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Design, engineering and structural integrity of electro-responsive carbon nanotube- based hydrogels for pulsatile drug release†

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Triggerable drug delivery from polymeric implants offers the possibility to generate remote-controlled drug release profiles that may overcome the deficiencies of conventional administration routes (intravenous injections and oral administration) including the toxicity due to overdose and systemic administration. An electro-responsive delivery system was engineered to deliver drug molecules in a pulsatile manner, controlled by the on/off application of electric voltage. Pristine multi-walled carbon nanotubes (pMWNTs) were incorporated into a polymethacrylic acid (PMAA)-based hydrogel matrix by *in situ* radical polymerisation. The effect of pMWNTs and cross-linker concentration on the electrical and mechanical properties of the hydrogel hybrids was thoroughly investigated. The incorporation of pMWNTs into the polymeric network improved the electrical properties of the hydrogel hybrids and drug release from the gels was significantly enhanced at high pMWNT concentrations, reaching 70% of the loaded dose after two short electrical stimulations. The presence of pMWNTs within the hydrogel matrix affected however the mechanical properties of the hydrogel by decreasing the pore size and therefore the swelling/de-swelling of the gels. The damage to the hybrid gel surfaces after electrical stimulation and the loss of the pulsatile release profile at high cross-linker concentrations suggested that the mechanism of drug release involved a compressing effect and intensified the stress on the polymeric network as a result of the electrical properties of pMWNTs.

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Introduction

Over the past two decades, controlled drug release from polymeric implants has attracted a lot of attention as an alternative to the conventional routes including oral administration and injections. Oral routes and injections (intravenous or intraperitoneal) allow the release of the maximum tolerable dose of drugs, however the amount of therapeutic agents reaching the target is often very low due to fast pharmacokinetics and clearance rates.[1,2] In contrast, polymeric implants allow a controlled drug delivery over long periods of time (up to months or years), provide an improvement in patient's compliance, and may help in achieving the targeted release of therapeutic agents with reduced toxic side effects.[3] To date, polymeric stimulus-responsive implants have been mainly engineered to produce a constant drug release achieving a zero-order release, which is not adequate for chronic disorders or diseases that require repeated administration.[4–7] One of the main drawbacks of this type of polymeric implant for generating pulsatile drug release profiles is related to the fact that after the first stimulation the implant would have released the whole content of the drug reservoir or would be too damaged to respond once again.[8–11] The development of implantable stimulus-responsive devices with higher mechanical capabilities that could achieve switchable and repeatable drug release appears therefore to be the best alternative.

Different types of external triggers for remote-controlled drug delivery have been explored to trigger drug release. In the form of field-based stimuli ultrasound,[12] radio-frequency,[13] magnetic,[14,15] electrical field[16] or near infra-red (NIR) illumination[17–19] have all been used. Hydrogels or hydrogel-based materials have been extensively studied as polymeric implants for controlled drug release due to their proven biocompatibility and their ability to respond to external stimuli such as temperature, pH or electrical field.[20] Also, the use of hydrogel matrices combined with field-responsive nanomaterials including carbon nanotubes,[21] iron oxide nanoparticles,[22] or metallic nanorods[23–25] to develop polymeric implants with multifunctional capabilities has been explored.

The application of an electric field as an external stimulus has been shown to be efficient and accurate in triggering drug release with good control of both the number of molecules released and the release rate by simply adjusting the voltage

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applied.[26] Electro-responsive systems have been developed using hydrogel matrices where pulsatile release profiles could be obtained upon the on/off application of an electric field.[27–30] However in many cases, the percentage of total drug release reached only a maximum of 50% of dose, even after several cycles of on/off electrical stimulation. In addition, the response to the electrical field was not sharp enough and an increase in drug release was obtained only after a minimum of 15 min of electrical field exposure as a consequence of the slow kinetics of the swelling/de-swelling process.

In addition to their numerous physical properties, pristine multi-walled carbon nanotubes (pMWNTs) are known to possess excellent electrical conductivity and to enhance synergistically the mechanical and electrical properties of polymer composites. These properties have been mainly exploited today for the development of actuators.[31–33] The combination of a hydrogel-based matrix with pMWNTs could lead to the generation of a novel class of highly electro-responsive materials that exhibit enhanced and sharper drug release profiles and improve the conductivity in an insulating type of polymeric material.

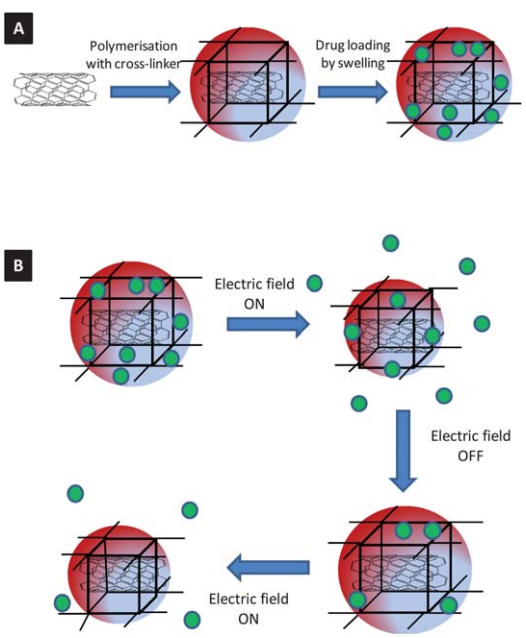
A limited number of studies have proposed the use of polymer–carbon nanotube hybrid gels as implants for controlled delivery, mainly focusing on the development of transdermal drug release. Wu *et al.* reported the fabrication of a carbon nanotube based membrane for programmable transdermal drug release of nicotine.[34] Controllable amounts of nicotine could be released upon the application of −300 mV field as a result of the presence of the carbon nanotubes. However, the release of useful amounts of nicotine was obtained only after the application of a 20 hour long electrical field. Such a pulsatile system would not be appropriate for the treatment of chronic diseases, whereby high concentrations of therapeutic agents are required within a short period of time. Similarly, Im *et al.* developed an alternative transdermal system based on polymeric hydrogels, using pMWNTs as additives to increase the electro-sensitivity of the matrix.[35] The amount of released drug was effectively increased for the hydrogel composites that contained pMWNTs. However, the percentage of total drug release of 70% could only be reached after 10 hours of constant electrical stimulation at a high voltage (15 V). The long-term application of an electrical field in that voltage range would lead to excessive heating and is not considered suitable for *in vivo* studies, as it could lead to tissue necrosis.

We have previously developed electro-responsive pMWNT–poly(methacrylic acid) (PMAA) hydrogel hybrids for 'on-demand' drug delivery.[36] The release of a model drug upon the application of a DC electric field was demonstrated *in vivo* following sub-cutaneous implantation of the hydrogel hybrids in a mouse model. This drug delivery device gave interesting results, greater amounts of drug were released from gels with high pMWNT contents; however the amount of drug released between each cycle of on/off electrical stimulation was not reproducible and decreased dramatically after the second electrical stimulation. Producing an adherent hydrogel hybrid is not trivial as the reversible swelling and de-swelling of the polymer matrix upon electrical exposure can lead to appreciable levels of shear stress that can result in structural damage. Although carbon nanotubes have been commonly used as additives to enhance the physical properties of various polymeric matrices, the mechanisms by which they improve these electrical, mechanical and thermal properties and their potential use in polymeric implants for controlled drug delivery systems have not yet been clearly investigated. In order to address these issues and to identify the optimal design parameters to achieve high and reproducible drug concentrations released repeatedly on electrical stimulation, the effect of varying pMWNT and cross-linker composition on the structural integrity of the matrix after successive electrical stimulations was investigated. We report in this study the engineering of PMAA–pMWNT hydrogel hybrids by varying pMWNT and cross-linker concentrations and the subsequent electrical, mechanical and thermal properties of the hybrid systems. The ability to release efficiently radio-labelled sucrose as the model hydrophilic drug molecule upon successive electrical stimulation using a DC electric field was also investigated.

Results

Preparation and characterisation of hydrogel hybrids

pMWNT–PMAA hydrogel hybrids were engineered for fabrication of an electro-sensitive drug delivery system with enhanced release properties as the result of the incorporation of pMWNTs within the hydrogel matrix as shown in Scheme 1A. This 'on-demand' delivery system was designed to release drug molecules in a pulsatile manner upon the on/off application of an electrical field in a similar manner to our previous study as illustrated in Scheme 1B.[36]



Scheme 1 pMWNT–PMAA hydrogel hybrids for pulsatile drug release. (A) Preparation of the pMWNT–PMAA hydrogel hybrids: methacrylic acid (MAA), *N*,*N*′-methylene bisacrylamide (BIS) and potassium persulfate were used as a backbone monomer, a cross-linker and an initiator, respectively. (B) Pulsatile drug release upon electrical stimulation.

The hybrid hydrogels were successfully prepared by *in situ* radical polymerisation in the presence of pristine multi-wall carbon nanotubes (pMWNTs) dispersed in distilled water. The use of surfactants such as sodium dodecyl sulfate (SDS) or sodium dodecyl benzenesulfonic acid sodium salt (SDBS), widely employed to facilitate the aqueous dispersibility of carbon nanotubes,[37] was discarded to minimize the parameters that may affect the gel characteristics. Transiently homogeneous carbon nanotube dispersions could be obtained in water after 30 min of bath sonication. The concentration of carbon nanotubes used for the gel synthesis ranged from 0 mg ml^{-1} to 0.5 mg ml^{-1} (Table S1†). Fig. S2† displays the magnified SEM pictures of the blank and hybrid gels (0.5 mg ml^{-1} of pMWNTs) prepared at 2 mol% of cross-linker. In the case of the hybrid gels, individual pMWNTs could be clearly seen in either an empty space inside a pore or within the mesh walls. This confirmed the successful incorporation of carbon nanotubes into the gel matrix. Above the concentration of 0.5 mg ml^{-1} of pMWNTs, the pre-polymerisation mixture became very unstable and aggregation occurred almost immediately after the start of the polymerisation process. Hybrid gels at different concentrations of cross-linker ranging from 0.5 mol% to 3 mol%, relative to the quantity of methyl methacrylic acid (MAA) monomer, were prepared. In order to evaluate the effect of pMWNT and cross-linker concentrations on the architecture of the gel matrix, its microstructure was investigated. Fully swollen gels obtained after three days of immersion in PBS buffer at pH 7.3 were freeze-dried and SEM images were obtained.

Fig. 1A shows the SEM images of hydrogel hybrids at two different concentrations of pMWNTs (0.05 mg ml^{-1} and 0.5 mg ml^{-1}) and of a blank hydrogel without carbon nanotubes. The cross-linker concentration was varied from 0.5 mol% to 3 mol %. The images of all of the gels, including hybrids, confirmed the presence of abundant pores with a size above 100 μm, which are typical of a macro-porous methacrylic acid-based hydrogel matrix in their swollen state.[38]

The size of the pores was measured for all the hybrid gels and blank gel from three different SEM images for each gel using ImageJ software (Fig. 1B). The size of the pores decreased significantly from 314 μm (S.E ± 45 μm) to 134 μm (S.E ± 32 μm) for the blank gel when increasing the cross-linker concentration from 0.5 mol% to 3 mol%. The same trend was observed for all of the hybrid gels with different amounts of pMWNTs. A significant decrease in pore size was observed at low cross-linker concentration (0.5 mol% and 1 mol%) with increasing the pMWNT concentration. This effect was not observed at high concentration of cross-linker (above 2 mol%).

Mechanical properties of the hybrid gels

The swelling degree of all the gels was determined at room temperature in PBS buffer at pH 7.3 that was selected to mimic physiological conditions. At this pH, PMAA-based hydrogels are known to reach complete swelling.[39] The final swelling $D_{s,F}$ was expressed as a function of cross-linker and pMWNT concentrations. The data are shown in Fig. 2.

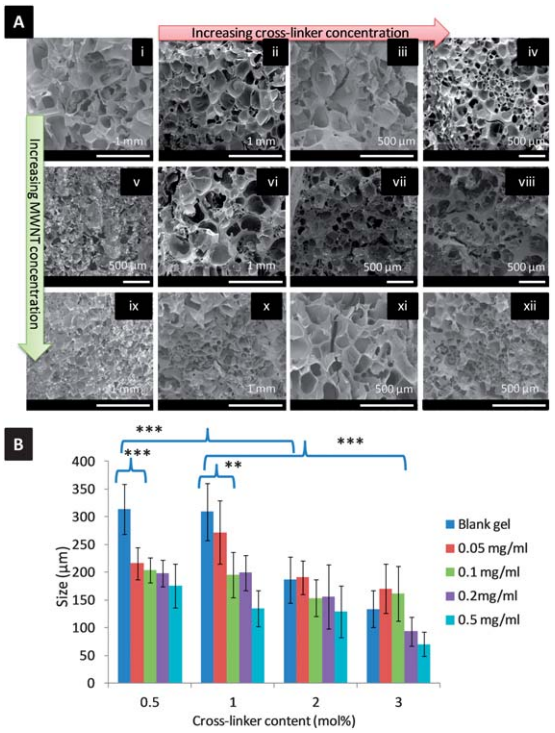


Fig. 1 Swollen hydrogel microstructure. (A) SEM images of the freeze-dried swollen hydrogels prepared at different concentrations of cross-linker 0.5 mol%, 1 mol%, 2 mol%, and 3 mol%: (i–iv) blank gel; (v–viii) hybrid gel at a pMWNT concentration of 0.05 mg ml^{-1}; (ix–xii) hybrid gel at a pMWNT concentration of 0.5 mg ml^{-1} and (B) pore size of swollen hybrid hydrogels at different concentrations of pMWNTs and with increasing cross-linker concentration from 0.5 mol % to 3 mol%. The size of the pores in each gel was determined from three different SEM images using the software ImageJ.

Increasing cross-linker concentration significantly decreased the swelling degree of the gels from approximately 10 at 0.5 mol % of cross-linker to approximately 3 at 3 mol% of cross-linker. The concentration of pMWNTs appeared to decrease insignificantly the swelling degree of the gels in each group.

Electrical properties of the hybrid gels

The bulk resistivity of the gels with various amounts of pMWNTs and cross-linker concentration was calculated from

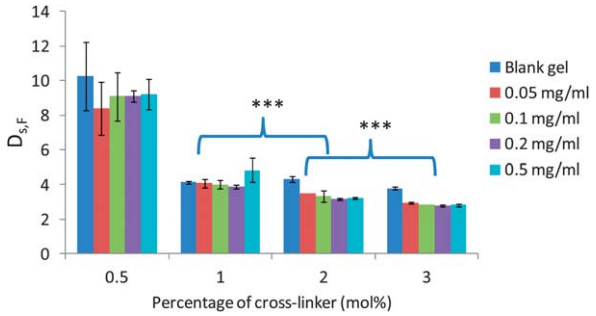


Fig. 2 Swelling properties of the hybrid gels. The final swelling of the gels was expressed as $D_{s,F}$, measured when the gels reached a constant weight and plotted *versus* the cross-linker concentration for all hybrids.

the measurements of the sheet resistance R_s on pre-cut 1 × 1 × 1 cm cubes of water-swollen gels, taking into consideration the distance between the electrical probes (Fig. 3).

Increasing the concentration of cross-linker seemed to decrease slightly the bulk resistivity of all the hybrid gels and blank gel, however the values of bulk resistivity reached a plateau for a concentration of 2 mol% and higher. At low cross-linker concentration (0.5 mol% and 1 mol%), increasing the pMWNT content appeared to significantly decrease the bulk resistivity of the gels, reaching a plateau for a pMWNT concentration of 0.1 mg ml^{-1}. At high concentration of cross-linker (2 mol% and 3 mol%) the incorporation of pMWNTs seemed to have no effect on the bulk resistivity of the hybrid gels.

The heating properties of the hybrid gels were evaluated by monitoring the temperature of the gels during exposure to the electrical field. These studies are considered critical for any *in vivo* translation of the system. The temperature of the gels should not increase above 42 °C, at which point tissue necrosis may start occurring. The change in temperature (ΔT) of the gels at different concentrations of pMWNTs and cross-linker was measured at room temperature during exposure to 10 V until equilibrium was reached (Fig. 4A). The change in temperature (ΔT) obtained for the gels ranged from 13 °C to 22 °C. Overall, increasing cross-linker concentration did not seem to have an effect on the heating properties in the gels. The temperature increase seemed to be greater at high concentrations of pMWNTs (above 1 mg ml^{-1}). In addition, the thickness of the gels appeared to have a significant effect on the heating properties of the gels. The change in temperature (ΔT) was measured for a 1 cm × 1 cm × 1 cm hybrid gel block prepared at 0.2 mg ml^{-1} of pMWNTs and 2 mol% of cross-linker and gradually reducing one dimension of the gels from 1 cm to 0.2 cm. As shown in Fig. 4B & C, the change in temperature decreased from 12 °C to 6 °C.

The de-swelling of the hydrogel hybrids upon exposure to 10 V was studied under aerobic conditions. All the gels were swollen until an equilibrated state was reached. Water release from the gel matrix was expressed as a percentage of weight change. The percentage of weight change for all of the gels at different pMWNT and increasing cross-linker concentrations was measured over 10 min (Fig. 5). Increasing the concentration of cross-linker significantly reduced the de-swelling of gels upon exposure to the electric field for all of the gels investigated. The percentage weight loss that reached 70% for the hybrid gels prepared at 0.5 mg ml^{-1} of pMWNTs and at 0.5 mol% of

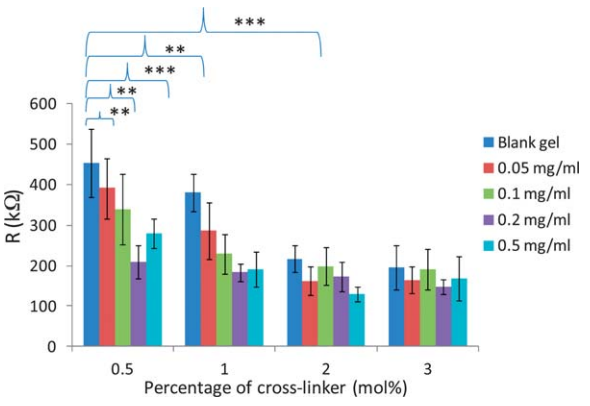


Fig. 3 Characterisation of the electrical conductivity of the hybrid gels: bulk resistivity R of all hydrogel hybrids of a volume of 1 cm^3 at different cross-linker concentrations (from 0.5 mol% to 3 mol%): the bulk resistivity was determined as the product between the thickness of the sample and the sheet resistance R_s.

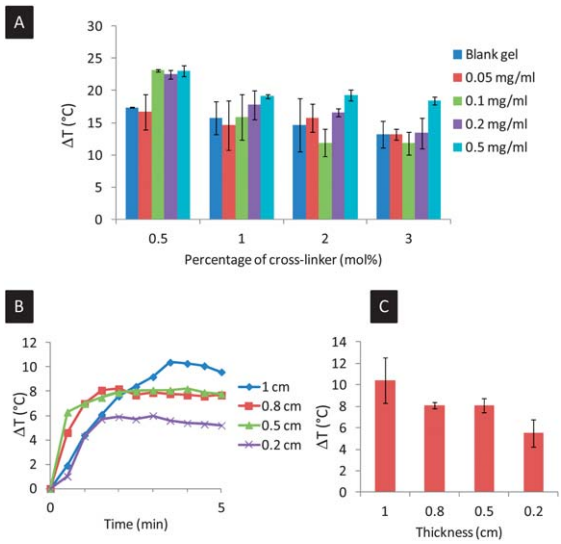


Fig. 4 Heating properties of the hybrid gels. (A) Heating properties of the gel hybrids: the temperature of the gel matrix was monitored over time during exposure to the electric field (10 V) until equilibrium was reached. The change in temperature (ΔT) was plotted as a function of cross-linker concentration for all hybrid gels. (B) Temperature profile over time of a selected hybrid gel prepared at 0.2 mg ml^{-1} of MWNTs and 2 mol% of cross-linker exposed to 10 V. (C) Temperature profile *versus* the thickness of the sample.

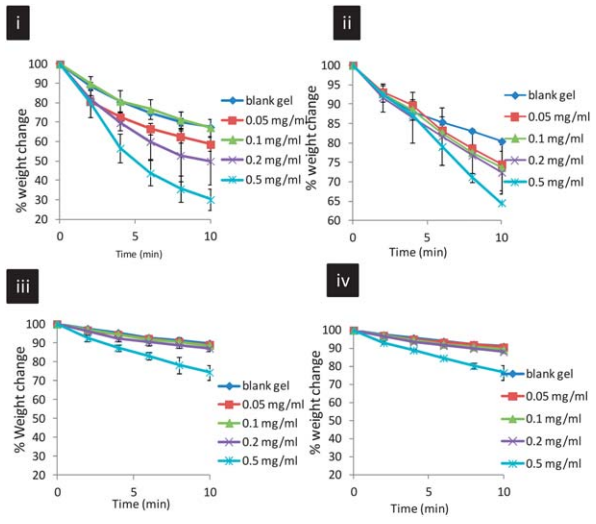


Fig. 5 Gel response upon exposure to an applied electrical voltage (10 V); de-swelling of hydrogel hybrids upon the application of an electrical voltage. Water release from the gel matrix corresponding to the percentage weight change of the gels was monitored over time for all hydrogels prepared at different cross-linker concentrations: (i) 0.5 mol%, (ii) 1 mol%, (iii) 2 mol% and (iv) 3 mol%.

cross-linker only reached 35%, 25% and 20% at 1, 2 and 3 mol% of cross-linker respectively. In addition, at a fixed cross-linker concentration, the de-swelling of gels upon electric field exposure was enhanced with increasing the concentration of pMWNTs.

Pulsatile drug release upon electrical stimulation

With the aim of optimising the electrically induced release behaviour of drugs from different gel hybrids, drug release experiments were performed on two different hybrid gels at 0.1 mg ml^{-1} and 0.2 mg ml^{-1} of pMWNT concentration and on the blank gel as control with increasing cross-linker concentration from 0.5 mol% to 3 mol% (Fig. 6).

Radio-labelled sucrose was selected as a model hydrophilic drug. The ^{14}C-sucrose release profile from the gels was obtained upon the on/off application of 10 V at 15 min exposure intervals. Radio-labelled ^{14}C-sucrose was loaded into the polymer matrix by swelling. The amount of ^{14}C-sucrose loaded in the gel was determined by weight difference between the swollen gel and the dry gel (Table S2†). For all of the gels prepared at low cross-linker concentration (0.5 mol% and 1 mol%) a pulsatile release profile was observed, while for higher concentrations of cross-linker, such as 2 mol% and 3 mol%, the pulsatile release profile was lost. At high concentrations of cross-linker, a rapid increase in the release of ^{14}C-sucrose was observed for all the gels as a result of the application of the electrical pulse. Upon the second application of the electric voltage no increase in ^{14}C-sucrose release was observed. It was apparent that the gels had lost their responsiveness to the electrical field. This is correlated with the reduced release of ^{14}C-sucrose at 2 mol% and 3 mol% of cross-linker.

In order to understand the mechanism of drug release from the gels and in particular the loss of their pulsatile capabilities

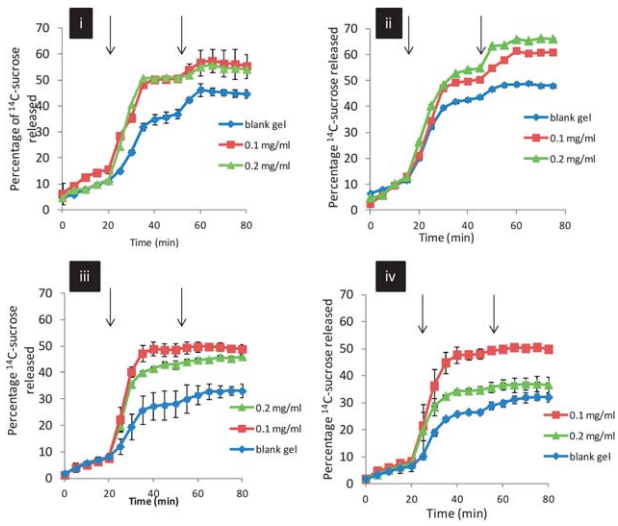


Fig. 6 Pulsatile drug release from hydrogel hybrids upon the on/off application of an electrical voltage. The pulsatile release of ^{14}C-sucrose was determined for the blank gel and the selected hydrogel hybrids (0.1 mg ml^{-1} and 0.2 mg ml^{-1} of MWNTs) at: (i) 0.5 mol%, (ii) 1 mol%, (iii) 2 mol% and (iv) 3 mol% of cross-linker. The black arrows indicate when the electric field is switched on. The electric field is then switched off after 15 min.

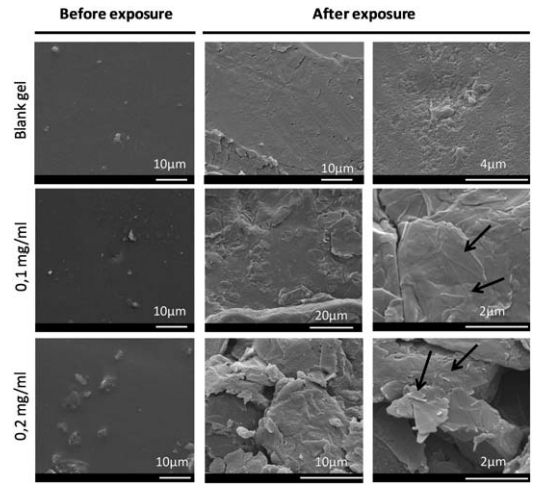


Fig. 7 Effect of electrical exposure on the gel microstructure: SEM images of the hybrid gels before and after exposure to the electrical voltage; blank gel, 0.1 mg ml^{-1} of MWNTs, 0.2 mg ml^{-1} MWNTs at 1 mol% of cross-linker. The exposure to the electrical voltage led to significant structural damage of the hybrid gels compared to the blank gel.

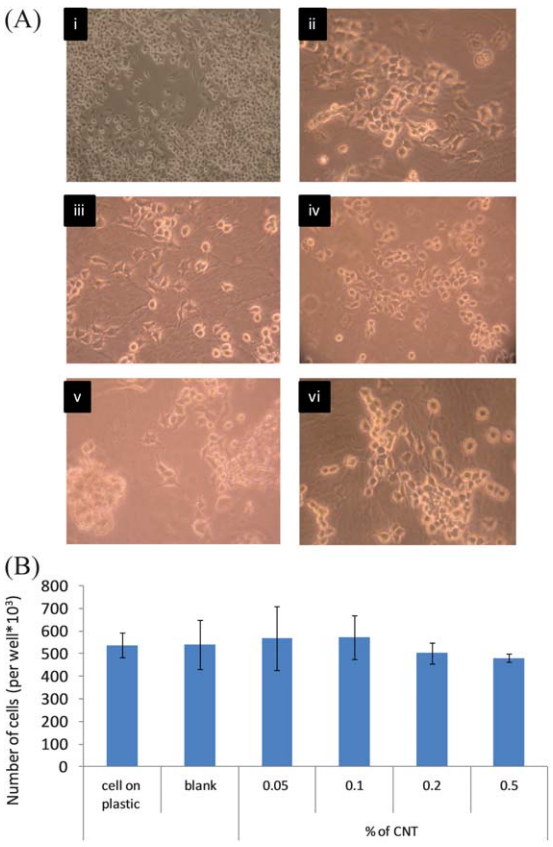


Fig. 8 Biocompatibility of hybrid gels. (A) Microscopy images of SHSY5Y cells after 96 hours of interaction with gels: (i) on a plastic surface; (ii) on blank gel-coated surfaces; (iii) on hybrid gel-coated surfaces containing 0.05 mg ml^{-1}; (iv) 0.1 mg ml^{-1}; (v) 0.2 mg ml^{-1}; and (vi) 0.5 mg ml^{-1} of MWNTs. The cells were cultured in normal cell culture media at 37 °C and 5% CO_2. (B) Cell viability of hybrid gels by counting cells stained with trypan blue.

at high concentrations of cross-linker, the gel surface before and after exposure to the electric field was studied. Fig. 7 shows the SEM images of the surface of dry gels prepared with 1 mol% of cross-linker (blank gel, 0.1 mg ml^{-1} pMWNTs and 0.2 mg ml^{-1} pMWNTs) before and after exposure to the electric voltage of 10 V for 15 min. The exposure to the electrical field led to significant damage in the gel structure. The damage was found to be more significant for the hybrid gels than for the blank gel. The presence of carbon nanotubes could not be clearly identified after exposure to the electrical field, however some individualised carbon nanotubes may be observed as straight lines (Fig. 7 black arrows) after exposure that were not present on the blank gel surface.

Biocompatibility of the hybrid gels

The biocompatibility of the gels was assessed by performing cytotoxicity studies. Human neuronal cell monolayers (SHSY5Y) were cultured on dishes coated with the gels in normal cell culture media and imaged with an optical microscope after 96 hours (Fig. 8A). No signs of toxicity or morphological and shape alterations were noticed on the cells. Cell viability was further assessed by counting cells deposited on the gel surface treated with trypsin and trypan blue (Fig. 8B). Exposure and culture to all gels showed high cell viability, an initial indication that confirmed that both the blank and hybrid gels were all biocompatible.

Discussion

Despite the growing interest in developing polymeric implants for 'on-demand' drug delivery, only a limited number of papers report the successful and remote-controlled release of therapeutic drug dose. Several studies have reported the use of conductive polymers such as polypyrrole or conductive additives such as carbon nanotubes inserted in a gel matrix for the fabrication of electro-responsive drug delivery devices.[9,40–44] These studies demonstrated the great potential of using such sophisticated materials capable of releasing drugs *in vitro* and *in vivo* as an alternative to traditional routes of drug administration.

In particular, in our previous study, we developed a pristine pMWNT–PMAA hydrogel hybrid system, where the use of pMWNTs improved the methacrylic acid-based hydrogel responsiveness to the electric field. This system showed encouraging results, in particular, in achieving pulsatile drug release profiles *in vivo*, detectable in mouse blood following subcutaneous implantation and on/off application of a DC electric field. However, this 'on-demand' delivery system displayed some important drawbacks such as a lack of reproducibility between each cycle of on/off electrical stimulation in drug release. A temperature increase of the overall gel upon exposure to the field was also observed. This temperature increase also known as 'resistive heating' could be explained by the high impedance of the poly(methacrylic acid) polymeric network. To the best of our knowledge, these issues that prevent any potential clinical translation have never been addressed in such systems. In order to overcome these problems, more thorough studies on the effect of pMWNTs and cross-linking content on the gel structure and response to the electric field are required.

In the present study, homogenous pMWNT–PMAA hydrogel hybrids were successfully prepared at different pMWNT and cross-linker concentrations in order to investigate the role of these two parameters in the gel structure and their response to electrical stimulation. This study aimed at optimising the successive release of therapeutically relevant drug doses upon the on/off application of the electric field and at providing an understanding of the mechanisms involved in the hybrid gel response.

Several factors are involved in the performance of hydrogel hybrids in releasing drug molecules in response to the electrical field such as the porous structure, the flexibility and the electrical properties of gel matrix.

One of the important parameters in the preparation of electro-responsive hydrogels is the porous structure of the gels. This parameter along with the flexibility of the gel matrix is critical to the mechanical properties of the gel matrix. The porous structure and in particular the pore size is related to water absorbency and influences the rate of swelling/de-swelling of the gel matrix. It was reported that larger pores increased significantly the rate of swelling/de-swelling of hydrogels.[45] Super-porous hydrogels with a pore size above 10 μm are able to reach complete swelling within minutes.[46,47] As a consequence, a decrease in the pore size would result in a decrease in the swelling/de-swelling rate that can compromise a pulsatile release profile.

The other parameter that affects the mechanical properties of hydrogels is their swelling degree. This is directly linked to the electrostatic repulsion between ionised groups of PMAA hydrogels. The results demonstrated that pMWNTs incorporated in the gel matrix do not interfere with the electrostatic interactions between the carboxylate groups. In fact a recent study demonstrated that well-incorporated carboxylated MWNTs could improve the swelling capabilities of pH-sensitive polyacrylamide-based hydrogel hybrids.[48] Nevertheless, the cross-linker concentration has a significant effect on the swelling/de-swelling properties of PMAA-based hydrogels by creating a tight network between the PMAA chains and reducing their flexibility and motion within the polymeric matrix.[49] Flexible hydrogel matrices with the ability to swell/de-swell quickly in response to the electrical stimulation were required for the system designed here. This meant that the optimum hydrogel composition would combine low cross-linker content and large pore size. The release profile data provided in Fig. 6 support the fact that gels with low concentrations of cross-linker (below 1 mol%) and large pore size are able to respond to several cycles of on/off electrical stimulation. At high concentrations of cross-linker and pMWNTs, the pulsatile release profile was lost, and the gels could not respond to a second electrical stimulation.

The conductivity of the polymeric matrix was significantly increased with the presence of the carbon nanotubes. This enabled an improved transfer of charge within the gel matrix that is crucial for the de-swelling mechanism of methacrylic

acid-based hydrogels upon electrical stimulation.[39] This important finding allows the application of lower electric voltages for the release of therapeutic doses of drugs using such hydrogel platforms. This was a major criterion in our thinking for possible *in vivo* translation of this system, since the use of electric voltages higher than 10.5 V could lead to unwanted tissue damage.[50]

In addition, multi-walled carbon nanotubes are known to be responsive to an applied electric voltage. Recently the mobility and orientation of pMWNTs in a polymer solution upon exposure to an electric field were reported.[51] Multi-walled carbon nanotubes were found to align themselves and to form an angle of 30° to the anode under a DC electrical stimulation. This tendency of the tubes to align combined with the high viscosity of the hydrogel matrix enhanced the expected anisotropic deformation of the gels at the anode under electrical stimulation. This led to an increase of water released from the hydrogel hybrid matrix. We hypothesize that this additional 'squeezing' of the gel matrix allowed the hybrids to overcome the decrease in their mechanical properties as a result of smaller pore size. However, the resulting stress exerted on the matrix upon electrical stimulation (more pronounced at high pMWNT concentrations) caused significant structural damage that further compromised its response following the first electrical stimulation. This was also evidenced from the ^{14}C-sucrose release profiles obtained from the hybrid gels that were not reproducible from cycle-to-cycle. We therefore suggest that the addition of pMWNTs into the PMAA hydrogel improved drug release by 'squeezing' of the gel matrix as a consequence of their alignment towards the anode. This is worsened at high cross-linker concentration, where the viscosity of the gel matrix becomes the dominant factor, leading to the self-destruction of the gel matrix.

The movement of pMWNTs upon electrical stimulation was also thought to be involved in the increased gel heating during exposure to the electrical field, in addition to Joule heating. The dependence between temperature increase and the alignment of semi-conducting nanoparticles (such as pMWNTs) in aqueous media has been recently reported.[52] The alignment of carbon nanotubes generated a 'resistive' heat that increased the temperature of the gel matrix upon the application of the electrical field. Once the temperature reaches values higher than 42 °C they would not be suitable for *in vivo* applications. This represents a major issue for the pre-clinical translation of all such electro-responsive systems. The degree of temperature increase could however be reduced by decreasing the thickness of the gel investigated, as final temperatures between 36 °C and 40 °C were observed for gels of a thickness of 0.2 cm upon electrical stimulation.

The studies of biocompatibility and cell viability for the hydrogel hybrids at all concentrations of pMWNTs confirmed that the hybrid gels remained non-toxic despite the incorporation of pMWNTs. The carbon nanotubes were encapsulated in a biocompatible gel matrix that prevents their direct exposure to cells or tissues. In addition, there was no evidence of release of pMWNTs in the release medium, confirming that pMWNTs were tightly enclosed in the gel matrix and could not escape.

This system represents great potential for the development of 'on-demand' delivery systems as it allows the remote controlled release of high quantity of drugs in a short amount of time and in the targeted area. However, further optimisation needs to be performed in order to obtain repeatable amounts of drug release after each cycle of electrical stimulation. The generation of an electro-responsive hydrogel hybrid with higher mechanical capabilities using carbon nanomaterials needs to be investigated further in order to overcome the rise of temperature and the structural deformities of the gel upon stimulation. Improving the pMWNT dispersion in the polymer matrix or the use of another carbon nanomaterial with higher electrical and thermal properties could be possible routes to follow.

Conclusions

An electro-sensitive drug delivery system was successfully prepared by '*in situ*' free radical polymerisation of methacrylic acid monomer in pMWNT dispersions in water. The incorporation of pMWNTs in the PMAA based hydrogels significantly enhanced the gel response to the applied electric field. A pulsatile release profile was obtained upon the on/off application of the electric field, and total drug release from the hybrid gels up to 70% was achieved after 80 minutes of on/off exposure to the electric field. This improvement compared to the blank gel resulted from an enhanced anisotropic de-swelling of the gel matrix upon exposure to the electric field due to the response of pMWNTs upon DC electric field stimulation.

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