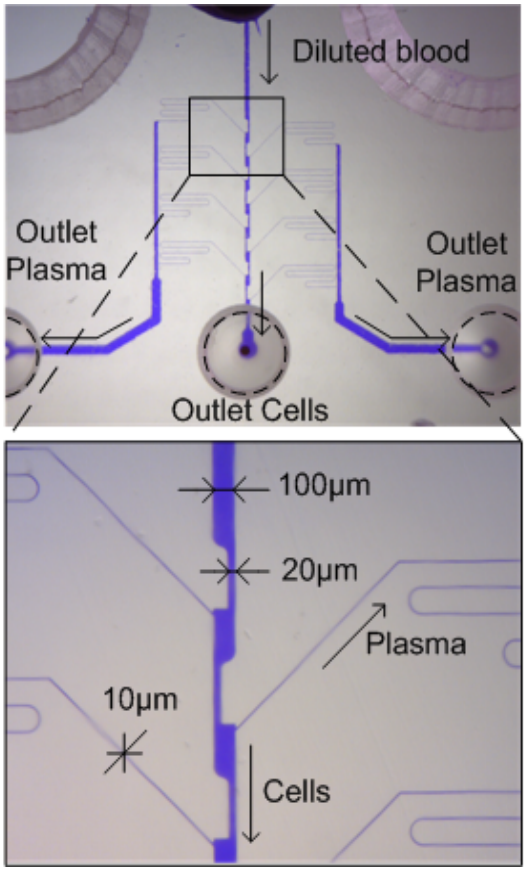

Figure 2: Optimised design of the blood plasma separator filled with a blue dye.

2.2 Fabrication and Testing.

Epigem Ltd (Redcar, UK), our industrial partner, has manufactured the chips using a standard lithography process. A PMMA lid and base layers are produced in order to create a bonded chip laminate. On top of the base layer is a further layer of SU8, into which are defined by photolithography the channel features. Further processing is carried out in the clean room following well defined

procedures. A photograph of the fabricated microfluidic chip is shown in Fig. 3

Mussel blood was used in preliminary testing of the device as it was easily obtained and the cells have relative sizes comparable to human blood cells. Human blood was collected from a healthy volunteer by finger prick using a sterile disposable lancet (Accu-check, Roche, Switzerland).The collected blood was suspended in 1mL of anticoagulant solution composed of Phosphate Buffered Saline (PBS) 1x and 10µL of ethylene diamine tetraacetic acid trypsin (EDTA-trypsin) (0.05M, Gibco). 200µL of the sample collections (cell and plasma) were screened using a flow cytometer (FC) (Partec CyFlow® SL, Germany). Flow cytometry allows accurate analysis of cell and particle populations.

The graph shown on Figure 3 is obtained from the FC results on the original design pictured at the top of Figure 3 (with 20 bifurcations on each side of the channel and only one constriction) and shows the number of events counted by the FC versus the size of the particle. The results, shows a purity efficiency of 56.6% at a flow rate of 20mL/hr.

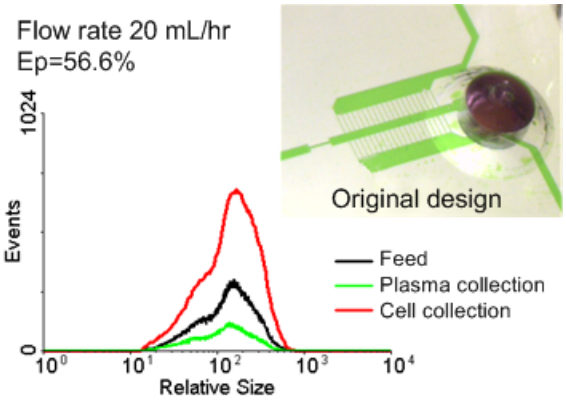

Figure 3: Flow Cytometry results. Graph of number of events in the feed, plasma and cell collection, versus the size of the particles in the original design (Kersaudy-Kerhoas, 2008).

The optimised design featured on Figure 2 is expected to give higher purity efficiencies at lower flow rates.

3. Micromagnetic Separator.

Magnetic separation of a pure cell population from a heterogenous starting sample is a well established technique in biomedical research. Superparamagnetic beads are commonly labelled with antibodies to specific receptors expressed on the surface of target cells. In most cases the use of beads is necessary in order to achieve magnetic separation. Recently however separation strategies have been investigated that do not require the use of expensive cell labels. One such method is magnetophoresis, which refers to the separation of biological cells based on their intrinsic magnetic properties (Zborowski, 2003). The use of magnetophoresis to separate blood has many advantages which include: low-cost manufacturing, simple structure, non-contact separation technique, hydraulic nature of magnetic field. Macroscale magnetic separators have had limited success as the generated fluxes are too small to have an effect on non-labelled biological cells (Han & Frazier, 2006; Pamme, 2006). To overcome this issue a micromagnetic separator is proposed in this paper for the separation of fetal nuleated red blood cells from maternal blood.

3.1 Theory and Design.

Most biological cells are diamagnetic by nature, however, deoxygenated red blood cells (RBCs) become paramagnetic due to the chemical interactions between the iron atom, the heme group and the globin domain of hemoglobin. In oxygenated hemoglobin the chemical bonds are covalent but in deoxygenated hemoglobin the bonds are ionic with 4 or 5 unpaired electrons present making it paramagnetic (Zborowski, 2003; Pauling, 2003). The magnetic susceptibility of deoxygenated RBCs in plasma has been noted in the literature to be approximately 3.9 10^{-6} (SI), which is much larger than other blood cells (Han & Frazier, 2006).

The proposed micromagnetic separator consists of a single microchannel, 100µm wide, with one inlet and 3 oulets. Electroplated

permalloy $Ni_{80}Fe_{20}$ elements are positioned in close proximity to the channel wall (Fig 4a). The fluidic channel has 2mm circular reservoirs for attachment of fluidic connectors. The external magnetic flux is provided by permanent neodymium iron boron (NdFeB) magnet (Fig 4b). The external magnetic flux provided by the permanent magnet magnetises the NiFe elements, creating a non-uniform field, which gives rise to a magnetic force on the deoxygenated blood as it flows through the microchannel (Fig 4c). Therefore, the magnetic forces in combination with the microchannel geometry allow blood cell separation.

3.2 Fabrication and Testing.

The micromagnetic separator is fabricated through micromachining of the photoresists AZ9260 and SU8 on a 50µm thick Kapton polyimide substrate. A glass wafer is used as a carrier substrate for the polyimide. Firstly, seed layers of titanium and nickel are deposited onto the polyimide substrate, using electron beam evaporation, to allow for uniform electroplating across the surface of the polyimide. The positive photoresist AZ9260 is spin coated and baked in 4 layers to achieve the required magnetic element height of 100µm. AZ 9260 is patterned with UV light with a Tamarac mask aligner at an exposure dose of 2000mJ/cm^2 followed by 20 minutes of development in AZ developer. The $Ni_{80}Fe_{20}$ permalloy is then DC electroplated. Once done, the AZ photoresist is stripped with acetone and the seed layer is etched.

The microfluidic channel is fabricated with the negative photoresist SU8-50 through UV photolithography. A layer of 100µm thick of SU8 photoresist is spin coated on top of the electroplated elements. A soft bake process at a temperature of 65^o C for 2 minutes and than 15 minutes at 95^{o}C is performed followed by sufficient cooling and relaxation time intervals. UV lithography with a Tamarac mask aligner is performed at an exposure dose of 960mJ/cm^2 with an I-line filter. The wafer is then post baked at 55^{o}C for 2 minutes and after sufficient relaxation time interval developed in EC solvent using a magnetic stirrer system for about 15 minutes. To seal the microchannels, SU8-50 is spun onto a similar polymer substrate and soft baked at 65^{o}C for 2 minutes and 95^{o}C for 5 minutes to form the cover for the microchannels.

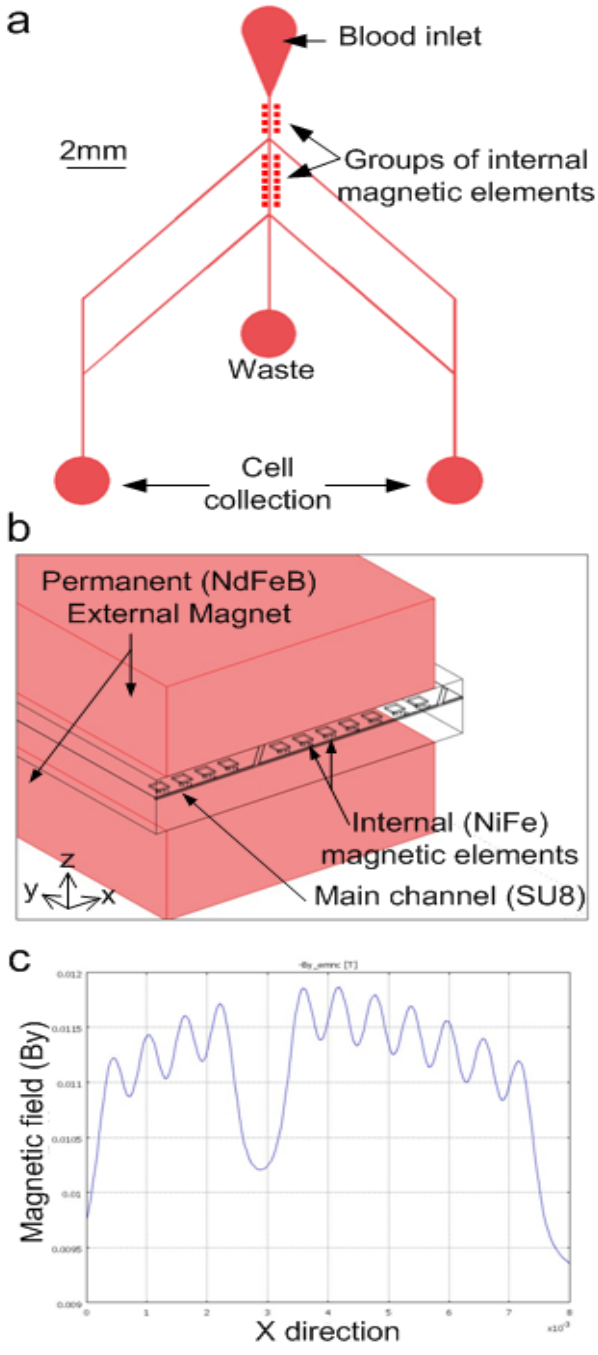

Figure 4: (a) Top view of the micromagnetic separator. (b) Cross-section in the (zx) plane of the 3D model. showing permanent magnet position to chip (c) The Y component of simulated magnetic field in the channel.

The substrate with the microfluidic channel is then bonded with the cover substrate. Exposure of the bonded structure is done through the backside of the adhesive layer. This is followed by a post exposure bake to complete the cross-linking at a temperature of 55^{o}C and with a force of 2Kgf for two minutes. The bonded substrates are then diced to form individual microfluidic channel packages of 40mm x 30mm. Figure 5 shows SU8 microchannels before bonding, auxiliary structures such as moats and stress relief lines are incorporated into the design to help with bonding and reduce cracking. PDMS connectors are then glued to the reservoirs at the end of the microfluidic channels and attached to a low velocity pump. The functionality of the prototype device will be tested using human blood and goose blood as models for fetal cells.

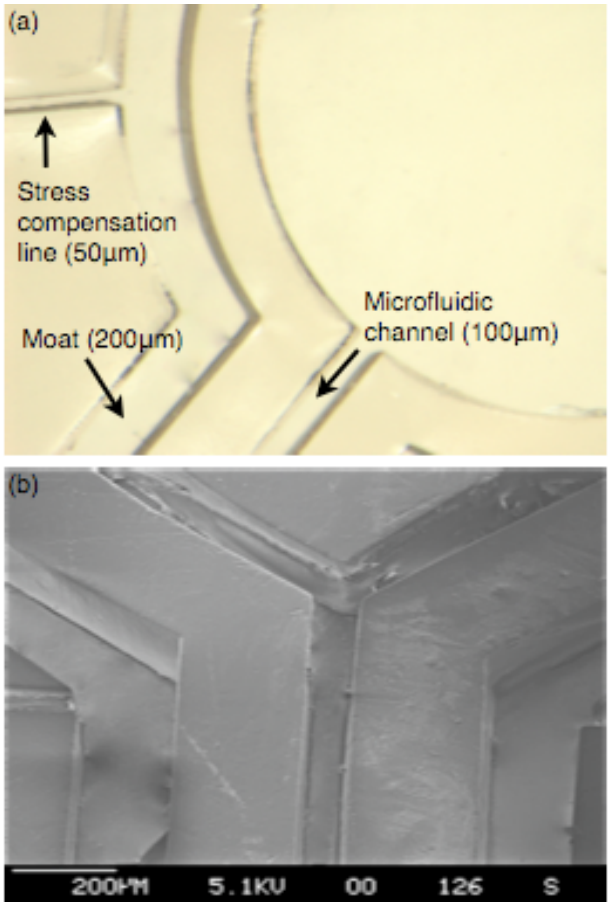

Figure 5: (a) Microchannels fabricated in SU8 (b) SEM image of SU8 channels

4. Conclusion and Future Work.

This paper has described the fabrication and testing of two microfluidic devices that will form the separation module of a lab-on-a-chip for non-invasive prenatal diagnosis. The first module, a blood plasma separator, has demonstrated that cells can be separated from plasma at high flow rate using biophysical effects. Experimental results show that the separation efficiencies obtained are in good agreement with previous studies, and with a better plasma yield. The second module, a micromagnetic separator, has shown promising and encouraging results in initial proof of experiments and simulations. Both chips have been manufactured with low-cost biocompatible materials.

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Heriot Watt University has strict policies on the use of animal and human blood for experimental testing, the university ethical committee and health and safety department was consulted before testing in both circumstances. Additional thanks go to Giuseppe Schiavone, Department of Electrical Engineering, Heriot-Watt University for his help on micromagnetic simulations.

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