Direct adhesion of endothelial cells to bioinspired poly(dopamine) coating through endogenous fibronectin and integrin $\alpha_5\beta_1$

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Introduction

Cell adhesion on biomaterial surface is crucial for tissue repair and regeneration. Mussel-inspired poly(dopamine) (PDA) coating has been proven to be a simple, versatile and effective strategy to promote cell adhesion onto various substrates. It was reported that the adsorption of adhesive serum proteins maintaining their native configuration contributed to the improvement of cell adhesion. [1] However, whether the PDA coating itself can directly promote cell adhesion remains unclear.

Herein, we evaluated the initial adhesive behavior of human umbilical vein endothelial cells (HUVECs) on PDA coating under serum-free condition.

Method

- Polycaprolactone (PCL) thin films were obtained by spin coating of a solution of PCL in chloroform (1% w/v) onto the silane-modified glass slides.
- For the PDA coating, glass-PCL substrates were immersed into a dopamine solution (2 mg/mL in 10 mM Tris, pH 8.5) for 24 h at room temperature.
- Adhesion, spreading and organization of the cytoskeleton of HUVECs on various substrates were studied by immunofluorescence microscopy and SEM.
- Expression of integrins and secretion of endogenous fibronectin were also identified by the indirect immunofluorescence method.
- The functional development and maintenance of endothelial phenotype on various substrates were analyzed by immunofluorescence of endothelial cell-specific markers.

Results

Table 1. Summary of the properties PCL coated glass (glass-PCL) and PCL coated glass further modified with polydopamine (glass-PCL-PDA).

	glass-PCL	glass-PCL-PDA
Thickness (nm)	66.0 ± 3.9	79.1 ± 3.8
Contact Angle (°)	72.6 ± 0.4	53.2 ± 1.8
RMS Roughness (nm)	4.62 ± 0.76	4.01 ± 0.37

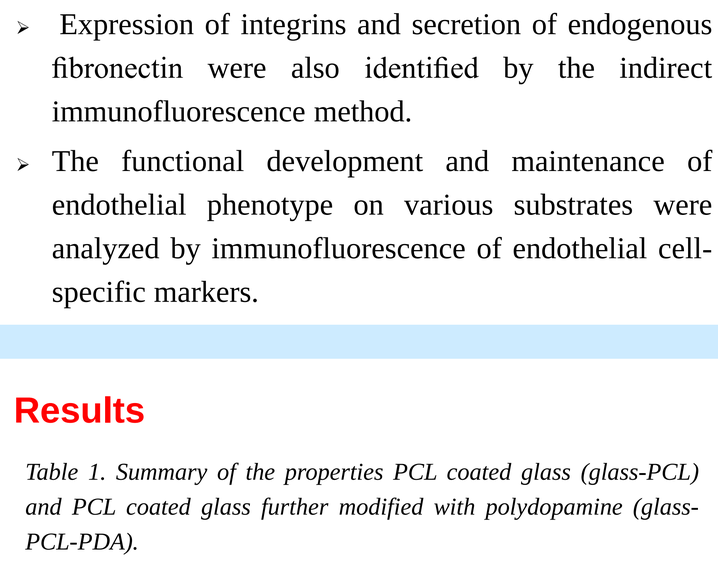

Fig. 1. F-actin staining for HUVECs adhered to PCL, PDA and collagen in serum free media for 0.5, 1 and 4 h. Scale bar = 200 μm. PDA coating itself can promote attachment and spreading of HUVECs.

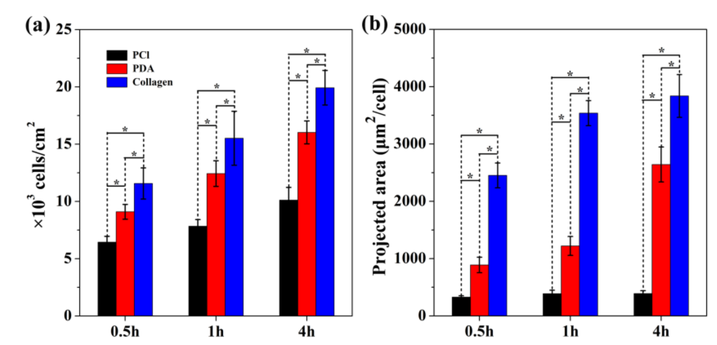

*Fig. 2. Quantification of cell density and spreading area of HUVECs after 0.5, 1, and 4 h of incubation on PCL, PDA and collagen. (mean ± SD, * p < 0.05, t test).*

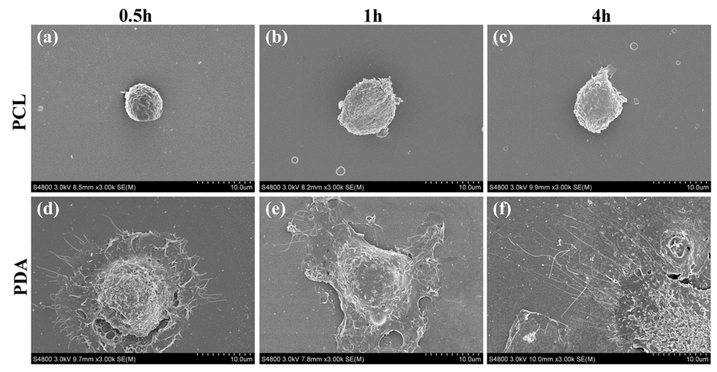

Fig. 3. SEM images of HUVECs adhered to PCL and PDA for 0.5, 1 and 4 h. Obvious lamellipodia and filopodia were observed during spreading of HUVECs on the PDA surface.

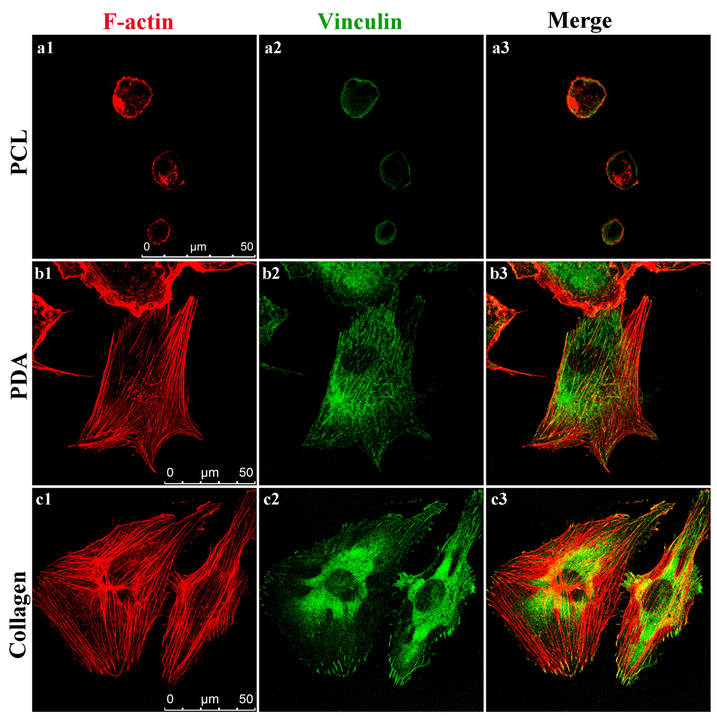

Fig. 4. HUVECs adhered to PCL, PDA and collagen for 4 h were stained for F-actin (red), vinculin (green). Scale bar = 50 μm.

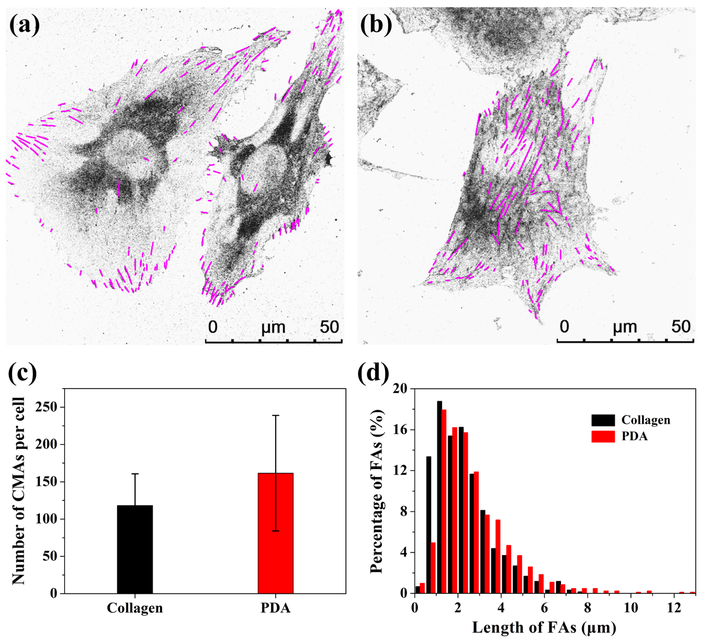

Fig. 5. Spatial localization and length distribution of cell–matrix adhesions (CMAs) in HUVECs after adhesion for 4 h. Magenta spots identifies the inverted fluorescent signal of CAMs on the surfaces of collagen (a) and PDA (b). Scale bar = 50 μm. Average number (c) and length distribution (d) of CAMs on collagen and PDA coatings. Data are expressed as mean ± SD.

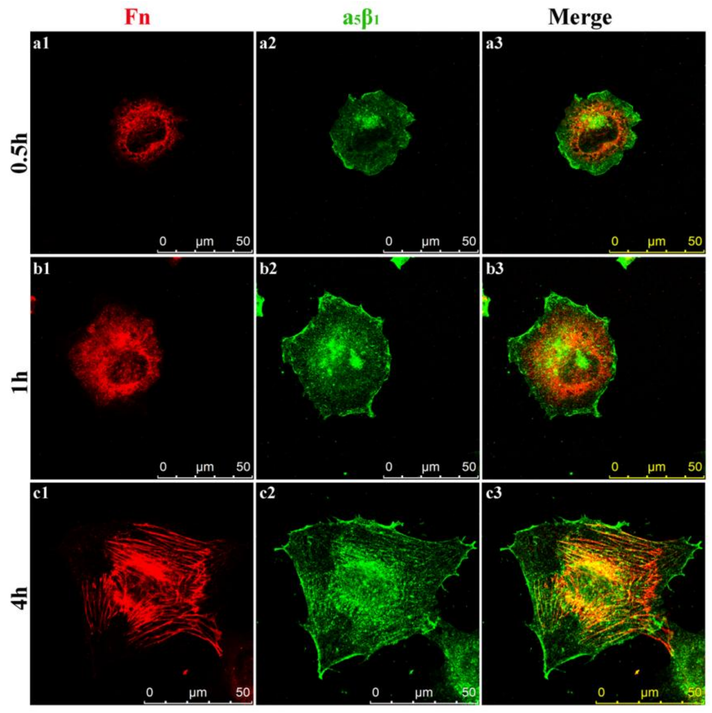

Fig. 6. Expression of endogenous fibronectin and its receptor, $\alpha_5\beta_1$ integrin, during the initial adhesion of HUVECs on polydopamine coating. Scale bar = 50 μm.

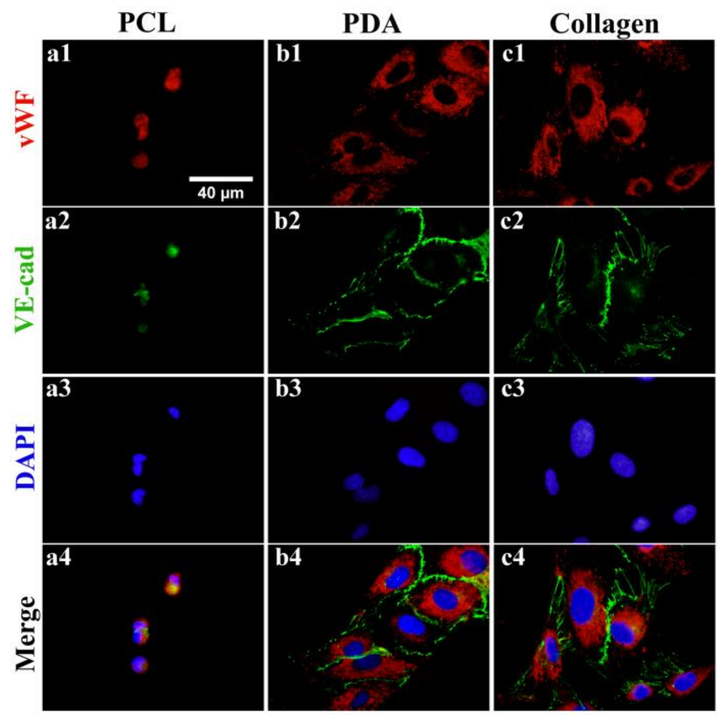

Fig. 7. Immunofluorescence detection of specific endothelial markers of HUVECs adhered to PCL, PDA and collagen for 4 h. Scale bar = 40 μm. HUVECs adhered to PDA coating were strongly positive for vWF and VE-cadherin.

Conclusion

Our findings highlight that the mussel-inspired PDA can facilitate the secretion, deposition and assembly of endogenous Fn, by which cell adhesion is triggered.[2] It provides new insight into the functions of bioinspired PDA coating in the field of cell-based biomedical applications.

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