Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Characterization of carboxylate nanoparticle adhesion with the human fungal pathogen *Candida albicans*

Amy Lyden,[a] Lisa Lombardi,[b] Wilfried Sire,[c] Peng Li, [a] Jeremy C. Simpson,[d] Geraldine Butler, [b] and Gil U. Lee [a]

[a.]School of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland.
[b.]Conway Institute, School of Biomolecular and Biomedical Science, University College Dublin, Belfield, Dublin 4, Ireland.
[c.]Grenoble-INP Phelma, 3 Parvis Louis Néel, 38000 Grenoble, France
[d.]School of Biology & Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland.

Email: amy.m.lyden@gmail.com, gil.lee@ucd.ie, gbutler@ucd.ie, gbutler@ucd.ie

pH Titration on Carboxylate Nanoparticles

Sample preparation: To prepare for zeta potential and diameter measurements, 100 µL of 200 nm polystyrene carboxylate particles stock solution at 20 mg/mL was diluted in 15 mL of distilled water. Solution was titrated to approximately pH 10 with 2M NaOH solution. 15 µL of 40 nm polystyrene carboxylate particle stock solution at 50 mg/mL was diluted in 10mL of distilled water. Solution was titrated to approximately pH 9 with 2M NaOH solution.

Titration and measurement: pH titrations were performed using Malvern Instruments Multi-Purpose Titrator (MPT-2). Sample was titrated with 0.1 M HCl solution in approximately 1 pH increments, from the starting basic pH to an acidic pH of less than 1.5. Zeta potential measurements were taken using electrophoretic light scattering (Malvern Instruments Zetasizer Nano Series). Each data point is an average of three measurements of 20 runs each. Hydrodynamic diameter measurements were taken using dynamic light scattering (Malvern Instruments Zetasizer Nano Series). Each data point is an average of three measurements of 11 runs each.

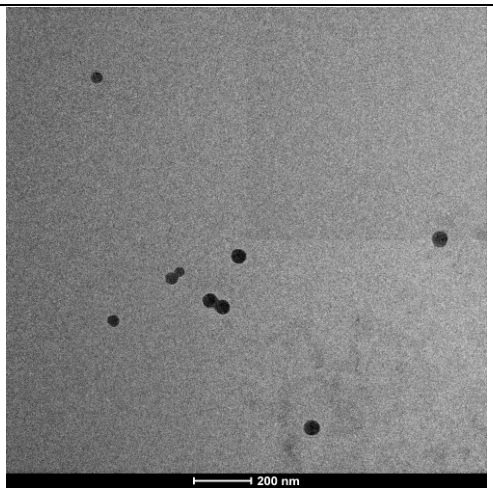
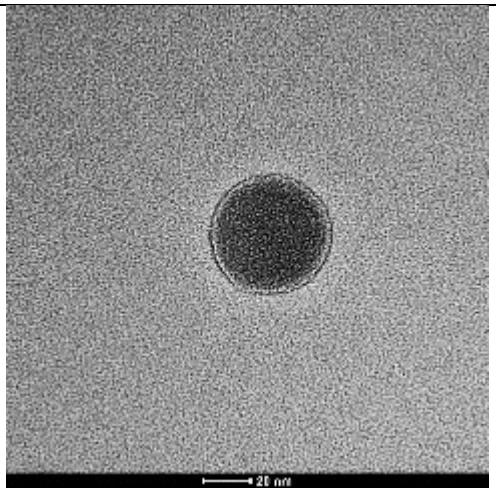
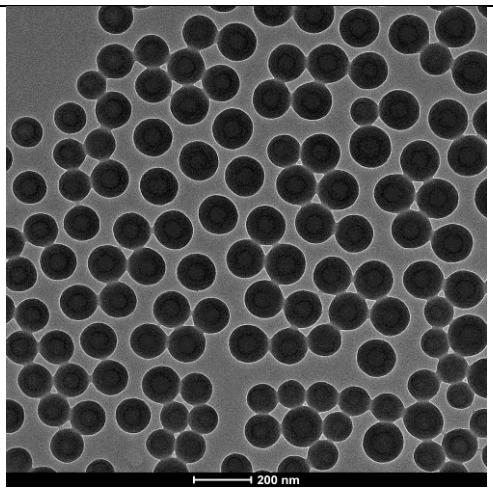
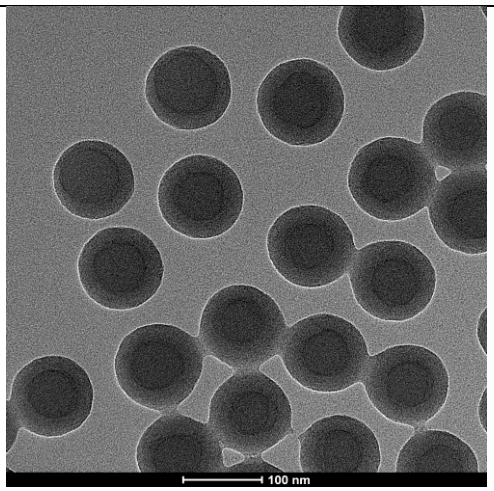
Table S1: DLS and zeta potential measurements of carboxylate polystyrene particles in distilled water at varying pH

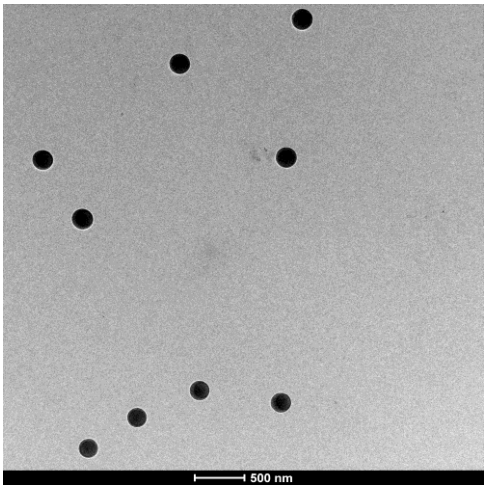
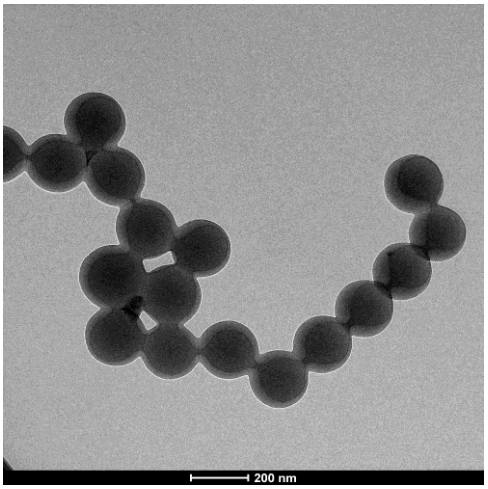
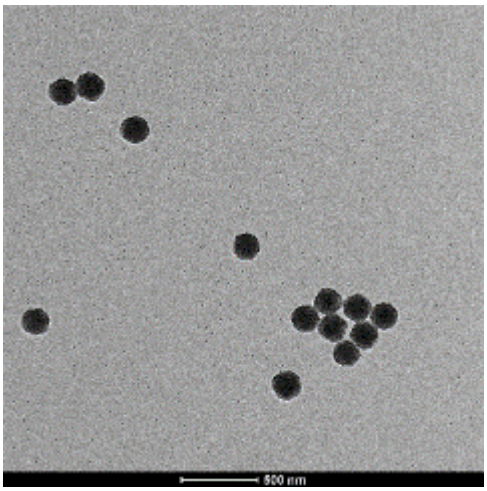
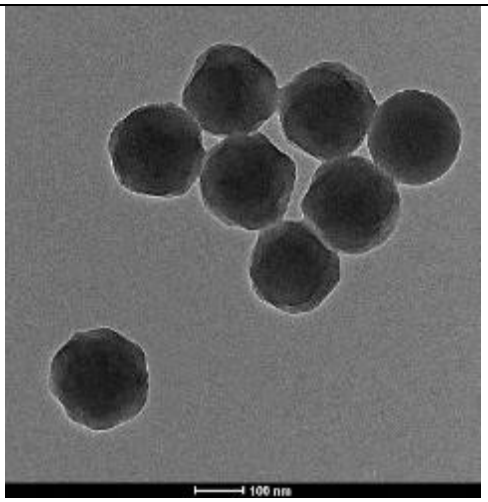
Sample	measured pH	Zeta Potential (mV)	Zeta Potential SD	Diameter (nm)	Diameter SD
200 nm Carboxylate Polystyrene Particles	10.2	-46.7	0.6	232.7	2.5
200 nm Carboxylate Polystyrene Particles	9.3	-46.6	1.2	232.0	2.8
200 nm Carboxylate Polystyrene Particles	8.3	-43.7	2.2	229.1	0.7
200 nm Carboxylate Polystyrene Particles	7.1	-47.1	0.9	219.1	1.6
200 nm Carboxylate Polystyrene Particles	6.4	-50.6	1.8	210.1	1.1
200 nm Carboxylate Polystyrene Particles	5.4	-54.2	1.0	205.1	0.2
200 nm Carboxylate Polystyrene Particles	4.6	-51.9	2.9	203.0	1.0
200 nm Carboxylate Polystyrene Particles	2.7	-46.0	0.5	202.1	0.7
200 nm Carboxylate Polystyrene Particles	2.1	-36.7	0.3	209.7	1.2
200 nm Carboxylate Polystyrene Particles	0.9	-11.6	0.2	1108.4	98.1
40 nm Carboxylate Polystyrene Particles	9.1	-57.1	3.9	88.4	0.9
40 nm Carboxylate Polystyrene Particles	7.9	-54.1	1.2	85.0	1.2
40 nm Carboxylate Polystyrene Particles	5.8	-46.4	0.2	81.7	0.4
40 nm Carboxylate Polystyrene Particles	5.2	-46.0	1.5	81.5	0.6
40 nm Carboxylate Polystyrene Particles	4.1	-33.9	0.3	80.0	0.5
40 nm Carboxylate Polystyrene Particles	3.6	-30.2	2.1	79.0	0.3
40 nm Carboxylate Polystyrene Particles	3.0	-20.8	1.9	79.6	1.0
40 nm Carboxylate Polystyrene Particles	2.2	-16.0	5.2	98.3	8.5
40 nm Carboxylate Polystyrene Particles	1.4	1.2	0.1	3158.0	677.3

TEM Images of Nanoparticles

TEM images were taken on FEI Company Technai $G^2$20 microscope. Diameters were analyzed in ImageJ.

Figure S1: Representative TEM images of ThermoFisher Red Fluorescent FluoSpheres used in this study. Diameters are averages from 50 particles measured in ImageJ. Coefficient of variation is percent of standard deviation divided by mean of the 50 particles measured in ImageJ.

Sample	TEM Diameter (Coefficient of Variation)	TEM Image 1	TEM Image 2
40 nm Carboxylate	51 nm (15%)		
100 nm Carboxylate	123 nm (13%)		

200 nm Carboxylate	199 nm (4%)		
200 nm Amine	192 nm (6%)		

Growth Rates of *C. albicans* Strains

Strains were grown in an overnight culture as previously described, diluted to 0.01 A_{600} in fresh YPD broth, and seeded on a clear 96-well tissue culture plate. The A_{600} was measured by a Biotek plate reader every 15 minutes for 24 hours. The doubling time was calculated using a linear regression in R of the linear region of the growth curve. The only statistically significant difference ($P<0.05$) in doubling time is between CAH7-1A1E2 and CAI4-URA3, the control strain– this could be due to natural aggregation of this strain as observed under microscope.

Table S2: Doubling time of *C. albicans* strains used in this study.

Strain	**Doubling Time (Hours)**	**SD**
SC5314	1.38	0.07
CAI12	1.35	0.12
CAI4-URA3	1.33	0.12
CAH7-1A1E2	1.83	0.21
1467	1.26	0.05
2757	1.24	0.07
1843	1.39	0.12
2034	1.31	0.12

Toxicity of carboxylate and amine nanoparticles to *C. albicans* in experimental conditions studied

C. albicans SC5314 was grown in an overnight culture as previously described, diluted to 1 A_{600} in Spider Medium, and incubated at 37C for 3 hours as previously described for hyphal development. Nanoparticles were added at indicated concentrations in PBS and cells and nanoparticles were incubated overnight at 4C on a rotator. Cells were diluted serially to 100 cells/mL, plated on YPD agar, and grown for 24 hours at 30C. Colony forming units were counted on two dilutions and averaged. Measurements represent the average and SD of three independent experiments. No statistically significant difference was observed between any samples with nanoparticles and control without nanoparticles (P>0.3).

Table S3: Colony forming units (CFU) of *C. albicans* SC5314 after incubation with nanoparticles

Sample	**Concentration**	**Colony Forming Units (10^6 CFU/mL)**
Carboxylate 200 nm	40 µg/mL	26.5 ± 1.72
Carboxylate 200 nm	20 µg/mL	24.2 ± 0.41
Amine 200 nm	40 µg/mL	24.7 ± 3.12
Amine 200 nm	20 µg/mL	27.3 ± 3.55
Control	0 µg/mL	27.5 ± 3.69

Confocal Images of *C. albicans* interaction with carboxylate nanoparticles in presence of serum

Figure S2: Confocal images of *C. albicans* SC5314 stained with 0.2 mM CFW after 3 hour hyphal development in Spider medium and reacted with 20 ug/mL of 40 nm or 200 nm red particles in PBS with 0% or 10% FBS, as indicated. All scale bars represent 10 µM in length.