RESEARCH ARTICLE

The use of Bacterial Cellulose Produced from the Local Isolate *Komagataeibacter xylinus* TELE8 as an Antibacterial for Wounds

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ABSTRACT

The present study aimed to use the bacterial cellulose pre-produced from local isolate *Komagataeibacter xylinus* TELE8 as antimicrobial dressings for wounds and compared their activity with specific artificial antibacterial. First, the toxicity of bacterial cellulose was examined *in-vitro* and *in-vivo* toward the shape and growth of human lymphocytes and then toward skin irritation, respectively. The results showed that there was no effect of bacterial cellulose dressings on the shape and numbers of human lymphocytes compared to the studied positive and negative control samples, also, the results of the study of the effect of bacterial cellulose dressings on the skin surface characteristics indicated that there was no erythema, edema, redness and dryness on the surfaces of the skin exposed to bacterial cellulose in test animals.

Thereafter, the effect of BC, anti, and BC + anti dressings were studied using 12 male rabbit of 6 months old and were randomLy distributed into four groups included: a control group (without treatment), BC group (treated with bacterial cellulose), BC + anti group (treated with bacterial cellulose and sodium fusidate), and anti group (treated with sodium fusidate), all wounds in animals were treated for 15 days according to their group and the observations were recorded. The results showed clear and rapid wound healing in two groups, including the BC group and BC +Anti group compared with the anti-group and control group, also, the scores of skin irritation in the two previous groups were significantly lower than the anti-group and the control group for all days of the experiment; therefore bacterial cellulose may provide a promising effect in enhancing wound healing and preventing wound infections that act in delaying wound recovery.

Keywords: Bacterial cellulose, *Komagataeibacter xylinus*, Wound healing, Wound infections, Wound recovery.

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INTRODUCTION

The skin is a key part of the body's defense against diseases, producing a barrier that stops infectious agents from entering and spreading throughout the body. A detailed chain of processes is set in action when microbes cause tissue damage, all with the goal of repairing the injured skin tissue. Hemostasis, inflammation, proliferation, and remodeling are all steps of the repair process.[1] Cellular, humoral, and molecular activities may be coordinated and regulated to achieve optimum tissue repair.

When bacterial infections, old age, and metabolic problems are present, the wound healing phase might become skewed, hindering proper healing and keeping the wound from fully sealing.

The frequency of skin infections is on the rise, especially in the elderly and those with diabetes, and chronic wounds are becoming more common all over the globe. Patient well-being suffers, and the public healthcare sector bears heavy financial load as a result of the time and money required to manage chronic wounds.[2,8]

There are two factors that affect how quickly an infection spreads and how long it takes to heal: first, the host's capacity to develop efficient immune responses, and second, the number of pathogens that make contact with the affected area. Microorganisms thrive in a wet, warm, and nutrient-rich microenvironment found under the skin surface. Many therapies are accessible to prevent the spread of germs and speed up recovery, but their impacts on the healing phase are

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yet unknown. Antibiotics are becoming more popular as a treatment option in this situation. According to Altoé *et al.*[9] antibiotic tendency for tissue and capillary development, which speeds up wound contraction and reduces infections, supports the regular utilization of antibiotics in injury treatment. Because the infection is a common problem of wound recovery, the right utilization of antibiotics may accelerate wound healing and greatly save healthcare expenses. The cytotoxicity of antibiotics on fibroblasts and keratinocytes has generated debate concerning their value in healing wounds that are not purposely infected. Additionally, antibiotic resistance might develop as a consequence of the widespread and uncontrolled usage of these medicines.[10,11] In order to find an alternative to antibiotics, this research was done to investigate the usage of bacterial cellulose (isolated by the authors using *Komagataeibacter xylinus* TELE8, (data not shown) as an antimicrobial for wounds.

MATERIALS AND METHODS

Bacterial Eellulose Production

The local isolate *K. xylinus* TELE8 was used to produce bio-cellulose, which was isolated from rotten lemon fruit, and then tested their ability to produce bio-cellulose in a previous study (results not shown in this paper).

Solutions of Bio-cellulose

The solution of bacterial cellulose with a concentration of 25% was prepared by taking a piece of bacterial cellulose weighing 25 g and placed in a glass beaker, then 50 mL of sterile distilled water was added and the bacterial cellulose was mashed using a glass slide, then the volume was completed to 100 mL by sterile distilled water and then different concentrations 5, 10 and 15% were prepared from the stock of bacterial cellulose solution.[12]

Toxicity of Bacterial Cellulose

The toxicity of bacterial cellulose produced by local isolate *K. xylinus* TELE8 was tested first in a *in-vitro* study based on the number of lymphocytes (growth viability) and in the second step based on the shape of lymphocytes, as shown below:

The lymphocytes were isolated according to (Boyle and Chow, 1969)[13] by taking 3 mL of human blood and placing it in a 5 mL ethylenediaminetetraacetic acid (EDTA) tube and mixing it well. Subsequently, 5 mL of phosphate buffer saline (PBS) was added to a new glass and then 3 mL from the EDTA tube was taken and added to a glass tube containing PBS and mixed briefly, thereafter, 3 mL of Ficoll solution (a colorless substance that is used to filter the blood and isolates lymphocytes from the rest of the blood components) was added in a new glass tube, and then 5 mL from the mixture of blood and PBS was carefully and gently added to the wall of the tube containing Ficoll solution until it formed a layer in a top of the glass tube.

This glass tube was centrifuged at 3000 rpm for 10 minutes. Then the cloudy middle layer (about 3 mL) was collected using a Pasteur pipette and transferred to a new glass tube to wash the lymphocytes, thereafter 5 mL of PBS was added and

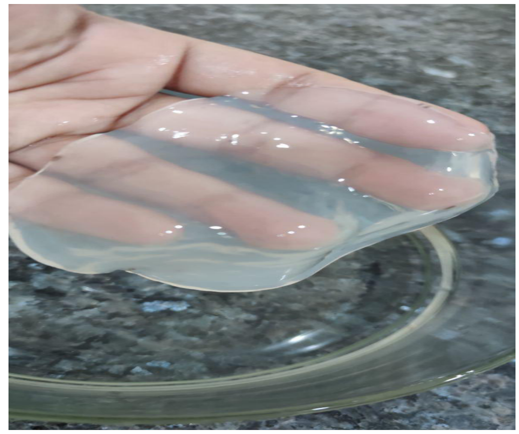


Figure 1: Bacterial cellulose produced from *K. xylinus* TELE8 and used as dressings to treat wounds.

shaken briefly, and then centrifuged again as above. Then the supernatant layer was removed by a Pasteur pipette leaving about 1-mL of PBS to mix the precipitated lymphocytes using a vortex.[13]

After the lymphocytes were isolated, 1-mL was added to 25 mL of RPMI 1640 medium and mixed well, then the number of lymphocytes in the mixture was counted using the hemocytometer chamber. Thereafter the mixture was distributed into 5 tubes (one tube as control and four tubes as a test), 1-mL from each bacterial cellulose solution 5, 10, 15, and 25% was added to the first, second, third, and fourth tube, respectively, while 1-mL distilled water was added to the control tube. All tubes were incubated at 37°C under 5% CO_2 for 24 hours, after the incubation period, the number of lymphocytes in the test and control was counted using a hemocytometer chamber, while the shape of the lymphocytes was examined using a light microscope.[12]

The second test of toxicity toward the bacterial cellulose was tested *in-vivo* based on the dermal irritation in the laboratory animals using three male rabbits (6 months old; 1200 to 1400 gm) for each experiment. After washing and sterilizing the animal with sterile distilled water, a specific selected area in the skin was shaved without occurring any wounds, thereafter this area was treated with a piece of bacterial cellulose (4x4 cm) as in Figure 1, and the data in each experiment were recorded in the test and control animal after 12, 24, and 48 hours, also the dressing and the bacterial cellulose material were changed after each studied period.[14]

Effect of Bacterial Cellulose on Wounds Healing

Twelve male rabbits (6 months old and weighing 1200 to 1400 gm) were used in this study, where the rabbits were distributed into four groups, with an average of three rabbits per group. The first group represented the control (without treatment), the second group was treated with bacterial cellulose dressings, and the third group was treated with sodium fusidate ointment dressings of 2%, while the fourth group is treated with dressings of bacterial cellulose and dressings of sodium fusidate ointment 2%.

Table 1: Effect of bacterial cellulose on the growth viability and shape of lymphocyte *in-vitro* after 24 hours at 37°C.

Concentration of BC (%)	*Lymphocytes number (cell/mL)*	*Cell shape under light microscope*
Negative control	450000	Normal
Positive control	540.000	Normal
5	540.000	Normal
10	540.000	Normal
15	510.000	Normal
25	490.000	Normal

Table 2: Effect of bacterial cellulose on the dermal irritation *in-vivo* after 12, 24, and 48 hours according to scoring criteria for dermal reactions.

Observation time (hours)	*Skin reaction*			
	Erythema	*Edema*	*Redness*	*Dryness*
12	-	-	-	-
24	-	-	-	-
48	-	-	-	-

No irritation= -

All rabbits used in the current experiment are first washed well and then sterilized with sterile distilled water, and then selected a specific area in the upper left shoulder of each rabbit, and then the animal was anesthetized (general anesthesia) by a veterinarian using a mixture of xylazine and ketamine. The selected area was shaved and made skin wounds (skin incisions with 6 mm in length and depth) using a new surgical blade for each animal.

All the proposed treatments were performed for the skin wounds in all groups according to the method mentioned previously, where the dressings are changed every 12 hours in the first 15 days of the experiment, and then the dressings are changed every 24 hours for the second 15 days of the experiment, where the skin wounds are washed with normal saline before each change of dressings. At the end of each day of the experiment and during the period of changing the dressings, the observations of erythema, edema, redness, and dryness were recorded to determine the wounds healing in the skin wounds of the test animals and compared with the control group, in addition to documenting this through daily photographs for all studied groups.[15,16]

RESULT

Toxicity of Bacterial Cellulose

The results of the toxicity study on the growth and shape of lymphocytes showed that the bacterial cellulose was not toxic to the lymphocytes when comparing the growth viability of lymphocytes in the treated samples with bacterial cellulose compared with the positive and negative control after 24 hours at 37°C, also there was no effect to bacterial cellulose on the shape of lymphocytes in treated samples compared to the control samples under the same above conditions. Table 1 shows that the shape of the lymphocytes was normal for all the studied treatments, and the differences between the numbers of lymphocytes, whether among the treatments or in the control samples, were few, which indicates the absence of a toxic effect of bacterial cellulose.

Table 2 indicates the results of the study of the effect of bacterial cellulose dressings on skin irritation after 12, 24, and 48 hours of treatment, where it was found that there was no erythema, edema, redness, and dryness in the skin at treated areas with bacterial cellulose dressings and for all periods studied, these results indicate that the bacterial cellulose doesn't have toxicity toward the skin.

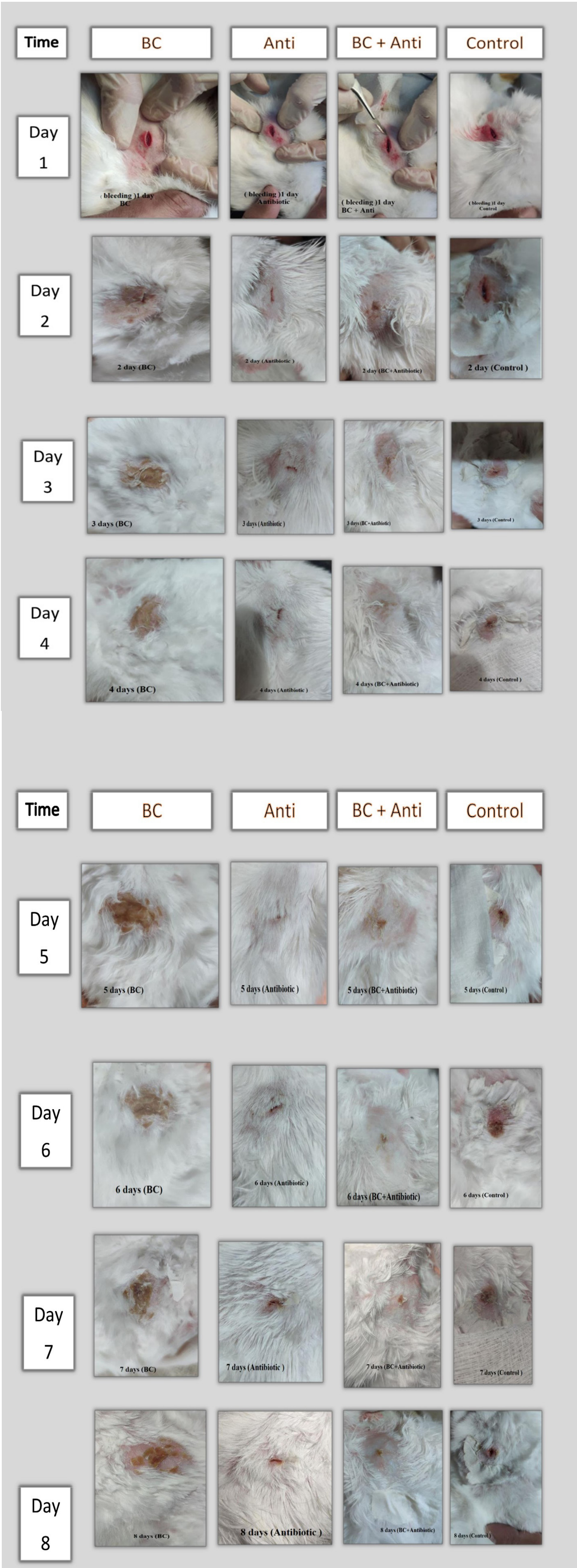
Effect of Bacterial Cellulose on Wounds Healing

The results and observations that were recorded daily on the skin surface characteristics in animals to detect the visible changes that determine the irritation scores in wounds areas and thus determine the recovery of the wounds in test and control animals, where the observations recorded from the second day of the experiment indicate that the wound areas in the three test groups were less red and hard compared to the control group and that these differences became greater with the progression of the experiment.

Figure 2 shows that the animal groups treated with BC dressings and those treated with BC + anti dressings showed a higher ability to heal wounds with slight visible changes in the wound areas compared with the animal group treated with anti dressings or with a control group. Also, it was noted that the wounds of animals treated with BC dressings, which were treated with BC + anti dressings were completely healed after 10 days, while complete recovery occurred for animals group treated with anti-dressings after 12 days, while the presence of wounds in the animals of control group continued until the 15th day of the experiment. According to the aforementioned observations, bacterial cellulose produced from local isolate *K. xylinus* TELE8 is a vital material not toxic and can be used to heal wounds and improve skin surface characteristics.

DISCUSSION

The fast expansion of important bio-based substance manufacturing and development has sparked a major interest in exploring cellulose as a significant natural supply for many uses, particularly its use in medicines. Cellulose has long been recognized for its distinctive characteristics, including biocompatibility, wettability, and nontoxicity, as well as its application in antimicrobial and hemostatic compounds[17]. Human skin covers the entire outer layer and serves as a barrier between the human body's internal environment and exogenous pathogenic microorganisms. Aside from these duties, the skin protects the body, aids in excretion, regulates temperature, and aids in sensory awareness of the environment. It also serves as a foundation system for blood vessels and nerves. Wound healing is a multi-stage process, of which the first stage is hemostasis, which originates at the location of blood leakage in the injury, is the initial response to damage[18].



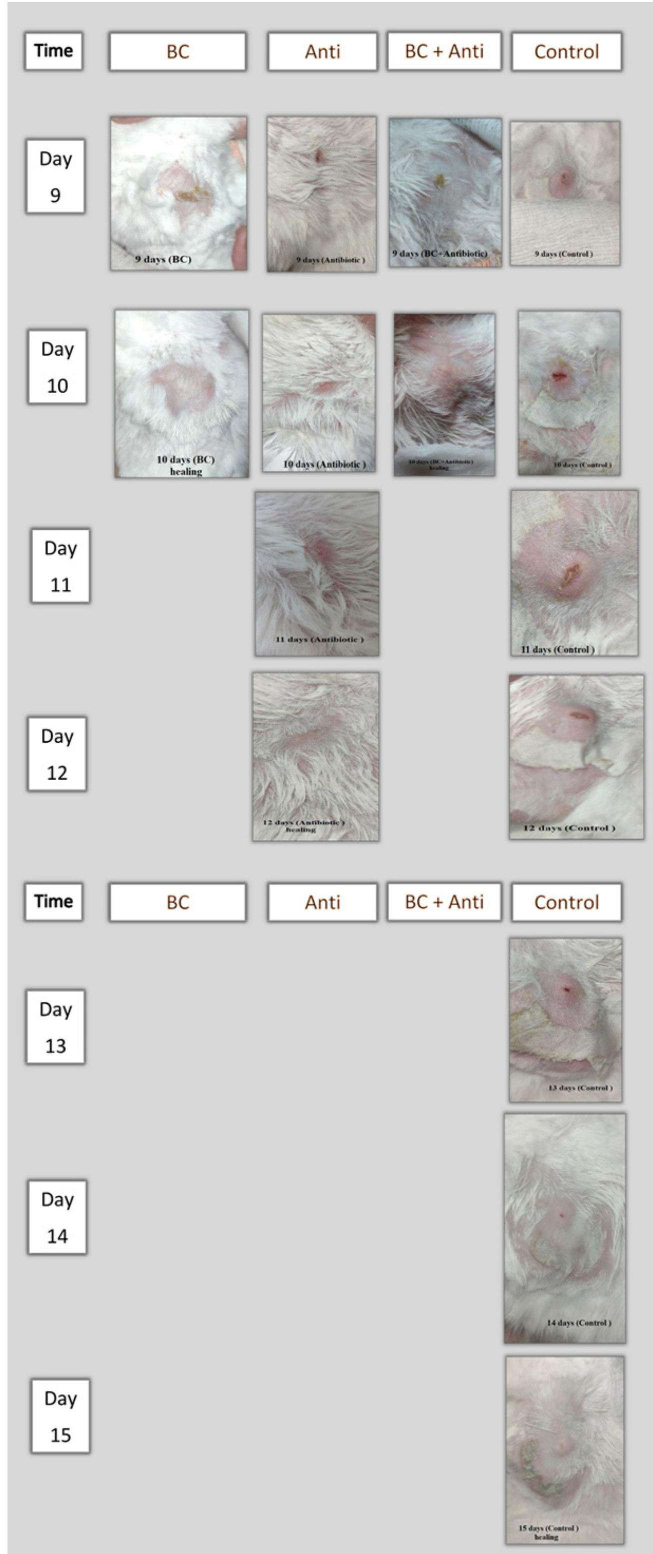


Figure 2: Healing of skin wounds induced in rabbits. BC group: treatment with bacterial cellulose dressings, Anti group: treatment with antibacterial dressings, BC+ Anti group: treatment with BC+ Anti dressings, and control group: without treatment.

The present study reveals high antimicrobial activity in preventing infection and promoting excellent wound healing. To the best of our knowledge, our study may be of the few studies that did not use bacterial cellulose as a solo-antimicrobial agent without the addition of any known antimicrobial agent. Fürsatz et al.[19] found that the use of ε-poly-l-lysine, an antimicrobial peptide, was effective in enhancing the effects of bacterial cellulose in wound healing care. Examples include vancomycin and ciprofloxacin being integrated into BC for wound dressing and skin repair. The preparation of antibiotic-containing BC composites using dried BC films was accomplished by soaking the films in various antibiotic solution concentrations. In addition, the mixtures have demonstrated antimicrobial action towards *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae*, and *Staphylococcus aureus*, making them suitable for utilization as wound dressings.

Furthermore, it was shown that the bilayer BC film produced from several carbon sources (sugarcane molasses, syrup, and fructose) could hold and gradually discharge the antibacterial medication ceftriaxone into the target tissues.[20,22] However, our work focused on using bacterial cellulose alone without any other substance and gave higher actions in wound healing than that presented by the use of both bacterial cellulose and antibiotic. Chitosan, gelatin, hyaluronic acid, and other biomass substances are often used in wound dressings. Despite the fact that these compounds have outstanding biocompatibility, degradability, and mechanical capabilities, bacterial cellulose has a distinct benefit when it comes to wound dressings. Because of its excellent tensile capacity, outstanding flexibility, powerful water-holding potential, substantial porosity to gas and liquid, and excellent friendliness with body tissues, BC is an ideal tissue repair product.[23,25]

CONCLUSION

Bacterial cellulose may provide a promising effect in enhancing wound healing and preventing wound infections that delay wound recovery. According to the current study results, this product can be used alone or in combination with other materials, such as antibiotics, in wound dressing to protect wounds from infections and provide better healing environment.

REFERENCES

1. Marrot L. Pollution and Sun Exposure: A Deleterious Synergy. Mechanisms and Opportunities for Skin Protection. Curr Med Chem [Internet]. 2018 Nov 20 [cited 2021 Dec 26];25(40):5469–86. Available from: https://pubmed.ncbi.nlm.nih.gov/28925870/
2. Whittam AJ, Maan ZN, Duscher D, Wong VW, Barrera JA, Januszyk M, et al. Challenges and Opportunities in Drug Delivery for Wound Healing. Adv wound care [Internet]. 2016 Feb 1 [cited 2021 Dec 26];5(2):79–88. Available from: https://pubmed.ncbi.nlm.nih.gov/26862465/
3. Phillips CJ, Humphreys I, Fletcher J, Harding K, Chamberlain G, Macey S. Estimating the costs associated with the management of patients with chronic wounds using linked routine data. Int Wound J [Internet]. 2016 [cited 2021 Dec 26];13(6):1193–7. Available from: https://pubmed.ncbi.nlm.nih.gov/25818405/
4. Nussbaum SR, Carter MJ, Fife CE, DaVanzo J, Haught R, Nusgart M, et al. An Economic Evaluation of the Impact, Cost, and Medicare Policy Implications of Chronic Nonhealing Wounds. Value Health [Internet]. 2018 Jan 1 [cited 2021 Dec 26];21(1):27–32. Available from: https://pubmed.ncbi.nlm.nih.gov/29304937/
5. Frykberg RG, Banks J. Challenges in the Treatment of Chronic Wounds. Adv wound care [Internet]. 2015 Sep [cited 2021 Dec 26];4(9):560–82. Available from: https://pubmed.ncbi.nlm.nih.gov/26339534/
6. Pop MA, Almquist BD. Biomaterials: A potential pathway to healing chronic wounds? Exp Dermatol [Internet]. 2017 Sep 1 [cited 2021 Dec 26];26(9):760–3. Available from: https://pubmed.ncbi.nlm.nih.gov/28094868/
7. Lindley LE, Stojadinovic O, Pastar I, Tomic-Canic M. Biology and Biomarkers for Wound Healing. Plast Reconstr Surg [Internet]. 2016 [cited 2021 Dec 26];138(3 Suppl):18S-28S. Available from: https://pubmed.ncbi.nlm.nih.gov/27556760/
8. Han G, Ceilley R. Chronic Wound Healing: A Review of Current Management and Treatments. Adv Ther [Internet]. 2017 Mar 1 [cited 2021 Dec 26];34(3):599–610. Available from: https://pubmed.ncbi.nlm.nih.gov/28108895/
9. Altoé LS, Alves RS, Sarandy MM, Morais-Santos M, Novaes RD, Gonçalves RV. Does antibiotic use accelerate or retard cutaneous repair? A systematic review in animal models. PLoS One [Internet]. 2019 Oct 1 [cited 2021 Dec 26];14(10):e0223511. Available from: /pmc/articles/PMC6786583/
10. Hirsch T, Koerber A, Jacobsen F, Dissemond J, Steinau HU, Gatermann S, et al. Evaluation of toxic side effects of clinically used skin antiseptics *in-vitro*. J Surg Res [Internet]. 2010 Dec [cited 2021 Dec 26];164(2):344–50. Available from: https://pubmed.ncbi.nlm.nih.gov/19726054/
11. Dreifke MB, Jayasuriya AA, Jayasuriya AC. Current wound healing procedures and potential care. Mater Sci Eng C Mater Biol Appl [Internet]. 2015 Mar 1 [cited 2021 Dec 26];48(3):651–62. Available from: https://pubmed.ncbi.nlm.nih.gov/25579968/
12. Wang J, Zhao L, Zhang A, Huang Y, Tavakoli J, Tang Y. Novel bacterial cellulose/gelatin hydrogels as 3D scaffolds for tumor cell culture. Polymers. 2018 May 24;10(6):581.
13. Boyle W, Chow A. Isolation of human lymphocytes by a Ficoll barrier method. Transfusion. 1969 May 6;9(3):151-5.
14. Dourado F, Gama M, Rodrigues AC. A review on the toxicology and dietetic role of bacterial cellulose. Toxicology reports. 2017 Jan 1;4:543-53.
15. Qiu Y, Qiu L, Cui J, Wei Q. Bacterial cellulose and bacterial cellulose-vaccarin membranes for wound healing. Materials Science and Engineering: C. 2016 Feb 1;59:303-9.
16. Rezvani Ghomi E, Khalili S, Nouri Khorasani S, Esmaeely Neisiany R, Ramakrishna S. Wound dressings: Current advances and future directions. Journal of Applied Polymer Science. 2019 Jul 15;136(27):47738.
17. Naomi R, Idrus RBH, Fauzi MB. Plant- vs. Bacterial-Derived Cellulose for Wound Healing: A Review. Int J Environ Res Public Health [Internet]. 2020 Sep 1 [cited 2022 Jan 12];17(18):1–25. Available from: /pmc/articles/PMC7559319/
18. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. Nat Rev Microbiol [Internet]. 2018 Feb 12 [cited 2022 Jan 12];16(3):143–55. Available from: https://pubmed.ncbi.nlm.nih.gov/29332945/
19. Fürsatz M, Skog M, Sivlér P, Palm E, Aronsson C, Skallberg A, et al. Functionalization of bacterial cellulose wound dressings

with the antimicrobial peptide ε-poly-L-Lysine. Biomed Mater [Internet]. 2018 Jan 30 [cited 2022 Jan 12];13(2):025014. Available from: https://pubmed.ncbi.nlm.nih.gov/29047451/

20. Vismara E, Bernardi A, Bongio C, Farè S, Pappalardo S, Serafini A, et al. Bacterial Nanocellulose and Its Surface Modification by Glycidyl Methacrylate and Ethylene Glycol Dimethacrylate. Incorporation of Vancomycin and Ciprofloxacin. Nanomater (Basel, Switzerland) [Internet]. 2019 Dec 1 [cited 2022 Jan 12];9(12):1668. Available from: https://pubmed.ncbi.nlm.nih.gov/31766754/
21. Volova TG, Shumilova AA, Shidlovskiy IP, Nikolaeva ED, Sukovatiy AG, Vasiliev AD, et al. Antibacterial properties of films of cellulose composites with silver nanoparticles and antibiotics. Polym Test. 2018 Feb 1;65(2):54–68.
22. Zheng L, Li S, Luo J, Wang X. Latest Advances on Bacterial Cellulose-Based Antibacterial Materials as Wound Dressings. Front Bioeng Biotechnol [Internet]. 2020 Nov 23 [cited 2022 Jan 12];8(11):593768. Available from: /pmc/articles/PMC7732461/
23. Sulaeva I, Henniges U, Rosenau T, Potthast A. Bacterial cellulose as a material for wound treatment: Properties and modifications. A review. Biotechnol Adv [Internet]. 2015 [cited 2022 Jan 12];33(8):1547–71. Available from: https://pubmed.ncbi.nlm.nih.gov/26253857/
24. İnal M, Mülazımoğlu G. Production and characterization of bactericidal wound dressing material based on gelatin nanofiber. Int J Biol Macromol [Internet]. 2019 Sep 15 [cited 2022 Jan 12];137(9):392–404. Available from: https://pubmed.ncbi.nlm.nih.gov/31233795/
25. Graça MFP, Miguel SP, Cabral CSD, Correia IJ. Hyaluronic acid-Based wound dressings: A review. Carbohydr Polym [Internet]. 2020 Aug 1 [cited 2022 Jan 12];241(8):116364. Available from: https://pubmed.ncbi.nlm.nih.gov/32507198/