

# Plasma expanders stabilize human microvessels in microfluidic scaffolds

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**Abstract:** Plasma expanders such as dextran and hydroxyethyl starch (HES) are important components of solutions designed to maintain vascular volume in the clinical setting and to preserve organs *ex vivo* before transplantation. Here, we show that these polymers also exert stabilizing effects on engineered microvessels in microfluidic type I collagen and fibrin scaffolds. Standard growth media, which did not contain dextran or HES, led to severe leakage, vascular collapse, and catastrophic failure of perfusion. Remarkably, vessels that were provided with 3% dextran or 5% HES had few focal leaks, maintained adhesion to the scaffold, and were typically viable and patent for at least 2 weeks. We found that the

junctional marker VE-cadherin localized to a wide band in the presence of plasma expanders, but only at concentrations that also stabilized vessels. In conjunction with a previous computational model (Wong et al., *Biomaterials* 2010;31:4706–4714), our results suggest that plasma expanders stabilize microvessels via physical mechanisms that enhance VE-cadherin localization at junctions and thereby limit vascular leakiness. © 2012 Wiley Periodicals, Inc. *J Biomed Mater Res Part A*: 100A: 1815–1822, 2012.

**Key Words:** microvascular tissue engineering, dextran, hydroxyethyl starch, microfluidic hydrogel, perfusion

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## INTRODUCTION

The development of techniques to vascularize engineered tissues is a necessary step toward the formation of viable constructs of thickness larger than the diffusion limit of oxygen.<sup>1</sup> To this end, we and others<sup>2–6</sup> have proposed methods to construct microfluidic scaffolds that can be vascularized and perfused immediately. The microfluidic channels in such scaffolds do enable immediate perfusion, whereas they do not guarantee that the initial levels of flow can be sustained. Our recent studies indicated that both chemical and mechanical signals—via modulation of cyclic AMP levels and flow rates, respectively—play crucial roles in promoting the function and stability of engineered vasculature in microfluidic gels.<sup>7,8</sup> Supplementation with  $\geq 80 \mu\text{M}$  dibutyryl cyclic AMP and flow equivalent to a shear stress of at least 10–15  $\text{dyn}/\text{cm}^2$  were required to obtain vessels that could maintain flow for at least 2 weeks; without either one of these signals, the endothelium delaminated from the wall of the scaffold.

In these previous studies, we routinely supplemented the culture media with 3% (w/v) 70 kDa dextran to increase the viscosity of the perfusate and thereby reduce the volume of media needed to generate the necessary shear.<sup>4,7,8</sup> Preliminary data from these studies suggested that microvessels that were grown in the absence of dextran did not survive as long as those in the presence of 3%

dextran. Dextran is an example of a plasma expander, a polymer used clinically to maintain or restore vascular volume (e.g., during hypovolemic shock).<sup>9</sup> By design, plasma expanders increase the oncotic pressure of plasma, which can promote retention of water within the bloodstream and thereby can maintain or increase blood pressure to counteract blood loss or the shift of water from blood to tissue compartments. Dextran and another plasma expander, hydroxyethyl starch (HES), are also used to help preserve organs for transplant (e.g., in the University of Wisconsin solution), in part by preventing the development of edema in cold storage.<sup>10,11</sup> Whether the beneficial effects of plasma expanders on vascular function *ex vivo* extend to engineered microvessels *in vitro* is unclear.

The current study formally investigates the relationship between the longevity and barrier function of a microvessel and the macromolecular content of the perfusate used to culture it. We treated vessels that were formed in type I collagen and fibrin scaffolds with different concentrations of plasma expanders to assess the effects of these polymers on long-term stability and barrier function. To uncover the mechanisms by which plasma expanders affected vascular phenotype, we also quantified the localization of the junctional marker VE-cadherin in endothelial cells grown in their presence.

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## MATERIALS AND METHODS

### Cell culture

We cultured blood microvessel-derived endothelial cells (BECs) from human dermis (lots 6F4144 and 5F1293 from Lonza and lots 7082905.1 and 0040804.2 from Promocell) on gelatin-coated dishes. The standard growth media was MCDB 131 (Caisson Labs) with 10% fetal bovine serum (Atlanta Biologicals), 80  $\mu$ M dibutyryl cAMP (Sigma), 1  $\mu$ g/mL hydrocortisone (Sigma), 2 U/mL heparin (Sigma), 25  $\mu$ g/mL endothelial cell growth supplement (Biomedical Technologies), 0.2 mM ascorbic acid 2-phosphate (Sigma), and 1% glutamine–penicillin–streptomycin (Invitrogen). We passaged BECs at a 1:4 ratio and discarded them at passage six.

### Vascularization of microfluidic collagen and fibrin scaffolds

We formed microfluidic type I collagen scaffolds ( $n = 143$ ) *in vitro* as described previously.<sup>4,7,8</sup> Briefly, type I collagen (7–7.5 mg/mL final concentration from rat tail; BD Biosciences) was gelled around an albumin-coated 120- $\mu$ m-diameter needle (Seirin) for 2 h at 22°C. Removing the needle after gelation formed a microfluidic collagen gel that contained a single open channel. Scaffolds were conditioned for 24 h by drop-wise perfusion with culture media.<sup>12</sup> For a given sample, the same formulation of culture media was used for conditioning the collagen as for perfusion of the endothelialized vessel later.

Microfluidic fibrin scaffolds ( $n = 62$ ) were formed in a similar manner. Fibrin gels consisted of 7.5 mg/mL fibrinogen (Aniara), 1 U/mL thrombin (Sigma), and 2 mM CaCl<sub>2</sub> in phosphate-buffered saline (PBS) and were formed around needles at 22°C. Thirty minutes after the start of gelation, we added PBS to both ends of each gel to maintain hydration. After an additional 90 min, we removed the needles and conditioned open channels for 24 h by drop-wise perfusion with culture media.

Collagen and fibrin scaffolds were endothelialized by trypsinizing BECs and seeding them into the channels in a dense suspension (one confluent 60-mm dish of cells in 20  $\mu$ L of culture media). For collagen and fibrin scaffolds, we set up flow in seeded channels at a pressure head of 5 cm H<sub>2</sub>O 1 and 24 h after seeding, respectively; the extended incubation period between seeding and perfusion in fibrin-based tubes was needed to ensure firm adhesion of BECs to the fibrin.

We perfused vascularized scaffolds with media that was supplemented with 0.1, 1, or 3% dextran (MW 70 kDa; Sigma) or 0.1, 3, or 5% HES (MW 130 kDa; AK Scientific), or with standard growth media (i.e., without dextran or HES). For fibrin-based vessels, the media was also supplemented with 6-aminocaproic acid (2 mg/mL; Sigma) to prevent fibrinolysis.<sup>13</sup>

### Measurement of vessel lifespan

We defined the day of vessel “death” as the earliest day on which the flow rate decreased to half of the maximum observed for that tube or on which endothelial delamination (e.g., detachment of endothelium as a sheet from the scaffold)

or denuding (e.g., shedding of individual cells) spanned a total of  $\geq 150$   $\mu$ m in the upstream half of the tube. A small amount of delamination near the outlet was common across experimental conditions and was not considered in the measurement of lifespan. For Kaplan–Meier lifespan analysis, we censored samples when the scaffold peeled away from the surrounding surfaces or when undesired particles (e.g., dust or air bubbles) occluded the lumen.

### Measurement of delamination

We mapped endothelial delamination from the collagen gel after 4 days of perfusion; samples that died on day 3 were not included in this analysis. We obtained phase-contrast images of each microvessel with a Plan-Neo 10 $\times$ /0.30 NA (Zeiss) objective, stitched the images together, and marked regions of delamination at the upper and lower walls manually using Adobe Photoshop. Thus, each vessel yielded two binary maps of delamination. To obtain a heatmap of the frequency of delamination for a given perfusate, the binary maps from multiple vessels were stacked and averaged using ImageJ (NIH).

### Assay of barrier function

We quantified the barrier function of the microvessels by using a permeability assay.<sup>14</sup> We added Alexa Fluor 488-conjugated 10 kDa dextran (Invitrogen) to the media at 20  $\mu$ g/mL and obtained time-lapse fluorescence images every minute until the lumen of the vessel was uniformly filled with fluorescence signal ( $\sim 20$  min). Focal leaks (i.e., localized sites of dye efflux) were counted manually and are reported as the number of leaks per frame per length of vessel.

### VE-cadherin staining and quantification of junctions

BECs were cultured on glass coverslips that were coated with 0.1% gelatin (Sigma) in standard media or in media supplemented with dextran or HES. On day 3 postseeding, cells were fixed with 4% paraformaldehyde for 15 min and then permeabilized and blocked in blocking buffer (5% donkey serum, 0.1% Triton X-100, and 10 mM glycine) for 30 min. The cells were then incubated with goat anti-VE cadherin (1:200, Santa Cruz Biotechnology) in blocking buffer for 1 h and then washed thrice with blocking buffer over the course of 30 min. Next, the cells were incubated with Alexa Fluor 488-conjugated donkey anti-goat IgG (1:200, Invitrogen) in blocking buffer for 1 h and then exhaustively washed. Fluorescence images were taken with a Plan-Neo 40 $\times$ /0.75 NA (Zeiss) or Plan Fluor 20 $\times$ /0.45 NA (Nikon) objective.

To determine the average width of VE-cadherin staining at endothelial cell–cell junctions, we used a custom MATLAB script to divide the total fluorescent area by the total perimeter of the junctions. Junctions that were not in focus and regions with nonjunctional staining (e.g., perinuclear background) were excluded from analysis.

### Measurement of viscosity

We measured the flow rate of MCDB 131 (with and without dextran and HES) and H<sub>2</sub>O through PE-50 polyethylene

tubing (Braintree Scientific) with a pressure head of 5 cm H<sub>2</sub>O at 22°C and calculated media viscosity by multiplying the viscosity of H<sub>2</sub>O by the ratio of the flow rate of H<sub>2</sub>O to the flow rate of media.

### Statistical analysis

We compared vessel lifespans by applying the log-rank test to Kaplan–Meier survival curves (Prism ver. 5, GraphPad) and considered differences to be significant for a Bonferroni-corrected *p* value less than 0.008 (i.e., 0.05 divided by six, the number of pairwise comparisons among four survival curves). To compare delamination frequencies, we calculated the mean pixel value for each pair of binary delamination maps and applied the Mann–Whitney *U* test with a *p* < 0.008 threshold. To compare focal leak densities, we used the *U* test with a *p* < 0.008 threshold. To compare average VE-cadherin junctional widths, we used the Friedman test (a nonparametric repeated-measures ANOVA) followed by Dunn's posttest, with *p* < 0.05 considered significant. Data uncertainties are given as ±SD.

## RESULTS

### Characterization of vessels

In microvessels that were formed in collagen scaffolds, the maximum flow rates were greatest for vessels that were perfused with standard media and 0.1% dextran and were lowest for vessels that were treated with 3% dextran (1.66 ± 0.43 mL/h for standard media, 1.74 ± 0.33 mL/h for 0.1% dextran, 1.41 ± 0.23 mL/h for 1% dextran, and 0.94 ± 0.11 mL/h for 3% dextran). Similarly, addition of HES decreased the maximum flow rates (1.87 ± 0.50 mL/h for 0.1% HES, 1.62 ± 0.26 mL/h for 3% HES, and 1.30 ± 0.21 mL/h for 5% HES). For most microvessels that were formed in collagen scaffolds, the maximum flow rate occurred within the first 3 days of perfusion. The average shear stress ranged between 10 and 15 dyn/cm<sup>2</sup> in all microvessels, regardless of the dextran or HES concentration in the media (13.4 ± 2.1, 12.9 ± 1.1, 11.9 ± 1.7, and 10.5 ± 0.9 dyn/cm<sup>2</sup> for 0, 0.1, 1, and 3% dextran, respectively; 14.0 ± 3.3, 13.4 ± 1.9, and 12.0 ± 3.7 dyn/cm<sup>2</sup> for 0.1, 3, and 5% HES, respectively).

Microvessels in fibrin scaffolds tended to be narrower than those were in collagen. The maximum flow rates were 0.75 ± 0.10, 0.96 ± 0.20, and 1.16 ± 0.20 mL/h for perfusion with 3% dextran, 5% HES, and standard media, respectively. The corresponding shear stresses were 11.7 ± 1.6, 12.6 ± 2.0, and 13.2 ± 2.4 dyn/cm<sup>2</sup> for 3% dextran, 5% HES, and standard media, respectively.

### Dextran promotes vascular function in microfluidic collagen scaffolds

Constant flow with standard growth media produced microvessels that frequently delaminated by day 4 of perfusion [Fig. 1(A)]. In many samples, the delamination was severe and occurred throughout the length of the vessel [Fig. 1(B)]. By day 7, essentially, all vessels that were provided with standard media had delaminated, and we considered them nonfunctional [Fig. 1(C)].

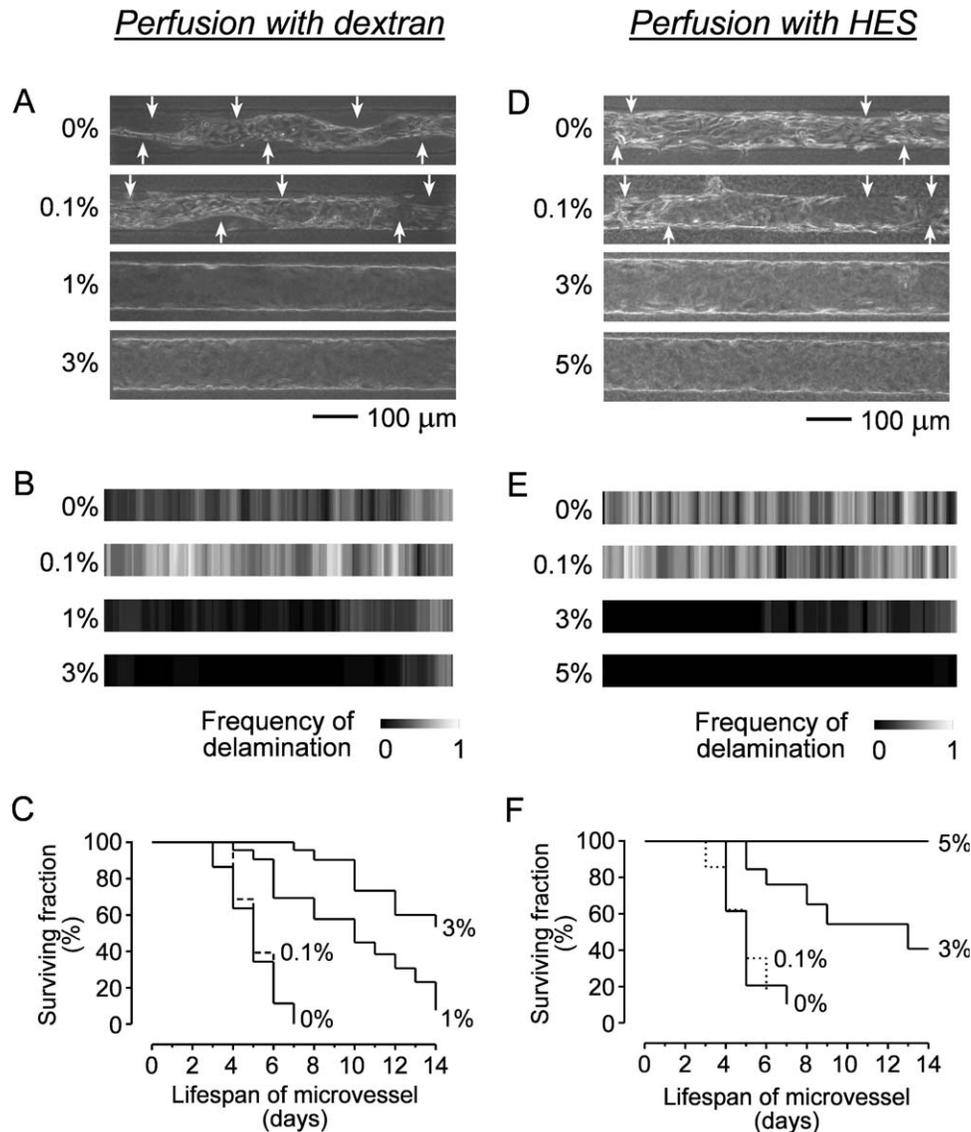
Supplementing the media with dextran strongly improved the function of the vessels in a dose-dependent manner [Fig. 1(B)]. At day 4 of perfusion, microvessels that were treated with 1% or 3% dextran had significantly less delamination than had vessels that were given lower concentrations (*p* < 0.0001 for 3% vs. 0.1%; *p* = 0.0032 for 3% vs. 0%; *p* = 0.0018 for 1% vs. 0.1%). We did not detect statistically significant differences in delamination frequency in vessels that were provided with 1 versus 3% dextran or with 0.1% dextran versus dextran-free media.

Notably, the spatial distribution of delamination differed among the different concentrations of dextran [Fig. 1(B)]. In contrast to the widespread delamination in vessels that were exposed to 0.1% dextran or to standard media, only minor delamination was present in the upstream half of vessels with 1% dextran; delamination was confined to the outlet regions of vessels with 3% dextran. Functionally, the decreased incidence of vascular delamination upon perfusion with 1% or (especially) 3% dextran enhanced the ability of the vessels to sustain long-term flow [Fig. 1(C)]. Microvessels that were perfused with these concentrations of dextran often remained patent and sustained flow for at least 2 weeks and survived significantly longer than those given 0.1% dextran or standard media (*p* < 0.0001). Consistent with the delamination results, perfusion with 0.1% dextran did not extend vascular lifespan. Microvessels that were provided with 0.1% dextran typically “died” from abrupt delamination throughout the entire vessel. With 1 or 3% dextran, on the other hand, vessels either lasted the full 2 weeks or experienced a gradual reduction in flow rate that eventually reached the 50% threshold. We attributed this slow reduction in flow rate to constriction at the outlet portion of the vessel.

To quantify the barrier function of microvessels, we counted focal leaks to trace amounts (20 µg/mL) of fluorescent 10 kDa dextran on day 3 of perfusion and before any delamination occurred (Fig. 2). Perfusion with standard media or with 0.1% dextran resulted in numerous large focal leaks that quickly saturated the surrounding collagen with fluorophore [Fig. 2(A)]. Surprisingly, addition of 1 or 3% dextran greatly reduced the numbers of leaks, and the ones that did form were small. Compared to standard media, 1 or 3% dextran decreased mean focal leak density by approximately three and sixfold (*p* = 0.0070 and 0.0025, respectively) [Fig. 2(B)]. As observed with delamination and lifespan, addition of 0.1% dextran was insufficient to affect vascular leak density.

### Hydroxyethyl starch promotes vascular function in microfluidic collagen scaffolds

Supplementation of the media with hydroxyethyl starch (HES) led to similar improvements in vascular stability in collagen scaffolds [Fig. 1(D,E)]. Microvessels that were perfused with 5% HES delaminated much less than did vessels that were given 0.1% HES or standard media (*p* = 0.0044 for 5% vs. 0%; *p* = 0.0013 for 5% vs. 0.1%). Delamination was confined to the outlet regions of vessels with 5% HES, while generalized delamination prevailed in the presence of 0.1% HES or standard media.



**FIGURE 1.** Dextran (A–C) and HES (D–F) inhibit vascular delamination and extend vascular lifespan in collagen gels. A: Phase-contrast images of microvessels that were perfused for 4 days with the indicated concentrations of dextran. B: Heatmaps of delamination (0% dextran,  $2n = 52$ ; 0.1%,  $2n = 42$ ; 1%,  $2n = 56$ ; and 3%,  $2n = 60$ ). C: Kaplan–Meier survival curves (0% dextran,  $n = 22$ ; 0.1%,  $n = 16$ ; 1%,  $n = 23$ ; and 3%,  $n = 26$ ). D: Phase-contrast images of microvessels that were perfused for 4 days with the indicated concentrations of HES. E: Heatmaps of delamination (0% HES,  $2n = 26$ ; 0.1%,  $2n = 24$ ; 3%,  $2n = 28$ ; and 5%,  $2n = 24$ ). F: Kaplan–Meier survival curves (0% HES,  $n = 14$ ; 0.1%,  $n = 14$ ; 3%,  $n = 14$ ; and 5%,  $n = 14$ ). White arrowheads point to regions of delamination, and flow is from left to right in all images.

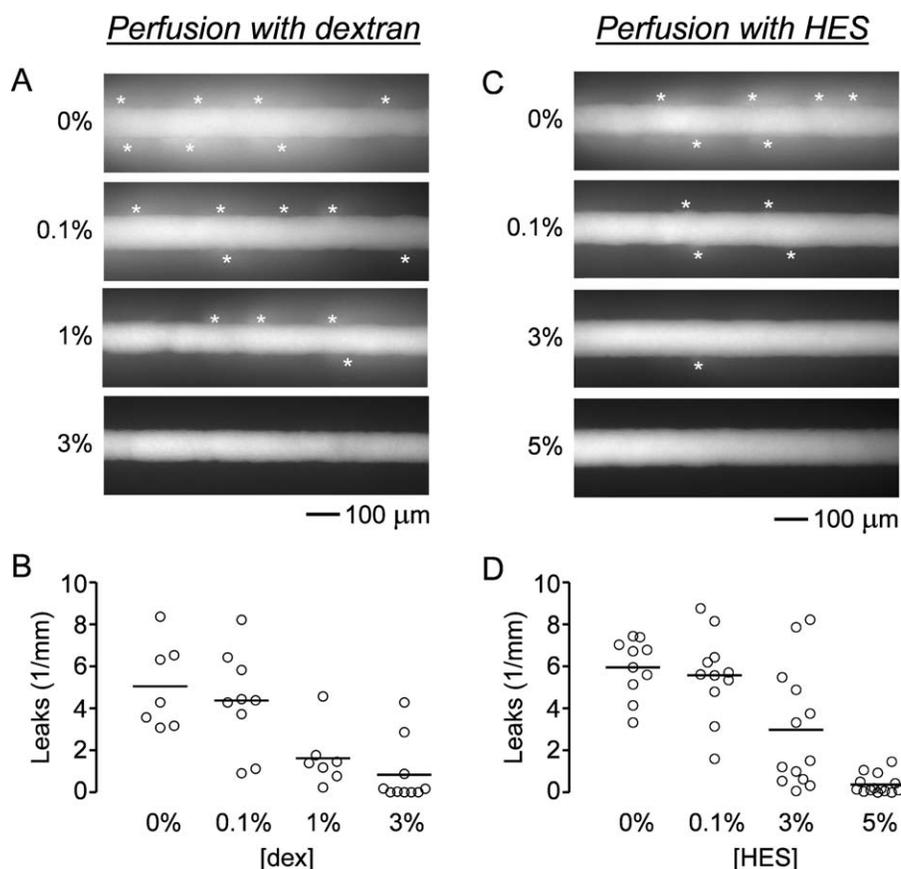
Perfusion with HES also extended the lifespan of microvessels [Fig. 1(F)]. Vessels that were given 3 or 5% HES survived significantly longer than did those with 0.1% HES or standard media ( $p < 0.0001$  for 5% vs. 0.1%;  $p < 0.0001$  for 5% vs. 0%;  $p = 0.0005$  for 3% vs. 0.1%;  $p = 0.0025$  for 3% vs. 0%). With 5% HES, all vessels maintained flow for at least 2 weeks. Addition of 0.1% HES had no effect on vascular lifespan.

Adding HES to the media improved the barrier function of microvessels in collagen gels [Fig. 2(C)]. Only small, rare focal leaks were present in microvessels that were perfused with 5% HES, and the leak density was significantly smaller than for vessels that were given 3% HES, 0.1% HES, or standard media ( $p = 0.0014$ ,  $< 0.0001$ , and  $< 0.0001$ ,

respectively) [Fig. 2(D)]. The addition of 3% HES also led to a decrease in leak density, but this difference did not reach statistical significance.

#### Dextran and HES promote vascular function in microfluidic fibrin scaffolds

To determine if the effects of dextran and HES were specific to collagen scaffolds, we formed microvessels in fibrin gels. Upon seeding, BECs bound less strongly to the fibrin gels compared to the collagen gels, and so we cultured the cells overnight in the fibrin channels under static conditions before initiating constant flow at a pressure head of 5 cm H<sub>2</sub>O. Microvessels in fibrin gels did not distend under perfusion as much as vessels in collagen gels did; as a result, for



**FIGURE 2.** Dextran (A, B) and HES (C, D) promote vascular barrier function in collagen gels. A: Fluorescence images of vessels that were perfused with the indicated concentrations of dextran and 20  $\mu$ g/mL of Alexa Fluor 488-conjugated 10 kDa dextran. B: Plot of focal leak density versus dextran concentration. C: Fluorescence images of vessels that were perfused with the indicated concentrations of HES and 20  $\mu$ g/mL of Alexa Fluor 488-conjugated 10 kDa dextran. D: Plot of focal leak density versus HES concentration. Asterisks denote focal leaks.

a given media composition, the flow rates were lower for vessels in fibrin scaffolds than in collagen scaffolds.

We found that delamination was minimal in microvessels within fibrin scaffolds, even when the perfusate consisted only of standard media. Without dextran or HES, however, approximately half of the vessels developed barren patches in the endothelium by 2 weeks of perfusion [Fig. 3(A)]. These denuded regions were not accompanied by delamination; they resembled “holes” in the endothelium rather than regions where a cohesive monolayer detached. We never found such barren regions in vessels that were grown in the presence of 3% dextran or 5% HES. Because we considered such defects in the endothelium to be indicators of gradual vascular breakdown, fibrin-based vessels had significantly shorter lifespans with standard media than with 3% dextran or 5% HES ( $p < 0.0001$  for both) [Fig. 3(B)]. Denuding was the primary reason for vessel “death” in microvessels formed in fibrin scaffolds.

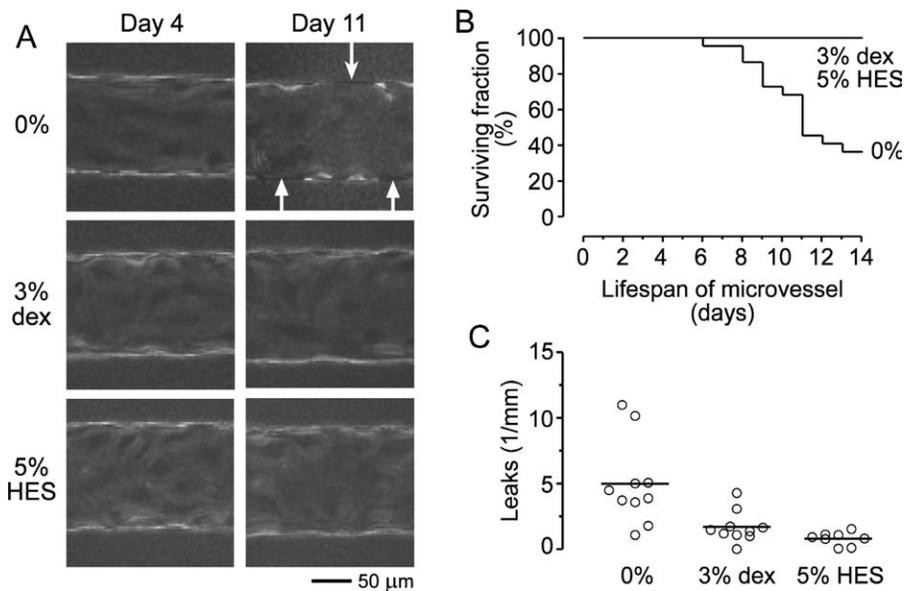
In the presence of standard media, microvessels in fibrin gels had leaky barriers. Addition of dextran or HES led to significant decreases in focal leak densities [Fig. 3(C)]. Supplementation with 3% dextran or 5% HES decreased mean focal leak density by approximately three and sixfold ( $p = 0.0051$  and  $0.0010$ , respectively).

#### Dextran and HES induce increased VE-cadherin junctional localization

To better understand the possible mechanisms by which dextran and HES stabilize vessels in microfluidic scaffolds, we treated BECs on coverslips with various concentrations of these polymers and stained the cultures for the junctional marker VE-cadherin; staining on coverslips rather than in intact vessels afforded greater imaging contrast and resolution. BECs that were grown in media that contained 3% dextran or 5% HES had greater VE-cadherin junctional localization compared to those grown in 0.1% dextran, 0.1% HES, or standard media [Fig. 4(A)]. The VE-cadherin junctions were thin and discontinuous when grown in the latter three media compositions. In media that contained 3% dextran or 5% HES, VE-cadherin formed wide, continuous bands at junctions. The average width of the fluorescent area was significantly higher when BECs were grown in 3% dextran or 5% HES ( $p < 0.001$  and  $p < 0.01$ , respectively, compared to standard media) [Fig. 4(B)].

#### DISCUSSION

This study demonstrated that the plasma expanders dextran and HES stabilize vascularized microfluidic scaffolds against



**FIGURE 3.** Dextran and HES improve vascular phenotype in fibrin scaffolds. A: Phase-contrast images of vessels that were perfused with 3% dextran, 5% HES, or standard media taken at day 4 and day 11 postperfusion. Arrows denote local areas of endothelial loss (“denuding”). B: Kaplan–Meier survival curves. C: Plot of focal leak density versus concentration of plasma expander.

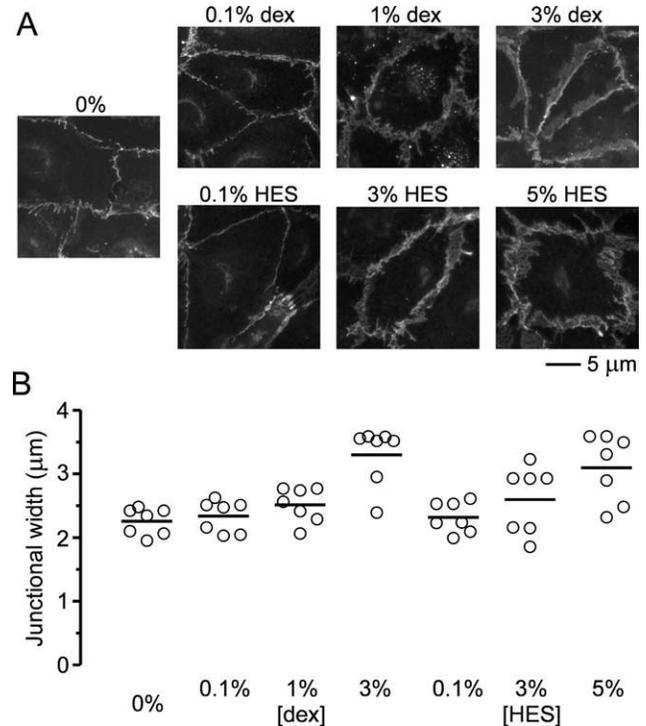
endothelial delamination or denuding, help sustain perfusion over the span of weeks, and improve vascular barrier function. These effects required polymer concentrations on the order of tens of milligram per milliliter. Thus, perfusion with dextran- or HES-containing solutions may be useful when engineering long-lived functional microvessels for the nutritive maintenance of tissues *in vitro* before possible implantation *in vivo*.

### Mechanism of vascular stabilization

How do dextran and HES enhance the stability of microvessels? Aside from both being large polysaccharides, the two polymers share few obvious chemical similarities. Dextran is a largely linear polysaccharide with  $\alpha$ -1,6 glycosidic linkages<sup>15</sup>; HES is a branched polysaccharide with  $\alpha$ -1,4 linkages.<sup>16</sup> Despite their chemical and structural differences, both polymers were capable of producing approximately the same effect if present at similarly large concentrations (3% for dextran, 5% for HES; equivalent to  $\sim 0.5$  mM polymer). The addition of 0.1% dextran or HES (i.e.,  $\sim 10$   $\mu$ M polymer), however, did not augment vascular stability. Thus, we think it is unlikely that a receptor specific for dextran or HES exists on the endothelial surface. Instead, our data point to a nonspecific physical mechanism by which dextran and HES exert their effects on vascular phenotype.

Recently, we have proposed that microvascular stability in microfluidic scaffolds is largely governed by the balance of physical forces.<sup>7,17</sup> In particular, the transmural pressure (vascular pressure minus pressure in the scaffold) must be sufficiently large and sustained to ensure that the endothelium does not detach from the scaffold wall. One possible physical mechanism by which dextran and HES could stabilize a microvessel is by promoting a tight vascular barrier, which enables a large transmural pressure to be

maintained.<sup>7</sup> Indeed, we found that microvessels that were perfused with 3% dextran or 5% HES had few focal leaks (Fig. 2). Several groups have shown that junctional localization of VE-cadherin correlates with vascular barrier function, and disruption of cadherin bonds results in vascular



**FIGURE 4.** Dextran and HES increase localization of VE-cadherin at junctions of BECs. A: Immunofluorescence images of VE-cadherin. B: Plot of average width of VE-cadherin junctions versus concentration of plasma expander.

leak.<sup>18,19</sup> Consistent with these studies, we found that exposure to 3% dextran or 5% HES increased the width of VE-cadherin staining at endothelial junctions (Fig. 4).

We observed that delamination in microvessels that were perfused with 0.1% dextran, 0.1% HES, or standard media showed no preferential localization along the entire length of the channel (Fig. 1). In contrast, microvessels that were perfused with 3% dextran or 5% HES only delaminated selectively near the outlet end of the channels. These results are consistent with computational models of the transmural pressure distribution within single vessels.<sup>8</sup> The agreement between our current experimental results and previous computational models of flow-induced stresses in vascularized scaffolds lends support to our theory that physical, rather than biochemical, mechanisms are largely responsible for dextran- and HES-mediated vascular stability.

We point out that changes in shear stress were not responsible for the observed differences in barrier function. Although the media viscosity varied with polymer concentration, the shear stress was maintained around 10–15 dyn/cm<sup>2</sup> for all media compositions, because the flow rates were higher for the less viscous solutions. In fact, the shear stresses in plasma expander-treated tubes were slightly lower than for untreated tubes, which would promote instability, contrary to observations.<sup>8</sup>

Dextran, HES, and other plasma expanders raise the colloidal osmotic pressure of solutions in which they are dissolved. Because we perfused scaffolds with the various concentrations of plasma expanders upon seeding (i.e., before confluence is reached), the plasma expander concentration in the scaffold is initially the same as that in the perfusate, and no transendothelial osmotic effect is present at this stage. Although we cannot rule out the possibility that a decrease in osmotic pressure in the scaffold can develop after endothelial confluence is reached, it is difficult to see how changes in osmotic pressure can account for the change in VE-cadherin staining upon addition of these polymers. The idea that dextran or HES can “plug” pores in the vessel wall<sup>20,21</sup> is inconsistent with the 0.1–1 μm size of leaks<sup>7</sup> and the 1–10 nm size of the plasma expander. Thus, reduced transendothelial flow due to osmosis is at most a minor mechanism for the observed stabilization.

What physical mechanism could account for the increase in the width of VE-cadherin junctions upon addition of dextran or HES? The explanation that we currently favor is that these polymers induce attractive depletion forces between adjacent membranes.<sup>22</sup> Such an effect has been observed in the polymer-induced aggregation of red blood cells and requires crowded aqueous conditions.<sup>23–26</sup> Following the analysis of Tsuchida and coworkers,<sup>23</sup> we measured the intrinsic viscosity of the dextran and HES and calculated the hydrodynamic radii to be 6.0 and 5.7 nm, respectively. At concentrations of 3% dextran and 5% HES, ideal polymer molecules would occupy ~94% and ~74% of the solution volume, respectively. The large excluded volume fractions are consistent with a crowded environment and suggest that polymer-mediated enhancement of junctional extent is

plausible. Alternatively, crowding-induced formation of membrane complexes<sup>27</sup> may lead to intracellular signaling that stabilizes endothelial junctions, although the identity of such pathways (if they exist) remains unknown.

### Implications for vascularization of microfluidic scaffolds

In this study, we found that microvessel leakiness correlated inversely with lifespan. This result agrees with the findings in microvessels that were treated with other chemical or mechanical signals<sup>7,8</sup> and suggests that stable vascularization of microfluidic scaffolds requires a tight vascular barrier. Supplementing the perfusate with dextran or HES is a straightforward way to achieve this requirement.

Our work also suggests that other large polymers may be able to stabilize vascular function. Notably, high-molecular-weight poly(ethylene glycol) has been found to broaden junctional VE-cadherin localization (see Fig. 5 in ref. 28) and to decrease endothelial leakiness.

### CONCLUSIONS

This study demonstrates how the plasma expanders dextran and HES affect the stability of engineered microvessels. Our work supports a physical theory of stabilization, in which the added polymers broaden junctional overlap (perhaps through depletion forces), which largely eliminates focal leaks and thereby enables the maintenance of a stabilizing transmural pressure. We did not find that a specific receptor for the plasma expanders was responsible for stabilization. Given that dextran and HES are inexpensive and relatively inert, we recommend that microvessels that are grown *in vitro* be routinely perfused with media containing plasma expanders to stabilize vascular channels and sustain nutrition. We note that such ideas may find parallels in devising perfusates for the vascular preservation of organs *ex vivo* and could explain the vascular benefits of plasma expanders seen in such studies.<sup>11,29</sup>

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