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# Supplementary Information

## Impact of confining 3-D polymer networks on dynamics of bacterial ingress and self-organisation

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## S1. Introduction

Studies of bacterial interactions occurring during motility, colonisation and culturing have been limited to surface-associated bacteria.[1] Agar, as a biopolymeric hydrogel consists of blocks of D-galactose and 3,6-anhydro-L-galactose and dominant culturing surface, together with other polysaccharides provide extremely hydrated surfaces that facilitate bacterial interaction, and motility. The material properties of such surfaces are well known to influence bacterial transport and colonisation, for instance, many bacteria swarm optimally over the very restricted range of agar concentration e.g. 0.5 – 0.7%.[2] The surface colonisation of bacteria has also been investigated with synthetic polymer hydrogels in terms of their modes of translocation and states aggregation and colony formation.[3] Such studies have examined, for example, oxidized poly(dimethylsiloxane),[4] polyacrylamide and poly(acrylic acid),[3] although as noted they have as yet not supplanted agar.

In general, the hydrodynamics of microorganism motion is frequently viewed as flow at low Reynolds number where at these sizes, inertia is unimportant and viscous forces are dominate.[5,6] In such a regime, hydrodynamic interactions, which may arise from cell – cell or cell – material interactions, play an important role bacterial surface colonisation. In this study, we explore the impact of these interactions within a defined synthetic polyelectrolyte hydrogel polyacrylic acid.

## S2. Experimental Methods

### S2.1 Synthesis of polyacrylic acid (K-PAA)

Poly(acrylic acid) was synthesized as the potassium form (K-PAA). Acrylic acid (4.76 mL) containing 180 – 200 ppm of monomethyl ether hydroquinone as inhibitor and methylene bisacrylamide (0.011 g) as the crosslinker were mixed at 85 °C under $N_2$ prior to the addition of ammonium persulphate initiator (0.042 g). The resultant product had a degree of neutralisation DN of 75% and a crosslinking degree of 0.066.

### S2.2 Modifications of polyacrylic acid

To obtain the protonated poly(acrylic acid) (H-PAA), polyacrylic acid was hydrogen exchanged with 10 mM HCl and washed until free of $K^+$, $H^+$, $Cl^-$.

Fluorescent nanoparticles were prepared as stabilized dye encapsulated silica nanoparticles according to Wang et al.[7] Ruthenium-tris(4,7-diphenyl-1,10-phenanthroline) dichloride was dissolved in dichloromethane with Pluronic F-127, dried and dispersed in 0.85 M HCl. Tetraethoxysilane was then added to the homogeneous solution and allowed to hydrolyse. Silica-particle growth was terminated by a drop-wise addition of dimethoxydimethylsilane (150 µL) and kept stirring for 24 h at room temperature. Unreacted components were then removed by dialysis with excess MilliQ water and filtered through a 0.2 µm Teflon filter. The product was then characterised by electron microscopy and fluorescent spectroscopy.

Fluorescent nanoparticles were incorporated in K-PAA hydrogel film by adding a nanoparticle dispersion to the final composition prior to thermal crosslinking. The homogeneous dispersion was then cast on a glass slide and maintained at 85 °C under a weak flow of nitrogen in dark condition for 1 hour yielding dry nanoparticle conjugated K-polyacrylate.

**S2.3 Nutrient and buffer solutions**

A 10 mM phosphate buffered saline (PBS) solution was prepared by dissolving 2.83 g $Na_2HPO_4$ and 1.36 g $KH_2PO_4$ in 1000 mL distilled water, and the pH value was adjusted to 7.2–7.4. The NB and PBS solutions were sterilized by autoclaving at 121 °C for 20 min.

**S2.4 Quantification of live-dead bacteria**

A mixture of SYTO® 9 and propidium iodide fluorescent dyes (Molecular Probes, Invitrogen, Grand Island, NY, USA). SYTO® 9 permeated both intact and damaged cell membranes, binding to nucleic acids and fluorescing green when excited by a 488 nm wavelength laser. On the other hand, propidium iodide only entered cells with significant membrane damage, binding with higher affinity to nucleic acids, fluorescing red and indicating non-viable cells.

**S. Supplementary Figures**

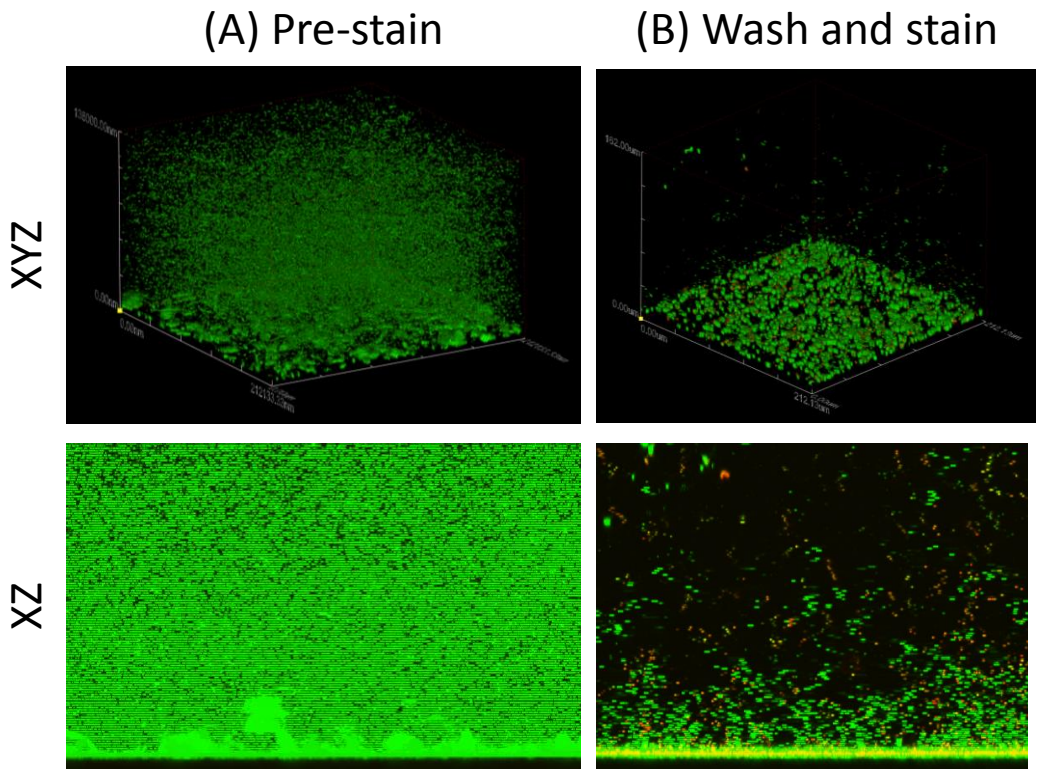


**Figure S1.** Comparison of two staining methods to visualise the ingress of *Pseudomonas fluorescens* into PAA pre-swollen with MilliQ $H_2O$ after 18 hours. (A) Prior to the incubation, *P. fluorescens* was prestained with Syto® 10 (indicating with green colour) then prestained bacteria were used to study its ingress into hydrogel, (B) The ingress of *P. fluorescens* was labelled according to Methods. It indicated that washing step is crucial to remove the non-attached bacteria.

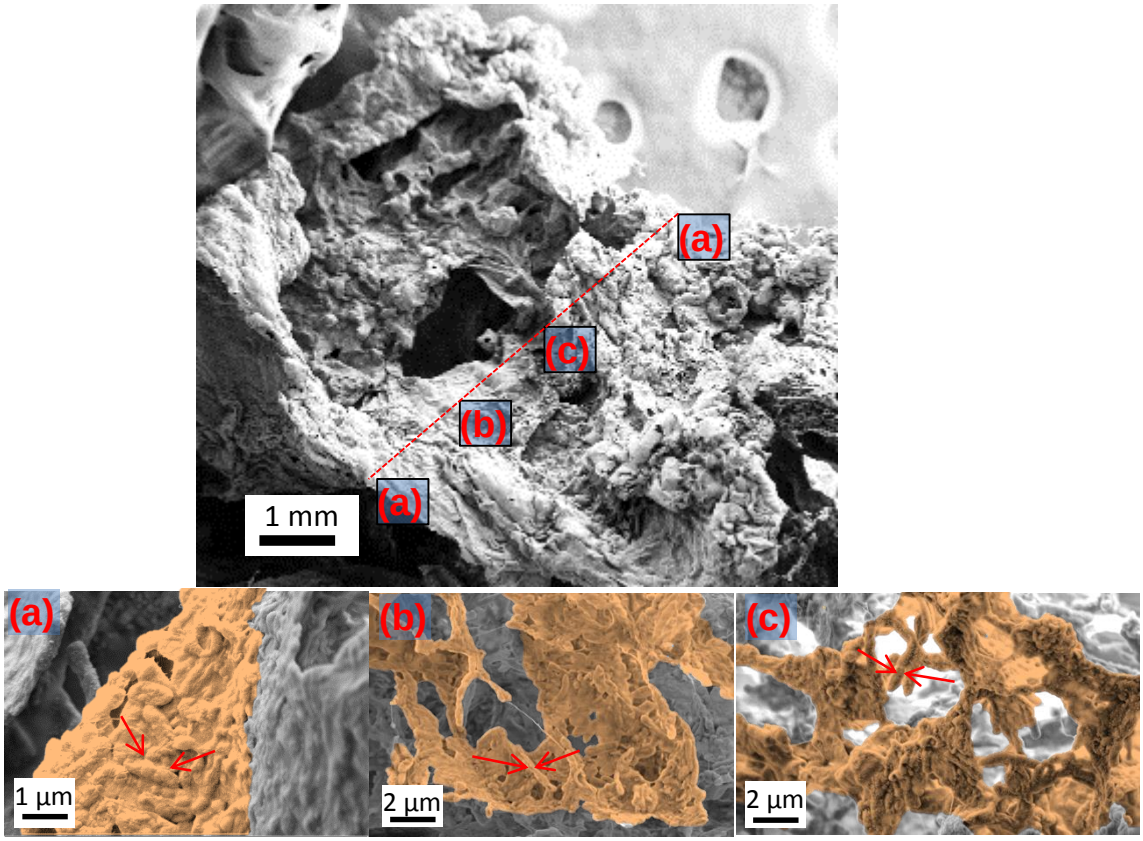


**Figure S2.** Scanning electron micrographs of *P. fluorescens* colonisation within K-PAA hydrogel pre-swollen with nutrient NB after 4 days.

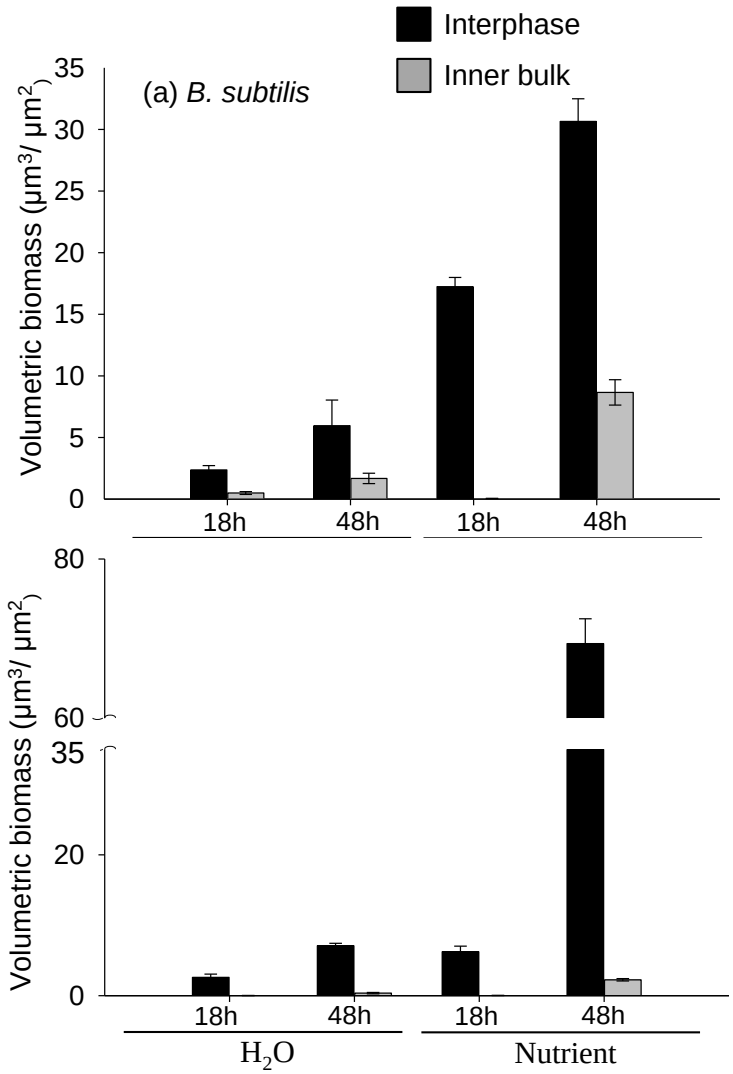


**Figure S3.** Total volumetric biomass of (a) *B. subtilis* and (b) *P. fluorescens* within (bio)interphase and inner bulk regions of swollen K-PAA after 18 h and 48 h.

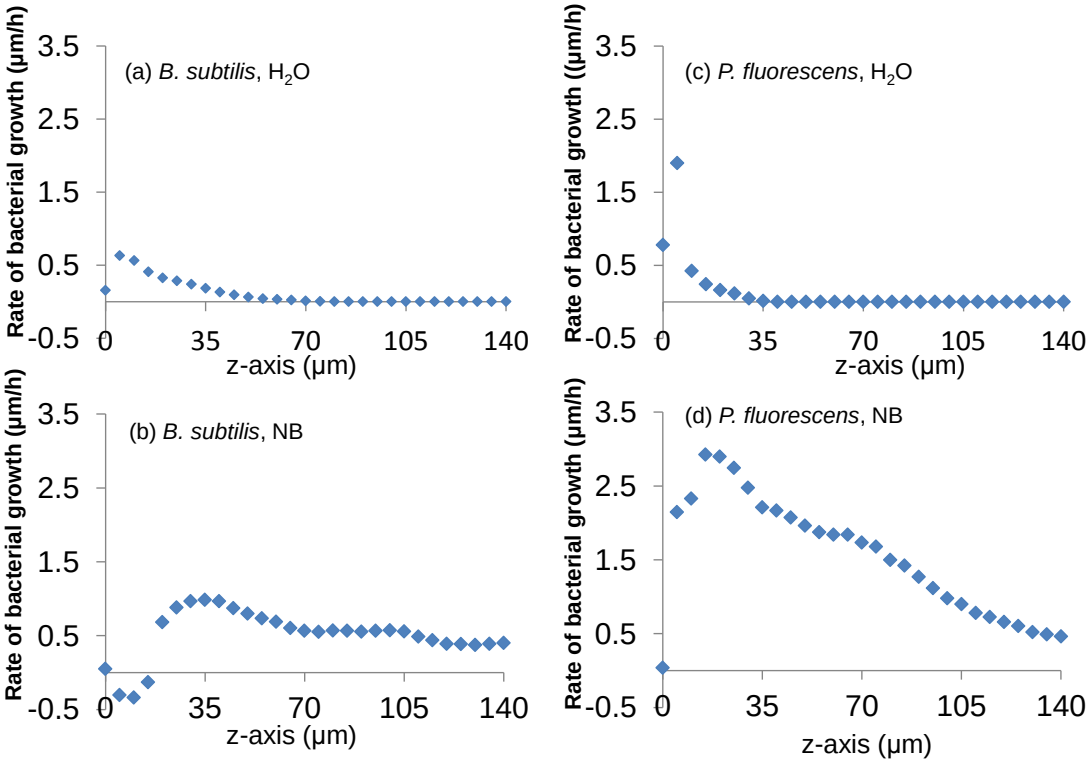


**Figure S4.** Rate of bacterial population, (a, b) *B. subtilis* and (c, d) *P. fluorescens* in swollen hydrogel preswollen with (a, c) $H_2O$ and (b, d) nutrient (NB) over the period of 30 h (18 – 48 h).

## Notes and references


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