The heterogeneous motility of the Lyme disease spirochete in gelatin mimics dissemination through tissue

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Edited* by Charles S. Peskin, New York University, New York, NY, and approved January 10, 2012 (received for review September 5, 2011)

The Lyme disease spirochete *Borrelia burgdorferi* exists in nature in an enzootic cycle that involves the arthropod vector *Ixodes scapularis* and mammalian reservoirs. To disseminate within and between these hosts, spirochetes must migrate through complex, polymeric environments such as the basement membrane of the tick midgut and the dermis of the mammal. To date, most research on the motility of *B. burgdorferi* has been done in media that do not resemble the tissue milieus that *B. burgdorferi* encounter in vivo. Here we show that the motility of *Borrelia* in gelatin matrices in vitro resembles the pathogen's movements in the chronically infected mouse dermis imaged by intravital microscopy. More specifically, *B. burgdorferi* motility in mouse dermis and gelatin is heterogeneous, with the bacteria transitioning between at least three different motility states that depend on transient adhesions to the matrix. We also show that *B. burgdorferi* is able to penetrate matrices with pore sizes much smaller than the diameter of the bacterium. We find a complex relationship between the swimming behavior of *B. burgdorferi* and the rheological properties of the gelatin, which cannot be accounted for by recent theoretical predictions for microorganism swimming in gels. Our results also emphasize the importance of considering borrelial adhesion as a dynamic rather than a static process.

The movements of microorganisms have fascinated researchers for >300 y, yet we are still just beginning to understand many aspects of these movements. As a prime example, the swimming of cells and microorganisms is almost universally driven by the undulation or rotation of thin filaments, whether it be the flapping of eukaryotic flagella, the beating of cilia, or the spinning of bacterial flagella (1). A baseline understanding of how these motions produce the thrust to propel a microorganism through water was developed by the pioneering work of G. I. Taylor and E. M. Purcell (2–4). In their natural environments, though, many microorganisms move through substances that do not behave like water. The nematode *Caenorhabditis elegans* lives in soil and undulates its body to move (5). In addition to soil, the nematode can move through viscous and viscoelastic fluids and gels and along the top of moist surfaces using similar undulatory motions (6). *C. elegans* changes its gait depending on the viscosity of the environment (6); viscoelasticity in the environment slows the swimming speed (7). Changes in the wave shape and frequency of the beating flagellum may enhance the ability of mammalian sperm to move through viscoelastic fluids, such as cervical mucus (8). Recent theoretical work has tried to explain how viscoelastic or gel-like media affect the swimming of microorganisms (9–14), but, to date, there have been very few empirical studies to test the theoretical predictions (7).

Here we focus on the spirochete that causes Lyme disease, *Borrelia burgdorferi* (*Bb*), which traverses complex environments during its enzootic cycle. The motility of *Bb* has been studied extensively in liquid media and methylcellulose solutions (see, for instance, ref. 15). Like most other swimming bacteria, *Bb* moves through these environments by rotating long, helical flagellar filaments. However, in spirochetes, the flagella are enclosed within the periplasmic space, the narrow region between the cell wall (i.e., cytoplasmic membrane plus peptidoglycan layer) and the outer membrane (16). The flagella are attached to 7–11 motor complexes positioned near the ends of the microorganism (17). The filaments originating from each end wrap around the cell body and are often long enough to overlap in the center of the bacterium (16). Forces between the cell cylinder and the flagella cause the cell body to deform into a planar, wave-like shape (18). When the flagella rotate, the cell body undulates as a traveling waveform (19), which drives the swimming of the bacterium.

Liquid media and methylcellulose solutions, though, are poor facsimiles for many of the environments that *Bb* encounter in nature. Lyme disease spirochetes transition between two markedly different hosts, the arthropod vector *Ixodes scapularis* and small mammals, such as *Peromyscus leucopus*, the white-footed mouse (20). To move from the tick to the mammal, *Bb* must migrate through many different tissues. In the tick, a small number of spirochetes exit the midgut during feeding by traversing a layer of epithelial cells and a thin, but dense, polymeric network known as a basement membrane (21). The spirochetes then swim through the hemocoel, a fluid environment containing hemocytes and hemolymph, where they attach to the salivary glands, penetrate another basement membrane, and enter the salivary ducts (22). *Bb* is then inoculated into the skin of its mammalian host where it translocates through the collagen-dense extracellular matrix (ECM) to access small vessels that provide portals for hematogenous dissemination. Cultured spirochetes injected i.v. into the vasculature undergo transient and dragging interactions before attaching firmly to the microvascular endothelium and working their way through interjunctional spaces separating endothelial cells (23). The tissue barriers that *Bb* navigates in ticks and mammals respond with a combination of viscous and elastic behavior to forces generated by the bacterium. These natural environments are differentiated further from liquid media and methylcellulose solutions because they contain cells and various ECM components, such as collagen, fibronectin, and decorin, to which *Bb* binds (24). How adhesion influences microorganism motility has not been explored from an experimental or theoretical perspective.

To begin to understand the motility of *Bb* in its natural environments, here we use gelatin matrices to bridge the gap

Author contributions: M.W.H., S.M.D.-E., M.J.C., A.A.B., L.K.B., J.D.R., and C.W.W. designed research; M.W.H., S.M.D.-E., A.A.B., and L.K.B. performed research; C.W.W. contributed new reagents/analytic tools; M.W.H., H.C.F., and C.W.W. analyzed data; and M.W.H., S.M.D.-E., L.K.B., J.D.R., and C.W.W. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1114362109/-/DCSupplemental.

between controllable, in vitro motility assays and the natural environments that *Bb* encounters. Gelatin, a denatured form of collagen, was chosen because it possesses many of the same binding sites as native collagen (25) and because it has been used as an in vitro equivalent to the ECM for the study of borrelial motility (21, 26). We find that *Bb* in the dermis of chronically infected mice and in gelatin exhibit three or four motility states, which are determined by transient adhesion between the bacterium and the matrix. In addition to adhering to these substrates, spirochetes can migrate through them, even though the pores in the gelatin matrices are significantly smaller than the diameter of the bacteria. The undulation and migration speed of the bacteria are found to depend strongly on the physical properties of the environment. This work shows that gelatin is a good facsimile for the ECM and reveals the biophysical effects of the environment on borrelial motility. In addition, we deduce that adhesion is a dynamic process that allows *Bb* to transition between stationary and migratory behaviors.

Results

***B. burgdorferi* Motility in the Mouse Dermis Includes Motility States Not Observed in Liquid Media or Methylcellulose Solutions.** Approximately 20% of the total dermal volume in mammals consists of an entangled meshwork of types I and III collagen fibers (27, 28). *Bb* can bind directly to type I collagen (26) as well as other ECM constituents. To determine whether the motility of *Bb* in the dermis differs from that documented in liquid media and methylcellulose solutions, we used two-photon intravital confocal microscopy to track pathogen movement in the ears of mice that had been tick inoculated with Bb914, a virulent strain 297 isolate that constitutively expresses GFP (21). Spirochete movement was characterized by traveling-wave undulations of the cell body (Fig. 1*A*) as observed previously in liquid media, in methylcellulose solutions (19), and in the mouse dermis after i.v. injection (23). However, we also observed a major subpopulation of spirochetes that were actively motile but did not move through the dermis (Fig. 1*A*). These stationary, but motile, spirochetes occurred in two different states. Spirochetes in the first state, which we term *wriggling*, undulated as a traveling wave but otherwise remained fixed in place. In the other state, termed *lunging*, the spirochetes appeared to have one fixed end, while the remainder of the organism bent and relaxed as the organism apparently attempted to break free from its tether. These stationary states are likely due to adhesion between the bacteria and the ECM, as the spirochetes are still motile and are exerting forces on the ECM but are unable to translocate. Although we cannot directly probe whether the spirochetes in these states are actually physically bound to the matrix, it is quite probable that they are. In fact, the lunging state resembles the movement of *Bb* when it is tethered to a coverslip (19). For this reason, we classify this impedance as adhesion. The remainder of the bacteria, referred to as *translocating*, demonstrated a net displacement within the dermis. In general, these translocating spirochetes appeared similar to motile spirochetes in liquid media or methylcellulose; however, the fraction of translocating bacteria in the dermis was lower than that in liquid media or methylcellulose, where nearly all of the bacteria translocate. In addition, our observations suggest that spirochetes reverse their swimming direction less frequently in the dermis than in standard in vitro motility assays. Furthermore, we do not observe flexing motions, which are characteristic of spirochetes in liquid media (19). Therefore, at least three states of motility exist in the mouse dermis, two of which are not observed in liquid media or methycellulose solutions. The motility states were dynamic, as spirochetes in the dermis were observed to transition between them. For example, Fig. 1*A* shows a spirochete that switches from lunging to translocating (Movie S1). Another interesting feature of these heterogeneous motility states is that spirochetes in the different states were observed in close proximity to one another (Fig. 1*A*).

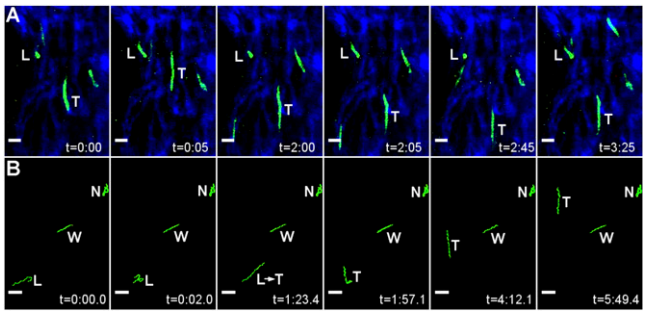

Fig. 1. The motility patterns of Lyme disease spirochetes (Bb914) in gelatin resemble those observed in mouse skin. (*A*) Time course of spirochetal (green) motility in the dermis of a tick-inoculated mouse (Movie S1). Lunging (L) and translocating (T) bacteria are shown. The dermal collagen fibers fluoresce blue due to second-harmonic generation. (*B*) Time course of *Bb* motility in 3% gelatin (Movie S2). All four motility states are shown: nonmotile (N), wriggling (W), lunging (L), and translocating (T). (Scale bars: 10 μm.)

***B. burgdorferi* Motility in Gelatin Resembles Motility in the Mouse Dermis.** To determine whether spirochetes within gelatin matrices show the same motility states as those observed in the dermis of the mouse, we examined the motility of Bb914 in 3% gelatin matrices using wide-field, epifluorescence, and confocal microscopy. In some experiments, microspheres were embedded into the matrix to guarantee that the spirochetes were within the gelatin (Movies S3 and S4). Spirochetes in the matrices exhibited all three types of motility seen in the dermis: wriggling, lunging, and translocating (Figs. 1*B* and 2*A*). In addition, high-speed acquisitions revealed a substantial fraction of spirochetes that were nonmotile, constituting a fourth behavior. This nonmotile state may also be present in the dermis but not seen due to the time interval between consecutive images in our intravital imaging (see *Adhesions to the Matrix Are Transient*).

Gelatin Concentration Affects Binding of Spirochetes to the Matrix. Having established that spirochete motility in our gelatin matrix system closely resembles that in tissues, we investigated the effect of gelatin concentration (2–5%) on the motility of *Bb*. We found that the fraction of bacteria in each motility state was strongly affected by the concentration of gelatin (Fig. 2*B*). Not surprisingly, increasing the concentration led to a larger population of nonmotile bacteria, which varied approximately linearly with gelatin concentration, ranging from 4.7 ± 2.4% in 2% gelatin to 35 ± 4% in 5% gelatin. The proportions of bacteria in the wriggling and lunging states decreased with increases in the gelatin volume fraction; however, the populations in the lunging state appeared to plateau at ~8% at the highest concentrations. The translocating population proved to be the most interesting state. As adhesion and confinement of the bacteria by the gelatin are more likely at higher gelatin concentrations, we had expected to find that the percentage of translocating bacteria would decrease with gelatin concentration. Instead, the fraction of translocating bacteria increased from 20.3 ± 0.5% in the 2% matrices to 35.5 ± 5.2% in the 3% matrices ($P = 0.042$); the populations then decreased to 24.5 ± 2.4% and 21.2 ± 4.2%, in the 4% and 5% matrices, respectively.

Swimming Speed of *B. burgdorferi* in Gelatin Matrices. Swimming speed in liquid media is determined by the wavelength, amplitude, and frequency of the undulating swimmer (2). Therefore, we measured speed and waveform of bacteria in the matrix system with a newly developed algorithm that parameterizes the centerline of the shape of bacteria in our time-lapse images (*SI Text S1* and Movie S5). From the parameterized shape, we computed the center of mass and the average orientation of the bacteria and then calculated swimming speed along the direction of the mean orientation. We found that translocating bacteria in the 3–5% gelatin matrices swam without slipping (i.e., the

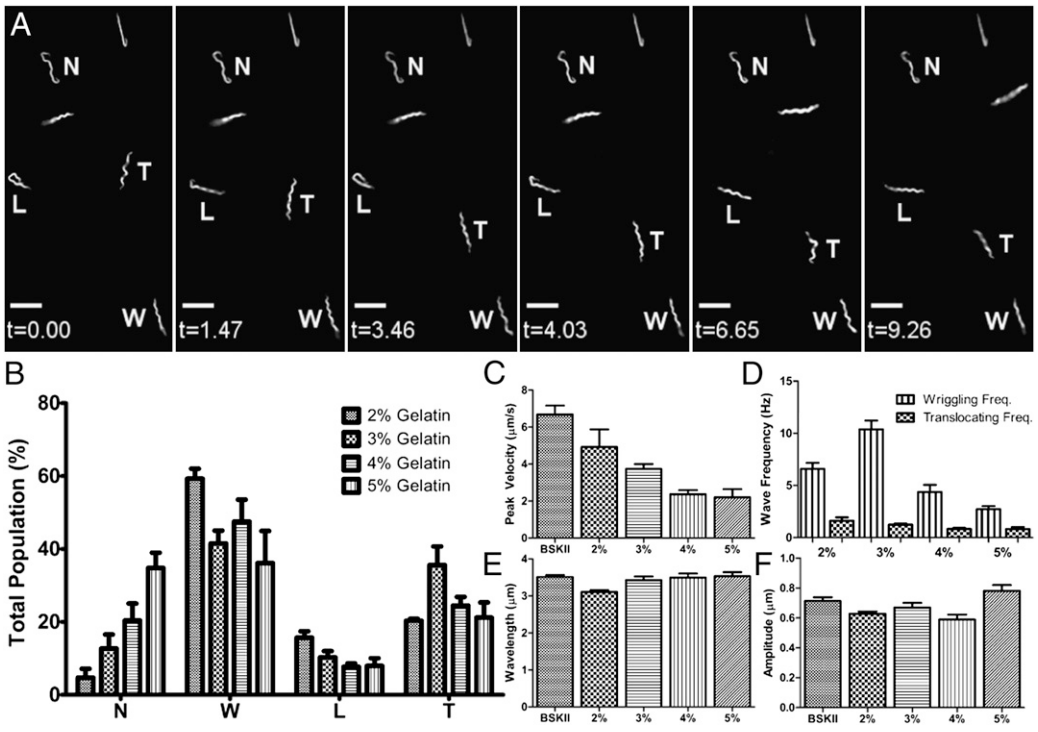

Fig. 2. (*A*) High-speed image acquisition reveals four distinguishable phases of spirochetal motility in gelatin (Movie S6): nonmotile (N), wriggling bacteria (W) that undulate without locomoting, lunging bacteria (L) that undulate and deform but maintain at least one fixed point along the cell length, and bacteria that translocate through the gelatin matrix (T). (Scale bar, 10 μm.) (*B*) The fraction of bacteria in each state depends on gelatin concentration. (*C*) The speed of translocating bacteria decreases with gelatin concentration and is always less than that in liquid medium. (*D*) The undulation frequency for translocating and wriggling bacteria as a function of gelatin concentration. The wriggling frequency always exceeds the translocating frequency and shows a peak in 3% gelatin solutions. (*E*) In all concentrations of gelatin and BSK, spirochete wavelengths were measured to be ~3.3 μm; (*F*) the amplitude is also independent of gelatin concentration.

swimming speed was equal to the wave speed), as has been observed for *Leptonema illini* in 1% methylcellulose solution (29) but is not predicted by any current theories for swimming in non-Newtonian fluids (9, 12, 13, 30). In the 2% matrices, translocating bacteria sometimes slip with respect to the matrix but still typically swim without slipping. The average swimming speeds of translocating bacteria in 2% and 3% gelatin matrices were 4.9 ± 1.0 μm/s and 3.7 ± 0.3 μm/s, respectively (Fig. 2*C*). Increasing the gelatin concentration further decreased the swimming speed (Fig. 2*C*). In comparison, the velocity of *Bb* in liquid medium [Barbour–Stoenner–Kelly II (BSK) medium] was 6.9 ± 0.7 μm/s, which is somewhat faster than previously reported for an avirulent 297 strain in salt buffer (19).

We also used the parameterized shape to compute the wavelength and amplitude of the flat-wave shape of *Bb*. We found that these cell shape parameters were unaffected by the concentration of gelatin, with an amplitude of 0.63 ± 0.03 μm and a wavelength of 3.31 ± 0.04 μm (combined mean ± SEM for all gelatin concentrations and BSK medium) (Fig. 2*D*). Our value of the amplitude is somewhat lower than that previously reported (19), which is likely due to segments of a spirochete's flat wave being out of the plane of focus. The wave frequency of the translocating bacteria is equal to the swimming speed divided by the wavelength and decreases from 1.6 ± 0.3·s^{-1} (2%) to 0.8 ± 0.2·s^{-1} (5%). We also measured the frequency of the wriggling bacteria. Interestingly, the wriggling frequency always exceeds the frequency of the translocators. The peak wriggling frequency was ~10 Hz in the 3% gelatin matrices (Fig. 2*E*), which is comparable to what has been measured in methylcellulose solutions (19). The wriggling frequency was slightly lower in 2% gelatin matrices (~7 Hz) and reduced further in 4% and 5% matrices (4 Hz and 3 Hz, respectively). The frequency in 3% gelatin was statistically significant compared with the frequencies in the other matrices ($P < 0.003$).

Microstructure of the Gelatin Matrices. From our high-speed imaging, it was not clear what determines the motility state of a given bacterium. One possibility is that the different states are determined by nonhomogeneity of the local environment. Another possibility is that adhesion to the matrix is stochastic, with the fraction of bacteria in a given motility state set by the kinetics of the binding and unbinding of adhesion proteins. Our time-lapse imaging favors the latter option, as we often see translocating spirochetes swimming very near wriggling or nonmotile bacteria. To investigate this question more fully, we used scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to examine the microstructure of the gelatin matrices. We found that all concentrations of the gelatin matrices were homogeneous on length scales comparable to or larger than the diameter of *Bb* (~300 nm) (Figs. S1 and S2) and estimated from these images that the average pore size of the gelatin is <100 nm in the 2% matrices, ~50 nm in the 3% matrices, and slightly <50 nm in the 4–5% matrices. In addition, at all concentrations, the gelatin appeared to be in close contact with the organisms, and there was no noticeable difference in the gelatin structure as a function of distance from the surface of the bacterium (Fig. S2). These results suggest that the bacterium must push aside the gelatin filaments to enter and move through the matrix and that the gelatin is in close contact with the bacterial surface. Interestingly, the structure of the gelatin matrices closely resembles the basement membrane that lines the midgut of the tick (Figs. S2 and S5). In the dermis of a mouse ear, *Bb* were found to be tightly surrounded by collagen fibers (Figs. S2*H* and S5), which provides further evidence that the gelatin matrices are a good facsimile for studying *Bb* motility through the ECM and basement membrane.

Rheological Properties of the Gelatin Matrices. As demonstrated by the TEM images (Fig. S2), the gelatin matrices are extremely tight environments. For the bacteria to penetrate into the matrix, they must displace the gelatin to accommodate the diameter of the cell. We, therefore, characterized the mechanical properties of the gelatin matrices using shear rheology and estimated the Young's modulus of the gelatin matrices using a Poisson ratio of 0.4 (31). We found that the 2% matrices [Young's modulus of ~3 Pa (Fig. S3*A*)] were very soft and only nominally a gel, with a loss modulus that was comparable to the storage modulus for all frequencies that we explored. In the other matrices, the storage modulus was significantly larger than the loss modulus, and the

Young's modulus increased linearly from ~20 Pa (3% matrices) to ~300 Pa (5% matrices) (Fig. S2 *A* and *B*).

The gelatin must push in against the spirochete, requiring the organism to withstand this environmental pressure. If the pressure from the gelatin is at least comparable to the bacterial turgor pressure, then the diameter of the spirochete should depend on the gelatin concentration. Because no such change in diameter was observed (Fig. S4), we can put a lower bound on the turgor pressure in *Bb*, which must be at least 5 kPa to prevent the cell diameter from changing by >10%.

Adhesions to the Matrix Are Transient. Our intravital imaging in the dermis examined *Bb* motility over tens of minutes, whereas our high-speed imaging in gelatin spanned only tens of seconds. We, therefore, used confocal microscopy to examine spirochete motility in the gelatin matrices over longer time intervals. These movies revealed a much larger fraction of motile organisms than in the short time interval movies (Fig. 3*A* and Movie S7). In fact, movement of spirochetes in these movies more closely resembled our observations in the dermis, where the nonmotile fraction was not observed. Because the longer time interval movies show a significantly smaller percentage of nonmotile bacteria than the short time interval movies, adhesions between the bacteria and the matrix are likely transient. Indeed, as noted earlier and shown in Fig. 2*B*, we frequently observe bacteria that transition between different motility states. These results strongly suggest that differences between the motility states are dictated by the degree of adhesion between the bacterium and the matrix and that adhesions turn over on timescales of minutes.

Because the adhesions are transient, bacteria translocate for several seconds to minutes before becoming stuck. After a while, the bacteria break free and translocate again. If the spirochete swims off in a new direction after becoming stuck, then one would expect the behavior of the spirochete to be diffusive on long timescales; [i.e., the mean squared displacement (MSD) should scale linearly with time]. In all gelatin concentrations, we found that the MSD was subdiffusive on short timescales and transitioned to superdiffusive behavior on timescales of ~100 s (Fig. 3*B*). The average log-log slopes of the MSD data showed largely subdiffusive behavior (slopes <1); interestingly, the 5% matrices had the largest population of bacteria exhibiting superdiffusive behavior (Fig. 3*C*).

We were unable to observe diffusive behavior on longer timescales because translocating bacteria often traversed the entire field of view without becoming stuck. We could, nonetheless, use our confocal gelatin matrix data to determine whether our short-time interval observations were consistent with those obtained over longer time intervals. We determined that the number of bacteria that were tracked in a given field of view for at least a given length of time decays approximately exponentially (Fig. 3*D*). The time constant for this exponential decrease should be inversely proportional to the fraction of translocating bacteria, Φ_t, multiplied by the translocation velocity and also should be proportional to the size of the field of view (*SI Text S2*). A plot of the time constant as a function of $1/\Phi_t V$ (Fig. 3*D*, *Inset*) yields a straight line with a slope of 114 μm, which is approximately equal to half the size of our field of view. Therefore, the fractions of bacteria in the short time interval movies reflect an equilibrium in the transitions between the motility states.

Discussion

In this paper, we set out to develop an in vitro system to study *Bb* motility that would better approximate the natural host environments that *Bb* encounters (ticks and mice). Real time intravital imaging of host-adapted spirochetes in the dermis of mice revealed qualitative differences in motility in comparison with standard in vitro motility assays performed in liquid media containing salts or methylcellulose (19). In addition to a translocating population that swims via an undulating waveform, similar to spirochetes observed in vitro, we also found two other forms of motility, which we termed lunging and wriggling. These two forms of motility were characterized by at least one stationary point along the length of the bacterium. We then showed that in gelatin matrices these same motility states are observed; in addition, we also observe a fourth, nonmotile state. Whereas the nonmotile state appears similar to the stopped state observed in standard motility assays (15), the dependence of this state on gelatin concentration suggests that it arises from adhesion, as opposed to flagellar dynamics. It is likely that spirochetes in the nonmotile state are present in the dermis but are not observed due to the longer acquisition time of these images, as supported by the difference between our high-speed and confocal imaging in gelatin. Collectively, these findings argue that standard motility assays with either liquid media or methylcellulose solutions may not provide an accurate description of the motility of *Bb* in its natural environments. Finally, we quantified the biophysics of borrelial motility in gelatin. We found that the fraction of bacteria in each motility state is strongly dependent on the concentration of gelatin and that *Bb* can transition between translocating and lunging, between lunging and wriggling, and between wriggling and nonmotile states (Fig. 4*A*). Although we did not observe any other transitions, it could be that these occur infrequently. Our data suggest that these transitions are due to

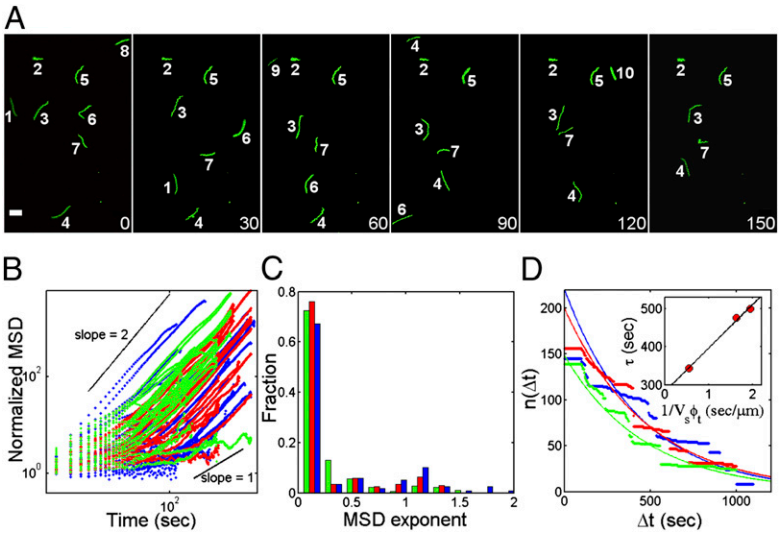

Fig. 3. (*A*) Time-lapse images of *Bb* in a 3% gelatin matrix show that adhesions to the matrix are transient (Movie S7). Individual spirochetes are numbered. Most are translocating (spirochetes 1, 3, 4, 6–10). Nonmotile (2) and wriggling (5) spirochetes also are shown. (Scale bar, 10 μm.) (*B*) Characteristic MSD vs. time plots for the 3–5% gelatin matrices. In all concentrations, the motility over short time intervals is subdiffusive, with a transition to superdiffusive behavior (slope between 1 and 2) that occurs at ~100 s. (*C*) Histogram of the slopes of the MSD for the different gelatin concentrations. The 5% matrices show the greatest persistence of motion (i.e., the largest fraction with slopes >1). Black lines show slopes of 1 and 2. (*D*) The number of bacteria tracked for at least a time Δt decays approximately exponentially. (*Inset*) Decay time as a function of $1/\Phi_t V$, which is linear with slope of 114 μm. (*B–D*) Plot coloring shows 3% (green), 4% (red), and 5% (blue) gelatin.

transient adhesions between the bacterium and the gelatin. One of the most interesting features of the population data is the peak in the translocating population in the 3% gelatin matrices (Fig. 2*B*). There is also a corresponding peak in the wriggling frequency in 3% matrices (Fig. 2*E*); however, the swimming speed and the wave frequency of the translocators decrease monotonically (Fig. 2*D*).

Adhesion between *Bb* and host tissues is believed to play an important role in the parasitic strategy of the bacterium and has been studied extensively (24). Typical adherence assays determine the number of spirochetes that remain on a surface after washing (see, for example ref. 26), which quantifies instantaneous adhesion. Our results suggest that adhesion in the dermis and gelatin is a dynamic process in which the bacteria can bind and unbind from the matrix. From our population data, we can characterize the effect of gelatin concentration on the adhesions between the bacteria and the gelatin (Fig. 4*B*) and can also estimate the time constants for these reactions. Our results suggest that the rate of binding to the matrix is affected by the concentration of adhesins, as the nonmotile state increases with gelatin concentration. The unbinding rate, however, is not likely affected by the concentration, because our MSD data show a change in slope at ~100 s at all gelatin concentrations, suggesting that the binding and unbinding rates are on the order of 10 ms. We presume that the translocating population possesses no fixed adhesions to the matrix, whereas the nonmotile population possesses the largest number of adhesions. If forces between the spirochete and the gelatin affect the unbinding of adhesion molecules, then the equilibrium rate constants K_{wl} and K_{lt} divided by the gelatin concentration should decrease monotonically with the square of the effective viscosity of the matrix, η, times the undulation frequency, f (*SI Text S3*). Our data agrees well with this prediction (Fig. 4*C*). Therefore, the binding rate is likely proportional to the gelatin concentration and the off rate increases with applied force from the motile spirochete.

It is often stated that spirochetes swim faster in gel-like environments than in liquid media, and this property is believed to be important for the dissemination of the bacteria through their hosts (16, 19, 32, 33). For example, *Bb* and *L. illini* show marked increases in speed in 1% methylcellulose solutions (29, 33). Our results, though, show a decrease in swimming speed with gelatin concentration that is caused by a substantial decrease in the undulation frequency. However, although *Bb* swims slower in gelatin, it swims without slipping. Therefore, the ratio of the swimming speed to the wave speed is larger by approximately a factor of 10 over that measured in liquid media (*SI Text S4*). Recent computational modeling has shown that viscoelasticity can enhance swimming speed for undulating bodies with a pronounced increase in amplitude at the tail (30), and experiments with rotating helices have shown a similar effect (34). Both of these cases, however, show only modest speed enhancement, and in neither case is swimming without slipping observed.

We did not expect that the wriggling frequency would always be substantially larger than the wave frequency of the translocating bacteria. However, wriggling bacteria dissipate energy only by undulating, whereas translocators also dissipate energy by moving through the matrix. Because the total power dissipated must be equal to the power input by the flagellar motors, the frequency of translocators should be lower than that of wrigglers. At low rotational speed, the torque from the flagellar motor is approximately constant (35). The power input is equal to the torque times the frequency. The power dissipated comes from two sources, rotating the flagellum in the periplasmic space and moving the bacterium through the environment. Using a simple model for swimming through a gel, we can estimate the total power dissipated (*SI Text S5*). The effective viscosity of the gelatin matrix should then be linearly proportional to the inverse of the undulation frequency, as $1/f = \alpha\beta\eta + c$, where β and c are constants and $\alpha = 1$ for wriggling and 7/2 for translocating (*SI Text S5*). This theoretical result predicts that the undulation frequency is largely independent of the matrix elasticity. We tested this prediction using our rheology measurements to define η (Fig. S2*B*) and found good agreement between the predicted behavior and our experimental measurements (Fig. 4*D*). In methylcellulose solutions, *Bb* swims without slipping, but the undulation frequency is seemingly unaffected. It is not clear why this behavior occurs, but it could be that these solutions are viscoelastic fluids, not gels, or that the concentrations that are used (<1%) are low enough that the bacterium is not always in direct contact with the polymer.

Dissemination of *Bb* within its arthropod vector and mammalian hosts, as well as transitioning between them, relies upon the spirochete's ability to move through dense tissues. It is interesting to speculate as to why the motility states that we observe in the dermis and in gelatin may be important during the enzootic cycle. The tick midgut seems to be a hospitable environment for *Bb*, as the spirochetes are found in high abundance at this site (21, 36, 37). However, during the blood meal, some spirochetes must leave the tick and transition to the mammalian host for the species to survive (38). Egress from the tick midgut is likely a difficult process, accomplished only by an astonishingly few spirochetes (21, 39). Traversal of the midgut into the hemolymph involves adhesion and wiggling given the tight spaces the spirochete has to maneuver through [i.e., epithelial cell junctions and the basement membrane (Figs. S2 and S5)]. Although our experiments do not directly probe the mechanism by which spirochetes penetrate dense matrices, it is clear that the undulatory swimming of *Bb* enables these bacteria to migrate through fairly dense polymer networks; however, motility is inhibited in dense matrices, which explains why so few organisms are able to escape the midgut (21).

Once in the mammal, *Bb* need to disseminate through the host to evade the host immune response, enter the bloodstream, find hospitable tissue in which to replicate efficiently, and return to the dermis to infect new ticks (38). The translocaters, therefore, play a vital role in spirochete dissemination throughout the mammal. Swimming solely in one direction, though, is not a good means for spreading within an environment. Rather, many

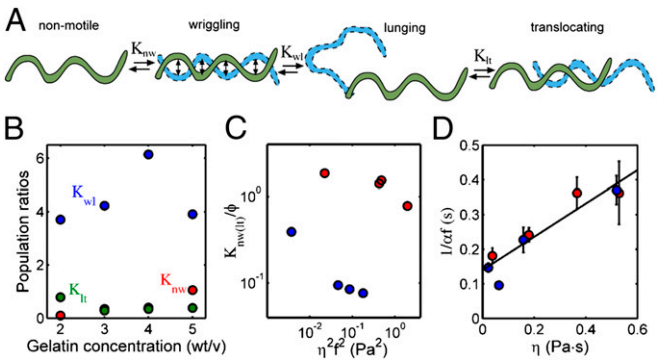

Fig. 4. A simple kinetic model determines the effective rate constants for binding and unbinding to the matrix as a function of gelatin concentration. Transitions occur between the nonmotile and wriggling states, between the wriggling and lunging states, and between the lunging and translocating states. If these transitions are in equilibrium, then the equilibrium rate constants are equal to the ratios of the fraction of bacteria in the states; (e.g., the equilibrium constant for the transition between nonmotile and wriggling, K_{nw}, is equal to the ratio of the fractions of nonmotile bacteria to lunging bacteria). (*B*) The equilibrium constants as a function of gelatin concentration. The transition from wriggling to nonmotile, K_{nw}, increases approximately linearly with gelatin concentration. (*C*) K_{wl} and K_{lt} are also proportional to gelatin concentration (ϕ) and also decrease with the force from the bacterium (which is proportional to the square of the effective viscosity η times the undulation frequency, f). (*D*) The inverse of the frequency of wrigglers (red) and translocators is proportional to the viscosity. The parameter $\alpha = 1$ for wrigglers and 7/2 for translocators (*SI Text S4*). The black line shows the best linear fit to the data.

microorganisms use random walks, such as the run-and-tumble behavior of *Escherichia coli* (35). In vitro experiments of *Bb* motility display two modes of swimming, runs and flexes, which were suggested to be analogs of runs and tumbles in *E. coli* (15). However, we did not observe flexing in the dermis or gelatin. We, therefore, suggest that lunging may be the equivalent of tumbling. Translocating bacteria move along relatively straight paths. When the spirochetes adhere to the matrix, they lunge by compressing along their longitudinal axis and then uncompressing in a new direction. Because the adhesions are transient, some lungers break free and swim off in a new direction. The lunging state then serves to periodically reorient bacteria, much like a tumble.

What roles might the wriggling and nonmotile states serve? To complete the enzootic cycle, spirochetes must strategically position themselves in the dermis to be acquired by another tick. The stationary states (nonmotile and wriggling) may be a mechanism for keeping higher burdens of spirochetes in collagen-rich tissues like the skin. In addition, these stationary states might be part of the spirochete's strategy for immune evasion. Stationary bacteria would be more susceptible to internalization by much slower moving resident and recruited phagocytes, effectively acting as decoys that would facilitate dissemination of translocating bacteria. However, whatever the functional role is of these different motility states, our data show a striking example of spirochete diversity. Transient adhesions act to subdivide the bacterial population into multiple motility states, even in fairly uniform tissue-like environments. This diversity may allow *Bb* to exploit changing environments and host-mediated environmental responses and is likely to be important in the enzootic cycle and the progression of disease.

Materials and Methods

Here we give a brief description of the methods. See *SI Text S1* for a complete description.

All experiments were done using Bb914 (26), which were grown to late log-phase in BSK II medium supplemented with 6% normal rabbit serum (Pel-Freeze Biologicals).

Mouse experiments used C57BL/6 mice (4–6 wk old), purchased from The Jackson Laboratory, and were conducted following the National Institutes of Health guidelines for housing and care of laboratory animals and were performed under animal protocols approved by the Yale University Institutional Animal Care and Use Committee. Infection was introduced into mice using laboratory-reared *I. scapularis* nymphal ticks infected as larvae with Bb914, as described in refs. 21 and 40. Mice were imaged 98 d after infection, using an Olympus BX61WI fluorescence microscope with a 20×, 0.95 NA water immersion Olympus objective and a dedicated single-beam LaVision TriM laser scanning system (LaVision Biotec) that was controlled by Imspector software.

Gelatin stocks were prepared by dissolving Type-A gelatin powder in sterile PBS, as previously described (21). A total of 5×10^7 spirochetes in BSK II were introduced onto the top of each gelatin matrix and incubated in the dark for 1 h. High-speed videos were captured on a BX41 microscope (Olympus) equipped with a Retiga Exi Fast CCD camera (QImaging) with StreamPix 3 software (Norpix). Long-term videos were captured on an LSM 510 Meta (Zeiss) with the aperture fully open.

ACKNOWLEDGMENTS. The authors thank E. Dufresne, R. Boltyanskiy, and C. Osuji for performing the rheology experiments and N. Charon for useful discussions. This research was supported by National Institutes of Health Grant R01GM072004 (to C.W.W.); National Institutes of Health Grant R01AI085798 and the Jockers Award (to L.K.B.); National Institutes of Health Grants R01AI029735 and 3R01AI029735-20S1 (to J.D.R. and M.J.C.); and National Institutes of Health Grants R01AI26756 (to J.D.R.), R03AI085248 (to M.J.C.), and U54AI057159 (to S.M.D.-E.).

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