Supplementary Information (SI) for Soft Matter.
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Supplementary Information for

Physico-chemical regulation of bacterial growth success under 3D confinement

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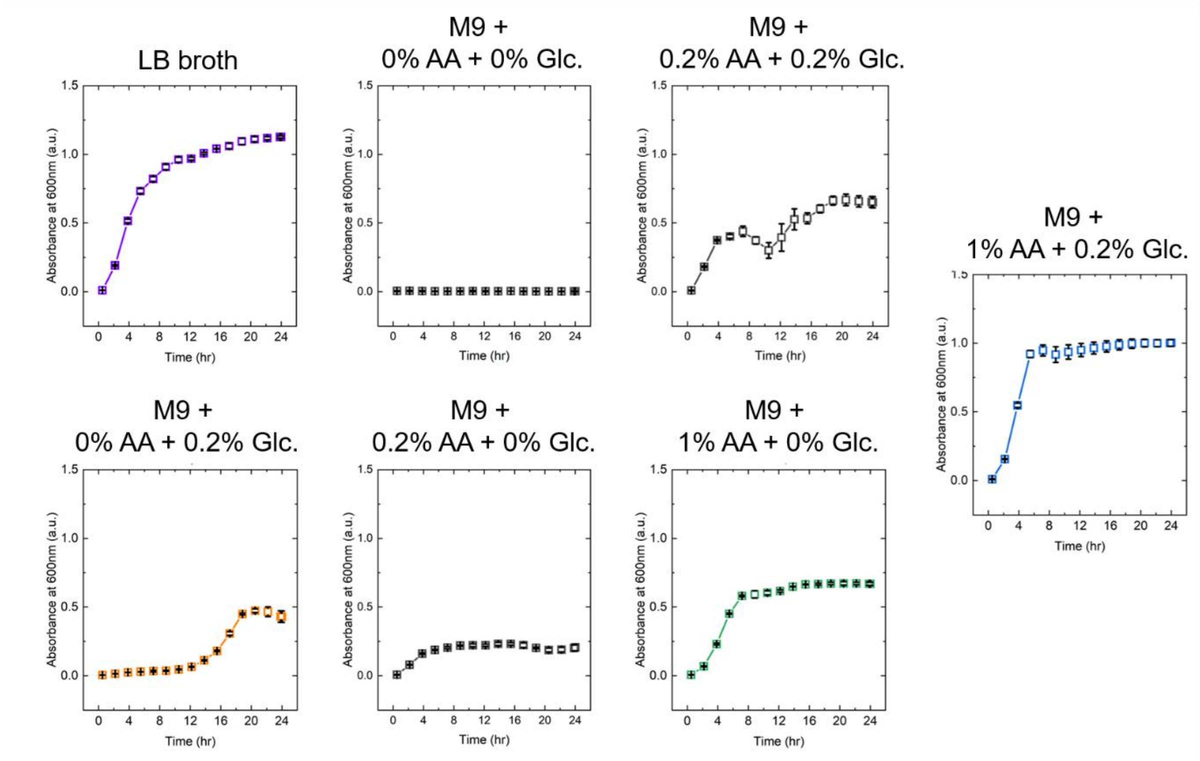
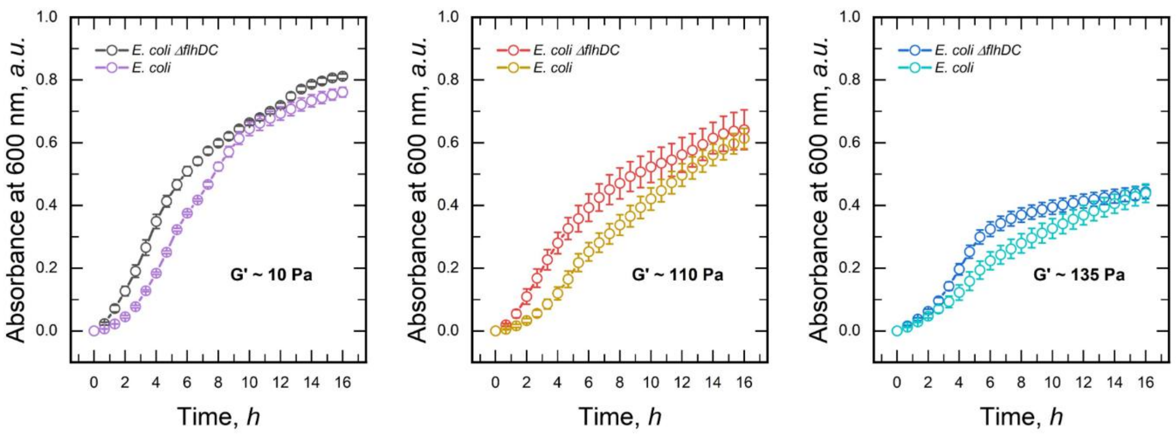
Material property	3D growth matrix	Mucosal samples	References
Stiffness (elastic modulus)	Low confinement, G’ ~ 10 Pa High confinement, G’ ~ 110 Pa	Pig small intestine mucus Soft, G’ ~ 10 Pa Stiff, G’ ~ 100 Pa	3D matrix[31,60,61]: Bhattacharjee & Datta, 2019b, 2019a; Sreepadmanabh et al., 2024 Mucosal samples[30,62,63]: Nordgård & Draget, 2015; Sardelli et al., 2019; Sellers et al., 1991
Porosity (nano-scale)	Polymeric mesh size ~40-100 nm	Nanometer-scale pores in human cervicovaginal mucus	3D matrix[31,60,61]: Bhattacharjee & Datta, 2019b, 2019a; Sreepadmanabh et al., 2024 Mucosal samples[64]: Lai et al., 2010
Porosity (micron-scale)	Interparticle pore spaces Low confinement (~5 microns) High confinement (~2 microns)	Micron-scale pores in pig jejunum, pig ileum, and human ileum	3D matrix[31,60,61]: Bhattacharjee & Datta, 2019b, 2019a; Sreepadmanabh et al., 2024 Mucosal samples[65]: Krupa et al., 2020
Charge density	Negatively charged polymeric network (Carbopol 980)	Negatively charged mucins	3D matrix[31,60,61]: Bhattacharjee & Datta, 2019b, 2019a; Sreepadmanabh et al., 2024 Mucosal samples[66,67]: Boegh & Nielsen, 2015; McShane et al., 2021

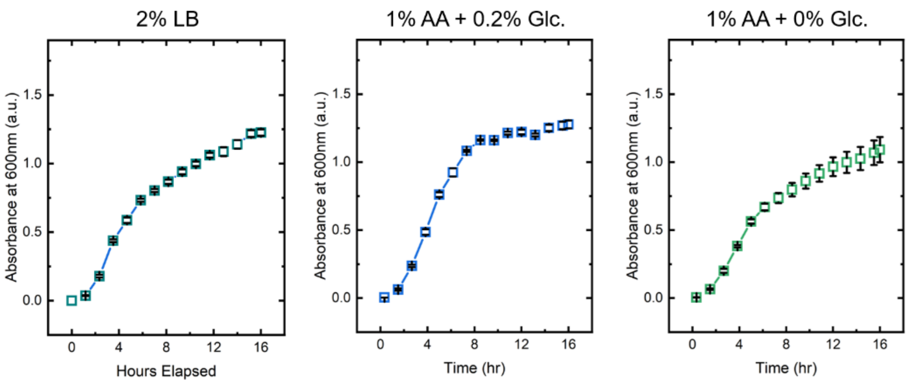
SI Table 1. Rheological properties comparing the 3D growth media against natural mucosal samples.



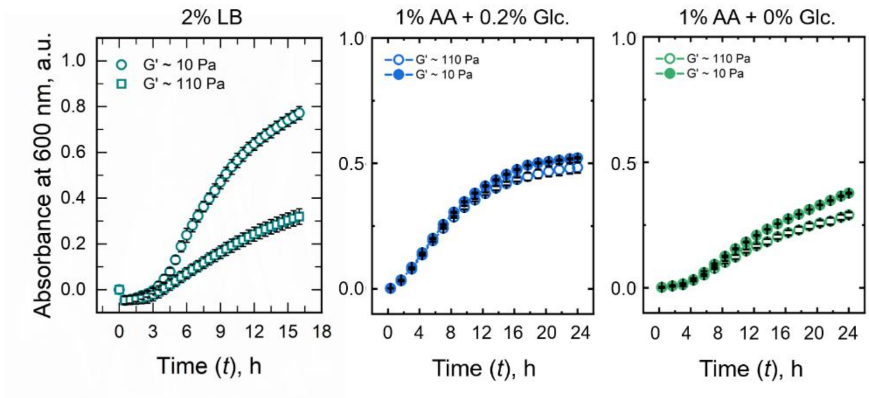
SI Fig. 1 Shaken liquid growth assays evaluating the growth of *E. coli* ΔflhDC across different nutrient conditions.



SI Fig. 2 Motility does not confer significantly greater fitness benefits under confinement, as tested using a motile and non-motile strain of *E. coli* (the latter carries a deletion in the flagellar protein-encoding gene flhDC), which show no significant differences between the strains across low, high, and very high degrees of confinement.



SI Fig. 3 Shaken liquid growth assays evaluating the growth of *E. faecalis* across different nutrient conditions.



SI Fig. 4 Growth assays performed using 3D matrices comparing the growth of *E. faecalis* between low and high degrees of physical confinement across different nutrient conditions.