STUDY ON THE DRUG LOADING AND RELEASE POTENTIAL OF BACTERIAL CELLULOSE

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Bacterial cellulose (BC) is known for its unique characteristic properties, which make it a versatile biomaterial for application in various domains. Benzalconium chloride, which is widely used as antimicrobial drug in wound dressing, was used to test the drug loading and release potential of BC. The drug loading ability increased with an increase in drug concentration. The BC gradually released up to 90% of the drug within 24 h. The antibacterial activity of drug loaded BC was tested against *Escherichia coli* and *Staphylococcus aureus*. The non-toxic biocompatible nature of BC was confirmed by an MTT assay on human PBMCs with 90% cell viability, which allows its application as a regenerative biomaterial. The liquid absorbing capacity and release rate were calculated as swelling ratio and water release rate, and the results supported its anticipated use as antimicrobial wound dressing material. Thus, BC was demonstrated as a promising candidate for application as modern wound dressing material due to its drug loading and release potential.

Keywords: biomaterial, biocompatible, antimicrobial activity, swelling ratio, drug loading potential

INTRODUCTION

Bacterial cellulose (BC) is a type of biocellulose synthesized by certain bacteria. Under stationary culture conditions, these bacteria produce a thick gel or pellicle of cellulose on the surface of the liquid medium, while under shaking, it is produced in the form of beads, spheres or stellates. BC differs from plant cellulose in its higher purity, crystallinity, degree of polymerization and tensile strength.[1–4] BC has Iα and Iß crystalline forms, unlike plant cellulose, which presents mainly the Iß structure.[5] BC presents many important characteristics, such as high water holding capacity, high porosity, gradual water release, high crystallinity and high Young's modulus, finer weblike network. Also, its biocompatible, non-toxic and biodegradable nature makes it valuable for biomedical applications, such as wound dressing, tissue scaffolds, artificial blood vessels and biomembranes.[6-9] Its properties could be modified during synthesis by varying culture and environmental parameters.[10-11] The present study is an effort to look for innovative applications of BC due to its drug loading and release potential in the field of modern antimicrobial wound dressing.

Bacterial cellulose has a promising application as wound dressing, but it does not have its own antimicrobial property, which is essential in preventing wound infection during wound healing, and hence it is imperative to include an antimicrobial agent within BC. Wound dressings have been developed mostly for chronic wound treatment, while dressings for acute conditions are rare. The present study has proposed modern acute antimicrobial wound dressing material containing a commercial drug – benzalconium chloride.

In our previous work, the production and characterization of BC, produced under shaking conditions was investigated.[12-14] The produced BC had a pure nature with high crystallinity (81%) and was highly of Iα type. The Z-average particle size was 1.44 µm with high porosity of 181.81% and high water absorption and holding capacity (400%).[14]

In the present work, the drug loading and release capacity of BC was investigated. In addition, the non-toxic biocompatible nature of BC was confirmed, along with its antimicrobial activity, water release rate and swelling ratio to

achieve its performance as a biomaterial in future biomedical applications.

EXPERIMENTAL

Microorganism

Gluconoacetobacter hansenii (NCIM 2529) from National chemical laboratory, Pune, India, was used in this study. The organism was maintained on Hestrin Schramm (HS) agar plates containing (in gram per liter): D-glucose, 20; peptone, 5; yeast extract, 5; citrate, 1.15; disodium phosphate, 2.7; pH 6.0. Then, it was incubated at 30 °C for 24-48 h. The distinct colony that appeared on the plate was isolated and purified by repeated streaking onto HS medium.[15]

Production and purification of BC

The *Gluconoacetobacter hansenii* from the HS agar plate was inoculated into an HS broth (pH 6.0). The flasks were incubated at 30 °C for 2 days at 120 rpm in an orbital shaking incubator. This was used as 5% (v/v) inoculum. A production medium statistically optimized in our laboratory[10,11] was used in this study, with the following composition (in gram per liter): sucrose, 28.1; KNO_3, 5; Na_2HPO_4, 0.1; $CaCl_2$, 12.6; $MgSO_4$, 1; and applying the following reaction parameters: temperature, 25 °C; incubation time, 5 days; agitation speed, 170 rpm. After an incubation of 5 days, the beads of BC were separated by filtration and rinsed with distilled water to remove excess medium, and then immediately boiled (at 90 °C) in 0.1 M NaOH solution for 30 min, to remove the cells and medium embedded in the cellulose material. After boiling, the beads of BC were purified by extensive washing in distilled water at room temperature until the pH of the water became neutral. The final purified cellulose was lyophilized.

Applications

Drug loading capacity

Benzalconium chloride is an antimicrobial cationic surfactant, which is widely used for commercial wound dressing, being effective against Gram positive bacteria. BC does not have an antimicrobial property, but due to its high water holding capacity and porosity, it can absorb and slowly release the antimicrobial solution. The drug loading capacity and steady release of benzalconium chloride, as well as the antimicrobial capacity of the drug-loaded BC, were tested.

Drug loading capacity and release ratio

BC was dried and cut into disc shape samples (6 mm diameter with average thickness of 350 µm) with similar weight. One piece was immersed into each feed with varying concentrations (0.5%, 1%, 2%, 4%, 6%, and 8%) of benzalconium chloride. The concentration of benzalconium chloride was determined spectrophotometrically by measuring λ max at 263 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 benzalconium chloride was determined as the difference in weight before and after soaking. The amount of adsorbed benzalconium chloride was expressed as milligrams of benzalconium chloride per g of BC. The drug loading capacity was calculated as the amount of adsorbed benzalconium chloride per area (πr^2) of the BC disc.

The release of benzalconium chloride was studied as follows: the BC disc with the respective feed concentration was removed and immersed into a sealed beaker containing 25 ml of deionised 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 263 nm for measuring the concentration.[16]

Antimicrobial assay

The antimicrobial activity of benzalconium chloride loaded BC disc 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 benzalconium chloride loaded BC discs were placed on the plates. The discs were slightly pressed and kept for diffusion at 4 °C in the refrigerator for 30 min. The plates were examined for a possible clear zone of growth inhibition after incubation at 37 °C for 24 h.[17]

Cytotoxicity assay

Peripheral blood mononuclear cells were isolated from human blood with the use of Histopaque. PHA stimulation of PBMCs was done by phytohemagglutinin (PHA) (Sigma) 5 µg/ml and human interleukin (IL–2) (PerkinElmer). The lyophilized BC was powdered and 2 mg/ml biocellulose stock was prepared in RPMI-1640 medium. The assay was run in duplicate. 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 (0.2×10^6 per well) were added and the plate was incubated for five days at 37 °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-diphenyl-tetrazolium bromide) (Sigma, USA). Optical density was measured at 550 and 630 nm wavelength, 630 nm was used as a reference wavelength.[18] Percent viability was determined as follows:

$$\% \text{ Viability} = \frac{\text{Test}}{\text{CC}} \times 100 \quad (1)$$

where CC is cell control.

Water release rate (WRR)

1 g of wet weight of BC sample 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 g of BC sample 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 4 h. The swelling potential was determined by measuring the initial weight (Gi) and the weight of the sample in swollen state (Gst) using the following formula:

$$\text{Swelling Ratio} = \frac{(Gst - Gi)}{Gi} \times 100 \quad (2)$$

The relation of swelling ratio against time was plotted.

RESULTS AND DISCUSSION

Drug loading capacity

Benzalconium chloride is a quaternary ammonium compound, which has been used for many years as an antiseptic and disinfectant. This drug was incorporated in 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.[19]

Release of benzalconium chloride from BC

An antimicrobial compound should show prolonged antimicrobial activity, which needs extended and stable release of the drug from the support. The elution of benzalconium chloride of different concentrations from BC was demonstrated in Fig. 1b. The elution ratio was calculated as follows:

$$\text{Elution Ratio} = \frac{\text{Eluted drug}}{\text{Adsorbed drug}} \times 100 \quad (3)$$

Elution ratio was increased with an increase in time. Benzalconium chloride was released gradually from BC up to 90% within 24 h. The nanostructured and three dimensional porous networks of BC fibers allowed a slow gradual release of benzalconium chloride in water due to the large surface area. Hence, the nanoparticle drug carriers are of importance for intramural drug transport and uptake.[20]

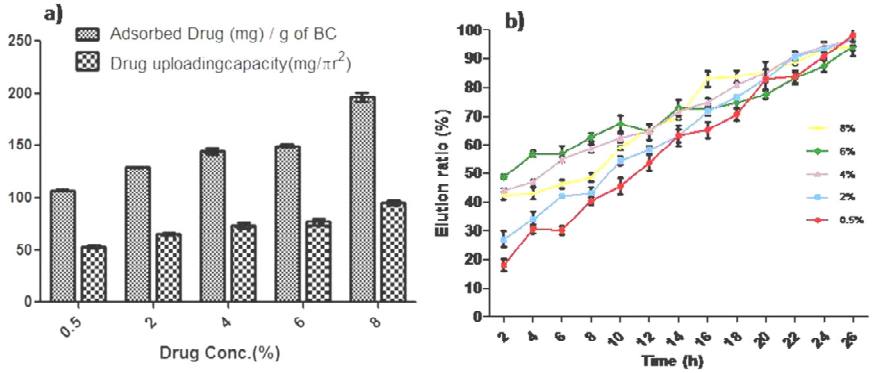

Figure 1: a) Relation between feed drug concentration with adsorbed drug and drug loading capacity; b) Release behavior of benzalconium chloride drug from BC

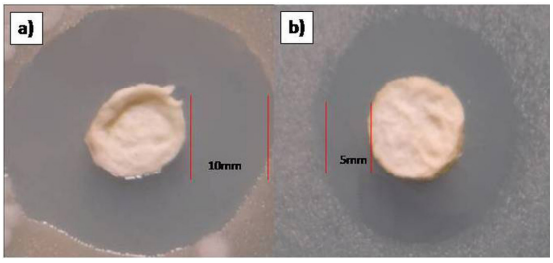

Figure 2: Antimicrobial activity of benzalconium chloride loaded BC disc against a) *Staphylococcus aureus* and b) *E. coli* at 8% drug concentration. The red lines show the inhibition zone

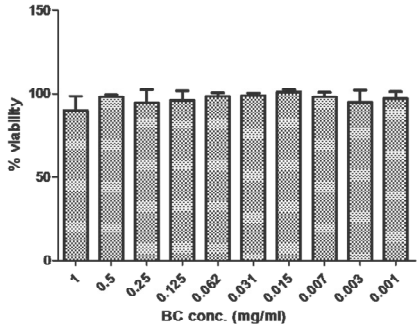

Figure 3: Non-toxic effect of BC on the viability of PBMCs using the MTT assay

Antimicrobial activity of drug loaded BC discs

Fig. 2 demonstrates a qualitative antibacterial activity against *E. coli* and *S. aureus*. The BC with higher benzalconium chloride concentration (8%) exhibited antimicrobial activity against *E. coli* and *S. aureus*. Based on the diameter of the inhibition zone, a higher antimicrobial activity was remarked against *Staphylococcus aureus*. No inhibitory zone was observed for the control unsoaked BC. Hence, it could be concluded that the inhibitory activity was attributed to benzalconium chloride only.

Cytotoxicity assay

The non-toxic effect of cellulose on human PBMCs was confirmed by a cytotoxicity assay. In this assay, 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 90% 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 assay confirmed the non-toxic biocompatible nature of BC. Therefore, this property of BC allows its use for various vascular grafting, as scaffold materials[21,22] and substrate for mammalian cell line cultures.[23]

Water release rate and swelling ratio

The gradual release of liquid is important in the area of drug delivery. The nanostructured web network of BC fibers 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 fibrils, 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 post-production treatment, in composites, similarly to, for example, chitosan.[24,25]

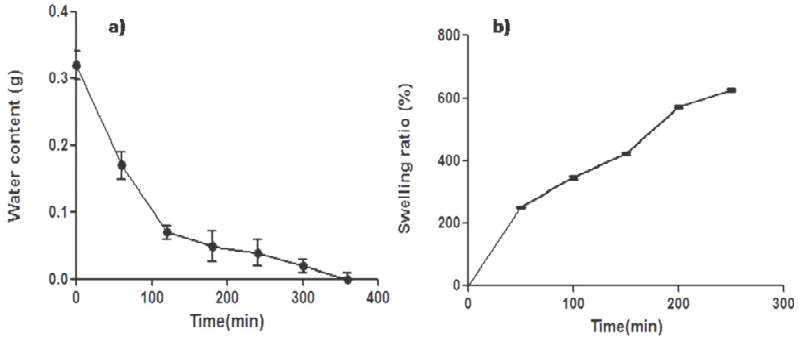

Figure 4: a) Water release rate of BC at varying time periods; b) Swelling ratio of BC in deionized water at varying time periods

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. The water absorption performance of BC was investigated as swelling ratio. 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.

CONCLUSION

BC has been investigated for its drug loading and release potential. Ideally, this material will be inexpensive, tasteless and chemically inert. A portable BC disc 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 discs 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. The non-toxic biocompatible nature of BC makes it a promising substrate for regenerative biomaterial. Thus, it has been demonstrated that BC is a promising candidate for applications as modern wound dressing material.

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REFERENCES

[1] M. Iguchi, S. Yamanaka, A. Budhiono, *J. Mater. Sci.*, **35**, 261 (2000).
[2] S. Bae, Y. Sugano, M. Shoda, *J. Biosci. Bioeng.*, **97**, 33 (2004).
[3] A. N. Nakagaito, S. Iwamoto, H. Yano, *Appl. Phys. A*, *Mat. Sci. Process*, **80**, 93 (2005).
[4] S. Bae, M. Shoda, *Biotechnol. Bioeng.*, **90**, 20 (2005).
[5] R. H. Atalla, D. L. Vanderhart, *Science*, **223**, 283 (1984).
[6] R. Jonas, L. F. Farah, *Polym. Degrad. Stabil.*, **59**, 101 (1998).
[7] D. Klemm, D. Schumann, U. Udhardt, S. Marsch, *Prog. Polym. Sci.*, **26**, 1561 (2001).
[8] A. Svensson, E. Nicklasson, T. Harraha, B. Panilaitis, D. L. Kaplan *et al.*, *Biomaterials*, **26**, 419 (2005).
[9] L. Hong, Y. L. Wang, S. R. Jia, Y. Huang, C. Gao *et al.*, *Mater. Lett.*, **60**, 1710 (2006).
[10] Q.-S. Shi, J. Feng, W.-R. Li, G. Zhou, A.-M. Chen *et al.*, *Cellulose Chem. Technol.*, **47**, 503 (2013).
[11] F. Mohammadkazemi, K. Doosthoseini, M. Azin, *Cellulose Chem. Technol.*, **49**, 455 (2015).
[12] B. V. Mohite, K. K. Kamalja, S. V. Patil, *Cellulose*, **19**, 1655 (2012).
[13] B. V. Mohite, B. K. Salunke S. V., Patil, *Appl. Biochem. Biotechnol.*, **169**, 1497 (2013).
[14] B. V. Mohite, S. V. Patil, *Carbohyd. Polym.*, **106** 132 (2014).
[15] S. Hestrin, M. Schramm, *Biochem. J.*, **58**, 345 (1954).
[16] B. Wei, G. Yang, F. Hong, *Carbohyd. Polym.*, **84**, 533 (2011).
[17] W. M. M. Kirby, G. M. Yoshihara, K. S. Sundsted, J. H. Warren, *Antibiotics Ann.*, **892**, 1956 (1957).
[18] M. Ferrari, M. C. Fornasiero, A. M. Isetta, *J. Immunol. Methods*, **131**, 165 (1990).
[19] T. R. Hoare, D. S. Kohane, *Polymer*, **49**, 1993 (2008).
[20] C. Song, J. Due, J. Zhao, S. Feng, G. Du *et al.*, *Chem. Mater.*, **21**, 1524 (2011).
[21] S. Yamanaka, E. Ono, K. Watanabe, M. Kusakabe, Y. Suzuki, European Patent 0396344 (1991).
[22] H. Bäckdahl, M. Esguerra, D. Risberg, B. P. Gatenholm, *J. Tissue Eng. Regen. Med.*, **2**, 320 (2008).
[23] K. Watanabe, Y. Eto, S. Takano, S. Nakamori, H. Shibai *et al.*, *Cytotechnology*, **13**, 107 (1993).
[24] W.-S. Chang, H.-H. Chen, *Food Hydrocol.*, **53**, 75 (2016).
[25] I. Sulaeva, U. Henniges, T. Rosenau, A. Potthast, *Biotechnol. Adv.*, **33**, 1547 (2015).