

Supplementary Information

Studies of release kinetics and antibacterial activity on pH-responsive core-shell microparticles loaded with lactoferrin

Teresa Paduano^{a,b,§}, Michela Salamone^{c,§}, Federica Carraturo^c, Simona Zuppolini^{a,}, Mauro Zarrelli^a, Aldobenedetto Zotti^a, Rosa Vitiello^b, Marika Avitabile^b, C. Valeria L. Giosafatto^b, Marco Guida^c, Riccardo Tesser^b and Anna Borriello^a*

^a Institute for Polymers, Composites and Biomaterials (IPCB)- National Research Council, I-80055 Portici, Italy

^b University of Naples Federico II, Department of Chemical Sciences, 80126, Naples, Italy

^c University of Naples Federico II, Department of Biology, 80126, Naples, Italy

[§] Equal contribution

*Correspondence: simona.zuppolini@cnr.it

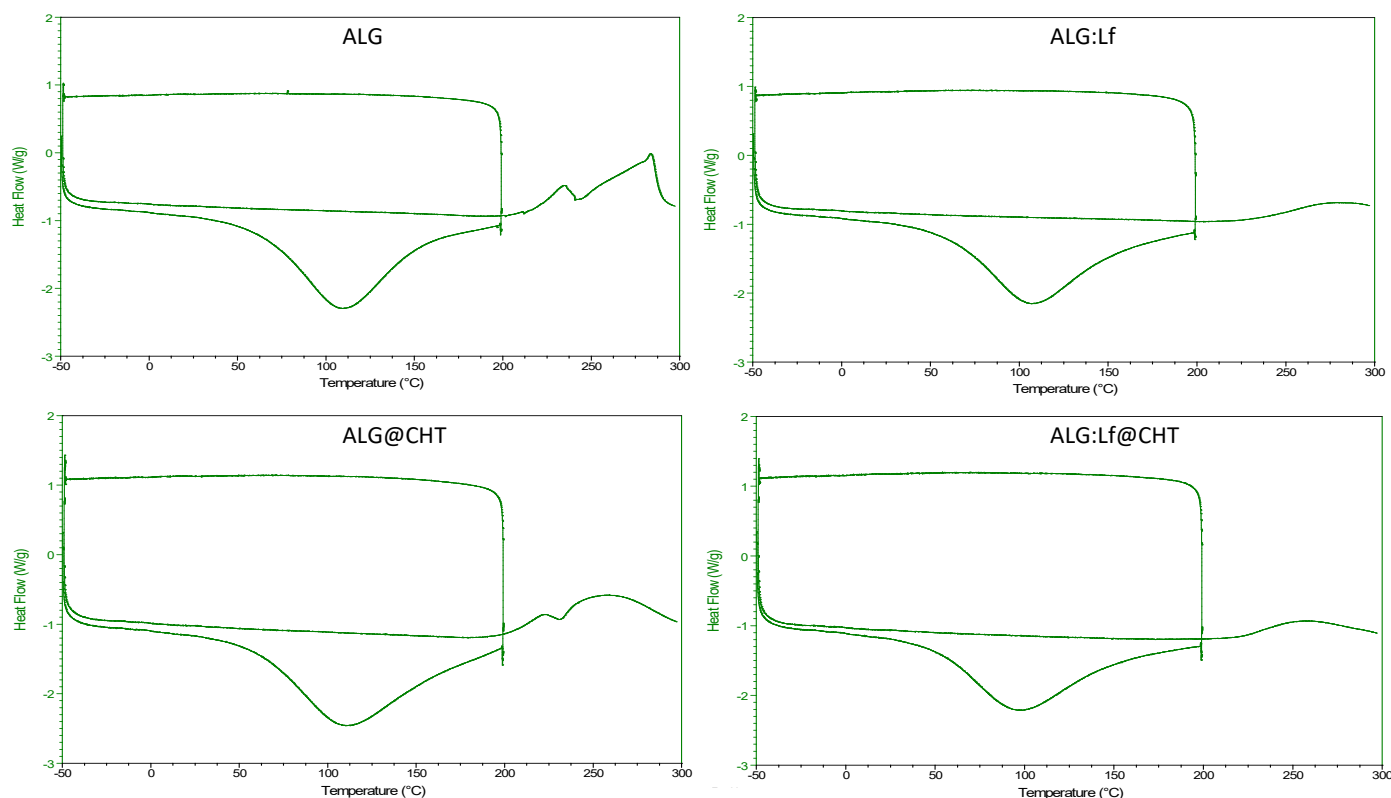


Figure S1. DSC thermograms of: (a) ALG, (b) ALG:Lf, (c) ALG@CHT, and (d) ALG:Lf@CHT formulations.

Table S1. *In vitro* Lf release timepoints from loaded core-shell ALG:Lf@CHT particles at neutral pH. Data are presented as mean concentration (C (%)) $\pm$ standard deviation (SD) of independent experiments performed in triplicate.

Time (min)	C(%)	SD $\pm$
0	0	0
1	3.9	3.6
2	3.9	2.2
5	4.7	1.8
10	4.9	2.2
20	4.6	1.8
30	5.3	3.4
60	5.6	3.7
120	6.8	4.2
180	9.2	4.8
240	9.5	3.6
1440	10.5	2.2
2880	12.1	1.8

Table S2. *In vitro* Lf release timepoints from loaded core-shell ALG:Lf@CHT particles at pH 5. Data are presented as mean concentration (C (%)) $\pm$ standard deviation (SD) of independent experiments performed in triplicate.

Time (min)	C(%)	SD $\pm$
0	0	0
1	7.3	3.3
2	9.6	3.2
5	23.9	2.2
10	89.6	1.9
12	91.3	1.4

Table S3. *In vitro* Lf release timepoints from loaded core-shell ALG:Lf@CHT particles at pH 8.5. Data are presented as mean concentration (C (%)) $\pm$ standard deviation (SD) of independent experiments performed in triplicate.

Time (min)	C(%)	SD $\pm$
0	0	0
1	6.9	1.3
2	7.1	1.1
5	8.9	0.9
10	9.2	2.1
15	11.5	0.7
25	13.0	1.2
35	20.6	0.8
60	43.4	1.4
100	97.7	2.1
120	98.0	0.9
180	98.1	1.8

Table S4. Microbial concentration and Die-off rate [%] of *S. aureus*^a

Viability assay at pH 5 on <i>S. aureus</i> ATCC® 6538 [10 ⁶ CFU/mL]			
Samples	t ₀	t ₁ (1h 5')	
	Microbial Concentration [CFU/mL]	Microbial Concentration [CFU/mL]	Die-off rate [%]
CN	1,25E+06 ± 7,42E+04	8,53E+05 ± 2,68E+04	31,97
CP	1,51E+06 ± 1,07E+04	1,37E+04 ± 1,37E+04****	99,10
ALG:Lf@CHT	1,15E+06 ± 2,54E+04	1,01E+06 ± 6,67E+03	12,41
ALG@CHT	1,23E+06 ± 3,00E+04	8,77E+05 ± 4,63E+04	28,67
Lf	8,29E+05 ± 2,93E+04	6,29E+05 ± 2,57E+04	24,20

^a*S. aureus* ATCC® 6538 was tested at pH 5 after 1 hour and 5 minutes with ALG:Lf@CHT and ALG@CHT at 0.5 mg/mL. Two-way ANOVA followed by Šidák's multiple comparisons test (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

Table S5. Microbial concentration and Die-off rate [%] of *E. coli*^a

Viability assay at pH 5 on <i>E. coli</i> ATCC® 25922 [10 ⁶ CFU/mL]			
Samples	t ₀	t ₁ (1h 5')	
	Microbial Concentration [CFU/mL]	Microbial Concentration [CFU/mL]	Die-off rate [%]
CN	3,06E+06 ± 1,31E+06	2,06E+06 ± 5,56E+05	32,61
CP	2,68E+06 ± 1,16E+06	0,00E+00 ± 0,00E+00****	100
ALG:Lf@CHT	3,35E+06 ± 1,19E+06	1,67E+06 ± 3,70E+05	50,24
ALG@CHT	3,46E+06 ± 1,05E+06	5,69E+05 ± 7,62E+04*	83,56
Lf	2,50E+06 ± 7,00E+05	8,03E+05 ± 7,13E+04	67,87

^a*E. coli* ATCC® 25922 was tested at pH 5 after 1 hours and 5 minutes with ALG:Lf@CHT and ALG@CHT at 0.5 mg/mL. Two-way ANOVA followed by Šidák's multiple comparisons test (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

Table S6. Microbial concentration and Die-off rate [%] of *P. aeruginosa* ^a

Viability assay at pH 5 on <i>P. aeruginosa</i> ATCC® 9027 [10 ⁶ CFU/mL]			
Samples	t ₀	t ₁ (1h 5')	
	Microbial Concentration [CFU/mL]	Microbial Concentration [CFU/mL]	Die-off rate [%]
CN	1,00E+06 ± 0,00E+00	8,00E+05 ± 0,00E+00	20,00

CP	1,00E+06 ± 0,00E+00	0,00E+00 ± 0,00E+00****	100
ALG:Lf@CHT	1,03E+06 ± 3,33E+04	6,07E+05 ± 6,67E+03	41,29
ALG@CHT	1,37E+06 ± 3,18E+05	2,33E+05 ± 5,24E+04****	82,93
Lf	1,00E+06 ± 0,00E+00	4,57E+05 ± 5,67E+04	54,33

P. aeruginosa ATCC® 9027 was tested at pH 5 after 1 hour and 5 minutes with ALG:Lf@CHT and ALG@CHT at 0.5 mg/mL. Two-way ANOVA followed by Šídák's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Table S7. Microbial concentration and Die-off rate [%] of *E. coli*^a

Viability assay at pH 7 on <i>E. coli</i> ATCC® 25922 [10 ⁶ CFU/mL]			
Samples	t ₀	t ₁ (25 h)	
	Microbial Concentration [CFU/mL]	Microbial Concentration [CFU/mL]	Die-off rate [%]
CN	2,56E+06 ± 1,47E+06	1,71E+06 ± 6,39E+05	33,22
CP	1,35E+06 ± 1,78E+05	0,00E+00 ± 0,00E+00****	100,00
ALG:Lf@CHT	1,24E+06 ± 2,35E+05	1,19E+06 ± 1,90E+05	3,98
ALG@CHT	1,47E+06 ± 2,04E+05	1,46E+06 ± 3,72E+05	0,14
Lf	2,39E+06 ± 6,99E+05	3,87E+05 ± 1,82E+05	83,84

E. coli ATCC® 25922 was tested at pH 7 after 25 hours with ALG:Lf@CHT and ALG@CHT at 0.5 mg/mL. Two-way ANOVA followed by Šídák's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Table S8. Microbial concentration and Die-off rate [%] of *P. aeruginosa*^a

Viability assay at pH 7 on <i>P. aeruginosa</i> ATCC® 9027 [10 ⁶ CFU/mL]			
Samples	t ₀	t ₁ (25 h)	
	Microbial Concentration [CFU/mL]	Microbial Concentration [CFU/mL]	Die-off rate [%]
CN	1,80E+06 ± 1,15E+05	2,54E+06 ± 2,68E+05	-40,93
CP	3,54E+06 ± 2,70E+05	0,00E+00 ± 0,00E+00****	100,00
ALG:Lf@CHT	1,37E+06 ± 3,18E+05	1,20E+06 ± 9,67E+04	11,95
ALG@CHT	1,40E+06 ± 3,06E+05	3,30E+05 ± 3,00E+04*	76,43
Lf	1,03E+06 ± 3,33E+04	1,91E+06 ± 9,00E+04	-84,84

P. aeruginosa ATCC® 9027 was tested at pH 7 after 25 hours with ALG:Lf@CHT and ALG@CHT at 0.5 mg/mL. Two-way ANOVA followed by Šidák's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Table S9. Microbial concentration and Die-off rate [%] of *S. aureus*^a

Viability assay at pH 8.5 on <i>S. aureus</i> ATCC® 6538 [10 ⁶ CFU/mL]			
Samples	t ₀	t ₁ (4 h)	
	Microbial Concentration [CFU/mL]	Microbial Concentration [CFU/mL]	Die-off rate [%]
CN	1,02E+06 ± 1,67E+04	8,69E+05 ± 5,07E+04	14,49
CP	2,17E+06 ± 1,20E+05	0,00E+00 ± 0,00E+00****	100,00
ALG:Lf@CHT	1,23E+06 ± 3,00E+04	6,60E+05 ± 2,98E+04****	46,37
ALG@CHT	1,23E+06 ± 3,33E+04	7,41E+05 ± 2,31E+04****	39,89
Lf	1,16E+06 ± 3,04E+04	1,03E+06 ± 2,67E+04	11,47

S. aureus ATCC® 6538 was tested at pH 8.5 after 4 hours with ALG:Lf@CHT and ALG@CHT at 0.5 mg/mL. Two-way ANOVA followed by Šidák's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Table S10. Microbial concentration and Die-off rate [%] of *E. coli*^a

Viability assay at pH 8.5 on <i>E. coli</i> ATCC® 25922 [10 ⁶ CFU/mL]			
Samples	t ₀	t ₁ (4 h)	
	Microbial Concentration [CFU/mL]	Microbial Concentration [CFU/mL]	Die-off rate [%]
CN	1,73E+06 ± 3,30E+05	1,96E+06 ± 4,23E+05	-13,05
CP	1,17E+06 ± 8,82E+04	0,00E+00 ± 0,00E+00****	100,00
ALG:Lf@CHT	1,24E+06 ± 1,49E+05	5,46E+05 ± 2,58E+04	56,06
ALG@CHT	1,15E+06 ± 1,37E+05	6,70E+05 ± 4,73E+04	41,57
Lf	1,43E+06 ± 8,65E+05	2,57E+05 ± 3,48E+04	82,09

E. coli ATCC® 25922 was tested at pH 8.5 after 4 hours with ALG:Lf@CHT and ALG@CHT at 0.5 mg/mL. Two-way ANOVA followed by Šidák's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).