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New Adhesion Mechanism in *Giardia*: Role of the Ventrolateral Flange in the Attachment of Trophozoites

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The protozoan parasite *Giardia*, an intestinal flagellate, has evolved an unique attachment organelle called the ventral adhesive disk (VAD). This attachment organelle mediates attachment of the trophozoite to the microvillous border (MVB) of intestinal absorptive cells, and production of lesions via attachment are thought to lead to diarrheal disorder characteristic of giardiasis [1]. The VAD has contractile proteins arranged around it's circumference and it has been suggested that contraction of this area might function like a purse-string suture. The resemblance of the VAD to a suction cup has led to the hypothesis that suction or a negative pressure produced under the VAD by a grasping action might produce the adhesive force regulating attachment..

In real-time interference reflexion video microscopy, the ventrolateral flange, a cytoplasmic rim located around the periphery of the VAD, has been observed to form transient interactions with the substratum that dynamically change from focal to close contacts in seconds or less. To test the hypothesis that the VLF may mediate attachment separate from the VAD, altered topographical substrates consisting of arrays of columnar pillars were created through photolithography and micromolding of polystyrene in a polysilicone mold [2]. Gi*ardia lamblia* were grown overnight at 37°C, and cells washed in Hank's balanced saline before attachment for 5 minutes to polystyrene chips containing arrays of columnar pillars. Distance (edge-edge separation) between arrays of pillars (1 μm high, 2 μm wide) ranged from 1 to 5 microns, a distance that would enable the pillar columns to prevent direct attachment of the VAD (~5 μm diameter) to the substratum. Cells were aldehyde fixed, postfixed in osmium, and critical point dried. Attachment of trophozoites to polystyrene substrates was evaluated using a Hitachi S-4700 field emission SEM operated at 2 keV..

Giardia trophozoites showed a high degree of adherence to topographically flat surface of the polystyrene chips (Figure 1) and attachment appeared to be mediated by the VAD. Trophozoite attachment to topographical arrays of pillars was greatly reduced, but was not eliminated. Examination of trophozoites adhering to pillar arrays revealed attachment of the VLF to the tops of the pillars (Figure 2). Because pillars spacing prevented "suction-like" attachment of the VAD, adhesion appeared to be mediated by interaction of the VLF with the pillar surface. This new mechanism of adhesion may provide new insights on transient interactions of trophozoites with the MVB.

1. S.L. Erlandsen and D.E. Feely *Giardia* and Giardiasis, Plenum Press, New York, 1984.
2. A.P. Russo et al., J. Biomed Microdevices 4 (2002) 277.

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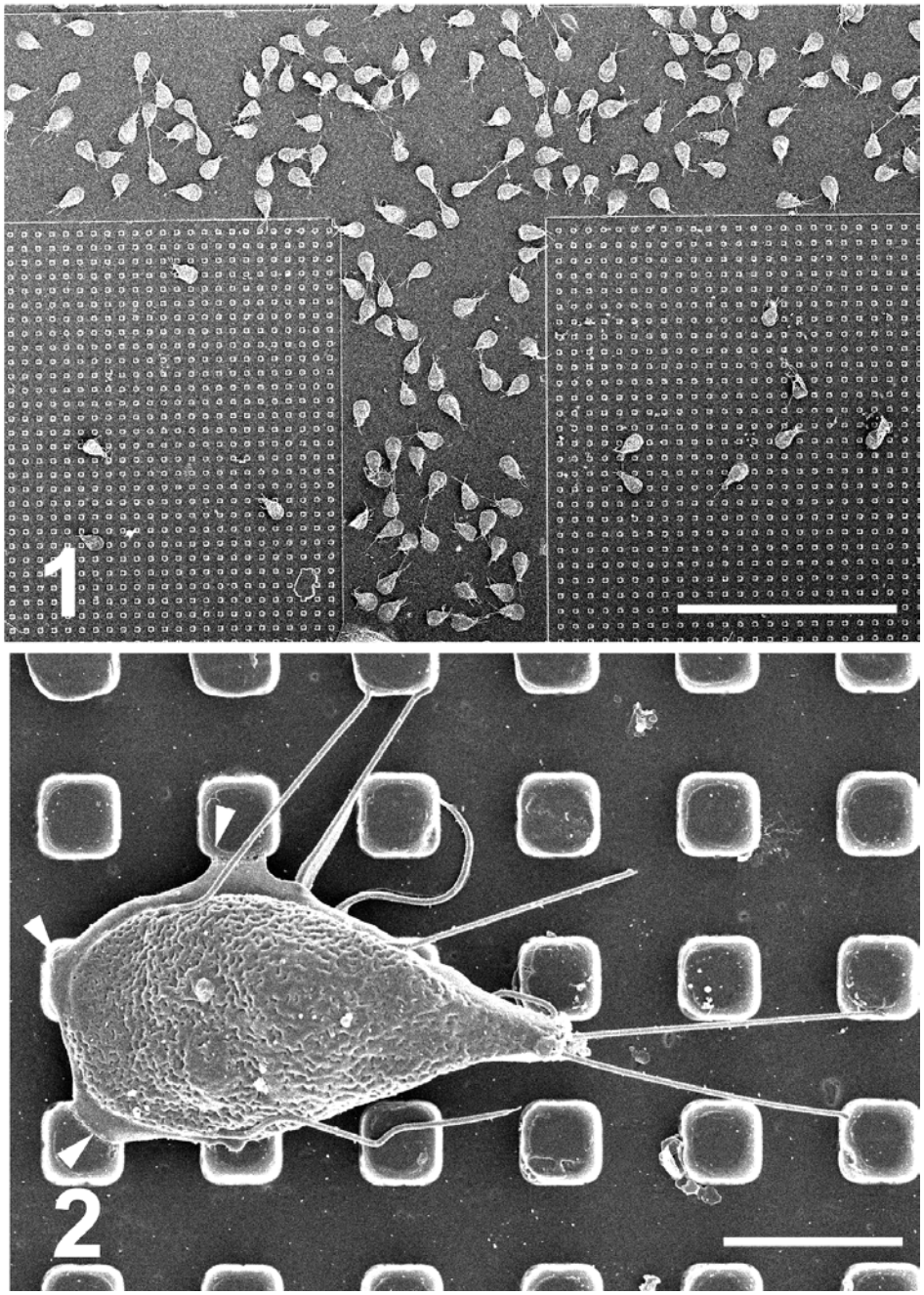

Figure 1. SEM of polystyrene chip showing pattern of adhesion of *G. lamblia* trophozoites. Attachment of trophozoites occurs in greater number on flat topographical surface than on arrays of pillars. Magnification bar, 100 µm.
Figure 2. SEM of individual *G. lamblia* trophozoite adhering to surface of pillar arrays. Observe interaction of VLF (arrowheads) with the tops of pillars. Interpillar spacing is 2 µm, which prevents the VAD from reaching the surface. Magnification bar, 5 µm.

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