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Synthesis of Electroconductive Hydrogel Films by Electro-controlled Click Reaction and its Application to Drug Delivery System†

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Electroconductive hydrogels are composed of 3-dimensionally structured hydrogels and conducting molecules with electrical, optical, and reversible redox properties. This hybrid material can be utilized as electrically-stimulated biomaterials in implantable biosensors or drug delivery systems. In this study, carbon nanotube-incorporated polyvinyl alcohol (PVA)-based hydrogels were synthesized by an electro-click reaction, which was controlled by an electrochemically generated Cu(I) catalyst. When the reduction potential of Cu(II) ions was applied, PVA-based hydrogels were deposited onto indium-tin-oxide-coated glass electrodes via Cu(I)-catalysed alkyne–azide cycloaddition (click reaction). When the hydrogels contained carbon nanotubes, thicker films were deposited because the embedded carbon nanotubes provided a larger electrochemical active area. In addition, the carbon nanotubes improved the electrical conductivity of the hydrogel systems. We investigated the electro-stimulated drug release behaviour with electro-click conductive hydrogels using tetracycline as a model drug.

1. Introduction

Electroconductive hydrogels are conducting polymeric networks composed of hydrogels and inherently conductive polymers.[1] These composites have a combination of their components` properties, which are hydrogels' softness, high degree of hydration, high diffusivity of small molecules and biocompatibility and the conducting polymers' electrical conductivity, optical switching, and reversible redox properties. Furthermore, their chemical and mechanical properties can be tuned easily such as changing the crosslinking density.[2,3] These hybrid systems can be fabricated into specific forms on various substrates according to the intended purposes. Electroconductive hydrogels have been applied to biomaterials with electrical stimuli such as those used in biosensors,[4] or tissue engineering.[5]

Drug delivery is the process of transferring a drug into the body over a period of time and at a specific rate, with the desired drug concentration maintained for effective therapy. There are several drug delivery methods such as oral dosing and transdermal drug delivery. Oral dosing is the simplest method, but it has disadvantages such as poor absorption. Transdermal drug delivery is an alternative that introduce drug molecules through the skin into the blood stream. It is attractive because it is safe and easy to apply,[6] but one of its disadvantages is that high molecular drugs have low permeability.[7] Iontophoresis is a method used to transfer drugs or proteins in patches into our bodies through the skin using an applied electric field.[8] Pillai et al. showed that insulin delivery through the skin was influenced by electronic parameters.[9] The charge applied during iontophoresis affected the electrochemical stability of insulin and the current strength also affected the permeation of insulin. Electroconductive hydrogels can be a good option for electro-stimulated drug delivery[10] because they have faster response due to their improved electrical properties. Pillay et al. reported ON-OFF drug release kinetics using polyaniline-incorporated poly(vinyl alcohol)-based hydrogel via electrical stimulus.[11] When the amount of polyaniline increased, the conductivity of polyaniline-poly(vinyl alcohol) hydrogel increased linearly. The erosion rate of the hydrogel also increased due to electro-actuation, leading to erosion. Torresi et al. reported the controlled release of safranin from semi-interpenetrating polyaniline-polyacrylamide networks.[12] The polyaniline grew inside the pores of swellable polyacrylamide hydrogel. The change in the oxidation state of the polyaniline expanded and contracted the hydrogel, indicating that the safranin storage in the hydrogel was liberated by electric stimuli.

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†Electronic Supplementary Information (ESI) available: SEM image of ITO-coated glass after gelation reaction without bis-azide crosslinker (Fig S1); Cyclic voltammogram of CNT/PVA click solution (Fig S2); Cyclic voltammogram of $Fe(CN)_6^{3-}$ with different scan rates (Fig S3); UV-vis spectra of tetracycline (Fig S4); The release profile of tetracycline from PVA hydrogel film without CNTs (Fig S5). See DOI: 10.1039/x0xx00000x

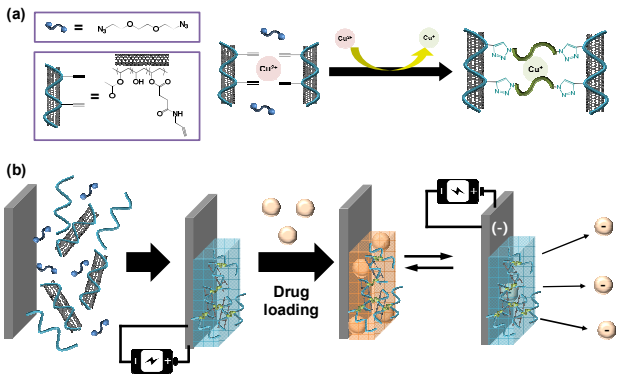

Fig 1. (a) In the presence of Cu(I) generated by an electric potential, CNT-wrapping alkyne-functionalized PVA chains[13-16] are crosslinked with bis-azdie via Cu(I) catalyzed alkyne–azide cycloaddition. (b) Schematic illustration of electroconductive hydrogel films synthesized by electrically controlled click reaction. CNT-embedded hydrogel films were synthesized onto the electrode at the reduction potential of Cu(II). These hydrogel films can be used in electro-stimulated drug delivery system.

Herein, carbon nanotube (CNT)-incorporated polyvinyl alcohol (PVA)-based hydrogels were synthesized by an electrochemically controlled click reaction, specifically, Cu-catalysed alkyne–azide cycloaddition (Figure 1). Click reaction has many advantages,[17] such as high selectivity and high yields, and thus this reaction has been widely used for polymer surface modification and film fabrication.[18] In previous research, we demonstrated that electroconductive hydrogels can be synthesized via click chemistry using a Cu(I) catalyst generated by a reducing agent (sodium L-ascorbate).[19] In this study, we utilized an electrochemical strategy to generate Cu(I) from Cu(II) *in-situ* instead of using a reductant.[20] This "electro-click" reaction has been exploited by several research groups in creating multifunctional polymer materials[21-26] since further stabilization of the easily oxidized Cu(I) during film deposition is not necessary and spatial and temporal control of the reaction is also possible. Using the electrochemically controlled click reaction, we were able to synthesize CNT-embedded electro-conductive hydrogel films on electrodes from alkyne-functionalized PVA and bis-azide. It should be noted that as pointed out in the recent review article,[27] it would be the first example that electro-click reaction is used for incorporating large building blocks such as CNTs on surface. The properties of the resulting hydrogel films were characterized by UV-vis spectroscopy, surface profilometer, and scanning electron microscopy, followed by measurements of its electrical properties using electrochemical impedance spectroscopy. Using the resulting electroconductive hydrogel films, we tested drug release with tetracycline as a model drug, confirming that electrical stimulation can indeed control the release behaviour.

2. Experimental section

2.1 Materials

Alkyne-functionalized poly(vinyl alcohol) (a-PVA),[19] 1,2-Bis(2-azidothoxy)ethane (bis-azide)[28] were prepared according to published procedures. The number-average molecular weight of a-PVA is 13~23 kDa, and the substitution of alkyne was roughly estimated to be 1/20 out of repeat units of PVA, which was proven by ^{1}H-NMR spectra(more detailed information in the reference 15). Multi-walled carbon nanotubes (CNTs) were purchased from Sigma-Aldrich, and tetracycline hydrochloride was purchased from TCI.

2.2 Instrumentation

Cyclic voltammetry were performed on a CHI electrochemical analyser in a three-electrode cell configuration consisting of an Ag/AgCl reference electrode, a Pt coil as a counter-electrode and an indium tin oxide (ITO)-coated glass, fluoride tin oxide (FTO)-coated glass. 0.1 M solution of potassium chloride in water was used as a supporting electrolyte. Scanning electron microscope images were obtained by using FE-SEM JEOL-7100. Hydrogel thickness were obtained by using surface profiler.

Electrochemical impedance spectra of hydrogels were measured using the potentiostat (CompactStat, Ivium Tech.). EIS measurements were performed with an amplitude of 10 mV and the frequency from 0.1 Hz to 2000000 Hz at open-circuit voltage. Swollen hydrogel films were placed between ITO-coated glass electrodes with 1 mm Teflon spacer.

2.3 CNT dispersion in a-PVA (CNT/PVA)

A solution of a-PVA was prepared from a-PVA powder (30 mg) in 1 mL of 0.1 M KCl aqueous solution. CNT (~1 mg) was added to the solutions, and sonicated with a probe-tip sonicator (26 W) for 30 min. The mixture was centrifuged (13 000 rpm) for 90 min, and then decants were collected.

2.4 Synthesis of hydrogel film

30 mg/mL of a-PVA, 6 mM of bis-azide and 10 mM $CuSO_4$•$5H_2O$ in aqueous solution was prepared with 0.1 M KCl as supporting electrolyte. Firstly, the reduction potential of Cu (II) was found by cyclic voltammetry using the ITO-coated glass electrode (width = 1.5 cm, height = 4 cm) from -1.3 V to 0.3 V vs Fe^{2+}/Fe^{3+}. The height of hydrogel film was decided to the amount of solution (dipping area of electrode). The reduction peak of Cu (II) was appeared around -0.3 V vs Fe^{2+}/Fe^{3+}. Next, hydrogel was deposited on the ITO-coated glass electrode by chronoamperometry applied the reduction potential of Cu (II), which was found by cyclic voltammetry. The hydrogel film was synthesized for 1, 3, 5, 7, and 9 minutes and the thicknesses of hydrogel film dried in air were compared in the presence of CNTs or not.

2.5 Drug delivery test

In this case, hydrogel film was synthesized onto the FTO-coated glass electrode (width = 0.75 cm, height = 5 cm) because of the better adhesion. The condition of film deposition was almost the same with using the ITO-coated glass electrode, but the reduction potential of Cu (II) was shifted to -0.4 V. Synthesized hydrogel film had 0.75 cm of width and 1.2 cm of height. 1 mg/mL of tetracycline hydrochloride dissolved in PBS was prepared and the hydrogel films were dipped into the solution.

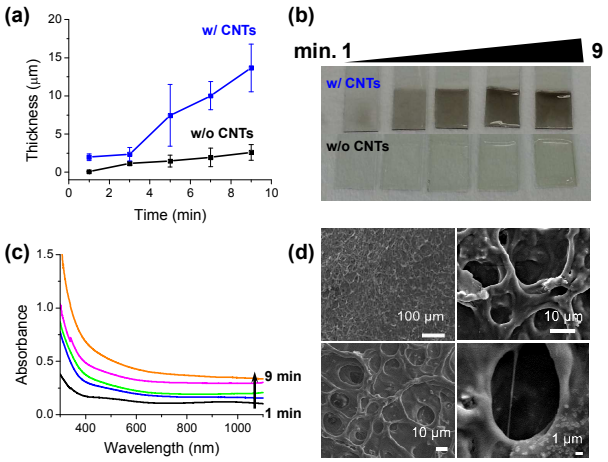

Fig 2. (a) The thicknesses of the hydrogel films were measured with the deposition time. When CNTs were incorporated, the hydrogel films were thicker than those without CNTs. (b) Pictures of the hydrogel films, with and without CNTs, were compared. (c) The UV-vis absorption spectra of CNT-embedded hydrogel films showed that thicker films contained more CNTs. (d) SEM images of CNT-embedded hydrogel films after lyophilisation showed porous structures.

After 1 hour, the hydrogel films was rinsed in the distilled water. The amount of release drug was chased by UV-vis spectroscopy and the absorbance was observed every minutes by repeated measure mode. The hydrogel-deposited FTO electrodes were dipping in the cuvette with 2.5 mL of PBS solution. Electrical stimulus was applied during 30 seconds every 2 minutes, performed on an Epsilon (repeated DC Potential Amperometry, DCPA). Applied potential on the electrode was determined by the open circuit voltage that was checked before the experiments. Also, as varying the pH of solution, we observed how the release profile was affected by the charge state of drug.

3. Result and discussions

3.1 Synthesis and Characterization of Electroconductive Hydrogel Films via Electro-controlled Click Reaction

Electroconductive hydrogel films were synthesized on the surface of electrodes by an electrically controlled click reaction. The hydrogel films were prepared using alkyne-functionalized poly(vinyl alcohol) (a-PVA) with or without carbon nanotubes (CNTs, multi-walled). Terminal alkyne groups of a-PVA were allowed to undergo Cu-catalysed click reaction with 1,2-bis(2-azidoethoxy)ethane (bis-azide). In previous research, the Cu(I) catalyst was generated by a reducing agent, sodium L-ascorbate, so that the crosslinking reaction occurred in the whole polymer solution.[19] In this research, the Cu(I) catalyst was generated by electrochemical reduction in the presence of Cu(II) within the solution. Consequently, the synthesis of hydrogels was allowed only when (and where) Cu(II) was reduced to Cu(I) in the presence of a crosslinker, bis-azide.

To control the click reaction by electrochemistry, the pre-gelation solution was prepared; the solution contained CNT (~1 mg/mL) dispersed with a-PVA (~30 mg/mL) along with bis-azide (~6 mM) as a crosslinker. Into this solution, Cu(II) ions were added (10 mM) and they were in an inactive form for the click reaction. To find the reduction potential of Cu(II), cyclic voltammetry was conducted using the pre-gelation solution. We found that the peak current occurred at the potential of -0.3 V versus Fe^{2+}/Fe^{3+} (**Fig S2**). The potential of -0.3 V (vs Fe^{2+}/Fe^{3+}) appeared suitable to reduce Cu(II) to Cu(I) since the more reduced potential resulted in the deposition of Cu metal as well. When the solution did not contain bis-azide, little hydrogel film was deposited on the surface of indium-tin-oxide (ITO)-coated glass electrode although cyclic voltammogram showed the reduction of Cu(II) (i.e., the generation of active Cu(I)). It is possible that the hydrophilic polymer PVA could be adhered to the surface of ITO-coated glass electrodes through hydrogen bonding or van der Waals interaction, but the deposition through non-covalent interactions appeared insignificant compared to that through the covalent bond-forming reaction (i.e., electro-click reaction, **Fig S1**). The above results indicate that the hydrogel films were synthesized via Cu-catalysed click reaction, which occurred only at the reduction potential of Cu(I). It would be possible that electro-click occurs near to the electrode surface and the macromerized or cross-linked polymeric materials (hydrogels) are deposited on to the electrode surface due to reduced solubility and the hydrophilic nature of ITO-coated electrode surface.

The thicknesses of the synthesized hydrogel films were regulated by the reaction time for applying the potential since the synthesis of the hydrogel films was electrically controlled by the generation of Cu(I) species from Cu(II). The thicknesses of the synthesized hydrogel films were measured by surface profilometry (**Fig 2a**). As the reaction potential was applied for a longer period of time, the synthesized hydrogel films became thicker, as expected with the longer reaction time. We found, however, that the growth rate of hydrogel films with CNT-embedded solutions was higher than those without CNTs, which we attributed to the increase in electrochemically active surface area (see below). We clearly observed the incorporation of CNTs inside the hydrogel films (**Fig 2b**), and the amount incorporated increased linearly with the reaction time as shown in the UV-vis absorption spectra of the synthesized hydrogel films (**Fig 2c**). The morphology of the hydrogel films was also observed by scanning electron microscopy (SEM, **Fig 2d**) with the hydrogel films fully dried by lyophilisation. The SEM images showed the 3-dimensional porous structures, which indicated that molecules could be diffused into and out of the hydrogel films.

3.2 Effects of Dispersed Carbon Nanotubes on the Electrical Properties of Hydrogel Films

First, we tested the hypothesis that the CNT-embedment increases the electrochemically active surface area during hydrogel synthesis. The peak currents of Cu(II) reduction in the pre-gelation solution with CNTs had larger values when compared to those without CNTs (**Fig S2**). We hypothesized that the dispersed CNTs induced the larger "apparent"

electrochemical active surface area (A_{app}), so that more Cu(II) ions were reduced on the same surface area of the working electrode. To prove this hypothesis, the A_{app}s of working electrodes were estimated with the Randles-Sevcik equation using ferricyanide ions, $Fe(CN)_6$.[3-29]

The cyclic voltammograms (CVs) of $Fe(CN)_6^{3-}$ (10 mM) in pre-gelation solutions with and without CNTs were measured and compared with Pt buttons as the working electrodes at different scan rates (**Fig S3**). In these experiments, Cu ions were not added, so the electroactivities in CVs were solely from $Fe(CN)_6^{3-}$. From the plot of redox peak currents versus square-root of the scan rates (**Fig 3a**), the apparent diffusion coefficients (D_{app}) of $Fe(CN)_6^{3-}$ were calculated using the Randles-Sevcik equation, which states that D_{app} is proportional to the peak current over the surface area (I_{peak}/A_{app}).[30] We assume that the inclusion of CNTs in the pre-gelation solutions does not change the diffusivity of ferricyanide ions much. Thus, if other conditions were the same, the peak current should be proportional to the surface area A_{app}. As shown in **Fig 3a**, the redox peak currents of ferricyanide ions with CNTs were 2.27 times larger than those without CNTs. Therefore, we conclude that the apparent electrochemically active surface area in the pre-gelation solution with CNTs was 2.27 times larger than without CNTs (**Fig 3b**), resulting in the generation of more Cu(I) catalysts and faster deposition of hydrogel films in the presence of CNTs. This indicated that dispersed CNTs may improve the electrical properties of hydrogel films.

The improved electrical conductivity of CNT-embedded hydrogel films was confirmed by electrochemical impedance spectroscopy (**Fig 3c**). Hydrogel films were synthesized on an ITO-coated glass electrode (10×10 mm) with a PTFE spacer (1 mm) and swelled with several drops of 0.1 M KCl (aq). The hydrogel films were then covered with Pt-sputtered ITO-coated glass. The measured impedance value of hydrogel films with CNTs at 0.1 Hz was almost half of the value without CNTs. Therefore, the incorporation of CNTs improved the electrical properties of the hydrogel films, which would help with transferring electrical stimulus.

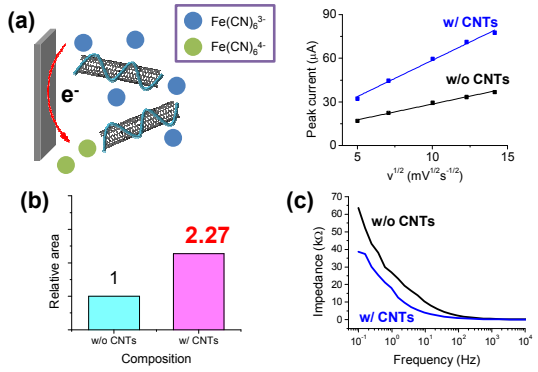

Fig 3. (a) Cyclic voltammograms of $Fe(CN)_6^{3-}$ in the solutions with and without CNT and their peak currents as varying scan rates. (b) Relative electrochemically active area between with and without CNTs calculated by Randles-Sevcik equation. CNTs cause the electrochemical reaction to occur on a larger active area. (c) The improved electrical conductivity of CNT-embedded hydrogels was confirmed by electrochemical impedance spectroscopy

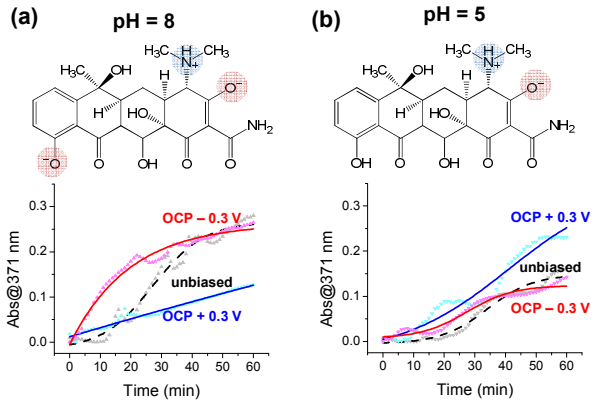

Fig 4. The release profiles of tetracycline under the different biased potentials (versus open circuit potential) were evaluated for 1 hour at pH 8 (a) and pH 5 (b) using drug-loaded CNT-embedded hydrogel films. Guiding lines are also drawn as dotted ones. The chemical structures of tetracycline with different charge states at different pHs are also drawn. Tetracycline is mostly in the neutral state or slightly cationic state at pH 5, and in the anionic state at pH 8.

3.3 Electrically Stimulated Drug Delivery Test with CNT-Incorporated Hydrogel Films

Electroconductive hydrogel films deposited on the FTO-coated glass electrodes were tested to determine their capability as an electrically controlled drug delivery system. Tetracycline, which is the component of antibiotics that inhibit proteins synthesis[31], was used as a model drug to test the electro-stimulated drug delivery. Tetracycline has different charge states: neutral or slightly cationic at pH 5 and anionic at pH 8 (**Fig 4**).[32] The pH levels of the releasing solutions (blank solutions) was maintained at pH5 and 8 with PBS (1x) buffer. The synthesized electro-click conductive hydrogel films were dipped in the PBS 1x (pH = 7.4) solution containing tetracycline (1 mg/mL) for 1 hour. They were then rinsed with distilled water to remove extra tetracycline on the surface of the hydrogel films. The release profiles of tetracycline at different pHs were evaluated by UV-vis spectroscopy, and the absorbance was measured every minute under 3 types of stimuli: unbiased (open circuit potential, OCP), positively biased (OCP + 0.3 V), and negatively biased (OCP – 0.3 V). Electrical stimuli were applied using a 3-electrode system consisting of Ag/AgCl as the reference, Pt wire as the counter, and the hydrogel films on FTO-coated glass electrodes as the working electrode. OCPs were normally observed in the range of -50 ~50 mV.

The releasing behaviour of tetracycline appeared largely dependent on the pH of the solutions, and we found that such the behaviour could be controlled by electrical stimuli. First, the releases of tetracycline under unbiased conditions were investigated (**Fig 4a and b**), and they showed the typical S-shape of simple diffusion at both pHs 5 and 8. The S-shape of release profiles suggests some induction periods require for the release of tetracycline. The presence of induction periods has been attributed to the physical/chemical changes of the hydrogel, such as swelling, deswelling, erosion, or actuation

induced by the applied electric fields.[33,34] It seems reasonable that our electroconductive hydrogel systems respond to the external electric fields in the presence of electrolyte molecules, which may cause the change of the physical state of the hydrogels. Without external electric fields (i.e., V = OCP), however, it is not clear why the induction period occurs. We suspect the hydrogel films may require a period of equilibrium since the tetracycline loading was conducted in solutions with a slightly different pH (~7.4). We observed that tetracycline was released at a faster rate under pH 8; the maximum release rate at pH 8 was 3.45±0.52 nmol/cm^2·min, but the maximum rate at pH 5 was only 2.16±0.48 nmol/cm^2·min. We attributed this difference to the increased charge state at pH 8 (negatively charged versus neutral). Interestingly, the maximum release rate of tetracycline with CNT-embedded hydrogel films was slightly lower than that with PVA hydrogel films, which was 4.32±0.82 nmol/cm^2·min (**Fig S5**). We suspect that additional π-π interactions between the carbon nanotubes and tetracycline may slow the release.

Next, the release profiles of tetracycline with electrical stimulus were investigated under different pH conditions. To determine the effect of electrical stimulus, the release tests under biased stimuli were processed and compared. At pH 8, where tetracycline exists in an anionic state, the negatively biased potential induced faster release from the CNT-embedded hydrogel films when compared to the release under unbiased potential (**Fig 4a**). However, tetracycline appeared to diffuse out slowly under positively biased potential. We attributed this to the anionic charge on tetracycline; negative bias on the electrode repelled the negatively charged tetracycline while positive bias attracted it. These results clearly show that CNT-embedded hydrogel films are able to control the release profile of tetracycline using charge-charge interactions.

At pH 5, the releases of tetracycline were somewhat retarded, but slight, albeit meaningful differences were observed (**Fig 4b**). Tetracycline drugs seemed to be released slightly faster under positively biased potential, and similar or slightly slower under the negatively biased potential at pH 5. This phenomenon could be explained by the fact that tetracycline is in the neutral (or slightly positive) state, and thus the electrical stimulus had little effect on the release behaviour. The slight acceleration of the release in the positively biased potential may be due to the slightly positive state of tetracycline, resulting in repulsive interactions. Overall, we concluded that the release behaviour of the model drug tetracycline can be controlled by external electrical stimuli, along with the pHs of the solutions, using charge-charge interactions.

Conclusions

In this study, we synthesized CNT-embedded, electro-conductive hydrogel films via electrically controlled click reaction. The active catalyst Cu(I) was generated in situ at the reduction potential and the hydrogel films were deposited onto electrodes through Cu(I)-catalysed azide-alkyne cycloaddition. CNTs-incorporated hydrogel films were deposited faster than hydrogel films in the absence of CNTs under the same deposition conditions. We attributed this to the increase of "apparent" electrochemically active surface area due to the incorporation of CNTs, which was confirmed by CVs of ferricyanide ions. The electrical conductivity of resulting hydrogel films was improved by the embedded CNTs, and they were utilized in electrically stimulated drug release. The release behaviour of the model drug tetracycline was enhanced under negatively biased potential at pH 8 due to charge-charge repulsion. We found that when tetracycline is in a neutral or slightly positive state at pH 5, the release profiles were barely affected by electric potentials, although slight acceleration was observed under positively biased potential. CNT-embedded hydrogel films seem to be a good platform for electro-stimulated drug delivery systems, and the "electro-click" reaction enables temporal and spatial control of the synthesis of such electro-conductive hydrogels.

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