

Development of a novel two-channel microfluidic system for biomedical applications in cancer research

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Structure: 1. Introduction / 2. Methods and Experiments / 3. Results / 4. Conclusion

Abstract

In this paper we present a novel two-channel microfluidic system which acts as an artificial blood capillary vessel to examine the migration steps of cancer cells in the microvasculature. The system consists of polycarbonate and is fabricated by combining hot embossing and thermal bonding. The optically transparent polycarbonate allows the use of live cell and fluorescence microscopy. The main feature of this device is that all processes take place under continuous laminar flow conditions at distinct tuneable shear rates.

1 Introduction

The most hazardous characteristic of cancer is its ability to form metastatic tumor cells which can penetrate the blood vessel system (intravasation) and be transported to distant regions where they may settle and colonize other organs as metastases. The aim of this work is to examine the entire migration steps of penetrated cancer cells in the bloodstream from overflowing, unstable attachment, firm adhesion and diapedesis up to migration into the matrix using live cell fluorescence microscopy. For this purpose we designed a two-channel microfluidic system based on polycarbonate which is used as an artificial blood capillary vessel. To examine the migration of cancer cells from the blood system into the surrounding tissue the microfluidic system consists of a two-layered channel system, separated by a porous membrane (cf. Figure 1).

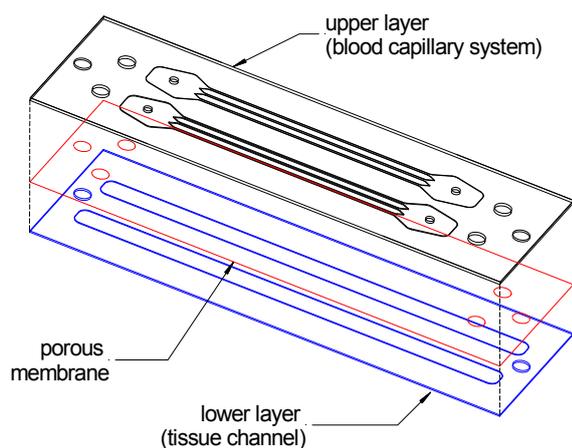


Figure 1: Schematic of the microfluidic two-channel system (exploded view)

The upper layer contains two groups of channels with a width of 300 μm each and heights of 50 μm and 100 μm , respectively, mimicking the microvasculature. In addition, the corresponding membrane surface is coated with a

monolayer of human endothelial cells to mimic the blood vessel wall (cf. Figure 2). The lower layer consists of two larger channels filled with matrix material to simulate the tissue.

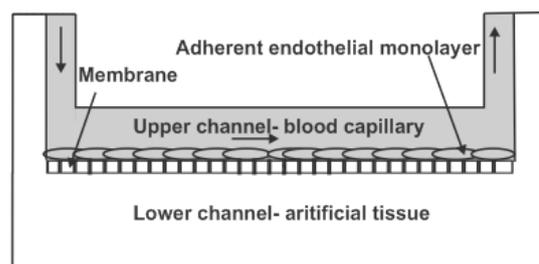


Figure 2: Cross section of the microfluidic system. The arrows inside the upper channel indicate the blood flow

In contrast to other groups, which e.g. use a Boyden chamber for static experiments [1] our design even allows the investigation of the influence of shear flow inside the upper channel system of the artificial blood vessel.

Even though microfluidic structures are often produced using polydimethylsiloxane (PDMS) [2], the two layers of our microfluidic system are fabricated by hot embossing [3] and assembled using a thermal bonding process. The use of thermoplastic polymer results in a more stable system which enables long term investigations.

2 Methods and Experiments

Prior to the fabrication of the complete system, we examined different polymer foils with regard to auto fluorescence and cell adhesion and finally polycarbonate (PC) was chosen due to its transparency, high strength and excellent biocompatibility. Furthermore porous membranes from PC with defined pore densities are commercially available.

For the biological experiments, the lower channel was filled with matrix components. Then human endothelial

cells (EC) were seeded into the upper channel system. After 48 hours, an almost homogeneous monolayer of endothelial cells was achieved, as shown in Figure 3(a) where the endothelial cells are stained orange-red. After this time an isomolar solution containing green-stained cancer cells was flooded over the EC layer inside the upper channel system as shown in Figure 3(b) to investigate the migration steps of the cancer cells.

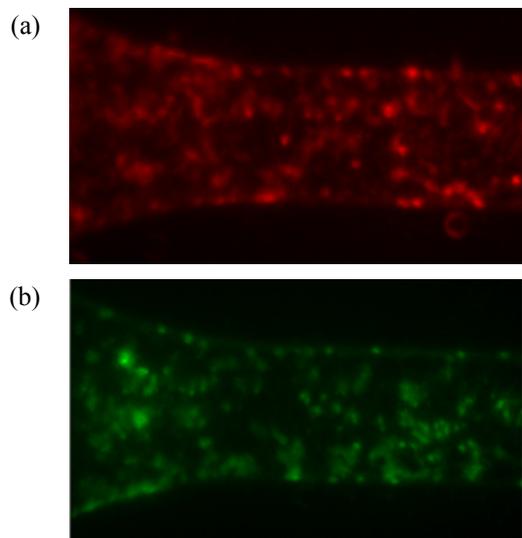


Figure 3: (a) Micrograph of the adherent EC monolayer (b) Micrograph of the floating cancer cells

In addition to the biological experiments we performed computer-aided simulations of the flow behavior of cancer cells considering cell diameter, density and viscosity of the blood.

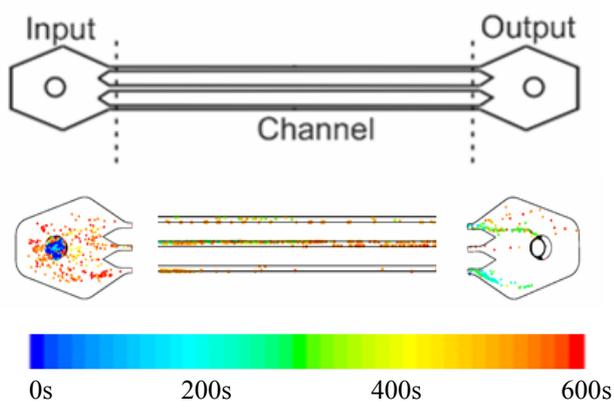


Figure 4: Schematic of the microfluidic channel and flow simulation of blood cells inside the channel system. The colors represent their positions at different times after the injection

It could be shown that about 9% of the injected cells adhere to the walls of the channels of the blood vessel system. Only those, which adhere to the endothelial monolayer on top of the porous membrane, can migrate into the artificial tissue. The result of simulations (cf. Figure 4) is consistent with the experiments.

3 Results

In addition to the sole observation of adhesion processes between endothelium and cancer cells under flow conditions [2], our microfluidic two-channel system allows to examine the entire migration steps of intravascular cancer cells from the overflowing, the unstable attachment, the firm adhesion and diapedesis up to migration into the matrix using live cell and fluorescence microscopy. A small number of cancer cells penetrates the EC monolayer and the porous membrane and enters the matrix of the lower channel. Focusing on the matrix below the membrane, migrated cancer cells can be identified as white points (cf. Figure 5). Due to the long exposure time the flowing cancer cells in the upper channel above the membrane generate a gray background.

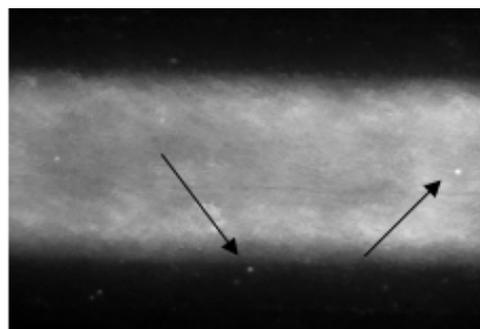


Figure 5: Micrograph of the visible cancer cells inside the artificial tissue of the lower channel

4 Conclusions

The development of this microfluidic channel system was mainly focused on the rheological simulation of the microvascular system. The combination of production technology, channel structure, tunable flow parameters and functionalized membrane enables unique new medical and scientific applicability. Therefore, this microfluidic system acts as an artificial blood vessel providing a direct insight into the process of cancer cell extravasation under flow conditions.

References

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