



A microfluidic chip platform with electrochemical carbon nanotube electrodes for pre-clinical evaluation of antibiotics nanocapsules

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ABSTRACT

This paper presents a microfluidic chip platform with electrochemical carbon nanotube electrodes for preclinical evaluation of antibiotics nanocapsules. Currently, there has been an increasing interest in the development of nanocapsules for drug delivery applications for localized treatments of diseases. So far, the methods to detect antibiotics are liquid chromatography (LC), high performance liquid chromatography (HPLC), mass spectroscopy (MS). These conventional instruments are bulky, expensive, not ease of access, and talented operator required. In order to help the development of nanocapsules and understand drug release profile before planning the clinical experiments, it is important to set up a biosensing platform which could monitor and evaluate the real-time drug release profile of nanocapsules with high sensitivity and long-term measurement ability. In this work, a microfluidic chip platform with electrochemical carbon nanotube electrodes has been developed and characterized for rapid detection of antibiotics teicoplanin nanocapsules. Multi-walled carbon nanotubes are used to modify the gold electrode surfaces to enhance the performance of the electrochemical biosensors. Experimental results show that the limit of detection of the developed platform using carbon nanotubes electrodes is 0.1 $\mu\text{g/ml}$ with a linear range from 1 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$. The sensitivity of the developed system is 0.023 $\text{mA ml}/\mu\text{g}$ at 37 °C. The drug release profile of teicoplanin nanocapsules in PBS shows that the antibiotics nanocapsules significantly increased the release of drug on the 4th day, measuring 0.4858 $\mu\text{g}/(\text{ml hr})$. The release of drug from the antibiotics nanocapsules reached 34.98 $\mu\text{g/ml}$ on the 7th day. The results showed a similar trend compared with the measurement result using the HPLC instrument. Compared with the traditional HPLC measurements, the electrochemical sensing platform we developed measures results with increased flexibility in controlling experimental factors for long-term preclinical measurement of nanocapsules in real time and at low cost.

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1. Introduction

In recent years, with advances in bioengineering and nanotechnology, many researchers have focused on developing new drug delivery methods for disease therapy. Currently, the development of nanocapsules for drug delivery applications in the localized treatment of disease has been attracting a great deal of interest (Jeong et al., 1997; Mora-Huertas et al., 2010). Drug delivery methods are moving from oral applications or local treatments to in vivo target drug delivery for people suffering from chronic illness or require long-term treatment. New drug delivery methods can sub-

stantially alleviate damage to the drugs by gastric, intestinal fluid. In addition, drugs carried to the target tissues by blood circulation are often diluted, thereby compromising efficacy. In addition, new drug delivery methods protect normal tissues from damage due to the toxicity of the drug and to increase the therapeutic efficiency of the drugs. Here is one example. In orthopedics, osteomyelitis is one of the most troubling clinical diseases for elderly patients infected by bacteria. Osteomyelitis is caused by bacterial infection resulting in inflammation of bone and bone marrow. Teicoplanin has been widely used as an antibiotic treatment of osteomyelitis, but traditional oral administration, intravenous injection, and intramuscular injection have shown very poor drug efficiency. Although injecting the drug is more effective than taking it orally, it causes long-term toxicity in the kidneys (nephrotoxicity), ototoxicity (ototoxicity), as well as other side effects. Normally, clinical treatment has been performed in the following manner – surgical debridement (the removal of necrotic and infected tissue), followed by the pasting of antibiotics drugs over

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the treated tissue for 4–6 weeks. Currently, bone cement (polymethylmethacrylate, PMMA) is used as an embedding material for antibiotics drugs. Antibiotic paste is implanted in the infected area to provide sustained release of antibiotics drugs to suppress inflammation. Even though implanted PMMA is biocompatible, it is not biodegradable and the material hinders the growth of bone tissue in the infected area. Therefore, secondary surgical operations are needed to remove the beads following completion of the drug treatment. In addition, the release rate of the drug from embedded antibiotic bone cement is very low. Several studies have demonstrated the making of antibiotics nanocapsules using multilayer polyelectrolytes/aminoglycoside deposition on zinc oxide particles (Khopade et al., 2005), polylactide–polyglycolide copolymers with antibiotic vancomycin and gentamicin (Liu et al., 2005), and poly(ethyleneglycol) monomethyl ether/polylactide-co-glycolide with antibiotic teicoplanin (Peng et al., 2010). Polylactide-co-glycolide (PLGA) is a popular biodegradable and temperature-sensitive copolymer used for implantable devices. In an aqueous solution, this copolymer self-assembles into a micro-packet structure. The use of such a mechanism can cause antibiotics drugs embedded in the copolymer to form antibiotics nanocapsules. The therapy efficiency of the treatments relies on the drug release profile. However, it is costly and risky to do the clinical trials before fully understanding the characteristics of nanocapsules.

So far, the drug release profile of nanocapsules is still characterized through the use of large-scale instruments. Traditionally, drug release profiles have been characterized by means of instruments such as HPLC or UV–vis spectroscopy for the development of new nanocapsule drugs. Using traditional instruments has several disadvantages, such as large sample volume, a lack of real-time and long-term measurement, complex processes, and the need for highly skilled clinicians. These days, the application of partial antibiotics for localized treatment of disease has been introduced as a possible solution; however, this provides only a portion of the drug release information of nanocapsules. During the process of drug development, long-term observation of drug characteristics, such as degree of acidity, drug encapsulation capability, and release stability, biocompatibility, side effects, particularly with regard to drug embedding capacity and the stability of drug release are crucial. In clinical treatment, drug dosage must be maintained at effective concentrations for a period of time. If the concentration of the drug in the target tissue is too low, it remains ineffective; and if the concentration is too high, it can be toxic to tissues. Nanocapsules require consistency and long-term stable delivery; therefore, the development of the biosensing platforms is necessary for the design and adjustment of drug release profiles and the stability of nanocapsules. A number of studies have demonstrated the detection of antibiotics using electrochemical magneto sensing (Zacco et al., 2007), a cell-based luminescence sensor (Möhrle et al., 2007), fluorescence sensing with magnetic beads (Piyasena et al., 2008), surface plasmon resonance sensing (Raz et al., 2009), and lab-on-a-chip with wavelength interrogated optical sensing techniques (Suárez et al., 2009); however, these systems tend to be complex and lack long-term measurement ability. Electrochemical detection with cyclic voltammetry has the advantages of ease of fabrication, low cost, and easy processing. To aid in the development of nanocapsules and understand drug release profiles prior to clinical experiments, it is important to establish a microfluidic chip platform capable of monitoring drug release profiles of nanocapsules in easy way. However, electrodes in electrochemical detection always have regeneration problems on the surfaces of electrodes. Mechanical stability, high sensitivity, and re-generable surface are three major concerns for ideal electrochemical sensing electrodes. They are strongly related to Joule heating, electrode surface morphology, and aging or contamination during the electrochemical sensing.

In the past years, carbon nanotubes have been attracting a great deal of attention, in the enhancement of neural recordings (Keefer et al., 2008), energy storage (Lee et al., 2010), and biosensors (Chen et al., 2010). This is due to their catalytic properties, signal sensitivity, and amplification. Especially, CNT has superior material properties in mechanical strength (Krishnan et al., 1998) and thermal conductivity (Berber et al., 2000). The cyclic tests of multi-walled carbon nanotube coated electrodes have been investigated to show good stability (Minnikanti et al., 2009). So far, several CNT deposition techniques at low temperatures have been successfully developed, such as electrophoretic deposition (Bocaccini et al., 2006), self-assembly monolayer (Su et al., 2006), transfer printing (Kim et al., 2010), ink-jet printing (Beecher et al., 2007), spraying coating (Kaempgen et al., 2009), PDMS cast molding (Wu et al., 2009), and drop coating (Sivaramakrishnan et al., 2008). The all above process are compatible with fabrication process of plastic chips. Recently, several lab on chip platforms with multi-walled carbon nanotubes have been studied, such as microfluidic chips with CNT detectors for detection of antioxidant profiles (Crevellén et al., 2008), microfluidic chips with CNT sensors for detection of sodium hypochlorite (Yang et al., 2009), and CNT-based immunoassay for optical detection of Staphylococcal Enterotoxin B (Yang et al., 2010). In this study, a new type of electrochemical biosensor platform using electrodes modified with carbon nanotubes for pre-clinical, real-time, and long-term evaluation of the drug release profile of nanocapsules will be developed and characterized. Multi-walled carbon nanotubes will be employed and studied on working electrodes of electrochemical biosensors to increase sensitivity and as a protective layer to increase the mechanical strength and address the regeneration problem.

2. Materials and methods

2.1. Materials and nanocapsules

Teicoplanin was obtained from Targocid (Cruppo Iepetit S.p.A.). Poly(ethyleneglycol) monomethyl ether (mPEG)(Mn, 550 g/mol) as an initiator was purchased from Polyscience, Inc., USA. D,L-lactide and glycolide were purchased from PURAC biomaterials, USA. Stannous 2-ethylhexanoate (Stannous Octoate) was purchased from Aldrich Chem. Co., Inc. Phosphate buffered saline (PBS) as a buffer solution was purchased from Aldrich Chem Co., Inc. Multi-walled carbon nanotube (MWCNT) (product no. 698849; composition, >99%; O.D. $\times$ length, OD, 6–13 nm $\times$ 2.5–20 μ m; average wall thickness, 7–13 graphene layers; surface area, $\sim$ 220 m²/g; CVD method produced) was purchased from Aldrich. All chemicals used in buffer preparation and in supporting electrolytes were HPLC grade. D.I. water used in the experiments was purified in a Barnstead water purification system. 4 inch cyclic olefin copolymers (Topas® COC 6015) wafers were prepared by injection molding and used as chip substrates.

Teicoplanin, a glycopeptide (glycopeptide) antibiotic against gram-positive bacteria (gram-positive bacteria), is one of the most common antibiotics drugs for the treatment of osteomyelitis. Teicoplanin is a time-dependent type antibiotic, effective when its drug concentrations exceed the minimum inhibitory concentration of 10 μ g/ml. PLGA is a popular biodegradable and temperature-sensitive copolymer used for implantable devices. In an aqueous solution, this copolymer self-assembles into a micro-packet structure. The use of such a mechanism can cause antibiotics drugs embedded in the copolymer to form antibiotics nanocapsules. In this study, PLGA is coupled with the hydrophilic mPEG to form nanocapsules. The hydrophobic site of this amphiphilic copolymer was attached to the antibiotics drug, teicoplanin, as shown in Fig. 1(a). Antibiotics nanocapsules were produced by co-author,

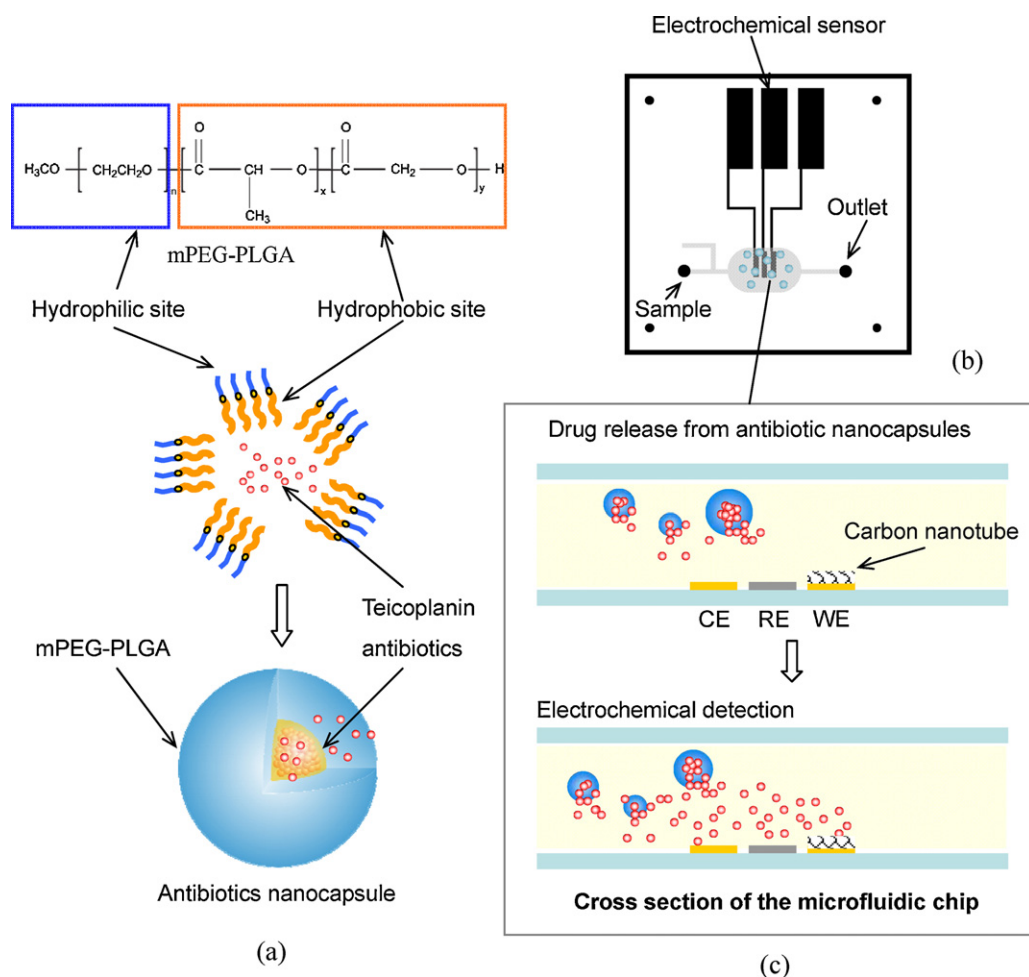


Fig. 1. Schematic illustration of the microfluidic chip platform with electrochemical carbon nanotube electrodes for preclinical evaluation of the drug release of antibiotics nanocapsules: (a) the drawing of nanocapsules, (b) the microfluidic platform, and (c) the cross section of the microfluidic chip (CE: counter electrode; RE: reference electrode; WE: working electrode).

Dr. Chu (Peng et al., 2010). mPEG-PLGA to be used as biomaterial for the nanocapsules. Teicoplanin was added to a 25 wt% copolymer aqueous solution. The final concentration of teicoplanin in the copolymer solution was 840 $\mu\text{g}/\text{ml}$.

2.2. Chip platforms and apparatus

In this study, a microfluidic chip platform with a carbon nanotube-coated working electrode was designed and developed, for preclinical evaluation of the drug release profile of nanocapsules, as shown in Fig. 1(b). The electrochemical biosensing platform comprises sensing electrodes and a microfluidic chip. Each electrode has 500 μm in width and 3 mm in length. The gap between the electrodes is 500 μm . The microfluidic chamber has 1 cm in length, 5 mm in width, and 100 μm in depth. The microfluidic chip platform provides the advantages of tiny samples, long-term detection of drug release, ease of fluids handling, and low cost. Antibiotics nanocapsules can be included in the electrochemical biosensing platform for preclinical evaluation of drug release profiles of antibiotics nanocapsules. The developed biosensor makes use of electrochemical sensing principles. The electrochemical biosensor includes the counter electrode, the reference electrode, and the working electrode, which were made through UV photolithographic techniques. The electrodes were patterned on a 4" COC wafer with a 400 nm-thick gold layer deposited by e-beam evaporation. After the reference electrode was made by

electroplating Ag/AgCl on the gold layer. The working electrode was modified by coating the gold layer with multiwall carbon nanotubes via the following steps. First, a carbon nanotube solution with a concentration of 500 $\mu\text{g}/\text{ml}$ was prepared by mixing multiwall carbon nanotubes in D.I. water in an ultrasonic sink for 30 min and the wafer was coated and patterned with S1818 positive photoresist. Except for the working area, the entire electrode was blocked by a pattern produced with the photoresist. A drop of mixed CNT solution was deposited on the surface of the Au working electrode, after baking the photoresist at 60 °C for 24 h. Following the baking of CNT, the patterned photoresist was removed by separate rinsing with acetone, methanol, and D.I. water. According to SEM observation, the thin CNT layer tended to attach to the gold electrode; afterward, a COC plastic microfluidic chip was integrated with the fabricated electrochemical biosensor. The antibiotic solution released from the nanocapsules in a PBS solution was injected into the biosensor platform for real-time electrochemical detection of teicoplanin release profiles. The detection system comprised a microfluidic chip platform, sensing circuit, a hotplate, a PC-based DAQ system (NI USB-6281), and a LabVIEW program, shown in Fig. 2. The layout of the sensing circuit is shown as Supplementary Fig. 1s. An oxidation potential of 0.95 voltages was applied to the electrochemical sensor, and the detection of antibiotic samples was performed by cyclic voltammetry scanning with a scanning rate of 50 mV/s, which was controlled by a PC-based DAQ system with a LabVIEW program. Phosphate buffered saline (PBS) solution and drug sample were

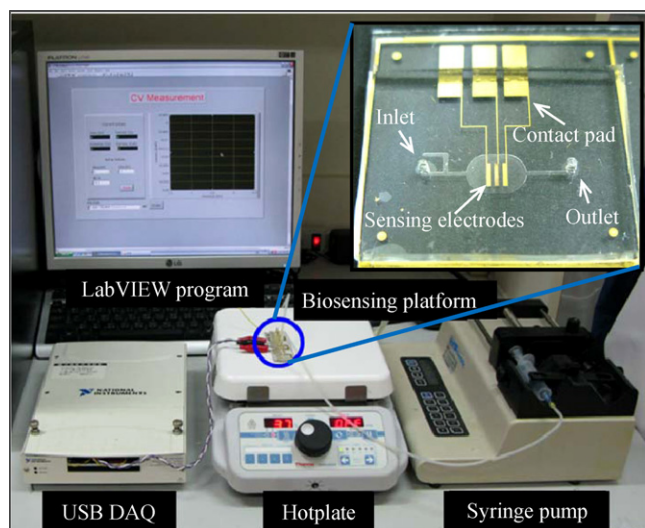


Fig. 2. Photograph of experimental setup for sensing of drug release profile of the antibiotics nanocapsules.

injected into the biosensor platform with a syringe pump. The platform was maintained at 37 °C over a hotplate. Following detection, the solution was removed and the sample was re-injected into the chip. The microfluidic chip platform with CNT-coated working electrodes can easily be operated following the above procedures for preclinical evaluation of antibiotics nanocapsules.

3. Results and discussion

3.1. Characterizations of CNT-coated electrodes

Mechanical stability, high sensitivity, and re-generable surface are three major concerns to work as an ideal electrochemical sensing electrode. They are strongly related to the Joule heating effect, surface morphology of electrodes, and electrode aging or

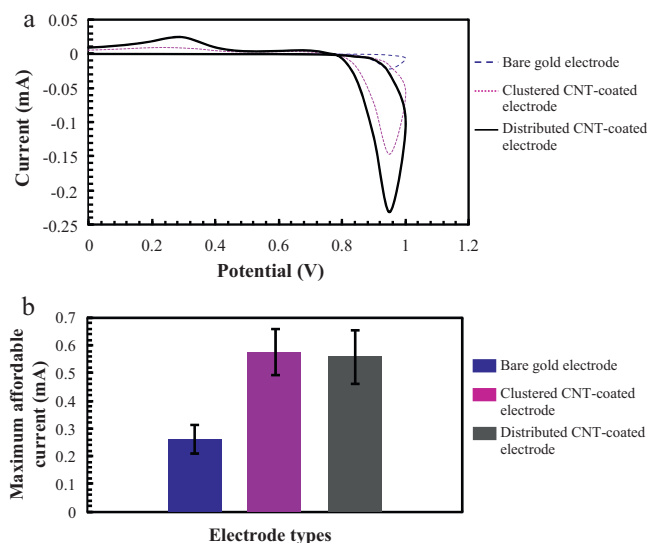


Fig. 4. Performance of different-type electrodes: (a) CV signals with different-type electrodes, and (b) maximum affordable currents with different-type electrodes.

contamination during long-term electrochemical sensing. In this study, the working electrodes were treated with multiwall carbon nanotubes using a drop coating method. The dispersion of CNT can be improved by ultrasonic mixing. In order to compare the performance of electrodes with/without CNT dispersion process, two types of CNT-coated electrodes were prepared in the experiments. Clustered CNT-coated electrodes were prepared by drop coating of CNT without ultrasonic dispersion process. Distributed CNT-coated electrodes were prepared by drop coating of CNT with ultrasonic dispersion process. The images of electrode surfaces with different treatments were shown in Fig. 3(a)–(d). Compared to a platform using bare gold working electrodes, the electrochemical oxidation voltage using the CNT-modified platform was kept at the same value. The measurement results showed that the sensing signals had been amplified from 0.0222 mA (bare

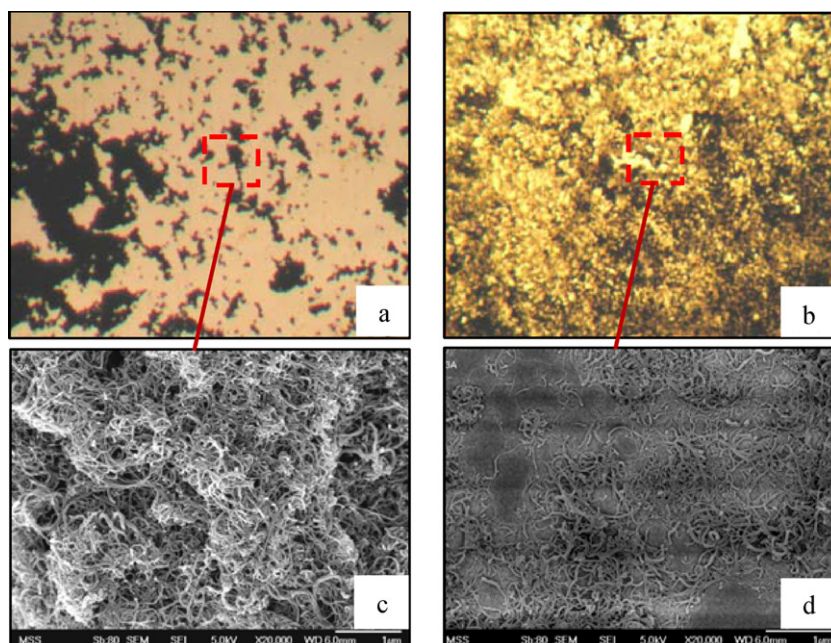


Fig. 3. Photographs of different-type electrodes: (a) microphotographs of the clustered CNT-coated working electrode surface, (b) SEM image of the clustered CNT-coated working electrode surface, (c) microphotographs of the distributed CNT-coated working electrode surface, and (d) SEM image of the distributed CNT-coated working electrode surface.

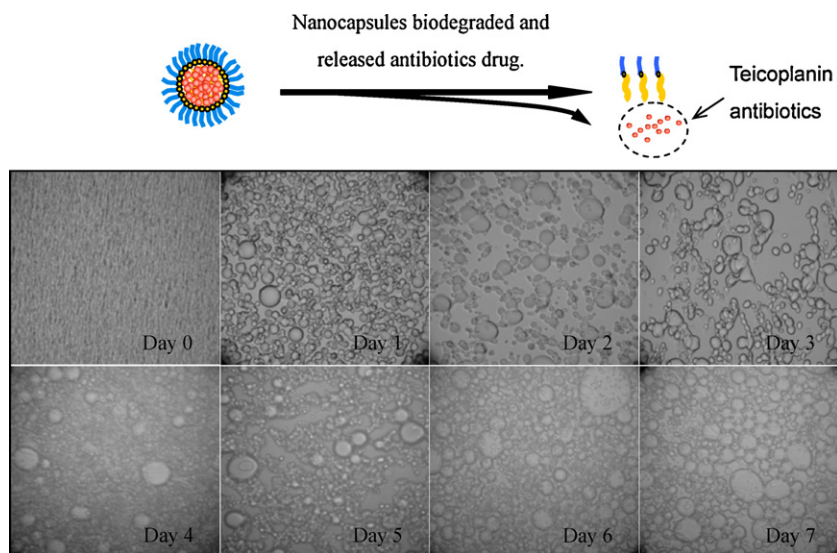


Fig. 5. Microphotographs of antibiotic teicoplanin nanocapsules during the drug release.

gold electrode) to 0.233 mA (distributed CNT-coated electrode). The amplification of the signal for the biosensor platform using working electrodes coated with carbon nanotubes was as high as 10.5 times, shown in Fig. 4(a). In addition, the experiments showed that bare gold electrodes are easily damaged by the sensing current due to the Joule heating effect. The maximum affordable current was improved from 0.208 mA (bare gold electrode) to 0.6680 mA (distributed CNT-coated electrode), following modification of the platform by coating the working electrodes with carbon nanotubes, as shown in Fig. 4(b). The maximum affordable current for the

biosensor platform using working electrodes coated with carbon nanotubes was as high as 3.2 times compared with the platform with bare gold working electrodes. The power losses in the electrode due to the Joule heating effect are a product of the square of the current and the resistance of the electrode. So, the maximum affordable power for the biosensor platform using the CNT working electrodes was as high as 10.3 times compared with the platform with bare gold working electrodes. The modified working electrodes provided increased surface area and rapid heat dissipation to increase signal strength and avoid heat damage.

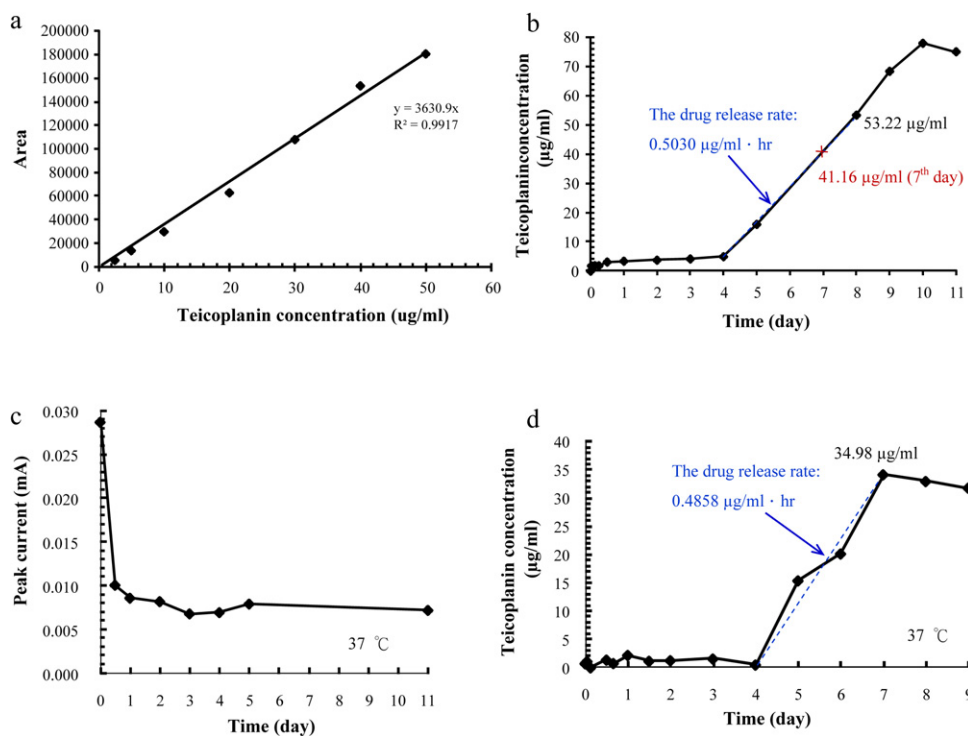


Fig. 6. Drug release profile of antibiotic teicoplanin nanocapsule samples: (a) the measurement results using HPLC with different teicoplanin concentrations for calibration use, (b) measurement results of antibiotic teicoplanin nanocapsule samples using HPLC, (c) measurement results using the chip platform with the bare gold working electrode, and (d) measurement results using the chip platform with the distributed CNT working electrode.

3.2. Measurements of the electrochemical sensing platforms

Different teicoplanin concentrations were prepared in phosphate buffered saline (PBS) for calibration measurements. The antibiotics teicoplanin nanocapsules will be implanted in the body, so the characterization of the electrochemical sensing platform at 37 °C must be considered. The electrochemical sensing platform was put on a hotplate for temperature control. Because the original pH of teicoplanin samples was 7, pH buffers were used to change the pH values of teicoplanin samples to 5 and 6. The calibration curves at various temperatures and pH values are illustrated in [Supplementary Fig. 2s](#). The measured peak currents were increased around 4–5% at the working temperature of 37 °C compared to the measured peak currents at 25 °C. The measurement results show that there is slight difference at different pH conditions. Calibration curves at 37 °C were used to convert the measured signal into concentrations.

Micrographs of morphological change in the antibiotics nanocapsules are shown in [Fig. 5](#). At first, the morphology of the nanocapsules appeared uniformly dispersed, but the nanocapsules began to change during the drug release experiments. In addition, according to the measurements, the pH of the nanocapsule solution shifted from pH 7 to pH 5 during the biodegradation, shown in [Supplementary Fig. 3s](#). In order to evaluate the developed platform, HPLC is used to measure the drug release profile. The calibration curve for HPLC is shown in [Fig. 6\(a\)](#). In [Fig. 6\(b\)](#), according to the measurements taken from HPLC, the antibiotics nanocapsules significantly increased the release of drug on the 4th day, measuring 0.5030 $\mu\text{g}/(\text{ml h})$. The release of drug from the antibiotics nanocapsules reached 53.22 $\mu\text{g}/\text{ml}$ on the 8th day. In addition, the platform with bare gold working electrodes was used to measure the drug release profile to compare with our developed platform with carbon nanotube-coated electrodes. The result from the bare gold electrode showed that the peak current gradually decreased, as shown in [Fig. 6\(c\)](#). This was due to the blocking by analytes or molecules from nanocapsule materials on the surface of the gold working electrodes during the experiments. The regeneration of the surface of the gold electrodes for electrochemical detection is a big issue in biosensors with regard to reuse and long-term measurements. However, the developed platform with carbon nanotube-coated electrodes showed good performance in electrochemical sensing. From the results, the limit of detection of the developed electrochemical biosensing platform using carbon nanotubes electrodes was 0.1 $\mu\text{g}/\text{ml}$. The linear range was from 1 $\mu\text{g}/\text{ml}$ to 10 $\mu\text{g}/\text{ml}$ ($R^2 = 0.9837$). The sensitivity of the developed system was 0.023 $\text{mA ml}/\mu\text{g}$ at 37 °C. The drug release profile of teicoplanin nanocapsules in PBS was measured using the developed electrochemical sensing platform, shown in [Fig. 6\(d\)](#). According to the measurements taken from our electrochemical sensing platform at 37 °C, the antibiotics nanocapsules significantly increased the release of drug on the 4th day, measuring 0.4858 $\mu\text{g}/(\text{ml h})$. The release of drug from the antibiotics nanocapsules reached 34.98 $\mu\text{g}/\text{ml}$ on the 7th day. The results showed a similar trend compared with the measurement result using the HPLC instrument. Compared with the traditional HPLC measurements, the electrochemical sensing platform we developed measures results with increased flexibility in controlling experimental factors for long-term preclinical measurement of nanocapsules in real time and at low cost.

4. Conclusions

In this work, electrochemical biosensing platforms with carbon nanotube-coated electrodes have been successfully developed and characterized for rapid detection of antibiotics teicoplanin

nanocapsules. Experimental results show that the limit of detection of the developed electrochemical biosensing platform using carbon nanotubes electrodes is 0.1 $\mu\text{g}/\text{ml}$ with a linear range from 1 $\mu\text{g}/\text{ml}$ to 10 $\mu\text{g}/\text{ml}$. The sensitivity of the developed system is 0.023 $\text{mA ml}/\mu\text{g}$ at 37 °C. The drug release profile of teicoplanin nanocapsules in PBS shows that the antibiotics nanocapsules significantly increased the release of drug on the 4th day, measuring 0.4858 $\mu\text{g}/(\text{ml h})$. The release of drug from the antibiotics nanocapsules reached 34.98 $\mu\text{g}/\text{ml}$ on the 7th day.

Furthermore, in orthopedics, osteomyelitis is one of the most troubling clinical diseases for elderly patients infected by bacteria, and applying partial antibiotics for localized healing of the disease is a recent solutions. Nanocapsules require consistency and long-term stable delivery. Through the use of the biosensor platform, we were able determine the characteristics of nanocapsules easily, to provide information for improving the synthesis of nanocapsules in the next stage prior to clinical experiments. The results from the developed platform show a similar trend compared with the measurement results using the HPLC instrument. Compared with the traditional HPLC measurements, the platform we developed measures results with increased flexibility in controlling experimental factors for long-term preclinical measurement of nanocapsules in real time and at low cost. The antibiotic biosensor platform could be further integrated with a micro fluidic platform for controlled synthesis of nanocapsules to provide feedback regarding the drug release profile, in the optimization of the synthesis process.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.bios.2011.02.017](https://doi.org/10.1016/j.bios.2011.02.017).

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