PULSATILE SHEAR STRESS AND HIGH GLUCOSE CONCENTRATIONS INDUCED CELL DEATH IN ENDOTHELIAL CELLS


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ABSTRACT
This paper presents the exposure of endothelial cells to high glucose concentration combined with shear stress variety, which mimicking the heart beat cycle stimulates reactive oxygen species (ROS) overproduction and mitochondria damage, and further leads to increased cellular apoptosis. The apoptosis and necroses level is studied using real-time fluorescence microscopy with the measurement of yopiro-1 iodide and propidium iodide dye. Results show the apoptosis rate matched well with the ROS overproduction and mitochondria damage rate and the study will further unclose the role of diabetes as a contributor to endothelial dysfunction and cardiovascular diseases.

KEYWORDS: Hemodynamic Lab-on-a-chip system, Endothelial cell, Cell death.

INTRODUCTION
Apoptosis is an induced cell suicidal process that allows the biological organisms to destroy damaged or unwanted cells in an orderly way [1-2]. It can be activated by many stimuli under different physical (shear stress) and chemical (glucose concentration) conditions. The mitochondria pathway is one of the major pathways of apoptosis which linked to reactive oxygen species overproduction [3]. The existed studies about hyper-glucose induced apoptosis are experimented under static condition in plastic dishes or constant shear stress and they are unable to study any physiological pulsatile shear-induced cellular responses [4]. This paper introduces a hemodynamic Lab-on-a-chip system with highly controllable microfluidic platform where shear-induced cellular response under different extracellular conditions are studied by investigating the ROS production level in the endothelial cell and monitoring the morphology of the mitochondrion for disfunctionalities via fluorescence imaging technique. Finally, the shear stress and glucose concentration induced cell apoptosis are detected.

Figure 1 shows the shear stress is imposed directly on the surfaces of ECs and the blood soluble molecules send chemical cues to cells. The abnormal microenvironments acts as exogenous ROS sources and elevate ROS production to overcome the antioxidant defense system, leading to the damage of mitochondria [5], which is part of the pathway of cell apoptosis.

MATERIALS AND METHODS
The microfluidic chip was fabricated in polydimethylsiloxane (PDMS) by using a standard soft lithography technique. Three conditions with different glucose concentration can be achieved with the concentration gradient network. A pulsation free precision pump was used for cell loading and media injection. The flow profile of the pump was configured to mimic the blood flow in a blood vessel. A heating plate was used to maintain the temperature of the microfluidic chip at 37°C for long-term observation under the microscope.

Figure 2(a-b) shows the ECs growing in the microchannel. Fig. 2(c-d) shows mitochondrial morphology as (c) filamentous reticular mitochondrial networks, and (d) mitochondrial fragmentation. The measurement of intracellular ROS level using a cell permeant fluorescent dye of 2', 7'-dichlorodihydrofluorescein diacetate (H$_2$DCFDA) is shown in Fig. 2(e), and measurement of endothelial cell apoptosis and necrosis using yopiro-1 and propidium iodide is shown in Fig. 2(f).

EXPERIMENTAL RESULTS AND DISCUSSIONS
Figure 3 shows the cell apoptosis and necrosis rates of ECs with 10 mM glucose concentration under different shear...
stress treatment conditions. The high glucose environment induced at least 15% cell death and the death rate rises with the time lapse. Adding the high shear stress effect to the endothelial cell further enhance both apoptosis and necrosis rate. 1-hour pulsatile shear stress of 30 dyne cm$^{-2}$ under this high glucose concentration does not have much change but if the 30 dyne cm$^{-2}$ shear stress time prolong to 4 hours, the induced cell death reach 30%.

Figure 4 shows when the glucose concentration elevated to 20 mM, the apoptosis and necrosis rate further increased. Compare with the glucose concentration of 10 mM, the effect of pulsatile shear stress is more obvious with the time lapse. 4-hour pulsatile shear stress of 30 dyne cm$^{-2}$ under this super-high glucose concentration induced almost 40% of cell death.

Figure 5 shows the time-dependent responses of the ROS level, mitochondria damage and cell apoptosis rate in ECs under 10 mM glucose concentration and 30 dyne cm$^{-2}$ shear stress. The ROS level is gradually increased in the first 60 min and stabilized afterward. By comparing the ROS level at 60-min time frame between the one with low concentration of glucose and the one with high concentration of glucose, the ROS level is elevated by 30%. For the endothelial cells under the sustained high glucose....

Figure 2: Microphotos of (a) seeding ECs in the microchannel; (b) culture for 48 hours; (c) filamentous reticular mitochondrial networks; (d) mitochondrial fragmentation; (e) measurement of intracellular ROS level using H$_2$DCFDA and (f) measurement of cell apoptosis and necrosis using yo-pro-1 and propidium iodide.

Figure 3: Cell apoptosis and cell necrosis rate of ECs with 10 mM glucose under different shear stress treatment conditions.

Figure 4: Cell apoptosis and cell necrosis rate of ECs with 20 mM glucose under different shear stress treatment conditions.
concentration, mitochondrial fragmentation is prevalent, which shows a mitochondrial fission ratio of 20% when being exposed under pulsatile shear stress of 30 dyne cm⁻². The further induced apoptosis rate through mitochondrial damage pathway was agreed with the damage rate.

Figure 6 shows the measurement results of ROS level, mitochondria damage and cell apoptosis under 20 mM which confirm the relationship between glucose concentration and cell death rate. Compare with the glucose concentration of 10 mM, the results show that high glucose level under exhaustive exercise increases the intracellular ROS level substantially. The chemical treatment (i.e. high concentration of glucose) produced a more dominant effect in promoting mitochondrial fission than the physical treatment (i.e. pulsatile shear stress), especially in such an extreme case of 20 mM of glucose.

CONCLUSIONS
In conclusion, The shear-induced cellular responses of endothelial cells under different glucose concentrations were realized by mimicking the physiological pulsatile flow profile in blood vessel. The results show that pulsatile shear stress is an essential element to mimic the physiological conditions in the blood vessel and ROS level was significantly elevated during exhaustive exercise (shear stress of 30 dyne cm⁻²) and with high glucose concentration (diabetes patient), which induce mitochondrial dysfunction and lead to cell apoptosis. The study will further unclose the role of diabetes as a contributor to endothelial dysfunction and cardiovascular diseases.

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