



Interpenetrating networks of bacterial cellulose and poly (ethylene glycol) diacrylate as potential cephalexin carriers in wound therapy

Iulia Elena Neblea¹ · Ana-Mihaela Gavrila¹ · Tanta-Verona Iordache¹ · Anamaria Zaharia¹ · Paul Octavian Stanescu² · Ionut-Cristian Radu² · Sabina Georgiana Burlacu¹ · Georgeta Neagu³ · Anita-Laura Chiriac¹ · Andrei Sarbu¹ 

Received: 31 January 2022 / Accepted: 22 August 2022 / Published online: 30 August 2022
© The Polymer Society, Taipei 2022

Abstract

In this work, hybrid hydrogels based on bacterial cellulose (BC) and poly (ethylene glycol) diacrylate (PEGDA) were synthesized by a radical polymerization reaction using a redox initiator system. The proposed interpenetrating networks (IPNs) were intended for developing a controlled release micro-vesicular system for wound therapy, through micro-colloidal architectures of hydrogels based on bacterial cellulose. Therefore, the hybrid hydrogels were first characterized to determine the influence of the BC concentration on the swelling degree and their mechanical stability. Further on, infrared spectroscopy (FTIR), thermo-gravimetric analysis (TGA/DTG), and scanning electron microscopy (SEM) were implemented to investigate their structure, composition, thermal stability, and morphology. The controlled release assay of cephalexin (CEX) was performed in buffer solution at pH 7.4 and 37 °C using CEX-loaded hydrogels and the release profiles were deciphered with the aid of UV–Visible spectroscopy. Cytotoxicity tests performed on simple and CEX-loaded samples indicated that the BC-PEGDA hydrogels containing the drug of interest were relatively non-toxic when exposed to murine fibroblasts, representing thus a potential candidate for materials used for wound treatment.

Keywords Hybrid hydrogels · Poly (ethylene glycol) diacrylate · Bacterial cellulose · Cephalexin · Drug delivery

Introduction

Hydrogels are some of the most interesting materials discussed in recent years, mainly due to their tunable properties and their hydrophilic nature. Hydrogels have a 3D insoluble network structure similar to soft tissue that can absorb large amounts of water or biological fluids. The absorption process occurs through counter-balanced forces such as capillarity, osmosis, or hydration, which cause the swelling of the material [1]. These interesting properties make their use possible in different fields such as biomedical, pharmaceutical, and technological fields with applications as controlled release systems [2, 3], encapsulation of cells [4], tissue repair [5], electrochemical capacitors [6], and sensors [7]. Among these applications, hydrogel-based drug delivery systems have become a research area of great interest and target some important characteristics such as biodegradability, high-swelling capacity and tissue-like mechanical properties.

One convenient way to prepare hydrogel delivery systems with desired properties is by combining synthetic polymers (e.g., polyacrylamides, polyvinyl alcohol, polyethylene glycols [9]) with natural polymers (chitosan, collagen,

Iulia Elena Neblea and Ana-Mihaela Gavrila authors with equal contribution.

✉ Anita-Laura Chiriac
raduanita@gmail.com

✉ Andrei Sarbu
andr.sarbu@gmail.com

¹ Advanced Polymer Materials and Polymer Recycling Group, The National Institute for Research&Development in Chemistry and Petrochemistry ICECHIM, Splaiul Independentei no. 202, 060021 Bucharest, Romania

² Department of Bioresources and Polymer Science, Faculty of Applied Chemistry and Materials Science, University “Politehnica” of Bucharest, 1–7 Gh. Polizu Street, 1st District, 011061 Bucharest, Romania

³ National Institute for Chemical Pharmaceutical Research and Development ICCF, 112 Calea Vitian, 031299 Bucharest, Romania

alginate, starch or gelatine [10, 11]). Of the variety of synthetic polymers, poly (ethylene glycol) diacrylate (PEGDA) is usually preferred for biomedical applications [14], due to its low toxicity, antimicrobial and antifouling properties [12], hydrophilicity, and resemblance to the extracellular matrix [13]. For instance, Frost and co-workers [8] developed a bioink with the use of poly (ethylene glycol) diacrylate and cellulose nanocrystals that can be used to create scaffolds for tissue engineering applications.

On the other hand, cellulose is a natural biopolymer found in plants with high potential for biomedical applications, due to its thermal stability, non-toxicity, and biodegradability, which led until now to extensive research. Biosynthesized cellulose by different Gram-negative bacteria like *Acetobacter* and *Agrobacterium*, known as biocellulose (BC), displays a different structure from cellulose found in plants, with soft microfibrils of less than 100 Å diameter, high crystallinity, and high water-absorption capacity [15, 16]. This is why, compared to cellulose from plants, biocellulose-based medical devices (e.g., artificial scaffolds for regeneration of cartilages, bones, urethra, heart valve prosthesis, artificial skin, and blood vessels, artificial cornea, and drug, hormone/protein delivery systems [18–20]), generally display higher chemical purity and porosity and increased strength in the wet state, being easier to sterilize without major changes of structure and properties [17]. Among the retrieved biomedical applications, several papers also nominate BC as an excellent material for wound-healing [21–23], due to its other unique properties that include water retention, film-forming, pain reduction, and autolytic debridement promotion [24, 25].

Thereby, the rationale behind the present study is to combine BC and PEGDA in the attempt to obtain a hydrogel material with synergic properties that can be loaded with the antibiotic cephalexin (CEX) and further used in wound therapy.

Cephalexin is a semi-synthetic antibiotic drug belonging to the class of β -lactam cephalosporins used to treat bacterial infections such as urinary tract infections, respiratory infections, skin infections, or other soft tissue wounds [26]. The class of cephalosporins is a group of commonly prescribed antibiotics being considered the safest and most effective bactericidal antimicrobial agents. CEX can be used as an active drug against both Gram-positive and Gram-negative bacteria, which act by disrupting the membrane integrity. CEX is almost completely absorbed in the gastrointestinal tract with a bioavailability of 95% and a half-life of about 1.1 h [27]. Cephalexin inhibits the production of essential structural components of bacterial cell wall and prevents infection with Gram-positive or Gram-negative bacteria. In this regard, the use of cephalexin has been reported in other studies for in vitro delivery from hydrogels [28].

Promising but limited results have been reported in the literature on the controlled release of cephalexin using hydrogels based on 2-hydroxyethyl methacrylate (HEMA), itaconic acid, and polyalkylene glycol methacrylate (BIS) [29] or using encapsulated nanohybrid materials based on carboxymethylcellulose and double-layered hydroxides for slow release into the gastrointestinal tract [27]. Even though the P(HEMA/BIS) hydrogels demonstrated a promising ability to deliver CEX at a controlled rate, cytotoxicity studies were not performed [29]. Another relevant study by Jilsha and Viswanad [30] reported the use of CEX formulated into nanosponge-loaded hydrogels to enhance in vitro skin permeation. This work suggested a two-step preparation protocol for hydrogels leading to time-consuming manipulation steps and numerous chemicals. Furthermore, Hajikarimi and Sadeghi also reported the use of CEX in drug delivery models for oral administration [31]. The authors presented the synthesis of a crosslinked poly N-vinyl pyrrolidone/acrylic acid/nanoclay hydrogel based on gelatine, but the release of the drug was under 75% at the ideal pH. It may also be relevant to mention that BC-PEGDA composites were studied as possible soft gel materials for cosmetic application [35], in which case the results show that an optimum ratio of BC and PEGDA can lead to materials with high biocompatibility and high adhesion to skin.—Hence, the literature survey provided evidence for the fact that studies of CEX release using BC-PEGDA hydrogels were not reported so far.

Recently, Zaharia et al. have established a direct protocol to prepare IPN hydrogels based on BC. In this respect, the study describes the synthesis of composite hydrogels based on BC and poly(acrylic acid-co-N,N'-methylene-bis-acrylamide) (PAA) with interpenetrating polymer networks (IPN), by radical polymerization, for the controlled release of liquid fertilizers for agriculture use [32]. Interpenetrating polymer network (IPN) hydrogels are formed by the combination of independent, yet interdigitating polymer networks at the molecular level [33]. According to IUPAC, IPN-based hydrogels differ from conventional composite hydrogels as IPN networks cannot be separated from one another without breaking crosslinks. Due to their improved mechanical strength, efficient drug loading capacity, and controlled swelling behavior, IPN hydrogels have been proven to be versatile and ideal matrices for the optimized release of various drugs [34].

To provide versatility to the previously developed method for IPN hydrogels preparation [32], this original study presents a simple and eco-friendly procedure to prepare IPN hydrogels, based on BC and PEGDA, that are suitable for treating surface dermal wounds. The novelty of this work considers a) the synthesis and characterization of IPNs from bacterial cellulose (BC) and poly (ethylene glycol) diacrylate (PEGDA) and b) the primary results

for CEX delivery using the novel BC-PEGDA delivery systems. The synthesis of the BC-PEGDA hydrogels was highlighted by Fourier transform infrared spectroscopy (FTIR) and the IPN formation and porosity of the hydrogel structure was confirmed by scanning electronic microscopy (SEM) [32]. Thermogravimetric analysis (TGA/DTG), rheology, and mechanical analysis were performed to investigate the physical–chemical properties of the CEX-loaded and unloaded hydrogels. The degree of swelling as well the ability to release CEX was also investigated for both loaded and unloaded hydrogels, while cytotoxicity of such BC-PEGDA hydrogels was investigated qualitatively to provide evidence for the potential application as drug delivery systems in wound therapy.

Experimental section

Materials

Poly (ethylene glycol) diacrylate (PEGDA) ($M_w = 700$ g/mol) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was used as received. The initiating redox system composed of potassium persulfate (KPS, 99%) and sodium metabisulfite (MS, 97%) was purchased from Acros Organics (US). Bacterial cellulose (BC) was synthesized by our collaborators from the University “Politehnica” of Bucharest in a static culture using pollen as nitrogen source and fructose as carbon source and delivered in acetic acid [36]. Cephalexin (CEX) was used as an active pharmaceutical ingredient in the loading/release process (St. Louis, MO, USA). All the products were used without further purification. Phosphate buffer (PBS) (Roti[®]-CELL) with pH = 7.4 was used as a dissolution medium, to quantify the release of the active substance loaded in hydrogels. Distilled water obtained in the laboratory (Distiller Liston A1204)- was used to prepare all aqueous solutions.

Synthesis and purification of BC-PEGDA-based hydrogels

The synthesis of IPN hydrogels based on non-lyophilized bacterial cellulose (BC) and poly(ethylene glycol) diacrylate (PEGDA) was performed by a radical copolymerization reaction, in aqueous media, using potassium persulfate (KPS) and sodium metabisulfite (MS) as redox initiator system, according to a previously described procedure [33]. The first step in the synthesis of the hybrid hydrogels was the preparation of the BC suspension. BC pieces were carefully shredded with a blender for 10 min until the particles reached 1–2 mm in size. Afterward, the particles were washed with distilled water and filtered on a filter paper for 2 h to reduce the water content to approx. 40% relative to the total amount of

water. In the next step, the aqueous solutions corresponding to the redox initiation system (1 wt. % of KPS rel. to PEGDA and 2 wt. % of MS rel. to PEGDA) were prepared. Subsequently, 10 wt % amount of PEGDA rel. to H₂O, distilled water and BC (0, 20, 40, 60, 80 and 100 wt. % rel. to PEGDA) were introduced into glass vials with a diameter of 10 mm. Thus, considering the BC amount, the resulted hydrogels were noted by analogy as *H0BC*, *H20BC*, *H40BC*, *H60BC*, *H80BC*, and *H100BC*. The obtained suspension was maintained at room temperature, under stirring in the ultrasonic bath for 2 h, to obtain a homogenous mixture of BC and PEGDA. Afterward, the redox initiation system (KPS/MS) was added and the mixture was further homogenized under stirring in a shaker (Benchmark Scientific MultiTherm[™] Shaker), 400 rpm at room temperature for 2 min. The vials were sealed with plastic caps and kept at 37 °C. After 24 h, the vials were broken and the obtained hydrogels (according to Fig. S1) were cut into small cylindrical pieces with a height of about 5 mm.

The purification of the hydrogel slices was performed by introducing the samples in glass vials with 100 mL of distilled water and kept for 7 days at room temperature. The water was changed daily to remove the unreacted compounds, such as potassium persulfate and sodium metabisulfite. After this purification step, the swollen hydrogels were introduced in an oven (LabTech Vacuum Drying Oven LVO-2030) at 40 °C until they reached a constant weight [37–39].

Characterization methods

BC and—BC-PEGDA hydrogels were analyzed by *Scanning Electron Microscopy (SEM)*, using the ESEM FEI Quanta 200 Instrument from Philips equipped with a secondary electron detector in the gaseous environment (GSED) and an acceleration voltage of 30 kV.

Fourier Transform Infrared Spectrometry (FTIR) spectra of hydrogels were recorded on a JASCO FTIR 6300 Spectrophotometer (Jasco Co. Japan) using 32 scans in the range 400–4000 cm⁻¹ with a resolution of 0.07 cm⁻¹, on KBr pellets.

Thermo-gravimetric Analysis (TGA/DTG) was assessed using TA Instruments Q5000 IR equipment by heating in nitrogen flow a sample of approx. 5 mg in the temperature range 25–700 °C, with a constant heating rate of 10 °C min⁻¹. The thermal stability of prepared hydrogels was investigated to underline the effect of different concentrations of BC upon the hybrid materials, as complementary results to FTIR measurements.

UV–vis spectroscopy was used to determine drug release profile. These were recorded at a temperature of 37 °C on Thermo Scientific[™] Evolution[™] 260 Bio UV–Vis

spectrometer (Waltham, MA, USA) in the range of 200–800 cm^{-1} using 10 mm quartz cuvettes filled with 2 mL of solution.

Swelling degree study

An important characteristic of hydrogels is their swelling degree, which represents the amount of liquid retained by the material. The gravimetric method that can be used to determine the swelling degree (SD), first involves weighing the xerogel (dry hydrogel), followed by successive weighing at different times after immersing the hydrogel in the liquid medium.

The swelling behavior of the synthesized hydrogels was determined by introducing a weighed xerogel disk (m_{dry}) into distilled water at a constant temperature of 37 °C. The swollen disks were removed from the medium at certain intervals. The excess water was removed with a filter paper, and the mass of the hydrogel (m_{wet}) was weighed and reintroduced into the used medium. The measurements were performed until the swollen gels reached a constant value, corresponding to equilibrium swelling.

$$\text{SD} = \frac{m_{\text{wet}} - m_{\text{dry}}}{m_{\text{dry}}} \times 100 \text{ (g water/g dry polymer)} \quad (1)$$

Load/release tests of cephalexin

The retaining capacity of hybrid BC-PEGDA-based hydrogels (noted as $H_{\text{CEX}}0\text{BC}$, $H_{\text{CEX}}20\text{BC}$, $H_{\text{CEX}}40\text{BC}$, $H_{\text{CEX}}60\text{BC}$, $H_{\text{CEX}}80\text{BC}$, and $H_{\text{CEX}}100\text{BC}$, respectively) and release profiles for CEX drug were investigated. To determine the release profiles of the active ingredient, the hydrogel disks were loaded with cephalexin into a phosphate-buffered saline solution (PBS) pH=7.4. Considering that the pH of infected surface wounds moves towards alkaline (above the normal pH of the skin 4.0–6.5) due to bacteria presence, the release experiments were performed in alkaline medium (in PBS at 7.4 pH) at a temperature of 37 °C in order to create similar minimum conditions of wound environment. Given the absorbent properties of hydrogels, the moist environment could potentially improve the healing process and stop the infiltration of bacteria [40, 41]. The molar concentration of the used drug solution was 7.196×10^{-3} mol/L (2.5 mg cephalexin/mL PBS). For this step, one disk from each xerogel was selected, weighed, and immersed in 3 mL of CEX loading solution for 48 h at room temperature. After swelling to equilibrium, the hydrogels were removed from the solution and dried in the oven at constant weight.

Considering the cephalexin loading/release studies in the literature, the choice of quantities was determined as a compromise between compliance with the solubility limit of

the drug at room temperature and optimal hydrogel loading [42]. The release of CEX was evaluated in PBS solution at pH=7.4 and 37 °C (measurements performed in triplicate). The release profile was visualized by UV–Vis analysis, considering the absorbance of the solution at regular time intervals and a wavelength of 260 nm for CEX. In this respect, CEX-loaded xerogels were placed in 50 mL Falcon centrifuge tubes containing 10 mL PBS and incubated in a water bath (Benchmark Scientific MultiTherm™ Shaker) at 350 rpm and 37 °C [43]. At different intervals, 5 mL solution was taken and analyzed using UV–VIS spectroscopy. After sampling, the solution in the tube was refilled with 5 mL of fresh solvent to maintain a constant release volume. Figure S2 shows the calibration curve for cephalexin used to calculate the drug concentrations released for the studied samples.

The analysis of the drug release pattern was performed by fitting the obtained data from the in vitro CEX release into four mathematical models for drug release [39, 44, 45]:

- (i) Zero-order model:

$$F = k_0 \cdot t \quad (2)$$

- (ii) First-order model:

$$F = 100 \cdot (1 - e^{-k_1 \cdot t}) \quad (3)$$

- (iii) Simple Higuchi model:

$$F = k_H \cdot t^{0.5} \quad (4)$$

- (iv) Linear logarithm form of Korsmeyer-Peppas model:

$$\log \frac{M_t}{M_\infty} = \log(k) + n \cdot \log(t) \quad (5)$$

where F is the amount of drug released in time t , k_0 is the zero-order release constant in units of concentration/time, k_1 is the first-order release constant, k_H is the Higuchi dissolution constant, M_t/M_∞ is the fraction of drug released at time t , k is the kinetic constant characteristic of the drug-polymer system, and n is the release coefficient that indicates the type of diffusion mechanism.

Indirect cytotoxicity assessment of hybrid BC-PEGDA hydrogels

Cell culture

The in vitro cytotoxicity evaluation of the hybrid BC-PEGDA-based hydrogels was performed by an indirect “extract” method using a culture chamber, in which case the material was separated from the cell culture through a diffusion membrane. The estimation of viable cells

was performed using the MTS assay. The samples were introduced in a culture medium (EMEM-ATCC), the sample: culture medium ratio being 1:10, being further incubated at 37 °C for 24 h. The “extraction medium” was harvested and filtered through 0.2 µm membranes and used for the incubation of cells for cytotoxicity tests [46, 47].

The L929 murine fibroblast cell line (ATCC CRL-6364) [46, 48, 49] was used as a test culture. The stock culture was prepared in flasks with a surface of approx. 25 cm² in EMEM medium with 10% horse serum and 1% antibiotic. Inoculation of the vials was performed at a density of 10⁶ cells/mL, 1 mL/vial, supplemented to 5 mL with the medium.

Culture harvesting and test culture preparation

The culture medium was removed, and the monolayer was washed with PBS. The next step was the treatment with 2 mL of trypsin–EDTA reagent for 2 min. After the detachment of the monolayer, 2 mL of fetal bovine serum was added and stirred to disperse the cells. The cell suspension was collected in a centrifuge tube, and sedimented at 1500 rpm for 5 min. The cell deposition was resuspended in a 5 mL serum-free EMEM medium and sedimented by centrifugation. Finally, the cells were suspended in an EMEM medium with 10% horse serum. The cell viability was counted and determined; the cell density was adjusted to 10⁶ cells/mL.

The testing procedure was performed using the “extract” method, which involves qualitative and quantitative evaluation of cytotoxicity. The procedure requires the cultures to be installed in 24-well plates and adding EMEM medium with 10% horse serum. The samples were incubated under standard conditions for 24 h. After 24 h, the medium was removed, and the test medium (extract) was added. The culture was incubated for 24 h under standard conditions. Again, after 24 h, the medium was removed, and replaced with a fresh medium and cell viability was determined by MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymetoxifenil)-2-(4-sulfophenil)-2H-tetrazol] method using the CellTiter 96 AQueous One Solution Cell Proliferation Assay kit (Promega, USA), Promega. Cultures that were incubated with a standard medium under the same conditions on the same culture plate were used as a reference. The blank was determined in 3 wells, in which no cell culture was performed.

Results and discussion

Synthesis of hybrid BC-PEGDA hydrogels

The research was aimed at obtaining bacterial cellulose (BC) and poly (ethylene glycol) diacrylate (PEGDA₇₀₀) as IPN hydrogels loaded with cephalexin (CEX) as a bioactive

substance. The BC-PEGDA hydrogels were synthesized by radical copolymerization reaction using potassium persulfate (KPS) and sodium metabisulfite (MS) as redox initiator systems. The schematic representation of the synthesis process and also the characterization of the IPN hydrogels can be observed in Fig. 1. The IPNs of BC and PEGDA were highlighted using SEM and the drug release tests showed that the materials were able to release up to 0.35 mg of drug under physiological conditions. In the following step, the materials were tested for cell viability. The results of cytotoxicity revealed that, under the described experimental conditions, the non-loaded samples showed a cytotoxic effect, while CEX-loaded hydrogels lack cytotoxicity leading to cell viability of up to 97% in a 24-h study period.

Swelling study of the unloaded hydrogels

The characteristic swelling degree (SD) of hydrogels increased with increasing BC amount. Figure 2 indicates that the H60BC sample shows the maximum degree of swelling with a value of approx. 230% as a result of excellent homogenization of the biopolymer. Although at a high concentration of BC the interpenetrated polymer network becomes more rigid, the H100BC hydrogel exhibits a rather high degree of swelling of about 210%. Furthermore, a low BC concentration and the absence of cross-linking agent determined weak mechanical properties. Therefore H0BC and H20BC samples broke during the swelling process. However, H80BC and H40BC samples displayed similar SD values (around 200%), meaning that an optimum ratio of BC should be in the range of 40–80%. According to other reported SD values for BC-based hydrogels, such as the one reported by Zaharia et al. (below 100% [32]), the SD values between 200–230% found in this study are quite impressive.

FTIR spectroscopy for loaded and unloaded hydrogels

FTIR analysis was used to characterize the synthesized BC-PEGDA hydrogels and specifically to prove the presence of CEX drug in the loaded hydrogels. FTIR spectroscopy of bacterial cellulose (BC), simple H0BC, and hybrid H20BC, H40BC, H60BC, H80BC, and H100BC hydrogels, respectively, presented some similarities and showed the main characteristic polymeric bands (Fig. S3). The bands, which indicate the presence of PEGDA polymers (-COOH, -CH₂) and bacterial cellulose (-C=O, -CH [48, 50]) are listed in detail in Table S1.

In Fig. 3, FTIR spectra of simple hydrogels (H0BC), hybrid hydrogels (H40BC), and loaded-hydrogels H_{CEX}0BC and H_{CEX}40BC, respectively, were chosen as representative models and further compared with the spectrum of CEX molecules. The FTIR spectra of CEX spectrum present a

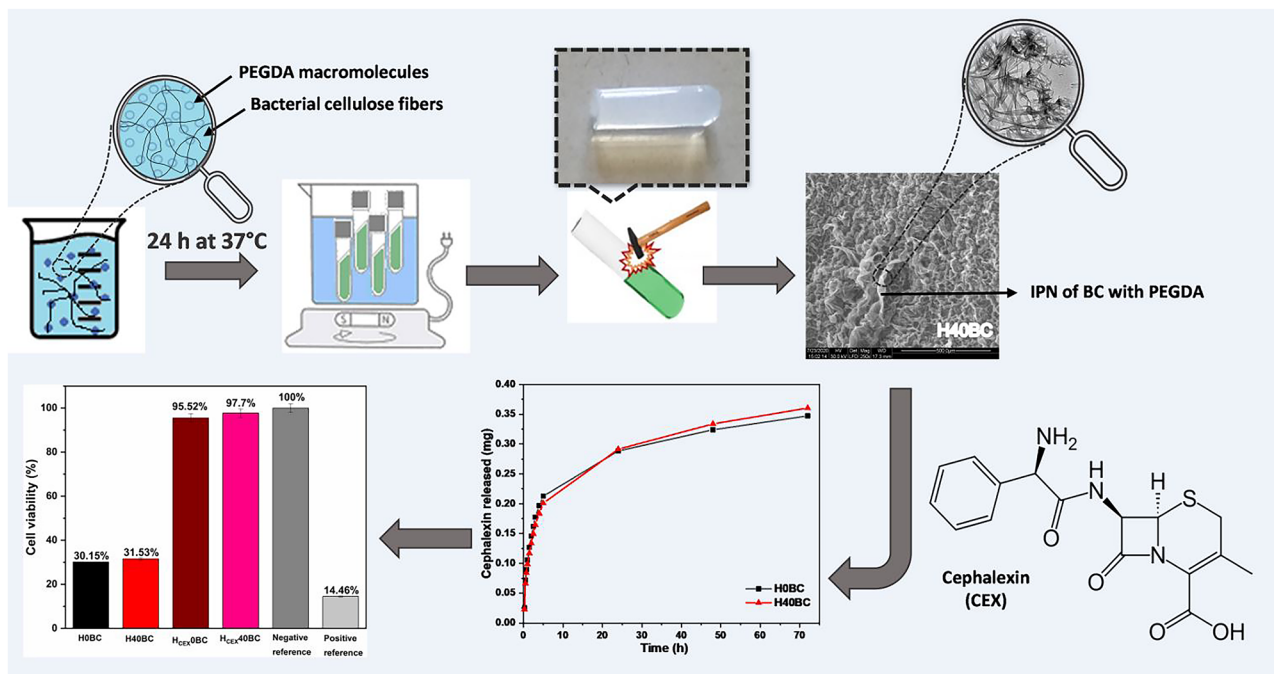


Fig. 1 Synthesis and analysis of the BC-PEGDA hydrogels

characteristic band at 3640 cm^{-1} attributed to OH group in carboxylic acid and other bands at 3420 and 3280 cm^{-1} due to amide N–H stretching vibrations, while a band at 3045 cm^{-1} corresponds to the acidic hydroxyl groups. Characteristic bands appearing at 1756 and 1692 cm^{-1} are attributed to four-membered lactam-carbonyl and secondary amide carbonyl groups, respectively [27]. The band at 1587 cm^{-1} is due to the antisymmetric and symmetric stretching vibrations of the carboxylate group; the bands appearing at 1401 and 1357 cm^{-1} are due to C–H bending vibrations. The C–N stretching vibrations are observed at

1280 cm^{-1} , whereas C–O stretching vibrations are observed at 1075 cm^{-1} . Characteristic bands of monosubstituted phenyl groups appeared at 696 and 755 cm^{-1} , whereas the bands assigned to the C–S stretching vibrations and S–H deformation vibrations have emerged at 755 cm^{-1} and 810 cm^{-1} respectively [51].

The FTIR spectra of the CEX-loaded hydrogels i.e., H_{CEX}0BC and H_{CEX}40BC indicate characteristic bands for the polymer and BC [52, 54], showing large similarity

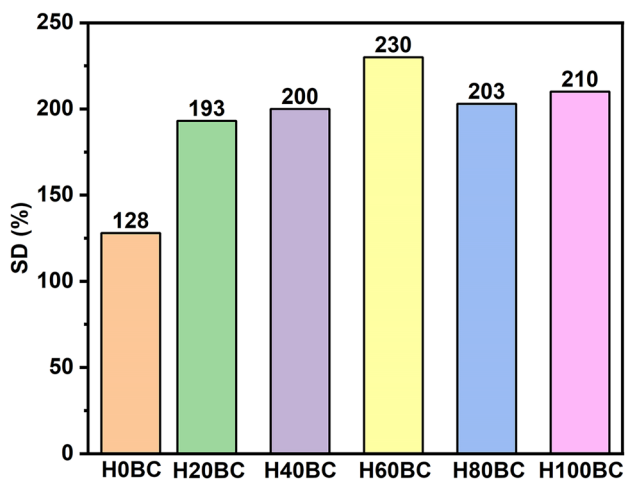


Fig. 2 Swelling degree (SD %) of the studied unloaded hydrogels

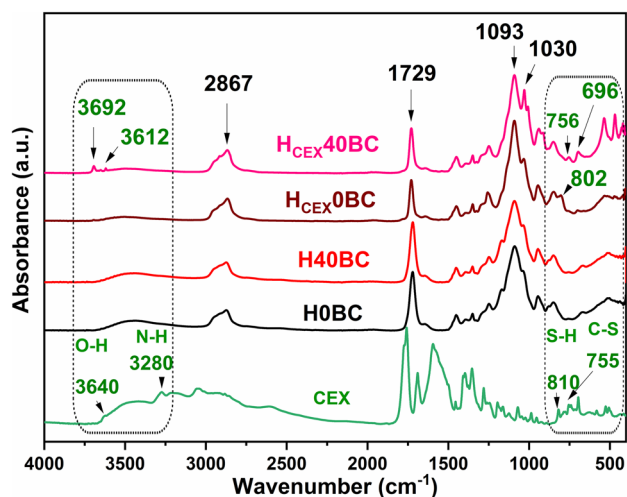


Fig. 3 FTIR spectra of H0BC, H40BC, loaded-hydrogels H_{CEX}0BC, and H_{CEX}40BC compared to that of CEX

and also exhibit some additional bands (as split peaks) in the region $1000\text{--}1300\text{ cm}^{-1}$ corresponding to C-O and C-N groups from CEX. As expected, the drug loading effect within the hydrogel matrix was better highlighted in the case of $H_{\text{CEX}40\text{BC}}$. Thus, a wide spectral dominium between 3695 and 3250 cm^{-1} having two bands around 3600 cm^{-1} and another flat one at around 3500 cm^{-1} in the $H_{\text{CEX}40\text{BC}}$ spectrum were attributed to the O-H and N-H groups from CEX after entrapment. The bands for C-S at 755 cm^{-1} and 696 cm^{-1} (in $H_{\text{CEX}40\text{BC}}$ spectrum) and for S-H group at 802 cm^{-1} (in $H_{\text{CEX}0\text{BC}}$ spectrum) indicate that CEX molecules were successfully loaded into the IPN network. However, other bands of interest appearing between $1450\text{--}1760\text{ cm}^{-1}$ and $1020\text{--}1260\text{ cm}^{-1}$ in drug-loaded hydrogels seem to overlap with the ones from the polymer.

Thermo-gravimetric analysis (TGA/DTG) for loaded and unloaded hydrogels

The thermal stability of BC-PEGDA hydrogels was investigated by TGA/DTG. Figure S4a, b depict the thermograms of simple, composite, and loaded hydrogels. Thermogravimetric analysis in the inert atmosphere of nitrogen showed that the organic part degrades almost completely in the studied temperature range. The values of the maximum decomposition temperatures corresponding to each degradation stage (Td) and the weight loss of the samples are summarized in Table S2.

All thermograms of analyzed samples exhibit a similar trend of thermal degradation. In the case of $H0\text{BC}$ sample (Fig. 4), the analysis indicates three stages of degradation: the first stage of degradation is observed from room

temperature to about $100\text{ }^\circ\text{C}$ due to water evaporation, the second stage takes place between $280\text{ }^\circ\text{C}$ and $350\text{ }^\circ\text{C}$ and is associated with the decomposition process of cross-linked diacrylate and polyethylene glycol molecules, and the third degradation stage between $350\text{ }^\circ\text{C}$ and $700\text{ }^\circ\text{C}$ is associated with the degradation process of the polymer backbone, remaining after the cleavage of the pendant groups.

As for BC-PEGDA hybrid hydrogels (e.g., $H40\text{BC}$ -Fig. 4), the thermal behavior is similar to that of $H0\text{BC}$. The characteristic stages of thermal degradation of PEGDA (approx. $390\text{ }^\circ\text{C}$) and also of the bacterial cellulose (between 300 and $320\text{ }^\circ\text{C}$, [53]) are observed. Thus, TGA/DTG investigation confirms the presence of both synthetic polymer and cellulose in the final structure of the synthesized hydrogels, indicating the specific degradation points of each component. The mass loss recorded in the case of hybrid hydrogels was approx. 96%. However, it can be seen that composite xerogels with high concentrations of BC present a higher total mass loss compared to that of $H0\text{BC}$ xerogels. By increasing the BC concentration, the thermostability of the final materials also increased; a fact highlighted by the improvement of the onset decomposition temperature characteristic of diacrylate and cross-linked polyethylene glycol molecules with low molecular weight. Consequently, the presence of BC led to the formation of cross-linking points between its fibrous structure and the PEGDA matrix, particularly for $H40\text{BC}$ (Fig. 4), which led to the formation of an IPN hydrogel with optimum thermal stability and increased mechanical strength.

Considering the overlapped thermograms and DTG profiles shown in Fig. S4, the representative CEX-loaded samples indicate an increased thermostability compared to the non-loaded samples. The CEX drug decomposes into

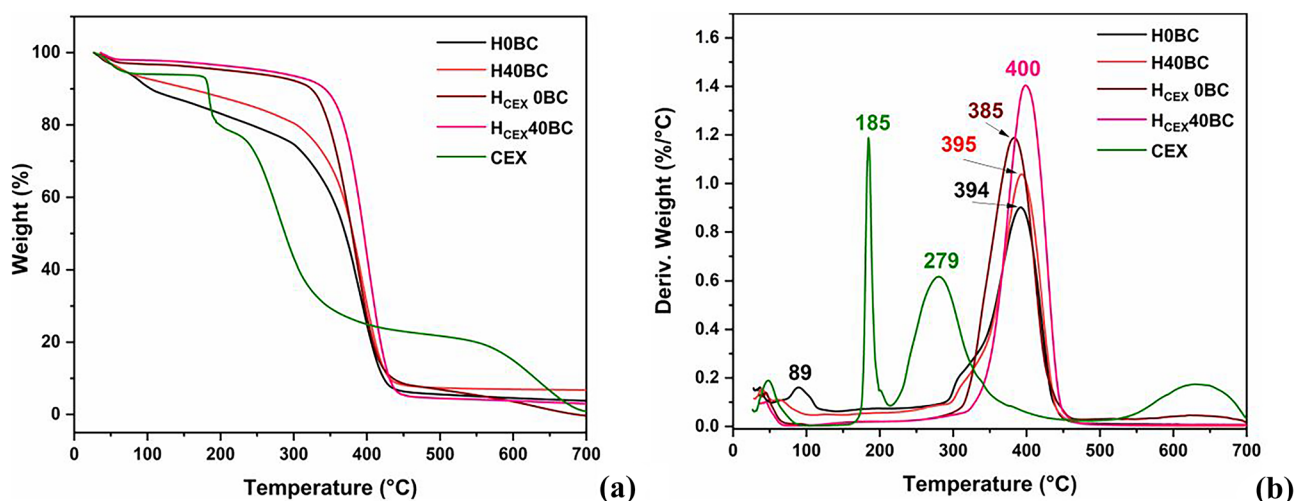


Fig. 4 TGA (a) and DTG (b) curves of the control hydrogels $H0\text{BC}$ and composite hydrogels $H40\text{BC}$, compared to CEX and the corresponding hydrogels loaded with CEX ($H_{\text{CEX}0\text{BC}}$, $H_{\text{CEX}40\text{BC}}$)

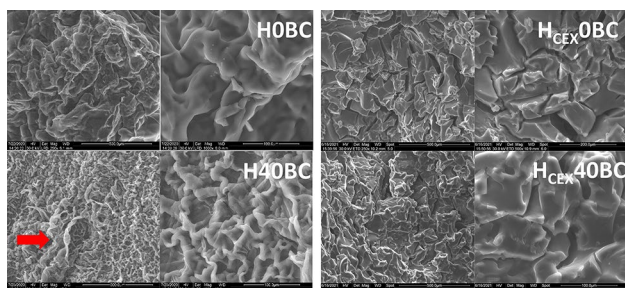


Fig. 5 Morphology of freeze-dried unloaded hydrogels H0BC and H40BC, and corresponding CEX-loaded hydrogels $H_{\text{CEX}0\text{BC}}$ and $H_{\text{CEX}40\text{BC}}$

three main stages, with a clear and relevant decomposition step occurring between 180–300 °C. According to the literature, after this step, the molecule is partially destroyed. Therefore, after 300 °C the range is unimportant [25]. The chosen samples ($H_{\text{CEX}0\text{BC}}$, $H_{\text{CEX}40\text{BC}}$) showed the optimal thermal behavior from the series of loaded hybrid materials (Fig. 4). As the main conclusion concerning the thermal behavior of CEX-loaded hydrogels, it can be stated that the introduction of CEX drug into the matrix has had a positive influence on the thermal stability of IPN hydrogel networks.

Morphology of hybrid hydrogels for loaded and unloaded hydrogels

The morphology of the chosen lyophilized hybrid hydrogels with and without bioactive CEX drug is presented in Fig. 5. The images indicated the presence of BC and the influence of CEX loading on the structure of hybrid hydrogels. H0BC shows a smoother and less porous structure, whereas the other SEM micrographs indicate the presence of bacterial

cellulose by showing a more rough and porous structure, thus highlighting the successful synthesis of an interpenetrated polymer network. The red arrows indicate the nanometric-sized BC fibers, which are randomly oriented and form a compact 3D network (Fig. S5). It can also be observed that the smooth surface of the polymer is covered with the multi-layered structure of BC, indicating that the cross-linking process took place inside the biopolymer's structure. The SEM images of the H100BC hybrid xerogel indicate an agglomeration of biocellulose fibers in its structure, determined by high concentrations and a low homogeneity (Fig. S6).

The morphology of unloaded hydrogels is quite different from that of CEX-loaded samples. This is probably due to the presence of CEX, as large organic species such as cephalixin can interfere with the IPN. For the hydrogels with incorporated CEX drug (Figs. 5 and S6), the surface was rather rigid and cracked, presenting a cubic morphology with open but disconnected channels. Coupled with the TGA results regarding the higher stability of loaded hydrogels, this stiff surface of loaded hydrogels indicated that CEX surely interacted with the polymer network, particularly with BC, which in turn can be an advantage for the following release behavior of the drug.

Loading/release profiles of CEX

The release profiles of CEX from loaded BC-PEGDA hydrogels into a physiological buffer solution (PBS, pH 7.4, at 37 °C) are illustrated in Fig. 6, whereas the controlled release of CEX drug was monitored using UV–VIS. For all the hydrogel formulations, a sustained release behavior was observed, with practically no burst effect. The slow-release rate indicated by samples with higher BC content

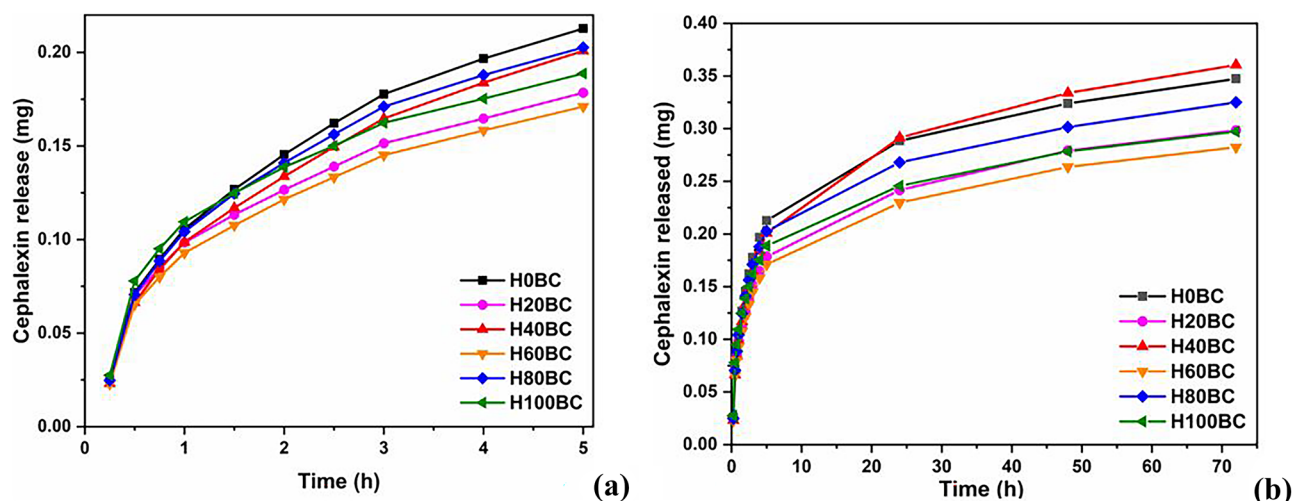


Fig. 6 The release profile of cephalixin in the first 5 h (a), release profile of cephalixin over 72 h (b)

Table 1 Fitted parameters of the kinetic models used in the CEX slow release

Mathematical models	H0BC	H20BC	H40BC	H60BC	H80BC	H100BC
Zero-order						
k_0 , $\mu\text{g/mL}$	0.589	0.461	0.556	0.448	0.554	0.474
R^2	0.894	0.860	0.901	0.864	0.880	0.835
First-order						
k_1 , min^{-1}	0.0054	0.0048	0.0055	0.0049	0.0053	0.0046
R^2	0.652	0.610	0.648	0.614	0.632	0.587
Higuchi						
k_H , $\mu\text{g/mL}\cdot\text{min}^{-1/2}$	13.212	10.412	12.429	10.128	12.464	10.776
R^2	0.974	0.952	0.976	0.956	0.967	0.937
Korsmeyer-Peppas						
k	2.557	2.496	2.612	2.544	2.557	2.419
n	0.628	0.571	0.638	0.584	0.621	0.551
R^2	0.921	0.889	0.916	0.893	0.909	0.875

certifies the presence of strong interactions between polymer chains, polysaccharide chains, and cephalixin molecules. In the first 5 h after immersion in PBS, H0BC, H20BC, and H40BC showed a similar behavior regarding the release ratio, unlike the other hydrogels from the series with higher BC concentrations, which tend to release CEX in lower amounts and slower (Fig. 6a). The same slow-release tendency can be observed up to 72 h (Fig. 6b), highlighting that H40BC sample releases the largest amount of drug from the studied series, at an optimum release rate. Thus, Fig. 6 reveals that the presence of a high BC concentration in the composite hydrogels determines a continuous release rate, a sharp release of cephalixin in the first part and continuing afterward with a rather slow release up to 72 h.

It can be concluded that the BC-PEGDA hydrogels as IPN systems are very promising drug carriers due to their good control over the release of CEX. Similar results were reported in the literature for other drugs by Barkhordari et al., by combining carboxymethyl cellulose with ibuprofen-intercalated LDH systems to develop bionanocomposite materials as drug delivery systems. They found that the new LDH–biopolymer nanocomposites are more effective than the LDH alone as drug delivery systems [27]. Preliminary drug release studies of ibuprofen liberation from bionanocomposites processed as beads show better protection against drug release at the acidic pH and a controlled liberation in the alkali conditions. Tomic et al. reported the in vitro release of Cephalixin in PBS (pH 7.4) at 37 °C from the 2-hydroxyethyl methacrylate, itaconic acid, and poly (alkylene glycol) methacrylates-based hydrogel. The hydrogel releases less than 80% of the drug within 10 h and ~100% is released after 90 h [42].

The simulated in vitro drug release from BC-PEGDA hydrogels was studied with the aid of four mathematical models, as described in Sect. 2.5; the final purpose being a more thorough investigation of the CEX release behavior.

The data was fitted to each model to determine R^2 and n coefficients for all the studied cases. According to the values of these coefficients, presented in Table 1, the Higuchi model was the most suitable mathematical model to describe the release in the first 5 h. The results were as expected because this model corresponds to the kinetic description of low-molecular weight compounds diffusing from porous matrices such as hydrogels. The Korsmeyer-Peppas model also describes this kind of release, but it resulted in lower fitting in BC-PEGDA based hydrogels. The n values of the studied samples fit in the 0.5–0.6 interval, which corresponds to a non-Fickian diffusion. Individual graphs for Zero-order, First-order, Higuchi and Korsmeyer-Peppas models are provided as Supplementary Data (Figs. S7, S8, S9, S10, S11 and S12).

Indirect cytotoxicity of unloaded and CEX-loaded hydrogels

Quantitative evaluation of cytotoxic effects of unloaded BC-PEGDA hydrogels i.e., H0BC and H40BC assessed to determine cell viability with MTS method was compared to that of loaded hybrid hydrogels meaning $H_{\text{CEX}0\text{BC}}$

Table 2 Evaluation of cytotoxicity of H0BC, H40BC, $H_{\text{CEX}0\text{BC}}$ and $H_{\text{CEX}40\text{BC}}$ samples (24-h exposure time), extract method

Sample	Average O.D.	Standard deviation
Negative reference	1.584	± 0.012
Positive reference (SDS)	0.229	± 0.003
H0BC	0.478	± 0.008
H40BC	0.499	± 0.016
$H_{\text{CEX}0\text{BC}}$	1.513	± 0.089
$H_{\text{CEX}40\text{BC}}$	1.548	± 0.038

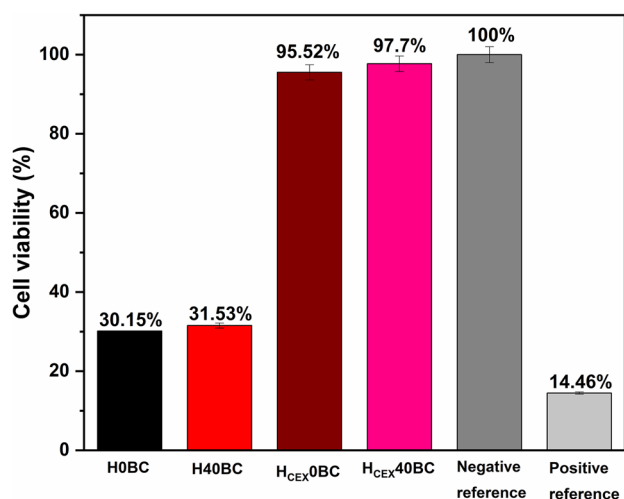


Fig. 7 Graphical representation of MTS viability data of unloaded H0BC and H40BC vs. loaded H_{CEX}0BC and H_{CEX}40BC after 24 h

and H_{CEX}40BC. Qualitative evaluation of the cytotoxic effects was performed by microscopic examination of cell morphology, degree of the display, vacuolation and detachment, cell lysis, and membrane integrity. For samples H0BC and H40BC, the examination of the cultures revealed morphological changes in the cells. The cell viability was significantly affected (Table 2).

Furthermore, when analyzing the viability of cells exposed to loaded hydrogels H_{CEX}0BC and H_{CEX}40BC, the low cytotoxic effect was attributed to the CEX molecules entrapped within the hydrogel matrix, which were released during the experiment (Fig. 7). The slight difference between the simple hydrogel, H_{CEX}0BC, and the composite one, H_{CEX}40BC, confirms the results obtained previously for the continuous release of CEX in high amounts. The cytotoxicity analysis also revealed a slight inhibition of cellular display and adhesion (less than 5%), without significantly affecting cell viability. Consequently, loaded BC-PEGDA hydrogels demonstrated their potential as drug delivery systems.

Conclusions

This study describes the successful synthesis of new IPNs based on bacterial cellulose (BC) and poly (ethylene glycol) diacrylate (PEGDA) by simple radical polymerization. The swelling degrees and the mechanical features of the hybrid hydrogels pointed out that 40% BC is an optimum amount for preparing the hydrogels. The observations during thermal analysis and microscopy indicated that CEX was also able to interact strongly with the hydrogel matrix, particularly with BC, which ultimately led to a more controlled release of the drug in the 5 h, with a very low burst effect. The controlled release profiles of

CEX also indicated a sharper release of the drug in the first 5 h and continued at a slower rate up to 72 h. The cytotoxicity test indicated that under certain conditions of exposure, the CEX-loaded samples displayed good cellular adhesion for murine fibroblasts without significantly affecting cell viability. Hence, the CEX-loaded BC-PEGDA IPNs may be used for wound treatment without affecting the integrity of the healthy cells.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10965-022-03250-9>.

Acknowledgements The study was funded by the Ministry of Research, Innovation and Digitization through the Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI)[Project no. 646PED/2022 DUACTIONMER and by EU and UEFISCDI in the frame of collaborative international project 157/2020 BIOSHELL, financed under the ERA- NET CofundBlueBio2019

Declarations

Conflict of interest The authors declare no conflict of interest.

References

- Roorda WE, Bodde HE, De Boer AG, Junginger HE (1986) Synthetic hydrogels as drug delivery systems. *Pharm Weekbl* 8:165–189. <https://doi.org/10.1007/BF01959775>
- Hajikarimi A, Sadeghi M (2020) Free radical synthesis of cross-linking gelatine base poly NVP/acrylic acid hydrogel and nanoclay hydrogel as cephalexin drug deliver. *J Polym Res* 27:57. <https://doi.org/10.1007/s10965-020-2020-1>
- Wang D, Yang X, Liu Q, Yu L, Ding J (2018) Enzymatically cross-linked hydrogels based on a linear poly (ethylene glycol) analogue for controlled protein release and 3D cell culture. *J Mater Chem B* 6:6067–6079. <https://doi.org/10.1039/C8TB01949E>
- Griffin DR, Kasko AM (2012) Photodegradable Macromers and Hydrogels for Live Cell Encapsulation and Release. *J Am Chem Soc* 134:13103–13107. <https://doi.org/10.1021/ja305280w>
- Nicodemus GD, Bryant SJ (2008) Cell encapsulation in biodegradable hydrogels for tissue engineering applications. *Tissue Eng Part B Rev* 14:149–165. <https://doi.org/10.1089/ten.teb.2007.0332>
- Sampath S, Choudhury NA, Shukla AK (2009) Hydrogel membrane electrolyte for electrochemical capacitors. *J Chem Sci* 121:727–734. <https://doi.org/10.1007/s12039-009-0087-7>
- Brinkman E, Van der Does L, Bantjes A (1991) Poly(vinyl alcohol)-heparin hydrogels as sensor catheter membranes. *Biomater* 12:63–70. [https://doi.org/10.1016/0142-9612\(91\)90134-V](https://doi.org/10.1016/0142-9612(91)90134-V)
- Frost BA, Sutliff BP, Thayer P, Bortner MJ, Foster EJ (2019) Gradient poly(ethylene glycol) diacrylate and cellulose nanocrystals tissue engineering composite scaffolds via extrusion bioprinting. *Front Bioeng Biotechnol* 7:280. <https://doi.org/10.3389/fbioe.2019.00280>
- Won N, Bok SH, Park JS, Na YH (2018) Nanocomposite hydrogel adhered to concrete material for aquaculture of marine organisms. *Macromol Res* 26:717–723. <https://doi.org/10.1007/s13233-018-6096-y>
- Memic A, Alhadrami HA, Hussain MA, Aldahri M, Nowaiser FA, Al-Hazmi F, Oklu R, Khademhosseini A (2016) Hydrogels 2.0: improved properties with nanomaterials composites for biomedical applications. *Biomed Mater* 11:014104. <https://doi.org/10.1088/1748-6041/11/1/014104>

- 11 Monteleale A, Kehr NS (2017) Nanocomposite hydrogels and their applications in tissue engineering. *Adv Healthc Mater* 6:1600938. <https://doi.org/10.1002/adhm.201600938>
- 12 Li L, Yan B, Yang J, Huang W, Chen L, Zeng H (2017) Injectable self-healing hydrogel with antimicrobial and antifouling properties. *ACS Appl Mater Interfaces* 9:9221–9225. <https://doi.org/10.1021/acsami.6b16192>
- 13 Krsko P, Libera M (2005) Biointeractive hydrogels. *Mater Today* 8:36. [https://doi.org/10.1016/S1369-7021\(05\)71223-2](https://doi.org/10.1016/S1369-7021(05)71223-2)
- 14 Numata Y, Kono H, Tsuji M, Tajima K (2017) Structural and mechanical characterisation of bacterial cellulose-polyethylene glycol diacrylate composite gels. *Carbohydrate Polym* 173:67–76. <https://doi.org/10.1016/j.carbpol.2017.05.077>
- 15 Iguchi M, Yamanaka S, Budhiono A (2000) Review – bacterial cellulose – a masterpiece of nature’s arts. *J Mat Sci* 35:261–270. <https://doi.org/10.1023/A:1004775229149>
- 16 Vandamme EJ, De Baets S, Vanbaelen A, Joris K, De Wulf P (1998) Improved production of bacterial cellulose and its application potential. *Polym Degrad Stab* 59:93–99. [https://doi.org/10.1016/S0141-3910\(97\)00185-7](https://doi.org/10.1016/S0141-3910(97)00185-7)
- 17 Keshk SM (2014) Bacterial cellulose production and its industrial applications. *J Bioprocess Biotech* 4:150. <https://doi.org/10.4172/2155-9821.1000150>
- 18 Oshima T, Taguchi S, Ohe K, Baba Y (2011) Phosphorylated bacterial cellulose for adsorption of proteins. *Carbohydr Polym* 83:953–958. <https://doi.org/10.1016/j.carbpol.2010.09.005>
- 19 Petersen N, Gatenholm P (2011) Bacterial cellulose-based materials and medical devices: current state and perspectives. *Appl Microbiol Biotechnol* 91:1277–1286. <https://doi.org/10.1007/s00253-011-3432-y>
- 20 Wang J, Gao C, Zhang Y, Wan Y (2010) Preparation and in vitro characterization of BC/PVA hydrogel composite for its potential use as artificial cornea biomaterial. *Mater Sci Eng C* 30:214–218. <https://doi.org/10.1016/j.msec.2009.10.006>
- 21 Zmejkoski D, Spasojević D, Orlovska I, Kozyrovska N, Soković M, Glamočlija J, Dmitrović S, Matović B, Tasić N, Maksimović V, Sosnin M, Radotić K (2018) Bacterial cellulose-lignin composite hydrogel as a promising agent in chronic wound healing. *Int J Biol Macromol* 18(Part A):494–503. <https://doi.org/10.1016/j.ijbiomac.2018.06.067>
- 22 de Sousa F, Moraes PR, Saska S, Barud H, de Lima LR, da Conceição Amaro Martins VD, de Guzzi Plepis AM, Ribeiro SJL, Minarelli Gaspar AM (2016) Bacterial cellulose/collagen hydrogel for wound healing. *Mater Res* 19(1):106–116. <https://doi.org/10.1590/1980-5373-MR-2015-0249>
- 23 Zmejkoski D, Marković Z, Budimir MD, Zdravković NM, Trišić D, Bugárová N, Danko M, Kozyrovska NO, Špitalský Z, Kleinová A, Kuzman SB, Pavlović VB, Todorović Marković BM (2021) Photoactive and antioxidant nanochitosan dots/biocellulose hydrogels for wound healing treatment. *Mater Sci Eng C* 122:111925. <https://doi.org/10.1016/j.msec.2021.111925>
- 24 Asanarong O, Quan VM, Boonrungsiman S, Sukyai P (2021) Bioactive wound dressing using bacterial cellulose loaded with papain composite: Morphology, loading/release and antibacterial properties. *Eur Polym J* 143:110224. <https://doi.org/10.1016/j.eurpolymj.2020.110224>
- 25 Swinger S, Gupta A, Gibson H, Kowalczyk M, Heaselgrave W, Radecka I (2021) Recent Advances and Applications of Bacterial Cellulose in Biomedicine. *Polymers* 13:412. <https://doi.org/10.3390/polym13030412>
- 26 Kundu D, Banerjee T (2020) Development of microcrystalline cellulose-based hydrogels for the in vitro delivery of Cephalexin. *Heliyon* 6:e03027. <https://doi.org/10.1016/j.heliyon.2019.e03027>
- 27 Barkhordari S, Yadollahi M (2016) Carboxymethyl cellulose encapsulated layered double hydroxides/drug nanohybrids for cephalexin oral delivery. *Appl Clay Sci* 121:77–85. <https://doi.org/10.1016/j.clay.2015.12.026>
- 28 Legnoverde MS, Simonetti S, Basaldella EI (2014) Influence of pH on cephalexin adsorption onto SBA-15 mesoporous silica: theoretical and experimental study. *Appl Surf Sci* 300:37–42. <https://doi.org/10.1016/j.apsusc.2014.01.198>
- 29 Tomic SLJ, Babić MM, Antić KM (2014) pH-sensitive hydrogels based on (meth)acrylates and itaconic acid. *Macromol Res* 22:1203–1213. <https://doi.org/10.1007/s13233-014-2172-0>
- 30 Jilsha G, Viswanad V (2015) Nanosponge loaded hydrogel of cephalexin for topical delivery. *IJPSR* 6(7):2781–2789. [https://doi.org/10.13040/IJPSR.0975-8232.6\(7\).2781-89](https://doi.org/10.13040/IJPSR.0975-8232.6(7).2781-89)
- 31 Hajikarimi A, Sadeghi M (2020) Free radical synthesis of cross-linking gelatin base poly NVP/acrylic acid hydrogel and nanoclay hydrogel as cephalexin drug deliver. *J Polym Res* 27:57. <https://doi.org/10.1007/s10965-020-2020-1>
- 32 Zaharia A, Radu AL, Iancu S, Florea AM, Sandu T, Minca I, Fruth-Oprisan V, Teodorescu M, Sarbu A, Iordache TV (2018) Bacterial cellulose-poly(acrylic acid-co-N, N'-methylene-bis-acrylamide) interpenetrated networks for the controlled release of fertilizers. *RSC Adv* 8:17635–17644. <https://doi.org/10.1039/C8RA01733F>
- 33 Dhand AP, Galarraga JH, Burdick JA (2021) Enhancing biopolymer hydrogel functionality through interpenetrating networks. *Trends Biotechnol* 39(5):519–538. <https://doi.org/10.1016/j.tibtech.2020.08.007>
- 34 Mankotia P, Sharma K, Sharma V, Kumar V (2020) Interpenetrating polymer networks in sustained drug-releasing. *Adv Biopolym Syst Drug Deliv*. https://doi.org/10.1007/978-3-030-46923-8_9
- 35 Numata Y, Kono H, Tsuji M, Tajima K (2017) Structural and mechanical characterization of bacterial cellulose-polyethylene glycol diacrylate composite gels. *Carbohydr Polym* 173:67–76. <https://doi.org/10.1016/j.carbpol.2017.05.077>
- 36 Dobre LM, Stoica-Guzun A, Stroescu M, Jipa IM, Dobre T, Ferdes M, Ciumpilic S (2012) Modelling of sorbic acid diffusion through bacterial cellulose-based antimicrobial films. *Chem Pap* 66:144–151. <https://doi.org/10.2478/s11696-011-0086-2>
- 37 Teodorescu M, Lungu A, Stanescu PO, Neamtu C (2009) Preparation and properties of novel slow-release NPK agrochemical formulations based on poly(acrylic acid) hydrogels and liquid fertilizers. *Ind Eng Chem Res* 48(14):6527–6534. <https://doi.org/10.1021/ie900254b>
- 38 Li H, Yang J, Hu X, Liang J, Fan Y, Zhang X (2011) Superabsorbent polysaccharide hydrogels based on pullulan derivative as antibacterial release wound dressing. *J Biomed Mater Res Part A* 98A:31–39. <https://doi.org/10.1002/jbm.a.33045>
- 39 Cursaru B, Radu A-L, Perrin F-X, Sarbu A, Teodorescu M, Gavrilă A-M, Damian C-M, Sandu T, Iordache T-V, Zaharia A (2019) Poly(ethylene glycol) composite hydrogels with natural zeolite as filler for controlled delivery applications. *Macromol Res* 28(3):211–220. <https://doi.org/10.1007/s13233-020-8029-9>
- 40 Tang N, Zheng Y, Jiang X, Zhou C, Jin K, Wu W, Haick H (2021) Wearable sensors and systems for wound healing-related pH and temperature detection. *Micromachines (Basel)* 12(4):430. <https://doi.org/10.3390/mi12040430>
- 41 Bialik-Was K, Pluta K, Malina D, Majka TM (2019) Alginate/PVA-based hydrogel matrices with Echinacea purpurea extract as a new approach to dermal wound healing. *Int J Polym Mater*. <https://doi.org/10.1080/00914037.2019.1706510>
- 42 Tomic SLJ, Micic MM, Filipovic JM, Suljovic EH (2010) Synthesis, characterization and controlled release of cephalexin drug from smart poly(2-hydroxyethyl methacrylate/poly(alkylene glycol)(meth)acrylates hydrogels. *Chem Eng J* 160:801–809. <https://doi.org/10.1016/j.cej.2010.03.089>
- 43 Radu I-C, Hudita A, Zaharia C, Stanescu PO, Vasile E, Iovu H, Stan M, Ginghina O, Galateanu B, Costache M, Languth P, Tsatsakis A, Velonia K, Negrei C (2017) Poly(HydroxyButyrate-co-HydroxyValerate) (PHBHV) nanocarriers for silymarin release as adjuvant therapy in colo-rectal cancer. *Front Pharmacol* 8:508. <https://doi.org/10.3389/fphar.2017.00508>

- 44 Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S (2010) DDSolver: an add-in program for modelling and comparison of drug dissolution profiles. *AAPS J* 12(3):263–271. <https://doi.org/10.1208/s12248-010-9185-1>
- 45 Bhuyan M, Okabe H, Hidaka Y, Dafader NC, Rahman N, Hara K (2018) Synthesis of pectin-N, N-dimethyl acrylamide hydrogel by gamma radiation and application in drug delivery (in vitro). *J Macromol Sci Part A* 55(4):369–376. <https://doi.org/10.1080/10601325.2018.1442177>
46. ISO 10993-12:2021 Biological evaluation of medical devices – Part 12: Sample preparation and reference materials, 2021. Edition 5:1–21
47. ISO 10993-5:2009 Biological evaluation of medical devices – Part 5: Test for in vitro cytotoxicity, 2009. Edition 3:1–34
48. Drabczyk A, Kudlacik-Kramarczyk S, Glab M, Kedzierska M, Jaromin A, Mierzwinski D, Tyliczszak B (2020) Physicochemical investigations of chitosan-based hydrogels containing Aloe Vera designed for biomedical use. *Materials* 13(14):3073. <https://doi.org/10.3390/ma13143073>
49. Ouasti S, Donno R, Cellesi F, Sherratt MJ, Terenghi G, Tirelli N (2011) Network connectivity, mechanical properties and cell adhesion for hyaluronic acid/ PEG hydrogels. *Biomaterials* 32(27):6456–6470. <https://doi.org/10.1016/j.biomaterials.2011.05.044>
- 50 Ullah MW, Islam MU, Khan S, Kim Y (2016) In situ synthesis of a bio-cellulose/titanium dioxide nanocomposite by using a cell-free system. *RSC Adv* 6:22424–22435. <https://doi.org/10.1039/C5RA26704H>
- 51 Agnihotri SA, Jawalkar SS, Aminabhavi TM (2006) Controlled release of cephalexin through gellan gum beads: effect of formulation parameters on entrapment efficiency, size, and drug release. *Eur J Pharm Biopharm* 63:249–261. <https://doi.org/10.1016/j.ejpb.2005.12.008>
- 52 Imani M, Sharifi S, Mirzadeh H, Ziaee F (2007) Monitoring of polyethylene glycol-diacrylate-based hydrogel formation by real time NMR spectroscopy. *Iranian Polym J* 16:13–20
53. Fulias A, Vlase T, Vlase G, Doca N (2010) Thermal behaviour of cephalexin in different mixtures. *J Therm Anal Calorim* 99:987–992. <https://doi.org/10.1007/s10973-010-0708-x>
- 54 Panaitescu DM, Frone AN, Chiulan I, Casarica A, Nicolae CA, Ghiurea M, Trusca R, Damian CM (2016) Structural and morphological characterization of bacterial cellulose nano-reinforcements prepared by mechanical route. *Mater Des* 110:790–801. <https://doi.org/10.1016/j.matdes.2016.08.052>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.