Electronic Supplementary Information

Biocompatible Multilayer Capsules Engineered with a Graphene Oxide Derivative: Synthesis, Characterization and Cellular Uptake†

Loretta L. del Mercato,*[a] Flora Guerra,[b] Gianpiero Lazzari,[c] Concetta Nobile,[a] Cecilia Bucci,[b] and Rosaria Rinaldi[c,d]

[a]CNR NANOTEC - Institute of Nanotechnology c/o Campus Ecotekne, Via Monteroni, 73100 Lecce, Italy.
Email: loretta.delmercato@nanotec.cnr.it
[b]Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (DiSTeBA), Università del Salento, Via Monteroni, 73100, Lecce, Italy.
[c]Istituto Nanoscienze-CNR, Euromediterranean Center for Nanomaterial Modelling and Technology (ECMT), via Arnesano, 73100, Lecce, Italy.
[d]Dipartimento di Matematica e Fisica "Ennio De Giorgi", Università del Salento, Campus Universitario Ecotekne, Via Lecce-Monteroni, 73047, Monteroni di Lecce, Italy.

1. Synthesis of GO-PEM Capsules

The aqueous dispersion of GO received from Sigma Aldrich possesses a zeta potential of -51 mV, making it suitable as strong polyanion layer during LbL. $CaCO_3$ cores, produced according to the protocol described in the Experimental Section, were alternatively exposed to 1 mL of GO aqueous solution (0.1 mg mL^{-1}) and 1 mL of PAH (2 mg mL^{-1}, NaCl 0.5 M, pH 6.5), as standard polycation. Two different geometries of deposition were tested in parallel, $(GO/PAH)_2$ and $(PAH/GO)_2$. The zeta potential of the particles changed between one layer and the next one (Figure S1), indicating the interaction of the individual layers, also when GO was tested as anchoring layer onto the $CaCO_3$ cores (zeta potential = -10 mV) (Figure S1a). The fact that GO adsorbed onto cores with similar charges is not unexpected since it is not excluded that some GO nanosheets can be able to diffuse trough the pores of the $CaCO_3$ particles leading to a further increase of the negative charge of the cores (zeta potential = -30 mV). However, regardless the order of addition of the GO and PAH solutions, $CaCO_3$ particles underwent a rapid aggregation and precipitation following the addition of GO to the test tube (Figure S2a-c). Extensive sonication of the GO solution, combined to the use of diluted solutions of GO (0.05 mg mL^{-1} and 0.025 mg mL^{-1}) did not help in reducing the particle aggregation (Figure S2d-f). After deposition of two bilayers of $(GO/PAH)_2$ and $(PAH/GO)_2$, the samples were incubated with EDTA solution (0.2 M, pH 7.0), for removal of the $CaCO_3$ cores. This last step led to complete damage of the LbL-coated particles and disassembly of the multilayer shells (Figure S2g-h). Thin layers of material, together with a few aggregates of capsules, could be detected in the final samples. This material can be ascribed to debris of GO flakes and PAH polymer used for LbL coating; their presence after EDTA treatment confirmed that highly unstable shells were deposited by adsorption of GO during LbL.

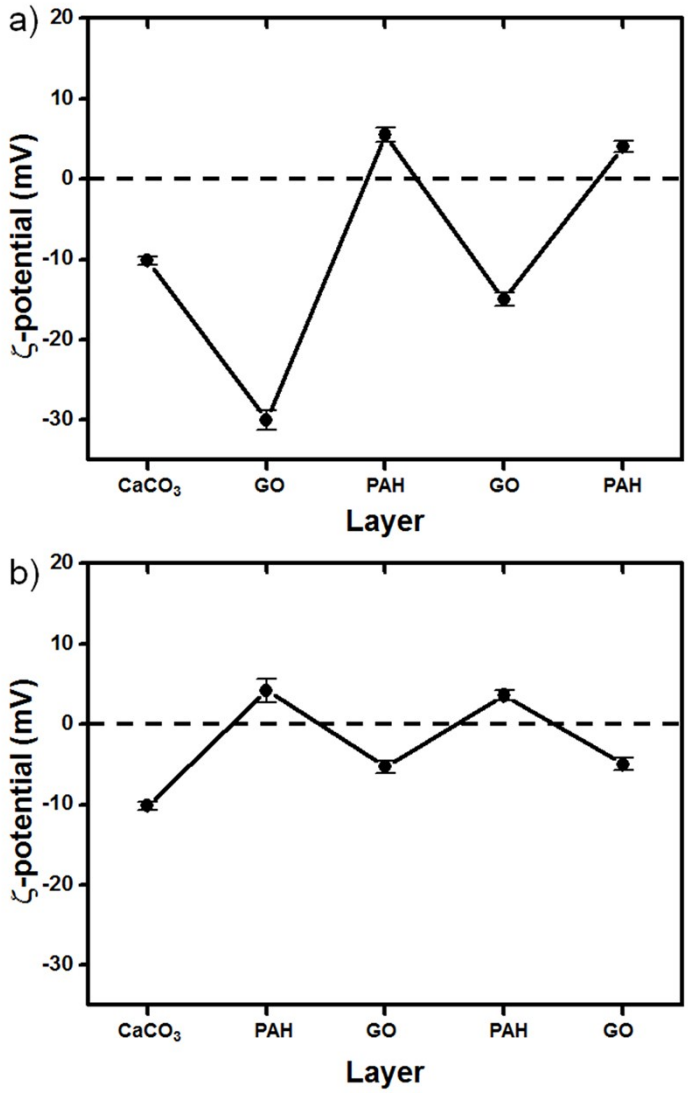

Fig. S1. Zeta potential of **(a)** $(GO/PAH)_2$ and (b) $(PAH/GO)_2$ multilayer films deposited on $CaCO_3$ microparticles.

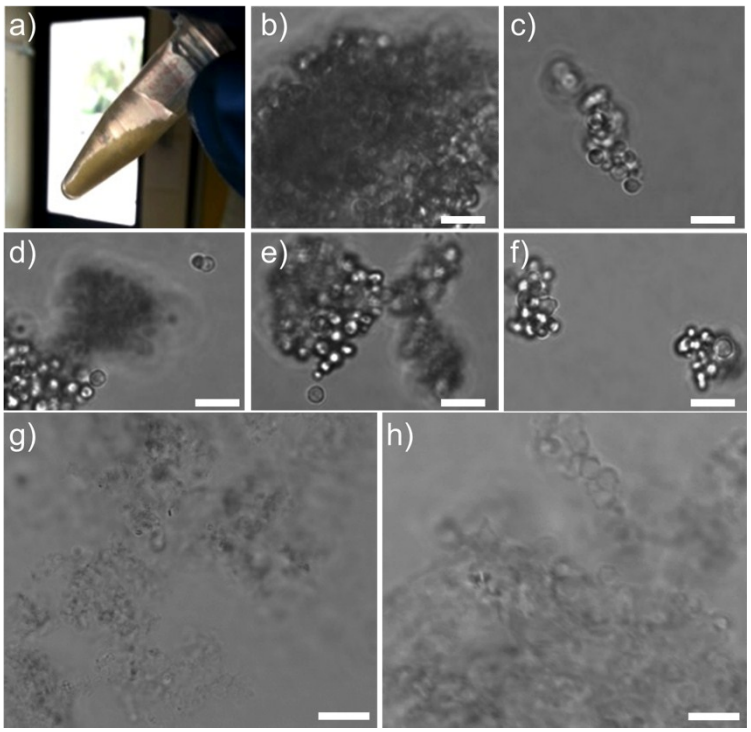

Fig. S2. (a) Photograph showing typical agglomeration of $CaCO_3$ coated particles and their sedimentation upon addition of 1 mL of aqueous solution of GO (0.1 mg mL^{-1}). **(b, c)** Representative bright field optical images of aggregates of $CaCO_3$ particles coated with 1 layer of GO. **(d-f)** Bright field optical images of $CaCO_3$(PAH/GO) particles at different concentration of GO (d: 0.1 mg mL^{-1}; e: 0.05 mg mL^{-1}; f: 0.025 mg mL^{-1}). **(g, h)** Representative bright field optical images of $(GO/PAH)_2$ and $(PAH/GO)_2$ samples after incubation with EDTA (0.2 M, pH 7.0). Scale bars: 5 μm.

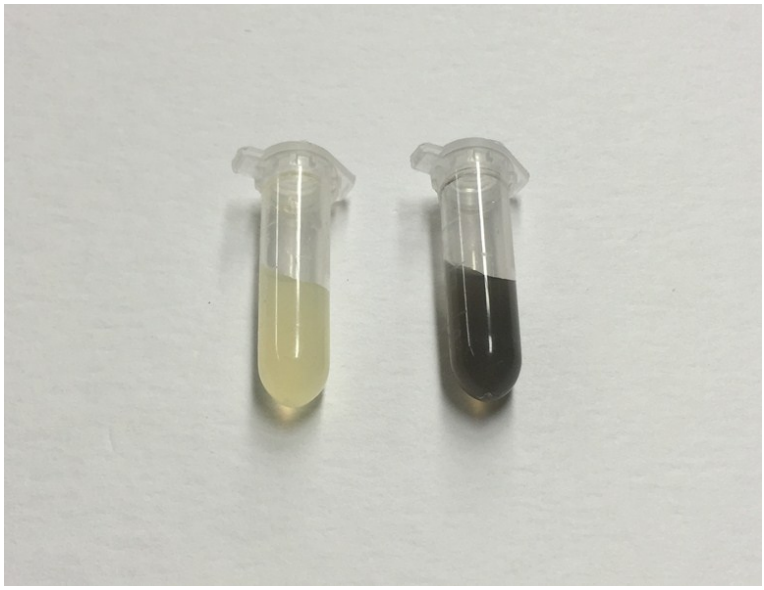

Fig. S3. The digital photo shows pellet color change following incubation of $CaCO_3$@pARG particles (left tube) with aGO suspension (0.1 mg mL^{-1}, in H_2O, pH 13) (right tube). Change in color from white to black corresponds to adsorption of aGO onto the oppositely charged pARG layer.

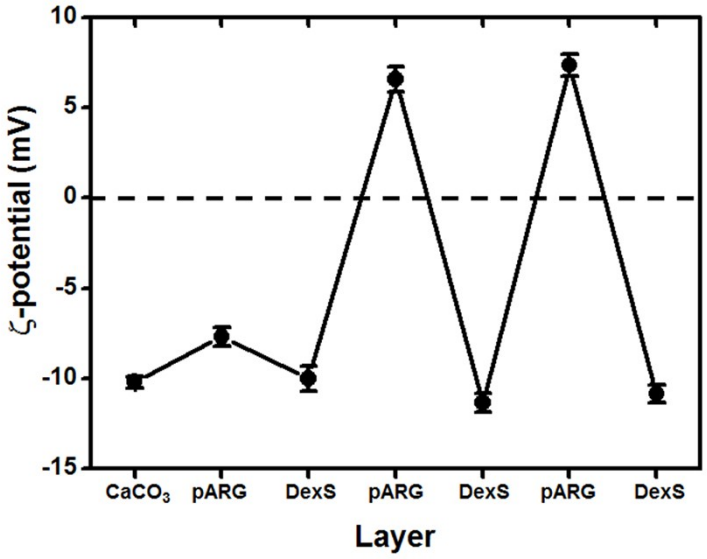

Fig. S4. Zeta potential of multilayer films made from pARG and DexS, deposited on $CaCO_3$ microparticles (CTR-PEM capsules).

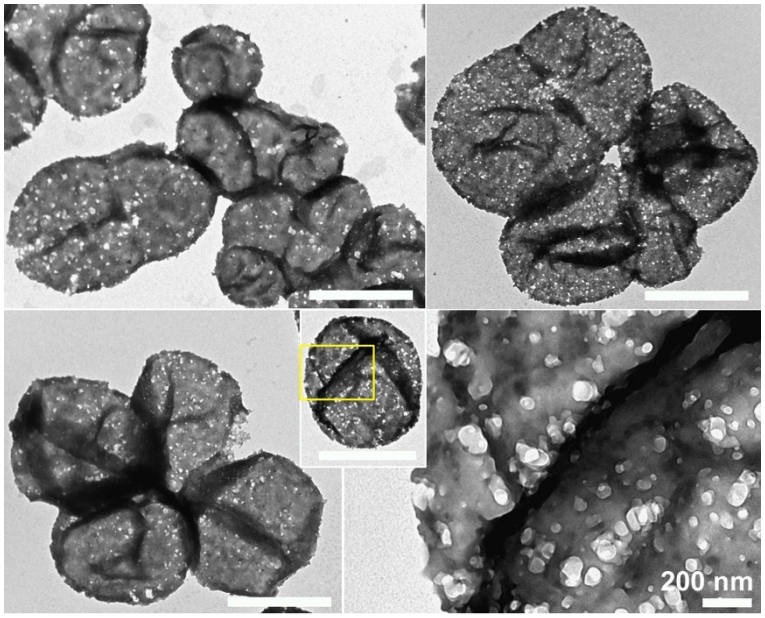

Fig. S5. TEM images of CTR-PEM capsules. Scale bars: 2 μm.

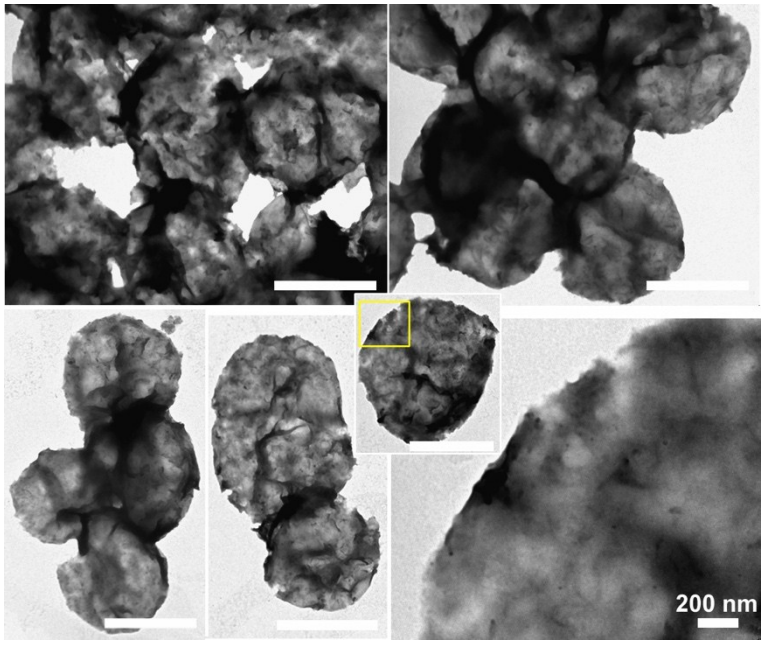

Fig. S6. TEM images of aGO-PEM capsules. Scale bars: 2 μm.

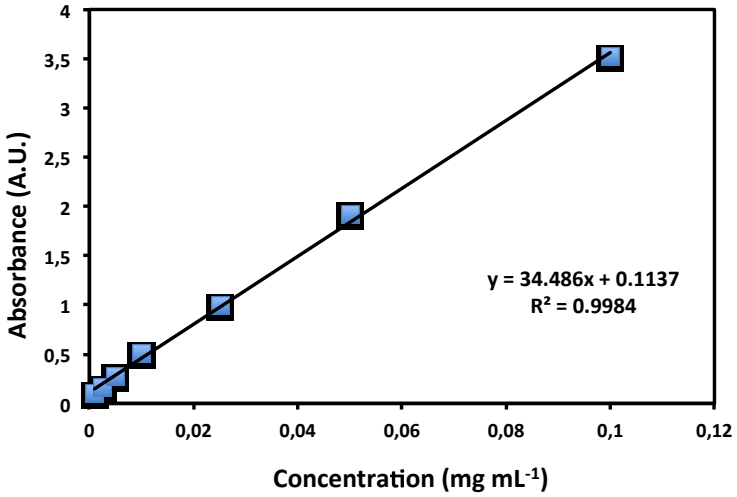

Fig. S7. Standard calibration curve of aGO from solutions of known concentration (0.1 mg mL^{-1}; 0.05 mg mL^{-1}; 0.025 mg mL^{-1}; 0.01 mg mL^{-1}; 0.005 mg mL^{-1}; 0.0025 mg mL^{-1}; 0.001 mg mL^{-1}). The total amount of aGO adsorbed onto the capsules was indirectly determined through comparing the aGO absorbance value at 265 nm, recorded in the supernatants collected from $CaCO_3$@(pARG/aGO) particles, with that of the calibration curve obtained from aqueous solutions of known concentrations of aGO (three readings for each concentration were recorded and averaged). Finally, the total amount of aGO measured per one batch of capsules (0.098 mg mL^{-1}) was divided by the total number of capsules per mL ($4.8x10^8$ capsules mL^{-1}) resulting in ≈ 0.204 pg of aGO per capsule.