Preparation of an Arg-Glu-Asp-Val Peptide Density Gradient on Hyaluronic Acid-Coated Poly(ε-caprolactone) Film and Its Influence on the Selective Adhesion and Directional Migration of Endothelial Cells

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ABSTRACT: Selective adhesion and migration of endothelial cells (ECs) over smooth muscle cells (SMCs) is very important in the rapid endothelialization of blood-contacting implants to prevent vascular restenosis. In this study, a uniform cell-resistant layer of methacrylate-functionalized hyaluronic acid (HA) was first immobilized on a poly(ε-caprolactone) (PCL) film via polydopamine coupling. Then, a density gradient of thiol-functionalized Arg-Glu-Asp-Val (REDV) peptide was prepared on the HA layer via thiol-ene click chemistry and the continuous injection method. The REDV gradient selectively enhanced EC adhesion and preferential directional migration toward the region of higher REDV density, reaching 86% directionality in the middle of the gradient. The migration rate of ECs was also significantly enhanced twofold compared with that on tissue culture polystyrene (TCPS). In contrast, the gradient significantly weakened the adhesion of SMCs to 25% of that on TCPS but had no obvious impact on the migration rate and directionality. Successful modulation of the selective adhesion and directional migration of ECs over SMCs on biodegradable polymers serves as an important step toward practical applications for guided tissue regeneration.

KEYWORDS: PCL film, REDV peptide, gradient materials, endothelial cells, smooth muscle cells, selective cell migration

1. INTRODUCTION

Selective cell migration is required for many important physiological processes. Undesired cell migration can cause disease or improper regeneration of tissues such as atherosclerosis, a chronic inflammatory disease of the arterial wall.1 During atherosclerosis, the endothelium, which is composed of endothelial cells (ECs), is damaged. Pathologically changed vascular smooth muscle cells (SMCs), which show more amplified growth potential and chemotactic activity than medial SMCs, subsequently migrate to the impaired vessels, leading to further damage to the vasculature.2 In-stent restenosis (ISR), a particular refractory form of neointimal hyperplasia, is another example.3 Stent implantation has become the main method for treating coronary artery diseases. However, the implantation may induce a series of pathological processes such as thrombosis and abnormal release of cytokines, which subsequently trigger the migration and proliferation of SMCs and thereby induce ISR.4 Therefore, it is of great importance to develop a material that can specifically enhance the migration of ECs rather than SMCs.

Chemical and physical signals have been utilized to enhance the mobility of ECs, such as extracellular matrix proteins and their derived peptides5−10 and micropatterns.11−14 Gradient materials, which have a gradually varying physical or chemical signal density, have been proven to be powerful tools for accelerating cell migration.15−18 Jiang et al. demonstrated that the mobility of ECs was largely enhanced by a vascular endothelial growth factor (VEGF) density gradient.19 More importantly, gradient signals can guide cells to move to a preferred direction.20,21 Wu et al. prepared a gradient density of VEGF onto silicon wafers/glass slides and found that over 80% of ECs migrated toward the region of higher VEGF density gradient.22 Paradise et al. prepared an extracellular pH gradient and found that cells are preferentially polarized and migrate toward the acidic end of the gradient.23

However, the majority of the materials are not cell-selective. A few studies have successfully used EC-specific ligands to specifically promote EC adhesion and rapid in situ endothelialization.24,25 Kushwaha et al. developed a material with EC-binding peptides and the ability to release nitric oxide (NO), which decreased the adhesion and migration of SMCs.26 However, selective enhancement of EC migration by tailored materials, which have a gradually varying physical or chemical signal density, have been proven to be powerful tools for accelerating cell migration.15−18 Jiang et al. demonstrated that the mobility of ECs was largely enhanced by a vascular endothelial growth factor (VEGF) density gradient.19 More importantly, gradient signals can guide cells to move to a preferred direction.20,21 Wu et al. prepared a gradient density of VEGF onto silicon wafers/glass slides and found that over 80% of ECs migrated toward the region of higher VEGF density gradient.22 Paradise et al. prepared an extracellular pH gradient and found that cells are preferentially polarized and migrate toward the acidic end of the gradient.23

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materi...oms has scarcely been achieved so far. Our previous study has been the only study to fabricate a complementary density gradient of poly(2-hydroxyethyl methacrylate) (PHEMA) brushes and YIGSR peptide, an amino acid sequence specifically improving the mobility of ECs on a silicon wafer/glass slide using a surface-initiated atom transfer radical polymerization (SI-ATRP) approach. In that study, the ECs exhibited preferred orientation and enhanced directional migration toward the region of lower PHEMA density and higher YIGSR density.27

Although a setup based on SI-ATRP is robust on model surfaces such as silica, the method is complicated, leading to inconvenience when transferred to real biomaterials such as biodegradable polyesters for artificial vessel engineering. The effect of the gradient may also be influenced because of the complicated structures and physicochemical properties of real biomaterials. Moreover, selective cell adhesion has not been addressed previously. Therefore, in this study, a more general and simpler method was developed to generate a gradient on biodegradable poly(ε-caprolactone) (PCL) films with defined functions (Figure 1). PCL was chosen because it is a biodegradable polymer with appropriate physiochemical properties, nontoxic degradation products, and a long degradation time appropriate for the development of a tissue-engineered vascular graft. Moreover, an ideal material will not only enhance the mobility of ECs but also reduce the adhesion and/or migration of SMCs, leading to a greater advantage of ECs over SMCs.

As shown in Figure 1, the PCL film was coated with a thin polydopamine (PDA) layer on which methacrylate-functionalyzed hyaluronic acid (MA-HA) molecules were covalently immobilized. Polydopamine, a typical catechol-based compound, is well-known for its excellent adhesion ability on both organic and inorganic surfaces.28 PDA contains many amino groups which can potentially be used for further molecular conjugation. Hyaluronic acid (HA) is a natural polysaccharide that has received tremendous attention in medical applications due to its superior inert properties as an anionic glycosaminoglycan and high antifouling ability against fibronecin adsorption.29 Therefore, the MA-HA layer functions to resist nonselective cell adhesion. Arg-Glu-Asp-Val (REDV) peptide is the smallest active sequence of fibronecin that can be identified by integrin αvβ3 receptor on ECs30 and has been widely used for designing EC-selective surfaces.31 In this study, a thiol (cysteine)-functionalized REDV peptide was further added onto the MA-HA layer via a thiol–ene reaction in a gradient manner, which was manipulated by continuous injection of the REDV peptide solution into the container of MA-HA-modified substrate.32 The REDV density gradient on the cell-resistant HA layer was investigated in terms of EC adhesion, polarity, adhesion force, mobility, and directionality by comparison with SMCs.

2. EXPERIMENTAL SECTION

2.1. Materials. Poly(ε-caprolactone) (PCL, Mn ~80 kDa), 3-methacryloyloxypropyltrimethoxysilane (TMSPMA), and 3-hydroxytyramine hydrochloride (dopamine) were purchased from Sigma-Aldrich, St. Louis, MO, United States. Hyaluronic acid (HA, Mn ~100 kDa) was purchased from Zhenjiang Dongyuan Biotech Co., Ltd., Zhenjiang, China. Cys-Arg-Glu-Asp-Val (REDV-SH) was synthesized by GL Biochem Co., Ltd., Shanghai, China. Tris(hydroxymethyl)aminomethane, N-hydroxy succinimide (NHS), and 1-ethyl-3-(3-dimethylamino)propyl)-carbodiimide hydrochloride (EDC) were purchased from Aladdin Inc. Na2CO3, NaHCO3, methacrylic anhydride (MA), 1,4-dioxane, ethanol, N,N-dimethylformamide (DMF), dimethylbenzene, and acetone were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. All chemicals were of analytical grade and used as received without special notice. The water used in this study was purified by a Milli-Q system (Millipore, Billerica, Massachusetts, United States).

2.2. Synthesis of Methacyrlylated Hyaluronic Acid (MA-HA). MA-HA was prepared according to a previously reported method.33 In brief, HA (1.0 g) was dissolved in 100 mL of mixed solvent of DMF/water (1/2, v/v), into which a 20-fold excess of methacrylic anhydride (7.4 mL) was added. The mixture was stirred for 24 h in an ice bath after the solution pH was adjusted to 8.0 using a 5 mol/L NaOH solution. The pH was maintained between 8–9 during the whole procedure. MA-HA was obtained by precipitation in excess ethanol and collected by centrifugation, which was further purified by dialysis for 3 d against distilled water and finally freeze-dried. The grafting ratio of methacrylate was 2.1 in each HA repeating unit with four --OH reaction sites according to 1H NMR (Figure S1, Supporting Information).

2.3. Preparation of PCL--PDA Substrate. Glass slides were cut into 1 × 1 cm pieces, which were further treated with “piranha” solution (a mixture of 70% sulfuric acid and 30% hydrogen peroxide (v/v)). Caution: this solution is highly corrosive and should be handled carefully), thoroughly washed with water, and dried under a nitrogen flow. TMSPMA was dissolved in dry toluene to obtain a 0.0025% (v/v) solution into which the glass slides were immersed for 30 min at room temperature to make the surface hydrophobic. Then, the slides were sequentially washed with toluene, acetone, and alcohol with the assistance of ultrasonication. They were dried under a nitrogen flow and then annealed at 60 °C for 1 h. The PCL membrane with a thickness of 200 nm was prepared by spin-coating a 2% (w/v) PCL/1,4-dioxane solution onto the glass slides under 1600 rpm. The PCL-coated glass slides were immersed in a 2 mg/mL dopamine/tris-HCl (10 mM, pH 8.5) solution at 37 °C. After a 12 h deposition, the PDA-coated slides were removed and washed with plenty of water.
Thiol-functionalized REDV peptides were dissolved in Na₂CO₃/NaHCO₃ buffer (pH 9) to reach a concentration of 0.4 mg/mL. The MA-HA-coated glass slides were incubated at 37 °C for 3 h and allowed to attach for 12 h. ECs and SMCs cultured on the substrates were fixed with 4% paraformaldehyde at 37 °C for 30 min. The cells were washed with PBS 3 times and then permeabilized using 0.5% Triton X-100 for 10 min. After being washed with PBS 3 times, the samples were incubated in a 1% bovine serum albumin (BSA) PBS solution for 30 min at 37 °C. After being washed twice, the cells were stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) and rhodamine-labeled phalloidin (Invitrogen) at room temperature for 1 h. After being washed three times in PBS, the cells were observed under a confocal laser scanning microscope (CLSM, LSM-510, Zeiss, Germany). Angles of cell alignment with the x-axis were calculated using ImageJ software.

2.8. Cell Migration. All samples were sterilized under UV light for at least 0.5 h. Four positions (0, 2.5, 5, and 10 mm) were selected on the gradient, corresponding to the REDV peptide grafting time of 0, 1.5, 30, and 60 min, respectively. The respective uniform surfaces with the same reaction time and thereby the same REDV peptide densities were used as controls. The accurately detectable width of each point was positioned ±0.5 mm along with gradient. ECs and SMCs were seeded at a density of 5 × 10⁴ cells/cm² and allowed to attach for 12 h. A low cell density can be used to obtain separated cells after cell adhesion to avoid cell–cell contact (>90%). There was only a small ratio of cell aggregates which were ruled out when calculating cell migration behaviors. Then, cell migration traces were recorded in situ for 12 h under a time-lapse phase-contrast microscope (DMI6000B, Leica) equipped with a cell culture chamber (37 °C and 5% CO₂ humidified atmosphere). The migration trace of each individual cell was tracked, and a series of (xᵢ, yᵢ) position coordinates within the observation period was recorded using ImageJ software with the manual tracking plugins. The cell trajectories were reconstructed based on exported cell positions. The starting position of each single cell was automatically defined as the original position (0, 0). The cell migration distance S was then calculated by the Chemotaxis Tool (Ibids, Germany) with the time gap of 15 min according to the following equation.

\[
S = \sum_{i=1}^{n} \sqrt{(x_i - x_{i-1})^2 + (y_i - y_{i-1})^2}
\]

At least 30 cells were analyzed for each data point. Proliferating and spherical detached cells were not included in the results. The cell migration rate was calculated based on the formula \(v = S/t\). The data were analyzed by Rayleigh test, which indicates an asymmetric distribution of the end points to present cell directional migration tendency with a significance level of \(p < 0.05\).

In the cell sheet migration experiment, the substrate was covered with a polydimethylsiloxane (PDMS) stamp with a slit of approximately 600 µm in width. The slit was placed vertically to the direction of the gradient. Cells were seeded onto the desired positions of the gradients at a density of 5 × 10⁴ cells/cm² and were allowed to form a confluent monolayer. The PDMS film was gently removed 12 h
after cell seeding to allow cell migration on the gradient. The cell sheets were observed under the time-lapse microscope for 12 h.

2.9. Statistical Tests. Three or more independent experiments were carried out to get reproducible data. Statistical analysis was performed based on one-way analysis of variance (ANOVA) with a Tukey post hoc method. A significance level of \( p < 0.05 \) was chosen for all of the tests without special explanation. A few comparisons were also made between individual groups via \( t \)-test with the same significance level.

3. RESULTS AND DISCUSSION

3.1. Preparation and Characterization of the Gradient and Grafting Mass of MA-HA and REDV. To construct the substrate for making the REDV gradient with cell selectivity, a PCL film was first prepared on a glass slide by spin-coating. The film had a thickness of approximately 200 nm as determined by ellipsometry, atop which a uniform layer of polydopamine (approximately 10 nm) was generated after a 12 h deposition. Furthermore, a homogeneous MA-HA layer (0.7 nm in a dry state) was covalently immobilized onto the polydopamine layer via the reaction between carboxyl groups and amine groups (Figure 1).

The grafting density of MA-HA increased with the reaction time and reached a plateau of 645 ng/cm\(^2\) after 3 h (Figure S2a). Thiol-functionalized REDV peptides were further conjugated via thiol-ene click chemistry. The density increased almost linearly along with the length of the reaction time (Figures 2 and S2b) and reached 46, 81, 151, and 191 ng/cm\(^2\) after 15, 30, 60, and 90 min reactions, respectively. The corresponding molar ratios of REDV peptides to MA-HA repeating units were 10.0, 17.6, 32.8, and 41.5\%, respectively. Further prolongation of the reaction time did not significantly enhance the REDV density. With these results, it is possible to prepare an REDV density gradient by controlling the solution level and thereby the reaction time by continuous injection of the peptide solution into a vial containing the MA-HA functionalized substrate. Preliminary results showed that the adhesion and migration behaviors of both ECs and SMCs were influenced by the REDV density but were almost identical on the surfaces with REDV densities of 151 and 191 ng/cm\(^2\) (Figures S3 and 4). Therefore, the REDV density was controlled between 0 and 151 ng/cm\(^2\), i.e., with a reaction time of 0–60 min, to prepare the gradient surface.

As illustrated in Figure 1, the density gradient of REDV peptides was created on the MA-HA functionalized surface with the lowest REDV density at the 0 mm position and the highest REDV density at the 10 mm position on the x-axis. The surface ratio between sulfur and carbon, which is positively correlated with the content of REDV peptides and are the only molecules containing sulfur, was measured by XPS at a depth of approximately 2.8 nm on the surface. As shown in Figure 2b, the surface content of REDV peptides increased along with the gradient position, confirming the successful generation of an REDV density gradient on a single substrate. The slopes of the QCM-d and XPS lines were 4.0 and 3.2, respectively, which represent similar increasing tendencies. It is worth mentioning that the highest REDV content reached only 6.4\% of the surface. However, the results obtained from QCM-d suggest that the REDV content (151 ng/cm\(^2\) in the wet state) should be much higher than 6.4\%, approximately 20\%, compared with that of HA (645 ng/cm\(^2\) in the wet state). The detectable depth of the surface was 2.8 nm, which was much thicker than that of the MA-HA layer (0.7 nm). Therefore, the apparent lower REDV content estimated by XPS may have been caused by the interference of the underlying PDA layer, which contributes a large portion of carbon.

3.2. Selective Adhesion of ECs. To evaluate the impact of the REDV density gradient on cell-selective adhesion, equal numbers of ECs and SMCs were seeded onto the same materials. After 8 h (Figure 3), the absolute numbers of SMCs (red color in Figures 3a and b, 20–42 cells/cm\(^2\)) on all the positions of the gradient were significantly smaller than that on TCPS (126 cells/mm\(^2\)), likely due to the cell-repulsion effect of HA molecules. Because of a limitation of the antifouling ability of HA molecules and/or their insufficient density on the surface, a small ratio of cells were still able to attach to the surfaces. Because REDV peptide specifically interacts with ECs, the number of SMCs on the gradient surface was not determined by the peptide density. The adherent ECs also decreased to an extent similar to that of SMCs close to the 0 mm position. Scale bar is 200 μm. (c) Numbers of ECs and SMCs cultured for 8 h on TCPS at different positions of REDV density gradient. The cells were seeded onto the surfaces at a density of 2.1 × 10\(^4\) cells/cm\(^2\). At least 10 images were analyzed for each data point, and 3 independent experiments were carried out. * indicates statistically significant difference at the \( p < 0.05 \) level.
For a more quantitative analysis of the bindings between substrates and cells, the cell adhesion forces of ECs and SMCs on homogeneous surfaces with different REDV densities were characterized. As shown in Figure 4, the adhesion force of ECs obviously enhanced along the REDV density, for instance, from 1.1 nN on the HA surface to 4.1 nN on the surface with the highest REDV density (151 ng/cm²), which leads to better adhesion of ECs along the gradient. In contrast, the adhesion forces of SMCs on all the positions of the gradient surfaces were smaller than 1.3 nN, which is significantly smaller than that on the glass slide (2.3 nN, data not shown).

3.3. Cell Morphology and Orientation. Previous studies have demonstrated that a gradient surface can polarize cells and align cells with the gradient direction, which is an important step for directional migration of cells. As shown in Figures S5a–d and S6, the ECs on the REDV gradient surface showed more stressed actin fibers and larger cell spreading area than their counterparts at the 0 mm position without REDV. Moreover, the ECs on the gradient surface showed a preferred tendency to align toward the gradient direction with higher REDV density. In contrast, the ECs on the uniform surfaces always exhibited a random orientation of actin fibers (Figures S7a–d). The SMCs always showed randomly organized actin fibers without a preferred direction on both the gradient surface (Figures S5e–h) and uniform surface (Figures S7e–h). Moreover, the spreading areas of the SMCs on the gradient surface were always significantly smaller than that on TCPS, also confirming the cell selectivity of the gradient (Figure S8).

The orientation of the ECs and SMCs was further quantitatively analyzed (Figure 6). It is well-known that on a uniform surface, cells orient randomly and statistically show a ±45° angle to the x-direction. The ECs showed a smaller angle to the x-direction, especially at the 2.5 and 5 mm positions, confirming better alignment of ECs with the gradient (Figure 6a). However, the ECs at the 0 and 10 mm positions were not as sensitive to the gradient because of too low and too high REDV density, respectively. In contrast, the alignment angle of SMCs toward the x-direction was around ±45°, indicating the randomly oriented nature of SMCs (Figure 6a). Furthermore, the ratio of cells aligned within ±30° to the x-direction was also calculated at different positions to demonstrate cell orientation intuitively (Figure 6b). The results showed that 52 and 61% of the ECs oriented at ±30° to the x-direction on the REDV gradient at the 2.5 and 5 mm positions, respectively. In contrast, less than 20% of SMCs oriented at ±30° to the x-direction at all the positions of the gradient. The results substantiate that ECs have the tendency to orient to the positive x-direction on the gradient surface with enhanced REDV density, and the strength can be mediated by the REDV density (gradient position).

3.4. Cell Migration on REDV-Grafted Gradient. During vascular repair and regeneration processes, selective adhesion and migration of ECs over SMCs is desired to promote fast endothelialization on biomaterials. In this research, surface grafting of HA was expected to significantly reduce the nonspecific adhesion of both ECs and SMCs, and the introduction of an REDV density gradient would lead to specific adhesion and directional migration of ECs with better mobility.

The migration behavior of ECs and SMCs on the REDV density gradient was followed and carefully analyzed. The cells were plated at a low density to avoid cell–cell interactions. Under such a condition, the migration behaviors of the cells can be controlled solely by the cell–substrate interactions. The cells were monitored in situ to obtain the migration trajectories. Both types of cells showed similar mobility on the PDA surface (data not shown) compared to those on the TCPS, suggesting that the underlying PDA layer does not have significant impact on cell migration. As shown in Figures 7b, c, 8b, and 8c, 84% of the ECs moved toward the positive x-direction of the gradient at the 2.5 and 5 mm positions, respectively. At the gradient positions of 0 and 10 mm, the percentage of ECs migrating directionally toward the positive x-direction of the gradient decreased to 60.5 and 75.5%, respectively (Figure 7d). In contrast, SMCs showed random migration along the whole gradient, as evidenced by only approximately 50% of SMCs moving toward the positive x-direction (Figures 7g–j). The directional movement of ECs was controlled by the directional polarization of ECs under the stimulation of the REDV peptide density gradient (Figure 6). This conclusion was further confirmed by the fact that the ECs moved randomly without a preferred direction on the uniform REDV surface (Figures S5a–d). The less-oriented cells positioned at higher REDV densities are most likely responsible for the weakened directionality.
Figures 7b–d also show that ECs traveled significantly longer distances on the gradient than those on the TCPS. Consequently, their migration rate (14.1 μm/h) at the 5 mm position on the gradient was significantly faster than that on TCPS (7.2 μm/h, Figure 8a) and on the uniform surface with the similar REDV density (7.7 μm/h, Figure 8b). At the 0 and 2.5 mm positions, ECs also showed enhanced migration rates, i.e., 10.1 and 12.5 μm/h, respectively, and both were faster than those on TCPS and corresponding uniform material surfaces. Therefore, the enhanced mobility of ECs on the gradient surface must be attributed to the cellular polarization and orientation induced by the underlying gradient signal. In contrast, the migration rate of SMCs remained unchanged on the gradient and even slowed slightly at the 5 mm position (11.8 μm/h) compared with that on TCPS (14.7 μm/h). At the 2.5 and 5 mm positions, ECs migrated as fast as SMCs. Therefore, the REDV gradient can also selectively enhance the mobility of ECs.

It is worth mentioning that ECs had a similar migration rate at the 10 mm position (7.8 μm/h) compared to that on TCPS. It is known that cell migration rate is a biphasic function of cell adhesion force. Cells have the largest migration rate on surfaces with moderate adhesion strength.41,42 Therefore, the reduced migration rate of ECs at the 10 mm position of the gradient might be attributed to strong cell adhesion force. Both ECs and SMCs are viable on the gradient, with similar viability compared to the uniform surfaces with the same REDV peptide density (Figure S10). The viability of the ECs is always higher than that of the SMCs at the same surface due to relatively lower cell adhesion of the SMCs.

The wound healing process is greatly influenced by changes in cell migration ability. Therefore, the cell sheet assay, in which cells generally maintain their various cell–cell contacts and migrate as a coherent sheet, was also performed (Figure 9 and Videos S1–3).43 In this case, the cells are not likely to move freely and thereby exhibit slower mobility. Therefore, the results in Figure 8 and Figure 9 are not directly comparable in terms of the absolute values.

The EC sheets only directionally migrated toward the positive x-direction on the REDV gradient with almost 140 μm
of movement in the positive x-direction and only a few micrometers in the negative x-direction (Figure 9). Because the cells do not duplicate very prominently during the 20 h period, the movement of the cell sheet is mainly attributed to the collective migration of cells, again proving the strong influence of the REDV gradient. In contrast, the EC sheet moved similar distances toward both directions (43 and 47 μm) on the uniform REDV surface and did not move on the HA surface (less than 10 μm for each direction). All the results demonstrated the success of the present REDV gradient, which could selectively induce the adhesion and directional migration of ECs with faster mobility while hampering the adhesion of SMCs.

Control over EC responses at the biomaterial interface is important for endothelialization of vascular prostheses. Traditional approaches build a bioactive surface that specifically supports EC adhesion or proliferation. In our opinion, a bioactive surface that can specifically induce the adhesion and directional migration of ECs will also lead to fast endothelialization of biomaterials. Several groups have obtained promising results with the migration behavior of ECs on bioactive surfaces. For example, Wu et al. prepared a density gradient of growth factor (VEGF) and found that although over 80% of ECs moved toward the direction with higher VEGF density, the cell migration rate (5.5 μm/h) was not significantly enhanced by the gradient. Liu et al. built a fibronectin density gradient together with a density gradient of VEGF in the same direction. They found that the cell migration rate increased fourfold compared with that on the single gradient. However, directionality was not mentioned. In this study, both the cell migration rate and directionality of ECs were enhanced synchronously, which furthermore showed selectivity over SMCs. In our previous study, a complementary density gradient of PHEMA and YIGSR peptides was fabricated to selectively induce directional migration of ECs with enhanced mobility. However, these promising results based on model substrates could not necessarily guarantee the successful design and preparation of functional biomaterials due to the complicated structure of real biomaterials and tedious methodology.

In this study, an REDV density gradient was generated on a cell-resistant layer, enabling the selective induction of EC adhesion and subsequent directional migration with a significantly enhanced migration rate (twofold). The gradient was constructed on the biodegradable PCL film and, with polydopamine as an adhesive layer, suggests that further in vivo evaluation of the gradient and potential application in regenerative medicine may be easily realized. The method is relatively simple, and the reaction processes are basically thermodynamically controlled except for the gradient generation step, leading to good control over the quality of samples in different batches. Moreover, this type of gradient can be easily transferred to other types of substrates, including metals, polymers, and ceramics. Furthermore, only approximately 25% of SMCs attached to the surface compared with that on TCPS. Therefore, the gradient can not only enhance the mobility of ECs but can also reduce the adhesion of SMCs, leading to a greater advantage of ECs over SMCs on the surface.

5. CONCLUSIONS

In this work, a bioactive surface was built on a biodegradable PCL film with the assistance of a polydopamine adhesive layer. Then, a density gradient of REDV peptides was successfully prepared on a uniform HA cell-resistant layer predeposited on PCL film. The REDV gradient on the HA layer could selectively enhance the adhesion, mobility and directional migration of ECs toward the direction of higher REDV density. In contrast, the adhesion of SMCs was significantly weakened on the gradient surface compared with that on TCPS, though the mobility was not obviously influenced. By constructing a cell-selective gradient on biodegradable biomaterials with a cell-resistant effect and signal peptide, a new avenue is paved for selective EC adhesion and migration, highlighting a new perspective on designing advanced and highly functional biomaterials for targeted tissue regeneration.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.6b09375.

Additional characterization data of the gradient surface (PDF)
Video 1 of EC sheet migration on HA surface (AVI)
Video 2 of EC sheet migration on uniform REDV surface with a density of 81 ng/cm² (AVI)
Video 3 of EC sheet migration on REDV gradient at the 5 mm position (AVI)
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