

ENDOTHELIAL CELLS EXPOSED TO FLUID SHEAR STRESS REGULATE MIGRATION OF COCULTURED SMOOTH MUSCLE CELLS

Naoya Sakamoto, Toshiro Ohashi, Masaaki Sato

Graduate School of Engineering
Tohoku University
Sendai
Japan

INTRODUCTION

Intimal hyperplasia, a typical feature of atherosclerosis, is found to occur in response to hemodynamic conditions such as shear stress. In the hyperplastic lesion, it is well known that smooth muscle cells (SMCs) migrate from media to intima. Migration of SMCs may be associated with endothelial cells (ECs) mediated shear stress. It is important to know the effect of shear stress on migration of SMCs combined with the cellular interaction between ECs and SMCs.

Several studies have reported that migration and locomotion of SMCs were related with the interactions between ECs and SMCs by using cocultured model [1,2]. However, there are few studies about the effect of shear stress on migration of SMCs in relation to interaction with ECs, because the cocultured model used in previous studies was not appropriate to be exposed to shear stress. Hence, it is still unclear that the effect of shear stress on migration of SMCs.

In this study, the effect of shear stress on migration of SMCs was investigated using a newly designed cocultured blood vessel model. Moreover, we examined activity of matrix metalloproteinases (MMPs) in cocultured model to reveal the relationship with the migration of SMCs.

METHODS

Cell culture

ECs and SMCs were isolated from the same bovine aorta and were cultured with Dulbecco's modified Eagle Medium containing 10% fetal bovine serum. When confluent, cells were passaged at 1:3 to 1:4 split ratios and were used from 5th to 9th generations of ECs and from 4th to 7th of SMCs.

Construction of cocultured mode

We constructed a multilayer-cocultured model composed of ECs, a collagen layer, a polycarbonate membrane filter with pore diameter of 0.6 μm , and SMCs as shown in Figure 1. The membrane filter was treated with 0.1% collagen type I.

SMCs were seeded on a culture dish and allowed to grow until they covered the bottom of the dish. After the culture medium was

removed, the space ring was placed on the dish. A solution of 0.5% collagen type I along with 10x concentrated Medium 199, and reconstruction buffer (0.05 M NaOH, 200 mM HEPES, and 0.26 M NaHCO_3) was subsequently layered over the SMCs. Immediately, the membrane filter was laid on the collagen layer. The collagen layer was then allowed to polymerize at 37 $^{\circ}\text{C}$ for 30 min. Finally, ECs were seeded on the membrane filter and cultured statically until use.

Flow exposure experiment

At 1 day after constructing the cocultured model, the model was incorporated into a parallel-plate flow chamber. The flow chamber was connected to a flow loop which allowed regulation of flow rate, culture medium temperature, and pH. The cocultured model was exposed to a steady shear stress of 0.1 or 1.5 Pa for 48 hours.

Observation of migrating SMCs

After flow exposure experiment, nuclei of SMCs were stained by incubation with 10 μM SYTO 13 (Molecular Probes). Following treatment with formalin, cross-sectional images of migrating SMCs

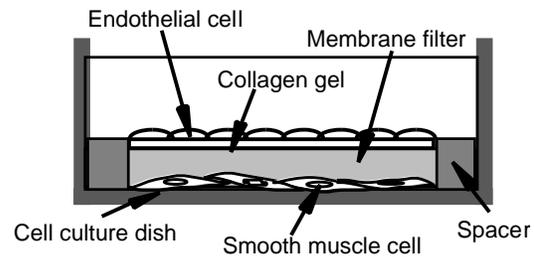


Figure 1. Schematic diagram of cocultured blood vessel model

were obtained in the area of 190 μm x 400 μm using confocal laser scanning microscope. From the images, we calculated migration ratio, which is defined by the number of migrating SMCs divided by total number of SMCs in the observed area. Migration ratios obtained from three areas in a dish and were averaged. The model without ECs was also cultured statically as control model.

Gelatin zymography

After exposed to shear stress, the cocultured model was washed with cultured with serum free medium for 12 hours. The conditioned medium used for the culture was concentrated by ultrafiltration. Proteins with gelatinolytic activity in the concentrated sample were identified by electrophoresis in the presence of SDS in 7.5% discontinuous polyacrylamide gels containing 1 mg/ml gelatin. Gels were stained with Coomassie Blue R-250 and destained until bands were visible. Gels were then dried and scanned.

RESULTS AND DISCUSSION

The effects of shear stresses on migration ratio were shown in Figure 2. The migration ratio of the static group was 1.8 times higher than that of the control. Shear stress of 0.1 Pa did not suppress the migration of SMCs compared with the static group. Shear stress of 1.5 Pa decreased significantly the migration ratio compared with the static group. These results indicated that ECs might have a stimulative effect on migration of SMCs, and that effect might be suppressed by higher shear stress.

Figure 3 showed a typical result of gelatin zymography. There are two types of MMP-2, which are pro-form and activated form. MMP-2 was secreted in an inactive pro-form and activated for digesting extracellular matrix. Activities of pro-form and activated form MMP-2 were found to be affected by experimental conditions. Changes in activity of activated form MMP-2 are given in Figure 4. Data were expressed as percentage of control. In the static condition, the activity of activated MMP-2 was increased by coculturing with ECs. The activity of activated MMP-2 decreased with exposure to shear stress compared with static group. At shear stress of 1.5 Pa, the activity of activated MMP-2 decreased significantly. These results indicated that ECs might induce activation of MMP-2 at the static condition and higher shear stress might downregulate the induction of activated MMP-2. In addition, since higher shear stress suppressed the migration of SMCs and also the activation of MMP-2, suppression of

MMP-2 activation might have a correlation with downregulation of SMC migration. Recent studies have reported that activation of MMP-2 in blood vessel associated with the formation of atherosclerosis [3,4]. Taken together, higher shear stress may suppress activation of MMP-2, resulting in prevention of atherogenesis.

CONCLUSIONS

In this study, we investigated the effects of shear stresses on migration of SMCs using a newly designed cocultured blood vessel model. The number of migration of SMCs was decreased by shear stress of 1.5 Pa, however 0.1 Pa shear stress did not suppress migration. In addition, shear stress of 1.5 Pa also suppressed the activation of MMP-2. These results suggested that higher shear stress might stimulate synthesis of inhibitor for activation of MMP-2 in ECs, then suppress migration of SMCs.

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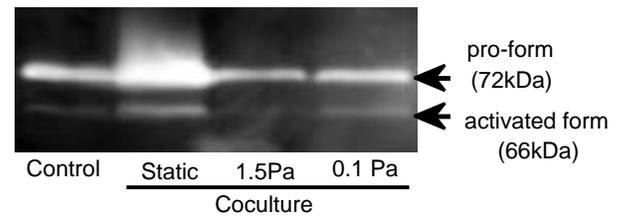


Figure 3. Identification of MMP-2 by SDS-PAGE gelatin zymography

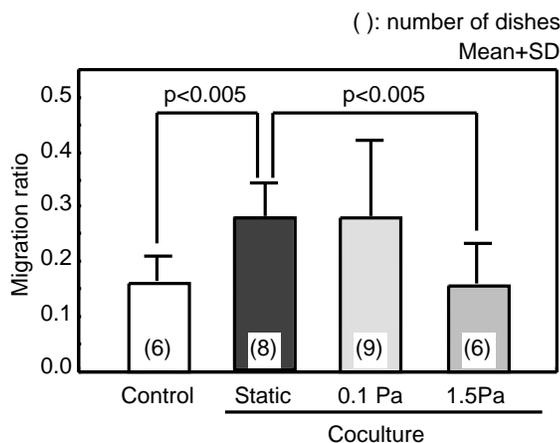


Figure 2. Changes in migration ratio of smooth muscle cells

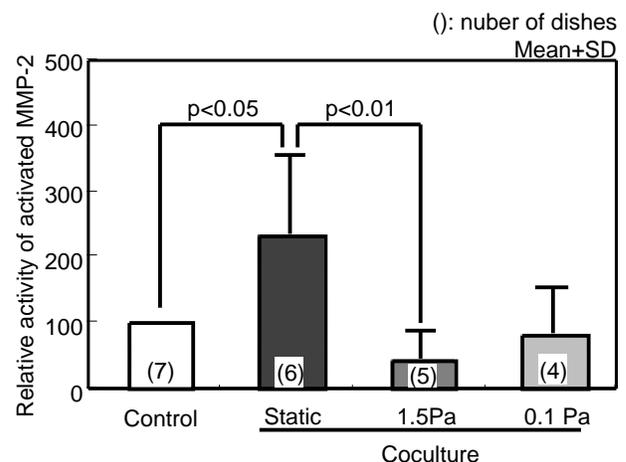


Figure 4. Changes in relative activity of activated MMP-2