Available online www.ijpras.com

International Journal of Pharmaceutical Research & Allied Sciences, 2019, 8(3):137-143

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Drug Release of Bacterial Cellulose as Antibacterial Nano Wound Dressing

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ABSTRACT

Bacterial cellulose (BC) is a natural polymer that can be utilized for many applications. Because of its renewable nature, good biocompatibility and excellent physical features of bacterial cellulose, it can be utilized in pharmaceutical, biomedical fields, and nanotechnology applications. In this study, we prepared antibiotic bacterial cellulose loaded with tetracycline hydrochloride and gentamicin, and its drug release, as well as antibacterial activity, were evaluated separately. The structure and morphology of the loaded bacterial cellulose were determined by scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). AATCC100 test was used for antibacterial properties against Escherichia coli and Staphylococcus aureus. Ultraviolet spectrophotometry device was used to detect absorption and release process of mentioned antibacterial cellulose. By these unique specifications of bacterial cellulose layer loaded with tetracycline hydrochloride and gentamicin, we found that they may successfully serve as a wound dressing and other medical biomaterials.

Key words: *bacterial cellulose, antibacterial, biocompatibility, drug release, gentamicin, tetracycline hydrochloride.*

INTRODUCTION

Skin is the largest, most complex, most interesting and most prolific living member of the body [1]. The skin is an essential organ that without it no creature is able to survive [2]. From ancient times, people have used wound dressings for the treatment of severe skin burns and injuries for centuries [3]. They utilized traditional gauze-based dressing, such as woven and non-woven sponges as well as natural and synthetic bandages to keep the wound dry. They believed exudate absorption and evaporation, together with the prevention of bacterial invasion play a key role in successful wound healing [4, 5].

World Health Organization (WHO) suggests that every human being has a right to take advantage of the most efficient, inexpensive, the safest and easiest techniques to cure illnesses [6]. Over the last few decades, the view on wound healing has been changed significantly. Wound healing process represents a complex series of biological events to restore skin barrier function, prevent dehydration and reduce the risk of bacterial infection [7, 8]. Nowadays, burn wound and skin treatments differ widely, and a large number of different wound dressing materials are available for their treatments. Wound dressing has been developed from both natural and synthetic materials. An ideal wound dressing must provide a moist environment, effective oxygen circulation, and thermal insulation. It must be easy to apply and painless to remove without providing allergic reactions. The process of wound recovery is affected by many factors such as the type of wound being treated, patient health conditions and the social environment. So, the selection of a suitable dressing depends on every individual occurrence [9, 10]. Bacterial cellulose is a polymer produced by some bacteria, which has high flexibility and compatibility with the immune system. According to mentioned specifications, extensive researches have been done on bacterial cellulose as a substitution for wound dressing. Modified and finishing bacterial cellulose is an

excellent dressing material for treating various kinds of wounds, burns, and ulcers. Cellulose polymers especially nanofibrillar cellulose are the most natural products for biomedical applications [11]. Tetracycline hydrochloride and gentamicin are common drugs with antibiotic effects. They are effective in most of the systemic infections that act against gram-positive and gram-negative bacteria, the intracellular pathogens chlamydia, mycoplasmas, and rickettsia as well as eukaryotic protozoan parasites. They are used to treat urinary tract infections, acne, gonorrhea, and other conditions. Maneerung et al. impregnated silver nanoparticles into bacterial cellulose and the freeze-dried BC film exhibited strong antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* [12]. Its high purity, the unique physical properties, and its biocompatibility triggered considerable interest in BC particularly in the biomedical era [13]. The aim of this paper is to consider the effect of antibacterial and drug release of bacterial cellulose that has been loaded with tetracycline and gentamicin materials.

EXPERIMENTS

Materials

Acetobacter xylinum ATCC23768 was used for bacterial cellulose production. Bacterial cellulose was supplied from Tarbiyat Moddares Medical laboratories (Tehran-Iran). Tetracycline hydrochloride and gentamicin were purchased from Hakim pharmaceutical Co. (Tehran-Iran). Acetic acid and other chemical materials such as sodium hydroxide (NAOH) and potassium phosphate monobasic were purchased from Merck Co. (Germany).

Methods:

- **Preparation of nano bacterial cellulose layers:**

The bacterium was grown in SH medium at 30 ℃ under static culture conditions. After 17 days of cultivation at 30 ℃, the cellulose nanolayers formed on the surface of culture broth. The nano cellulose layers were removed after cultivation and rinsed with distilled water. They were cut into 1g pieces and used for drug bio-sorbents in wet and dry states. The bacterial cellulose layers in the wet state before and after bleaching are shown in figure 1 (a, b) and dry state in figure (c).

A B C

Figure 1: (a) Bacterial cellulose layers in wet state before bleaching. (b) Bacterial cellulose layers in wet state after bleaching. (c) Bacterial cellulose layers in dry state.

- **Drugs loading on bacterial cellulose layers:**

Bacterial cellulose wet nanolayers were cut into 1 g pieces and divided into three groups. The first wet group pieces immersed in Tetracycline (0.08 & 0.1 g/cc) and gentamicin (0.1 & 0.5 g/cc) solvent for 24 hours. Wet pieces were investigated for drug release. The second groups were dried. Then dry pieces immersed in Tetracycline (0.08 & 0.1 g/cc) and gentamicin (0.1 & 0.5 g/cc) solvent for 24 hours. The second groups were investigated for drug release. The third group immersed in Tetracycline (0.08 & 0.1 g/cc) and gentamicin (0.1 & 0.5 g/cc) solvent for 24 hours and then dried. After drying the third group, they were also investigated. The coded samples have been shown in table 1.

Table 1: The coded samples with drugs in various states.

group	Concentration (gr/10cc)	Drug	Coding	Sample
first	0.1	Gentamicin	G-W-0.1	A
second	0.1	Gentamicin	G-D-0.1	**B**
third	0.1	Gentamicin	G-W-D-0.1	**C**

first	0.5	Gentamicin	G-W-0.5	**D**
second	0.5	Gentamicin	G-D-0.5	**E**
third	0.5	Gentamicin	G-W-D-0.5	**F**
first	0.1	Tetracycline	T-W-0.1	**G**
second	0.1	Tetracycline	T-D-0.1	**H**
third	0.1	Tetracycline	T-W-D-0.1	**I**
first	0.08	Tetracycline	T-W-0.08	**J**
second	0.08	Tetracycline	T-D-0.08	**K**
third	0.08	Tetracycline	T-W-D-0.08	**L**

- **Scanning electron microscopic (SEM)**

The samples of bacterial cellulose (BC), BC impregnated with tetracycline hydrochloride (BC-TCH), and BC impregnated with gentamicin (BC-gentamicin) were characterized by SEM operating at an accelerating voltage of 24 KV (Kato Tech Co., LTD, Japan) to investigate the surface morphologies of these samples that had been coated with a thin layer of gold under high vacuum conditions.

- **Fourier transmission infrared spectroscopy (FTIR)**

Bacterial cellulose (BC), BC impregnated with tetracycline hydrochloride (BC-TCH), and BC impregnated with gentamicin (BC-gentamicin), pure tetracycline hydrochloride, and gentamicin drugs were analyzed on a BIORAD-FTS-7pc type of FTIR spectrometer.

- **Assessment of antibacterial properties**

The antibacterial activity was characterized according to the reported method AATCC100 test. *Escherichia coli* and *S. aureus* were selected as the representatives of the gram-negative and gram-positive bacterium. They were cultured in nutrient broth medium at 37 ℃ for 24 hours before usage. The BC layers and BC impregnated layers with tetracycline hydrochloride and BC layers impregnated with gentamicin were cut. The disks were put in sterile nutrient agar plate and inoculated with bacterial suspension, respectively. Then, the agar plates were examined and the numbers were counted. The percentage reduction was calculated using equation 1:

$$R(\%)=\frac{A-B}{B}\times 100 \qquad \text{(Equation 1)}$$

Where A is the number of bacteria on the untreated wound after 24 hours and B is the number of bacteria on the pre- and post-treated wounds with tetracycline and gentamicin.

- **In vitro drug release studies:**

The samples of BC, BC-TCH, and BC-gentamicin were immersed in 20 ml of phosphate buffer solution (ph 5.5) and distilled water (ph 7.4) at 37 ℃ as release cultures for 24 hours. The tested samples were collected from each solution of the buffer. These samples were immersed in a new solution of mentioned buffer and distilled water. The amounts of released drugs of immersed samples were measured by ultraviolet spectrophotometry device.

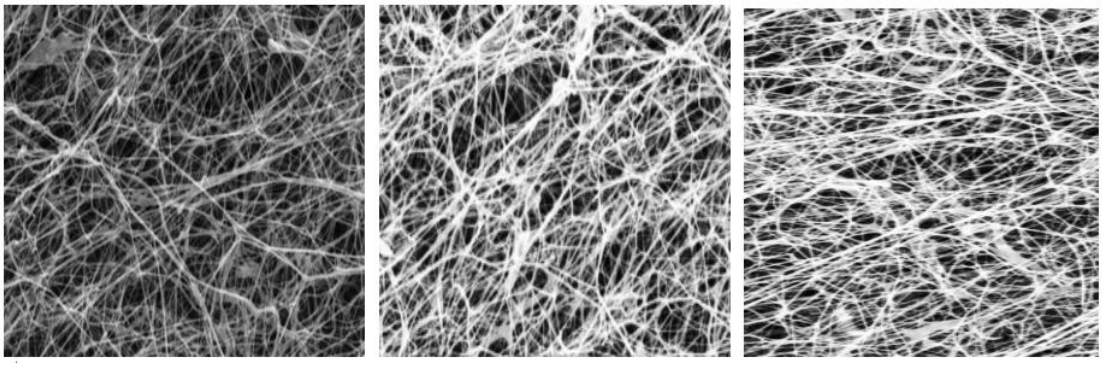
The ultraviolet (Uv) of Tetracycline in the buffer and distilled water were measured at λ_{max}=276 nm and λ_{max}=213 nm. The Uv of gentamicin in the buffer and distilled water were measured at λ_{max}= 211 nm and λ_{max}= 214 nm. Specific Uv was adjusted for each group of BC mentioned in section 2.2.2, according to the calibration curve of tetracycline and gentamicin in the same solution of buffer and distilled water. The results were reported for each group of bacterial cellulose as follows.

RESULTS AND DISCUSSIONS

Surface morphology

The morphologies of bacterial cellulose (BC), BC impregnated with tetracycline hydrochloride (BC-TCH), and BC impregnated with gentamicin (BC-gentamicin) were analyzed using SEM (fig. 2A, B and C). Fig 2A shows the morphology of BC that exhibited a nanoporous three-dimensional network structure. Bacterial cellulose was smooth and ribbon-like and diameter of nano cellulose fibers was around 90 nm, fig 2B shows the morphology of BC impregnated with tetracycline hydrochloride (BC-TCH) and fig 2C shows the morphology of BC

impregnated with gentamicin (BC-gentamicin) that has an irregular surface, with the pre-scan of numerous naps that made with drugs after drying. The average diameter of coated nano cellulose fibers was around 100-220 nm.

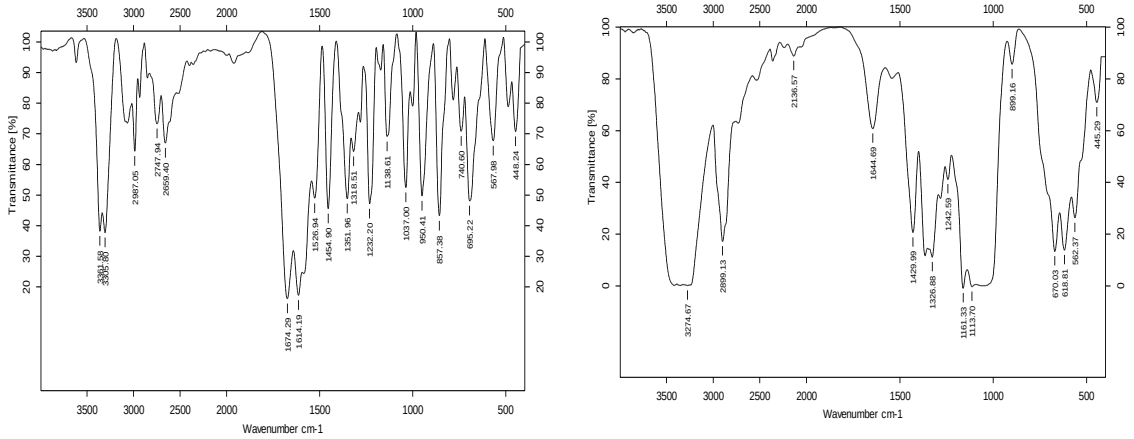


A B C

Figure 2. SEM reported Bacterial cellulose wounds (A) image of BC, (B) BC-TCH, (C) and BC-gentamicin

FTIR spectroscopy

FTIR was performed to determine the structure of the composite dressing identifying the presence of below-mentioned specific functional groups within a sample. Chemical reactions were involved in each step of graft. FTIR spectra of cellulose sample were measured. The main intensity of the band at around 2900 cm^{-1} comes from the C-H stretching vibrations. The FTIR absorption band at 3400 cm^{-1} assigned to O-H stretching. The absorption band at 1400 cm^{-1} assigned to CH bending. Cyclohexane peak was observed at 1050 cm^{-1} and C=O peak was observed at 1650 cm^{-1} (fig. 3A). The characteristic peaks of tetracycline hydrochloride were showned for NH and OH stretching at 3361-3308 cm^{-1} and aromatic CH stretching at 2500-3000 cm^{-1}. The vibration peaks at 2987 cm^{-1} and 1614-1674 cm^{-1} were assigned to CH_3 stretching and C=C stretching respectively. Aromatic C-H bending was appeared at 1454 cm^{-1} (fig. 3B). The FTIR spectra of gentamicin showed the peaks at 3200-3500 cm^{-1} to OH stretching. The peak for N-H and amino peak were observed at 1633 cm^{-1} and 1050 cm^{-1} respectively (fig. 3C). FTIR spectra of coated cellulose with tetracycline revealed the band at around 2904 cm^{-1} whose main intensify comes from ample C-H. The FTIR absorption band at 1249 cm^{-1} was assigned to C-N amino Acid in tetracycline. The intense increase around 1500 cm^{-1} showed the peak for C=C aromatic in nano cellulose and tetracycline (fig. 3D). FTIR spectra of coated cellulose with gentamicin showed the strong peaks for C-H alkaline at 2890 cm^{-1}. That was related to banding with gentamicin and nano cellulose. In addition, the FTIR absorption band for cellulose ether at peak 1158 cm^{-1} showed linking (fig. 3E).



A **B**

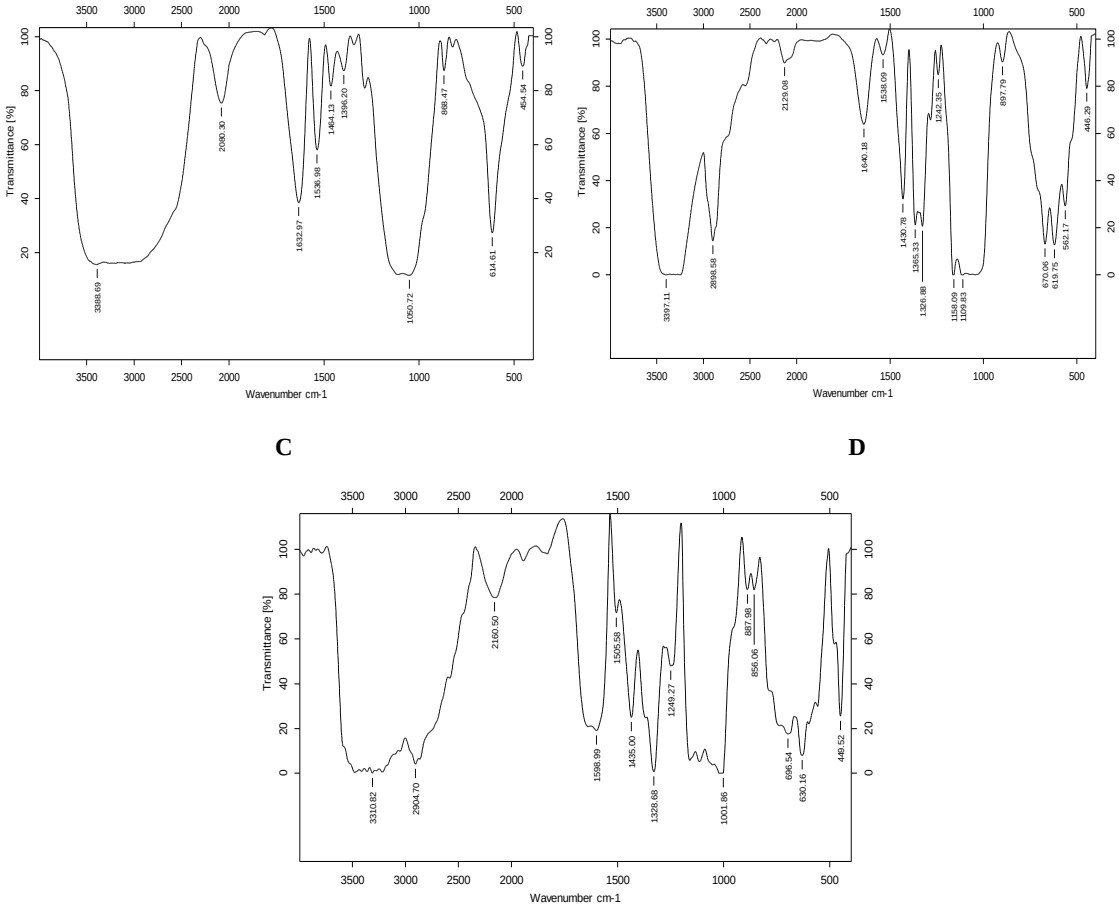
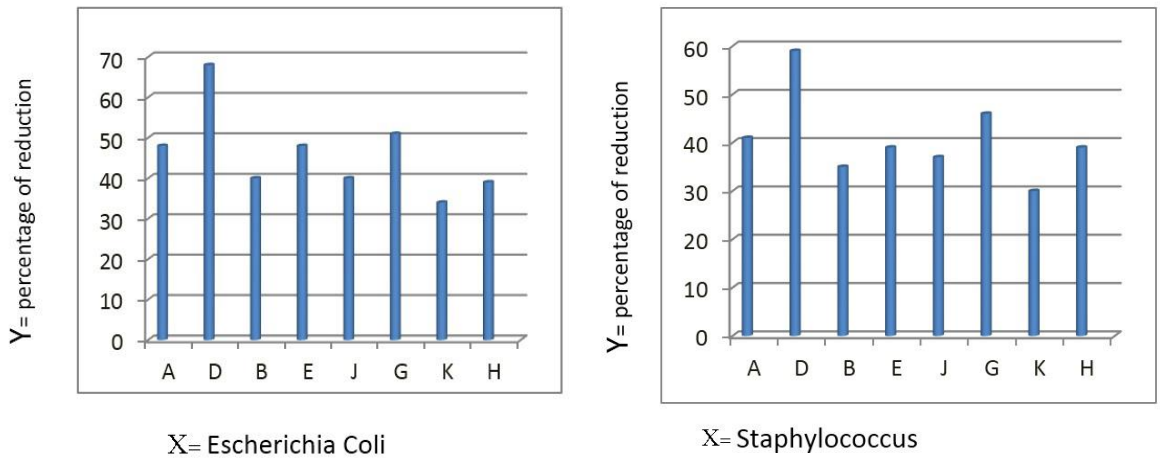


Figure 3: A) FTIR spectra of cellulose: spectra of Tetracycline, C) spectra of gentamicin, D) spectra of cellulose with Tetracycline, E)cellulose with Gentamicin.

Antibacterial test results:

The samples have a broad antibiotic spectrum that includes gram-negative and gram-positive bacteria. In this work, the antibacterial activities of BC, BC-TCH, and BC-gentamicin were investigated using *E. coli* and as the model bacterium by AATCC100-1999.tes. The growth rate of bacteria in a wound is highly dependent on the rate of drug release from the nanofiber surface in a wound dressing material. After consideration of nanofibers with antibiotic drugs, antibacterial testing was done. Percentage reduction of bacteria was calculated and its results are shown in (chart 1). As you can see in the graph in (chart 1) in *E. coli* 18% better treatment was shown than *S. aureus*.



Consideration of drug release

The kinetic drug release profiles are illustrated in chart 2. The drug release study was carried out for about 8 hours. The charts a and b are related to the drug release of gentamicin in distilled water and buffer phosphate respectively. Charts c and d are related to the drug release of tetracycline in distilled water and buffer phosphate respectively. The different concentrations of gentamicin with dry nano cellulose in buffer phosphate was used when the drug release rate rapidly fell to zero.

We observed inconsiderable drug release in the different concentrations of gentamicin in distilled water.

The investigation of various concentrations of tetracycline in distilled water and buffer phosphate showed the same action. Since tetracycline has large molecules, so it is not easy to separate and release the drug.

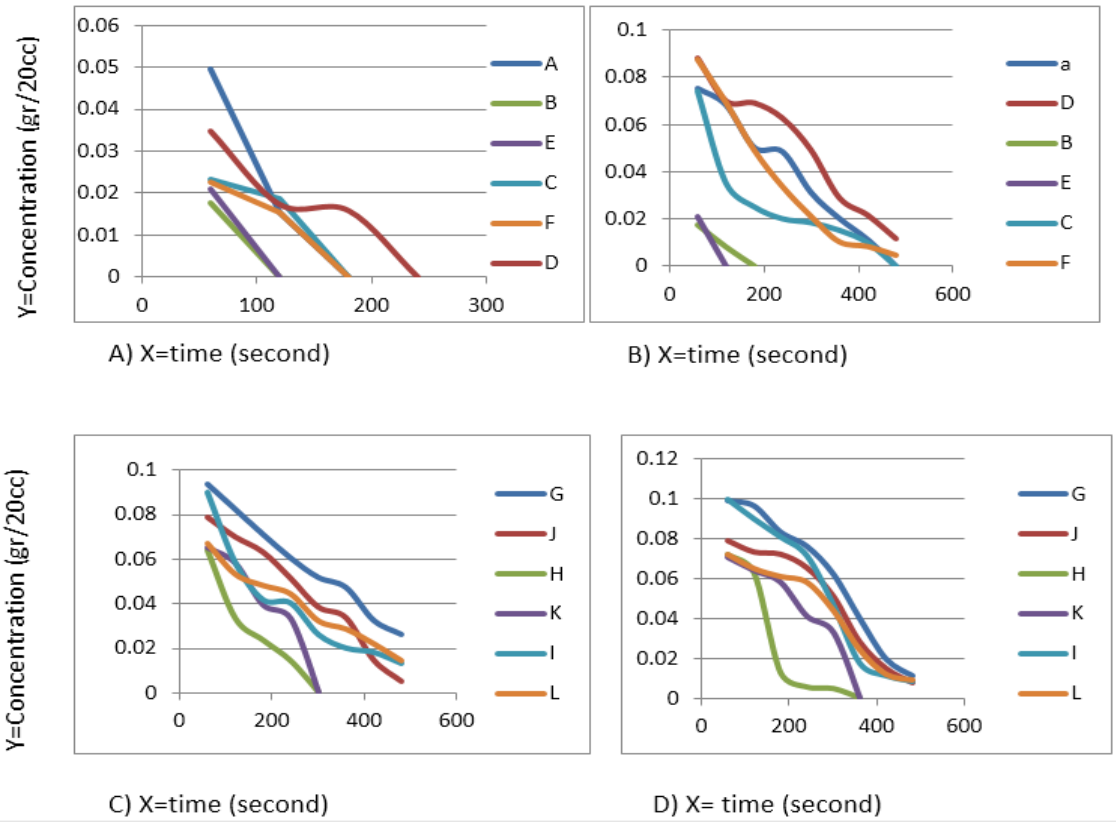


Chart 2: a) gentamicin in distilled water- b) gentamicin in buffer phosphate- c) tetracycline in distilled water- d) Tetracycline in buffer phosphate

CONCLUSION

In the research, controlled release and antibacterial activity of T-CH and gentamicin loaded BC were prepared and investigated. Bacterial cellulose is a natural polymer with unique characteristics such as the ability to maintain a moist environment, strong antibacterial properties resisting against *E. coli* and *S. aureus*, and high water absorption capability.

It was found that samples of the first and third groups showed a continuous drug release for about one day. The rate of drug release of both low and high concentrated drug seems to be the same. But the low concentrated drug has fewer side effects and saves the amount of drug usage.

Therefore, low concentrated drug usage is recommended. We also found that nanolayers can play an important role in disinfecting the wounds. Nanosheets coated with tetracycline can stay longer on the wound due to slower release into the buffer phosphate environment. The drug release in a wet state (first group) was much more controlled.

As a result, the BC-TCH and BC-gentamicin have a high potential application in wound dressing and can be used in any wound treatment especially patients with bedsores and burnt skin.

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