Supporting Information

Design, engineering and structural integrity of electro-responsive carbon nanotube-poly(methacrylic acid) hydrogels for pulsatile drug release

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Materials and Methods

Materials

Pristine multi-walled carbon nanotubes (MWNTs) were purchased from Nanostructures and Amorphous Materials Inc. (Houston, TX, USA; stock no 1240XH, 95%, O.D 20-30 nm). Methacrylic acid (MAA) was purchased from Sigma-Aldrich (Dorset, United Kingdom) and distilled under vacuum before use. N,N'-Methylenebis(acrylamide) (MBAA) and potassium persulfate were purchased from Sigma-Aldrich (Dorset, United Kingdom) and were used as cross-linker and radical initiator respectively. MBAA was recrystallized in water before use. Phosphate buffer saline (PBS) solution was prepared from PBS tablets purchased from Sigma Aldrich (Dorset, United Kingdom). Radio-labeled [^{14}C]-sucrose was purchased from PerkinElmer (PerkinElmer UK, Seer Green, HP9 2FX, United Kingdom).

Methods

Preparation of the gels. Cross-linked water swollen PMAA-MWNT hydrogel hybrids were prepared by *in-situ* radical polymerization with increasing MWNT concentrations (0 mg/ml, 0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml, 0.5 mg/ml) and at different cross-linker concentration ranging from 0.5 mol% to 3 mol%. The monomers MAA (2.54 ml, 3 mol.l^{-1}) and MBAM (0.5 mol%, 1.0 mol%, 2 mol% or 3 mol% relative to MAA) were added to 10ml homogenous dispersion of pristine MWNTs in water. The radical initiator potassium persulfate (1 mol% of the combined quantities of MAA and MBAA) was added and the mixtures were sonicated for 15 min to ensure complete dissolution and uniformity. The pre-polymerisation solutions were heated at 70°C for 20 hours. The blank gel was prepared under the same conditions as the hybrid gels without the presence of MWNTs. The resulting gels were immersed in water for at least a week, changing the water every day, in order to remove any unreacted monomer and initiator molecules. Afterwards, the gels were then cut into 1 cm^3 cubes and allowed to dry at room temperature for three days until they reached a constant weight.

Hybrid gel characterisation. Scanning electron microscopy (SEM) of the swollen hybrid gels was performed using FEI Quanta 200 FEG ESEM. The dried gels were immersed in PBS buffer at pH 7.3 for three days until they reached a constant weight. The swollen gels were freeze-dried in order to remove any water from the polymer matrix and to preserve the swollen microstructure of the gels. The size of the pores of each gel was determined by calculating the average size of the pores from three different SEM images using Image J software. SEM images of dry hydrogel hybrids were recorded before and after exposure to the electric voltage.

Swelling studies. Swelling studies were carried out for the hydrogel hybrids with increasing MWNT concentration (0 mg/ml, 0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml, 0.5 mg/ml) and cross-linker

concentration from 0.5 mol% to 3 mol% by immersing the dry gels in 10 ml of PBS buffer at pH 7.3 at room temperature. The weight of the swollen gels was recorded at regular intervals, until they reached a constant weight. Excess water on the surface of the gels was removed with a filter paper. The swelling degree D_s was then evaluated by using the following equation:

$$D_S = \frac{(W_t - W_0)}{W_0}$$

Where W_t is the weight of the gels at time t and $W_0$is the weight of the dry gels at time 0. $D_{s,F}$ was also defined as the final swelling degree after three days when the weight of the gels was found to be constant.

Resistance measurements. The sheet resistance R_s (Ω/square) was measured at least five times at different locations at the surface of the gels using a Multimeter (Beckman Industrial™ T100B RS component LTD). The electrical resistance was calculated using the following equation:

$$\rho = R_s \times l$$

Where ρ is the bulk resistivity of the gels (Ω.cm) and l is the thickness of the sample.

De-swelling studies under an electric field. The response to a DC electric field of the hybrid gels was monitored by weight change upon exposure to an electric voltage of 10V. Fully swollen hydrogel hybrids of a volume of 1 cm × 1 cm × 1 cm were placed into contact with two carbon electrodes with dimensions of (20× 20× 5 mm) connected to a DC power supply. Water release from the gel matrix corresponding to the percentage weight change of the gels was monitored over time for all of the hybrid hydrogels prepared at different cross-linker concentrations (0,5mol% , 1mol%, 2mol% and 3mol%). The de-swelling of the gel matrix upon exposure to the electric voltage was expressed as a percentage of weight loss over time using the following equation.

$$W_{\%} = \frac{W_t}{W_0} \times 100$$

Where W% is the percentage of change, W_t is the weight of the gel after exposure to the electric voltage at time t and W_0 is the weight of the fully swollen gel at time 0.

Electrical voltages ranging from 5V to 15 V were applied onto either control gel (cross-linker concentration of 1mol%) and hybrid gel prepared with a MWNT concentration of 0,2 mg/ml (cross-linker concentration of 1 mol%) and water release from the gel matrix was monitored overtime and expressed as percentage of weight change.

Temperature studies. The temperature of the gel matrix was monitored over time during exposure to the electric field (10V) until equilibrium was reached using a thermocouple. The change in temperature (ΔT) was plotted as a function of cross-linker concentration for all the hybrid gels (0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml and 0.5 mg/ml of MWNTs) and blank gel. The heating properties of a selected hydrogel hybrid (0.2 mg/ml MWNT content and 1 mol% of cross-linker) were measured for decreasing thickness. The volume of the gel was reduced by decreasing one dimension and the gel was placed between the two electrodes so that the distance between the two electrodes decreased with the thickness of the gels.

Release of radio-labelled sucrose [^{14}C]-sucrose. [^{14}C]-sucrose was selected as a hydrophilic drug model and was loaded into the gel matrix by immersing the dry gels in 10 ml of 3 μCi [^{14}C]-sucrose solution in PBS buffer at pH 7.3 for 3 three days, until the gels were completely swollen. The loading efficiency of the gel matrix was determined by weight difference between the dry gel and the fully swollen gel after loading the drug. Drug release was performed by inserting the radio-labelled loaded gels between a pair of carbon electrodes and immersing the gel and electrodes in a 10 mM solution of HEPES buffer at pH 7.3. A voltage of 10V was applied ON and OFF every 15 min. The release of [^{14}C]-sucrose from the hybrid gels at increasing concentration of MWNTs and cross-linker concentration was monitored by sampling 150 μl aliquots from the release medium every 5 min and the radioactive content was analysed by liquid scintillation using a LS 6500 liquid scintillator (PerkinElmer, Seer Green, HP9 2FX). The quantity of 14C-sucrose in the release medium was normalised with the amount 14C-sucrose initially loaded in the gel matrix and expressed as a percentage of the loaded 14C-sucrose.

Cell culture and cytotoxicity and adhesion studies. After synthesis, the dried gels were immersed in anti-microrganisms saline buffer (2% peniciline/streptomycine, 2% fungizone in PBS;Gibco-Invitrogen-Life technologies, UK) in sterile tissue culture treated multiplates for 7 days at room temperature. Following a bath-washing in PBS in a different tissue culture treated multiplate for 48hrs at room temperature, disinfected gels were transferred into another multiplate, immersed in complete cell culture medium (10%FBS in DMEM:F12 medium; Gibco-Invitrogen-Life technologies, UK) and incubated at 37°C (humidified CO_2 incubator) for at least one week . During this time, cell culture medium was changed every day until gels swell to their final size and pH of the medium reach 7, as indicated by the phenol red component present in the cell culture medium. A square-shaped piece of gel fitted in a well of a 12 wells plate.

After this preparation, cell culture medium was removed from the well and immediately a80μL cell suspension containing 40.000 neuroblastoma cells SHSY5Y (ATCC #CRL2266) was deposed on top of the swelled gel. After 3 hours incubation for adhesion, complete cell culture medium (10%FBS, 1%pen/strep in DMEM:F12 medium; Gibco-Invitrogen-Life technologies, UK) was added to immerse gels completely (about 1.5mL per well). Cell viability by mean of trypan blue internalization was then evaluated after a 96 hours incubation during which cells' behavior on gel was monitored via optical microscopy.

Table 1: Gel compositionand formulation

Samples[a]	**Cross-linking degree MAA/MBAM (mol%)**	**Volume of MAA (ml)**	**Mass of MBAM (mg)**	**Mass of PPS (mg)**
ASG 18	99.5:0.5	2.54	23.1	81.9
ASG 19	99:1	2.54	46.3	82.7
ASG 16	98:2	2.54	92.5	83.6
ASG 17	97:3	2.54	138.8	85.9

[a] The monomers were dissolved in 10 ml of distilled water. For the preparation of the hybrid gels the monomers were dissolved in a dispersion of MWNTs at the appropriate concentration of carbon nanotubes

Table 2: Loading content of 14C-sucrose within the hydrogel hybrids

Samples[a]	Cross-linking degree MAA/MBAM (mol%)	MWNT concentration (mg/ml)	Loading content of drug[b] (mg)
ASG 18	99.5:0.5	0	1301 ± 672
ASG 19	99:1	0	1639 ± 122
ASG 16	98:2	0	1759 ± 479
ASG 17	97:3	0	1359 ± 17
HA11	99.5:0.5	0.1	2117 ± 145
HA 15	99:1	0.1	2080 ± 108
HA32	98:2	0.1	2284 ± 232
HA73	97:3	0.1	1018 ± 204
HA12	99.5:0.5	0.2	1730 ±200
HA 14	99:1	0.2	1722.25 ±95
HA74	98:2	0.2	1487 ±20
HA75	97:3	0.2	1416 ±217

[a] ^{14}C-sucrose was selected as model of hydrophilic drug
[b] Mean ± standard deviation (n = 3 for each sample)

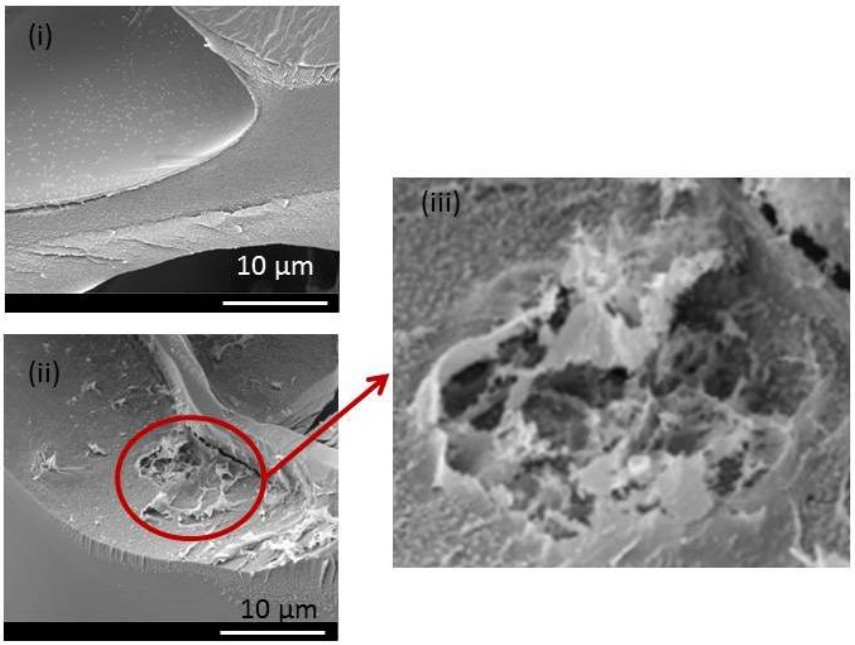

Figure 1: **Hydrogel microstructure**. Magnified SEM pictures of hybrid gel at 0.5 mg/ml of MWNTs and 3 mol% of cross-linker. Individual MWNTs were clearly visible both inside pores and in mesh walls.

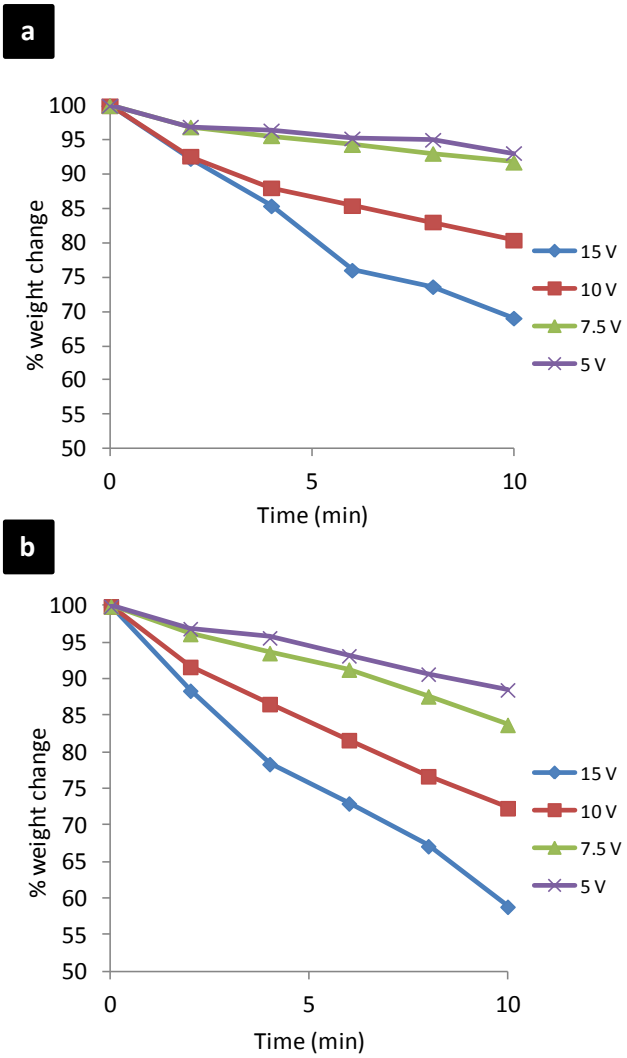

Figure 2: Effect of applied electrical voltage on gel de-swelling. Electrical voltages ranging from 5V to 15 V were applied on either **(a)** control gel (cross-linker concentration of 1 mol%) and **(b)** hybrid gel prepared with a MWNT concentration of 0,2 mg/ml (cross-linker concentration of 1 mol%). Water release from either control gel and hydrogel hybrids was dependent on the electrical voltage applied.

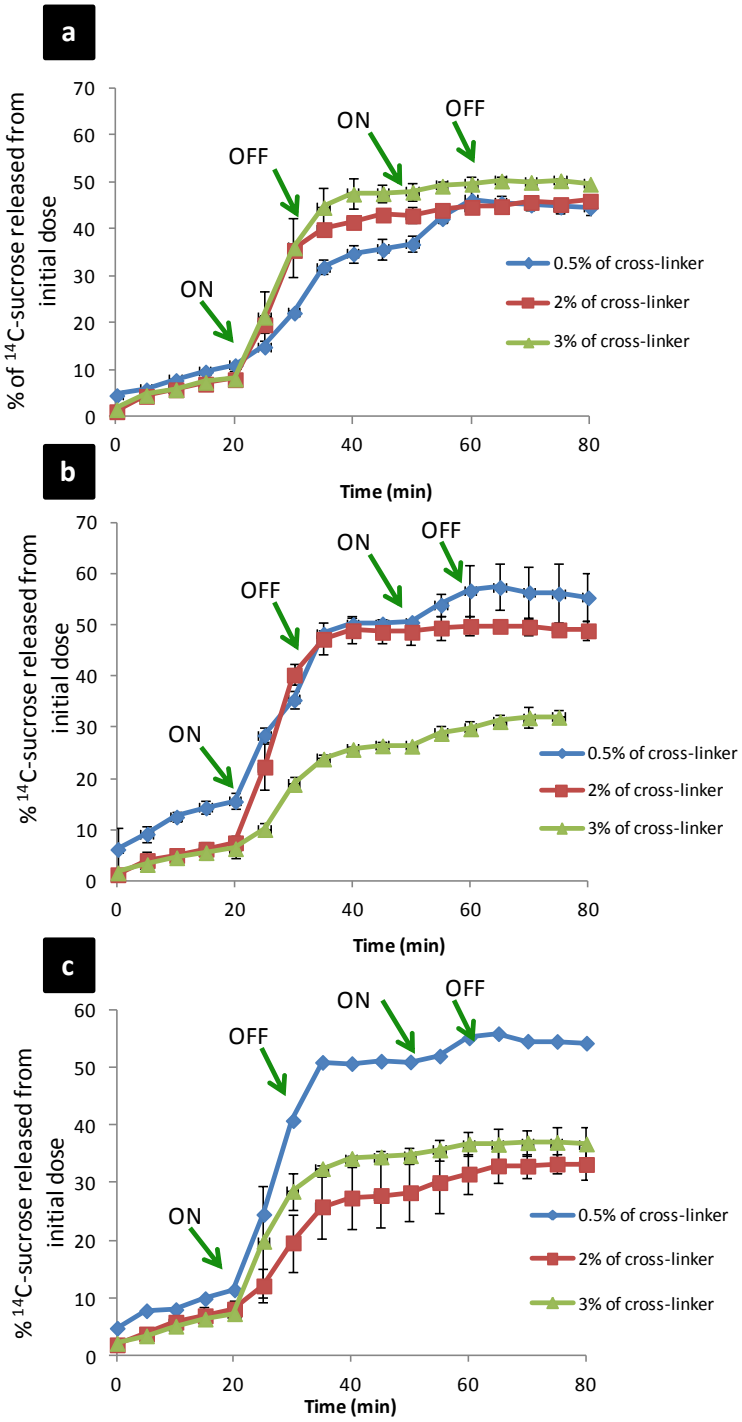

Figure 3: **Pulsatile drug release from hydrogel hybrids upon the ON/OFF application of an electrical voltage and the effect of pMWNT concentration.** ^{14}C-sucrose was selected as a model hydrophilic drug. Drug release was monitored over time while applying ON/OFF electrical field at 15 min exposure interval, illustrated by the plain arrows (ON) and dashed arrows (OFF). The pulsatile release of ^{14}C-sucrose was determined for (a) the blank gel and 2 selected hydrogel hybrids ((b) 0,1 mg/ml and (c) 0,2 mg/ml of MWNTs) at different cross-linker concentration. The increase in cross-linker concentration led to a decrease of ^{14}C-release and a loss of the pulsatile release profile.