*Chemistry Research Journal*, 2020, 5(1):99-105

*Available online www.chemrj.org*

**Research Article**

**ISSN: 2455-8990**
**CODEN(USA): CRJHA5**

# Biological Wound Dressing for Anti-Cancer Drug Loading and Release

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**Abstract** Bacterial cellulose (BC) as a biological Nano fabric with unique properties possesses the essential features for applications in drug loading and controlled drug release, such as large surface area and excellent loading capacity. In this study, tamoxifen, which is widely used as anticancer drug in skin cancer, was used to test the drug loading and release potential of BC.

The loaded drug on BC increased with increasing in drug concentration. Up to 95% of the drug released from BC within 24h. The antibacterial property of treated BC was tested by inhibition zone method and the results supported its anticipated use as antibacterial wound dressing material. The non-toxic of BC was approved by MTT test on human with 92% cell viability, which shows its application as a compatible material for wound dressing. The release rate and absorbing capacity were calculated with water release rate and swelling ratio. Thus, BC can be used for application as novel aqueous film coating with lower cost due to its anticancer drug loading and release potential.

**Keywords** Antibacterial properties, Bacterial cellulose, Drug loading, Swelling ratio, Wound Dressing

## Introduction

The use of biopolymers for drug release is an active area of research because they are readily available, relatively inexpensive, potentially degradable and biocompatible [1, 2]. The increased use of biofilm-forming materials as vehicles for medicaments, specialized coatings on medications or as packaging agents has prompted the researchers to develop novel materials for these applications. Several biopolymers like bacterial cellulose (BC) obtained from renewable resources were developed as alternatives to remnant resources [3]. BC is an unbranched polysaccharide, comprised of linear chains of β-1, 4-glucopyranose residues and is generally produced by microorganisms as carbon source for their growth [4]. Microorganisms (Acetobacter, Achromobacter, Aerobacter, Agrobacterium and Pseudomonas) when cultured under specific conditions produce cellulose, which has molecular structure identical to cell-wall plant cellulose but differs in crystalline arrangement and properties [5].

BC exhibits many unique structural and biochemical properties such as ultrafine nanofiber network structure (1.5-nm width) [6], bioadaptibility, inert, biodegradability, hypoallergenity, bio consistency and chemical stability [7, 8]. BC possesses good water absorbance and increase capacity due to its reticulated structure, which provides large surface area and a capacity to absorb water (approximately 200 times its weight) [9-11]. The above-mentioned properties make BC a suitable candidate for application as tablet film coating agent.The molecular formula of BC $(C_6H_{10}O_5)_n$is the same as that of plant cellulose, but their physicaland chemical features are different [12]. BC is preferred over the plant cellulose asit can be obtained in higher purity and exhibits a higher degreeof polymerization and crystallinity index. It also has highertensile strength and water holding capacity than that of plantcellulose,

making it more suitable raw material for wound dressing. A number of studies in the literature report the successful use of BC membranes in biomedical applications [13] and, more specifically, drug delivery systems due to their unique physical and mechanical properties [14] Such membranes are particularly advantageous in topical or transdermal drug delivery systems, as they have the ability to absorb exudates and adhere to irregular skin surfaces, such as the oral mucosa [15, 16].

One of the drugs used in skin cancer and melanoma is tamoxifen [17]. This drug has similar effects to estrogen in some tissues (agonists) and has similar effects in other tissues relative or block of estrogen (antiagonists) [18]. In the present work, the non-toxic biocompatible nature of BC was confirmed. The tamoxifen loading and release capacity of BC was investigated. The antibacterial activity, water release rate and swelling ratio were tested to achieve its performance as a biomaterial in future biomedical application.

## Material and Methods

### Chemicals

Acetobacter Xylinum (ATCC23760) was obtained from microbial laboratory of Tarbial modaress university of Tehran, Iran. Tamoxifen was purchased from Iran Hormone Company. Glucose (96% purity), glycerin (99.5%), and $Na_2HPO_4$ were purchased from Sigma–Aldrich. Yeast extract and bacteriological peptone were purchased from Himedia, and citric acid (99.6%purity) was obtained from Acros Organics. Glycerin solution (99%) was purchased from Fagron, and sodium lauryl sulfate (SLS, 99% purity) was obtained from Fluka. All other chemicals were of analytical grade.

### BC Production

BC membranes were produced using Acetobacter Xylinum (ATCC23760).The pre-inocula were prepared at 30 °C during 48 h, in static conditions, in Hestrin and Shramm (HS) liquid medium (20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 2.7 g/L $Na_2HPO_4$, 1.15 g/L citric acid, agar, 15 g/L, pH 5), before inoculation (10% v/v) of 50 mL into 500 mL liquid production medium in Erlenmeyer flasks. The flasks were kept at 30 °C, in static conditions, for 96 h.

### BC Purification

After the incubation time, BC membranes were withdrawn from the culture medium and treated with a 0.1 mol $L^{-1}$ NaOH solution, at 80 °C, for 30 min to eliminate all attached cells. Then, the membranes were washed with distilled water to remove components of the culture media and other residues until its whitening and reaching pH 7.0. BC membranes were dried at 30 °C and then stored in a desiccator. Measurements of the thickness of the membranes were performed in a Form tracer profilometer, model SV-CS25 (Mitutoyo, Kawasaki, Japan).

### Drug Loading Capacity and Release Ratio

Tamoxifen is a common anticancer drug, which is used for anticancer wound dressing, being effective against some bacteria. BC has high water holding capacity and porosity; it can absorb and slowly release the antimicrobial solution. The drug loading capacity and steady release of tamoxifen, as well as the antimicrobial capacity of the drug-loaded BC, were tested.

BC films were dried and cut into disc shape samples (6 mm diameter with average thickness of 400μm) with similar weight. One piece was immersed into each feed with varying concentrations (0.5%, 1%, 2%, 4%, 6%, and 8%) of tamoxifen. The concentration of tamoxifen was determined spectrophotometrically by measuring λ max at 326 nm. The BC disc was accurately weighed before and after soaking. After overnight soaking, the BC disc was removed from the solution and the excess of solution was wiped out with filter paper. The amount of adsorbed tamoxifen was determined as the difference in weight before and after soaking. The amount of adsorbed tamoxifen was expressed as milligrams of tamoxifen per g of BC. The drug loading capacity was calculated as the amount of adsorbed tamoxifen per area ($лг^2$) of the BC disc.

For investigation on tamoxifen release: the BC with the respective feed concentration was removed and immersed into a sealed beaker containing 25 ml of deionized water. The flasks were vigorously shaked and were incubated for 24 h at room temperature on a rotary shaker at 120 rpm. The sample was drawn after every 30 min and analyzed spectrophotometrically at 326 nm for measuring the concentration.

**Antibactrial Effect**

The antimicrobial effect of treated BC with tamoxifen was tested against *Escherichia coli* and Staphylococcus aureus by the disc diffusion method. The Mueller-Hinton agar plates were spread with a test culture suspension and the tamoxifen loaded BC discs were placed on the plates. The discs were slightly pressed and kept for diffusion at 3 °C in the refrigerator for 40 min. The plates were examined for a possible clear zone of growth inhibition after incubation at 37 °C for 22 h.

**Cytotoxicity Test**

Blood mononuclear cells were isolated from human blood. PHA stimulation of PBMCs was done by phytohemagglutinin (PHA) 5 μg/ml and human interleukin (IL–2). The lyophilized BC was powdered and 2 mg/ml BC stock was prepared in RPMI-1640medium. Serially double diluted stock (100 μl) was used as assay concentrations in 96-U bottom plate with an equal volume of RPMI-1640 medium (100 μl). The dilution suspension of stimulated PBMCs ($0.2\times10^6$ per well) were added and the plate was incubated for five days at37 °C with humidified $CO_2$ (5%) atmosphere. After incubation, cell viability was determined by the MTT assay [3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazoliumbromide). Optical density was measured at 550 and 630 nm wavelength, 630 nm was used as a reference wavelength. Percent viability was determined as follows:

**(1)**

$$\%\,Viability = \frac{Test}{X} \times 100$$

Where X is cell control.

**Release Rate**

BC sample (1 g of wet weight) was measured, followed by continuously weighing the samples stored under ambient conditions on a bench in air (relative humidity 30%) at different time intervals. Finally, the dry weight of the BC sample was taken when there was no further decrease in weight. This dry weight was subtracted from all the readings of weight taken during the drying process of the BC sample. Similarly, the loss of water at different time intervals was plotted against time.

**Swelling Ratio**

1 gr of BC was taken and cut into equal size pieces and dried to constant weight. The initial weight was measured and the sample was then immersed in deionized water at room temperature and allowed to swell for 4h. The swelling potential was determined by measuring the initial weight (wi) and the weight of the sample in swollen state (wst) using the following formula:

**(2)**

$$Swelling\ Ratio = \frac{(\mathrm{wst} - wi)}{wi} \times 100$$

The relation of swelling ratio against time was plotted.

**Result and Discussion**

**Drau Loading Capacity**

Tamoxifen has been used for many years as an anticancer drug. This drug was treated on BC. The relation between feed drug concentration and drug uploading capacity was determined as shown in Fig. 1a. Drug uploading capacities

of BC increased exponentially with an increase in drug concentration. The drug loading capacity depends on the porous structure of the loading material and the subsequent release depends upon the diffusion coefficient of the molecule.

**Release Tamoxifen from BC**

An anticancer drug may show prolonged antimicrobial activity, which needs extended and table release of the drug from the support. The elution of tamoxifen of different concentrations from BC was demonstrated in Fig. 1b. The elution ratio was calculated as follows:

**(3)**

$$Elution\ Ratio = \frac{Eluted\ drug}{Adsorbed\ drug} \times 100$$

Elution ratio was increased with an increase in time. Tamoxifen was released gradually from BC up to 95% within 24 h. The nanostructured and three dimensional porous networks of BC fibers allowed a slow gradual release of tamoxifen in water due to the large surface area. Hence, the nanoparticle drug carriers are of importance for intramural drug transport and uptake.

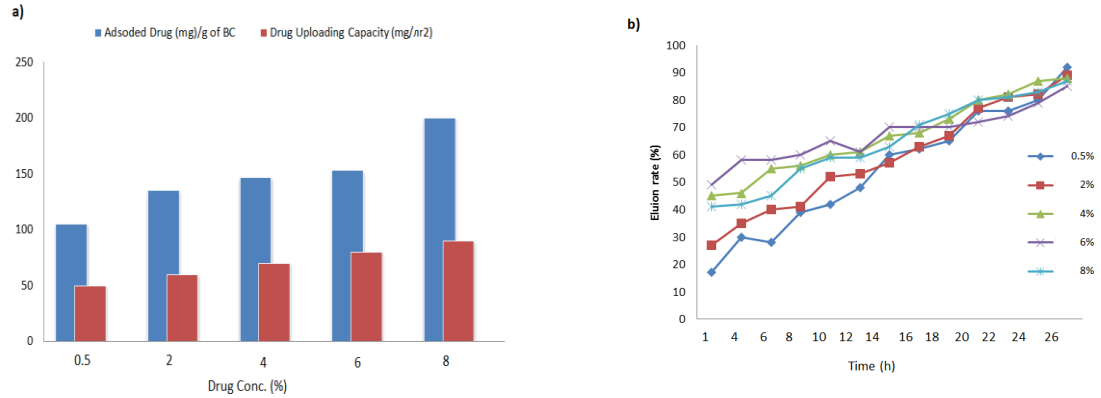


*Figure 1: a) Adsorbed drug and drug loading capacity relation between feed drug concentrations; b) Release tamoxifen from BC*

**Antibactrial Activity Treated BC**

Antibacterial activity against E. coli and S. aureus are shown in Fig. 2. The BC with higher tamoxifen concentration (8%) exhibited antimicrobial activity against *E. coli* and *S. aureus*. Based on the diameter of the inhibition zone, a higher antimicrobial effect was remarked against staphylococcus aureus. No inhibitory zone was observed for the control BC. Hence, it could be concluded that the inhibitory activity was attributed to tamoxifen only.

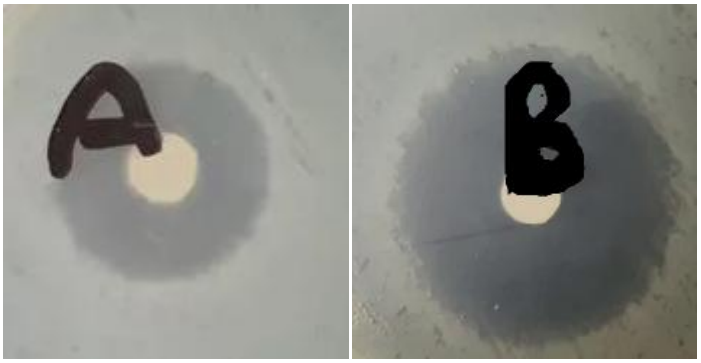


*Figure 2: Antimicrobial activity of tamoxifen loaded BC against a) Staphylococcus aureus and b) E. coli at 8% drug concentration*

### Cytotoxicity Test

Cytotoxicity test was used for non- toxic effect of BC on human PBMCs. In this test, the measured absorbance was proportional to the viable cell number and inversely to the degree of cytotoxicity. Even at the highest concentration (1 mg/ml) almost 92% cell viability was observed (Fig. 3). We concluded that the nanostructured fiber of BC used in this study was not toxic to human PBMCs. This test confirmed the non-toxic biocompatible nature of BC. Therefore, this property of BC allows its use for various vascular grafting, as scaffold materials and substrate for mammalian cellline cultures.

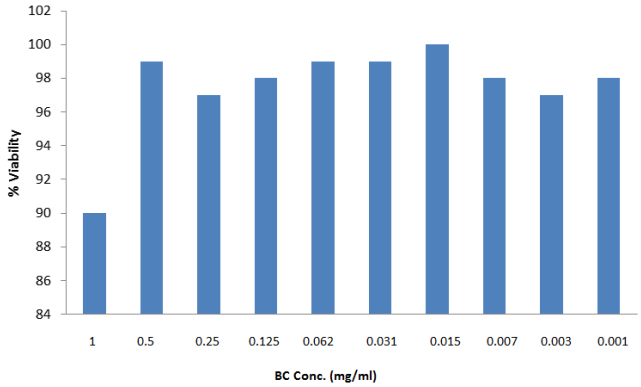


*Figure 3: Non-toxic of BC on the viability of PBMCs using the MTT test*

### Water Releas Rate and Swelling Ratio

Nano structured of BC fabrics allows its faster release. The water release from BC was steady. After 6 h, almost all of the water was released (Fig. 4a). Water molecules are physically entrapped at the surface and on the inside of the particles composed of reticulated fibers. The BC fibers, which act as a shield for water molecules, resist the fast flow of water molecules out of BC. The highly porous nature of BC permits a faster release of the drug, although it also depends on the diffusion coefficient of the molecules of the drug compound. BC could be tailored for improved controlled release of medicinal substances by in situ modification or by postproduction treatment, in composites, similarly to, for example, chitosan. The water absorption of BC was investigated as swelling ratio. The liquid absorbing capacity is important for wound a dressing material, as it can hold the moisture on the wound site, which promotes wound healing. BC showed 100% swelling ratio, with about 25 min release (Fig. 4b). The swelling ability was extended up to 600% with an increase in the time period.

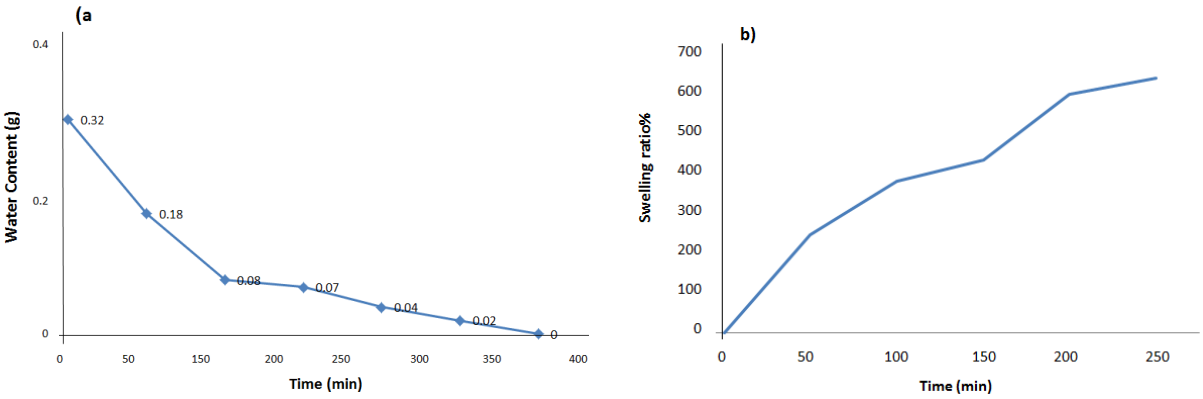


*Figure 4: a) Water release rate of BC; b) Swelling ratio of BC in deionized*

### Conclusion

This study showed the feasibility of using BC as a bio fabric for incorporation and controlled release of tamoxifen. The non-toxic biocompatible nature of BC makes it a promising substrate for regenerative biomaterial. A portable BC with high water absorption and antibacterial property was developed. A steady and long-lasting release of the

antimicrobial agent(within at least 24 h) was achieved by the produced BC, thus providing a sustained release of the antimicrobial compound, which allowed us to conclude that BC can be used as a wound dressing material. Antimicrobial BC films could be a prospective commercial wound dressing material for acute trauma treatment. The high water holding capacity of BC and its gradual release of liquid correspond to high drug loading and release ability. Thus, it has been demonstrated that BC is a promising candidate for applications as modern wound dressing material.

**Acknowledgment**

Thanks and appreciation from the Microbial laboratory of Tarbial Modaress University of Tehran, Iran and the Iran hormone pharmaceutical company to supply drug tamoxifen.

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