A Novel Fluorophore-tagged RGD Peptide to Monitor and Enhance Endothelial Cell Adhesion to Micropatterned Surfaces

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Introduction:

Cardiovascular disease is the leading cause of death in Canada. Artificial blood vessels are used to replace or bypass diseased blood vessels. Half of the small-diameter (<6 mm) grafts fail within 5 years[1], which could be prevented if healthy endothelial cells colonized the graft surfaces.

Fibronectin is an extracellular matrix protein that is essential for vascular development[2, 3]. The RGDS and WQPPRARI fibronectin-derived peptides respectively promote endothelial cell adhesion and motility[4, 5]. We have previously conjugated these peptides to polytetrafluoroethylene, a material used to fabricate vascular grafts, in order to enhance endothelial cell adhesion and growth[6, 7]. Peptide micropatterns consisting in 10 µm diameter RGD peptide spots covering 20% of the surface, with the remaining surface functionalized with WQPPRARI led to higher cell yields than surfaces functionalized with a single peptide. However, the local effects of the micropatterns on endothelial cell adhesion and growth had not been investigated.

We hypothesized that the RGDS-rich regions would locally enhance cell adhesion, whereas the RGDS:WQPPRARI surface coverage ratio would affect cell expansion. The objective of this work was to generate fluorophore-tagged micropatterns to investigate the local effects of the peptides on human saphenous vein endothelial cell (HSVEC) adhesion and expansion.

Materials and Methods:

Glass surfaces were micropatterned as previously described[7], but replacing the RGDS peptide by a fluorophore-tagged RGD peptide, termed RGD-TAMRA. Unless otherwise stated, the RGD-TAMRA:WQPPRARI ratio was 35:65. HSVECs were obtained with the informed consent of donors at the Saint-François d'Assise Hospital. Cells were cultured in M199 medium containing antibiotics, bFGF, heparin and fetal bovine serum (FBS). Cells were seeded at 5000 cells/cm^2 in M199. After 3 h of time-lapse phase contrast imaging, EGM medium supplements (Lonza) were added, except for replacing FBS by bovine albumin, insulin, transferrin and selenium. The cells were cultured for up to 6 days before fixing and staining.

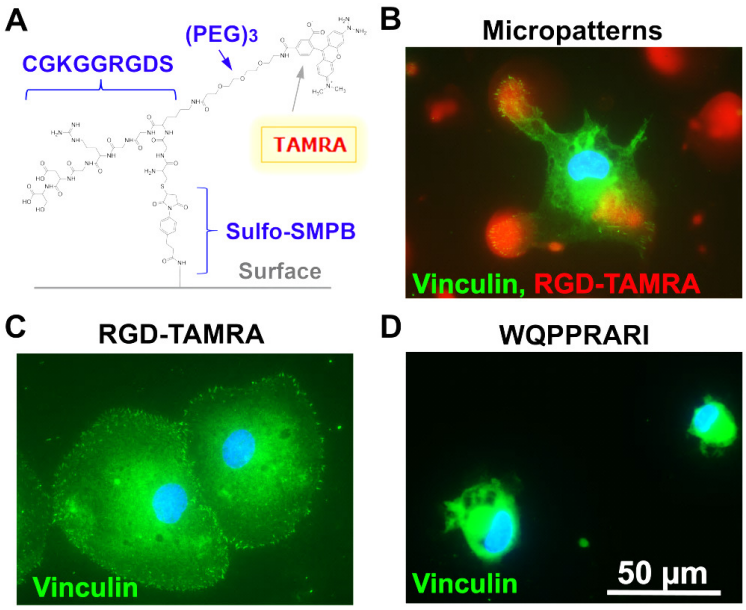

Figure 1. Effect of surface micropatterning on the distribution of focal adhesions. (A) The RGD-TAMRA molecule. Identification of focal adhesions 3 h after seeding on (B) micropatterned surfaces or surfaces uniformly treated with (C) RGD-TAMRA or (D) WQPPRARI. RGD-TAMRA functionalized regions are shown in red except for (C).

Results:

To observe the local effects of peptide micropatterns on HSVECs, a novel fluorophore-tagged RGD peptide was designed (Fig. 1A). The fluorophore-tagged peptide micropatterns were visualized by fluorescence microscopy during live cell imaging and after fixing the cultures (Fig. 1B). During cell adhesion, the direction of HSVEC cell spreading on the micropatterned surfaces was guided by the position of the RGD-TAMRA spots. After 3 h of adhesion, focal adhesions were co-localized with RGD-TAMRA functionalized regions (Fig. 1). The rate of cell spreading was correlated with the fraction of the surface area covered by RGD-TAMRA (Fig. 2A). Both the RGD-TAMRA treated and the micropatterned surfaces led to significant HSVEC cell expansion in serum-free medium, contrary to the WQPPRARI-treated surfaces (Fig. 2B). However, the focal adhesions were not co-localized with the RGD-TAMRA functionalized regions after cell expansion.

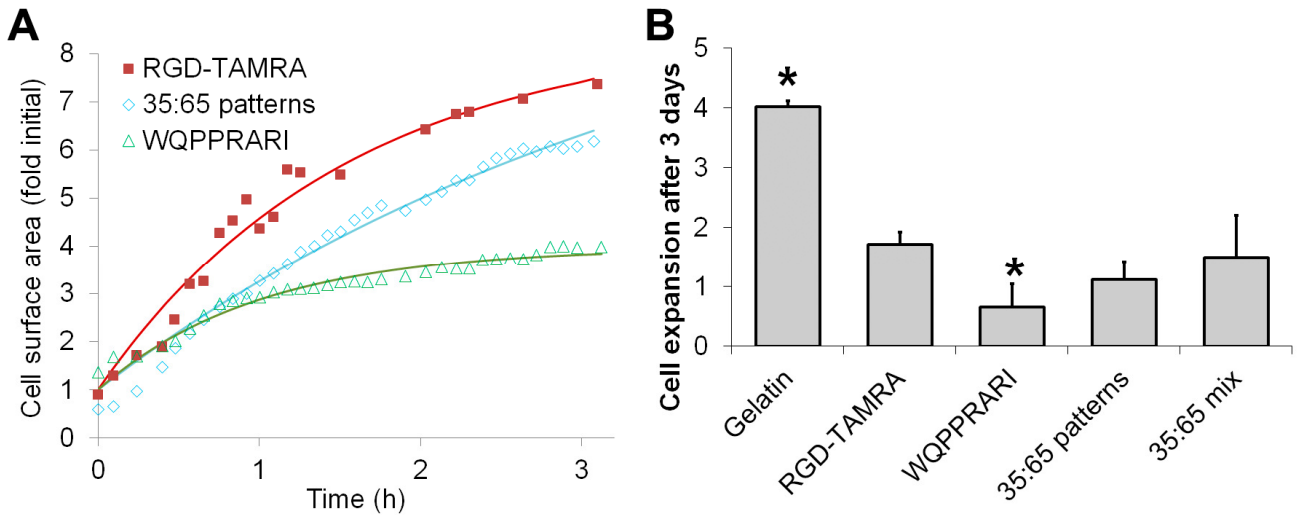

Figure 2. Cell spreading and growth on micropatterned or uniformly treated surfaces. (A) Change in cell surface area as a function of time. (B) Cell expansion after 6 days. Data represent the mean ± SEM of cultures from N=3 HSVEC donors. *p<0.05 compared to RGD-TAMRA

Discussion:

A fluorophore-tagged RGD peptide was designed to observe the effects of peptide micropatterns on HSVEC adhesion and growth. The location of the RGD-functionalized regions influenced the rate and direction of cell spreading, whereas cell expansion was similar on micropatterned and RGD-functionalized surfaces. RGD and WQPPRARI micropatterning could be used to control endothelial cell adhesion and mimic the anisotropic distribution of integrin ligands in the normal vasculature[8].

References:

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