Supplementary Information

Mucus-on-a-chip: Investigating the barrier properties of mucus with organic bioelectronics

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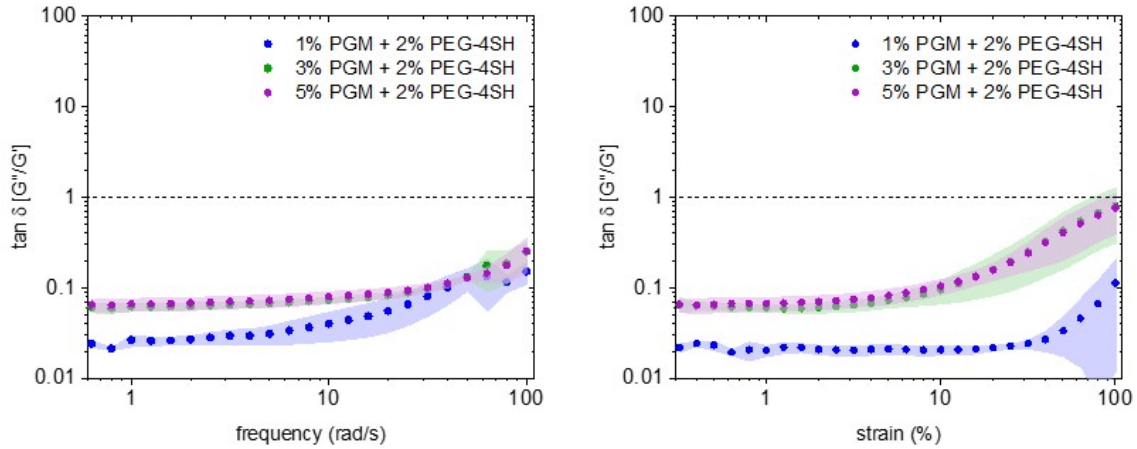
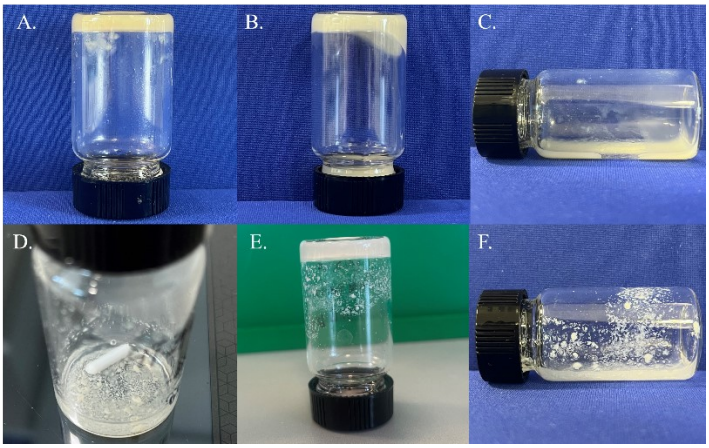
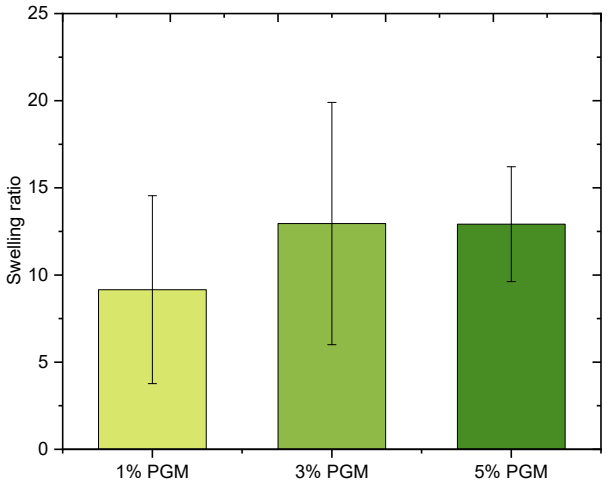


Supplementary Figure 1. Graph of loss factor, tan δ, defined as the ratio of G" to G', for frequency sweep and strain sweep. Graphs are supplementary to Figure 1C and 1D within the main text. Dominant elasticity is observed across the entire region investigate where the loss factor remains below the flow point, denoted by the dashed line at tan(δ) = 1 in both graphs.

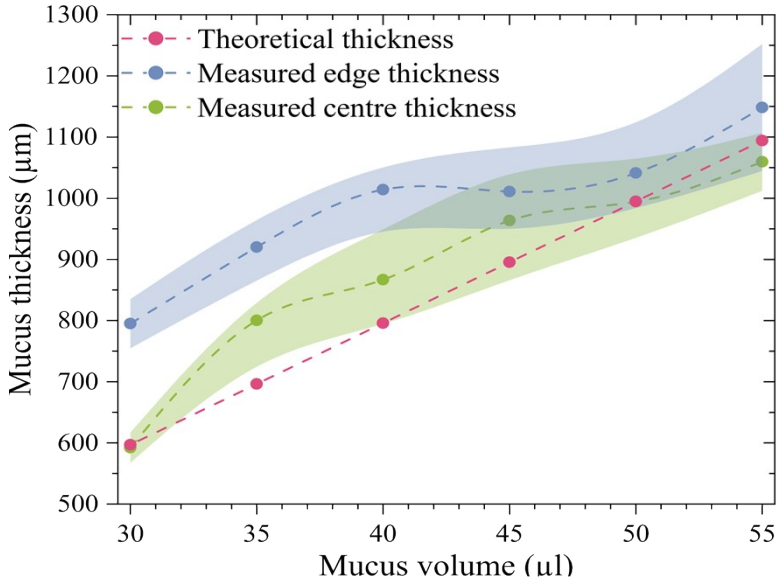


Supplementary Figure 2. A-C Images of semi-synthetic PGM-PEG-4SH mucus upon gelation, post 90 minutes NAC treatment and post 24 h NAC treatment (these first three images are provided in the main text Figure 3D but repeated here for ease of comparison). D-F Images of mucus extracted from HT-29-MTX cells upon addition of PEG-4SH showing before gelation, post gelation and 24 h post NAC treatment. Visible aggregates are observed in the HT29-MTX crosslinked mucus gel showing higher heterogeneity than the PGM equivalent.



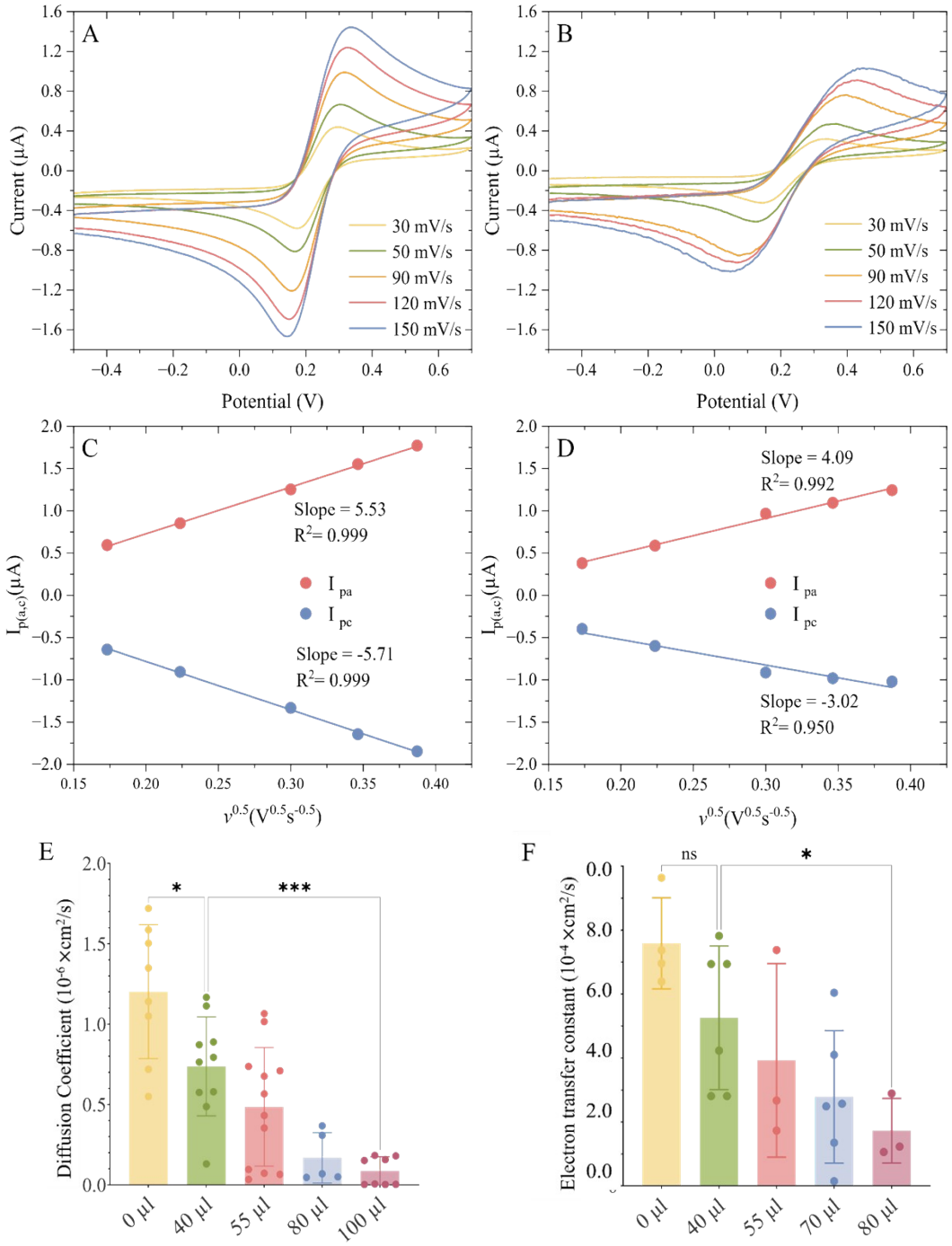
Supplementary Figure 3. Graph of swelling ratio for semi-synthetic mucus gels with varying PGM percentage. Error bars show standard deviation from n = 3 independently prepared gels at each PGM concentration investigated.

To determine swelling ratios, each samples were prepared in 2 mL quantities and allowed to gel overnight. 5 mL PBS was added to each sample and allowed to equilibrate overnight. Excess PBS was discarded and the wet samples weighed (M_w) before undergoing lyophilisation. The dried mass (M_d) was then measured. The swelling ratio was determined by calculating the relative mass increase due to water intake to the dry mass (swelling ratio = $(M_w - M_d) / M_d$).



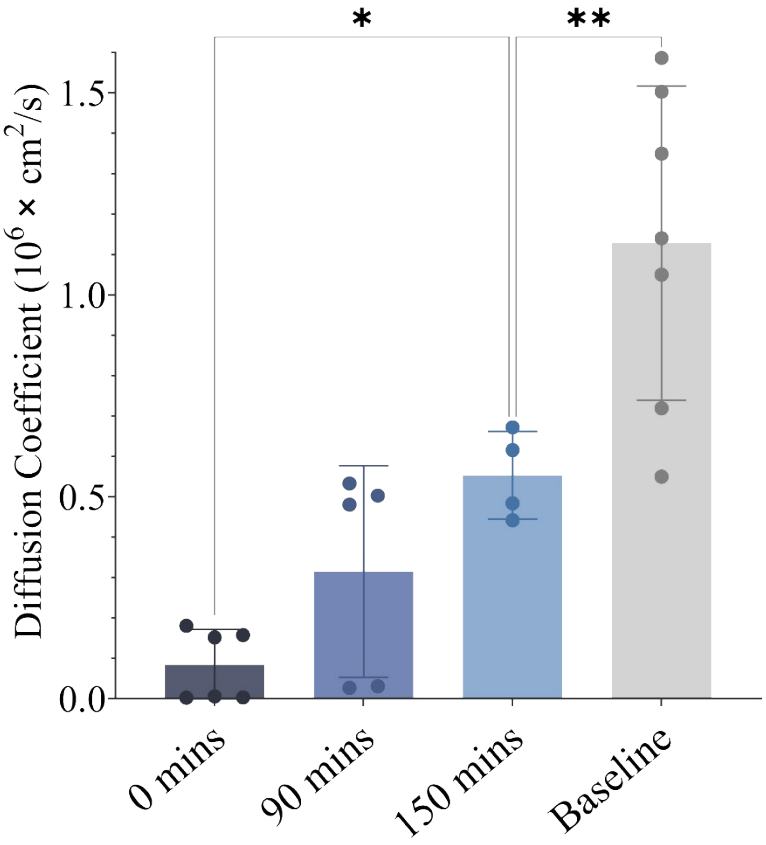
Supplementary Figure 4. Thickness of mucus corresponding to volumes of mucus added to the well atop the microelectrode arrays. Difference observed on outer electrodes vs inner electrodes due to wetting of the well wall by the mucus leading to a concave meniscus. Error

band denotes standard deviation.



Supplementary Figure 5. Cyclic voltammetry (scan rates 30, 50, 90, 120, and 150 mV/s) of 10 mM [Fe(CN)6]3-/4- with 0.1 M KCl, in phosphate-buffered saline carried out with A. 55 µL and B. 70 µL mucus, (5 % PGM + 2 % PEG) coated on the electrode surface. C. Anodic and cathodic current peak positions extracted from A as a function of the square root of the scan rates. D: Anodic and cathodic current peak positions extracted from B as a function of

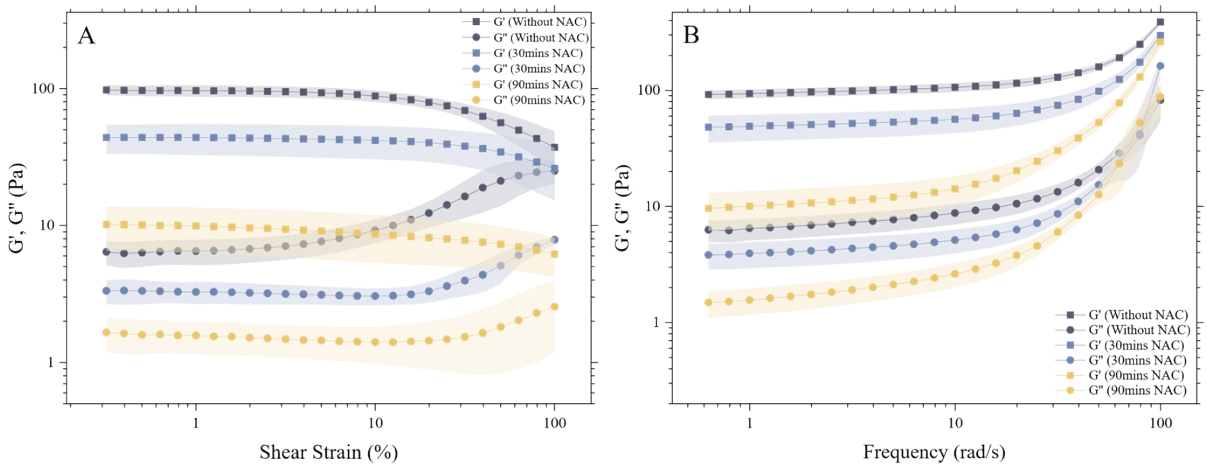
the square root of the scan rates. E and F: Diffusion coefficient and electron transfer rate constant of ferricyanide molecules through mucus layer computed from CV measurement.



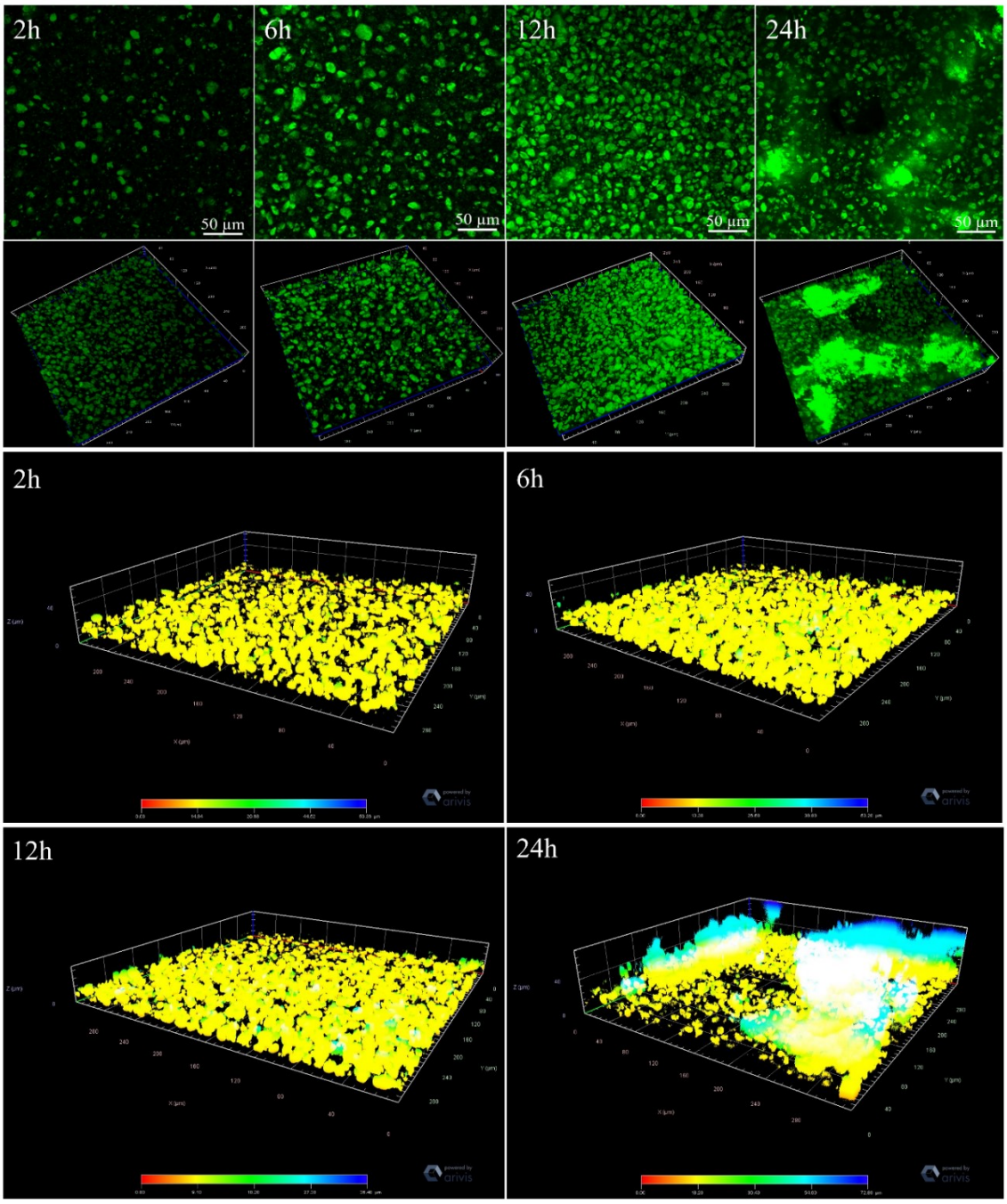
Supplementary Figure 6. Diffusion coefficient (10^6×cm^2/s) change after NAC treatment calculated from CV data using the Randles-Sevcik which describes the relationship between the scan rate (v) in CV to the peak current (I_p). which can be employed to calculate the diffusion coefficient of the reversible redox probe from a CV curve:

$$I_p = 0.4463nFAC^0(\frac{nF\nu D}{RT})^{0.5}$$

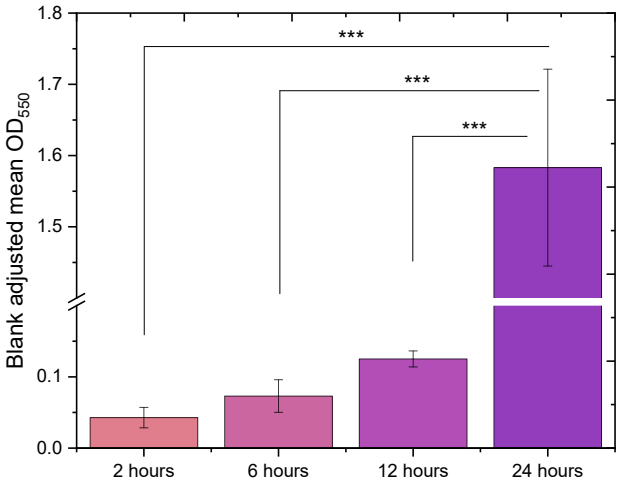
I_p= the cathodic or anodic peak current (μA)
n= number of electrons transferred in the redox event (n = 1 for ferricyanide ions)
F= Faraday's constant (96485.34 C/mol)
A= electrode surface area (cm^2)
C^0= bulk concentration of the redox probe species (mol/cm^3)
v= scan rate (V/s)
D= diffusion coefficient of $K_3[Fe(CN)_6]$ ions (cm^2/s)
R= ideal gas constant (8.314 J/(K·mol))
T= absolute temperature (K)



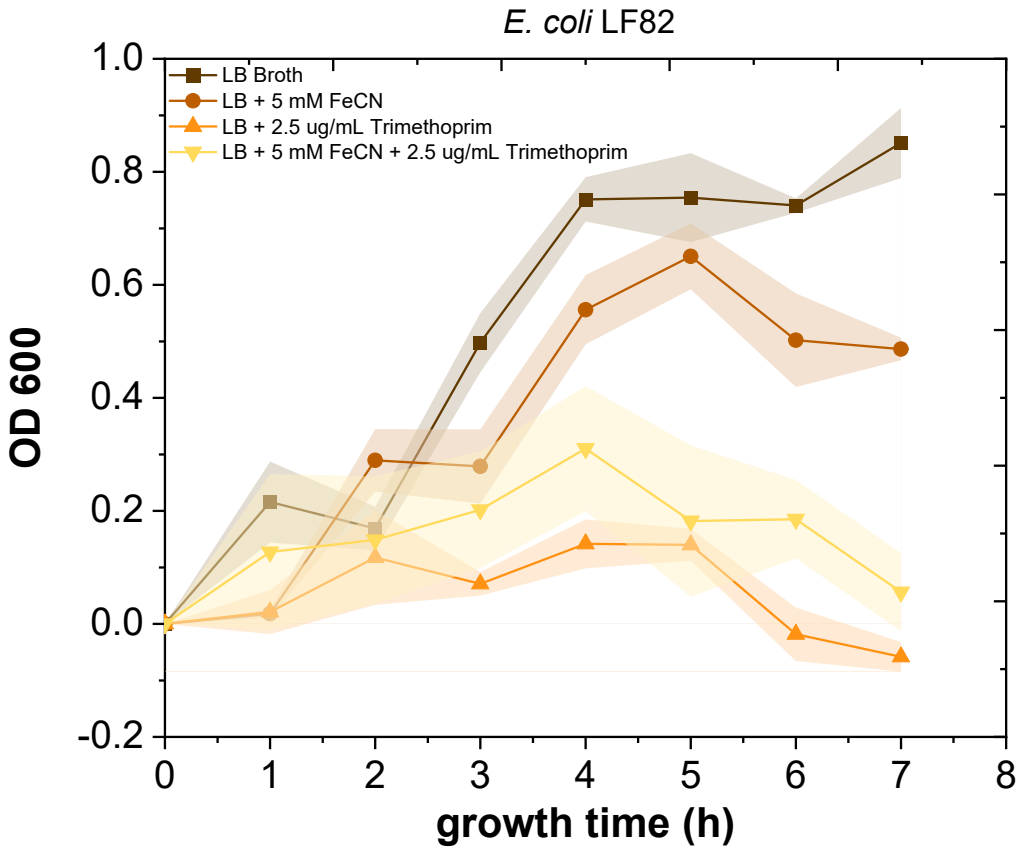
Supplementary Figure 7. A. Bulk rheological measurements of synthetic mucus with exposure to 50 mM N-acetyl-L-cysteine.. G' represents elastic modulus (squares) and G" represents viscous modulus (circles). B. Frequency sweep at strain=1% from 0.1 to 100 rad/s;



Supplementary Figure 8. Green fluorescent protein (GFP)-expressing *E. coli* BL21 grown to early log phase in LB broth. Added to mucus at 8 x 10^7 per well in LB and allowed to attach. Confocal imaging of the observed biofilm on mucus at 2h, 6h, 12h and 24. Imaging performed by confocal laser scanning microscopy on the ZEISS LSM 800 with the fluorophore as GFP on the ZEISS Zen Blue software. Scale bar is 50 µm. 3D depth coded images are provided for the 2,6,12,24 hour timepoints corresponding to the fluorescence z-stacks above.



Supplementary Figure 9. Crystal violet microtiter assay to determine LF82 *E coli* biofilm growth on the mucus mimic. This experiment was performed analogously to the bacterial incubation study shown in Figure 4 of the main text. Upon reaching the growth time investigated, bacterial suspension was removed and wells gently washed twice. Crystal violet staining and quantification was performed according to well established procedure[46]. Quantification by OD_{550} was performed on a plate reader (BMG Labtech CLARIOstar) and all samples were diluted equally so all wells could be quantified within the range of the detector. Data shown are from a minimum of three technical triplicates and from three biological replicates. *** = $p < 0.001$.



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107 **Supplementary Figure 10.** Optical density at 600 nm as a measure of *E. coli* LF82 growth. An

108 overnight culture of *E. coli* LF82 was grown in LB broth and used to inoculate fresh LB broth

109 at 1% (v/v). Samples were collected every hour and optical density measured for seven hours

110 (BMG Labtech CLARIOstar). Error band denotes standard deviation, N=3.

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