Phloroglucinol-enhanced whey protein isolate hydrogels for tissue engineering

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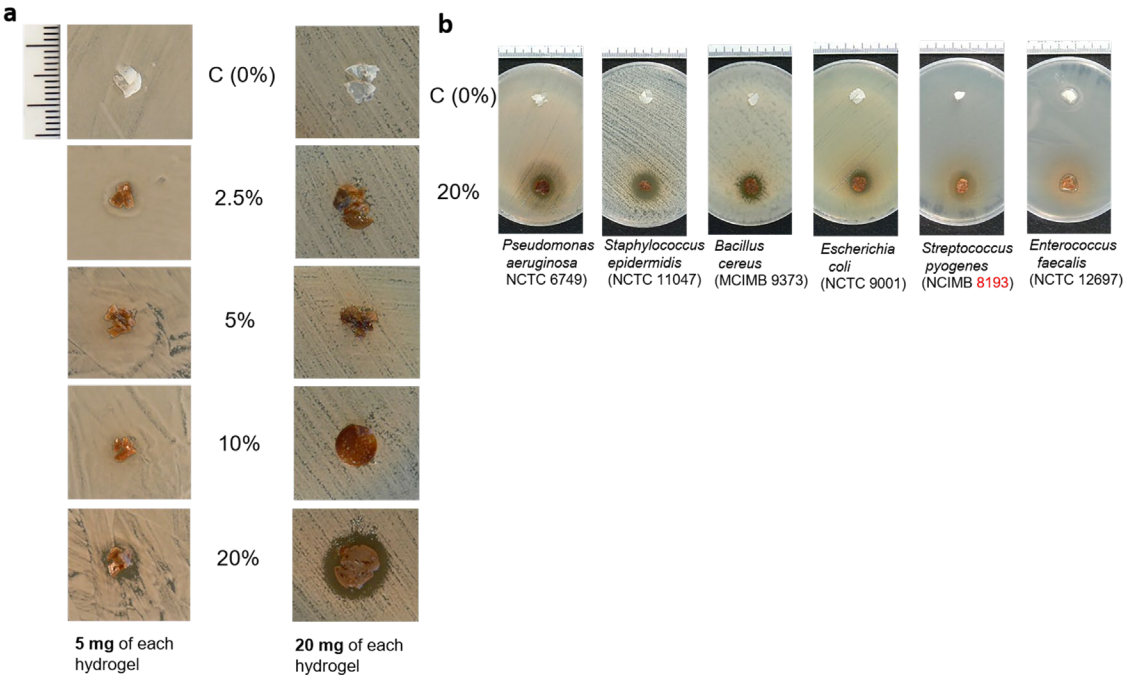
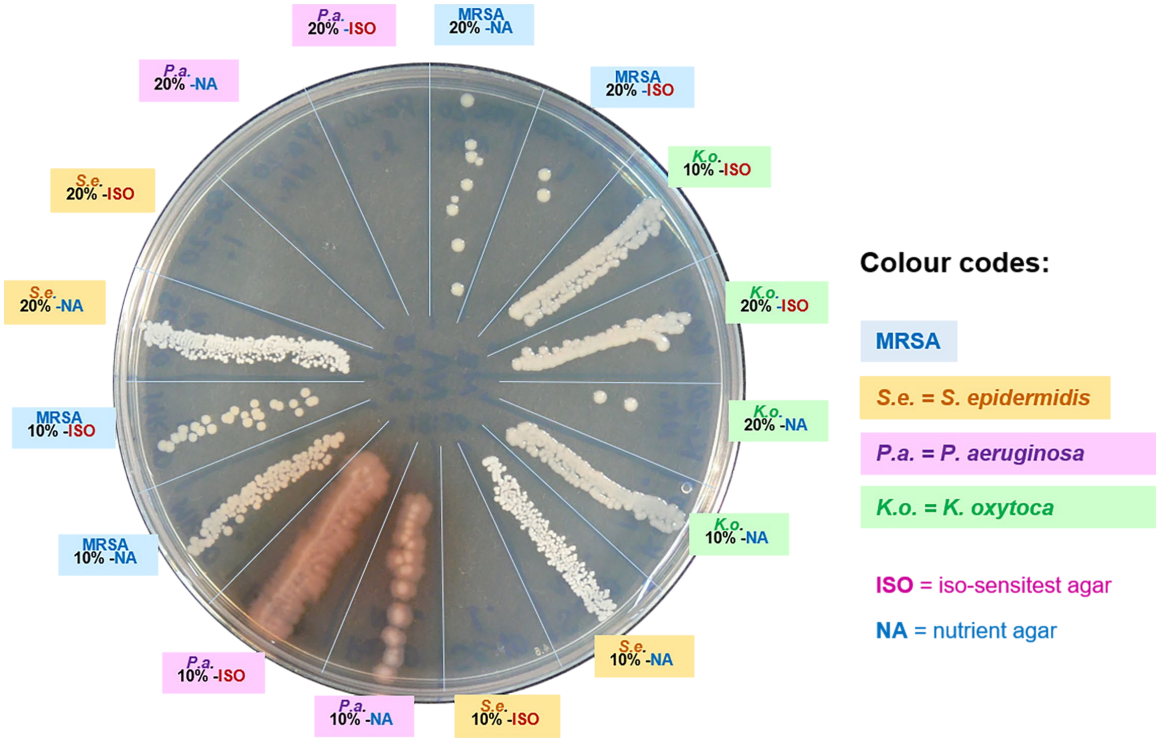
Supplementary

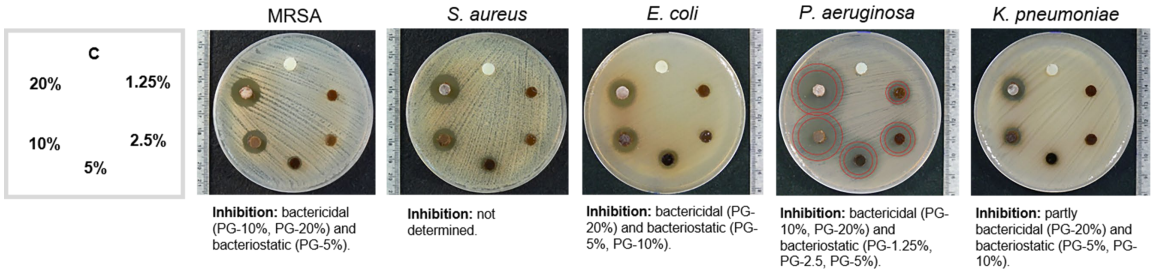


Supplementary Fig. 1: Testing of WPI-PG hydrogels. (**a**) Cuts of hydrogels with various concentrations of phloroglucinol (in total weight 5 mg and 20 mg for each hydrogel) were placed on the MRSA strain (NCTC 13552) swabbed onto ISO-sensitest agar. (**b**) Cuts of

hydrogels with 20% of phloroglucinol (in total weight 30 mg for each hydrogel) were placed on six bacterial strains swabbed onto ISO-sensitest agar. All plates were incubated overnight at 37 °C. The ruler with 1 mm scale is attached.



Supplementary Fig. 2: Testing modes of growth inhibition after exposure of bacteria to disc-shaped hydrogels containing 10% and 20% of phloroglucinol. After sensitivity tests on Iso-sensitest (ISO) and nutrient agar (NA), areas of zones of inhibition (ZOI) without bacterial growth were touched and streaked out onto sectors of nutrient agar plates and incubated overnight at 37 °C. The absence of growth indicates bactericidal inhibition; abundant growth results from bacteriostatic inhibition; reduced number of colonies implies combination of bactericidal and bacteriostatic effects.



PG	Average ZOI size (mm)	SD
5%	8.5	0.6
10%	13.2	0.7
20%	17.5	0.4

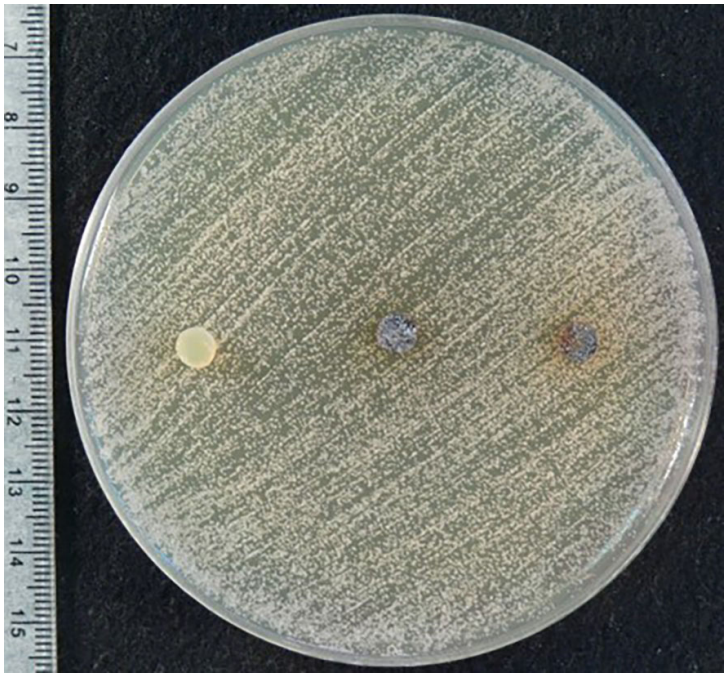
PG	Average ZOI size (mm)	SD
5%	8.2	0.7
10%	13.1	0.4
20%	16.9	0.6

PG	Average ZOI size (mm)	SD
5%	9.6	0.6
10%	13.8	0.4
20%	15.5	0.3

PG	Average ZOI size (mm)	SD
1.25%	9.8	0.8
2.5%	13.5	2.0
5%	17.5	0.6
10%	21.7	0.3
20%	22.7	1.3

PG	Average ZOI size (mm)	SD
5%	6.9	0.4
10%	10.0	0.8
20%	12.5	0.7

Supplementary Fig. 3: Quantification of dose-dependent inhibition of bacteria after exposure to disc-shaped WPI-PG hydrogels. The range of phloroglucinol concentrations was from 1.25% to 20%. Sensitivity testing was carried out on Iso-sensitest agar at 37 °C. Average size of ZOI and SD were calculated based on data from 4-6 measurements in replicated assays. ZOI for *P. aeruginosa* had internal areas of complete inhibition of growth (surrounded by internal circles) and areas of partial inhibition (external circles) where bacterial growth was observed at low densities. Only areas with complete growth inhibition were used for measuring ZOI.



Supplementary Fig. 4: WPI-PG-20% hydrogels do not affect the growth of *C. albicans*. On the left: a disc-shaped WPI hydrogel without phloroglucinol. The assay was carried out on YEPD agar (a medium for yeasts) at 30 °C for two days.