# USING CO-CULTURE MICROSYSTEM FOR CELL MIGRATION UNDER FLUID SHEAR STRESS

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## ABSTRACT

This study has developed a new method to co-culture the two different cells in the specific gap and the cells migration is observed in the fluid shear stress device. Our strategy is using the capillary principle to seed two different cells separately in the co-culture microchip. Smaller gap sizes (100 μm and 50 μm) would cause the migration phenomenon due to the mutual induction between ECs and SMCs. When the fluid shear stress is raised up to 12 dyne/cm$^2$, it restrains both the velocities of the SMCs and ECs migration.

**KEYWORDS:** co-cultivate, micro-molding in capillaries, fluid shear stress, cell migration.

## INTRODUCTION

The dysfunction of vascular cells including endothelial cells (ECs) and smooth muscle cells (SMCs) is involved in the development of atherosclerosis. In addition, blood flow-induced shear stress, a major hemodynamic force acting on the vessel wall, is also an important contributing factor for this disease [1, 2]. In this study, in order to investigate the cells migration and induced phenomenon of the ECs and SMCs, shear stresses the of 7 dyne/cm$^2$ and 12 dyne/cm$^2$ [3] were applied by the fluid shear stress device since the influence of the share stress is of importance.

## EXPERIMENTAL

Our strategy is to use the MEMS technology to fabricate the co-culture microchip and cultivate the two different kinds of cells in the channels with the specific gap (500 μm, 200 μm, 100 μm, and 50 μm). Moreover, the fluid device of the various shear stresses (0 dyne/cm$^2$, 7 dyne/cm$^2$, and 12 dyne/cm$^2$) has been combined with the co-culture microchip, on which the mutual induction and migration phenomenon between the cells under various shear stresses would be observed. The experimental processes are shown in Fig. 1.

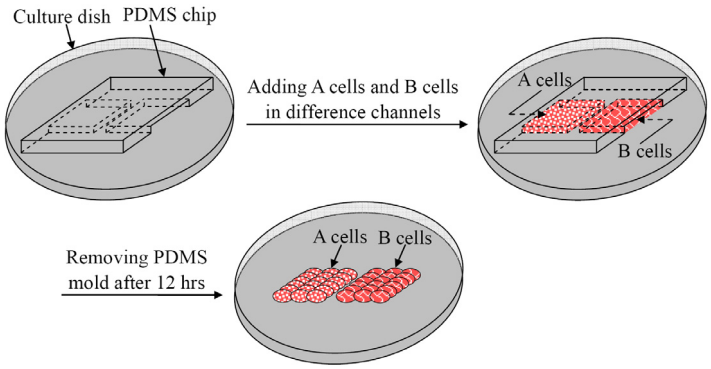


*Figure 1: The experimental processes for co-culture cells.*

## RESULTS AND DISCUSSION

The SU-8 molds were measured using Scan Electronic Microscope to get accurate microstructure size and surface (Fig. 2). It is interesting to observe the effect of the different gap sizes on cell migration. After being co-cultivated for 12 hr at gap size of 500 μm, the migration distance of the ECs is 113.5 μm and that of the SMCs is 208.7 μm. when gradually decreasing the gap size of the co-culture microchip, the migration distance of the cells is increased at the same time interval. We have discovered the migration distance of the SMCs is bigger than the ECs (Fig. 3). At the gap size of 500 μm and co-culture time of 12 hr, the migration velocities of the ECs and the SMC are 11.4 μm/hr and 25 μm/hr, respectively. The migration velocity of the SMCs is faster than that of the ECs in various gap sizes (Fig. 4). When the observation time is increased in various size gaps, the migration velocities of the ECs and the SMCs are gradually increased. Conclusions were drawn from above experimental results: (1) the migration velocity of the co-cultivated cell is faster than that of mono-cultivated cell, (2) when the gap size is decreased, the migration distances of ECs and SMCs are increased at the same time interval, and (3) the migration velocity of the SMCs is bigger than that of ECs regardless of the gap size.

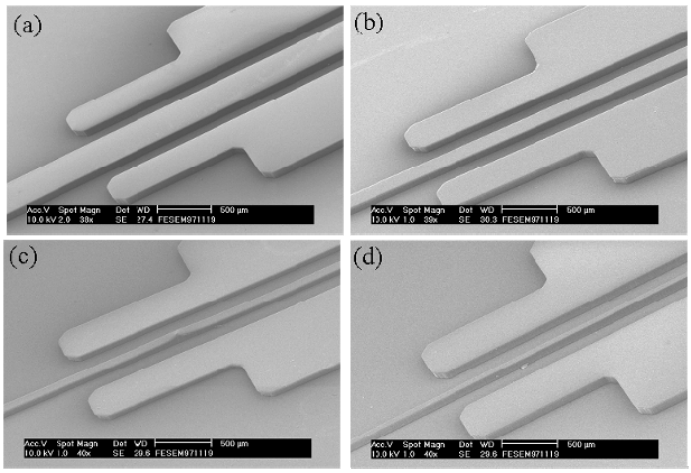


*Figure 2: The SEM pictures of the SU-8 mold, (a) 500 μm, (b) 200 μm, (c) 100 μm, and (d) 50 μm.*

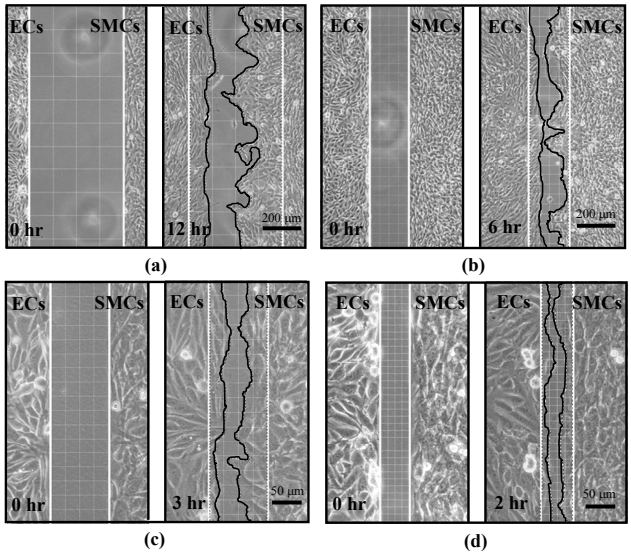


*Figure 3: The ECs and SMCs were co-cultured at various gap sizes under no shear stress. (a) The 500 μm gap size for 12 hr observation time, (b) the 200 μm gap size for 6 hr observation time, (c) the 100 μm gap size for 3 hr observation time, and (d) the 50 μm gap size for 2 hr observation time.*

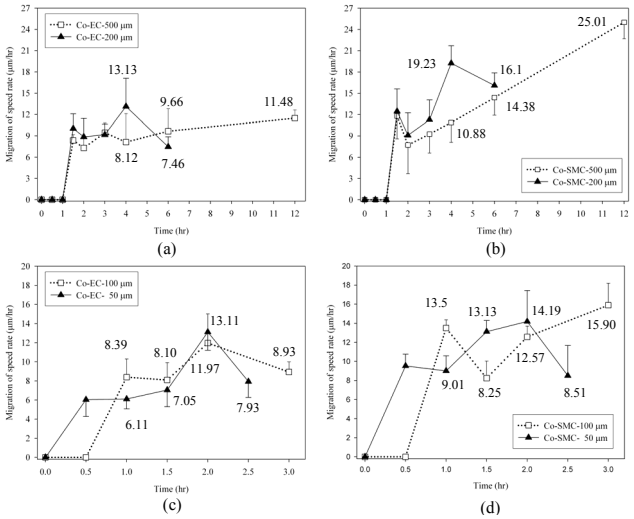


*Figure 4: The relationship between the reaction time and migration velocity at various gap sizes under no shear stress. (a) The migration velocity of the ECs at gap sizes of 500 μm and 200 μm, (b) the migration velocity of the SMCs at gap sizes of 500 μm and 200 μm, (c) the migration velocity of the ECs at gap sizes of 100 μm and 50 μm, and (d) the migration velocity of the SMCs at gap sizes of 100 μm and 50 μm.*

With the gap sizes of 500 μm, 200 μm, 100 μm, and 50 μm under the shear stresses of 7 dyne/cm$^2$ and 12 dyne/cm$^2$, we have found the ECs and SMCs have not touched with each other at 12 hr, 6 hr, 3 hr, and 2 hr, respectively. The shear stress causes the cells to touch each other more slowly than without shear stress. Because the shear stress has disturbed the message communication between the SMCs and the ECs, it would be more obvious under the shear stress of 12 dyne/cm$^2$(Fig. 5). It is found that when increasing the shear stress, the migration velocities of the SMCs and the ECs are slower. Conclusions were drawn from above experimental results: (1) the migration velocity of the SMCs is bigger than ECs under various shear stresses, and (2) the fluid device disturbs the message communication between the SMCs and the ECs, and it is very obvious under 12 dyne/cm$^2$.

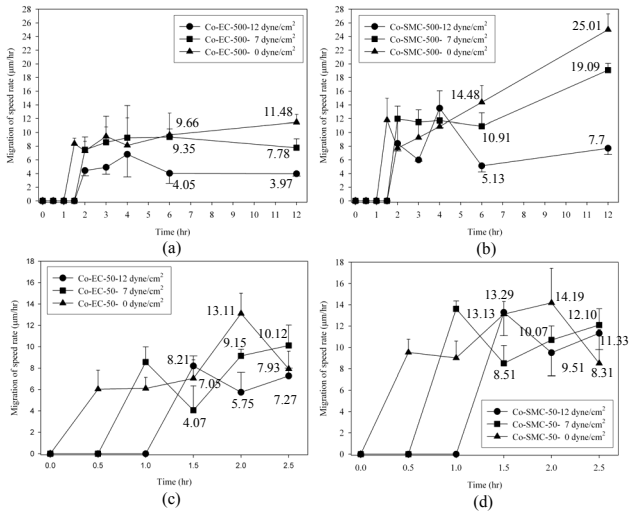


*Figure 5: The relationship between the reaction time and migration velocity under various shear stresses, including 0 dyne/cm$^2$, 7 dyne/cm$^2$, and 12 dyne/cm$^2$. (a) The migration velocity of the ECs at gap size of 500 μm, (b) the migration velocity of the SMCs at gap size of 500 μm, (c) the migration velocity of the ECs at gap size of 50 μm, and (d) the migration velocity of the SMCs at gap size of 50 μm.*

## CONCLUSION

We have successfully developed the co-culture microchip and separated two kinds of cells in the specific gap (the minimum gap size is 50 μm). In order to observe the cell migration phenomenon in the fluid condition, we also successfully combined the fluid device with the co-culture microchip. It is found that the migration distance of the SMCs is bigger than the ECs at various gap sizes. In the smaller gap (100 μm and 50 μm), the ECs and SMCs would generate the mutual induction to cause the cell migration. The various shear stresses are found to disturb the message communication between the SMCs and the ECs, and the migration velocity of the cells would become slower when the shear stress is gradually increased. The developed co-culture microsystem will provide a convenient way to cultivate two kinds of cells in a specific gap under the various shear stresses, and it would have more applications for bio-manipulation and tissue repair engineering in the future.

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