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Comment on "The interaction of cells and bacteria with surfaces structured at the nanometre scale"

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In a recent article in *Acta Biomaterialia* Anselme et al. [1] published an interesting, complete and exhaustive review of the interaction of cells and bacteria with nanostructured surfaces. In this review the authors referred to our article [2] and stated that the retention of bacteria in the grooves of the surfaces was probably due to dewetting phenomena, as the images were taken in air on dried substrates. From this affirmation the readers of the journal might infer that the structure of the aggregates and bacterial distribution are a consequence of capillary forces acting during sample drying. It is well known that dewetting forces have a strong influence on the distribution of colloids on surfaces [3] and some researchers have attempted to describe bacteria as colloidal systems [4]. However, this approach fails to explain a considerable number of cases [5]. In fact, results recently reported indicate that the adhesion of bacteria differs markedly from colloidal particles due to the presence of appendages that rearrange during adhesion until the bacteria are positioned in the energetically most favorable position [6].

Experiments carried out in liquid environments have demonstrated that capillary forces do not drive the organization of *Pseudomonas fluorescens* on gold substrates having sub-micrometer sized surface features consisting of a grid of ~550 nm wide rows separated by 650 nm and on average 120 nm deep [7]. Fig. 1 shows the organization of *P. fluorescens* on this substrate using two different experimental tools. The image in Fig. 1a was obtained by atomic force microscopy after drying the sample in air at 70% relative humidity, while Fig. 1b shows an epifluorescence image of substrates kept immersed in sterile water throughout the experiment [7]. It is evident that both images exhibit the same trapping and orientation and, consequently, the distribution of bacteria is not significantly influenced by dewetting processes. In addition, we have studied the aggregation of bacteria on microstructured surfaces with grooves wider than the diameter of the bacteria and found that dewetting forces do not drive cell aggregation [8]. Consequently, it should be emphasized that recent experimental results [7] show that: (a) the structure of the aggregates and bacterial size and distribution are not a consequence of capillary forces during sample drying; (b) bacterial behavior is very different from abiotic particles, such as colloid particles, due to their self-organization on the surface using flagella and pili and the production of extracellular polymeric material.

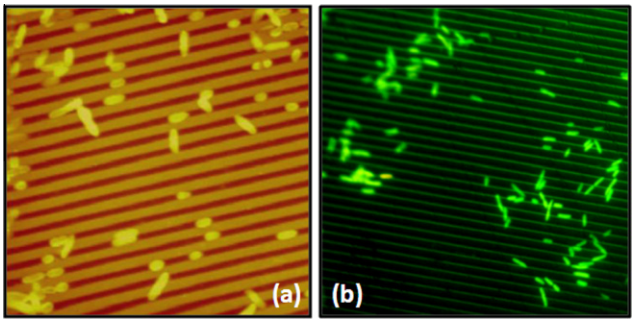

Fig. 1. Images of *P. fluorescens* attached to sub-microstructured gold surfaces. (a) AFM image (topographic contact mode, 25.2 × 25.2 μm) after drying the sample in air at 70% relative humidity. (b) Epifluorescence image (50 × 50 μm) after keeping the substrate immersed in sterile water throughout the experiment. Note that channels are dark in the AFM image and bright in the epifluorescence image. For the experimental conditions see supporting information in Diaz et al. [7]. Adapted with permission from Diaz et al. [7]. © Copyright 2010 American Chemical Society.

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