BIOCELLULOSE: ANTISEPTICS WOUND DRESSINGS TO MITIGATE EPIDERMIS INFECTIONS

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ABSTRACT

Bacterial Nanocellulose (BNC) stands out as a promising biomaterial with unique properties such as biocompatibility, non-cytotoxicity, and the ability to reduce healing time. However, its lack of antibacterial activity limits its broad application. Recent attention has been drawn to antiseptics like Chlorhexidine digluconate (CHX) and Povidone-iodine (PVP-I) for local hygiene. This study aims to synergize the BNC' properties with these substances by incorporating them into a matrix to create an antiseptic wound dressing that may prevent the development of epidermis infection. The BNC films were incorporated with 1 mL of CHX, PVP-I, then incubated overnight at 4 °C. Evaluation of antiseptic was conducted through disk diffusion tests against *Staphylococcus epidermidis*, yielding inhibition halos measuring 17.65 ± 1.64 mm for the positive control (amikacin antibiotic), 28.28 ± 1.17 mm for CHX, and 15.72 ± 0.67 mm for PVP-I. These results, suggest the potential functionalization of BNC for use as an antiseptic wound dressing, demonstrating the BNC films' ability to combat epidermal infections by effectively inhibiting *S. epidermidis* growth in response to the incorporated antiseptics.

Keywords: Bacterial Nanocellulose. Wound care. Wound dressings. Infections. Antiseptics.

1 INTRODUCTION

The human skin serves a critical role as a protective barrier, shielding against pathogens, physical trauma, and excessive water loss, thereby safeguarding the body's internal environment. However, skin injuries caused by traumas, burns or ulcers can compromise this vital defense mechanism, leading to challenging wounds that resist healing[1]. Infections, commonly instigated by gram-positive or gram-negative bacteria, including drug-resistant strains, further exacerbate the healing process. Notably, *Staphylococcus aureus* (39.3%), *Escherichia coli* (30.4%), *Pseudomonas aeruginosa* (19.6%), *Staphylococcus epirdermidis* (17.8%), *Klebsiella spp.* (12.5%) and *Enterobacter spp.* (10.7%) are among the primary microbial agents implicated in surgical wounds[2-4].

S. epidermidis, a ubiquitous inhabitant of human skin and mucous membranes, has emerged as a significant opportunistic pathogen, particularly implicated in infections associated with implanted medical devices and surgical wounds[4-7]. When upon invading wounds, microorganisms trigger an inflammatory cascade, rapidly colonizing and forming biofilms. These biofilms impede re-epithelization, prolonging healing times, exacerbating tissue damage, and predisposing patients to chronic infections, often necessitating drastic measures like amputation[8]. Conventional antibiotics face challenges in penetrate biofilms, rendering them less effective. Hence, antiseptics such as Chlorhexidine digluconate (CHX) and Povidone-iodine (PVP-I) have become mainstays in wound care protocols[9-11].

Cellulose-based polymers, sourced from diverse organisms like plants, algae, and bacteria, offer immense potential for biomedical and biotechnological applications[12-14]. Bacterial Nanocellulose (BNC), characterized by its nanostructure network, exhibits properties like mechanical robustness, biodegradability, biocompatibility, low toxicity and high porosity[15,16]. As a result of these characteristics, BNC is a promising biomaterial particularly in the medical field, like as wound dressings for treatment of burns [17-20]. However, the lack of inherent antibacterial activity in BNC poses a limitation. To address this, our research aims to enhance the effectiveness of BNC by integrating antiseptic agents. Drawing upon BNC's fluid-absorption capability and its versatility in incorporating diverse substances, our objective is to develop an antiseptic wound dressing tailored to mitigate epidermal infections caused by *S. epidermidis*.

2 MATERIAL & METHODS

2.1 Microbial strain, growth medium, biosynthesis and BNC purification

BNC films were produced as films. The process involved inoculating Hestrin-Schramm (HS) medium with *Komagataeibacter xylinus* (ATCC® 53524™). HS medium was formulated with 20.0 g/L glucose, 5.0 g/L yeast extract, 5.0 g/L bacterial peptone, 2.7 g/L disodium phosphate, and 1.15 g/L citric acid, adjusted to pH 6.5 using sodium hydroxide solution and sterilized via autoclaving (121 ºC for 20 minutes)[21].

K. xylinus was inoculated onto HS agar plates and incubated at 28 ºC for a period of seven days. Following incubation, bacterial colonies were randomly selected and suspended in HS medium. Currently, a 10 % (v/v) of bacteria inoculum was prepared and transferred to 96-well plates. After seven days of incubation, the films were retrieved and subjected to purification with 0,1 M NaOH at 50 ºC for 24 hours. Subsequently, they were rinsed with distilled water until reaching a neutral pH and then sterilized.

2.2 Functionalization of BNC films with antiseptics

The methodology was adapted from Dydak (2021). Functionalization was achieved by adding 1 mL of 2% Chlorhexidine digluconate (Rioquímica, São Paulo, Brazil), 10% Povidone-iodine (Rioquímica, São Paulo, Brazil) to sterile BNC films with a diameter of 6.4 mm. Subsequently, they were incubated overnight at 4 ºC to facilitate the chemisorption of antiseptics[22].

2.2 Disc diffusion test

Susceptibility tests were conducted following the adapted method of disk diffusion outlined in the Clinical and Laboratory Standards Institute guidelines (2015)[23]. The strain of *Staphylococcus epidermidis* (CBAM 0609) was sourced from the "Coleção de Bactérias da Amazônia - Oswaldo Cruz Foundation (Manaus, Brazil)" and cultured on nutrient agar plates at 35 ºC for 24 hours.

A standardized suspension of microorganisms was prepared by inoculating four colonies of *S. epidermidis* in 5 mL of physiological solution (0.9% NaCl) and adjusting it to 0.5 McFarland turbidity standard. This suspension was then spread onto Mueller Hinton agar plates. Negative control films (BNC without antiseptics incorporation) were inserted onto the plates, while positive controls were established using paper disc filters impregnated with 30 µg of amikacin (Laborclin, Paraná, Brazil). Finally, BNC films incorporated with CHX and PVP-I were placed on the plates and incubated for 24 hours. The diameter of the zones of growth inhibition was measured in triplicate using a pachymeter, and the mean values, along with standard deviations, were calculated.

3 Results and discussion

The availability of inhibition zones was compared with the biological reference standard, represents by paper disc filters impregnated with 30 µg of amikacin. Notably, *S. epidermidis* strains exhibited and inhibition zone of 17.65 ± 1.64 mm, indicative of sensitive to the tested antibiotic. The values obtained with the antiseptics were interpreted in accordance with the guidelines proposed by CLSI (2015) and OSTROSKY et al. (2008). Sensitive was defined as a difference of ± 3 mm or greater from the positive control; while moderately sensitive results displayed a difference of up to ± 2 mm. In contrast, resistance was identified by the presence of a halo equal to or smaller than 2 mm[23,24].

Analysis of the results revealed the formation of inhibition zones with 2% CHX and 10% PVP-I, consistent with the anticipated outcomes. Notably, no inhibition zones were observed in the negative control as shown in Figure 1.

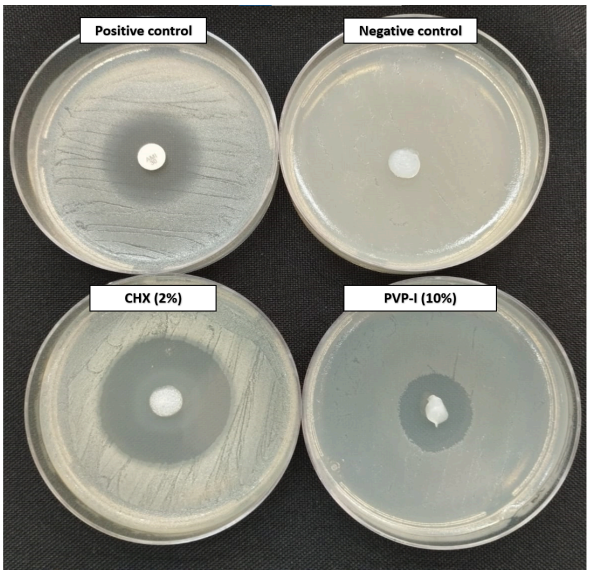

Figure 1 Susceptibility testing of BNC films incorporated with antiseptics.

The results presented in Table 1 demonstrate the susceptibility of *S. epidermidis* to Chlorhexidine digluconate and Povidone-iodine, with differences of 10.63 mm and 1.93, respectively, compared to the positive control.

Table 1 Inhibition zones obtained from disk diffusion

Staphylococcus epidermidis	**Inhibition zones (mm) ± Standard deviations**
Positive control	17.65 ± 1.64
Chlorhexidine digluconate (CHX)	28.28 ± 1.17
Povidone-iodine (PVP-I)	15.72 ± 0.67

4 Conclusion

In conclusion, our findings support the potential of BNC as a versatile platform for developing antiseptic wound dressing. Both Povidone-iodine and Chlorhexidine digluconate were effectively integrated into BNC films. Importantly, the presence of these antiseptics rendered the films effective against *S. epidermidis*, which often causes wound infections. This underscores the promise of BNC-based materials in mitigating epidermal infections and highlights their prospective role in advancing wound care strategies.

REFERENCES

1 SEM, C. K.; GORDILLO, G. M.; ROY, S.; KIRSNER, R.; LAMBERT, L.; HUNT, T. K.; GOTTRUP, F.; GURTNER, G. C.; LONGAKER, M. T. 2009. Wound Repair Regen. 17 (6). 763–771.

2 GUO, S.; DIPIETRO, L. A. 2010. J Dent Res. 89 (3). 219-229.

3 METCALF, D.G; BOWLER, P. G. 2013. Burns Trauma. 1 (1). 5-12.

4 DOS SANTOS, W. B.; DA SILVA, J. C.; BERNARDO, T. H. L.; BASTOS, M. L. A.; VERÍSSIMO, R. C. S. S. 2016. Revista SOBECC. 21 (1), 46–51.

5 SEVERN, M. M.; HORWILL, A. R. 2023. Nat. Rev. Microbiol. 21 (2). 97–111.

6 VUONG, C.; OTTO, M. 2002. Microbes and Infect. 4. 481–489.

7 BRESCÓ, M. S.; HARRIS, L. G.; THOMPSON, K.; STANIC, B.; MORGENSTERN, M.; O'MAHONY, L.; RICHARDS, R. G.; MORIARTY, T.F. 2017. Front. Microbiol. 8 (401).

8 HAN, G.; CEILLEY, R. 2017. Adv. Ther. 34. 599–610.

9 PANG, Q.; LOU, D.; LI, S.; WANG, G.; QIAO, B.; DONG, S.; MA, L.; GAO, C.; WU, Z. 2020. Adv. Sci. 7 (6).

10 KRAMER, A.; DISSEMOND, J.; KIM, S.; WILLY, C.; MAYER, D.; PAPKE, R.; TUCHMANN, F.; ASSADIAN, O. 2018. Skin Pharmacol. Physiol. 31 (1). 25-58.

11 MURPHY, C.; ATKIN, L.; SWANSON, T.; TACHI, M.; TAN, K. Y.; CENIGA, M. V.; WEIR, D.; WOLCOTT, R.; ČERNOHORSKA, J.; CIPRANDI, G.; DISSEMOND, J.; JAMES,G. A.; HURLOW, J.; MARTÍNEZ, J. L. L.; MROZIKIEWICZ-RAKOWSKA, B.; WILSON, P. 2020. J. Wound Care. 29. s1-s26.

12 LIU, Z.; LI, X.; XIE, W.; DENG, H. Carbohydr. Polym.173. 353–359.

13 JI, K. W.; WANG, W.; ZENG, B.; CHEN, S.; ZHAO, Q.; CHEN, Y.; LI, G.; M. A. 2016. T. Sci Rep. 6 (21863).

14 MACHADO, B. A. S.; REIS, J. H. O.; CRUZ, J. L.; LEAL, I. L.; BARBOSA, J. D. V. B.; AZEVEDO, J. B.; DRUZIAN, J. I. 2017. African J. Biotechnol. 16 (2). 1567-1578.

15 FISCHER, M. R.; GARCIA, M. C. F.; NOGUEIRA, A. L.; PORTO, L. M.; SCHNEIDER, A. L. S.; PEZZIN, A. P. T. 2017. Matéria (Rio de Janeiro). 22 (1).

16 POWELL, L. C.; KHAN, S.; CHINGA-CARRASCO, G.; WRIGHT, C. J.; HILL, K. E.; THOMAS, D. W. 2016. Carbohydr. Polym. 10 (137). 191-197.

17 ALVAREZ, O. M.; PATEL, M.; BOOKER, J.; MARKOWITZ, L. 2014. Wounds. 16 (7). 224–233.

18 LEGEZA, V. I.; GALENKO-YAROSHEVSKII, V. P.; ZINOV'EV, E. V.; PARAMONOV, B. A. ; KREICHMAN, G. S.; TURKOVSKII, I. I.; GUMENYUK, E. S.; KARNOVICH, A. G.; KHRIPUNOV, A. K. 2004. Bull Exp. Biol. Med. 138 (3). 311-315.

19 VIEIRA, R. G. P.; FILHO, G.; ROSANA, A. 2007. Carbohydr. Polym. 2. 182-218.

20 YAGUISHITA, N. 2007. J. Vasc. Bras. 6 (2). 193-194.

21 HESTRIN, S.; SCHRAMM, M. 1954. Biochem J. 58. 345–352.

22 DYDAK, K.; JUNKA, A.; DYDAK, A.; BROŻYNA, M.;PALECZNY, J.; FIJALKOWSKI, K.; KUBIELAS, G.; ANIOŁEK, O.; BARTOSZEWICZ, M. 2021. Int. J. Mol. Sci. 22 (3996).

23 CLSI. 2015. Performance standards for antimicrobial disk susceptibility tests. In: CLSI document M02-A12. 12nd ed. Wayne, Pennsylvania.

24 OSTROSKY, E. A.; LIMA, M. L.; KANEKO, T. M.; NISHIKAWA, S. O.; FREITAS, B. R. 2008. Rev. Bras. Farmacogn. 18, (2). 301-307.

ACKNOWLEDGEMENTS

This work has been supported by CAPES and the authors would like to thank the Universidade Tecnológica Federal do Paraná (UTFPR), Campus Dois Vizinhos, to the Graduate Program in Biotechnology (PPGBIOTEC) and to the Research Group - NanoBioCell.