3D human vasculature-on-a-chip: The biological effect of combustible cigarette smoke and vapor from three heated tobacco products on monocyte adhesion to vessels comprising coronary artery endothelial cells

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INTRODUCTION

Heated tobacco products (HTPs) are potentially reduced-risk products because aerosols have fewer harmful constituents than cigarette smoke. Research on the reduced-risk potential of HTPs has mainly focused on the respiratory tract, but the cardiovascular system should be done. In this study, we used a human-mimic microfluidic vascular-on-a-chip to assess the effect of HTP aerosols on monocyte adhesion, which is an early-stage biological event of atherosclerosis.

MATERIALS AND METHODS

Tobacco products

Three types of HTPs (devices/consumables) were obtained in Japan, and the Kentucky reference 1R6F cigarette, as a representative combustible cigarette, was purchased from the Kentucky Tobacco Research and Development Center, University of Kentucky (Table1). The total particulate matter (TPM) from 1R6F cigarette smoke (CS) and aerosol collected mass (ACM) from the vapor of three commercial HTPs were collected on a Cambridge filter pad, dissolved in dimethyl sulfoxide (DMSO), and added to the culture medium at concentrations from 100 to 400 µg/mL and 200 to 3000 µg/mL, respectively (Table 2).

Cells and 3D microfluidic cell culture platform

Human primary coronary artery endothelial cells (HCAECs) were purchased from PromoCell and cultured on a two-lane OrganoPlate® (Mimetas), a three-dimensional (3D) microfluidic cell culture platform, for 3–4 days until tube-like structures formed (Fig. 1).

Preparation of exposure samples

Human monocytic leukemia cells (THP-1) were differentiated into macrophages by stimulating with 300nM PMA for 48 h and then incubated in the fresh medium for 16 h. Media containing particle phase extracts of CS from 1R6F and vapor from three commercially available HTPs, including our proprietary DT3.0a, were applied to the macrophages. After 1 h of exposure, the medium was refreshed and incubated for 3 h to prepare the conditioned medium, which was then applied to HCAEC tubules, and incubated for 24 h.

Biological assays

- Proinflammatory cytokines released by macrophages were measured with Human IL-1 beta/IL-1F2 Quantikine ELISA Kit and Human TNF-alpha Quantikine ELISA Kit (R&D systems).
- The expression of intracellular adhesion molecules-1 (ICAM-1) was evaluated by immunostaining with anti-ICAM primary antibody and goat anti-mouse IgG conjugated with Alexa Fluor 488 secondary antibody (Abcam). The fluorescence intensity was analyzed with Operetta CLS (PerkinElmer).
- Monocyte adhesion was evaluated by applying fluorescently labeled (calcein AM solution, DOJINDO) monocytic THP-1 into HCAEC tubules and counting the number of monocytes adhered to the endothelial surface.

Table 1. Product information

Product Name	Heating system	Component	Heating/Combusting temperature
1R6F	Combustion	Tobacco stick	Approx. 800 ℃
DT3.0a	Peripheral and convection	Battery, Tobacco stick	Approx. 295 ℃
THS	Blade	Battery, Tobacco stick	Approx. 300 ℃
THP	Peripheral	Battery, Tobacco stick	Approx. 260 ℃

Table 2. Test sample preparation

1R6F TPM Concentration	HTP ACM Concentration	Positive Control (PC)	Negative Control (NC)
100–400 µg/mL (with 1% DMSO)	200–2000 µg/mL (with 1% DMSO) 3000 µg/mL (with 1.5% DMSO)	Medium with 100 µg/mL lipopolysaccharide (LPS, FUJIFILM Wako)	Medium with 1% or 1.5% DMSO

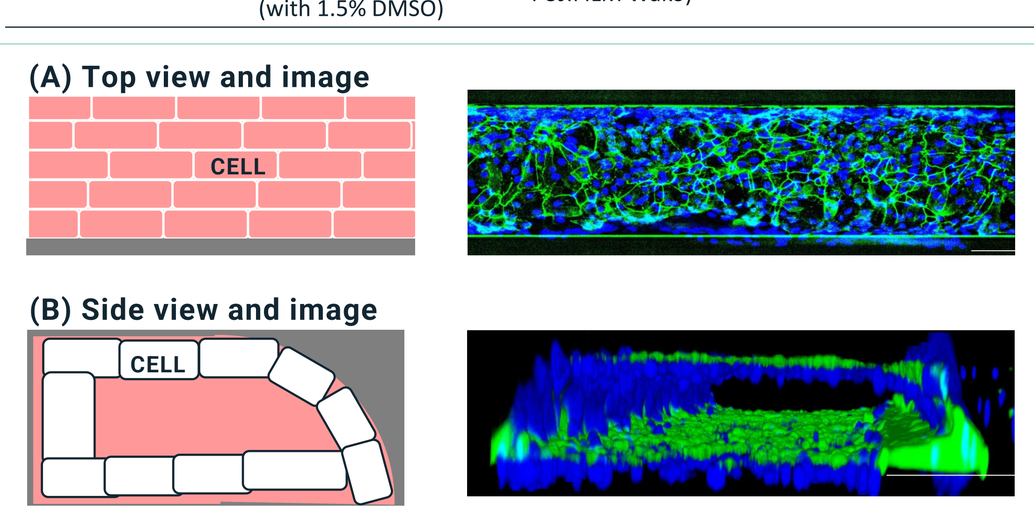
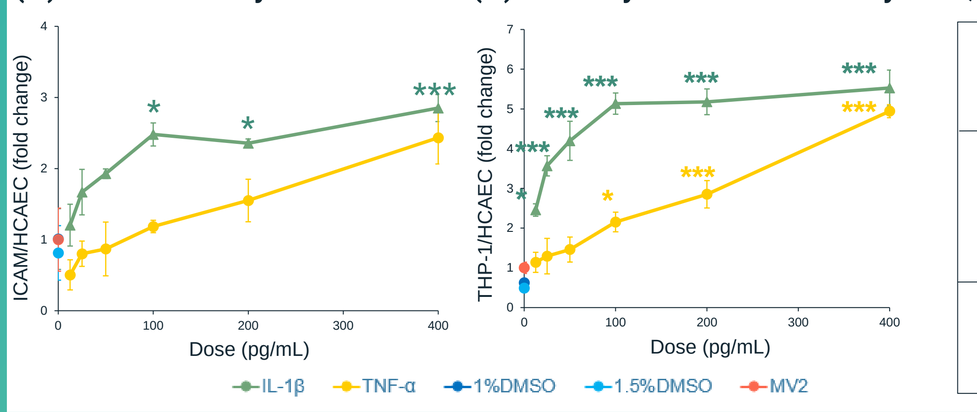


Fig. 1. Schematic and fluorescence images of the OrganoPlate®.
(A) Top and (B) side views. DNA (blue) and ZO-1 (green) were stained with Hoechst and immunofluorescent labels, respectively. Scale bar = 100 µm.



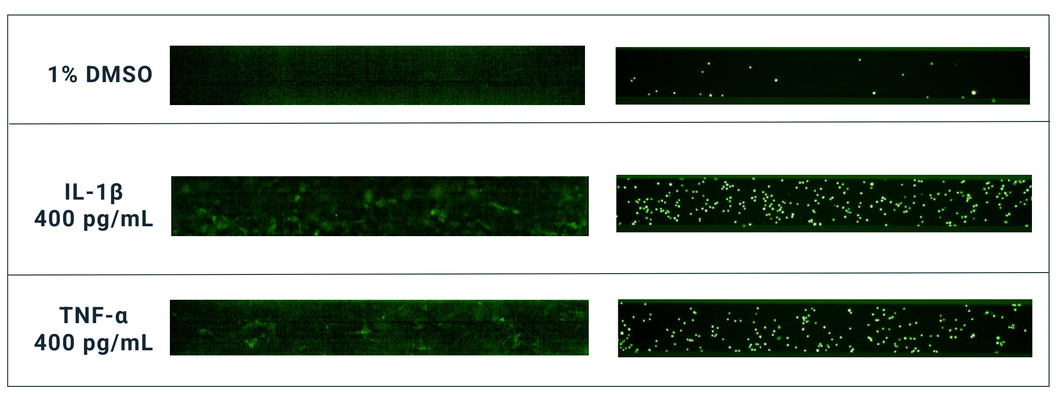
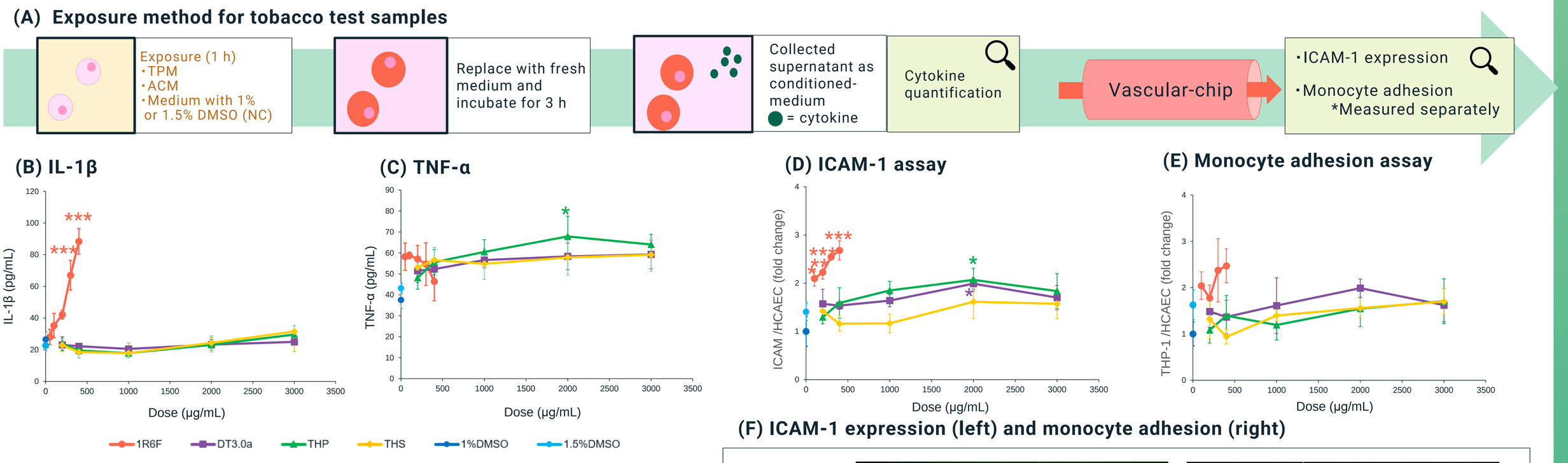


Fig. 2. Cellular responses to proinflammatory cytokines. (A) ICAM-1 expression measured by immunostaining and normalized by NC (MV2 medium). (B) Number of adhered monocytes normalized by NC (MV2 medium). Results represent the mean ± standard error of three independent experiments performed. *p < 0.05, **p < 0.01, and ***p < 0.001 compared with NC condition (Dunnett's test). (C) Vascular-on-a-chip fluorescence images of ICAM-1 immunostaining and labeled-monocyte adhesion.



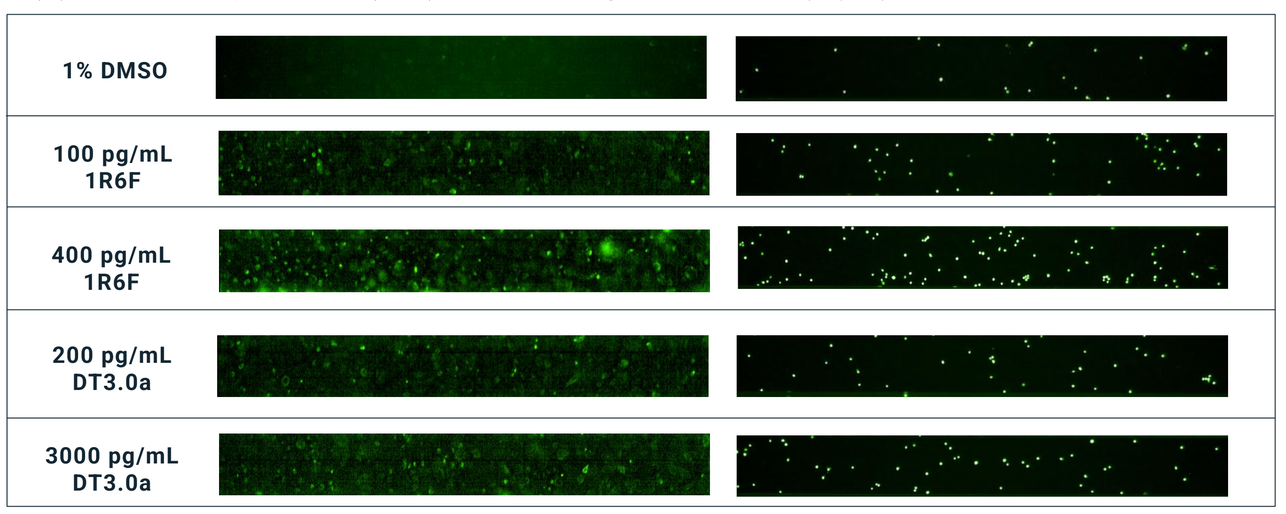


Fig. 3. Biological effect of the conditioned medium from macrophages exposed to tobacco extracts. (A) Stimulation of macrophages by test samples and collection of conditioned medium. (B, C) IL-1β and TNF-α in conditioned medium quantified with ELISA. (E) Fold change of ICAM-1 expression measured by immunostaining. (F) Fold change of the number of adhered monocytes. Results represent the mean ± standard error of three independent experiments performed. Fold changes were calculated as the ratio between exposure conditions and the negative control (NC) condition. *p < 0.05, **p < 0.01, and ***p < 0.001 compared with NC condition (Dunnett's test). (F) Vascular-on-a-chip fluorescence images of ICAM-1 immunostaining and labeled-monocyte adhesion.

RESULTS AND DISCUSSION

In this study, we established a new method to assess the effect of CS from 1R6F and ACM from HTPs by using a human-mimic microfluidic vascular-on-a-chip. As preliminary data, proinflammatory cytokines elicit ICAM-1 expression and monocyte adhesion in a dose-dependent manner. In the case of products, all HTP vapors had a lower effect on ICAM-1 expression and monocyte adhesion than 1R6F CS, even though different heating methods were employed to generate vapor from each HTP. This suggests that HTPs have a low impact on the medium-stage events of atherosclerosis. However, owing to the complex pathogenesis of atherosclerosis, we need to assess the effect of HTPs on the late-stage events of pathogenesis, thus allowing accurate evaluation of the reduced-risk potential of HTPs.

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