SUPPORTING INFORMATION Triggered Release of Bacteriophage K from Agarose/Hyaluronan Hydrogel Matrices by *Staphylococcus aureus* Virulence Factors

Jessica E. Bean[1], Diana R. Alves[1], Maisem Laabei[1], Patricia P. Esteban[2], Naing Tun Thet[1], Mark C. Enright[1], A. Toby A. Jenkins[1*].

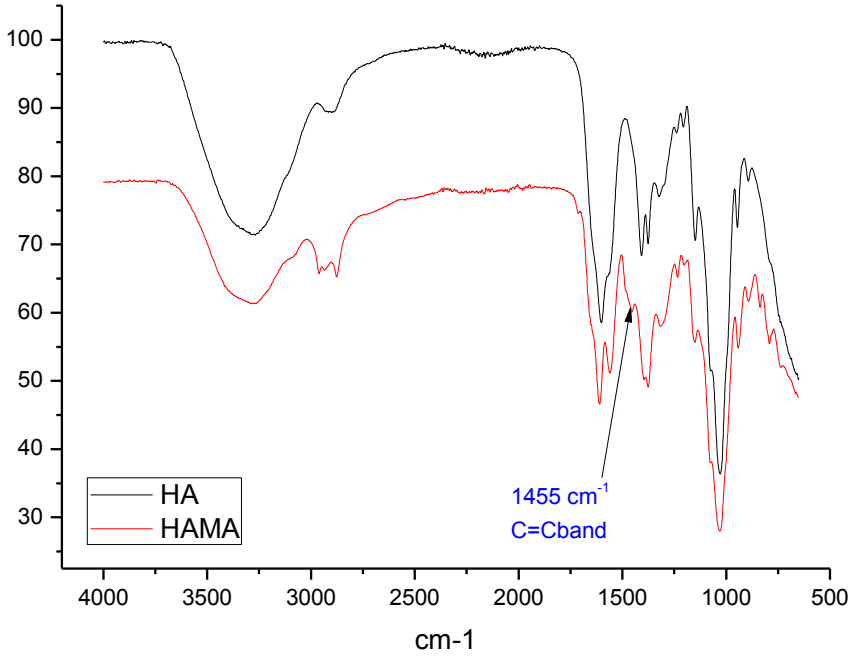


Figure S1, FTIR spectra of hyaluronic acid and hyaluronic acid methacrylate (HAMA), with appearance of peak at 1455 cm^{-1} giving evidence of methacrylation

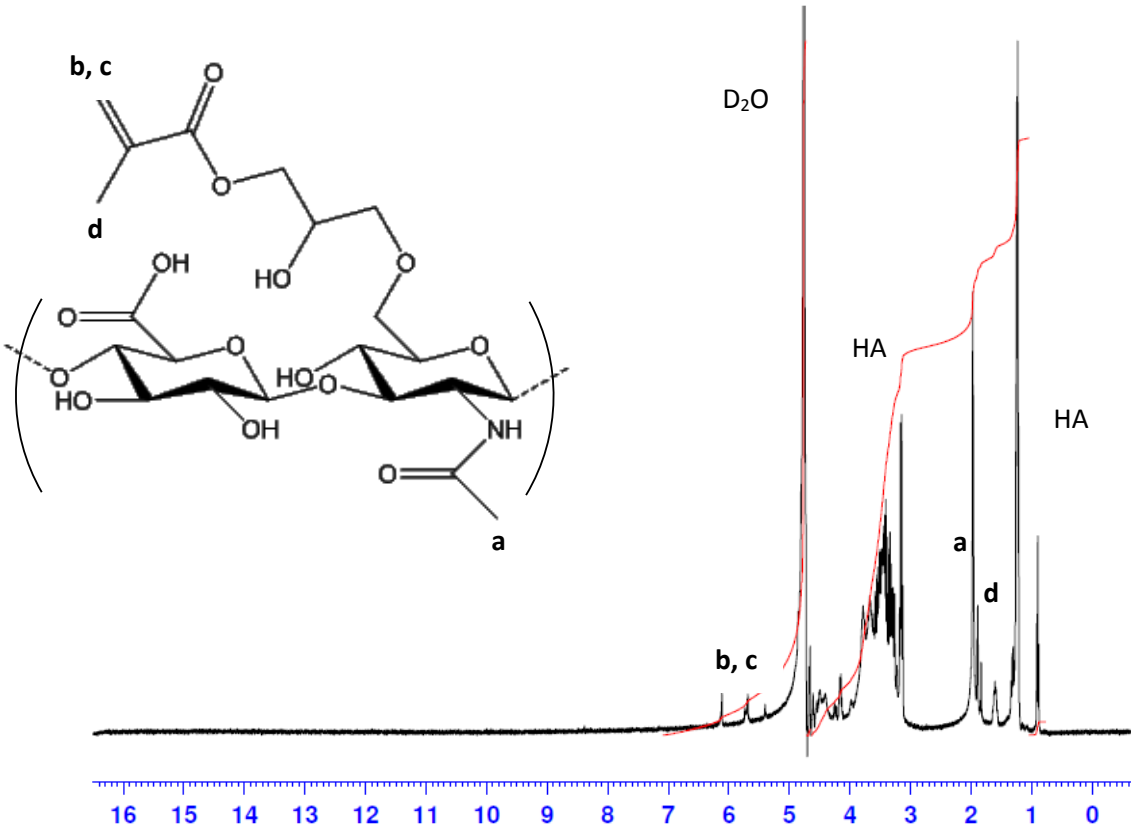


Figure S2 1H NMR spectrum of Hyaluronic Acid Methacrylate (HAMA). Resonances at 5.6 and 6.2 ppm verified the presence of methylene protons.

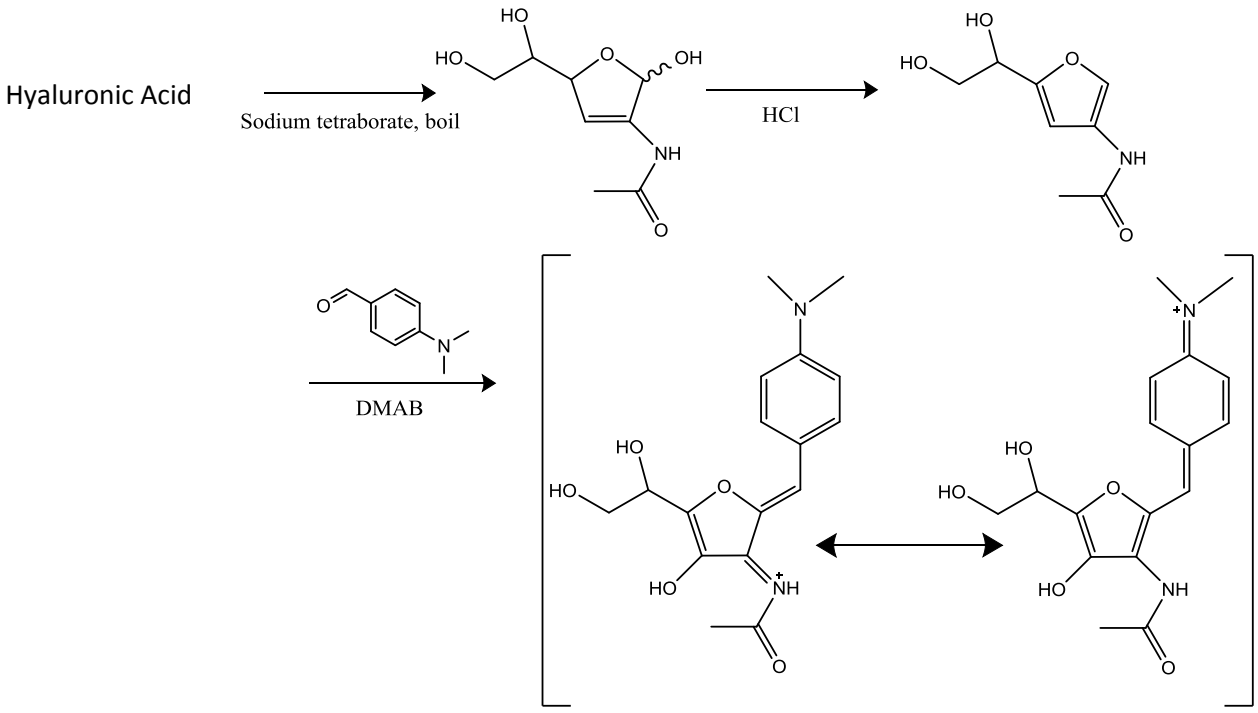
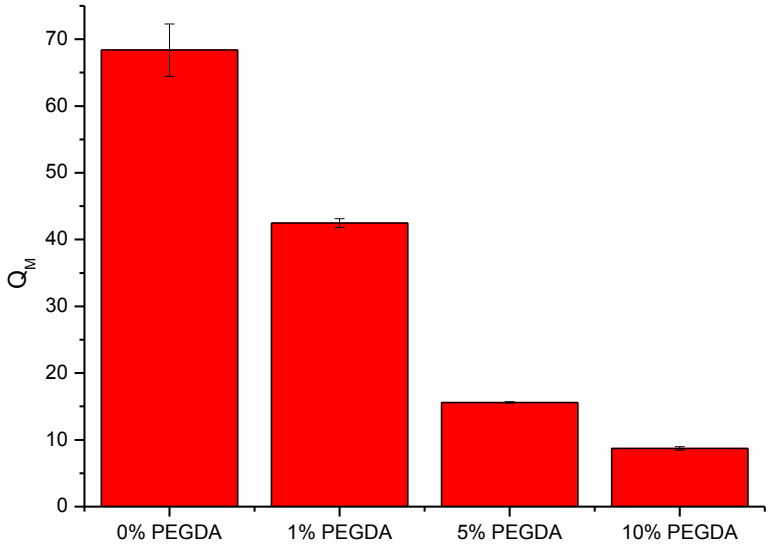


Figure S3 Carbazole assay reaction mechanism



	Swelling ratio, Q_M	Volumetric Swelling Ratio, Q_V	Florey Parameter, χ	Specific volume of dry polymer, $v(cm^3/g)$	Average molecular weight between XL, $M_c(g/mol)$	Effective Crosslink Density, $V_e(mol/cm^3)$	Mesh Size, ξ(nm)
0%PEGDA	68.4±3.9	83.8	0.473	0.575	7.45E+07	1.65E-08	6604
1%PEGDA	42.5±0.7	52.0	0.473	0.575	2.26E+07	5.45E-08	3099
5%PEGDA	15.6±0.1	19.0	0.473	0.575	1.81E+06	6.78E-07	627
10%PEGDA	8.7±0.3	10.5	0.473	0.575	4.13E+05	2.98E-06	246

Figure S4 Swelling ratio Q_M of HAMA-co-PEG hydrogels

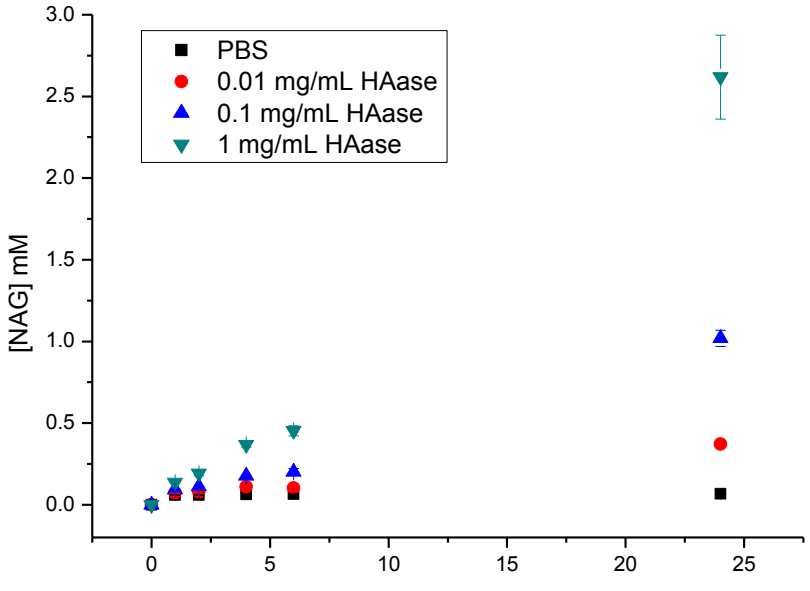


Figure S5 Quantitative analysis of HAase breakdown of 2% HAMA /1% PEGDA gels, measured via the release of NAG (measured using the Carbazole assay) as a function of incubation time and concentration of hyaluronidase.

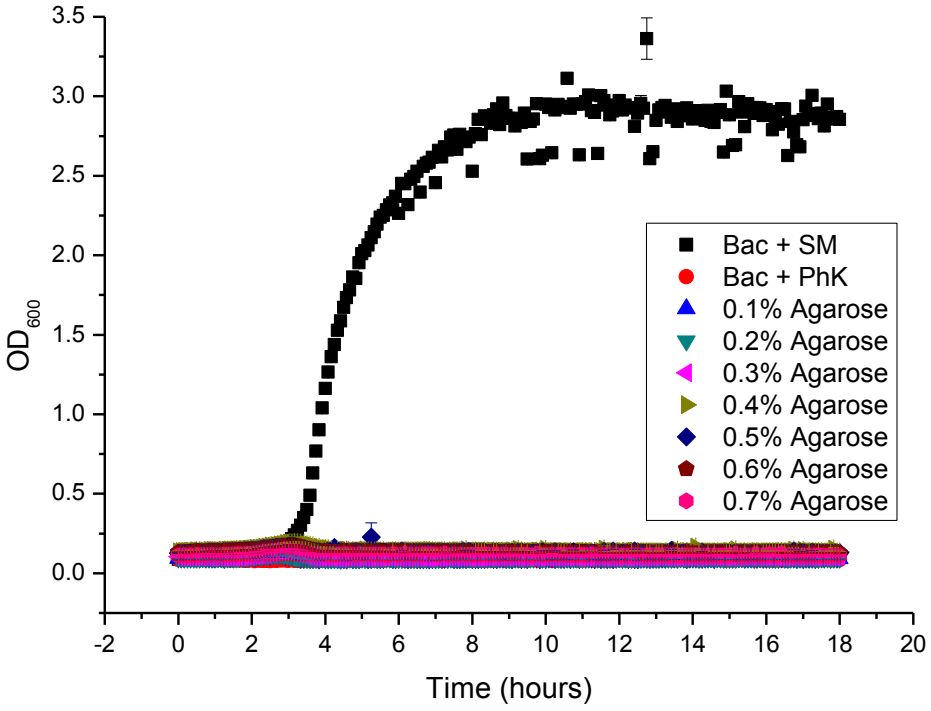


Figure S6 Phage K in agarose – killing of *S. aureus* H560: Complete suppression of growth / killing seen at all agarose concentrations.

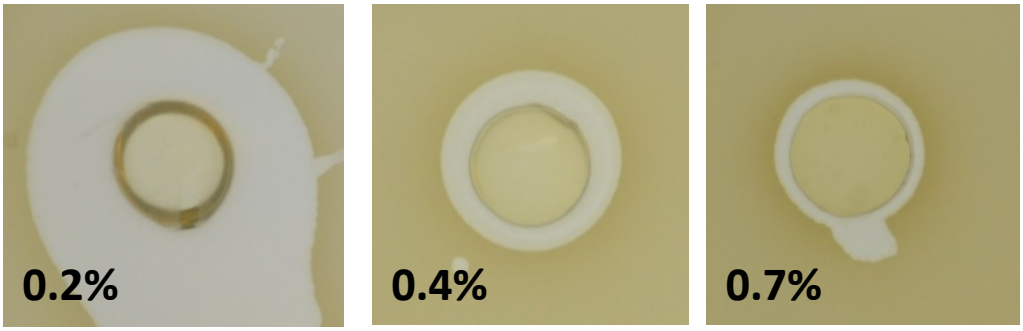


Figure S7 Bacteriophage K lysis of S. aureus H560 bacterial lawns on agar in 0.2%, 0.4% and 0.7% agarose

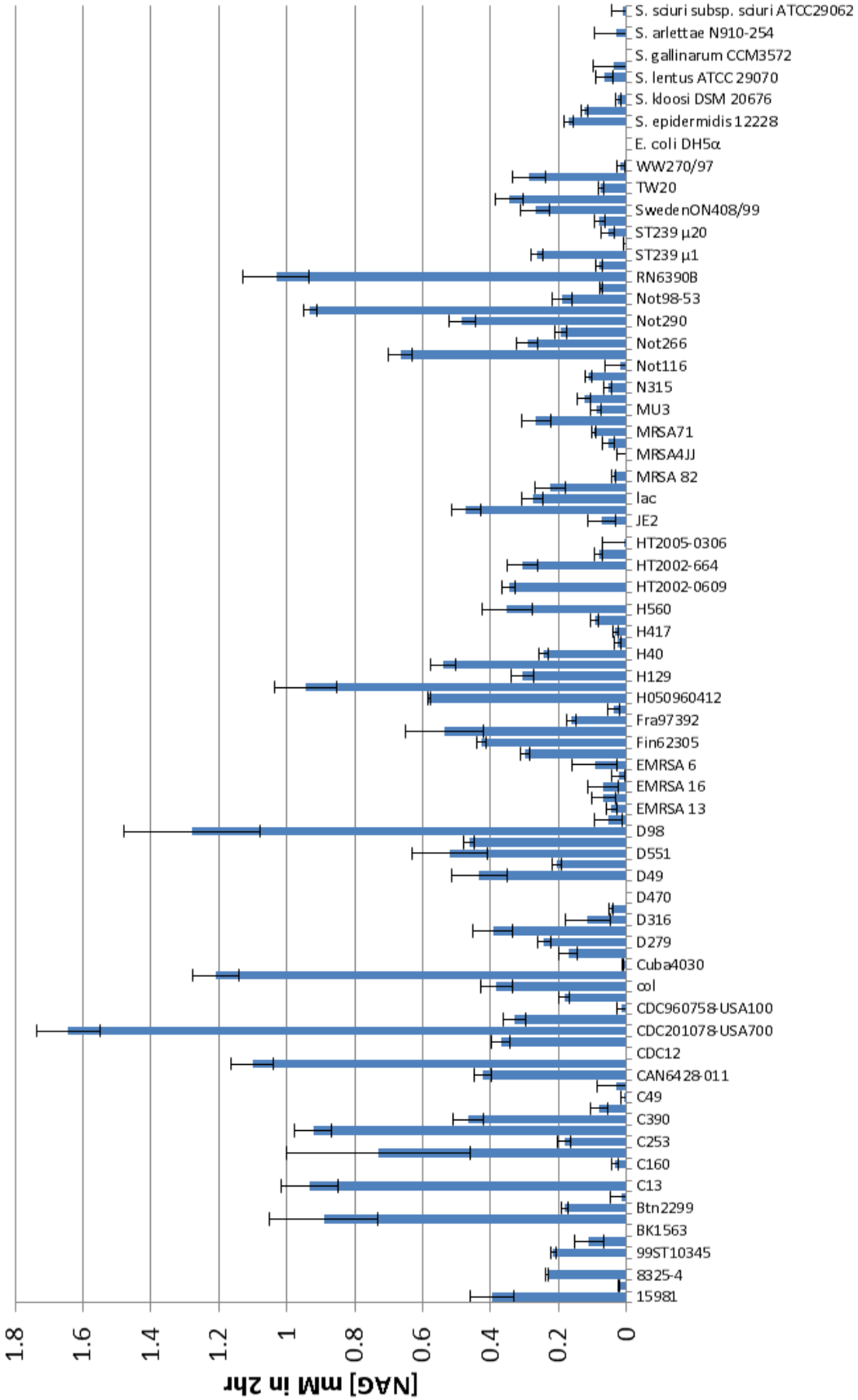


Figure S8 Screen of 116 *Staphylococcus sp.* and strains for HAase activity. Concentrations of NAG > 0.4 mM are considered very high, under 0.01 mM low and between these values intermediate, but sufficient to trigger phage release.

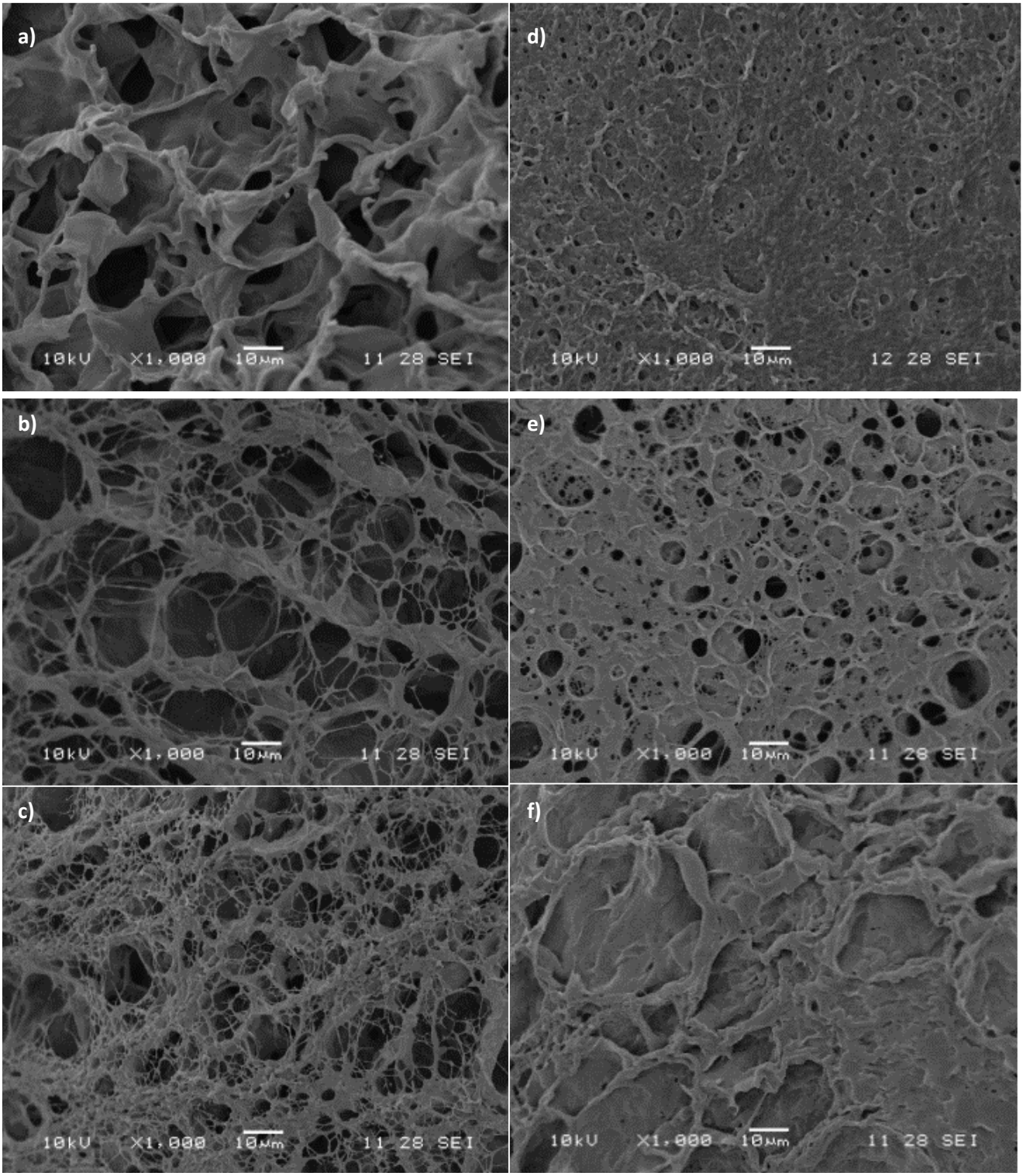


Figure S9 SEM images of HAMA hydrogels after 2h incubation with *S. aureus* supernatant. HAase positive strains: a) RN6390B, b) H560, c) lac. HAase negative strains: d) hys-, e) Mμ2, and f) TSB growth medium.