Electroresponsive Polymer-Carbon Nanotube Hydrogel Hybrids for Pulsatile Drug Delivery In Vivo

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Controlled drug delivery from polymeric implants has attracted significant attention over the last few decades in an attempt to minimize possible adverse reactions from systemic (oral, intravenous) administration.[1–3] The majority of polymeric implants today achieve zero-order release, which is a release at a rate that is independent of time and the concentration of drug. This is not acceptable for many therapeutic applications. Many disorders could benefit from pulsatile drug delivery, particularly post-surgery pain relief or treatment of infections.[4,5] On-demand drug delivery systems, where an external trigger is employed to release a drug from an implant, could offer remote controlled drug release according to patient needs. This mode of administration could significantly improve drug safety, patient compliance, possibly minimize resistance to medication, improve overall drug efficacy, and help design more personalized treatment modalities and protocols.[6]

In order to achieve drug release from a non-invasive external trigger, many efforts have been invested in the development of "smart" materials that can respond to field-based stimuli such as ultrasound, magnetic, near infrared (NIR), radio-frequency, and electrical fields.[7] Electroresponsive materials have attracted great attention as potential remote-controlled delivery systems. Indeed, the use of an electrical field offers the possibility to accurately regulate drug release levels according to the strength of the field applied. Popular smart materials, such as hydrogel-based polymers, have seen their properties and sensitivity to external stimuli enhanced by the use of nanoparticulate additives such as iron oxide,[8] gold,[9] silver[10] or carbon nanotubes.[11] Carbon nanotubes as additives in hydrogel preparation for the fabrication of electroresponsive systems have been previously explored for the development of actuators and biosensors, greatly enhancing the electrical and mechanical properties of hydrogel.[12–15] However, only a limited number of studies have utilized the electrical properties offered by carbon nanotubes in an electrosensitive matrix, such as polyelectrolyte hydrogels, as polymeric implants for controlled drug delivery and generally they reported zero-order release.[16] To date, only one study has reported on the in vivo use of electroresponsive hydrogels (without carbon nanotubes) for pulsatile drug release.[17] The in vivo profile, including pharmacokinetic data, of such pulsatile drug release systems based on electroresponsive hydrogel matrices is still needed.

Here, we have developed an electroresponsive polymer hydrogel containing pristine multiwalled carbon nanotubes (pMWNT) for pulsatile drug release as illustrated in **Figure 1**A. Radio-labelled sucrose was selected as a model hydrophilic small drug molecule for the determination of the release profile. Our hypothesis is that this system will combine the electrosensitivity of a poly(methylacrylic acid) (PMAA)-based hydrogel matrix with the enhanced electrical conductivity of pMWNTs for improved responsiveness to the electrical field.

PMAA/pMWNT hydrogel hybrids were successfully prepared by in situ radical polymerization, which consisted of adding the monomer (methacrylic acid (MAA)) and the cross-linker (*N,N'*-methylene bisacrylamide (BIS)) in an aqueous dispersion of pMWNT without the use of any added surfactants. Hybrid gels were prepared at different pMWNT concentrations (Figure 1B) and showed a homogeneous morphology at the macroscopic level, however the gels containing the highest concentration of pMWNTs (0.5 mg/mL) exhibited a degree of carbon nanotube aggregation at the bottom of the gel. Surface characterization of the gels was performed using scanning electron microscopy (SEM) to confirm that the pMWNTs were well-incorporated into the polymeric network (Figure 1C). The carbon nanotube backbone did not seem to have been damaged during the polymerization process. This was confirmed by collecting the Raman spectra of the carbon nanotubes incorporated into the gel matrix. The G/D peak ratio was not significantly changed on comparison with free pMWNT dispersed in water (Supporting Information Figure 1). An increase in the roughness of the gel surface was observed as the pMWNT concentration increased, due to nanotubes not being fully individualized throughout the polymer matrix but rather arranging themselves in clusters.

The electrical responsiveness of the hydrogel hybrids was then evaluated. The first parameter investigated was the swelling degree of the gels. The gel swelling degree (D_s) was monitored over time and the final swelling degree ($D_{s,F}$) the point at which the gels were saturated, was determined as shown in **Figure 2**A(i,ii). The incorporation of pMWNTs into the gel matrix did not have any significant effect on the swelling degree (all gels displayed a $D_{s,F}$ value of ≈4). The next parameter investigated was the release of water upon gel de-swelling as described in Figure 2A(iii). There was improvement in the gel response as the pMWNT concentration increased from 0 to 0.5 mg/mL that correlated with increased conductivity of the gels. The gel responsiveness to the applied electrical field was also voltage-dependant and water release was greater as the potential difference increased from 5 V to 15 V (Supporting Information

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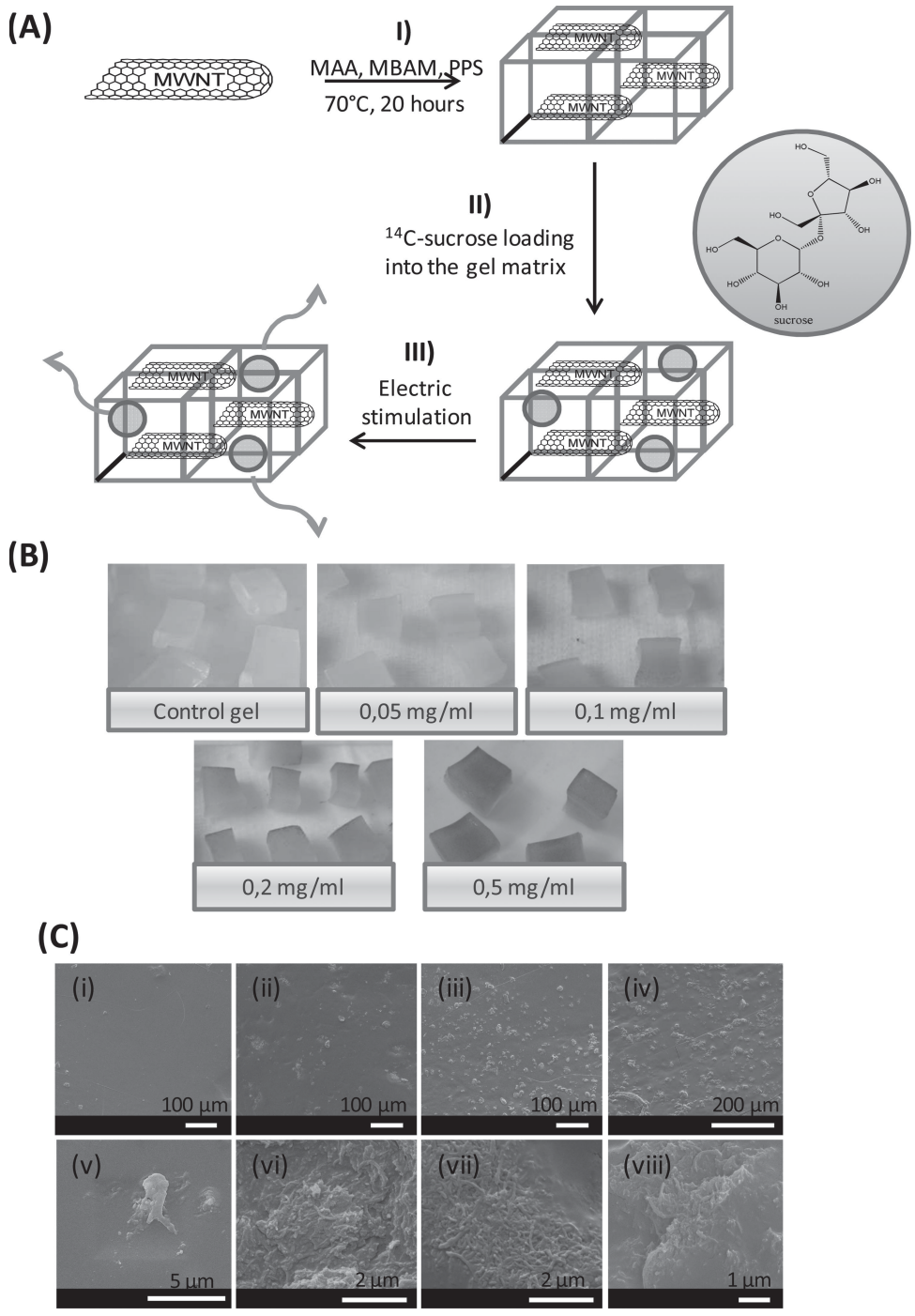

Figure 1. A) Preparation of poly(methacrylic acid)-pMWNT hydrogel hybrids and proposed mechanism of drug release upon electrical field application: I) Synthesis of PMAA-pMWNT hydrogel hybrids. In-situ radical polymerization (70 °C; 20 h) was used in the presence or absence of pMWNTs. Methacrylic acid (MAA), *N*,*N*′-methylene bisacrylamide (MBAM) and potassium persulfate (PPS) were used as a backbone monomer, a cross-linker and an initiator, respectively. II) Drug loading: [^{14}C]-sucrose was loaded into the gel matrix by immersion in radio-labelled sucrose solution (3 µCi) for three days. III) Drug release: [^{14}C]-sucrose was released from the gel matrix upon application of the DC electrical field. B) Hydrogel preparation. Images of hydrogels prepared at increasing concentrations of MWNTs (0–0.5 mg/mL). C) Hydrogel surface characterization: SEM images of dry hydrogels with increasing MWNT concentration at low and high magnification: i,v) blank gel, ii,vi) hybrid gel at 0.05 mg/mL MWNT, ii,vii) hybrid gel at 0.2 mg/mL, and iv,viii) hybrid gel at 0.5 mg/mL.

Figure 2A). The incorporation of pMWNTs reduced significantly the bulk resistivity of the pMWNT/PMAA hydrogel hybrids compared to the blank PMAA hydrogel matrix (Figure 2A(iv)). The electrical properties of the carbon nanotubes combined with the electrosensitivity of the PMAA gel matrix led to hybrid gels with higher sensitivity and an enhanced de-swelling of the gel matrix.

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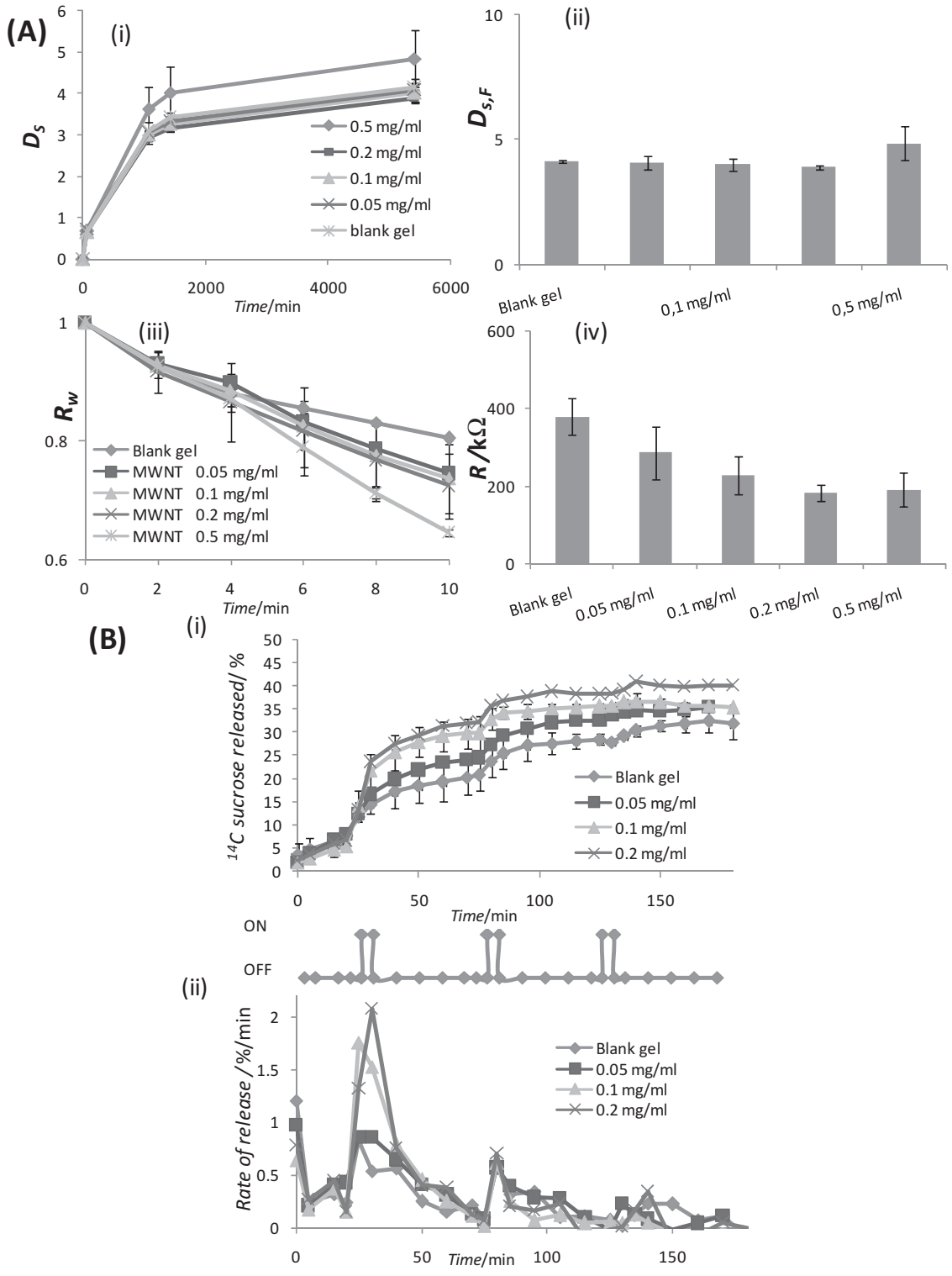

Figure 2. Characterization of gel swelling and electric properties: A) Swelling properties and de-swelling upon the application of a DC electrical field. i) Swelling degree of gels over time. ii) Final swelling degree of gels. iii) De-swelling properties on application of electrical voltage. Swollen hydrogel hybrids were placed in contact with two carbon electrodes. Water release from gel matrix was monitored over time and found to be dependent on pMWNT content. iv) Electric properties of hybrid gels: Bulk resistivity R of 1 cm^3 volume gel. B) Pulsatile drug release from hydrogel hybrids upon ON/OFF application of electrical voltage. i) Effect of pMWNT content. Drug release was monitored over time while applying electric field ON for 5 min and OFF for 60 min. Pulsatile release of ^{14}C-sucrose was determined for blank gel and 3 hydrogel hybrids (0.05 mg/mL, 0.1 mg/mL, and 0.2 mg/mL pMWNTs). ii) ^{14}C-sucrose release from gels. Rate of release was calculated over time obtained upon ON/OFF exposure to a DC electric field (10 V).

As these materials were mainly constituted of a PMAA matrix, which has fairly high impedance, it was expected that they would release heat when electrically stimulated. The gel matrix heating upon electrical stimulation was monitored at 37 °C over different time intervals relevant to an in vivo setup. It was found that during electrical stimulation of less than one minute the overall temperature of the gels increased by approximately 4 to 6 °C, reaching a temperature of around 41–42 °C (Supporting Information Figure 2B). For an electrical stimulation greater than one minute, the gel matrix temperature reached values around 50 to 52 °C that would not be appropriate for in vivo studies.

The ability of the electroresponsive pMWNT/PMAA hydrogel hybrids to release controllable drug doses repeatedly upon ON/OFF application of an electrical field was then investigated. Radiolabelled (^{14}C) sucrose was used as a model hydrophilic drug and was loaded into the matrix during swelling. All of the gels were immersed in a concentrated solution of ^{14}C-sucrose in HEPES buffer (pH 7.3, 25 mM) until they reached complete swelling and the quantity of loaded ^{14}C-sucrose was subsequently determined by the gel weight difference (Supporting Information Table 1).

An electric field of 10 V was applied for short time intervals (5 min) and then switched off for an hour. These stimulation characteristics were selected since electrical potential differences above 10 V are more likely to cause tissue damage.[18]

Figure 2B(i) shows the ^{14}C-sucrose release profile obtained after three cycles of ON/OFF electrical stimulation. For all gels, a pulsatile release profile was observed, followed by an increase in ^{14}C-sucrose concentration in the released media upon application of the electrical field. Sucrose release was significantly reduced after removal of electrical stimulation. ^{14}C-sucrose release was increased for all hybrid gels compared to the blank gel and was dependent on the pMWNT concentration. However, a significant decrease in sucrose release rate was observed after the second application of the electric field, particularly for gels that contained high pMWNT concentrations (Figure 2B(ii)). Although the hybrid gel at low pMWNT concentration (0.05 mg/mL) and the blank gel released lower quantities of sucrose upon the first electrical stimulation, they displayed a pulsatile profile with similar doses of sucrose released equally after each stimulation. This could be explained by the enhanced stress exerted on the matrix during the de-swelling process damaging the surface of gels that contained a high concentration of pMWNT. Interestingly, pMWNTs are known to respond to DC electrical stimulation by aligning themselves to form an angle of 30° in relation to the anode.[19] We can therefore speculate that the addition of pMWNTs in the PMAA hydrogel matrix improved drug release by "squeezing" of the gel matrix at the anode resulting from the straightening and alignment of the carbon nanotubes. At high pMWNT concentration, the high viscosity of the polymer matrix becomes the dominant factor, leading to structural damage of the gel matrix.[20] The development of improved hydrogel hybrids with higher mechanical capabilities allowing the polymer matrix to conserve its structural integrity after each electrical stimulation and to provide cycle-to-cycle reproducibility is currently under investigation.

The potential of this hybrid PMAA-pMWNT hydrogel to release small drug molecules in vivo was studied next. The ^{14}C-sucrose release profile in blood without any electrical stimulation was first investigated. Radiolabelled sucrose-loaded gels were subcutaneously implanted by a simple surgical incision on the upper dorsal region of the mouse and blood samples were collected (from the tail vein) at regular time intervals thereafter. The release profile obtained was compared to the blood profile of ^{14}C-sucrose solution injected intravenously (i.v.) or subcutaneously (s.c.) (**Figure 3**A). The release profile of the subcutaneous

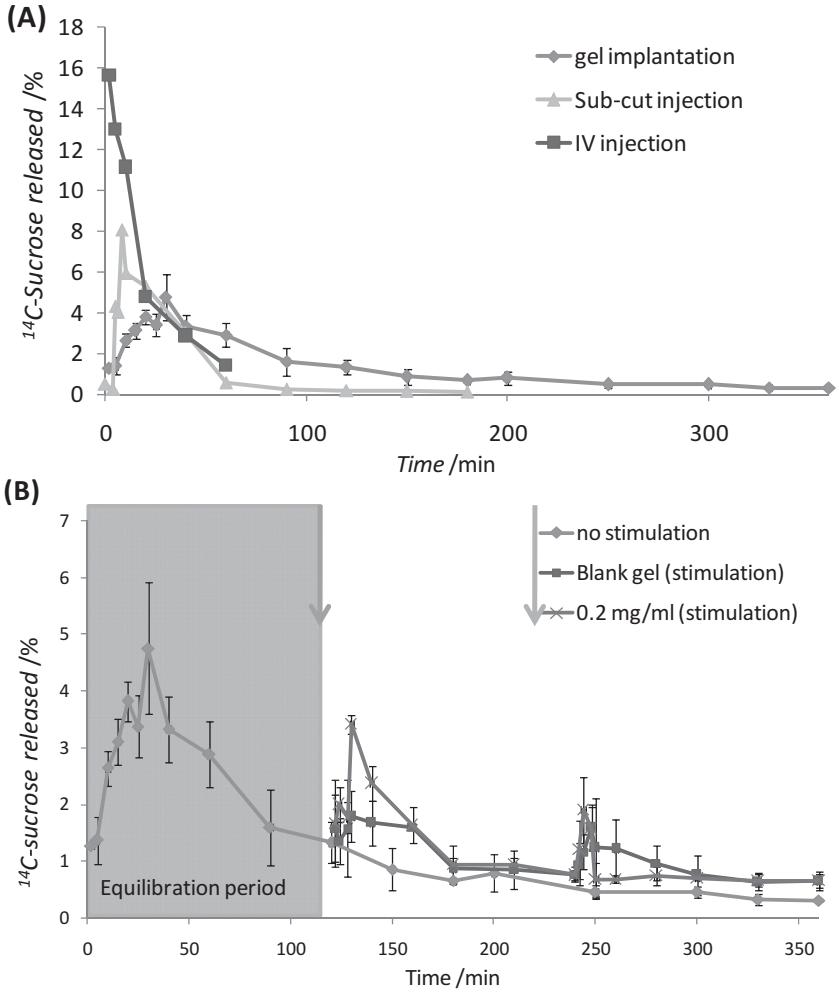

Figure 3. In vivo drug (sucrose) release in systemic circulation. A) Pharmacokinetic profiles of ^{14}C- sucrose via different routes of administration. Release profile of ^{14}C-sucrose in systemic blood circulation following intravenous injection (squares), subcutaneous injection (triangles), and subcutaneous gel implantation (diamonds). Unstimulated release from subcutaneous gels stabilized 2 h post-implantation. B) Release profile of ^{14}C-sucrose from hybrid gels. Sucrose release in systemic blood circulation was compared to blank gels and 0.2 mg/mL pMWNT hybrid gels upon electric stimulation. Gels were stimulated (10 V for 1 min) at 2 h intervals (vertical arrows). The first stimulation was performed following a 2 h equilibration period.

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hybrid gels demonstrated a much slower sucrose release compared to freely administered sucrose, as expected. The release continued over the course of 6 h for the implanted gel, while i.v. and s.c. injected ^{14}C-sucrose could not be detected in the blood beyond 1 h post-injection (Figure 3A). It must be appreciated that sucrose is indeed a molecule of small molecular weight, so it is rapidly excreted or metabolized. Although an initial burst of sucrose release (reaching around 5% of the injected dose) could be observed for the implanted gel, the release was found to be quite stable after 2 h of implantation. This initial release would correspond to an equilibration period during which the gel was adapting to the biological environment. Following this 2 h time period, the gels remained impermeable and stable for a long period of time after implantation, releasing negligible amounts of sucrose.

Electrical stimulation was initiated after this equilibration period. The hybrid gels prepared at 0.1 mg/mL and 0.2 mg/mL of pMWNT were subcutaneously implanted in the upper dorsal region of CD-1 mice and electrically stimulated for 1 min at 10 V at 2 h intervals. The data for the 0.2 mg/mL pMWNT hybrid gel and blank gel are shown in Figure 3B (data for the 0.1 mg/mL pMWNT are in Supporting Information Figure 3). For both the hybrid and blank gels, a pulsatile release profile was obtained, with a significant increase in the released sucrose concentration observed upon application of the electrical field for 1 min, followed by a progressive decrease upon removal of the electrical field. The quantity of sucrose released for all gels reached the baseline level of the control (non-stimulated) gel 1 h after the first electrical stimulation. Upon the second electrical stimulation, the sucrose release profile increased again demonstrating the ability of the gels to respond to the electrical field for a second time. In addition, maximum levels of sucrose were detected in blood 10 min post-stimulation, demonstrating the rapid responsiveness of the gels to the electrical field. The release profile was in fact similar to the one obtained by subcutaneous injection suggesting that the release of sucrose upon electrical stimulation can be as efficient as repeated subcutaneous injections.

In line with the in vitro data, the release profile from the hybrid gels showed an enhanced ^{14}C-sucrose release and sharper responses to the electric field, significantly outperforming the blank gel during the same time period and under the same conditions of stimulation. However, the quantity of sucrose released upon the second electrical stimulation was found to be significantly lower for the hybrid gel, comparable to that of the blank gel. This was also observed in our in vitro release investigations and confirmed the occurrence of surface damage on the gels after electrical stimulation, particularly in the case of hybrid gels. Gel surface damage following the electrical stimulation was studied using SEM (see Supporting Information Figure 4). Overall, the addition of carbon nanotubes significantly improved the drug release performance of PMAA hydrogels in vitro and in vivo, delivering higher quantities of drug through stimulation of only 1 min, and using relatively low electrical potential differences. This is considered a significant improvement on existing electroresponsive hydrogel technologies, through which high drug doses cannot be delivered repeatedly at an electric voltage of 10 V without stimulating for 10 min and often increasing the drug loading into the gel matrix.[21–23]

In order to evaluate their in vivo biocompatibility, gels (hybrid and blank) were implanted subcutaneously and monitored in the absence of electrical stimulation for 48 h. The skin around the implantation site was removed and analyzed by histology (Supporting Information Figure 5A). No significant signs of inflammation or necrosis were observed, which indicates that the gels (with or without MWNTs) were well-tolerated. The potential tissue damage induced by application of electrical stimulation was also investigated by examining the tissue proximal and distal to the electrical stimulation site (Supporting Information Figure 5B). The dermal tissue directly in contact with the electrode showed signs of necrosis and inflammation, but no adverse signs were observed either distal to the stimulation site or in the underlying tissue. The necrosis and inflammation around the stainless steel electrodes is most likely to be due to oxidation of the electrodes in contact with the skin and the gel. This response was investigated further and gels (blank and those containing pMWNT) were subcutaneously implanted for longer periods of time after electrical stimulation (48 h, 7 and 30 days). Animals did not show any side effects and animal body weight consistently increased during the period investigated. Histological analysis was performed on the tissue around the implantation site (Supporting Information Figure 5B). No histopathological abnormalities were observed confirming that the hybrid gels were well tolerated for a long period of time.

This study illustrated the in vivo efficiency of an implanted electroresponsive polymer-MWNT hybrid system capable of pulsatile drug release. The most relevant previous attempt to demonstrate pulsatile drug release in rodents was performed using NIR-induced release of ibuprofen from polymer implants, however in vivo investigations were performed only at a pilot stage.[24] In the present study, the addition of pMWNTs improved the performance of the PMAA blank gel matrix and allowed the in vivo delivery of a therapeutically relevant drug dose under short stimulation times and low electrical voltage. This previously unreported electroresponsive hydrogel system was also found to be biocompatible, with minimal tissue damage caused due to heat generated by the electrodes used for the electrical stimulation. Substitution of the invasive nature of electrode-induced electrical stimulation with wireless technologies is thought to completely resolve invasiveness and risk for tissue damage while enabling remote-controlled and pulsatile drug release. The development of polymer-MWNT hybrid hydrogels for electroresponsive drug delivery offers the possibility of delivering drug molecules in a controllable manner using short electrical stimulation times. Such systems therefore have the potential to contribute to the personalized management of chronic illnesses that require multiple dosage regimes.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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