Supplementary File for

A Complementary Density Gradient of Poly(hydroxyethyl methacrylate) and YIGSR Selectively Guides Migration of Endotheliocytes

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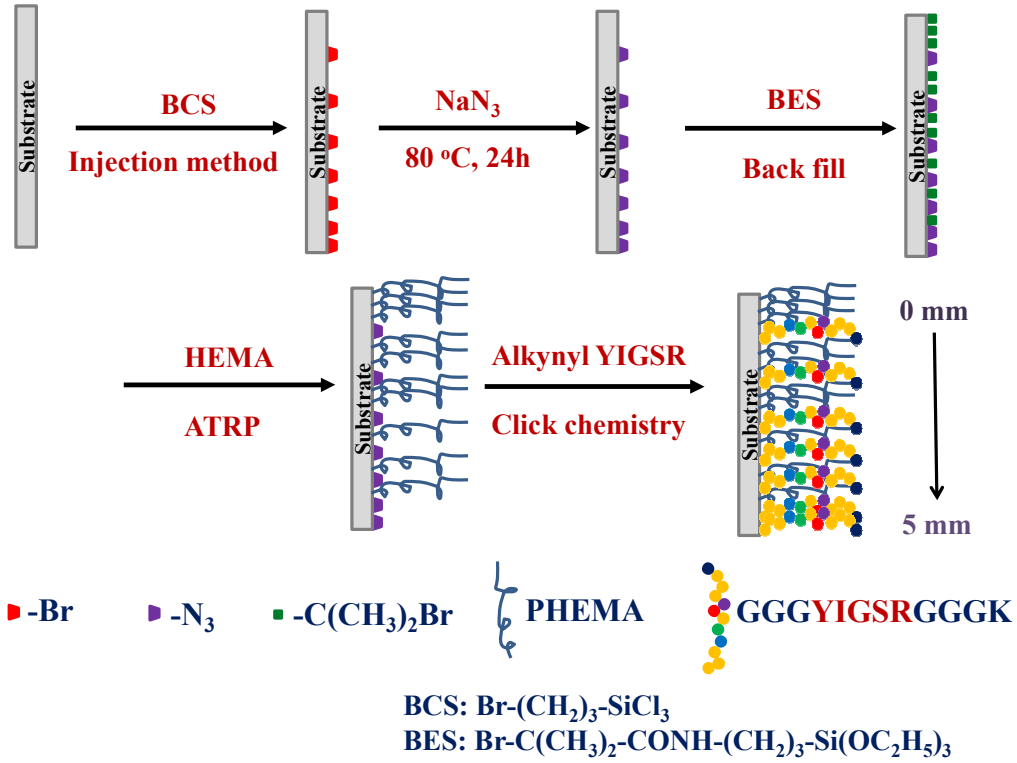
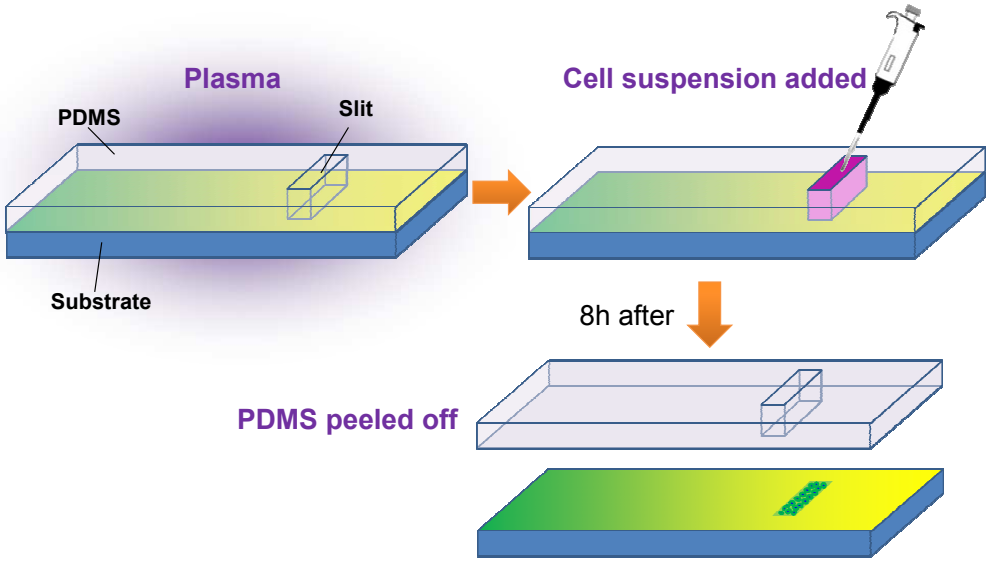


Scheme S1 Schematic illustration to show the fabrication of complementary density gradient of PHEMA and YIGSR, whose densities are controlled by the precursory immobilized BES and BCS, respectively.



Scheme S2 The scheme shows the preparation of a stripe of cells on the gradient. Firstly, a polydimethylsiloxane (PDMS) stamp with a slit (around 500 μm× 1cm) was used to cover the substrate with a gradient. The slit was placed vertical to the direction of gradient, exposing a desired stripe for cell culture. The stamps were pretreated with plasma to increase the surface hydrophilicity. Then, the cells were seeded onto the desired positions of the gradients at a density of 5×10^5 cells/cm^2 and were allowed to form a confluent monolayer. 8 h post cell seeding, the PDMS films were gently removed and the cells were allowed to migrate freely on the gradients.

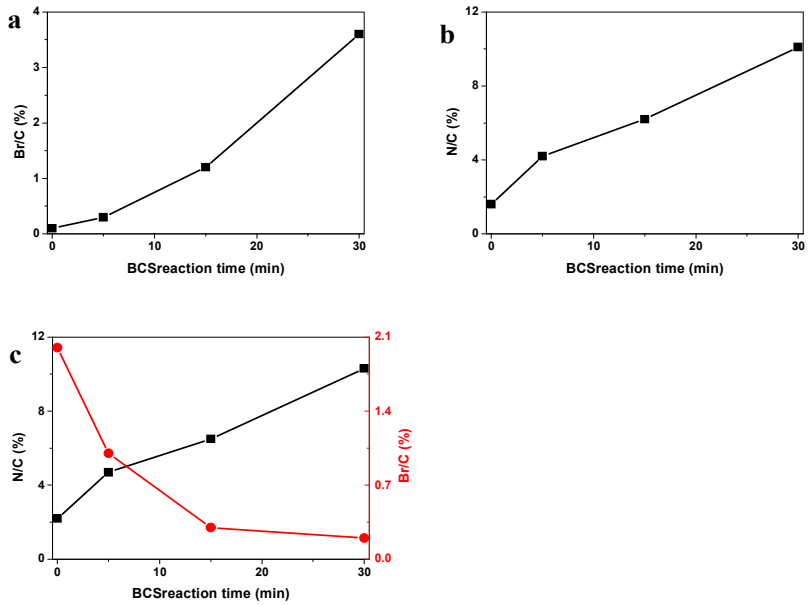


Figure S1 (a) Elemental molar ratios of Br/C, (b) N/C, and (c) Br/C and N/C as a function of BCS reaction time, on (a) -Br group density gradient, (b) $–N_3$ group density gradient, and $–N_3$/BES complementary density gradients, respectively.

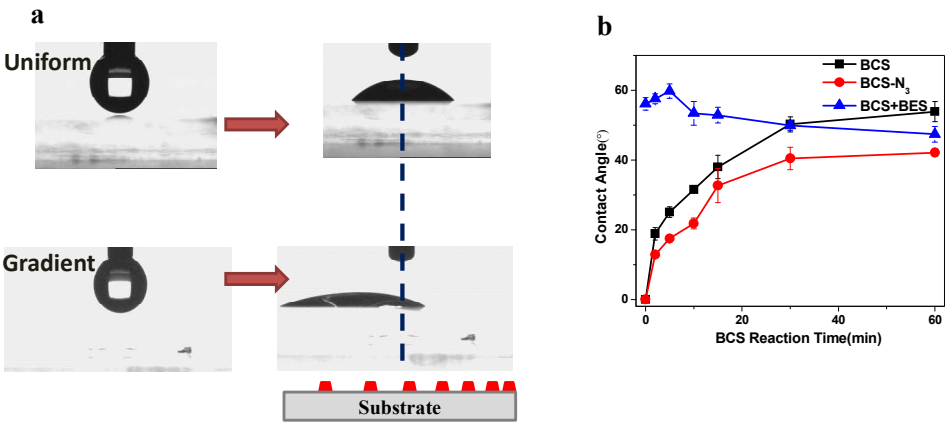


Figure S2 (a) Photos of water droplets on the surface uniformly reacted with BCS (the red ladder) for 15 min (upper panel) and the surface with BCS density gradient (lower panel, corresponding to the reaction time of 15 min). (b) Water contact angles measured on different uniform surfaces as a function of BCS reaction time.

The static contact angles of water were measured by a sessile-drop method on a DSA 100 contact angle measuring system (Krüss, Germany). The volume of each droplet was 2 μL. The results were averaged from 5 independent measurements.

On the uniform surface, the water droplet stayed at the center position (Figure S2a). When a water drop contacted with the gradient surface, it could not stay at its original position but moved to the direction with a shorter BCS reaction time (Figure S2b), suggesting the gradient nature of the wettability on the surface.

The water contact angles were measured on different uniform surfaces as a function of BCS reaction time (Figure S2b). The water contact angle was raised rapidly to ~20^{o} upon reaction with the hydrophobic BCS molecules, and then almost linearly increased along with the BCS reaction time at the earlier stage. It reached a plateau at about 50^{o} after 30 min, suggesting the surface was most covered by the BCS molecules. After the –Br groups were substituted by azide groups, the water contact angle decreased slightly, suggesting the successful introduction of the azide groups with stronger hydrophilicity. After backfilled with the hydrophobic BES molecules, the water contact angle of the surfaces reached to 55^{o} and became independent on the gradient position, suggesting the successful immobilization of the second silane molecules.

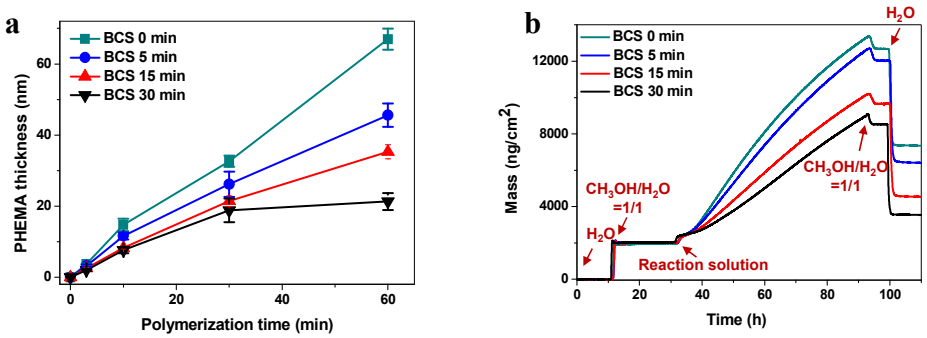


Figure S3 (a) PHEMA thickness and (b) mass as a function of polymerization time on various $-N_3$/BES surfaces with variable BCS reaction time (namely, initiator density), respectively. The arrows indicate the time points when different solutions were injected into the QCM chambers.

For QCM-D experiment, water was first injected into the chambers to get a base line showing the original state of the sensor. 10 min later, the water was changed to the reaction solvent (CH_3OH/H_2O=1/1). After flux for 20 min, the real reaction solution was added and polymerization started. When the reaction finished, the sensors were washed with solvent and then water until a horizontal line was observed. The difference between the original state and the final state represents the mass immobilized on the surface.

The thickness and mass of PHEMA brushes on uniform surfaces were measured by ellipsometry and QCM-D, respectively. They increased almost linearly along with the polymerization time. The thickness and mass of PHEMA brushes were higher when the surfaces were treated with BCS solutions for a shorter period of time, i.e. a lower density of azide groups but a higher density of initiators.

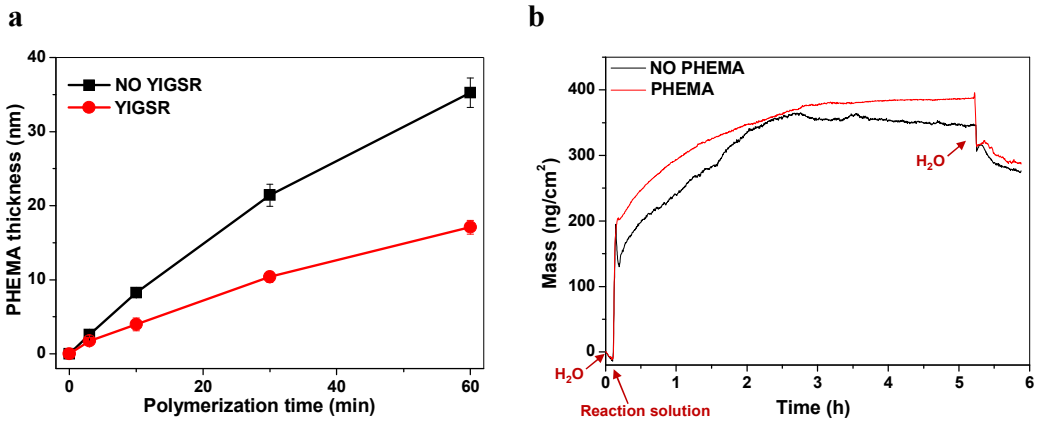


Figure S4 (a) Thickness of PHEMA brushes as a function of polymerization time on the surfaces with and without pre-immobilized YIGSR peptides, respectively. (b) QCM-D data showing the immobilization of YIGSR peptide on the substrates with or without PHEMA brushes.

The results show that the polymerization rate of PHEMA brushes is slower on the surface pre-immobilized with the peptides, suggesting that the peptides can hamper the polymerization to some extent. The PHEMA brushes on substrate did not interfere with the immobilization of peptide. Therefore, in our experiment the PHEMA brushes were built first, and then the peptides were clicked onto the surface.

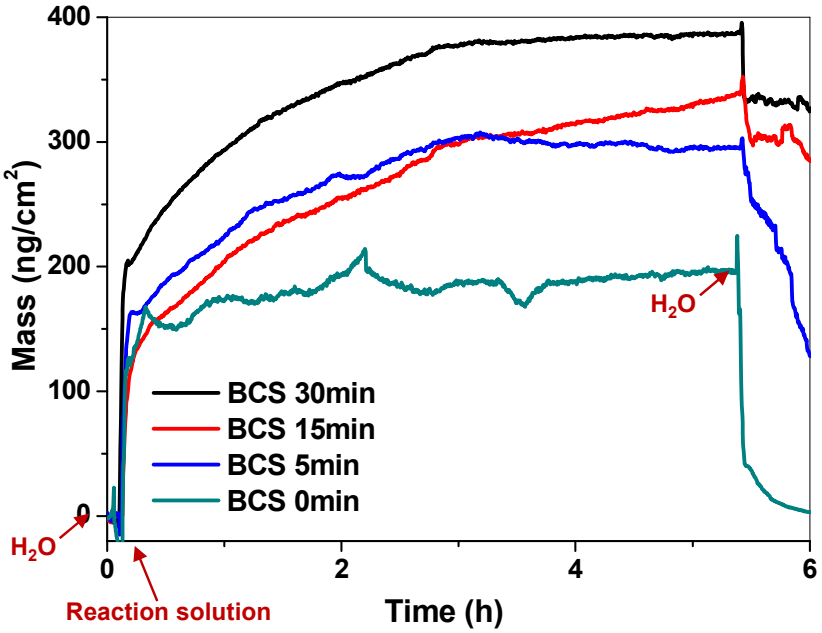


Figure S5 QCM-D data showing the immobilization of YIGSR peptide on the substrate at different BCS reaction time (namely, azide group density). The arrows indicate the time points when different solutions were injected into the QCM chamber.

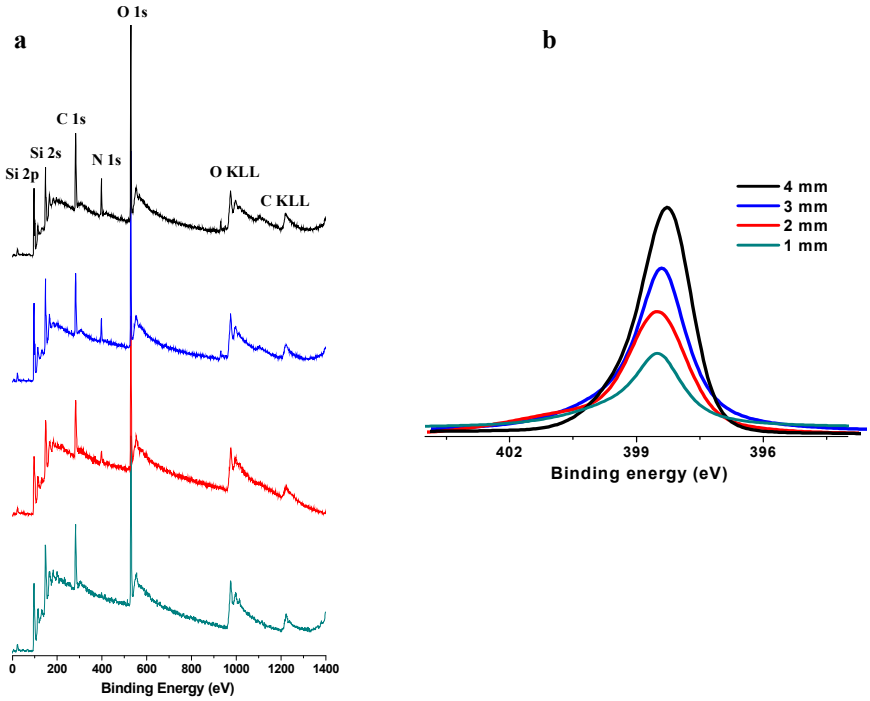


Figure S6 (a) XPS survey spectra and (b) core-level spectra of N1s on the complementary PHEMA/YIGSR density gradient surfaces with variable positions.

XPS was used to characterize the relative density of peptide molecules on the PHEMA/YIGSR gradient surface. The characteristic peaks of N element of peptide bonds appeared near 399.2 eV on the XPS spectra of PHEMA/YIGSR gradients. The intensity increased along with the gradient position, suggesting the increase of peptide density.

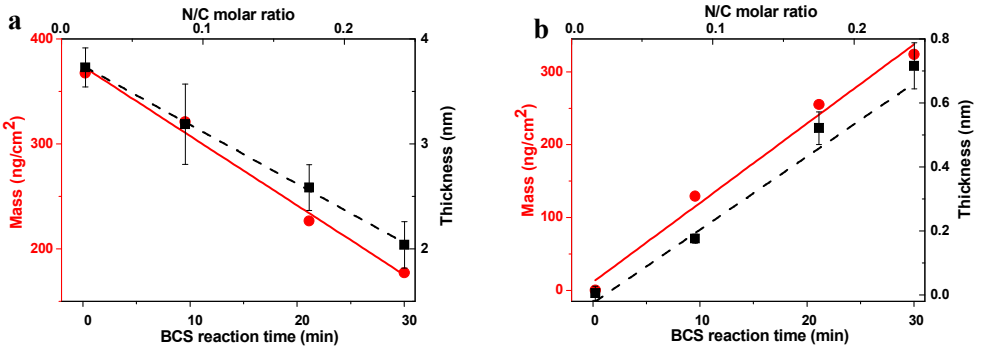


Figure S7 Grafting mass and thickness of PHEMA (a) and YIGSR (b) as a function of N/C ratio or BCS reaction time on uniformly silanized surfaces. The mass and thickness were measured by QCM-D and ellipsometry, respectively.

As shown in Figure S7a, the grafting density of PHEMA brushes decreased almost linearly along with the prolongation of BCS reaction time, and reached to 369, 321, 228, 177 ng/cm^2 on the PHEMA/azide surfaces which reacted with BCS for 0, 5, 15, and 30 min, respectively. Correspondingly, the thickness of PHEMA brushes decreased from 3.7 to 1.8 nm. Conversely, the grafting density of YIGSR peptides increased along with the prolongation of BCS reaction time (Figure S7b), as expected, and reached to 0, 129, 255, and 316 ng/cm^2 on the PHEMA/YIGSR surfaces which reacted with BCS for 0, 5, 15, and 30 min, respectively. These results confirmed the feasibility to control the density of PHEMA and YIGSR peptide brushes by the proposed strategy.

Since the gradient BCS reaction results in a similar -Br density to the corresponding uniform surface when the reaction time is the same, it is reasonable to assume that the PHEMA densities on the gradient surfaces at 1, 2, 3, 4 mm are equal to their counterparts on the uniform surfaces, respectively.

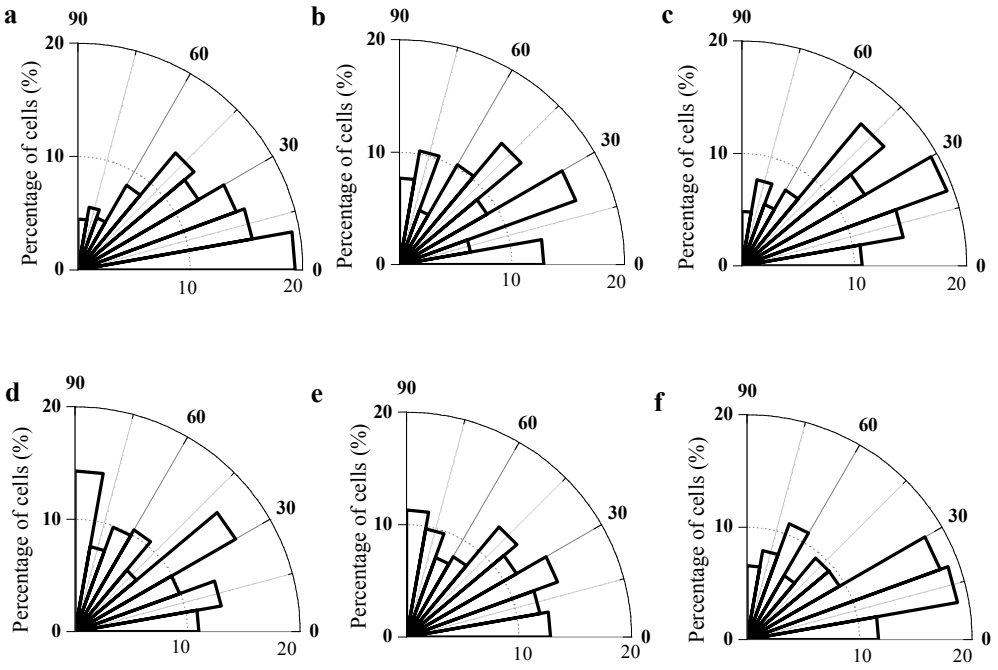


Figure S8 Distribution of the (a-c) ECs and (d-f) SMCs angles to the gradient direction on (a, d) PHEMA/YIGSR complementary gradient, (b, e) YIGSR, and (c, f) PHEMA gradient, respectively.

The images of ECs and SMCs were recorded under a phase contrast microscope 24 h post cell seeding on the gradient surfaces. The cell orientation of at least 300 cells (from 3 individual experiments) was analyzed using the Image Pro Plus software to calculate the orientation angle of each cell.

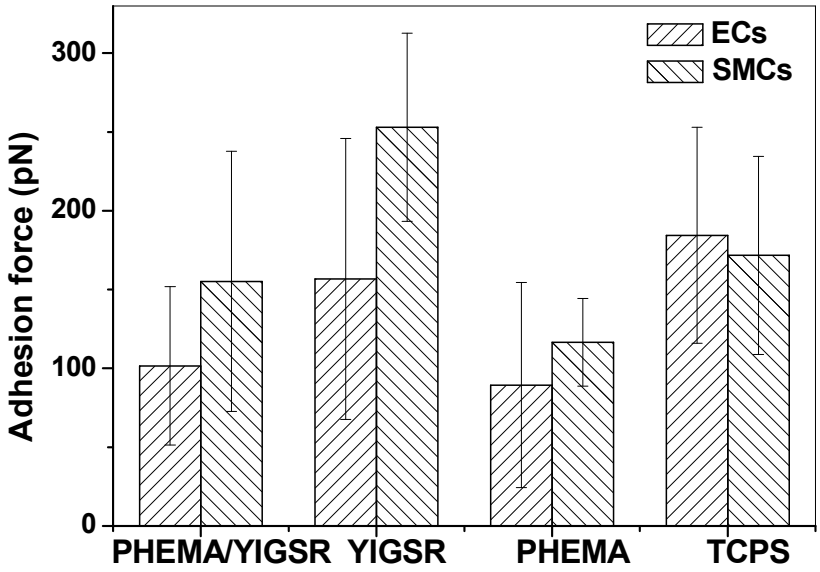


Figure S9 Adhesion force of ECs and SMCs cultured on different surfaces.

The cell adhesion force of ECs and SMCs on the substrates was measured according to the method suggested in Reyes's work.[1] After cell seeding for 24 h, the substrates were gently washed with PBS to remove the floating cells. The cell number was counted under a microscope. Then the glass slides were placed vertically at the bottom of centrifuge tubes which were filled with PBS. The numbers of cells remained on the glass slides were counted, and the fraction of adhesion cells was calculated after being centrifuged at 800 and 1500 rpm for 5 min, respectively. The forces exerted on cells were calculated according to the theory described previously.[2]

Video S1 Migration traces of (a) ECs, and (b) SMCs on PHEMA/YIGSR complimentary density gradient.

References

(1) Reyes, C. D.; Garcia, A. J. *J. Biomed. Mater. Res., Part A* **2003,** *67*, 328-333.

(2) Wu, J.; Mao, Z.; Gao, C. *Biomaterials* **2012,** *33*, 810-20.