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ARTICLE TYPE

Dual Stimulus Responsive Drug Releasing under the Interaction of pH Value and Pulsatile Electric Field for Bacterial Cellulose/Sodium Alginate/Multi-walled Carbon Nanotubes Hybrid Hydrogel

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A novel hybrid hydrogel composed of sodium alginate (SA), bacterial cellulose (BC) and multi-walled carbon nanotubes (MWCNTs) was synthesized by using $CaCl_2$ as a crosslinking agent. The hydrogel (BC/SA/MWCNTs) was proposed as a pH and electric field dual-stimulus responsive drug delivery system. Various amounts of MWCNTs were doped into the BC/SA in order to obtain the highest electric sensitivity of the composite hydrogel. The releasing profile of the drug from the hybrid hydrogels demonstrated a dual pH-/electric-sensitive property of composite hydrogel. The amount of drug release, which was found to be dependent on the applied electric current strength, was greater under the electrical stimulus compared with that under the passive diffusion. The electric-enhanced releasing behaviour was selective to the pH value of the surrounding culture. In neutral condition, release curves under electric voltage showed an obviously electric-sensitive. In acidic or alkaline condition, release curves with or without applied electric voltage had little difference. A pulsatile pattern of drug release was observed by switching "on and off" electric stimulus. In neutral condition, drug release from the hydrogel showed a significant pulsatile characteristic. The overall performance of the BC/SA/MWCNTs hybrid hydrogel demonstrated that MWCNTs as additives played a synergistic role in the control release performance of the drug delivery system.

1. Introduction

The conventional delivery systems have limitations of minimal synchronization between the required time for therapeutically effective drug plasma concentrations and the actual drug release profile exhibited by the dosage form. In recent years, invention of novel controlled drug delivery systems has become one of the most rapidly advancing areas in human health care field[1, 2]. Such delivery system has advantageous on releasing the drug at a specified rate, a proper temporal interval and an appropriate amount with the purpose of improving drug efficacy, reducing toxicity, and improving patient compliance and convenience during the entire treatment[3, 4]. In addition, intelligent drug delivery systems have been paid significant attention owing to their response to external stimuli, such as temperature[5], pH[6, 7], electric/magnetic field[8, 9], and light[10]. Among several intelligent drug delivery systems, "intelligent" and "smart" hydrogels has gained increasing interests with the ongoing enhancements on this technique[11]. pH-/electro-stimuli responsive hydrogels drug delivery systems have been widely reported.[3, 12, 13]. Because pH-responsive and electro-responsive can be regulated easily by external triggers, the pH-/electro-responsive hydrogels have many therapeutic applications due to a large variations of physiological pH exists not only in normal conditions such as the gastrointestinal tract, but also in pathological conditions such as tumoral culture.

Bacterial cellulose which is synthesized in abundance by acetobacter xylinum, has demonstrated its biocompatibility biomedical research. As BC has nanofiber-network structure, its various perfect properties such as high water holding capacity, high crystallinity, high tensile strength, good biocompatibility, low production cost made it to be one of the most promising biomedical materials. At present, BC has a good application in tissue engineering scaffold, wound healing process, skin of tissue engineering, renovating artificial blood vessels[14, 15].

Alginate extracted from brown algae is an anionic polysaccharide and can form hydrogel by Ca^{2+}, which positions in the interstices between G blocks, leading to an ordered con-formational structure called "egg-box " array. It can be used in wound healing, drug delivery, and tissue engineering owing to its favorable properties, including biocompatibility, biodegradability, non-toxicity, transparency, and ease of gelation[16-18]. As the hydrogel possesses large number of ionizable $-COO^-$ groups, alginate is prepared as component of stimuli-responsive delivery system [3, 19, 20]. A pH-responsive SA and its derivative network have been exploited to prepare enteric dosage forms using the network as coating or as a matrix, performing slow releasing rate at low pH condition due to limited swelling of the particles and drug diffusion, but rapid releasing rate at neutral condition due to erosion of the network and enhanced diffusion rate. However, low mechanical strength of SA hydrogels and relatively short effective drug release time limit its practical applications. It is also reported that pure SA hydrogel is nearly absent in electrical responsiveness under electric field[21].

Nevertheless the electric conductivity of many electro-stimuli

responsive hydrogels used for the intelligent release is not high enough to achieve an effective modulation of drug release. Thus, hybrid hydrogels with incorporation of conducting materials has been proposed to enhance the electro-sensitivity of materials[22-24]. Carbon nanotubes as additives into polymeric networks to fabricate electro-responsive hydrogels have been previously explored for the development of drug delivery systems, greatly enhancing the electrical and mechanical properties of hydrogels[25-27]. Bhunia et al. have obtained an increase of 201% in tensile strength and 196% elongation with 1wt% carboxy-functionalized multiwalled carbon nanotubes (c-MWCNTs) in Poly(vinyl alcohol) (PVA) matrix[28]. Yun et al. reported a drug delivery system of pH-/electro-responsive hybrid composite microcapsules containing MWCNTs[13]. Luo et al. reported the development of an electrically controlled drug release system based on conducting polymer and carbon nanotubes. Both groups found the drug nanoreserviors could effectively load drug and release drug in bioactive form under electrical stimulations[29]. However, the majority of intelligent polymeric hydrogels showed zero-order release, which was a release pattern with a settled rate and was independent of the concentration of drug and time. This is not effective for many therapeutic applications, such as post-surgery pain relief or treatment of infections which could benefit from pulsatile drug delivery. To the best of our knowledge, few studies has reported on the use of electro-responsive hydrogels (with or without carbon nanotubes) for pulsatile drug release in vivo. Ge et al. reported a new stimulus responsive nanoparticles (without carbon nanotubes) for pulsatile drug release[30]. Servant et al. developed an electro-responsive hydrogel containing pristine MWCNTs for pulsatile drug release[31]. A systematical study on carbon nanotubes doped into SA hydrogel form in order to explore its potential as a new pH and electric field dual-stimulus responsive drug release system has yet to be done.

In our previous work, we focused on developing bacterial cellulose (BC)/ sodium alginate (SA) composites hydrogel as a control drug release system in response to environmental stimuli [32]. In current study, MWCNTs as an additive was doped into BC/SA composites. Novel hybrid hydrogel BC/SA/MWCNTs was synthesized using ionic crosslinking method. SA and BC were used as polymer matrix to afford pH and electro-responsive property to hybrid matrix. MWCNTs were applied as active ingredients to enhance electro-responsive of the hydrogel system. The pH and electro-responsive swelling behaviors of hybrid hydrogel BC/SA/MWCNTs were studied, respectively. Drug release behavior of BC/SA/MWCNTs hybrid hydrogel was studied in different culture by varying pH value, electric field strength. The interaction of pH value and electric field strength in combined stimuli-release process was discussed. The pulsatile release behaviors under an "on and off" repeated electrical pulsatile stimulation from the BC/SA/MWCNTs hybrid hydrogels were also performed. Research on the variation of pulsatile releasing behavior of BC/SA composites before and after doping MWCNTs was carefully carried out. Meanwhile, the pH value selective pulsatile releasing behaviours of BC/SA/MWCNTs were also studied.

2. Materials and methods

2.1 Material

MWCNTs(diameter 40-60nm, length 1-2nm, amorphous<3%, Shenzhen Nanotech Port Co. Ltd.), Poly(vinyl pyrrolidone) (PVP, K-30 with the average molecular weight of 40,000, Yily Fine Chemicals Co. Ltd.) and SA (Tianjin Guangfu Fine Chemical Research Institute) were used in this study. BC used in this study was a gel-like semitransparent pellicle formed by A. xylinum AGR 60 (Hainan Yida Food Co., Ltd.). The rest of the regents used in the experiments were analytical reagents.

2.2 Preparation of hybrid hydrogels

The preparation procedure of hybrid hydrogel composed three stages. BC pellicles were firstly immersed in 0.1mol/L sodiumhydroxide solution for 60min at 90°C in water bath to remove the bacterial cell debris, then thoroughly washed by de-ionized water until the pH value was constantly at 7.0 and stored in distilled water at 4°C. BC pellicles were then crushed to form BC slurry at room temperature by homogenizer. A 2.0% (w/v) SA solution was prepared by dissolving SA in distilled water at room temperature to form a gel-like solution. Finally BC slurry was mixed by a magnetic stirrer to obtain homogenous BC/SA dispersions with weight ratios 2:1.

Ibuprofen was selected as a model drug to research the release characteristics. MWCNTs, premixed PVP (dispersing agent) with various weight ratio (0.05, 0.1, 0.25 and 0.5 wt%, respectively) were mixed with BC/SA under vigorous stirring and sonicated for 30min. The mixtures were placed in Petri dish, cross-linked by an aqueous solution of 1.5% (w/v) $CaCl_2$ and rinsed by de-ionized water to remove the excess cross-linking agents. Then the composites were frozen at -40°C for 12h prior to experience a freezing-dry process for 24h.

2.3 Conductivity measurement

The electrical conductivity of the hybrid hydrogel was measured at room temperature by the standard four-probe instrument (RTS-8, Probes Tech., China). According to the four-point probe method, conductivity can be obtained as follow:

The electrical conductivity of the hybrid hydrogel was measured at room temperature by the standard four-probe instrument (RTS-8, Probes Tech., China). According to the four-point probe method, conductivity can be obtained as follow:

$$\sigma = \frac{I}{\rho X} \quad \text{Eq.1}$$

where resistivity:

$$\rho = 2\pi S \quad \text{Eq.2}$$

where S is the probe spacing (mm), which was kept constant, I is the supplied current in microamperes, and the corresponding voltage is measured in millivolt[33].

2.4 Swelling behaviors

The swelling behaviors of the hybrid hydrogel under different pH conditions and electric voltages applied were determined by a gravimetric method. Briefly, the weight of dry hydrogel (Wo) was firstly obtained. The swelling behaviors of dry samples were investigated by their swelling ratios (SRs) in PBS solutions with pH=1.48-12.0 at 37°C. The dried samples were immersed into the PBS (pH=7.4) for a certain period under different voltage using power supply. At a predetermined time point, the swollen

samples were taken out to remove excess of buffer solution on the surface by wiping them with tissue paper and were weighed (Wt) immediately. After each weighing, the samples were returned to the containers with refreshed buffer solution. All tests were run in triplicate; the equilibrium swelling ratio of the hydrogels was calculated as follows:

$$\text{swelling ratio (Sw)} = \frac{W_t - W_o}{W_o} \times 100\% \quad \text{Eq.3}$$

2.5 Drug encapsulation and release in vitro

Drug loaded hydrogels samples were fabricated in a template (15mm in diameter) by drug loaded BC/SA/MWCNTs suspensions as described before and freeze-dried for 24h.

2.5.1 Drug release under different pH conditions

The drug loaded hydrogels were added into 100ml solution and vibrated at 37 °C with pH value ranging from 1.48 to 12.0, respectively. 5ml solution was taken for test and 5ml of fresh solution was replenished to refill the release solution. The concentrations of released drugs were quantified every 2h using UV/visible spectrophotometer at 266nm to measure the drug release profile and their established standard curves obtained in various pH values solutions. The amount of released drug was determined by cumulative curve. The drug release experiments were performed three times. Results were presented in terms of cumulative percentage release as a function of time using the following formula:

$$\text{Cumulative percentage release(\%)} = \frac{Q_t}{Q_m} \times 100\% \quad \text{Eq.4}$$

where Qt is the amount of Ibuprofen released from the conjugates at time t and Qm is the total amount of Ibuprofen loaded onto the hydrogels.

2.5.2 Drug release under applied voltage

Two circular platinum electrodes were kept in contact with the opposite surface of the swollen drug-loaded hydrogels in the 100ml PBS (pH=7.4), and electric voltages varied between 0-0.4V were applied. At different time interval, 5ml solution was extracted and analyzed. Results were presented by cumulative percentage release as a function of time using Eq.4.

To study the effect of electrode polarity on the release of the drug, the hydrogels were placed under the negatively charged electrode (anode in donor), the positively charged electrode (cathode in donor), and under no current system delivery over a period of 48 h.

2.5.3 Drug release under pulsatile stimulus

Apart from monophasic release pattern, in which drugs are released under a constant condition such as an invariable applied voltage. The pulsatile electrical stimulus was then applied on the hydrogel system with regulated DC power source to carry out the drug release behaviour under "on and off".

3. Results and discussion

3.1 Formation of the drug loaded BC/SA/MWCNTs composites

Fig.1 shows the preparation scheme for BC/SA/MWCNTs hybrid hydrogels. A stable aqueous MWCNTs dispersion was obtained by using nonionic surfactant polyvinyl Pyrrolidone (PVP)[34]. BC slurry was mixed with the alginate sol (2.0% w/v) through strong stirring to obtain homogenous BC/SA dispersions with the weight ratios 2:1. The guluronate blocks of the alginate chains were ionic crosslinked by Ca^{2+} ions. The MWCNTs were covered and fastened up by crosslinked polymer chains, which formed a continuous distribution in the BC/SA composite. In addition, the $CaCl_2$ solution was sprayed on the surface of BC caused inverse-phrase crosslinked by the alginate adhered to the BC and formed a corrugated egg-box-like structure of calcium alginate dispersed in the network. The drug was encapsulated into the BC/SA/MWCNTs composites as proposed in Fig. 1.

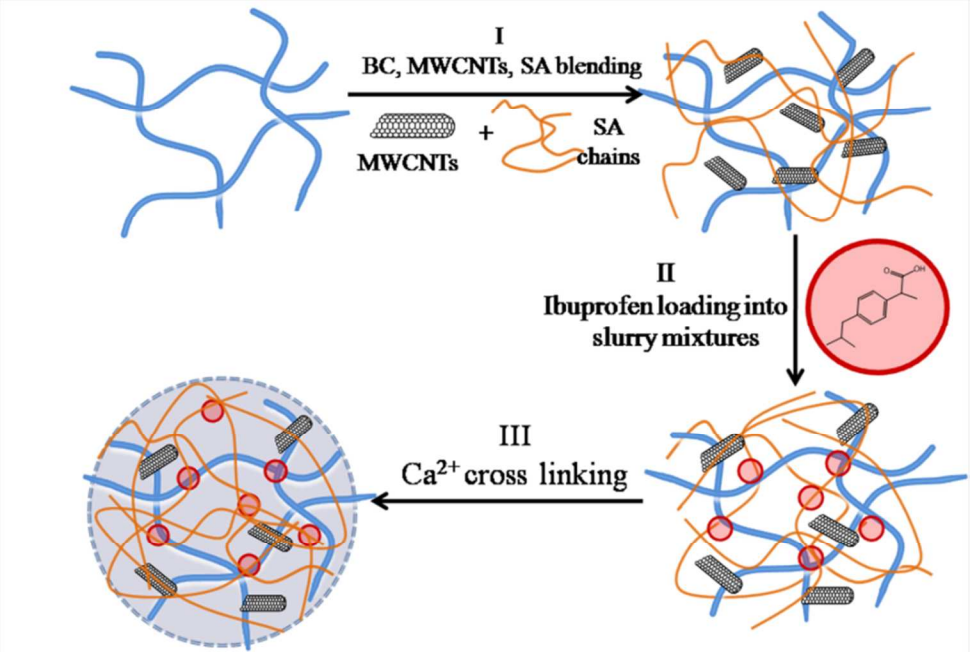

Fig.1 Schematic of the preparation of the drug loaded BC/SA/MWCNTs hybrid hydrogels

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3.2 Conductivity Measurement

Conductive properties of BC/SA/MWCNTs composites were investigated as a preliminary evaluation of their suitability for electro-responsive release. Fig.2 illustrates that the electrical conductivity of BC/SA/MWCNTs hybrid hydrogels were strongly dependent upon the doping amount of MWCNTs. There was an initially profound increase in the electrical conductivities of composite hydrogels with an increase of the MWCNT content, which peaked at the MWCNT content around 0.25 wt%. However, the conductivity declined with a further increase of the MWCNT content to 0.5wt%. Compared with BC/SA hydrogels, the electrical conductivity of BC/SA/MWCNTs hydrogels with MWCNT doping amount of 0.25 wt% had a highest electrical conductivity of $(4.67\pm0.57)\times10^{-3}$S/cm, a 640% improvement over BC/SA hydrogels. However, the value decreased to $(2.41\pm1.07)\times10^{-3}$S/cm when the MWCNT content was increased to 0.5wt%. This phenomenon was probably attributed to 0.25wt% doping content of MWCNTs allowed the formation of continuous conducting network in the BC/SA poly matrix. When the MWCNTs content increased to 0.5 wt%, the network of the composite hydrogels became less uniform with some CNT-rich domains, resulting in a decreased conductivity. Similar phenomenon was reported by some researchers[35, 36]. These results revealed that the adequate reaction time to prepare BC/SA/MWCNTs composites with higher electrical conductivity at about MWCNT content 0.25wt%

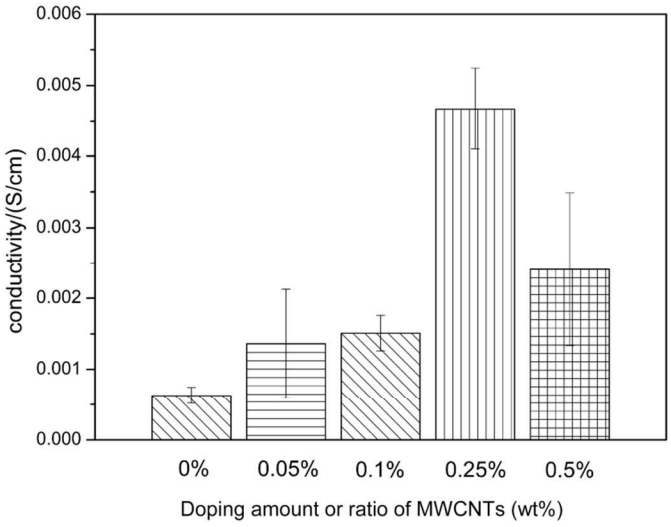

Fig.2 Electrical conductivity (S/cm) of BC/SA and the BC/SA/MWCNTS with 0.05%, 0.1%, 0.25%, 0.5%

3.3 Morphological analysis

The homogeneous mixture of BC/SA with different doping ratio MWCNTs was poured into Petri dish and cross-linked by 2% (w/v) $CaCl_2$ aqueous solution. After freeze-drying, black nanocomposites with inflexible structure was obtained. The surface morphology of the hybrid hydrogels are presented in Fig.3. There exited microporous three-dimensional interconnection network structure within BC/SA/MWCNTs hydrogels (Fig.3a-e). It could be observed that doping MWCNTs did not affect the pore structure of the hydrogels and size changed little. the pore structure and size changed little (Fig.3a-e). The pore size of the BC/SA/MWCNTs (0-0.5%) was in the range of 100–380μm, which played an essential role for drug encapsulating and diffusing. As shown in Fig.3f, calcium alginate hydrogels were found interpenetrate on the BC network and formed semi-interpenetrating matrix. As shown in Fig.3 (g-i), when the doping amount of MWCNTs was 0.05 to 0.25wt%, carbon nanotubes were well dispersed in hydrogels matrix and could formed semi-interpenetrating matrix with calcium alginate and BC fibers. When the doping amount of MWCNTs increased to 0.5wt% (Fig.3j), the micro-morphology of the composites hydrogels became less uniform with some CNT-rich domains, resulting in a lower enhancement in conductive properties in Fig.2.

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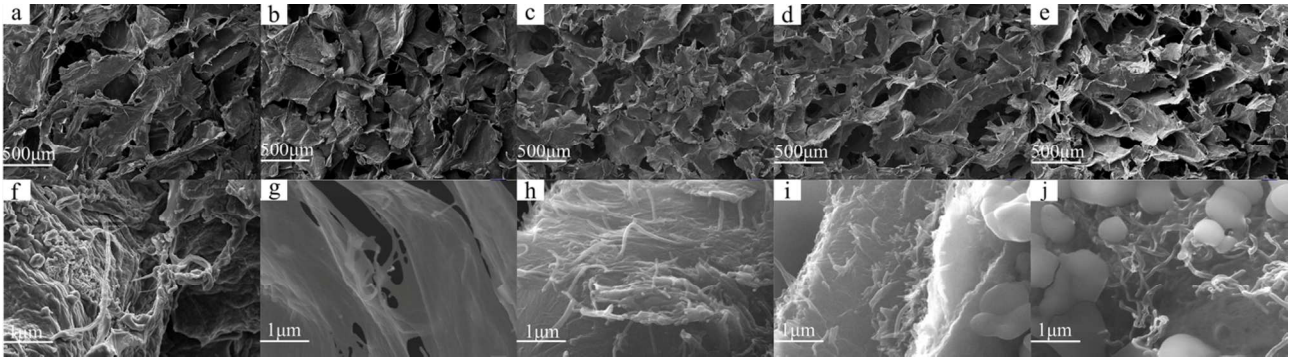

Fig.3 SEM images of surface (top) and cross-section (bottom) of BC/SA/MWCNTs0% (a, f), BC/SA/MWCNTs0.05% (b, g), BC/SA/MWCNTs0.1% (c, h), BC/SA/MWCNTs0.25% (d, i), BC/SA/MWCNTs0.5% (e, j)

3.4 Swelling studies

3.4.1 Electro-responsive swelling behaviors of the BC/SA/ MWCNTs composites

The electrical responsiveness swelling behaviors of the hybrids hydrogels were evaluated. The swelling behaviors of the hybrid hydrogels with different amounts of additive MWCNTs are illustrated in Fig.4a. The incorporation of MWCNTs into the hydrogels matrix did not have any significant effect on the swelling ratio (all hydrogels displayed SRs of≈1200%), which was in accordance with the result obtained by Servant[31]. Next, the SRs of the hydrogels (with MWCNTs of 0%, 0.05%, 0.1%, 0.25%, 0.5%) under application of electric voltage (0.4V) was investigated as described in Fig.4b. There was improvement in the hydrogels electro-response as the MWCNTs concentration increased from 0 to 0.5 wt%, which was correlated with increased conductivity of the gels. This observation clearly demonstrated that the incorporation of MWCNTs in the hydrogel improved the swelling ratio under application of electrical voltage of the samples[13, 37]. Fig.4c shows that the maximum electrical responsiveness swelling behaviors of the hybrids hydrogels was achieved by the BC/SA/MWCNTs0.25%. As evidenced from Fig.4d, the SRs of BC/SA/MWCNTs0.25% increased from 1130% to 1710% with the increasing electric voltage. The hydrogels responsiveness to the applied electric voltage was voltage-dependant and SRs was greater as the potential difference increased from 0V to 0.4 V. This could be contributed to electric voltage which accelerated the ionization of functional groups in the hydrophilic polymers. These characteristics indicated that BC/SA combined with the electro-sensitivity of MWCNTs led to BC/SA/MWCNTs with a higher sensitivity and an enhanced swelling.

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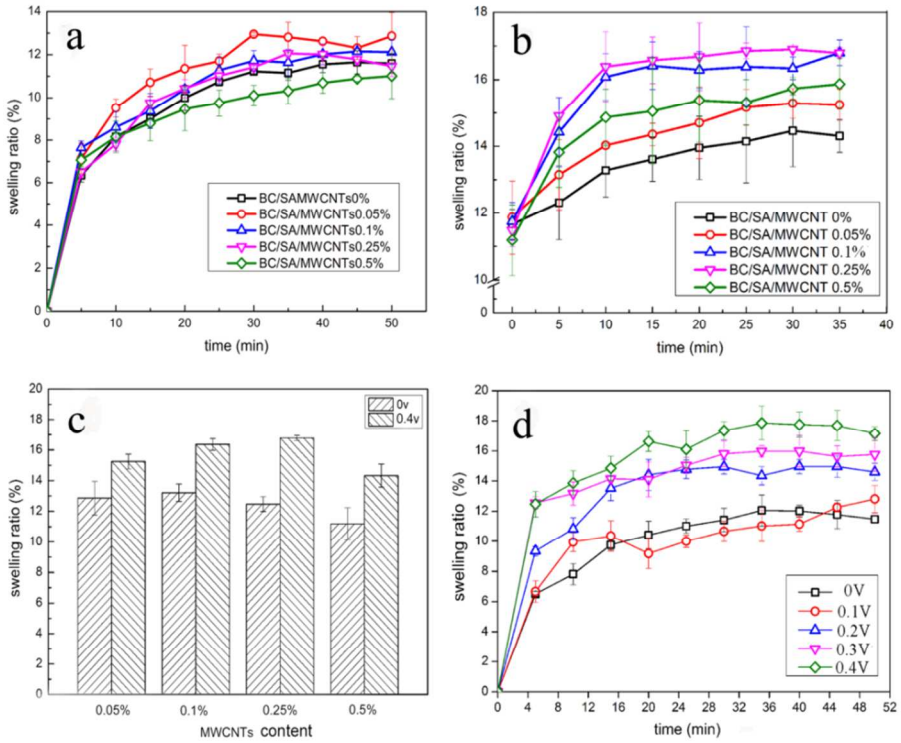

Fig. 4 Equilibrium swelling behaviors of BC/SA/MWCNTs composites with 0%, 0.05%, 0.1%, 0.25%, 0.5% in neutral solution (a), equilibrium swelling behaviors of BC/SA/MWCNTs composites with 0%, 0.05%, 0.1%, 0.25%, 0.5% under 0.4V electrical voltage (b), comparison of swelling behaviors of BC/SA/MWCNTs composites with 0%, 0.05%, 0.1%, 0.25%, 0.5% with and without 0.4V electrical voltage (c), equilibrium swelling behaviors of BC/SA/MWCNTs0.25% composites in different electrical voltage (d)

3.4.2 pH-responsive swelling behaviors of the BC/SA/ MWCNTs composites

BC/SA/MWCNTs0.25% hybrid hydrogels was chosen to study the swelling behaviors in PBS with pH value 1.48, 7.1 and 11.6 as shown in Fig.5. The SRs was 810% in pH=1.48. As reported in previous work, alginate was protonated into insoluble form of alginic acid by the H^+ ion in acidic condition which led to decrease of swelling ratio. In alkaline condition, the OH^- ion caused the deprotonation of alginic acid which resulted in a break of hydrogen bands and the generation of the electrostatic repulsion among polymer chains, which led to the increment of swelling ratio[19, 30] . As pH increased to 7.1, SRs reached about 1150% when the swelling process achieved equilibrium. When pH increased to 11.6, the SRs increased to more than 1500% since the carboxyl groups with SA dissociated into carboxyl anion in alkaline condition, which caused the ionic repulsion between anionic groups in the network resulting in the conformational stretching and the matrix swelled[38]. It was concluded that the alkaline condition led to more notable swelling more, while the acidic condition hindered the swelling degree.

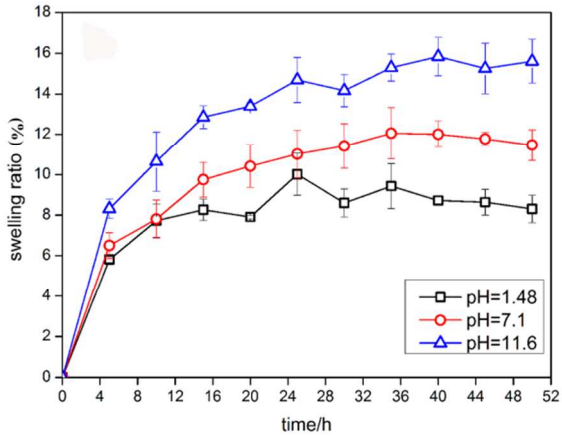

Fig.5 Equilibrium swelling behaviors of the BC/SA/MWCNTs0.25% in different pH values

3.5 In vitro drug release

3.5.1 pH-responsive drug release

Based on pH-responsive swelling behaviors of BC/SA/MWCNTs hydrogels, the drug release behaviours under various pH conditions were investigated. It was noticed that the drug was

released fast at alkaline conditions but slowly under acidic conditions. Previous study indicated that alginate could underwent a pH-induced change from a low releasing rate (low pH) to a high releasing rate (high pH) [16, 38]. Based on the result of these researches, we considered that the major control mechanism for the distinct release of drugs might be the pH responsive swelling behaviour of alginate. In acidic conditions, alginate was protonated into insoluble form which hindered the process of drug diffusion. In alkaline pH conditions, deprotonation of alginic acid caused a break of hydrogen bands and the generation of the electrostatic repulsion among polymer chains, which led to a faster release rate (Fig.6a, b).

The Korsmeyer-Peppas mechanism is a semi-empiric model, in order to understand the release kinetics of the drug from the hydrogels to the elapsed time t:

$$\frac{Q_t}{Q_m} = kt^n \qquad \text{Eq. 5}$$

Qt/Qm is the fractional release of drug in time t, k is a kinetic constant of the drug delivery system and n is the diffusion exponent that characterizes the drug release mechanism [39, 40]. This equation was used to the linearization of release data from several formulations. The n and k values were calculated from the slope and intercept of the plot of log(Qt/Qm) against lnt and represented in Fig. 6c along with the regression coefficients. For cylindrical hydrogels, if the exponent n<0.45, then the drug release mechanism is a Fickian diffusion and if 0.45<n < 0.89, then it is a non-Fickian or anomalous diffusion. The release of drugs in pH 1.48-12 was fitted with the Korsmeyer-Peppas model. The exponent "n" revealed that the drug release mechanism of drug in pH=11.6 was more diffusion-based (n= 0.489) than the one in pH=7.1 (n=0.682) or the one in pH=1.48 (n=0.689) as shown in Table.1. As the value of pH increased, the "n" exponent decreased. Therefore, the results showed that the samples obtained by loading the drug in porous matrices were controlled by a non-Fickian diffusion mechanism. Similar to this phenomenon, previous works reported that some pH-responsive hydrogels demonstrated a same releasing mechanism[41, 42] .

Table.1 Release parameters of Ibprofen sustained release from BC/SA/MWCNTs0.25% hybird hydrogels in vary pH conditions

	pH=1.48	pH=7.1	pH=11.6
n	0.689	0.682	0.489
k	0.198	0.370	0.518
R^2	0.998	0.993	0.981

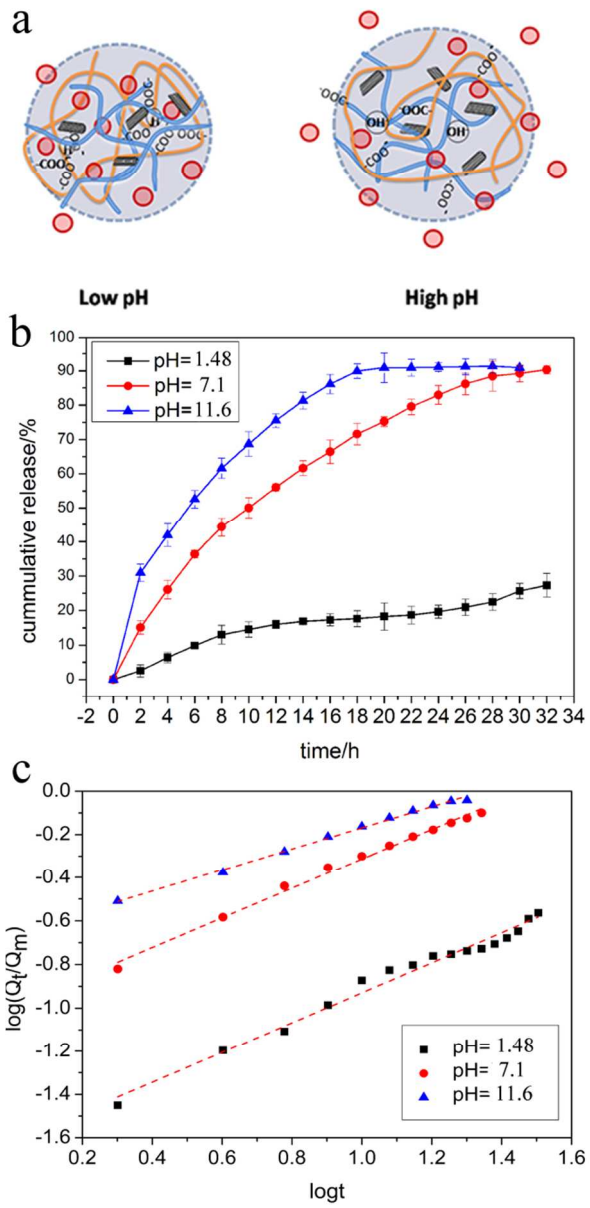

Fig.6 Schematic illustration of pH-sensitive release of Ibuprofen molecules from the BC/SA/MWCNTs0.25% (a), amounts of Ibuprofen released from BC/SA33 hydrogels at time t (h) under different pH values at 37oC (b), Korsmeyer-Peppas model for mechanism of drug release under different pH values (c)

3.5.2 Electro-responsive drug release

The release behaviors of Ibuprofen from BC/SA/MWCNTs0.25% hybird hydrogels under various applied voltage are shown in Fig.7a and b which indicated the drug release behaviors depended strongly on the applied electric voltages. During the first 2h, the drug released about 12% under 0V due to the relatively lower swelling ratio of hydrogels. Under 0.2V, 0.3V and 0.4V, drug releasing amount reached to 23%, 29% and 38% respectively. A higher applied electric voltage was a main factor for electro-responsive swelling behavior and could induce a more rapid release process. The diffusion exponent "n" was calculated from the slopes of the plots (Fig.7c) over a limited period of time using the Peppas' semi-empirical model (Eq.5). The n value of BC/SA/MWCNTs hydrogels without electric voltage was 0.689

(Table.2) showed a non-Fickian diffusion. The diffusion exponent n under 0.4V was 0.494, closed to the Fickian exponent value of n=0.45. The exponent n revealed that the drug release mechanism of drug under 0.4V was more diffusion-based (n=0.494) than the others (n=0.682, 0.693, 0.693, 0.698). From the releasing data, the electrical properties of the carbon nanotubes combined with the electro-sensitivity of BC/SA gel matrix led to the BC/SA/MWCNTs gels with a higher sensitivity and an enhanced electrical response releasing behaviour. In previous works, the similar phenomenon was reported to be electro-responsive with a same releasing mechanism. According our previous research about drug-loaded BC/SA hydrogels, the drug releasing rate showed an increasing trend with the increasing of electric voltage strength an electrical stimulus. Upon application of an electrical stimulus, the H^+ ion located in the hydrogels matrix, preferred to escape and move to the cathode surface. The absencen of H^+ ion resulted in the presence of more number of ionizable $–COO^-$ groups, thus enhancing swelling behaviours of hydrogels and increasing the drug releasing rate[32]. With doping MWCNTs into BC/SA matrix, BC/SA/MWCNTs had an increasing electrical conductivity which showed a more sensitive electric response in drug releasing. Luo, et al also reported drug-loaded CNTs sealed with polypyrrole (PPy) films could release drug in a controlled manner using electrical stimulation[29]. Yun, et al reported drug-loaded poly(vinyl alcohol) (PVA)/ poly(acrylic acid) (PAA)/ MWCNT nanofibers and MWCNT/ poly(vinyl alcohol) (PVA)/ poly(acrylic acid) (PAAC) composite microcapsules, partly. These two system both proved that MWCNTs could increase the conductivity of polymer matrix and improve the electro-responsive release behavior of the composites[13, 43].

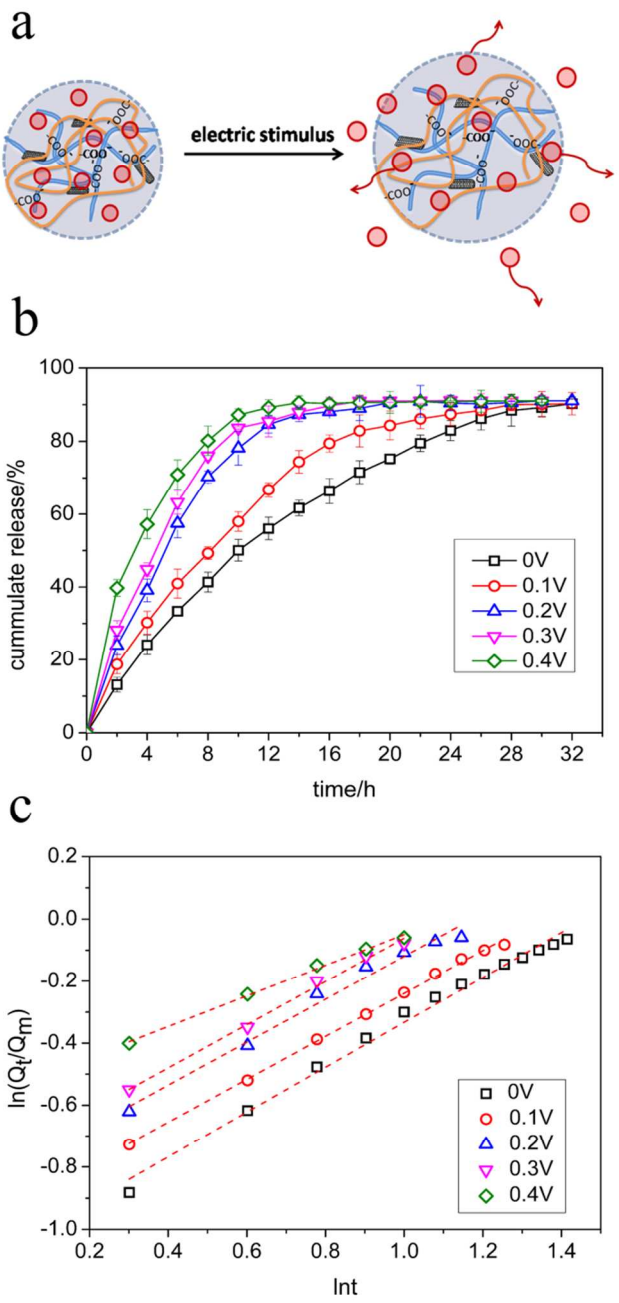

Fig.7 Schematic illustration of electro-sensitive release of Ibuprofen molecules from the BC/SA/MWCNTs0.25% hydrogels (a), amounts of Ibuprofen released from BC/SA/MWCNTs0.25% hydrogels at time t (b) under different electric voltage at 37°C (b), Korsmeyer-Peppas model for mechanism of drug release under different electrical voltage (c).

Table.2 Release parameters of Ibprofen sustained release from BC/SA hydrogels in various electric field strengths

voltage	0V	0.1V	0.2V	0.3V	0.4V
n	0.682	0.693	0.693	0.698	0.494
logk	0.347	0.394	0.443	0.468	0.580
R^2	0.996	0.993	0.979	0.998	0.991

Recently, multiple stimuli-response dosage forms had become a potential drug-delivery systems for use, a new drug delivery system that could release under complicated conditions was desired[41, 44]. Thereby, we further compared the releasing

behaviors of the drug under combination stimuli-release(varying pH with and without 0.4V applied electric voltage). As shown in Fig.8, unlike at pH=1.48 or pH=11.6 which had similar release curves with and without 0.4V applied electric voltage, releasing behaviour at pH 7.1 exhibited a relatively faster release rate under the electric streth applied.

The possible reason for this phenomenon was that when pH<7 alginate was protonated into insoluble form of alginic acid 0.4V applied electric voltage were not great enough to influence the conformation of the hydrogels. When pH>7, the deprotonation of alginic acid made a loose structure which allowed a fast release of drug. It was observed that applied electric voltage could not accelerate the drug release from hydrogel, which might be caused by alkaline conditions leading to increased mesh size could not restrict the diffusion of drug molecules thereby the effect of electro-response was slight.

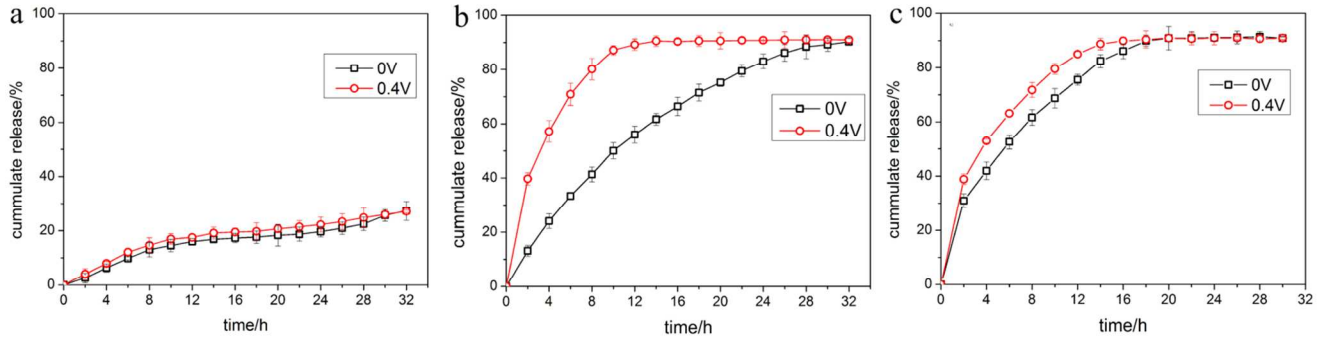

Fig.8 Combined drug release from BC/SA/MWCNTs0.25% hydrogels with varying pH under 0.4V electrical voltage pH=1.48(a), pH=7.1(b), pH=11.6(c)

3.5.3 Pulsatile drug release

Apart from conventional release in which drug was released under a sustaining condition such as an invariable pH or apllied voltage, release under varying conditions was required in disease states to present a rhythmic pattern[21, 45]. The drug release behaviours of BC/SA/MWCNTs hydrogels with an "on and off" electrical pulsatile stimulation (repeated stimulation of 0.2V for 2h followed by 0V for 2h) were investigated for 30h. We compared the sustaining and pulsatile stimulus release behaviors of BC/SA composites. Fig.9a shows that, drug releasing behaviours from BC/SA complex hydrogels under sustaining and pulsatile stimulus were practically same. The histograms revealed the release increments of model drug under sustaining stimulus were about 3-5% higher than the pulsatile stimulus. Then, we compared the pulsatile release behaviors of BC/SA/MWCNTs hybrid hydrogels and BC/SA complex hydrogels. Fig.9b showed the results of release profile under "on/off" electrical stimulation. The curves showed the cumulative amount of drug in the whole releasing process and the histograms revealed the release increments of model drug under each switch. Compared with the undoped hydrogel, the BC/SA/MWCNTs hybrid hydrogels showed a significant pulsatile release profile, followed by an increase in ibuprofen concentration in the released media upon application of the electric voltage. From histograms of Fig.9b shows that, ibuprofen releasing from BC/SA/MWCNTs showed an accelerated release behaviours under the application of the electrical field. While the electric stimulus was off, ibuprofen release rate was significantly reduced, and this conversion could be repeatedly cycled by adjusting the strength of electric voltage. The possible reason for this type of switching pattern was due to the higher electro-sensitivity by combining the MWCNTs into the polymer matrix[29, 31].

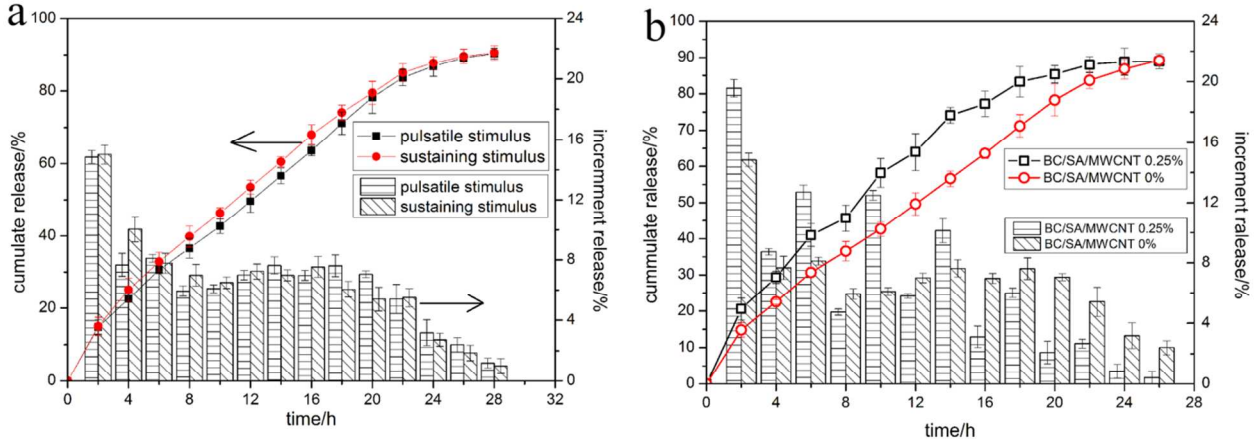

Fig.9 Pulsatile and passive drug release from BC/SA hydrogels under 0.2V electrical voltage (a), pulsatile drug release BC/SA/MWCNTs and BC/SA hydrogels (b)

Furthermore comparing the sustaining or pulsatile release behaviors under vary pH conditions, we found that unlike release

behavior under neutral pH which had an obviously pulsatile release profile under pulsatile stimulus, pulsatile releasing behaviors under acidic or alkaline pH exhibited a similar releasing trend. At pH=1.48, the release behaviors with or without stimulus were practically identical (Fig.10a) since alginate was protonated into insoluble form in acidic condition and hindered the process of drug diffusion. As shown in Fig.10b, it was clearly that a relatively faster release behavior was obtained under stimulus condition compared with the data under passive condition(pH=11.6), but pulsatile release behavior was not so distinct. The pulsatile release studies under vary pH conditions were effectively accelerated at neutral pH but suppressed at acidic or alkaline pH, which were in accordance with observations in sustaining release. Therefore, it could be concluded that the pulsatile release of drug was also effectively affected by the pH value.

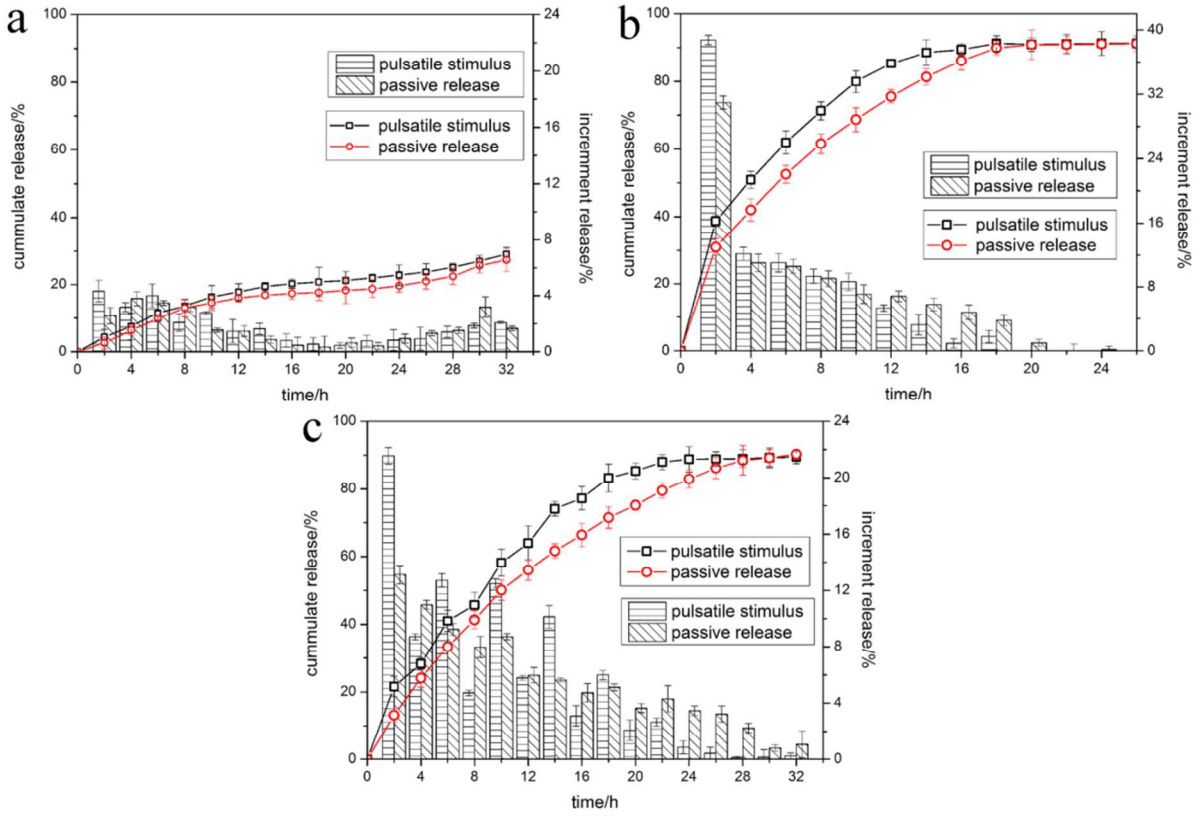

Fig.10 Pulsatile drug release from BC/SA/MWCNTs0.25% hydrogels under varying pH: pH=1.48 (a), pH=11.6 (b), pH=7.1 (c)

Conclusions

A novel hybrid hydrogels based on BC/SA incorporated with MWCNTs was successfully prepared as a controlled drug release system. Swelling studies showed that the BC/SA/MWCNTs hybrid hydrogels had pH-responsive and electro-responsive swelling behavior, in which drug release was based on the response of the system to environmental changes. In vitro, hybrid hydrogels system exhibited a pH-sensitive drug release behaviors with varying pH. This was because the drug release was mainly affected by the pH-induced protonation and deprotonation of SA. Meanwhile, the drug release could be enhanced in the presence of electric stimulus when compared with the passive release, and it was found to be dependent on the applied electric strength. The combination stimuli-release studies demonstrated that the electric-enhanced releasing behaviours had selectivity for the pH value of the condition, i.e. fast in neutral media but slow in acidic and alkaline media.

Pulsatile patterns of drug release were observed as electric stimulus was switched on and off. The pulsatile drug release studies indicated that embedded MWNTs as a conductive additive could effectively increased the electro-sensitivity of BC/SA/MWCNTs hybrid hydrogels. Furthermore, the pulsatile drug release studies also demonstrated that the pulsatile release behaviors were affected by the pH value. Only in neutral condition, drug release from BC/SA/MWCNTs hybrid hydrogels showed a significant pulsatile characteristic. Such systems therefore had the potential to contribute as a novel platform for pH-responsed gastrointestinal drug delivery or triggered by electric signal to get on-demand drug release.

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Notes and references

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