Core-Independent Approach for Polymer Brush-Functionalised Nanomaterials with Fluorescent Tag for RNA Delivery

Supporting information

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Materials:

2-(Dimethylamino)ethyl methacrylate (DMAEMA, Mn = 157.21), oligo(ethylene glycol methyl ether methacrylate) (OEGMA, M_n = 300), copper chloride (Cu(I)Cl), copper bromide ($Cu(II)Br_2$), 2,2'-bipyridyl (bipy), anhydrous toluene, triethylamine (Et_3N) and poly(styrenesulfonate) were purchased from Sigma-Aldrich and used as received. All chemicals and solvents were analytical grades unless otherwise stated. Cu(I)Cl was kept under vacuum until used. The fluorescent conjugated polyelectrolyte was synthesised according to a procedure reported literature[6]. Silicon wafers (100 mm diameter, <100> orientation, polished on one side/reverse etched) were purchased from Compart Technology Ltd and cleaned in a Plasma System Zepto from Diener Electronic, for 10 min in air. Silica particles (300 nm, unfunctionalised) were purchased from Bangs Laboratories (supplied as powder). Silica particles (70 nm) were synthesised according to previous reports[1]. Calcium carbonate particles were synthesised according to previous reports[2]. Triton X-100, gelatin, phallodin–tetramethylrhodamine B isothiocyanate, PFA (paraformaldehyde), DAPI (4,6-diamidino-2-phenylindole), phosphate buffered saline (PBS, 150 mM) were purchased from Sigma Aldrich. Dulbecco's modified eagle medium (DMEM) medium, OPti-MEM™ medium, fetal bovine serum (FBS), trypsin, versene, penicillin-streptomycin, L-glutamine and hoechst 33342 were from Thermo-Fisher.

Collagen type I was from BD Bio-science. GFP siRNA (target sequence CGG CAA GCT GAC CCT GAA GTT CAT) and negative control (NC) siRNA (N/A) were purchased from Qiagen®.

Synthesis of macroinitiator (MI):

The synthesis of macroinitiator was reported previously by Chen *et al*.[3] Briefly, it was prepared in a three-step reaction in Figure S1. First, a copolymer of PDMAEMA and PHEMA was synthesised *via* ATRP, then the hydroxyl groups of PHEMA was esterified with 2-bromoisobutyryl bromide and finally PDMAEMA was quaternised by methyl iodide. A typical procedure for preparation of the macroinitiator was described below.

Firstly, a solution of DMAEMA (16.9 g, 107.6 mmol), HEMA (3.43 g, 26.4 mmol), 2-propanol (50.39 g), ethyl α-bromoisobutyrate (α-BIB, 1.3 g, 6.6 mmol), Cu(II)Br (0.15 g, 0.6 mmol) and 2,2'-bipyridine (bpy, 2.60 g) was degassed with argon for 40 min with continuous stirring at room temperature. The Cu(I)Br catalyst (0.97 g, 6.7 mmol) was added under nitrogen to start the polymerisation. After 15 h, the reaction was terminated by exposure to air. To remove the spent Cu(II) catalyst, the reaction mixture was diluted with DCM and passed through a basic Al_2O_3-colume. The resulting solution was then concentrated and precipitated into diethyl ether. The purified white PDMAEMA-PHEMA copolymer was filtered, dried under vacuum at room temperature, and characterised by ^{1}H NMR spectroscopy. Based on ^{1}H-NMR, the monomer conversion exceeded 95% and the ratio of incorporated PDMAEMA: PHEMA was approximately 4:1, as expected. Secondly, esterification of the hydroxy groups of the PDMAEMA-PHEMA with excess 2-bromoisobutyryl bromide was carried out. Thus triethylamine (TEA, 26.4 mmol), DMAP (26.4 mmol), and THF (50 mL) were mixed and cooled to 0 °C, followed by the addition of 2-bromoisobutyryl bromide (0.0528 mol). A solution of the PDMAEMA-co-PHEMA (12 g) in THF (50 mL) was added dropwise to this yellow reaction solution over a period of 1 h under dry nitrogen. Subsequently, the reaction temperature was allowed to rise slowly to room temperature and the esterification was stirred for a further 18 h. The reaction was terminated by addition of EtOH and the white HBr-salt was removed by centrifugation (8000 rpm, 10 min and 15 °C). The product was carefully concentrated using a rotary evaporator below 30 °C and the product was precipitated in diethyl ether (cooled by dry ice). The off-white product was re-dissolved in THF and the purification procedure was repeated once. The product was characterised by ^{1}H-NMR. The reaction was efficient, with full conversion of the OH-groups according to ^{1}H-NMR as the corresponding peaks (peaks 4 and 5 in Figure S1) were de-shielded, consistent with the formation of an ester moiety. Finally, quaternisation was achieved by dissolving this esterified copolymer (6 g) in DMF (50 mL). Then, methyl iodide (2 mL) was added to this stirred solution and quaternisation was allowed to continue for 24 h at 20 °C. This reaction mixture was added to a large excess of THF, and the isolated cationic

macroinitiator was redissolved in water and freeze-dried overnight to obtain an off-white solid and then characterised with ^{1}H NMR. Quaternised amines are distinguishable in ^{1}H-NMR as peaks centred at 3.2 ppm. Overall, our results are in good agreement with previous reports.

Layer-by-layer MIs on silicon wafers

A piece of plasma-oxidised silicon wafer was immersed in a solution of positively charged MIs (2 mg/mL in 0.5 M NaCl) for 1 h at room temperature. Then the wafer was rinsed with copious amounts of DI water and dried under nitrogen stream. The resulting sample was named Si-MI. A mixed solution of negatively charged polyelectrolytes was prepared by mixing 10 mL PSS (2 mg/mL in 0.5 M NaCl) with 1 mL FCP (0.5 mg/mL in DMSO), with votexing. Then Si-MI was immersed in the above solution for 1 h at room temperature before washing with DI water and drying with nitrogen stream. The resulting sample was named Si-MI-FCP. By repeating the process of each polyelectrolyte, different layers of MIs coated silicon wafers can be obtained. Herein, one layer of MIs (Si-MI), two layers of MIs (Si-MI-FCP-MI, Si-2MI) and three layers of MIs (Si-MI-FCP-MI-FCP-MI, Si-3MI) were prepared to study polymer brush growth kinetics. The dry thickness of each step was measured *via* spectroscopic ellipsometry (JA Woollam, -SE), as presented in Figure S2.

Polymer brush growth kinetics on different layers of macroinitiators

To study PDMAEMA brush growth and the evolution of its thickness as a function of time, a solution of $CuBr_2$ (18 mg, 80 µmol), bipy (320 mg, 2.05 mmol), and DMAEMA (42 mmol, 6.6 g) in water/ethanol (4/1 (v/v), 30 mL) or in DMF/water (4/1 (v/v), 30 mL) was degassed using argon bubbling for 30 min. CuCl (82 mg, 828 µmol) was added into this solution quickly and the resulting mixture was sonicated to ensure fully dissolve of CuCl and further degassed for 30 min before polymerisation. MI-coated silicon wafers (~1 $\times$ 1 cm^2 each) were placed in reaction vessels and degassed *via* four cycles of vacuum/nitrogen. Subsequently, 1 mL of DMAEMA solution was transferred to reaction vessels under inert atmosphere *via* a syringe. The polymerisation was stopped at different time points (2.5, 5, 10, 20, 30, 60 and 120 min) by immersing the substrates in deionised water, followed by washing with ethanol and drying under a nitrogen stream. The dry thickness of PDMAEMA brush was measured *via* ellipsometry afterwards.

Synthesis of 70 nm silica nanoparticles (SiO_2)

Detailed synthesis methods of SiO_2 nanoparticles fabrication through a water-in-oil microemulsions system have been described in previous publications[1]. The microemulsion was prepared from 3.99 g tergitol NP-9, 1.22 mL 1-hexanol, 345 mg NH_4OH (35 %), 532 mg

H_2O and 30.3 mL cyclohexane. Two millilitres of tethaethyl orthosilicate (TEOS, 99 %) liquid was injected dropwise into the stirred microemulsion matrix at a constant rate. The reaction was kept at room temperature for 24 h to eventually form monodisperse SiO_2 nanoparticles. The resulting nanoparticles were characterised through dynamic light scattering using a Malvern Zetasizer Nano ZS.

Synthesis of calcium carbonate particles ($CaCO_3$)

Detailed synthesis method of $CaCO_3$ was described by previous method.[4] Briefly, equal volumes of 0.33 M solutions of $CaCl_2$ and Na_2CO_3 were rapidly mixed and thoroughly agitated on a magnetic stirrer for 30 s at room temperature. After that the agitation was stopped, and the reaction mixture was left without stirring for 10 min, during which time the formed amorphous primary precipitate of $CaCO_3$ transforms slowly into cubic microparticles. Finally, the particles were thoroughly washed with water.

Preparation of graphene oxide (GO)

GO (0.5 mg/mL) in deionised water was diluted from 4 mg/mL stock solution purchased from Graphenea and prepared as previously described[5]. The average lateral dimensions of graphene flakes are 1-2 μm and their thickness was measured as 1.3 nm by AFM. Therefore, the aspect ratio of these nanomaterials is near 1000.

Layer-by-layer macroinitiators and fluorescent conjugated polyelectrolytes assembly from different nanomaterials

Particles of different size, shape and surface chemistries were investigated to coat nanomaterials with MIs and FCPs, in order to enable the subsequent growth of polymer brushes. Spherical silica nanoparticles with different sizes (SiO_2, 70 nm and 300 nm), micro-sized cubic calcium carbonate particles ($CaCO_3$) and graphene oxide nanosheets (GO) were applied used in this LBL process. Briefly, 10 mL MI solution (2 mg/mL in 0.5 M NaCl) was prepared and stirred vigorously. 10 mL particle suspension (0.5 mg/mL in DI water) was added to MI solution dropwise, slowly. The resulting suspension was left stirring at RT for 4 h. The particles were then centrifuged (8000 rpm x 15 min for 70 nm SiO_2 and GO, 4000 rpm x 15 min for 300 nm SiO_2, 2000 rpm x 5 min for $CaCO_3$) before the supernatant was aspirated out. Particles were then washed with 10 mL DI water. The above process was repeated three times. MI coated particles (SiO_2(70)-MI, SiO_2(300)-MI, GO-MI and $CaCO_3$-MI) were resuspended to 10 mL DI water and added dropwise to 10 mL FCPs/PSS mixed solution (mixing 10 mL PSS (2 mg/mL in 0.5 M NaCl) with 1 mL FCP (0.5 mg/mL in DMSO) with a vortex), with vigorous stirring

for 4 h at RT. The washing and centrifuging process was the same as described above. Eventually, all the particles were coated with two layers of MI, namely SiO_2(70)-2MI, SiO_2(300)-2MI, GO-2MI and $CaCO_3$-2MI and subsequently used for brush polymerisation.

Polymer brush growth on different particles

PDMAEMA brush. The polymerisation solution was prepared as described previously by dissolving DMAEMA (6.6 g, 42 mmol), bipy (320 mg, 2.05 mmol), $CuBr_2$ (80 mmol) and CuCl (0.082 g, 828 μmol) in polymerisation solvent (DMF/water 4/1 (v/v), 15 mL). 10 mL nanomaterials-2MI dispersion (2 mg/mL) in DMF/water 4/1 (v/v) o were degassed for 30 minutes with argon bubbling while stirring. An equal volume of DMAEMA monomer solution was added to the suspension. Polymerisation was allowed to proceed under argon at RT for 60 min. To terminate the polymerisation, the particle dispersion was diluted using deionised water and bubbled with air until the colour changed from dark brown to blue (oxidation of CuCl). The particles were recovered *via* centrifugation, washed successively with water to get rid of the catalysts and residual monomer and finally the particles were dispersed in 10 mL deionised water and stored in the fridge.

POEGMA brushes. The procedure of POEGMA brush synthesis was similar to that used for PDMAEMA brushes except for the difference in monomer solution and polymerisation time. For OEGMA polymerisation, the monomer solution was: OEGMA (12.6 g, 42 mmol), bipy (320 mg, 2.05mmol), $CuBr_2$ (80 mmol) and CuCl (0.082 g, 828 μmol) in 15 mL solvent (DMF/water 4/1 (v/v), 15 mL). The reaction was kept in room temperature for 120 min before termination.

Polymer brush coated particle characterisation

Size and ζ-potential measurement. ζ-potential measurement was applied to track the change of polyelectrolyte layer on all the particles after each LBL process and polymerisation. Size measurement was applied to only silica nanoparticles (both 70 nm and 300 nm) after each LBL step and polymerisation with a Malvern zetasizer nano ZS. Samples were prepared by dispersing particles in DI water until obtaining a slightly cloudy solution and then sonicated for 10 min with shaking at regular intervals. Each sample were measured in triplicates at 25°C and the average result was taken as the final hydrodynamic diameter or ζ-potential. After polymerisation of POEGMA brushes, the ζ-potential became nearly neutral in agreement with previous reports[6]. The residual positive ζ- potential may result from the only partial screening of the cationic MI layers by POEGMA brushes. In contrast, nanomaterials coated with PDMAEMA brushes displayed highly positive ζ-potentials. The hydrodynamic diameter of silica nanoparticles was measured after each step of the process. The size of silica nanoparticles increased slightly whilst retaining good dispersity after the deposition of each

layer of polyelectrolytes in Fig. S3 and Table S1. More pronounced size changes were observed after PDMAEMA polymerization, in agreement with the associated thickness and swelling of the brush in water, at neutral pH20. The final hydrodynamic diameters of SiO_2(70)-PDMAEMA and SiO_2(300)-PDMAEMA nanomaterials were found to be 310 and 950 nm, respectively, in agreement with the expected brush swelling, but indicative of a small fraction of dimers or trimers, mediated by weak interactions.

Thermogravimetric (TGA) measurement. By using TGA, the dry mass of polymer particles was determined. Herein, the TGA was performed in air using a TA Instruments Q500. All samples were heated from room temperature to up to 1000 °C at a heating rate of 10 °C/min and dried under vacuum at room temperature prior to TGA runs.

Fourier transform infrared - attenuated total reflectance (FTIR-ATR). FTIR was used to characterise the different chemical groups expected within the respective materials through obtaining an infrared spectrum. ATR-FTIR spectroscopy in this study was carried out using a Bruker Tensor 27 with an MCT detector (liquid N2 cooled). Spectra were taken at a resolution of 4 cm^{-1} with a total of 128 scans per run. FTIR spectroscopy was carried out to confirm the coating of nanomaterials with PDMAEMA and POEGMA brushes from MI LBL assemblies. The corresponding spectra displayed characteristic peaks of PDMAEMA at 1730 cm^{-1} (C=O stretching), 2767 cm^{-1} and 2820 cm^{-1} (-CH_2 stretching of –$N(CH_3)_2$).[7] Similarly, vibrations at 1730 cm^{-1} (C=O stretching) and 2870 cm^{-1} (-CH_2 stretching), typical of POEGMA brushes, were observed in the corresponding spectra (Fig. S5).[7,8] For $CaCO_3$, as the volume fraction of polymer brush was significantly lower (owing to the larger diameter of the core, only weak C=O stretching could be observed.

Scanning electron microscopy (SEM). SEM measurements were carried out using a JEOL 2010 transmission electron microscope with a LaB6 filament, operated at 200 kV. Samples were prepared by dropping the diluted brush coated silica nanoparticle suspension on a copper grid with porous carbon film and drying at room temperature.

Transmission electron microscopy (TEM). TEM measurements were carried out using a JEOL 2010 transmission electron microscope with a LaB6 filament, operated at 200 kV. Samples were prepared by dropping the diluted brush coated silica nanoparticle suspension on a copper grid with porous carbon film and drying at room temperature. It is worth noting that soft polymer brushes can form bridges between particles that show local inhomogeneity in the coating, but this is not observed for single isolated particles.

Fluorescent imaging. Due to the coating of fluorescent conjugated polyelectrolyte, particles were able to be visualised by fluorescent microscope. Herein, Zeiss LSM710 Confocal and an Elyra supperresolution microscope were applied to detect the fluorescent particles after polymerisation. Briefly, samples were prepared by dropping the particles suspension on a glass slide and drying at RT before mounting to another coverslip.

Cell viability

HaCaT Cell culture and passage. DMEM media supplied with 10 % FBS, 1 % Penicillin-Streptomycin (P/S) and 1 % glutamine was used to culture HaCaT cells in 37°C/5 % CO_2 incubator. To harvest HaCaT cells (T75), cells were washed twice with pre-warmed PBS solution and then cells were detached from the flask by trypsinisation (versene/trypsin, 4/1 v/v, 5 mL, 37°C). 15 mL of DMEM medium was then added to the flask to quench the trypsin. Cells were transferred to a 50 mL centrifuge tube and centrifuged at 1200 rpm for 5 min. After discarding the supernatant solution, the pellet was resuspended in 10 mL FAD medium and the concentration of cells was measured with a haematocytometer.

Cell viability test. Cells were seeded at a density of 35 k cells per well (in 500 μL of DMEA medium) in 24-well plates 24 h prior to the assay. Particles coated with PDMAEMA and POEGMA brushes with final concentration of 10 μg/mL were added into each well for 4 h in 0.5 mL serum free OPTI-MEM medium and then the medium was replaced by full culture DMEM medium for further 24 h incubation. Cell viability was characterised via a live/dead assay in which cells were incubated in 500 μL DMEM medium of 4 mM calcein AM, 2 mM ethidium homodimer and hoechst 33342(for staining cell nucleus) for 30 min at 37 °C prior to imaging. Fluorescence imaging was used to characterise the densities of live and dead cells. Counting was *via* ImageJ to obtain the percentage of live cells. In our previous report, POEGMA brush coated silica nanoparticles showed good cell viability even at high particle concentration, whereas cationic PDMAEMA brush coated silica nanoparticle displayed poor stability in complex protein solutions and relatively high cytotoxicity due to the highly positive coatings. These results correlated with other literatures where free PDMAEMA[9] and POEGMA[10] polymer were tested for their cell viability.

Particle-cell interaction

Fluorescence microscopy. The protocol for culturing and passaging HaCaT-GFP cells was the same as for HaCaT cells. SiO_2(70), SiO_2(300) GO and $CaCO_3$ coated with PDMAEMA and POEGMA brush with final concentrations of 10 μg/mL were added into each well for 4 h in 0.5 mL serum free OPTI-MEM

medium and then the medium was replaced by full culture DMEM medium for further 24 h incubation before imaging.

SEM. After allowing the uptake of silica nanoparticles with different polymer brush coating, samples were characterised *via* SEM to study the morphology of the cell-nanomaterials interface. Cells seeded on class coverslips were fixed after 24 h uptake, with 2.5% glutaraldehyde in PBS for 2 h at room temperature. The samples were then washed 3 times with 0.1 M PBS and dehydrated with a series of ethanol washings by increasing the ethanol content from 20% to 100%, each wash repeated twice for 5 min. Critical point drying was then performed (EMS 850 Critical Point Dryer) to dry the samples and they were then coated with gold (SC7620 Mini Sputter Coater, Quorum Technologies) for 60 s coating at 20 mA process current before SEM imaging.

Transfection assay

HaCaT-GFP cells were seeded at a density of 35 k/well on glass cover slips pre-treated with collagen in 24-well plates, 24 h prior to the transfection assay. A final siRNA concentration of 50 nM/well was used. 100 µL SiO_2(70)-PDMAEMA/GFP siRNA, SiO_2(300)-PDMAEMA/GFP siRNA, GO-PDMAEMA/GFP siRNA and $CaCO_3$-PDMAEMA/GFP siRNA complexes were prepared at N/P=5, 10 and 15, in serum free OPTI-MEM medium. After removing the DMEM medium, cells were washed twice with pre-warmed serum free OPTI-MEM medium and another 400 µL was added. 100 µL siRNA solution was then added dropwise to each well and mixed by shaking gently. Cells were incubated with siRNA complexes for 4 h in an incubator and the medium was then replaced by 500 µL full culture DMEM medium for a further 24 h of incubation. Lipofectamine® 2000 complexed with GFP siRNA/negative control (NC) siRNA (protocol according to the manufacturer's instruction with a final siRNA concentration of 50 nM/well) was used as a positive/negative control. The transfected cells were washed with PBS three times, fixed in paraformaldehyde (PFA, 4 %, 10 min) and permeabilised with Triton X-100 (0.2 %, 5 min). Cells were then stained with TRITC-phalloidin (1:1000) and DAPI (4,6-diamidino-2-phenylindole, 1:1000) in blocking buffer (10% FBS and 0.25% gelatin from cold water fish skin, Sigma-Aldrich) and kept at room temperature for 1 h. Cover slips with fixed cells were mounted on glass slides before imaging with a Leica DMI4000 fluorescence microscope.

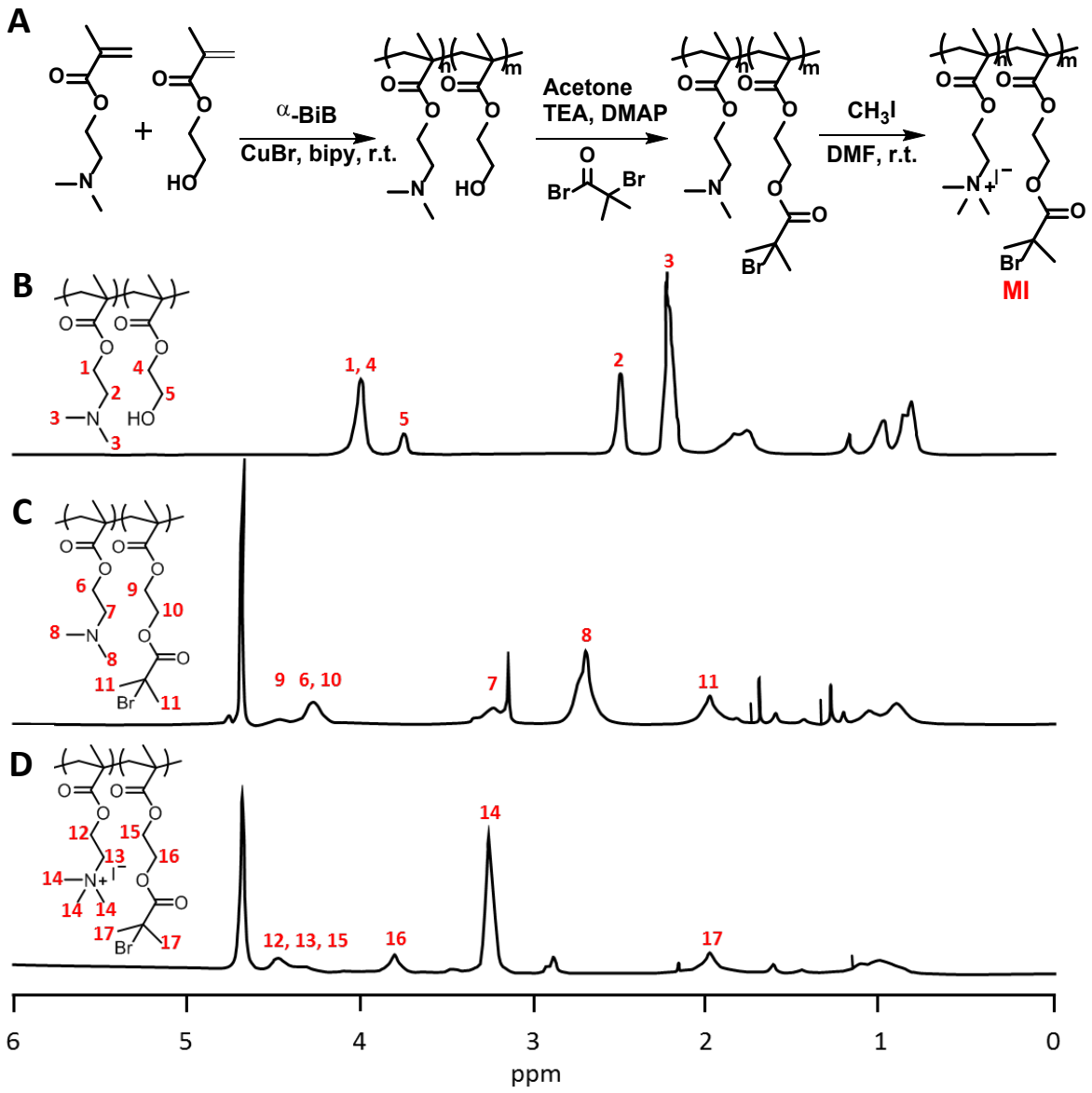

Fig. S1. Reaction scheme for the three-step synthesis of the cationic macroinitiator, a random copolymer of bromo-isobutyryl bromide-functionalised PHEMA and PDMAEMA at the molar ratio of 1:4 (A); ^{1}H NMR spectra for the synthesis of the cationic macroinitiator: PDMAEMA-PHEMA statistical copolymer (B, deuterated chloroform), copolymer after esterification with α-BiB (C, deuterium oxide), and the quaternised macroinitiator (D, deuterium oxide).

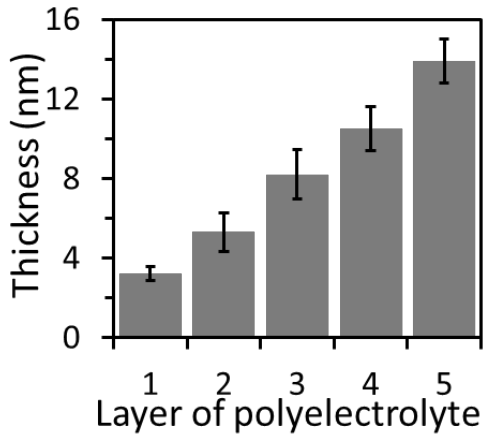

Fig. S2. Polyelectrolyte dry thicknesses on silicon wafers measured by ellipsometry, during layer-by-layer process. Layer 1: MI, layer 2: MI-FCP/PSS, layer 3: MI-FCP/PSS-MI, layer 4: MI-FCP/PSS-MI-FCP/PSS, layer 5: MI-FCP/PSS-MI-FCP/PSS-MI.

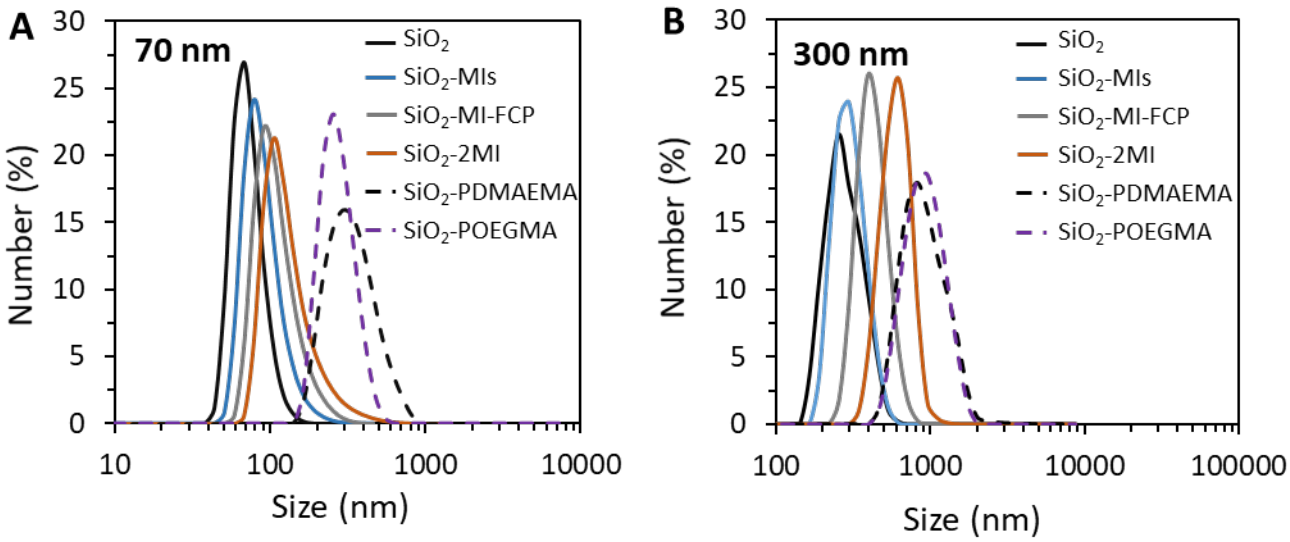

Fig. S3. Evolution of the hydrodynamic diameter of silica nanoparticles, characterised by dynamic light scattering, after layer-by-layer deposition of PEMs (at different steps) and generation of a PDMAEMA brush, on 70 nm SiO_2 (A) and 300 nm SiO_2 (B).

Table S1. Evolution of the hydrodynamic diameter of silica nanoparticles at different functionalisation stages. A comparison is made between small (70 nm) and large (300 nm) silica cores.

Evolution of the hydrodynamic diameter of silica nanoparticles at different functionalisation stages						
Sample	SiO_2	SiO_2-MI	SiO_2-MI-FCP	SiO_2-2MI	SiO_2-PDMAEMA	SiO_2-POEGMA
Small core SiO_2	70 ± 10 nm	90 ± 20 nm	110 ± 30 nm	140 ± 30 nm	310 ± 50 nm	270 ± 40 nm
Small core PDI	0.026	0.056	0.075	0.084	0.164	0.123
Large core SiO_2	280 ± 20 nm	300 ±20 nm	430 ± 30 nm	610 ± 40 nm	950 ± 50 nm	940 ± 50 nm
Large PDI	0.115	0.102	0.063	0.199	0.391	0.393

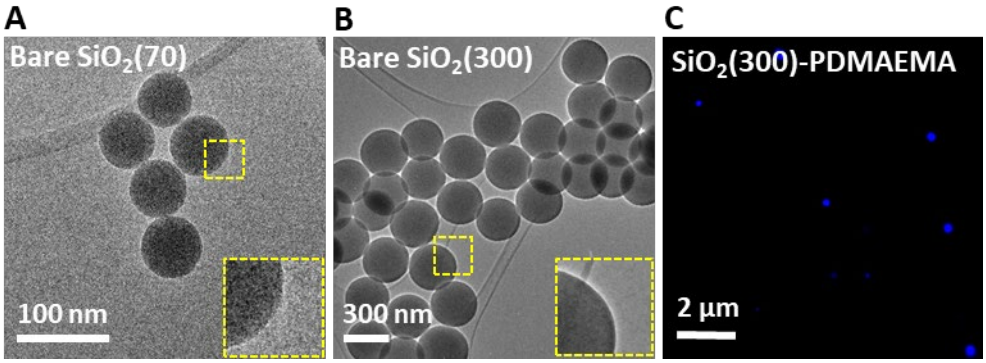

Fig. S4. TEM images of bare silica nanoparticle of 70 nm (A) and 300 nm (B); fluorescent image of SiO_2(300)-PDMAEMA (C).

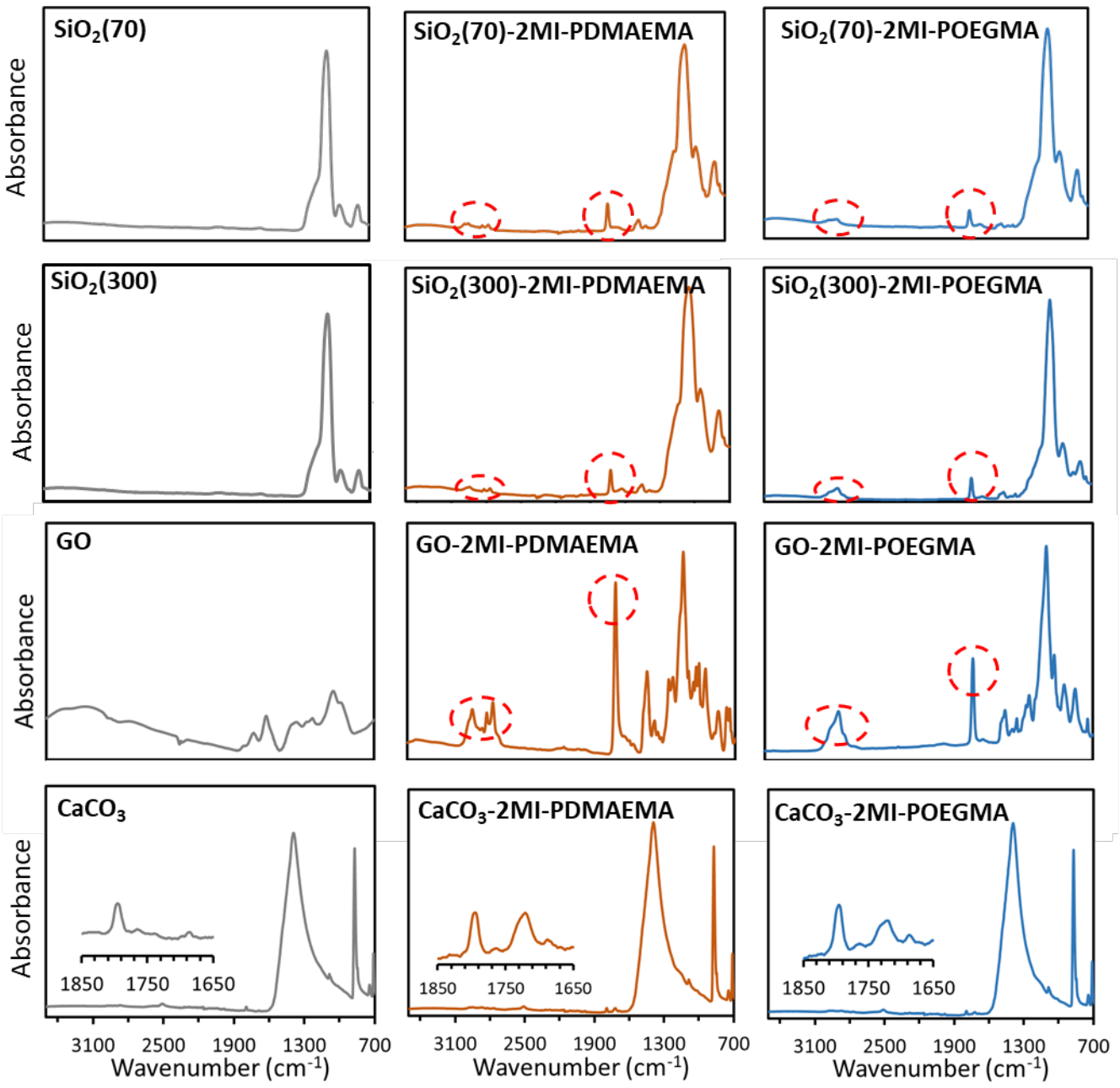

Fig. S5. ATR-FTIR of bare SiO_2(70), SiO_2(300) GO and $CaCO_3$ nanomaterials, and after coating with PDMAEMA and POEGMA brushes from two layers of MIs.

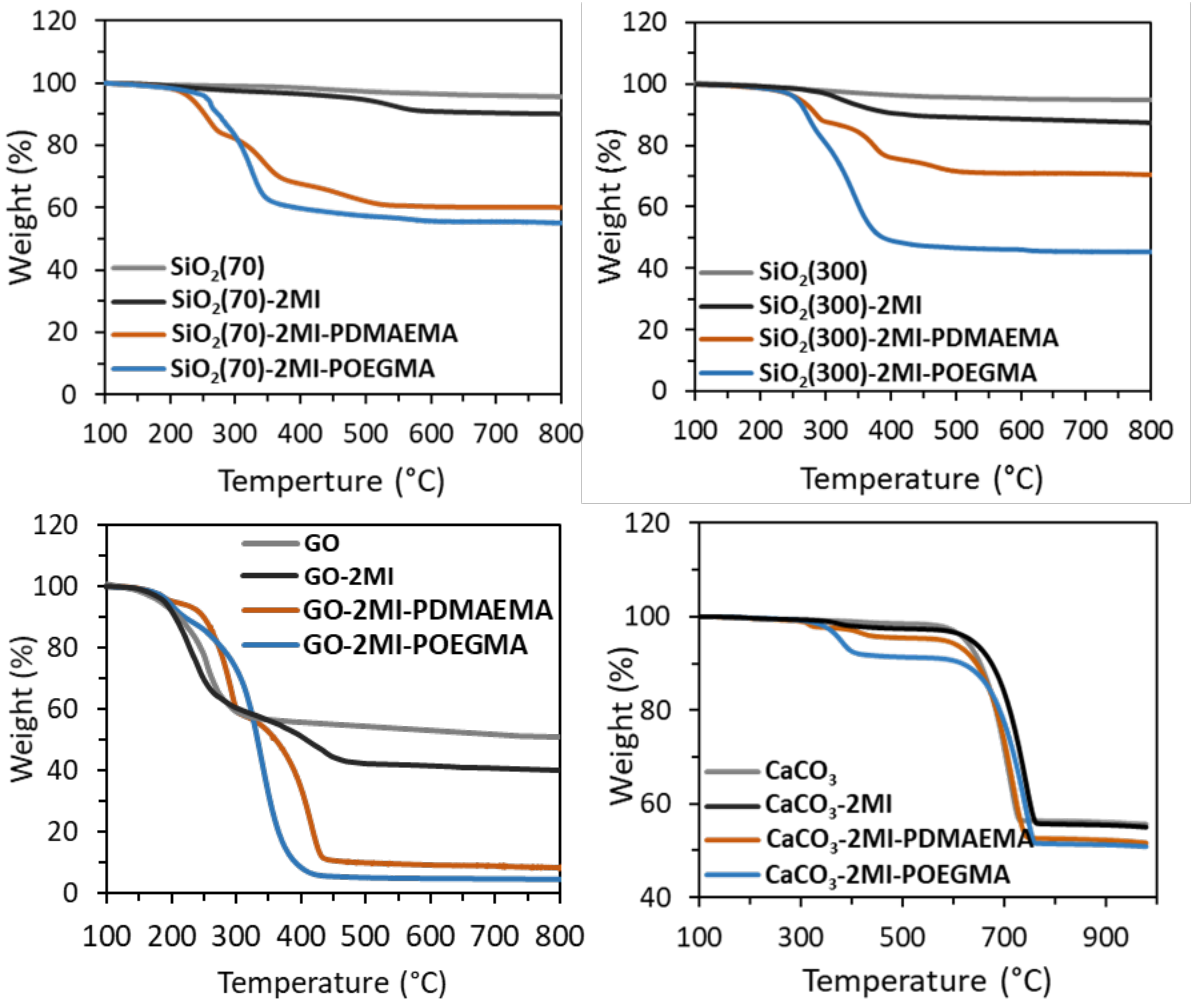

Fig. S6. TGA measurements recorded for bare SiO_2(70), SiO_2(300), GO and $CaCO_3$ and after polymerisation with PDMAEMA and POEGMA brushes on two layers of MIs.

Table S2. Calculation of brush thickness on different cores using equation S1-S3 based on TGA measurements.

Materials	Core wt %	Brush wt %	Brush thickness nm	Equation
SiO_2(70)-PDMAEMA	60	30	8.4	S1
SiO_2(70)-POEGMA	56	34	11.3	S1
SiO_2(300)-PDMAEMA	70	20	22	S1
SiO_2(300)-POEGMA	47	43	66	S1
GO-PDMAEMA	48	30	7	S3
GO-POEGMA	48	34	16	S3
$CaCO_3$-PDMAEMA	94.3	2.3	75	S2
$CaCO_3$-POEGMA	90.6	6.0	278	S2

$$h = R\left(\frac{W_{brush}\rho_{SiO_2}}{W_{SiO_2}\rho_{brush}} + 1\right)^{\frac{1}{3}} - R$$

Equation S1. Determination of the brush thickness h on silica nanoparticles: W_{brush} is the percentage of weight loss corresponding to the decomposition of polymer brushes, W_{SiO2} is the residual weight fraction, ρ_{brush} is the density of polymer brush ($\rho_{PDMAEMA,}$ 1.318 g/cm^3, $\rho_{POEGMA,}$ 1.105 g/cm^3), ρ_{SiO2} is the density of bulk SiO_2 (2.4 g/cm^3), R is the radius of SiO_2.[11]

$$h = \frac{W_{brush}\rho_{GO}}{2\,W_{GO}\rho_{brush}}$$

Equation S2. Determination of the brush thickness h on graphene oxide: W_{brush} is the percentage of weight loss corresponding to the decomposition of polymer brushes, W_{GO} is the weight fraction of GO, ρ_{brush} is the density of polymer brush ($\rho_{PDMAEMA,}$ 1.318 g/cm^3, $\rho_{POEGMA,}$ 1.105 g/cm^3), ρ_{GO} is the density of bulk GO (1.8 g/cm^3), here we assume that GO is a nanosheets with a thickness of 1 nm, in agreement with measurements.

$$h = \frac{W_{brush}\rho_{CaCO_3}a}{6\,W_{SiO_2}\rho_{brush}}$$

Equation S3. Determination of the brush thickness h on cubic calcium carbonate particles: W_{brush} is the percentage of weight loss corresponding to the decomposition of polymer brushes, W_{CaCO3} is the residual weight fraction, ρ_{brush} is the density of polymer brush ($\rho_{PDMAEMA,}$ 1.318 g/cm^3, $\rho_{POEGMA,}$ 1.105 g/cm^3), ρ_{CaCO3} is the density of bulk $CaCO_3$ (2.7 g/cm^3), a is the length of side.

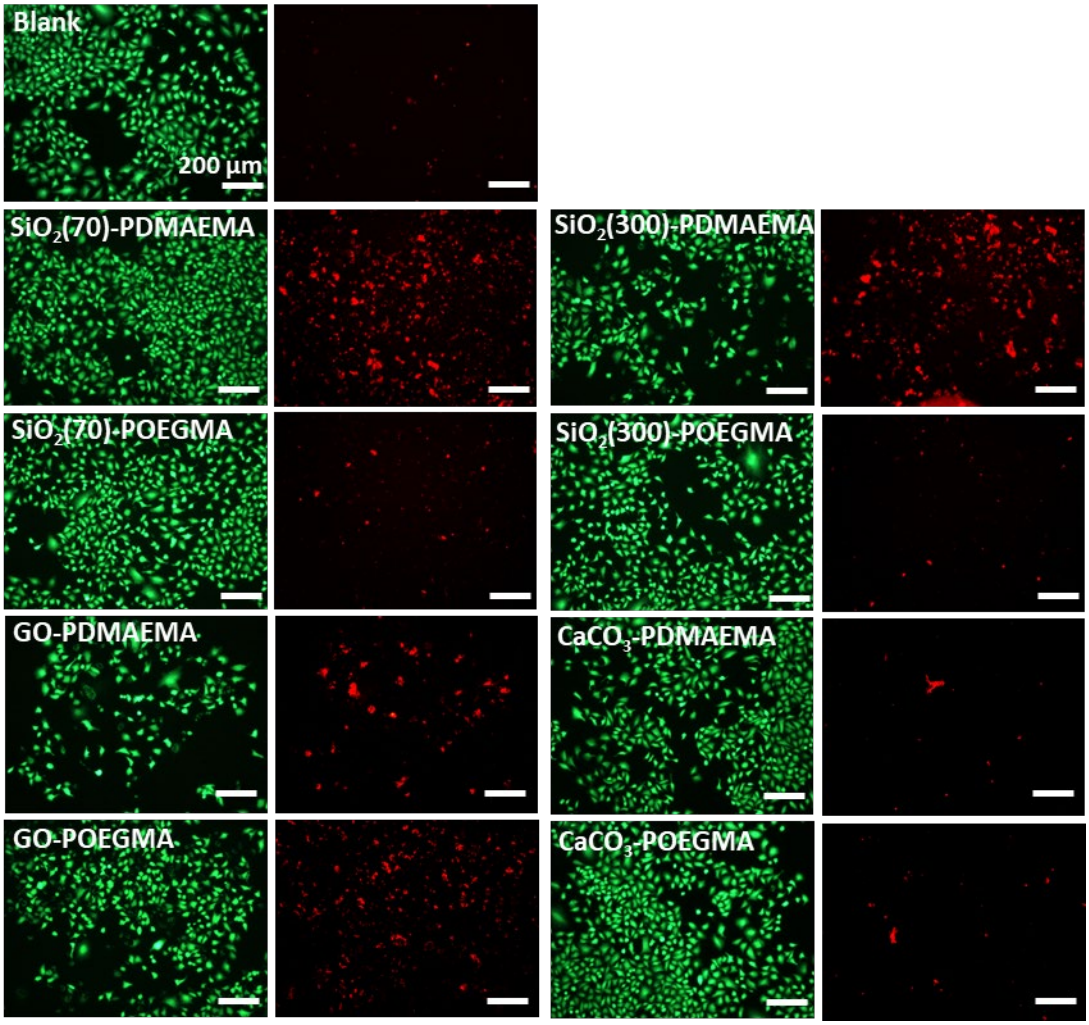

Fig. S7 Representative images from cell viability experiments with PDMAEMA/POEGMA brush-coated nanomaterials incubated on HaCaT cells. Live/dead assay: live, green; dead, red.

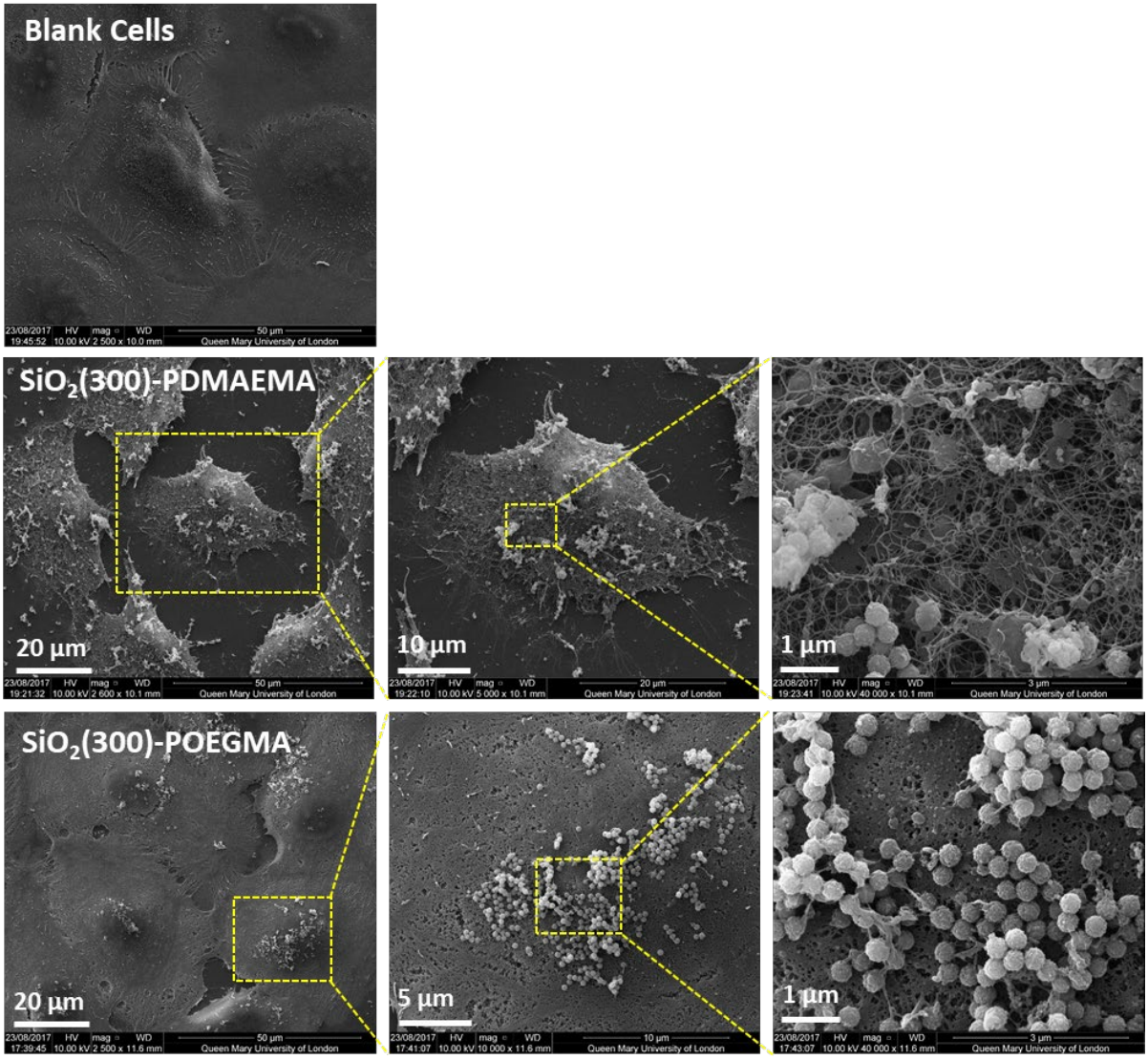

Fig. S8 SEM images of Blank HaCaT cells and HaCaT cells incubated with SiO_2(300)-PDMAEMA and SiO_2(300)-POEGMA for 4 h, in serum free OPTI-MEM medium and then by further culturing in full growth medium for 24 h.

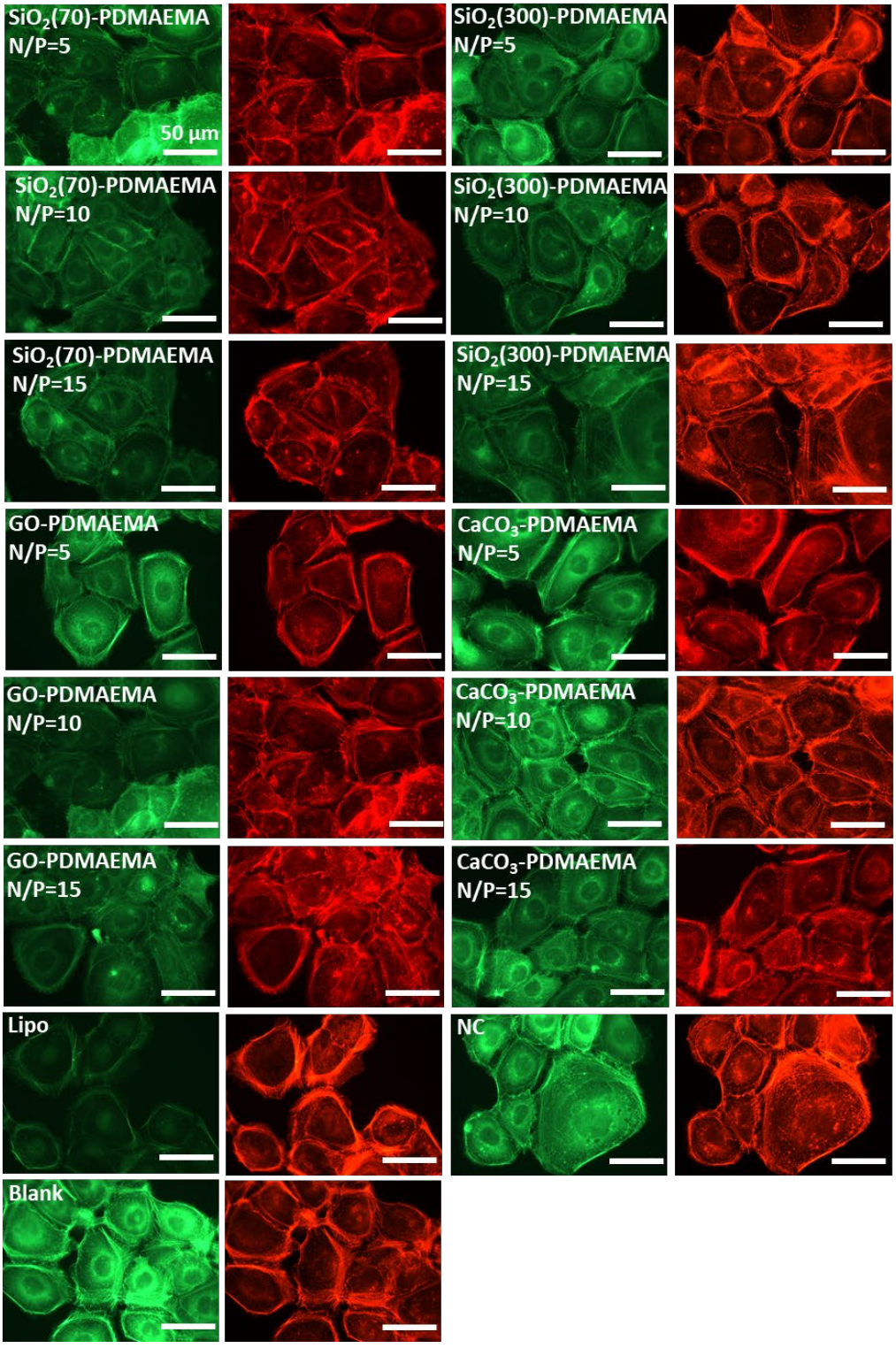

Fig. S9. Representative images for knock down efficiency of SiO_2(70)-PDMAEMA/GFP siRNA, SiO_2(300)-PDMAEMA/GFP siRNA, GO-PDMAEMA/GFP siRNA and $CaCO_3$-PDMAEMA/GFP siRNA with HaCaT-GFP cells at different N/P ratios.

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