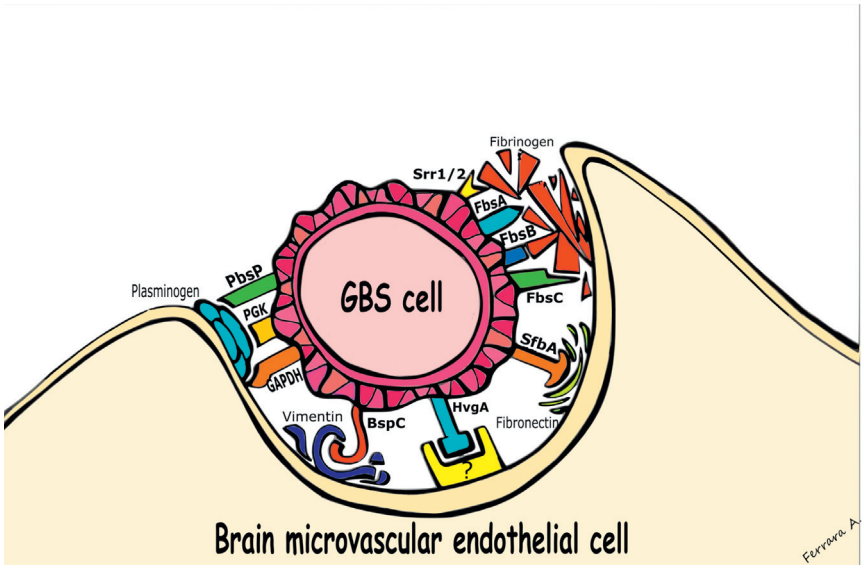

Figure 1. Interactions between Receptors on Brain Endothelial Cells and Cell-Wall Adhesins of *Streptococcus agalactiae* (GBS). Abbreviations: BspC, group B *Streptococcus* surface protein C; FbsA, FbsA, FbsC, fibrinogen-binding surface protein A, B, C; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HvgA, hypervirulent GBS adhesin; PbsP, plasminogen-binding surface protein; PGK, phosphoglycerate kinase; SfbA, streptococcal fibronectin-binding protein A; Srr1/2, serine-rich repeat proteins 1 and 2.

under certain circumstances to immune signaling, resulting in activation of NF-κB and the production of neutrophils attracting chemokines CXCL-1 and interleukin (IL)-8. This is an intriguing observation that deserves further investigation, particularly in view of the ability of vimentin to activate the ERK kinase pathway, as shown in a previous study using *E. coli* [8]. The authors note that proinflammatory cytokine/chemokine levels are lower in the brains of mice infected with a GBS *bspC* deletion mutant, but the interpretation of these data is difficult since they might simply reflect the lower bacterial load in the organs. Similarly, the slightly lower chemokine levels observed in BMEC cultures stimulated with the *bspC* deletion mutant might reflect the lower number of cell-associated bacteria. Also, the doses of recombinant BspC used to stimulate cells *in vitro* is rather high (10–20 mg), and further investigations are warranted to ascertain whether these concentrations are attainable in the proximity to immune cells *in vivo*. Despite these limitations, the new data raise important questions on the role of vimentin in innate immune responses against GBS.

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Forum

Fluidic Force Microscopy Captures Amyloid Bonds between Microbial Cells

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Fluidic force microscopy (FluidFM) is a recent force-controlled pipette technology that enables manipulation of single cells. FluidFM can be used for quantification of forces between single cells, and a novel mode of cell–cell adhesion was uncovered: amyloid-like interactions that mediate homophilic adhesion in the fungal pathogen *Candida albicans*.

Single-Cell Manipulation of Pathogens

Single-cell microbiology is a fast-growing research field. It uses single-cell manipulation tools for studying the behavior of individual cells to unravel cellular properties and interactions in an unprecedented manner. The past decade has witnessed exciting progress in the use of atomic force microscopy (AFM) for single-cell

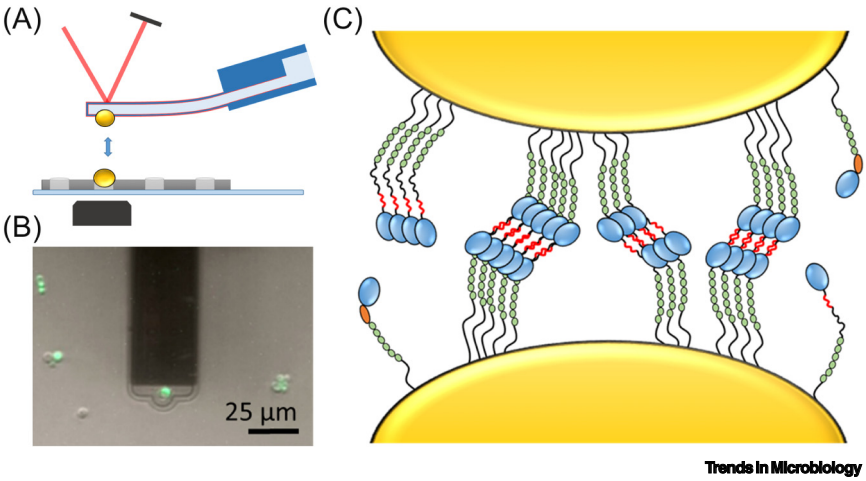

Figure1. Fluidic Force Microscopy: A Single-Cell Manipulation Tool for Studying Cell–Cell Adhesion in Microbiology. (A) A single yeast cell is immobilized on the cantilever aperture by applying a pressure difference. Polymer coating (in red) is used to prevent contamination of the cantilever. The cell probe is moved towards a target cell immobilized in a porous membrane, and forces between interacting cells are measured. (B)Labeling of the attached cell (in green) demonstrates that cell integrity is preserved, and thus that the method is nondestructive. (C)Proposed mechanism of amyloid-like homophilic adhesion in *Candida albicans*. Force-induced unfolding of Als5 proteins leads to the exposure of hidden amyloid sequences, which trigger the lateral assembly (*cis* interactions) of the proteins on the cell surface. Strong homophilic adhesion (*trans* interactions) between adhesins on opposing cells is mediated by amyloid-like bonds. Green, orange, and blue colors represent the folded TR, T, and Ig regions, respectively. Shown in red is the amyloid sequence of the force-induced unfolded T region. Adapted, with permission, from [11].

microbiology [1]. Specifically, AFM has enabled us to understand how pathogens use their surface proteins to guide cell adhesion and biofilm formation. Although powerful, classical AFM-based adhesion assays are time-consuming, which limits their use, especially for screening applications. In classical single-cell force measurements, the generation of a statistically significant number of measurements is hampered by the irreversible (chemical) immobilization of individual cells onto AFM probes. Hence, there is much interest in increasing the throughput of AFM-based single-cell manipulation.

FluidFM

FluidFM offers quick and reversible (physical) immobilization of cells onto the opening of microchanneled cantilevers via underpressure exerted by means of a pressure controller [2–4]. Thus, the hollow AFM cantilever becomes a force-controlled and force-recording pipette that can be operated in liquid under optical control [5], allowing a broad spectrum of applications, beyond adhesion measurements like single-cell injection and extraction [4,6]. Single-cell force microscopy by FluidFM was previously used to quantify the forces with which yeast and mammalian cells attach to abiotic surfaces to examine the impact of surface characteristics on adhesion [2]. In addition, the technology has been adapted to measure bacterial adhesion using pyramidal tips [3], and more recently using functionalized colloids to probe the adhesion of bacteria to environmentally relevant surfaces [7].

Unraveling Amyloid Bonds between Fungal Cells

The opportunistic fungal pathogen *C. albicans* is an emergent problem, with mortality rates as high as 40% in patients with systemic infections. The organism readily forms persistent drug-tolerant biofilms, including in abscesses and on implanted medical devices [8–10]. Biofilm formation is initiated by adhesion of *C. albicans* cells to a biological or artificial surface, followed by aggregation of fungal cells. These processes involve fungal cell surface Als (agglutinin-like sequence) proteins, which are made of an N terminal immunoglobulin (Ig)-like region, followed by a threonine-rich region (T), a tandem repeat (TR) region and a stalk region projecting the molecule away from the cell surface. Strikingly, the T region contains a short amyloid-forming core sequence which enables Als proteins to form functional amyloids that are critical for cell adhesion and biofilm formation [9]. Amyloids are protein aggregates composed of ordered β-sheet structures resulting from the interaction of identical amino acid sequences in multiple molecules. Fungal surface amyloids are of clinical importance because they occur during colonization of human tissues and can reduce macrophage-dependent inflammatory reactions. Earlier studies have shown that, under mechanical stress, structural changes in the adhesins lead to the exposure of hidden amyloid sequences which favor *cis* β-sheet interactions between Als proteins laterally arranged on the cell surface. This leads to the formation of nanodomains that promote cell aggregation.

In a recent study, Dehullu *et al.* [11] identified a previously undescribed adhesive function for Als proteins, that is, mediating *trans* β-sheet interactions between neighboring cells. FluidFM was key to this finding, and enabled noninvasive manipulation of single live cells to quantify cell–cell adhesion forces at increased throughput (~20 cell pairs per day). The authors first improved existing FluidFM protocols for the reliable and serial measurement of forces between yeast cells (Figure 1A,B). Following Potthoff *et al.* [2], coating the FluidFM cantilever with polylysine grafted polyethylene glycol improved quick attachment and detachment of single probe cells by reducing undesired cell

aggregation, thus increasing the throughput of the analyses. Target cells were noninvasively immobilized on microporous polymer membranes.

Using this methodology the authors measured the forces in cell–cell adhesion, focusing on Als5, the most widely investigated Als protein. Strong cell–cell adhesion involved the protein's small amyloid core sequence, and required expression on both interacting cells at high surface density. Intercellular forces were strongly inhibited by a short antiamyloid peptide. Forces were much weaker between cells expressing amyloid-forming Als5 and cells with a nonamyloid form of Als5, revealing that homophilic adhesion between proteins on adhering cells is the major mode of cell aggregation, rather than protein–ligand binding. Collectively, these observations favor a mechanism whereby amyloid-like β-sheet interactions play a dual role in cell–cell adhesion (Figure 1C), that is, in the formation of adhesin nanoclusters (*cis*-interactions) and in homophilic bonding between amyloid sequences on opposing cells (*trans*-interactions). Amyloid-forming sequences are found in many microbial proteins [12]. It is therefore possible that this mechanism of amyloid-based homophilic adhesion might be widespread.

Concluding Remarks

In summary, FluidFM is a powerful platform for quantifying interactive forces in single cells, at increased throughput and without the need for chemical fixation. Cell–cell experiments directly address the molecular interactions of functional adhesins in their native cellular environment. The technique enabled discovery of a novel function for *C. albicans* Als proteins, that is, driving homophilic cell–cell adhesion through amyloid bonds. In the future, FluidFM may contribute to the identification of small peptide inhibitors for antiadhesion therapy.

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