Visualization of the Dynamics of Invasion and Intravasation of the Bacterium That Causes Lyme Disease in a Tissue Engineered Dermal Microvessel Model

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Lyme disease is a tick-borne disease prevalent in North America, Europe, and Asia. Despite the accumulated knowledge from epidemiological, in vitro, and in animal studies, the understanding of dissemination of vector-borne pathogens, such as Borrelia burgdorferi (Bb), remains incomplete with several important knowledge gaps, especially related to invasion and intravasation into circulation. To elucidate the mechanistic details of these processes a tissue-engineered human dermal microvessel model is developed. Fluorescently labeled Bb are injected into the extracellular matrix (ECM) to mimic tick inoculation. High resolution, confocal imaging is performed to visualize the sub-acute phase of infection. From analysis of migration paths no evidence to support adhesion-mediated interactions between Bb and ECM components is found, suggesting that collagen fibers serve as inert obstacles to migration. Intravasation occurs at cell–cell junctions and is relatively fast, consistent with Bb swimming in ECM. In addition, it is found that Bb alone can induce endothelium activation, resulting in increased immune cell adhesion but no changes in global or local permeability. Together these results provide new insight into the minimum requirements for Bb dissemination and highlight how tissue-engineered models are complementary to animal models in visualizing dynamic processes associated with vector-borne pathogens.

1. Introduction

Lyme disease is prevalent in North America, Europe, and Asia,[1] and is the most common vector-borne disease in the United States.[2] The most recent data from the Centers for Disease Control and Prevention (CDC) estimates 476,000 new cases every year.[3] While antibiotic treatment is effective, some individuals experience symptoms for months or years following treatment.[4] Lyme disease can lead to health problems associated with the skin, joints, central nervous system (neuroborreliosis), and, to a lesser extent, the heart.[4,5]

Lyme disease in North America is caused primarily by the spirochete Borrelia burgdorferi (Bb) and is transmitted to humans by a bite from an infected tick.[3] Dissemination of vector-borne pathogens, such as Bb, involves several critical steps, including inoculation in the dermis, proliferation, and migration in the local tissue, intravasation into circulation or the lymphatic system, and extravasation into and colonization of distant...
tissues and organs. Most of our knowledge about dissemination comes from analysis of tissue samples in mouse models, e.g., enumeration of \( Bb \) at the inoculation site or in other tissues. Since the processes associated with dissemination are dynamic, visualization is key to elucidating mechanisms. A relatively small number of intravital microscopy (IVM) studies in mouse models have been key in beginning to unravel the details of \( Bb \) extravasation. These studies have been complemented by novel studies of spirochete adhesion in flow chambers, 3D migration studies, and membrane feeding assays. While these studies have been able to establish important links to results from microbiological studies, the biological and microenvironmental factors that regulate \( Bb \) dissemination, especially related to intravasation, remain poorly understood.

To address the knowledge gaps associated with \( Bb \) invasion and intravasation during the early sub-acute phase of infection, we developed a tissue-engineered human dermal microvessel model in a type I collagen extracellular matrix (ECM), the main structural component of the loose connective tissue of the dermis and matched to its stiffness. GFP-labeled \( Bb \) (B31 strain) were inoculated in the ECM to mimic a tick bite. High resolution, confocal imaging was then performed to visualize \( Bb \) migration in the ECM and intravasation into circulation. Based in vitro studies it has been postulated that \( Bb \) migration is directed by chemotactants, and that both invasion and interactions with the endothelium are regulated by adhesins. We show that in a 3D microvessel model, there is no evidence for chemoattraction, and that adhesins do not play a significant role in migration. \( Bb \) in the 3D matrix exhibited the same modes of motion as reported in 2D: forward, backward, and stationary. However, we show that the distribution of reversal events is significantly increased in 3D, which we attribute to the collagen fibers that serve as obstacles. We show that intravasation occurs at cell–cell junctions following a trial and error searching process. While \( Bb \) swimming through cell–cell junctions can occur with little resistance, in many cases, the rear end of the spirochete cell body is transiently tethered to the endothelium (for up to 100 s) prior to release into circulation. Finally, we show that \( Bb \) can induce endothelial activation in the absence of systemic inflammatory cytokines while maintaining normal barrier function. While these results remain to be further refined by independent measurements (e.g., knock-out studies of \( Bb \) adhesins) and in more complex models (e.g., inclusion of tick saliva), we show how the ability to independently control variables in a reducible tissue-engineered human model can advance our understanding of the complexity of \( Bb \) dissemination in humans.

2. Results

2.1. Creation of Borrelia burgdorferi Local Invasion Model

The critical steps in tick-borne pathogen dissemination, e.g., invasion, intravasation, arrest, and extravasation, take place at vascular interfaces. Therefore, we developed a tissue engineered dermal microvessel model to enable visualization of invasion and intravasation of \( B. burgdorferi \) (\( Bb \)) in a platform that enables independent control of key experimental variables (Figure 1a–d). The dermal microvessel was formed by seeding human dermal microvascular endothelial cells (HDMECs) into a 150 \( \mu \)m diameter channel in a type I collagen matrix (Figure 1a,b). Following adhesion and spreading, the HDMECs form a confluent monolayer within 24 h. To simulate tick inoculation, fluorescently labeled \( Bb \) (B31-A3 GFP strain) were injected into a small cavity \( \approx 900 \mu \)m away from the microvessel (Figure 1c,d and Figure S1a, Supporting Information) at 48 h after seeding (Figure S1b, Supporting Information). During experiments, the dermal microvessel was perfused with endothelial cell medium at a shear stress of \( \approx 4 \) dyn cm\( ^{-2} \), corresponding to typical values in post-capillary venules.

Following inoculation (Figure 1c), \( Bb \) migrate within the ECM (Figure 1d) and were observed beyond the microvessel within 1 h. At 48 h following inoculation, a large number of \( Bb \) were observed localized around the microvessel (Figure 1e,f, Figure S2, Supporting Information). To assess differences in the vicinity of the microvessel, we define the region within 20 \( \mu \)m of the microvessel, approximating the length of a typical \( Bb \) spirochete, as the perivascular region, and the region >20 \( \mu \)m from the endothelium as bulk ECM. The concentration of \( Bb \) in the perivascular region at the equatorial plane (Figure S1c, Supporting Information) increased over time, and at all time points was highest at the point closest to the inoculation site and decreased laterally in the upstream and downstream regions (Figure 1g). In addition, the density of \( Bb \) on the anterior side of the vessel was higher than that on the posterior side relative to the inoculation site (Figure S1d,e and Note S1, Supporting Information).

2.2. Characterization of Borrelia burgdorferi Migration

The migration paths of individual \( Bb \) in the ECM were tracked by determining the location of the midpoint along the \( Bb \) cell body (Figure 2a and Movie S1, Supporting Information). Each migration path consisted of a series of individual segment vectors describing distance and angle at each time point (\( At = 1 \) s). Tracking was limited to \( Bb \) with a residence time within the focal plane of at least 20 s. The median tracking time was \( \approx 100 \) s and, in total, we recorded 10 131 segments for 43 individual \( Bb \). From analysis of the migration paths (Figure 2b), we found negligible net displacement perpendicular (along \( y \)-axis, \( -0.60 \pm 23.5 \) \( \mu \)m) or parallel (along \( x \)-axis, \( 1.11 \pm 23.3 \) \( \mu \)m) to the microvessel (Figure 2c), showing that migration was random and that factors such as chemotaxis or interstitial flow did not play a significant role in \( Bb \) migration under the experimental conditions used here.

The instantaneous speed for \( Bb \) migration was determined from the elapsed time for each segment along the migration path. For comparison, we performed experiments with \( Bb \) in 1% methylcellulose solution (denoted as 2D) (Movie S2, Supporting Information), which is commonly used to study \( Bb \) “swimming” in vitro. The instantaneous speed in 3D was significantly higher than in 2D (5.14 ± 1.18 \( \mu \)m \( s \)\(^{-1} \) vs 2.07 ± 0.51 \( \mu \)m \( s \)\(^{-1} \), \( p < 0.0001 \)) (Figure 2d). In contrast, the net speed (the net displacement divided by the elapsed time for each migration path) in 3D was significantly lower than that in 2D (0.58 ± 0.37 \( \mu \)m \( s \)\(^{-1} \) vs 1.01 ± 0.65 \( \mu \)m \( s \)\(^{-1} \), \( p < 0.05 \)). The instantaneous speed in perivascular region was 3.39 ± 7.91 \( \mu \)m \( s \)\(^{-1} \), which is slightly lower than in the ECM (5.16 ± 3.75 \( \mu \)m \( s \)\(^{-1} \), \( p < 0.001 \)) (Figure S3a, Supporting Information).
Figure 1. Invasion of *Borrelia burgdorferi* (*Bb*) in a tissue-engineered dermal microvessel model. a) Human dermal microvascular endothelial cells (HDMECs) seeded into the microfluidic device in collagen type I extracellular matrix. b) HDMECs form a confluent monolayer after 2 days under shear stress (4 dyne cm$^{-2}$). c) Inoculation of GFP-tagged *Bb* into the dermal microvessel. d) Following inoculation, *Bb* migrate from the injection site. e) Fluorescence images of dermal microvessels at 0 and 48 h following inoculation with fluorescently labeled *Bb* (green). HDMECs (magenta). Flow was maintained at 4 dyne cm$^{-2}$. f) Fluorescence images in the vicinity of the inoculation site (see red box in panel (e)) over time. g) Heat map showing quantification of *Bb* in the perivascular region along the vessel over time. Imaging was performed at the equatorial plane of the microvessel with a depth of field of $\approx 4.3$ μm. The *Bb* concentration was obtained in a region within 20 μm of the endothelium at the equatorial plane on both sides of the vessel (i.e., anterior and posterior to the inoculation site). The location along the microvessel is relative to the point closest to the inoculation site and extends about 3 mm in both upstream and downstream directions.
Next, to assess the mode of *Bb* migration, we determined the angle (θ) between consecutive segments along the migration paths (Figure 2a). In all 3 conditions (ECM, perivascular region, and 2D), the distribution of θ was asymmetric: in 2D the distribution was biased toward small θ (relatively straight trajectories), whereas in the perivascular region or in the ECM, the distributions were biased toward large θ (i.e. large changes in direction) (Figure 2e). In general, θ is mainly distributed between 150° and 180° in 3D, compared to 0–30° in 2D (Figure 2e). In 3D, backward motion (θ > 90°) accounts for about 2/3 of segments (65.4% in ECM, 68.8% in the perivascular region), whereas in 2D forward motion (θ ≤ 90°) accounts for about 2/3 of segments (forward 69.2%) (Figure S3b, Supporting Information). Further analysis confirmed that *Bb* in 3D exhibited less persistent forward motion (Figure S3d,e,h, Supporting Information) but more back-and-forth motion (Figure S3f–h) compared to 2D. The frequency of persistent back-and-forth motion was slightly higher in the perivascular region compared to the ECM (see Note S2, Supporting Information). The larger frequency of back-and-forth motion explains why the net velocity is lower in 3D compared to 2D. In 3D, the collagen fibers provide obstacles to forward motion, resulting in a larger number of direction changes and more persistent back-and-forth motion. The higher instantaneous velocity in 3D is due to the lower viscosity of the interstitial fluid compared to 2D. All of the “stationary” segments (vector length ≤ 0.4 μm) were excluded from analysis, but only account for a small fraction of time in both 2D and 3D. Stationary states represented 1.1% of events (23 of 2104) in the ECM, 4.3% (87 of 2035) in the perivascular region, and 3.0% (106 of 3576) in 2D. In addition, most stationary events were transient (<2 s) (Figure S3c, Supporting Information): longer stationary events represented ≈1% of all stationary events and less than 0.1% of all segments. In the perivascular region the instantaneous speed parallel to the endothelium was lower (2.62 ± 1.86 μm s⁻¹) compared to perpendicular to the endothelium (4.37 ± 2.89 μm s⁻¹) (Figure S4a, Supporting Information), and the fraction of stationary events was higher and more persistent (Figure S4b,c and Note S3, Supporting Information). This could be associated with an increase in viscosity associated with basement membrane proteins around the endothelium.

We next characterized the microstructure of the collagen matrix in the bulk and the perivascular region. Scanning electron microscope images of lyophilized gels (Figure 2f) were very similar to decellularized vascular grafts with fiber diameters of 100–200 nm. The porosity of hydrated gels with the same collagen concentration is ≈95% and the pore size is estimated to be several hundred nanometers. There was no visible difference in ECM structure in the perivascular region compared to the bulk.
Figure 3. Dynamics of *Borrelia burgdorferi* (Bb) transmigration. a) Example of direct intravasation where the initial contact of a Bb (white arrow) with the endothelium is at a cell–cell junction. Transmigration occurs over 2 s. HDMECs (magenta), Bb (green). b) Example of direct intravasation. The red line indicates the position of the endothelium (obtained from phase images, not shown). c) Example of indirect intravasation where a Bb (white arrow) arrives at the microvessel, touches the endothelial cell body several times prior to migration to a nearby cell–cell junction and intravasation. A second Bb (red arrow) also shows back and forth motion on arriving at the endothelium. d) Transient tethering during transmigration. Although most of the Bb cell body has transmigrated into the lumen, the rear remains transiently tethered to the endothelium for several seconds prior to intravasation. e) Quantification of tethering time following transmigration. For 4 events there was no transient tethering.

the bulk ECM (Figure 2f), suggesting that the difference in migration speeds between ECM and the perivascular region is not related to ECM density. Immunofluorescence images of laminin confirmed the presence of a layer of basement membrane around microvessels (Figure 2g). When Bb contacted the microvessel endothelium, some interactions resulted in insertion into the endothelium (Figure 2h), a prerequisite for intravasation. Interactions with the endothelium are described in the next section.

2.3. Transendothelial Migration and Intravasation

Although the number of Bb in the vascular region following inoculation was relatively high (Figure 1g), intravasation events were rare. Intravasation events were analyzed if the Bb remained in the field of view at the equatorial plane of the microvessel during the initial interaction with the endothelium, transmigration, and intravasation. Across 19 analyzable intravasation events, two general mechanisms were observed: direct and indirect intravasation. In the direct mechanism, Bb contacted the microvessel and immediately transmigrated (Figure 3a,b), a process occurring over a few seconds. Microvessels with fluorescently labeled HDMECs showed that Bb inserted directly into the cell–cell junction prior to intravasation (Figure 3a and Movie S3, Supporting Information). In the indirect mechanism, Bb arrived at the endothelium, but underwent one or more cycles of back-and-forth motion, either parallel or perpendicular to the microvessel/ECM interface, prior to transmigration (Figure 3c, Figure S5a–d; Movies S4–S6, Supporting Information). The lateral displacement during this process was typically less than the length of an endothelial cell body. From analysis of videos with fluorescently labeled endothelial cells (Figure 3a,c and Figure S5e, Supporting Information), we confirmed that all transmigration events occurred at cell–cell junctions. In summary, over 19 events, 5 displayed direct transmigration, while 14 showed at least 1 cycle of back-and-forth motion while locating a cell–cell junction. Once most of the spirochete cell body had crossed the endothelium, Bb often remained transiently tethered for up to 100 s to the endothelium prior to complete intravasation (Figure 3d,e, and Movie S7, Supporting Information).

From imaging at 48 h post-inoculation, the rate (N h⁻¹) of Bb contacting the endothelium was ≈109 per hour, of which 2.33 ± 0.70% (n = 3 microvessels) resulted in intravasation. Since the region of imaging corresponds to ≈1% of the entire microvessel, we estimate an intravasation rate of around 250 per hour from the simulated tick bite. Assuming a Bb lifetime in circulation of ≤1 h, this corresponds to a blood concentration (assuming 5 L) of 0.05 per mL. This is in agreement with the fact that Bb are rarely detected in blood samples of individuals with Lyme disease where the upper limit would be <1 in a 10 mL blood sample or 0.1 per mL.²
Figure 4. Inoculation with *Borrelia burgdorferi* (*Bb*) induces endothelium activation but does not influence global or local barrier function. a) Fluorescence images of HDMEC microvessels perfused with leukocytes (1 × 10^6 mL^−1 THP-1 and 1 × 10^6 mL^−1 HL-60) for 10 min at 48 h following inoculation with *Bb* or vehicle. Imaging was performed 15 min following perfusion with leukocytes. THP-1 (red), HL-60 (green). b) The number of adherent THP-1 (left panel) and HL-60 (right panel) cells was significantly higher in the presence of *Bb* (*n* = 4 independent microvessels). c) Representative confocal z-axis maximum intensity projection images of ICAM-1 (magenta) and DAPI (blue) in microvessels 48 h following inoculation with *Bb* (green) or vehicle. d) ICAM-1 fluorescence intensity measured from confocal z-axis maximum intensity projection images of microvessels 48 h following inoculation with *Bb* (green) or vehicle. *Bb* inoculation: *n* = 6 microvessels. Vehicle: *n* = 7 microvessels. Each point represents an independent experiment relative to the averaged value from vehicle controls. e) Maximum intensity projection image (top panel) and slice at the equatorial plane (bottom panel) of ICAM-1 (magenta) inoculation with *Bb* (green). The inoculation site was toward the top of the microvessels in this orientation. f) Fluorescence images of dermal microvessels 48 h after inoculation with *Bb* or vehicle during perfusion with 2 MDa dextran. g) Permeability of 2 MDa dextran was the same 48 h following inoculation with *Bb* or vehicle. h) Immunofluorescence images of VE-cadherin at 48 h following inoculation with *Bb* or vehicle.

2.4. *Borrelia burgdorferi* and Endothelium Activation

To assess the effects of *Bb* on the endothelium, we perfused microvessels with monocytic (THP-1 cells) or promyelocytic (HL-60) cells 48 h following inoculation, and quantified the density of adherent cells following wash-out of suspended cells (Figure 4a). Microvessels inoculated with *Bb* showed a significant increase in the number of adherent leukocytes (Figure 4a,b) suggesting that *Bb* alone, in the absence of extrinsic inflammatory molecules, can activate endothelial cells and elicit an inflammatory immune re-
sponse. Immunofluorescence images confirmed upregulation of the adhesion molecule ICAM-1 by 3.68 ± 2.17-fold (Figure 4c–e, Figure S6, Supporting Information) following inoculation with \( Bb \) compared to its corresponding vehicle.

To determine whether \( Bb \) induced changes in barrier function, we measured microvessel permeability by perfusing with fluorescently labeled 2 M Da dextran (Figure 4f). The permeability was \( 4.3 \times 10^{-6} \text{ cm s}^{-1} \), and there was no difference between conditions (Figure 4g). Since the size of 2 M Da dextran is around 50 nm, these results suggest that the paracellular gaps are sufficiently large to allow \( Bb \) migration. Immunofluorescence imaging of VE-cadherin in microvessels showed well-formed adherens junctions, with no difference between \( Bb \) and vehicle (Figure 4h, Figure S6, Supporting Information).

3. Discussion

Dissemination of vector-borne pathogens involves several critical steps, including inoculation in the dermis, proliferation and migration in the local tissue, intravasation into circulation or the lymphatic system, and extravasation and colonization of distant tissues and organs. Much of our current knowledge of the interactions of pathogens with the vascular system comes from IVM studies in mouse models and in vitro models (e.g., Boyden chamber). 2D Transwell models capture some aspects of the dynamics of dissemination, but are reductive and do not allow real-time imaging. 3D tissue-engineered models vary in complexity and physiological relevance, but provide a diverse toolkit for the study of vascular phenomena.

We have developed a 3D tissue-engineered dermal microvessel model to visualize invasion and intravasation of \( Borrelia burgdorferi \) (\( Bb \)), the causative agent of Lyme disease, following inoculation into the ECM. Tissue-engineered models recapitulate the cylindrical vessel geometry, physiological flow rates, and incorporate human endothelial cells in contact with basement membrane embedded within an ECM.

3.1. \( Borrelia burgdorferi \) Migration in ECM

Following inoculation in the human dermal microvessel model, \( Bb \) exhibited the three modes of motion observed in vitro: forward motion, backward motion, and a stationary state. The instantaneous speed in ECM (5.1 \( \mu \text{m s}^{-1} \), Figure 2d) is very close to values reported in vitro in gelatin hydrogels (3.5–5 \( \mu \text{m s}^{-1} \)), and in vivo in the ear dermis in a mouse model (up to 4.0 \( \mu \text{m s}^{-1} \)), but faster than \( Bb \) swimming in 2D (2.1 \( \mu \text{m s}^{-1} \), Figure 2d) (viscosity of 1% methylcellulose: \( \approx 200 \text{ mPa s} \)). The distribution of the modes of motion were different between conditions. In 2D, forward motion was persistent and there was a relatively small fraction of reversal events. In contrast, in 3D there was a much larger fraction of reversal events, likely due to the collagen fibers which act as obstacles for forward motion. The average porosity of 3–7 mg mL\(^{-1}\) type I collagen gels is around 95%. For an average fiber diameter of \( \approx 150 \) nm (Figure 2f), this corresponds to an average spacing of around 400 nm. Therefore, during 1 s (imaging frequency), a \( Bb \) is expected to encounter more than 10 collagen fibers, some of which may result in large displacement angles between segments.

\( Bb \) express adhesins on their outer membranes, including proteins such as BBO406 and BmpA, which bind to the basement membrane protein laminin, and BBK32 which binds to the ECM protein fibronectin. While \( BBO406^- \) and \( BBK32^- \) mutant \( Bb \) result in reduced numbers of spirochetes in tissues in mice, their exact role in invasion and intravasation remains unknown. Our results suggest that adhesins do not play a significant role in \( Bb \) migration in the ECM. The instantaneous velocity in the collagen I matrix is faster than in methylcellulose suggesting that any binding interactions with fibronectin in the ECM is negligible. Studies of BBK32 binding to fibronectin found \( K_D = 0.019 \mu \text{m L}^{-1} \) and \( k_{off} = 0.10 \pm 0.054 \text{ s}^{-1} \), corresponding to an average residence time of around 10 s, much longer than the average duration of stationary states found here (\( \leq 1 \) s). Although we observed a decrease in instantaneous velocity in the perivascular region, this difference is only about twofold and is not consistent with the hypothesis that immobilization of \( Bb \) by laminin binding in the basement membrane is a key step in intravasation. Studies of BBO406 binding with laminin found \( K_D = 0.4 \mu \text{m L}^{-1} \) and \( k_{off} = 0.31 \pm 0.021 \text{ s}^{-1} \), much shorter than the average residence time of around 3 s. Under static conditions, the residence times would be expected to result in a population of relatively long-lived stationary states during migration in extracellular matrix or basement membrane. Since the duration of stationary states is relatively short, it is likely that the momentum generated by the flagella motors is sufficient large to overcome the binding to ECM components. The large fraction of reversal events in the perivascular region (\( \approx 40\% \) of segments \( >170^\circ \)) is likely due to the presence of the endothelial cell bodies, which provide large obstacles to migration.

3.2. Transmigration and Intravasation

We observed that intravasation occurs exclusively at cell–cell junctions via direct or indirect transendothelial migration. If the initial point of contact with the endothelium was at a cell–cell junction, then the \( Bb \) continued to migrate through the junction. If the first trial failed, the \( Bb \) exhibited back-and-forth motion and either located a cell–cell junction, or completely changed direction to a nearby location until a cell–cell junction was found. These results are consistent with the hypothesis that \( Bb \) and other spirochetes (e.g., \( Leptospira \)) use back-and-forth motion as a trial and error method to circumvent obstacles in tissues. We found no directional bias in the migration of \( Bb \) (Figure 2b,c), implying that the initial point of contact at the endothelium was purely random. However, this remains to be verified since we could not determine the fraction of contact events that resulted in intravasation due to \( Bb \) migration out of the field of view.

Although transmigration at cell–cell junctions was relatively fast, \( Bb \) often remained tethered to the endothelium at their rear for up to 100 s prior to intravasation. A possible explanation is that insertion into the lumen triggers reversal of the rear flagella motors to provide a resistance to the fluid shear force in the lumen. \( Bb \) are typically around 0.3 \( \mu \text{m} \) in diameter and 10–20 \( \mu \text{m} \) in length. In the examples shown here, transmigration occurred over a few seconds suggesting that the process involves free swimming through the cell–cell junctions in the endothelium in the absence of biochemical interactions. At a swimming
speed to 5μm s\(^{-1}\), a 10–20μm long \(Bb\) would take 2–4 s to cross the endothelium, consistent with the transmigration times observed here. In summary, live cell imaging (Figure 3 and Movies S4–S7, Supporting Information), fluorescence profiles (Figure S5e, Supporting Information), and the fast transit time provide strong evidence in support of transmigration occurring at cell–cell junctions.

3.3. Influence of \(Borrelia burgdorferi\) on the Endothelium

Local inflammation at the site of a tick bite is a hallmark of early Lyme disease (erythema migrans), and is characterized by upregulation of cytokines and chemokines associated with recruitment and activation of immune cells. Here we found that \(Bb\) alone can induce activation of the endothelium resulting in increased expression of adhesion molecules (e.g., ICAM-1) and leukocyte adhesion. These results show that \(Bb\) can elicit an inflammatory response in the endothelium even in absence of resident immune cells or stromal cells.

Despite activation of the endothelium following inoculation with \(Bb\), we found no change in the global permeability to 2 MDa dextran (hydrodynamic size 50 nm), and no evidence of focal leaks associated with local disruption of cell–cell junctions. These results suggest that barrier dysfunction is not required for intravasation and that \(Bb\) can transmigrate by swimming through normal gaps in cell–cell junctions.

4. Conclusions

In summary, we developed a tissue-engineered dermal microvesSEL platform to study processes associated with invasion and intravasation of \(Borrelia burgdorferi\) (\(Bb\)), the causative agent of Lyme disease. High resolution, confocal imaging was employed to visualize \(Bb\) migration in the ECM and invasion of the dermal microvessel model. Using this reductive model we report several key findings. (1) Migration was random with no directional bias implying that there was no chemoattraction to blood vessels. (2) We confirmed the same modes of migration as observed in 2D swimming: forward, backward, and stationary. (3) The distribution of angles between segments revealed a much higher fraction of reversal events in 3D, likely due to encountering collagen fibers during migration. (4) There was no evidence to support adhesion-mediated interactions between \(Bb\) and components of the ECM or basement membrane, suggesting that collagen fibers serve as inert obstacles to migration. (5) Transendothelial migration occurred at cell–cell junctions: initial contact with the endothelium away from cell–cell junctions resulted in cycles of back-and-forth motion or migration to a cell–cell junction. (6) Intravasation occurred over several seconds, consistent with \(Bb\) swimming through the cell–cell junctions. (7) Following transmigration, transient tethering of the rear end of the spirochete cell body occurred in some cases, with residence times up to 100 s. (8) \(Bb\) alone can induce endothelium activation, resulting in increased immune cell adhesion but no changes in global or local permeability. Together these results provide new insight into \(Bb\) dissemination at a tick bite, and highlight how tissue-engineered models are complementary to animal models in enabling a reductive approach to addressing key mechanistic questions in a model with human cells. The reductive approach has many advantages in studying complex dynamic processes, however, to establish physiological relevance our results remain to be refined (e.g., other \(Bb\) strains and adhesin knockout studies) and systematically extended to include other important variables (e.g., tick saliva and supporting cells).

5. Experimental Section

Fabrication of Dermal Microvessels and Borrelia Inoculation: Microvessels were formed using previously published protocols.[10c,19a] Briefly, \(\cong 1 \times 10^5\) human dermal microvascular endothelial cells (HDMECs, Lonza, CC-2543) were seeded into \(\cong 150\) μm diameter channels in a 7 mg mL\(^{-1}\) type I collagen matrix (Corning, 354249) and perfused in endothelial cell medium (ECM-2) medium for 2 days to achieve a confluent monolayer (see Supplementary Information for details). Next, \(1 \times 10^7\) Bb-GFP (B31-A3 GFP strain, passage 4) were injected into a side port to simulate a tick bite, and imaging or functional testing performed in a live cell chamber (In Vivo Scientific, LLC, St. Louis, USA) at 37°C and 5% CO\(_2\).

Tracking \(Borrelia burgdorferi\) Dissemination, Migration, and Intravasation: To track the dissemination of \(Bb\) from side port to the microvesSEL confocal imaging at single time point were acquired with 20x magnification at 0, 4, 24, 40, 48 h after inoculation. The entire microvessel (7102μm × 1782μm, 20×5 images) were scanned and the number of \(Bb\) in the vicinity of microvessel (20μm) were counted manually. To track the migration and intravasation events, time lapsed images were acquired at 20x magnification on a swept field confocal microscope (Nikon Eclipse Ti, Japan) for 1 h with image rate of 1 s per frame, and performed at 24 h or 48 h after \(Bb\) inoculation, focusing the region with intermediate \(Bb\) density. Endothelial cells were labelled with CellTracker Deep Red (Thermo Fisher Scientific, C34565) to visualize the endothelium during \(Bb\) intravasation. Tracking the migration of \(Bb\), calculation of \(Bb\) migration speeds and angles (θ) between two consecutive vectors along the migration path that determining forward or backward moving was described in detail in the Supporting Information Section.

Functional Assays of Microvessel: To assess immune cell adhesion, microvessels were perfused with \(1 \times 10^6\) HL-60 cells (ATCC) and \(1 \times 10^6\) THP-1 cells (ATCC) for 10 min at a shear stress of 0.2 dyne cm\(^{-2}\) 48 h after inoculation with \(Bb\) or vehicle (BSK-II medium). After washing out the non-adherent immune cells, adherent HL-60 and THP-1 cells were manually counted separately in each device, and the number of adherent cells normalized to the area of the microvessel.

To assess endothelium barrier function microvessels with 2μm Alexa Fluor647-conjugated 2 MDa dextran (Thermo Fisher Scientific, cat. no. D22914) in ECM-2 medium 48 h following inoculation with \(Bb\) or vehicle (BSK-II medium) inoculation were perfused. Phase contrast and fluorescence images (8107μm × 664μm) were acquired every 30 s for 2 min before and 5 min following perfusion with the fluorescent solutes. Permeability of microvessels was calculated from \(P = \Delta\phi/(1/\Delta A)/d/t\), where \(r\) is the vessel radius, \(\Delta\phi\) is the increase in fluorescence intensity upon initiation of perfusion of the solute, and \(d/t\) is the rate of increase of fluorescence increase as the solute permeates into the collagen gel. Sections from −700 to +2100 μm (relative to the inoculation site) were selected for calculation of permeability as \(Bb\) mainly accumulated within these regions with or without \(Bb\) inoculation.

Immunocytochemistry and Image Analysis: 48 h after \(Bb\) inoculation, immunocytochemistry was performed with protocol described in detail in the supplemental methods. Confocal z-stacks (0.4 μm in thickness) were acquired at 40x magnification on a swept field confocal microscope system (Prairie Technologies) and reconstruction of microvessels were assembled from ≤400 slices. To quantify the expression level of ICAM-1 (Figure 4g), maximal intensity projection of z stack of 400 slices was performed and fluorescence intensity was measured in ImageJ with background subtraction. Reported data were normalized to the averaged fluorescence intensity of microvessels (n = 7) from control group.
Keywords
The data that support the findings of this study are available in the supplementary material of this article.

Conflict of Interest
The authors declare no conflict of interest.

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Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Statistics: All experimental values were reported as mean ± standard deviation (S.D.). A student’s unpaired t-test (two-tailed with unequal variance) was used for comparison of two groups. Differences were considered statistically significant for \( p < 0.05 \).

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
P.S., Z.G., U.P., and J.D. conceived the original idea. Z.G. and P.S. wrote the manuscript. Z.G., T.C., N.Z., A.S., J.P., L.W., X.G., A.A., and R.L. contributed to the data acquisition and interpretation. P.S. supervised all work.

Data Availability Statement
The data that support the findings of this study are available in the supplementary material of this article.

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Keywords
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