A depth localization gradient of bioactive peptides regulates migration of smooth muscle cells

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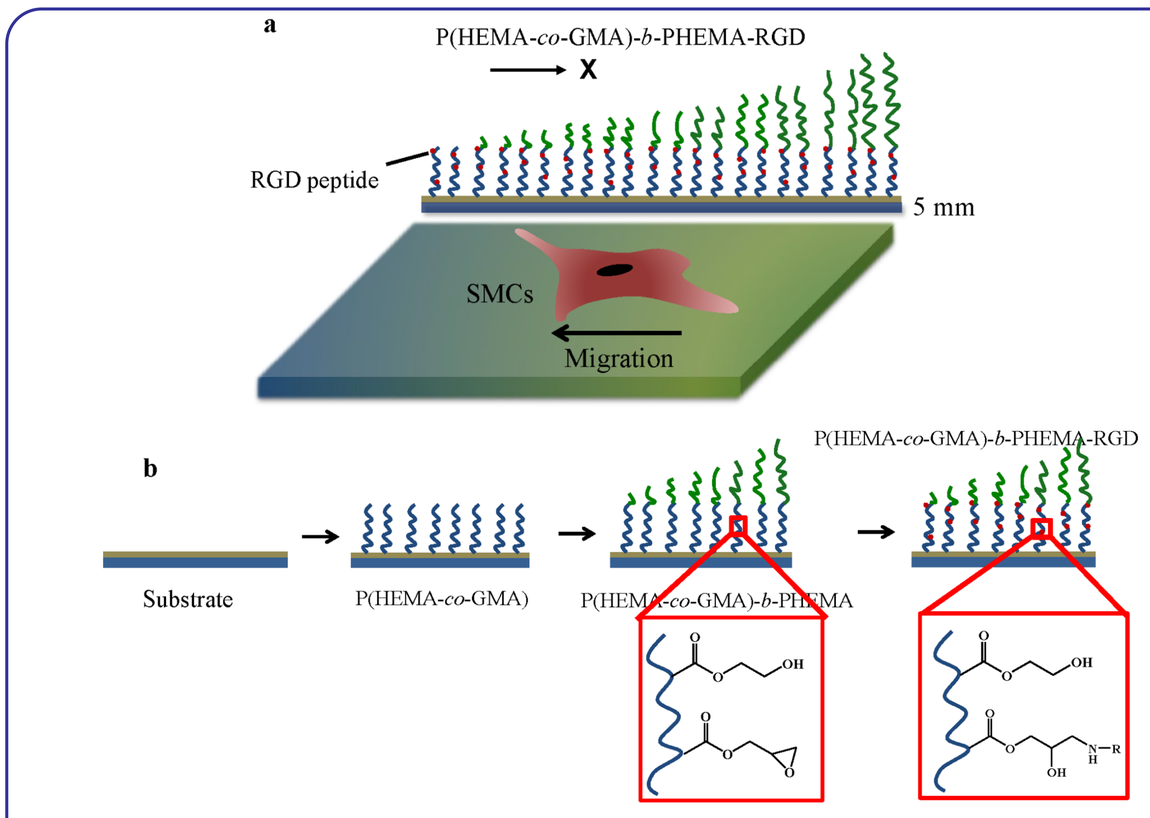
Abstract: The gradient localization of biological cues is of paramount importance to guide cell migration behaviors[1,2]. In this manuscript, P(HEMA-*co*-GMA)-*b*-PHEMA brushes with a uniform underneath P(HEMA-*co*-GMA) layer and gradient thickness of PHEMA segment were prepared by using surface-initiated atom-transfer radical polymerization. Adhesion and migration processes of smooth muscle cells (SMCs) were studied as a function of different depth localization of RGD peptides. Cell adhesion strength, arrangement of cytoskeleton, and gene and protein expression levels of adhesion-related proteins were studied to unveil the intrinsic mechanisms. It was found that the cell mobility was regulated by the complex and synergetic intracellular signals, which resulted from the difference in surface properties.



Scheme 1 (a) Schematic illustration to show the structure of depth localization gradient of RGD peptides and its mediation of directional cell migration. The direction of increased PHEMA thickness is defined as "+X" direction. (b) Preparation of P(HEMA-*co*-GMA)-*b*-PHEMA with a PHEMA thickness gradient, and the covalent immobilization of RGD peptides by reaction with epoxy groups.

Cell Orientation

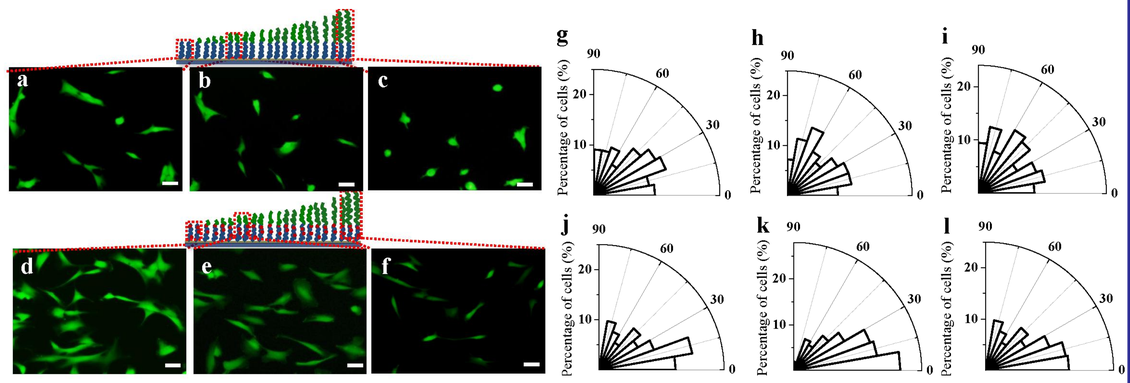


Fig. 2 (a-f) Fluorescent FDA staining images and (g-l) corresponding distribution of the SMCs angles to the X-axis of SMCs adhered on P(HEMA-*co*-GMA)$_{30min}$-*b*-PHEMA gradient surfaces (a-c) without and (d-f) with RGD immobilization after being cultured for 16 h at the positions of 1 mm, 2 mm and 5 mm, respectively. Scale bar=50 μm.

Cell Migration

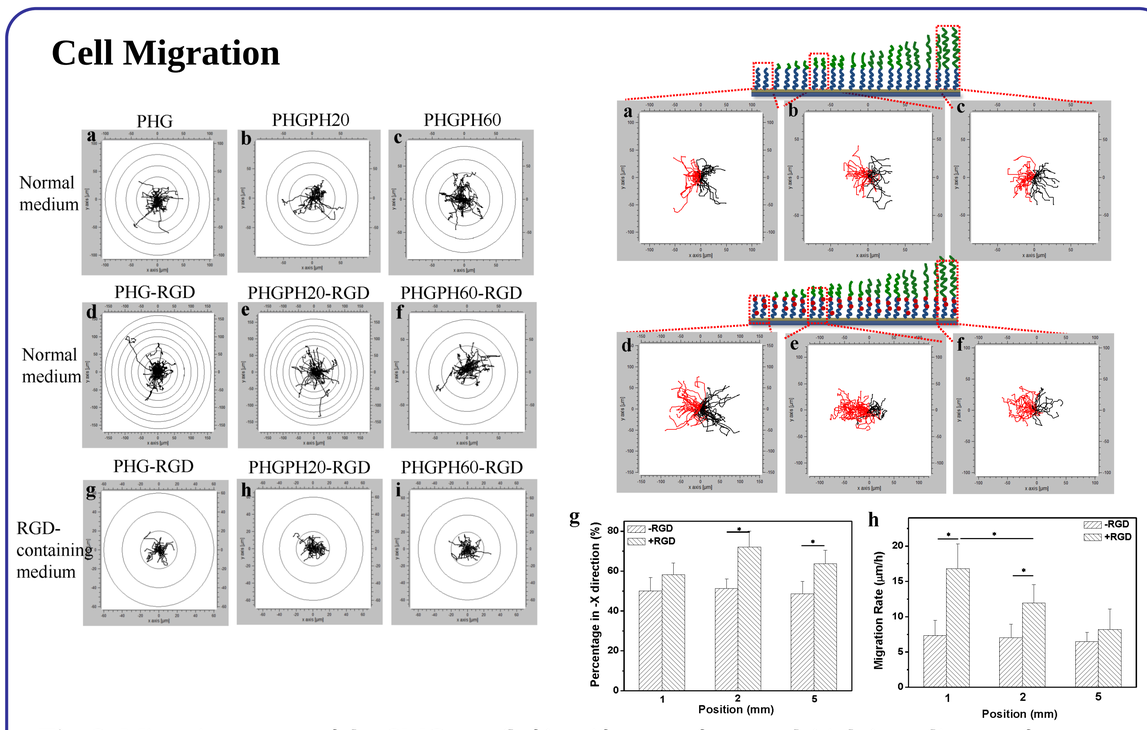


Fig. 3 Migration traces of the SMCs on (left) uniform surfaces and (right) gradient surfaces with and without immobilized RGD peptide.

Cell Adhesion

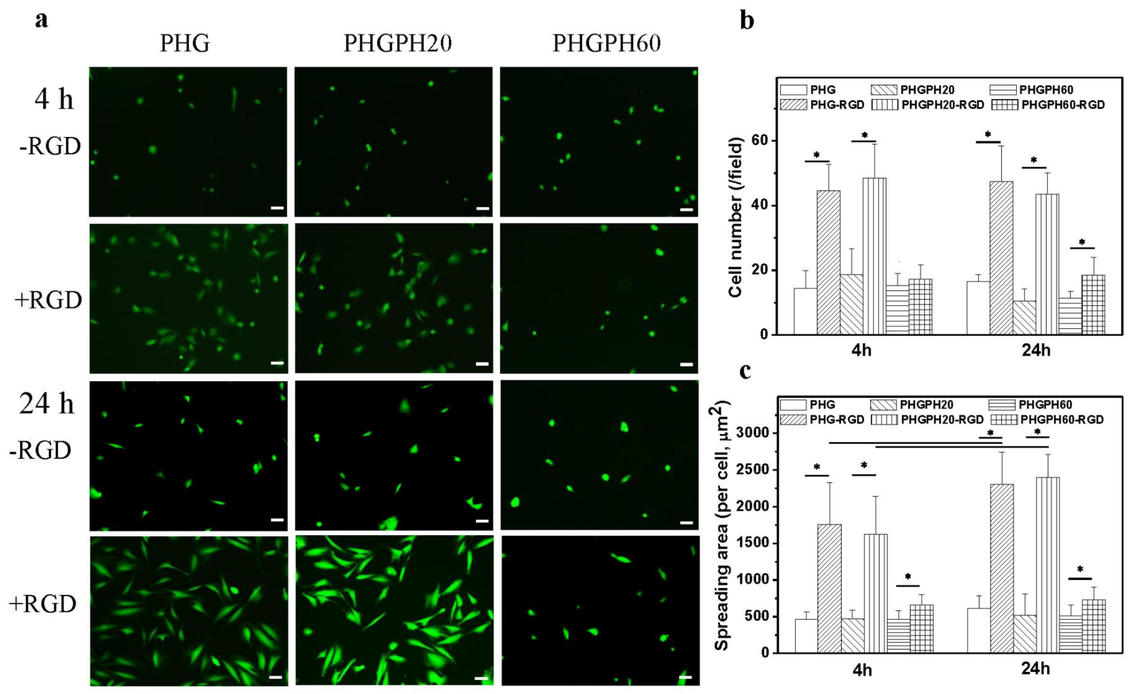


Fig. 1 (a) Fluorescent FDA staining images (b) Cell number and (c) cell spreading area of SMCs adhered on surfaces with and without RGD immobilization after being cultured for 4 h and 24 h, respectively. Scale bar=50 μm..

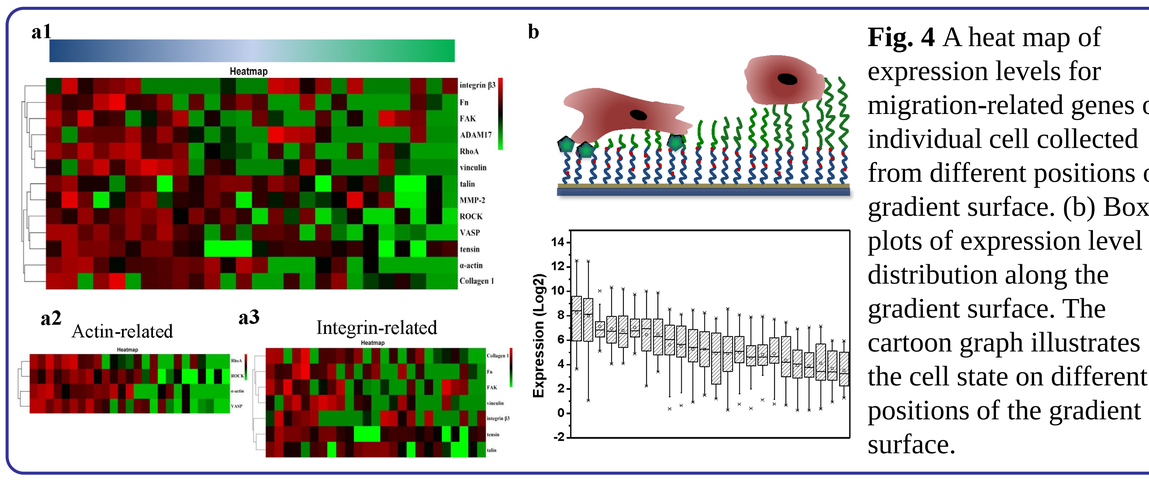


Fig. 4 A heat map of expression levels for migration-related genes of individual cell collected from different positions of gradient surface. (b) Box plots of expression level distribution along the gradient surface. The cartoon graph illustrates the cell state on different positions of the gradient surface.

Conclusions: A depth localization gradient of RGD peptides was successfully fabricated. Along with the increase of depth distance, the cell adhesion number, spreading area, adhesion force, and expression level of actin filaments and focal adhesion decreased. The cell migration rate increased with the decreasing depth distance of RGD peptide from the substrate surface. The SMCs exhibited directional migration behaviors toward the reverse gradient direction with a preferential orientation. Immunofluorescence staining, real-time PCR, as well as single-cell gene expression analysis were used to detect the migration-related proteins and genes to offer deeper insights into the formation of focal adhesions and their regulation of cellular responses by the Rho GTPase signaling. The insights obtained from the study may provide a new perspective on designing and improving the scaffold materials for desired tissue regeneration.

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