A CAPILLARY-ENDOTHELIUM-MIMETIC MICROFLUIDIC CHIP FOR THE STUDY OF CHEMOTACTIC RESPONSE
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ABSTRACT
We have developed a microsystem which mimics the dynamic, three dimensional micro environment in the blood vessel. The capillary-endothelium-mimetic microfluidic chip was used to study the chemotactic behavior of neutrophils by using quantitative approach. The quantitative study shows that at lower flow rates the chemotactic factor dominates over the flow rate increasing the extravasation of neutrophils whereas at higher flow rates the neutrophil extravasation minimizes.

KEYWORDS: Atherosclerosis, Biomimetics, Microfluidics, Chemotaxis, Neutrophil extravasation

INTRODUCTION
Atherosclerosis, the underlying cause of cardiovascular disease is caused due to chronic inflammatory response. Disturbed flow and abnormal shear stress in arterial branches cause leukocytes to adhere on the endothelial cells and subsequently transmigrate across the endothelial cell lining, contributing to the early events in atherogenesis [1]. Transwell migration system is a well established method for studying the chemotactic behavior of leucocytes but in such systems the chemotactic gradient cannot be kept constant. Microfluidic technology offers great possibilities over the conventional methods for the systematic study of in vivo systems [2]. Chemotactic behavior of leukocytes have been studied using a microfluidic channel wherein spatial and temporary controlled gradients were generated [1, 3]. In this paper, we report a three dimensional reusable microfluidic chip which mimics the capillary endothelial lining, imitating the hemodynamic factor to study the extravasation behavior of neutrophils.

THEORY
Leukocyte extravasation is the movement of neutrophils from the blood vessels to the site of injury or infection [4]. The goal of our research is to use the capillary-endothelium-mimetic chip to observe the neutrophil migration. Microfluidic chip was fabricated by sandwiching a micro pore array silicon chip between two polydimethylsiloxane (PDMS) chips, as elucidated in Figure 1. The silicon chip was fabricated by etching 5μm x 5μm size holes. In the upper compartment the micro pores were coated with fibronectin to enhance the adhesion of endothelial cells and further culture, representing the endothelial lining in the blood circulatory system. The fibronectin represents the connective tissue surrounding the blood vessels. The lower compartment is coated with collagen to trap the neutrophils which enter the lower channel after extravasation.

Figure 1: Schematic illustration of the PDMS & silicon based chip used for endothelial cell culture and neutrophil extravasation
EXPERIMENTAL

Endothelial cells were cultured in a continuous flow environment for two days and the neutrophil extravasation phenomena was observed after injecting interleukin-1β (IL-1β) and interleukin-8 (IL-8) in the upper and the lower channel respectively followed by neutrophil loading in the upper channel as illustrated in Figure 2.

![Figure 2. Sideview of the chip. (a) Cell loading and Culture (b) Injection of Interleukin-1β (c) Neutrophil loading (d) Injection of Interleukin-8 (e) Neutrophil extravasation](image)

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Figure 3a depicts a fluorescence image wherein anti-VE-cadherin is used to fluoresce the intercellular protein junction to confirm the integrity of the endothelial lining. Figure 3b shows collagen fixed on the bottom channel, used to trap the neutrophils after extravasation.

![Figure 3. (a) Anti VE-cadherin fluorescing the intercellular protein junction. (b) Bottom view: Indicates collagen coating used for trapping the neutrophils](image)

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RESULTS AND DISCUSSION

The encircled area in Figure 4a illustrates that after neutrophil adhesion the fluorescence decreases with time due to neutrophil extravasation, at 300 μm/s flow rate. Whereas at higher flow rate, 1500 μm/s the neutrophils are concentrated near the channel walls as shown in Figure 5. The quantitative analysis in Figure 6 shows that neutrophil extravasation is high at lower flow rates. The quantitative study for neutrophil diapedesis carried out in the presence and the absence of endothelial lining at different flow rates shows that at lower flow rates the chemotactic factor dominates over the flow rate increasing the extravasation of neutrophils whereas at higher flow rates the neutrophils get concentrated near the side walls which minimizes the possibility of extravasation.

![Figure 4. Images indicating Neutrophil adhesion and extravasation at 300 μm/s flow rate (a) Encircled area indicating the adhesion of neutrophils (b, c) Encircled area indicating the dimming of fluorescence (d) Bottom view indicating the neutrophils after extravasation](image)

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CONCLUSION

The proposed biomimetic chip serves a better option over the transwells to study the migration behavior of cells due to its continuous flow system. This can serve a better tool for studying cancer metastasis, inflammation, immunity, atherosclerosis and other blood vessel related issues.

REFERENCES


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