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Electroconductive nanocomposite hydrogel for pulsatile drug release

C.J. Pérez-Martínez [a,b], Sergio Daniel Morales Chávez [c], T. del Castillo-Castro [c,*], Tania Ernestina Lara Ceniceros [d], M.M. Castillo-Ortega [c], D.E. Rodríguez-Félix [c], Juan Carlos Gálvez Ruiz [e]

[a] Centro de Investigación en Materiales Avanzados, S.C., CP 31109 Chihuahua, Chihuahua, Mexico
[b] Departamento de Ciencias Químico Biológicas, Universidad de Sonora, CP, 83000 Hermosillo, Sonora, Mexico
[c] Departamento de Investigación en Polímeros y Materiales, Universidad de Sonora, CP, 83000 Hermosillo, Sonora, Mexico
[d] Centro de Investigación en Materiales Avanzados, S.C., CP 66600 Apodaca, Nuevo León, Mexico
[e] Departamento de Ciencias de la Salud, Universidad de Sonora, CP, 85010 Ciudad Obregón, Sonora, Mexico

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ABSTRACT

Polyacrylamide (PAAm) hydrogel containing nanofibers of polyaniline (PANI) has been prepared in order to evaluate it as electric stimuli-responsive material. Amoxicillin was loaded onto chemically synthesized PANI nanofibers of large-aspect-ratio. Composite hydrogel was obtained by the *in situ* incorporation of amoxicillin-loaded PANI during polymerization and reticulation of acrylamide. TEM images of cross sections of PAAm/amoxicillin-loaded PANI composite revealed a continuous 3D nanofiber network of PANI supported by the hydrogel matrix. The antibiotic molecules were accurately released (or sustained) from composite hydrogel in response to application (or removal) of cathodic electrical stimulation. *In vitro* cytotoxicity evaluation of composite hydrogel extract on mouse subcutaneous connective tissue has shown cell viability higher than 80%. The tuning release profile and minimal toxicity of the material evidenced its potential for electrically controlled drug delivery applications such as implantable devices and transdermal drug delivery systems.

1. Introduction

Electroconductive hydrogels (EHs) are polymeric blends or co-networks constituted by hydrated hydrogels and intrinsically conductive polymers. These systems combine the high electrical conductivity and electrochemical redox properties from conductive polymer with the swelling capacity and high molecular diffusivity associated to hydrogel in aqueous media.

The composition and the preparation method of EHs influence their structure at micro- and nano-scale, determining the final material properties. Suitable procedures have been developed for preparing electroconductive nanocomposite hydrogels of *N*-isopropylacrylamide and polyaniline (PANI) [1], polyvinyl pyrrolidone/PANI [2] and polyacrylamide (PAAm)/PANI [3], among others.

The creative combination of the inherent properties of constituent materials gives rise to technological applications of EHs [4,5]. In the biomedical field, these multifunctional smart materials have been evaluated as biorecognition membranes in biosensors [6,7], scaffolds for tissue engineering [8,9] and for electro-stimulated drug release systems [10,11].

Electro-stimulated drug release devices are engineered devices that produce a programmed drug release profile influenced by the application of voltage or current [12]. The design of these systems has become one of the promising advanced technology areas of polymers as well as in modern-day medical, agricultural and pharmaceutical sciences [12,13]. Unlike controlled drug delivery systems that involve spontaneous release patterns, the stimuli-responsive materials offer the promise of new treatments that require accurate and on-demand doses of medication at a specific patient's physiological conditions.

The literature shows some reports of EHs as electric stimuli-responsive materials for drug delivery. Madou and et al. [14] prepared a sphincter actuator of poly(2-hydroxy-ethylmethacrylate)-poly(*N*-vinylpyrrolidone) and PANI which opens and closes, corresponding to the shrinking and swelling conditions of the polymer system, in response to specific electrical potentials.

In a different approach, Niamlang and Sirivat [15] studied the release mechanisms of salicylic acid from drug-doped poly(phenylene vinylene)/PAAm hydrogels in the presence of electric fields. De Torresi et al. [16] electropolymerized aniline inside PAAm porous matrix for tetracycline release upon electrical stimulation; they observed that the use of positive potentials led to PANI expansion with the concomitant opening of whole network, facilitating the water uptake and the exit of higher amounts of antibiotics. Recently, Ge et al. [17] synthesized electric responsive nanoparticles of polypyrrole (PPy) loaded with pharmaceuticals, which were subcutaneously localized *in vivo* with the assistance of a temperature-sensitive hydrogel. In this case, after molecules were released from PPy nanoparticles by electrochemical

* Corresponding author.
E-mail address: terecat@polimeros.uson.mx (T. del Castillo-Castro).

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reduction/oxidation processes, electric-field-driven migration triggered the movement of charged entities toward the electrode of opposite charge, which resulted in the escape of drugs from the hydrogel.

Despite studies confirming the potential of EHs as electric stimuli-responsive materials, major limitations need to be overcome. These limitations include the passive loss of loaded drug molecules by diffusion and poor control of the release kinetics by electrical activation.

This study presents the evaluation of PAAm/PANI nanocomposite hydrogel as electric stimuli-responsive material. In a novel approach, amoxicillin was loaded onto chemically synthesized PANI nanofibers of large-aspect-ratio. The drug-loaded PANI fibers were encapsulated into hydrogel matrix during polymerization and reticulation of acrylamide monomer. The aim of this work is to show the switch ON/OFF capabilities of the material for drug release by applying electrical stimulations.

2. Experimental

2.1. Materials

Aniline (99.5%; Sigma-Aldrich) was distilled under vacuum before use and stored in the dark below 0 °C. Ammonium persulfate (APS, 98.7%; J. T. Bayer), L-glutamic acid (GA, 98.99%; J. T. Bayer), amoxicillin (potency ≥900 μg/mg; Sigma-Aldrich), acrylamide (AAm, ≥ 99%; Sigma), N,N′-methylenebis(acrylamide) (MBAAm, 99%; Sigma-Aldrich) and N,N,N′,N′-tetramethyl-ethylenediamine (TEMED, 99%; Sigma-Aldrich) were used as received without further purification.

2.2. Polymerization of aniline

PANI was synthesized by chemical-oxidative polymerization of aniline in the presence of GA, as reported in previous work [18]. Briefly, aniline was dissolved in an aqueous GA solution and the polymerization proceeded by slowly adding the APS solution to the monomeric solution placed in an ice bath under nitrogen atmosphere. The molar ratio of aniline:GA:APS was 1:0.25:1. The reaction mixture consisting of dark-green suspension of PANI was thoroughly rinsed with deionized water in a Buchner funnel until the filtrate became neutral. This procedure allows obtaining fibrillar structures of PANI coated with the amino acid with diameters in submicro- and nanometric scale [18].

2.3. Loading of amoxicillin to PANI

For the loading of amoxicillin, 25 mL of PANI suspension (14.8 g L^{-1}) was mixed with 5 mL of an aqueous solution of the drug (0.2 g mL^{-1}). After stirring for 24 h, the resultant mixture was carefully transferred to dialysis tubing (acetate of cellulose, purification capacity M.W. > 12,000). The sealed dialysis tubing was then put into 500 mL of deionized water at room temperature for removing the drug that was not adsorbed on PANI structures. The dialysis solution was periodically replaced with fresh deionized water until the amoxicillin loss was below 0.1%. After the dialysis process, 797 mg of amoxicillin remained into the 30 mL suspension.

The concentration of amoxicillin was determined by recording the absorbance at 273 nm in the Perkin-Elmer Lambda 20 UV–Vis spectrophotometer and the subsequent interpolation of the value in a calibration curve.

2.4. Incorporation of amoxicillin-loaded PANI into polyacrylamide hydrogel

In a cylindrical mold of 26 mm diameter chilled in an ice bath (3 °C), 10 mL of AAm/MBAAm aqueous solution (58 g of AAm and 2 g of MBAAm in 100 mL) was mixed with 8 mL of the amoxicillin-loaded PANI suspension. The gelation process was initiated by adding 1 mL of APS solution (0.1 g mL^{-1}). After 2 min of mixing, 200 μL of TEMED reactant (accelerator) was added with further stirring for 2 min. Then, a thin copper electrode with an active area of 40 mm × 15 mm was axially introduced in the center of the circular cross-section area of composite hydrogel. Once the mold containing the reaction mixture and the electrode were removed from the ice bath to room temperature (25 °C), the gelation instantaneously occurred (20–30 s). Finally, the composite hydrogel with the incorporated electrode was removed from the mold and it was immediately used in the experiment of electrically controlled drug release. Fig. 1 illustrates the preparation of PAAm/PANI/amoxicillin hydrogel. The theoretical mass ratio of PAAm to PANI–amoxicillin complex was 58:3. Each composite hydrogel contained 213 mg of amoxicillin before the studies of drug release under electrical stimulus were performed.

2.5. Morphology characterization of PAAm/PANI/amoxicillin hydrogel

Ultrathin sections of composite hydrogel were cut at −150 °C with a cryo-ultramicrotome RMC Products and placed on copper grids. After sample drying at room temperature, the morphology was analyzed by TEM using a JEOL JEM-2200FS microscope.

2.6. Controlled release of amoxicillin by electrical stimulus

The experiments of drug release were performed in 0.1 M sodium phosphate buffer (PB, pH 7, $I = 0.4$ M), containing a mixture of monobasic and dibasic forms of sodium phosphate. The hydrogel-coated electrode was immersed in 80 mL of PB (25 °C) together with an uncoated identical electrode. The distance between the hydrogel surface and coplanar free electrode was approximately 4 mm. For the release study, potentials were applied between two electrodes with a DC power supply Agilent, model E3632A for 1 min in intervals of 30 min. Samples of 1 mL were withdrawn at specific time intervals for measuring the released amoxicillin. Sink condition was maintained by replacing equal volume of buffer. The release studies were performed in triplicate and the average results were plotted versus time.

2.7. Cytotoxicity assay

Mouse normal subcutaneous connective tissues (L–929) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% heat-inactivated fetal calf serum in controlled atmosphere (80–90% humidity, 37 °C, 5% CO_2), using an Isotemp Fischer Scientific incubator.

The *in vitro* viability of mouse cells was explored by MTT assay when hydrogels of PAAm or PAAm/PANI were incubated in cell culture medium. Three cylindrical hydrogels (7 mm thick, 3 mm diameter) of each composition were placed into 10.2 mL of complete cell culture media at 37 °C for 24 h. Then, the hydrogels were removed and the conditioned media were reserved to the viability assays.

Cells were seeded in a 96-well plate with 50 μL of DMEM medium (high glucose, supplemented with 5% FBS) at a density of 10,000 cells/well. After 24 h, 50 μL of pure or diluted conditioned media was added to each well. The final concentrations of conditioned media in cell reservoirs were 50% (*v*/*v*), 25% (*v*/*v*), 12.5% (*v*/*v*) and 6.25% (*v*/*v*). Negative controls were obtained by adding 50 μL of standard cell culture medium instead of conditioned media solutions.

10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL) was added to each well at the end of the treatment period (48 h) and incubated at 37 °C for 4 h. Formazan crystals were dissolved with acidic isopropanol, and the plates were read in an ELISA plate reader, using a test wavelength of 570 nm and a reference wavelength of 630 nm. Plates were normally read within 10 min after adding isopropanol. All reported values were the means of triplicate samples. The morphology of cells was observed by a Nikon Eclipse inverted microscope.