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Dual functionalized PVA hydrogels that adhere endothelial cells synergistically

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ABSTRACT

Cell adhesion molecules govern leukocyte-endothelial cell (EC) interactions that are essential in regulating leukocyte recruitment, adhesion, and transmigration in areas of inflammation. In this paper, we synthesized hydrogel matrices modified with antibodies against vascular cell adhesion molecule-1 (VCAM1) and endothelial leukocyte adhesion molecule-1 (E-Selectin) to mimic leukocyte-EC interactions. Adhesion of human umbilical vein ECs to polyvinyl alcohol (PVA) hydrogels was examined as a function of the relative antibody ratio (anti-VCAM1:anti-E-Selectin) and substrate elasticity. Variation of PVA backbone methacrylation was used to affect hydrogel matrix stiffness, ranging from 130 to 720 kPa. Greater EC adhesion was observed on hydrogels presenting 1:1 anti-VCAM1:anti-E-Selectin than on gels presenting either arginine-glycine-asparagine (RGD) peptide, anti-VCAM1, or anti-E-Selectin alone. Engineered cell adhesion - based on complementing the EC surface presentation - may be used to increase the strength of EC-matrix interactions. Hydrogels with tunable and synergistic adhesion may be useful in vascular remodeling.

1. Introduction

Adhesion is an important regulator of cell proliferation, migration, apoptosis, and differentiation [1]. Previous research has demonstrated that adhesion is dependent on matrix chemistry and stiffness. For example, cell adhesion and migration is influenced by varying the density of cell adhesion peptides on surfaces [2], and cell spreading and density is regulated by material stiffness [3–5]. In addition, matrix stiffness is observed to direct human mesenchymal stem cell differentiation toward neurogenic, myogenic, or osteogenic commitment [6]. Previous reports have tailored adhesion by adjusting a single parameter. In contrast, biology utilizes multiple cues to regulate cell behavior.

Leukocyte-EC adhesion is regulated via multiple interactions between cell adhesion molecules (CAMs), such as vascular cell adhesion molecule-1 (VCAM1) and endothelial leukocyte adhesion molecule-1 (E-Selectin), and their binding ligands. Their role in leukocyte rolling, adhesion, and transmigration has been elucidated [7]. Antibodies that target CAMs have been used to modify drug delivery vehicles [8] or surfaces [9] to target inflamed ECs and cancer cells, respectively.

We have recently exploited the use of multiple CAMs that exhibit synergistic adhesion in targeted drug delivery [10–12]. Increased vehicle-cell binding was achieved at optimal anti-VCAM1:anti-E-Selectin ratios that complemented cytokine-activated EC surface expression. Cytokine-activated CAM expression is essential in harnessing the immune response; dysfunction of this response can lead to diseased states, such as atherosclerosis [13], ischemic cerebrovascular disease [14], cerebral aneurysms [15], and rheumatoid arthritis [16].

In this paper, we hypothesized that cell adhesion could be engineered via hydrogel surface chemistry and elasticity. Polyvinyl alcohol (PVA) was chosen because it is non-degradable, non-adhesive, and can easily be modified [17]. We mimicked adhesive interactions between cells via the presentation of antibodies that bind VCAM1 and E-Selectin on PVA hydrogels. We additionally investigated the effect of surface elasticity on cell adhesion by varying the methacrylate content of PVA gels. Understanding how to engineer cell adhesion is a fundamental problem in the development of materials for regenerative medicine.

2. Materials and methods

2.1. Materials

PVA (MW 13–23 kDa, 88% hydrolyzed), dimethyl sulfoxide (DMSO), 2-isocyanoethylmethacrylate (2-ICEMA), 2,6-di-tert-butyl-4-methylphenol (DTBMP), 4-aminobutyraldehyde diethyl acetal (4-ABA), hydrochloric acid (HCl), ammonium hydroxide (NH_4OH), *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (sulfo-NHS), 2-(N-morpholino)ethanesulfonic acid hydrate (MES), and sodium chloride (NaCl) were purchased from Sigma Aldrich (St. Louis, MO). Irgacure 2959 was purchased from Ciba Specialty Chemicals (Basel, Switzerland). Deuterium oxide (D_2O) was purchased from Cambridge Isotope Laboratories (Andover, MA). Hank's Balanced Salt Solution (HBSS) and phosphate buffered saline (PBS) were purchased from Invitrogen (Carlsbad, CA).

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2.2. Methacrylated, aminated PVA synthesis

PVA was dissolved in DMSO in a 20% w/v solution at 60 °C [18]. The solution was purged with N_2(g) for 30 min. 1, 2, 5, or 10 mol% of 2-ICEMA was added dropwise. 1% DTBMP was added to inhibit polymerization of methacrylates. The reaction was kept at 60 °C for 4 h. The methacrylated PVA was precipitated in acetone and collected and dried under vacuum. Table 1 shows the nomenclature used for each PVA formulation.

Methacrylated PVA was aminated using a method previously described [17,19]. Briefly, a 12% w/v solution of methacrylated PVA was dissolved in water at 40 °C. 4-ABA was added dropwise to a final concentration of 10 mol% for all formulations. HCl was then added dropwise to the solution to bring the pH down below 1, and the reaction was continued for 30 min. The pH was then increased rapidly to 8.0 with NH_4OH, and the final solution was dialyzed (MWCO 2000) and lyophilized.

^{1}H nuclear magnetic resonance (NMR) spectroscopy was performed to confirm the successful conjugation of methacrylate and amine groups to the PVA backbone. Specifically, samples were dissolved in D_2O and spectra were obtained using a Varian M300 Spectrometer.

2.3. Hydrogel fabrication

Aminated, methacrylated PVA solutions were made at 10–30% w/v in water with 0.75% Irgacure 2959 photoinitiator. Solutions were exposed to UV light (21.7 mW/cm^2, 365 nm) for 90 s, resulting in hydrogels. Hydrogels were synthesized either in Teflon molds (10 mm × 1 mm × 30 mm) for use in mechanical studies or in well plates and then cut to an appropriate size using a cork borer for use in cell studies.

2.4. Mechanical properties

The Young's moduli of various hydrogel formulations were determined using an Instron BioPuls machine (Instron, Norwood, MA). Hydrogels (10 mm × 1 mm × 30 mm) were extended at a rate of 1 mm/min at room temperature immediately after polymerization. Swelling properties were also evaluated. Hydrogels were swollen in HBSS to equilibrium. Mass swelling ratios (Q) were calculated by $Q = W_S/W_D$, where W_S and W_D are the masses of the swollen and dry hydrogels, respectively.

2.5. Cell culture

Human umbilical vein endothelial cells (Lonza, Walkersville, MD) were cultured in Endothelial Cell Growth Medium-2 (EGM-2; Lonza). ECs were maintained at 37 °C with 5% CO_2 in a humidified incubator and grown to confluence before seeding onto hydrogels or 12 well plates for gene expression studies. All hydrogel formulations used with cells were 20% w/v solutions of the precursor methacrylated and aminated PVA.

2.6. qRT-PCR

EC expression of E-Selectin and VCAM1 was examined as a function of proinflammatory cytokine interleukin-1-alpha (IL-1α) concentration and incubation time. Cells were treated with 1–10 ng/mL IL-1α for 2–24 h. RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA). Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed to evaluate the expression of E-Selectin and VCAM1 under inflammatory conditions. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the endogenous control. All primers were obtained from Applied Biosystems (Carlsbad, CA).

2.7. Covalent surface modification of hydrogels

Primary monoclonal mouse anti-human antibodies (R&D Systems, Minneapolis, MN) were activated for conjugation to the amine group on synthesized precursor hydrogels using 2 mM EDC and 5 mM sulfo-NHS in 50 mM MES buffer (pH 6.0, 0.5 M NaCl) for 1 h at room temperature [20,21]. An arginine-glycine-asparagine (RGD)-containing peptide (arginine-glycine-asparagine-serine, Tocris Biosciences, Ellisville, MO), anti-E-Selectin (R&D Systems) antibody only, anti-VCAM1 (R&D Systems) antibody only, or a 1:1 ratio of anti-VCAM1:anti-E-Selectin were added at 0.5 mg antibody/g amine group on the PVA backbone to the EDC/sulfo-NHS solution [22]. Hydrogels were added to the solution and allowed to react at 4 °C overnight. Gels were rinsed with PBS before use to remove excess reactants.

Table 1
Functionalization of PVA backbone as determined by ^{1}H NMR.

	Target methacrylation (mol%)	Measured methacrylation (mol%)	Target amination (mol%)	Measured amination (mol%)
PVA-1	1	0.68	10	4.3
PVA-2	2	1.15	10	4.6
PVA-5	5	1.73	10	4.6
PVA-10	10	3.41	10	4.4

2.8. Cell staining

ECs were activated for 6 h with 5 ng/mL IL-1α and then seeded onto PVA-2 gels (20% w/v). After a 24 h incubation, cells were fixed in cold acetone for 5 min at −20 °C. Nuclear and F-actin stains were performed by concurrent addition of 0.2 μg/mL 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Millipore, Billerica, MA) and 0.33 μM Alexa Fluor 546 phalloidin (Invitrogen) to cells for 1 h at room temperature. Fluorescent images were acquired using confocal microscopy (Zeiss LSM 510 META).

2.9. Centrifugation assay

Adhesion of cells onto hydrogels was assessed using a centrifugation assay adapted from previously described methods [23–25]. Gels were seeded with 0.5×10^6 activated ECs per gel for 24 h. Gels undergoing centrifugation were placed in 24 well plates filled completely with media in order to avoid the potential deleterious effects of air bubbles on cell retention. Plates were sealed with Titer-Top Plate Sealant (Electron Microscopy Sciences, Hatfield, PA), inverted, and centrifuged at 300× g for 10 min at 4 °C. Hydrogels in plates of identical set-up that were inverted at 4 °C but not centrifuged were used as controls for all conditions. Following centrifugation or inversion, cells were rinsed once with PBS and trypsinized. A Z2 Coulter counter (Beckman Coulter, Brea, CA) was used to determine the number of cells adhered to gels after either centrifugation or inversion. A cell retention ratio was calculated as the total number of cells remaining after centrifugation divided by the number of cells retained after inversion. Statistical significance between samples was determined by 2-way ANOVA analysis.

3. Results

In this report, we investigated cell adhesion as a function of surface stiffness and chemistry. We synthesized PVA hydrogels with increasing percentages of methacrylation. This generated a series of hydrogels with differing mechanical properties. Methacrylated PVA was functionalized with an amine moiety for subsequent conjugation of antibodies. PVA hydrogels presenting three ratios of antibodies, either 1:0, 1:1, or 0:1 anti-VCAM1:anti-E-Selectin, were used to study cell adhesion strength. EC binding to functionalized PVA hydrogels was quantified using a centrifugation assay and compared to unmodified, aminated PVA and RGD-modified PVA.

3.1. Methacrylated, aminated PVA synthesis

Fig. 1 depicts the chemical reaction scheme for synthesizing methacrylated, aminated PVA. Representative ^{1}H NMR spectra for methacrylated and aminated PVA are shown in Fig. 2. PVA designated with 1, 2, 5, and 10 refer to 1, 2, 5, and 10% methacrylation, respectively. Peaks at chemical shifts of 5.6–6 ppm (vinyl) and 2.8 ppm (amine) confirm successful methacrylation and amination, respectively. Table 1 describes the efficiency of methacrylation and amination for the four PVA formulations. This is calculated from the ratio of vinyl or amine peaks to the −CH and $-CH_2$ groups in the NMR spectra as determined by the area under the curve (AUC).

3.2. Characterization of mechanical properties

The concentration of methacrylate groups was altered to vary the elastic and swelling properties of synthesized gels. Crosslinking occurs with the formation of poly (methacrylate) chains that connect two or more PVA chains. The distance between crosslinks depends on the degree of substitution of the PVA chain. Young's moduli varied between 130 and 720 kPa for gels synthesized from a 10% w/v PVA-1 polymer solution and 30% w/v PVA-10 polymer solution, respectively (Fig. 3A). Young's moduli increased with increasing percentage of methacrylation or weight percent of PVA in polymer solutions. Mass swelling ratios reflected the extent of

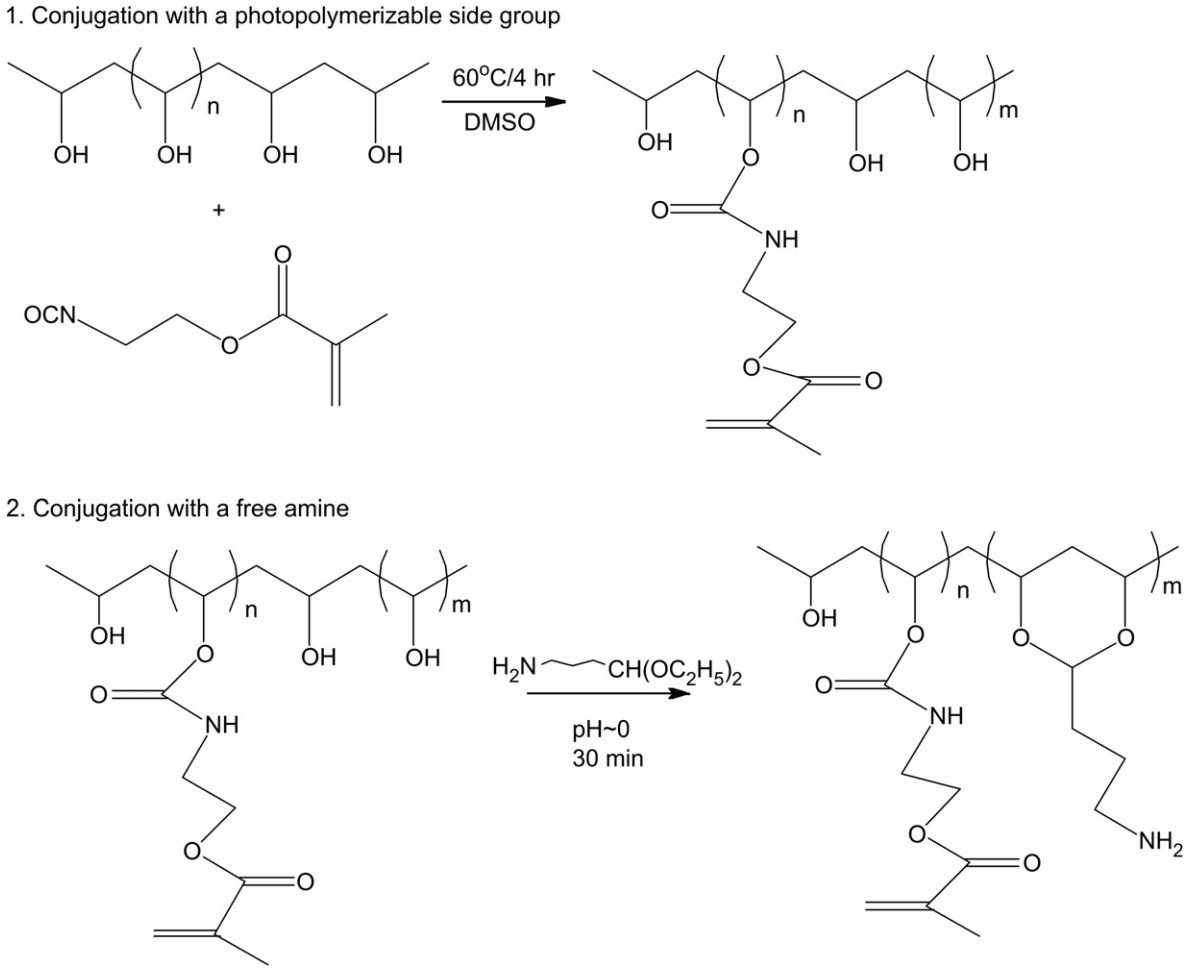


Fig. 1. Reaction scheme for methacrylation (1) and amination (2) of polyvinyl alcohol.

crosslinking of the gels (Fig. 3B); swelling ratios increased as the amount of methacrylate groups or weight percent of PVA decreased.

3.3. Endothelial cell adhesion to antibody-modified hydrogels

ECs temporally upregulate VCAM1 and E-Selectin in well-characterized patterns. We confirmed increased expression of VCAM1 and E-Selectin in ECs exposed to 1, 5, or 10 ng/mL IL-1α for 2, 6, or 24 h (Supplementary Fig. S1). The results were similar to documented reports of cytokine-activated EC expression [26], including our previous publication that correlated gene expression with surface CAM presentation [10]. EC stimulation with 5 ng/ml IL-1α for 6 h resulted in the highest upregulation of both E-Selectin and VCAM1. ECs were activated to maximize cell surface expression prior to being seeded on functionalized PVA hydrogels.

The relative cell adhesion abilities of PVA hydrogels with varying mechanical and chemical properties were investigated. Hydrogels (20% w/v) exhibiting sequentially increasing Young's moduli and decreasing mass swelling ratios were prepared from PVA-2, PVA-5, and PVA-10 solutions. Adhesion onto PVA-1 hydrogels was not evaluated due to the fragility of these hydrogels upon handling. Cell adhesion onto hydrogels modified with one of three ratios of anti-VCAM1:anti-E-Selectin was tested and compared to that on unmodified, aminated PVA and RGD-modified PVA. Activated ECs were seeded onto hydrogels for 24 h. More ECs adhered to functionalized PVA hydrogels than unmodified, aminated PVA hydrogels (Fig. 4). Gels modified with a 1:1 ratio of anti-VCAM1:anti-E-Selectin had the highest cell density on their surface 24 h post- seeding (2×10^5 cells/cm^2). Surface cell densities (1×10^5 cells/cm^2) were comparable between gels presenting RGD, anti-E-Selectin, or anti-VCAM1. Surface cell spreading was not observed on unmodified, aminated PVA hydrogels; cell spreading was qualitatively lower on hydrogels modified with anti-VCAM1 than on hydrogels modified with RGD, anti-E-Selectin, or 1:1 anti-VCAM1:anti-E-Selectin (Supplementary Fig. S2).

Strength of cell adhesion onto hydrogel surfaces was examined via the centrifugation assay. We determined the cell retention ratio by dividing the number of cells adhered after gel centrifugation at 300× g for 10 min by the number of cells adhered after gel inversion (1× g) for 10 min. Cell adhesion onto PVA hydrogels modified with 1:1 ratio of anti-VCAM1:anti-E-Selectin was higher than on unmodified hydrogels and those modified with RGD, anti-E-Selectin, or anti-VCAM1. No significant differences in adhesion onto PVA hydrogels with varying mechanical properties (Young's moduli ranging from 170 to 450 kPa) were identified. However, differences in adhesion between the antibody-functionalized hydrogels were most pronounced on hydrogels with greater stiffness (PVA-10 vs. PVA-2) (Fig. 5).

4. Discussion

We have synthesized a series of PVA hydrogels with different mechanical and chemical properties. We modified hydrogels with

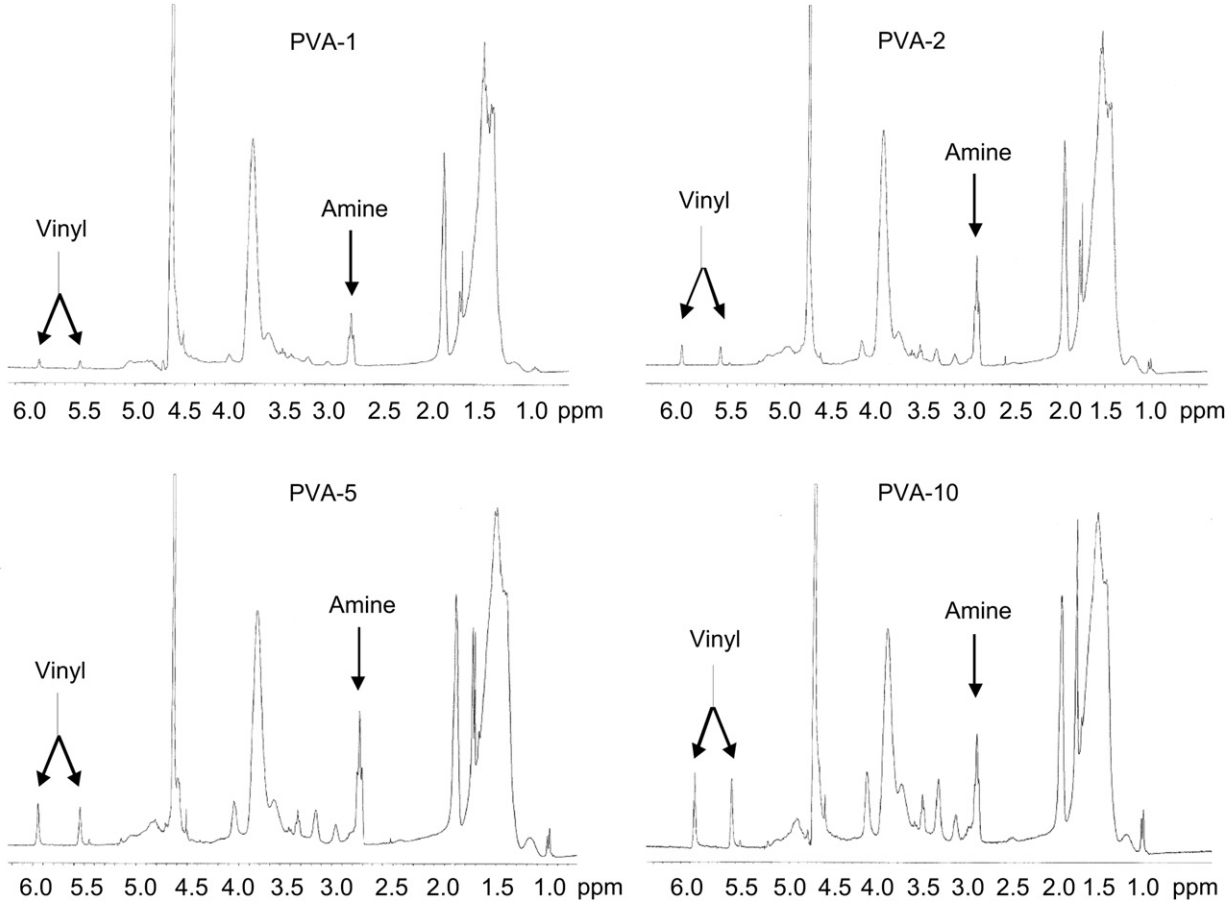


Fig. 2. ^{1}H NMR spectrum of methacrylated, aminated PVA-1, 2, 5, and 10. Successful backbone modification is verified by vinyl peaks at 5.6 and 6 ppm as well as an amine peak at 2.8 ppm.

either antibodies against cell adhesion molecules upregulated on inflamed endothelium or RGD, a peptide that binds the $\alpha_5\beta_1$ integrin domain [27]. Our interest in cell adhesion molecules derives from the fact that these molecules are regulated by cytokine-activation in a temporal and reproducible manner [26] and are localized within lipid rafts on the cell surface [28]. Mimicking cell–cell interactions by modifying hydrogels with antibodies against cell adhesion molecules may allow for the engineering of the strength of cell adhesion onto polymeric materials.

Cell adhesion molecules participate in leukocyte-EC interactions while integrins mediate cell–matrix interactions. RGD signaling involves the FAK pathway [29] whereas VCAM1 and E-Selectin signaling are mediated by the Rac pathway [30] and activation of the ERK1/2 pathway [31], respectively. Rac and ERK1/2 are downstream of FAK; these pathways regulate various cellular processes, including adhesion, migration, and actin polymerization [32]. Thus, directing adhesion through cell adhesion molecules is similar to the use of RGD. However, our approach is unique because we can tune adhesion based on the molecular density and organization of VCAM1 and E-Selectin.

The mechanical properties of our synthesized PVA hydrogels ranged from 130 to 720 kPa. This range reflects the Young's moduli exhibited by soft tissues, including thoracic aorta and femoral arteries (126–433 kPa [33]), and articular cartilage (500–1000 kPa [34]). Our hydrogels were stiffer than collagen (0.1–0.4 kPa [35]) and conventional alginate hydrogels (13–45 kPa, 0.21 g/mL calcium sulfate ($CaSO_4$) [36]) but were softer than hydroxyethyl methacrylate (HEMA) gels (1600 kPa, 3 mol% tetraethylene glycol dimethacrylate (TEGDMA) [37]). Additionally, the mechanical properties of our gels fell within the range of previously described methacrylated PVA hydrogels, which span from 55 to 838 kPa [17,18,38]. Cell adhesion was not significantly altered within the range of Young's moduli examined in this study (Fig. 5). However, cell adhesion has been shown to be affected by stiffness across a larger range: greater cell spreading and adhesion was found on polyacrylamide gels when moduli increased from 5 to 70 kPa [3], on poly(L-lysine)/hyaluronan films (3–400 kPa) with moduli greater than 300 kPa [4], and on polyelectrolyte films (0.15–150 MPa) of 150 MPa [5].

Notably, synergistic binding onto PVA hydrogels presenting both anti-VCAM1 and anti-E-Selectin was observed. Synergy may be defined as two antibodies that function together to produce a result not independently obtainable. We observed synergistic binding between IL-1α activated ECs and liposomes that presented an optimal ratio of antibodies to CAMs, a 1:1 ratio of anti-VCAM1:anti-E-Selectin [10]. Liposome binding was inhibited by disrupting lipid raft formation and blocking of either CAM [12]. This previous work suggested that cell surface density and organization is important in cell–material interactions. In the present study, synergistic binding was observed on PVA hydrogels presenting 1:1 anti-VCAM1:anti-E-Selectin. Synergy was demonstrated by the increased relative retention of cells on gels presenting 1:1 anti-VCAM1:anti-E-Selectin versus PVA hydrogels presenting anti-VCAM1 or anti-E-Selectin alone.

All hydrogels used for cell adhesion studies had similar antibody surface densities. Antibodies were conjugated onto aminated PVA

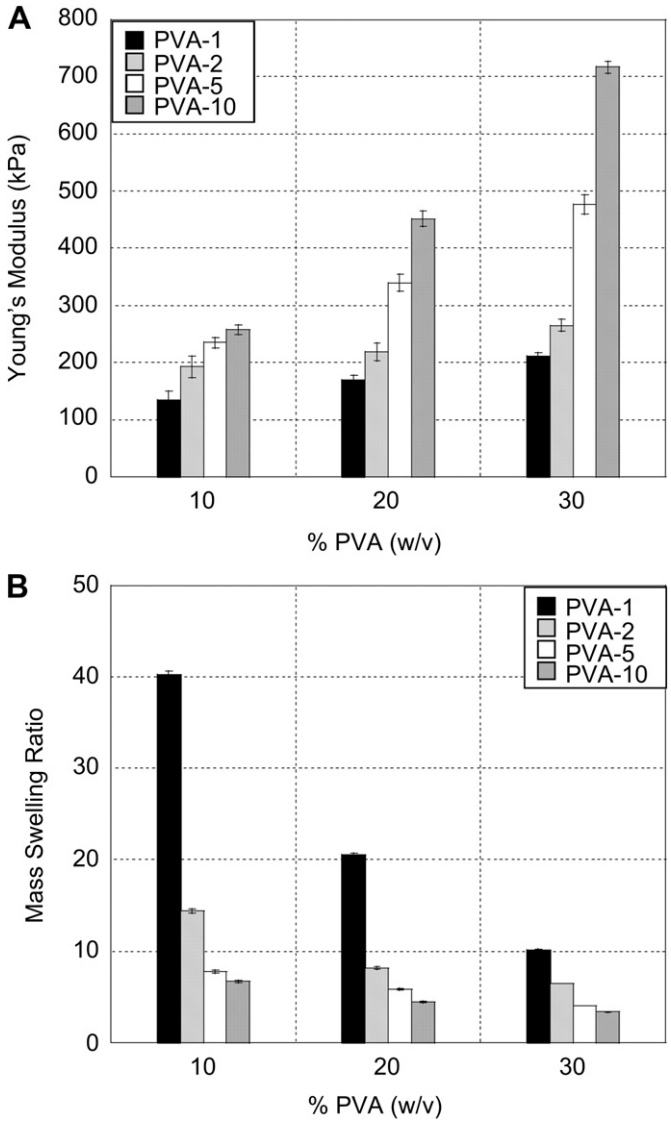


Fig. 3. Characterization of PVA hydrogel mechanical properties. The Young's modulus (A) and mass swelling ratio (B) of PVA hydrogels is dependent on the degree of methacrylation and the weight percent of PVA. Error bars reported are standard error.

hydrogels (as determined by ^{1}H NMR, Table 1) using carbodiimide chemistry. Our previous work has shown that this chemistry results in nonpreferential conjugation of antibodies to surfaces at 70% efficiency; the molecular density was confirmed by flow cytometry [11]. We estimate that our conjugation confers a density of 700 molecules/μm^2 based on the number of surface amine groups available for antibody conjugation and the conjugation efficiency. This is comparable to the 20-200,000 molecules/μm^2 modification density reported in previous studies on RGD-modified hydrogel surfaces [2,39].

Strength of cell adhesion onto hydrogel surfaces was measured by a centrifugation assay where we compared the number of cells that remained adhered after centrifugation at $300\times$ *g* relative to inversion, $1\times$ *g*. This force is equal to or greater than that used in previous analyses of cell adhesion strength [2,23,24]. The PVA hydrogels modified with 1:1 anti-VCAM1:anti-E-Selectin strongly adhered ECs; a 0.9 cell retention ratio was observed. In comparison, the previously studied systems that have most effectively adhered cells have shown lower cell retention. For example, RGD-modified interpenetrating networks composed of poly(acrylamide-co-ethylene glycol/acrylic acid) have exhibited a cell retention ratio of 0.6 for rat calvarial osteoblast cells centrifuged at $57\times$ *g* [24] and dinitrophenol functionalized acrylamide surfaces have demonstrated a 0.8 cell retention ratio for rat basophilic leukemia cells centrifuged at $300\times$ *g* [23]. The 1:1 anti-VCAM1:anti-E-Selectin PVA hydrogels presented here thus enable stronger binding than functionalized materials reported in the literature and demonstrate that hydrogel chemistry that mimics at least two types of cell–cell interactions can facilitate stronger cell adhesion than that which resembles cell–matrix interactions.

Presentation of either RGD, anti-VCAM1 or anti-E-Selectin alone resulted in similar EC adhesion (Fig. 5). The dissociation constant of RGD-integrin binding has been shown to be approximately 10^{-4}-10^{-6} M depending on the length of the peptide evaluated [40] whereas the disassociation constant of antibody-antigen binding is 10^{-9} M [41]. The differences in the disassociation constants did not correlate with overall EC adhesion; this is most likely because we are measuring several interactions and not a single interaction. Since anti-VCAM1 and anti-E-Selectin presenting PVA hydrogels had similar binding to RGD-modified surfaces, we concluded that the stronger adhesion observed with 1:1 anti-VCAM1:anti-E-Selectin is due to the synergy between anti-VCAM1 and anti-E-Selectin.

PVA hydrogels are non-cytotoxic, can conform to any geometry, and can be photopolymerized in situ on short time scales [17,42]. These qualities make PVA suitable as a vascular embolic agent. In comparison to ionically crosslinked alginate hydrogels that have been investigated for use in endovascular embolization [43,44], functionalized PVA hydrogels can not only adhere to ECs but also be tuned to match vascular mechanical properties. Future work will evaluate functionalized PVA hydrogels in vascular remodeling applications.

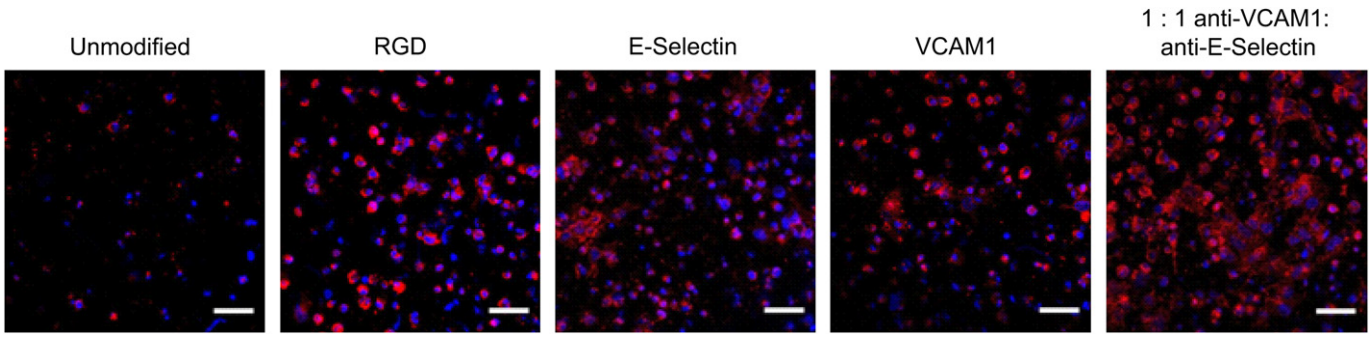
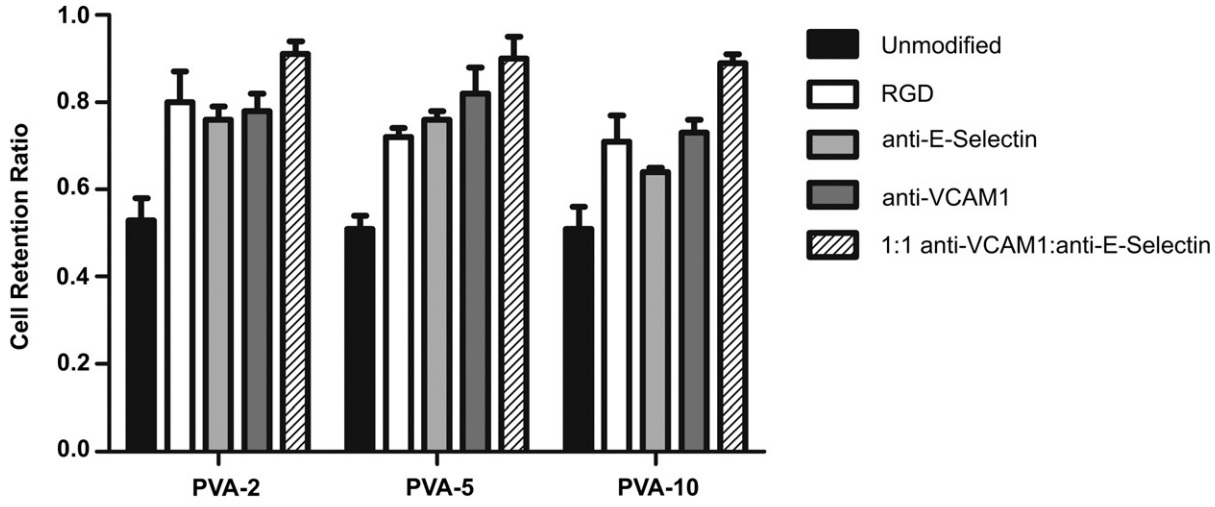


Fig. 4. Confocal microscopy images of activated ECs (5 ng/mL IL-1α treatment for 6 h) seeded onto PVA-2 hydrogels (20% w/v PVA) for 24 h and stained with F-actin (red) and nuclear stains (blue). Scale bar is 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



	Unmodified vs. RGD	Unmodified vs. anti-E-Selectin	Unmodified vs. anti-VCAM1	Unmodified vs. 1:1 anti-VCAM1:anti-E-Selectin	RGD vs. 1:1 anti-VCAM1:anti-E-Selectin	E-Selectin vs. 1:1 anti-VCAM1:anti-E-Selectin	VCAM1 vs. 1:1 anti-VCAM1:anti-E-Selectin
PVA-2	***	**	***	***	--	--	–
PVA-5	**	**	***	***	*	--	–
PVA-10	*	--	**	***	*	***	*

Fig. 5. Retention of ECs stimulated with IL-1α (5 ng/mL for 6 h) seeded onto antibody-modified hydrogels. All PVA hydrogels tested were synthesized from 20% w/v PVA solutions. Cell retention ratio is defined as ratio of cells remaining on gels after centrifugation at 300× *g* divided by cells retained on non-centrifuged samples. Error bars are reported as standard error. Statistical significance was calculated using a 2-way ANOVA analysis with $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

5. Conclusions

We have synthesized photopolymerizable, mechanically tunable, functionalized PVA hydrogels. PVA hydrogels presenting a 1:1 anti-VCAM1:anti-E-Selectin ratio exhibited strong, synergistic adhesion to ECs. These functionalized hydrogels may serve as ideal candidates for tissue engineering applications.

Acknowledgments

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biomaterials.2012.02.017.

References

[1] Ingber D. Mechanochemical control of cell fate switching. In: Lanza RP, Langer R, Vacanti J, editors. Principles of tissue engineering. Burlington, MA: Elsevier Academic Press; 2007.
[2] Maheshwari G, Brown G, Lauffenburger DA, Wells A, Griffith LG. Cell adhesion and motility depend on nanoscale RGD clustering. J Cell Sci 2000;113:1677–86.
[3] Pelham Jr RJ, Wang Y. Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc Natl Acad Sci U S A 1997;94:13661–5.
[4] Schneider A, Francius G, Obeid R, Schwinte P, Hemmerle J, Frisch B, et al. Polyelectrolyte multilayers with a tunable Young's modulus: influence of film stiffness on cell adhesion. Langmuir 2006;22:1193–200.
[5] Thompson MT, Berg MC, Tobias IS, Rubner MF, Van Vliet KJ. Tuning compliance of nanoscale polyelectrolyte multilayers to modulate cell adhesion. Biomaterials 2005;26:6836–45.
[6] Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006;126:677–89.
[7] Walzog B, Gaehtgens P. Adhesion molecules: the path to a new understanding of acute inflammation. News Physiol Sci 2000;15:107–13.
[8] Omolola Eniola A, Hammer DA. In vitro characterization of leukocyte mimetic for targeting therapeutics to the endothelium using two receptors. Biomaterials 2005;26:7136–44.
[9] Myung JH, Launiere CA, Eddington DT, Hong S. Enhanced tumor cell isolation by a biomimetic combination of E-selectin and anti-EpCAM: implications for the effective separation of circulating tumor cells (CTCs). Langmuir 2010;26:8589–96.
[10] Gunawan RC, Almeda D, Auguste DT. Complementary targeting of liposomes to IL-1alpha and TNF-alpha activated endothelial cells via the transient expression of VCAM1 and E-selectin. Biomaterials 2011;32:9848–53.
[11] Gunawan RC, Auguste DT. The role of antibody synergy and membrane fluidity in the vascular targeting of immunoliposomes. Biomaterials 2010;31:900–7.
[12] Gunawan RC, Auguste DT. Immunoliposomes that target endothelium in vitro are dependent on lipid raft formation. Mol Pharmacol 2010;7:1569–75.
[13] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135–43.
[14] Frijns CJ, Kappelle LJ. Inflammatory cell adhesion molecules in ischemic cerebrovascular disease. Stroke 2002;33:2115–22.
[15] Chyatte D, Bruno G, Desai S, Todor DR. Inflammation and intracranial aneurysms. Neurosurgery 1999;45:1137–46. discussion 1146–1137.
[16] Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation 2003;108:2957–63.
[17] Schmedlen RH, Masters KS, West J. Photocrosslinkable polyvinyl alcohol hydrogels that can be modified with cell adhesion peptides for use in tissue engineering. Biomaterials 2002;23:4325–32.
[18] Bryant S, Davis-Arehart K, Luo N, Shoemaker R, Arthur J, Anseth K. Synthesis and characterization of photopolymerized multifunctional hydrogels: water-soluble poly(vinyl alcohol) and chondroitin sulfate macromers for chondrocyte encapsulation. Macromolecules 2004;37:6726–33.
[19] Sanjust E, Cocco D, Curreli N, Rescigno A, Solliai F, Bannister J. Flavin-grafted poly(vinyl alcohol): preparation and properties. J Appl Polym Sci 2002;85:2471–7.
[20] Maraldo D, Mutharasan R. Optimization of antibody immobilization for sensing using piezoelectrically excited-millimeter-sized cantilever (PEMC) sensors. Sensor Actuat B 2007;123:474–9.

[21] Hermanson G. Bioconjugate techniques. San Diego, CA: Academic Press; 1996.
[22] Dhoot NO, Tobias CA, Fischer I, Wheatley MA. Peptide-modified alginate surfaces as a growth permissive substrate for neurite outgrowth. J Biomed Mater Res A 2004;71:191–200.
[23] Chu L, Tempelman L, Miller C, Hammer D. Centrifugation assay of IgE-mediated cell adhesion to antigen-coated gels. AIChE J 1994;40:692–703.
[24] Harbers GM, Gamble LJ, Irwin EF, Castner DG, Healy KE. Development and characterization of a high-throughput system for assessing cell-surface receptor-ligand engagement. Langmuir 2005;21:8374–84.
[25] McClay DR, Wessel GM, Marchase RB. Intercellular recognition: quantitation of initial binding events. Proc Natl Acad Sci U S A 1981;78:4975–9.
[26] Bevilacqua MP, Nelson RM, Mannori G, Cecconi O. Endothelial-leukocyte adhesion molecules in human disease. Annu Rev Med 1994;45:361–78.
[27] Burridge K, Turner CE, Romer LH. Tyrosine phosphorylation of paxillin and pp125FAK accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. J Cell Biol 1992;119:893–903.
[28] Kiely JM, Hu Y, Garcia-Cardena G, Gimbrone Jr MA. Lipid raft localization of cell surface E-selectin is required for ligation-induced activation of phospholipase C gamma. J Immunol 2003;171:3216–24.
[29] Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999;285:1028–32.
[30] van Wetering S, van den Berk N, van Buul JD, Mul FP, Lommerse I, Mous R, et al. VCAM-1-mediated Rac signaling controls endothelial cell-cell contacts and leukocyte transmigration. Am J Physiol Cell Physiol 2003; 285:C343–52.
[31] Hu Y, Szente B, Kiely JM, Gimbrone Jr MA. Molecular events in transmembrane signaling via E-selectin. SHP2 association, adaptor protein complex formation and ERK1/2 activation. J Biol Chem 2001;276:48549–53.
[32] Parsons JT. Focal adhesion kinase: the first ten years. J Cell Sci 2003;116: 1409–16.
[33] Zhang X, Kinnick RR, Fatemi M, Greenleaf JF. Noninvasive method for estimation of complex elastic modulus of arterial vessels. IEEE Trans Ultrason Ferroelectr Freq Control 2005;52:642–52.
[34] Armstrong CG, Mow VC. Variations in the intrinsic mechanical properties of human articular cartilage with age, degeneration, and water content. J Bone Joint Surg Am 1982;64:88–94.
[35] Borene ML, Barocas VH, Hubel A. Mechanical and cellular changes during compaction of a collagen-sponge-based corneal stromal equivalent. Ann Biomed Eng 2004;32:274–83.
[36] Boontheekul T, Hill EE, Kong HJ, Mooney DJ. Regulating myoblast phenotype through controlled gel stiffness and degradation. Tissue Eng 2007;13: 1431–42.
[37] You JO, Rafat M, Ye GJ, Auguste DT. Nanoengineering the heart: conductive scaffolds enhance connexin 43 expression. Nano Lett 2011;11:3643–8.
[38] Martens P, Anseth KS. Characterization of hydrogels formed from acrylate modified poly(vinyl alcohol) macromers. Polymer 2000;41:7715–22.
[39] Xiao Y, Truskey GA. Effect of receptor-ligand affinity on the strength of endothelial cell adhesion. Biophys J 1996;71:2869–84.
[40] Steiner B, Cousot D, Trzeciak A, Gillessen D, Hadvary P. Ca2+-dependent binding of a synthetic Arg-Gly-Asp (RGD) peptide to a single site on the purified platelet glycoprotein IIb-IIIa complex. J Biol Chem 1989;264:13102–8.
[41] Friguet B, Chaffotte AF, Djavadi-Ohaniance L, Goldberg ME. Measurements of the true affinity constant in solution of antigen-antibody complexes by enzyme-linked immunosorbent assay. J Immunol Methods 1985;77: 305–19.
[42] Bader RA, Rochefort WE. Rheological characterization of photopolymerized poly(vinyl alcohol) hydrogels for potential use in nucleus pulposus replacement. J Biomed Mater Res A 2008;86:494–501.
[43] Becker TA, Kipke DR, Brandon T. Calcium alginate gel: a biocompatible and mechanically stable polymer for endovascular embolization. J Biomed Mater Res 2001;54:76–86.
[44] Becker TA, Preul MC, Bichard WD, Kipke DR, McDougall CG. Preliminary investigation of calcium alginate gel as a biocompatible material for endovascular aneurysm embolization in vivo. Neurosurgery 2007;60:1119–27. discussion 1127–1118.