The Effectiveness of Chitosan as an Antimicrobial on Bacterial Cellulose-based Scaffold Skin Tissue Engineering

Khatarina Meldawati Pasaribu[1], Saharman Gea[2*], Safruddin Ilyas[3] and Tamrin[2]
[1]*Postgraduate Chemistry Study Program, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jl. Bioteknologi No. 1 Kampus USU, Medan, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia*
[3]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia*

Keywords: Bacterial Cellulose, Collagen, Chitosan, Antimicrobial, Composite.

Abstract: It is recognized that bacterial cellulose (BC) is used as a scaffold for tissue engineering. However, pristine BC is not ideal enough to be applied as a scaffold because bacterial cellulose does not have antimicrobial activity. The aim of this study was to evaluate the antmicrobial activity of bacterial cellulose and their composites. BC gel, produced by Acetobacter xylinum with HS medium as a carbohydrate resources, was immersed into chitosan (Ch) and collagen (Co) by ex-situ approach to produce BC/Ch/Col. The same procedures were repeated for BC/Ch, BC/Col, and BC/Col/Ch. The effectiveness of antimicrobial activity was carried out using disk paper to inhibit the growth of pathogen bacteria such as Escherichia coli and Staphylococcus aureus. The results showed that BC/Ch has the highest antimicrobial activity against E. coli and S. aureus with the inhibition zone of 10.15 mm and 7.9 mm, respectively.

1 INTRODUCTION

Cellulose is the most abundant biopolymer on earth and has been used for a broad range of implementations, such as filtration, food, medicine, healthcare, and cosmetics due to its low-cost, low-toxicity, hydrophilicity, biocompatibility, and flexibility. Since cellulose is so commonly used in biomedical fields and food packaging, it is appropriate to address its activity against pathogenic bacteria (Tsai et al., 2017). Unlike cellulose that is isolated from the plant, BC is more interested in studying because it is free of other polymers. In addition to being used as an ideal matrix for medical devices, it can be dried using freeze drying to mould it into three-dimensional structures. Its construct can make BC capable of retaining high water levels, mechanically resistant and biocompatible. BC has a nanofibrillary structure that supports cell regeneration either as an assistance in the healing of skin lesions 3 or in tissue engineering (Ataide et al., 2017).

The BC fibrous woven is made of three-dimensional nanofibres that are well-arranged, resulting in high surface area and porosity hydrogels (Esa, Tasirin and Rahman, 2014). Acetobacter xylinum is regarded as the most researched starter and the most effective bacteria as a BC producer that capable to assimilating different sugars and producing elevated cellulose levels in culture medium (Esa, Tasirin and Rahman, 2014).

Previous study shows BC can be used as a scaffolding for the growth of cells such as skin fibroblast, ligament, cartilage and others that do not contain blood vessels by in-vitro (Gea et al., 2018). BC has been demonstrated to be biocompatible with living tissues. Bacterial cellulose has high hydrophilic characteristics and never dries, which is the required property, as it has been shown that when the wound is constantly moisturized, wounds cure better and quicker (Kucińska-Lipka, Carayon and Janik, 2015).

Tissue engineering devices, mainly used as implantable scaffolding, is usually made from biomaterials with distinct structures and characteristics. To this end, many biomaterials – both synthetic and naturally occurring – have been used in tissue engineering (TE) applications, where extra scaffolding material changes such as anchoring of biologically active entities are generally needed. Materials such as cellulose, chitosan, hyaluronic acid and collagen have recently drawn considerable

Pasaribu, K., Gea, S., Ilyas, S. and Tamrin, .
The Effectiveness of Chitosan as an Antimicrobial on Bacterial Cellulose-based Scaffold Skin Tissue Engineering.
DOI: 10.5220/0008864701780181
In *Proceedings of the 1st International Conference on Chemical Science and Technology Innovation (ICOCSTI 2019)*, pages 178-181
ISBN: 978-989-758-415-2

interest as prospective materials for TE applications. (Ozdil and Aydin, 2014). Due to its outstanding biocompatibility and distinctive physiochemical characteristics, bacterial cellulose (BC) has appeared as a promising biomaterial for tissue engineering applications.

To this end, ex-situ approach for BC production has been the focus of much research as this approach can further improve BC characteristics in order to address the precise requirements for TE applications. While there has been a current trend in the expansion of BC implementations in TE, BC major TE implementations remain in wound dressing and bone regeneration. However, this is likely to be a chance, as the full potential of BC as a biomaterial and in TE is continually being explored (Stumpf et al., 2018). In this study, the BC is reinforced by collagen and chitosan to enhance its antimicrobial activities which can be applied for scaffold material in skin tissue engineering.

2 METHODS

2.1 Bc Preparation

Acetobacter xylinum as a sort of bacterial strain used to synthesize BC, was acquired from the Material and Polymer Postgraduate Labolatorium of Universitas Sumatera Utara, Indonesia. This aerobic gram-negative bacteria actively fermented at pH 4.5 and temperature between 25 and 30˚C using carbohydrate as carbon resources (Esa, Tasirin and Rahman, 2014). BC production in HS medium containing glucose (20 g/L), peptone (5 g/L), yeast extract (5 g/L), citric acid (1.15 g/L), and disodium hydrogen phosphate (2.7 g/L). The culture medium pH then was adjusted to 4.5 by using CH3COOH glacial. The culture medium was then autoclaved to remove potential contaminants and then permitted to reach room temperature. The Acetobacter xylinum starter was then put and inoculated for 7 days inside the incubator in a static culture medium at 28ºC. The cellulose fiber would be sythesized by Acetobacter xylinum during this inoculated period. After that, the gels obtained was immersed in NaOH (2.5 g/L) for 24 h to purified the BC from the bacteria and culture medium. The BC gel then bleached overnight in NaOCl (2.5 mL/L). Finally, the BC gel was washed by using aquadest until the BC reach neutral pH.

2.2 Production of BC/Ch Composites

The purified BC gel were immersed in 20 mL aqueous of chitosan (0.2 g/L) in acetic acid solution 1% for 24 h. Then they were dried in freeze dryer for 24 h. The final composite film have been marked as BC/Ch.

2.3 Production of BC/Col Composites

The purified BC gel were immersed in 20 mL aqueous of colagen (0.2 g/L) for 24 h. Then they were dried in freeze dryer for 24 h. The final composite film have been marked as BC/Col.

2.4 Production of BC/Ch/Col Composite

The purified BC gel were immersed in 20 mL aqueous of chitosan (0.2 g/L) in acetic acid solution 1% for 24 h. BC/Ch composite then immersed in 20 mL aqueous of colagen (0.2 g/L) for 24 h. Then they were dried in freeze dryer for 24 h. The final composite film have been marked as BC/Ch/Col.

2.5 Production of BC/Col/Ch Composite

The purified BC gel were immersed in 20 mL aqueous of colagen (0.2 g/L) for 24 h. BC/Ch composite then immersed in 20 mL aqueous of chitosan (0.2 g/L) in acetic acid solution 1% for 24 h. Then they were dried in freeze dryer for 24 h. The final composite film have been marked as BC/Col/Ch.

2.6 Antimicrobial Activity

The antimicrobial activity of BC, BC/Ch, BC/Col, BC/Ch/Col, BC/Col/Ch composites were assessed using a technique of disk diffusion against pathogenic bacteria such as Escherichia coli and Staphylococcus aureus. The technique of disk diffusion was conducted on medium nutrient agar in petri dish. All the samples tested were then shaped into a 10 mm diameter disk and sterilized for 5 min on each side using a low-power UV lamp. Then, the disks were placed on the agar plate inoculated. The plates were then placed in an incubator of 37 ° C for 24 hours. The efficacy of the inhibitory action of the evaluated samples on againts the bacterial was determined by measuring the inhibition zone diameter.

3 RESULT AND DISCUSSION

Medium culture methods on producing BC would determine the macrostructure morphology of BC. Static culture medium would produce a pristine solid woven fiber gelatinous cellulose film that formed on the interface of the medium. In agitation culture medium, cellulose is synthesized spread in medium. BC gels that produced by agitation medium culture was usually formed as of fibrous suspensions, pellets and have ir-regular masses. In this work the BC gels, that would use to made composites was produced in static culture medium conditions in HS medium for 7 days. After 7 days BC gels that formed on the surface of the medium a BC gel film was treated with NaOH and NaOCl. The treated BC gel film would form as colorless and transparent gel as showed at figure 1.

Figure 1: BC gel film.

In this study, the disk diffusion method was used to evaluate the antimicrobial acitivity of the tested samples, which is known as one of the popular methods of antimicrobial activity. Since the inhibition zone can be measured directly. Antimicrobial properties of the sampels have been observed to inhibit the growth of Escherichia coli and Staphylococcus aureus.

Prepared composites were placed on the surface of the Escherichia coli and Staphylococcus aureus bacteria lawn. The efficiency of antimicrobial activity of BC, BC/Ch, BC/Col, BC/Ch/Col, BC/Ch/Col composites was evaluated by measured the size of appeared clear zones of inhibition around the samples after 24 h of exposure.

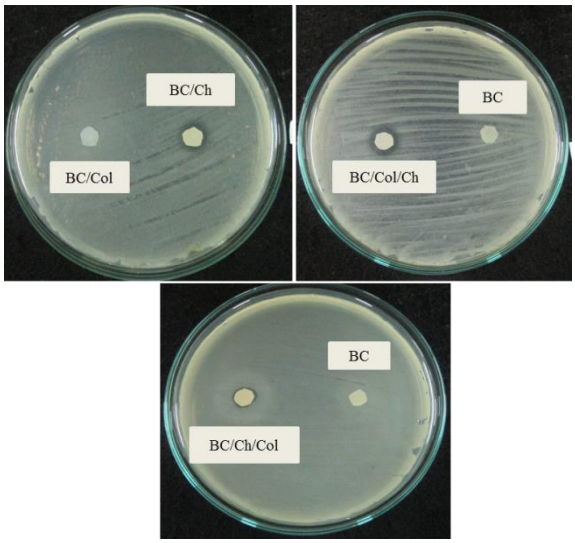

Figure 2: The inhibition zones of tested samples against pathogen bacteria Escherichia coli.

Pure BC is known to be a natural scaffolding material because it can be a suitable cell growth environment due to higher water retention that prevents tissue dehydration and cell death, resulting in faster skin repairs. But pure BC shows no antimicrobial activity to prevent infection in the affected area. In our case, pure BC as a control sample showed no antimicrobial activity where no inhibition zone was present in Figure 2.

After 24 h exposure diameters of inhibition zones measured as 10.15 mm, 6.4 mm, 7.9 mm and 8.8 mm for BC/Ch, BC/Col, BC/Ch/Col, BC/Co/Ch respectively. The best antibacterial activities are carried out by BC / Ch. The findings acquired showed that composite BC / Ch has great antimicrobial activity against Escherichia coli. This result also indicated that BC/Ch have better antimicrobial activities rather than Ag/BC, where showed bacterial colony-forming clear zone about 6.5 mm, which is in this range, it can be said that its good enough as an antimicrobial substrate (Pal et al., 2017).

This finding was also supported by another study that showed that the composite of BC/AgNPs displayed clear inhibition areas against both model bacteria tested (i.e. 2 mm for E. coli. and 9 mm for S. aureus), while no inhibition area for pristine BC was reported (Stumpf et al., 2018).

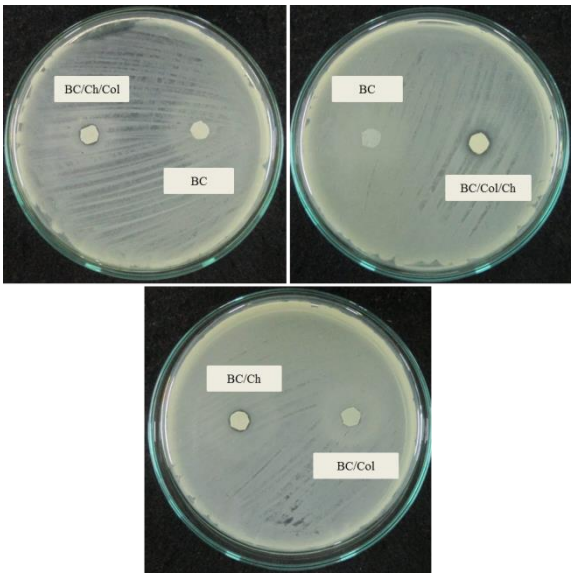

Figure 3: The inhibition zones of tested samples against pathogen bacteria Staphylococcus aureus.

Chitosan has been previously reported have antimicrobial effects against some pathogens such as E. coli and S. aureus. The polymers in BC films have been reported to be susceptible to colonization by bacteria. BC alone does not have antimicrobial activity, but the incorporation of chitosan into the BC films could prevent bacterial adhesion. After 24 h exposure diameters of inhibition zones measured as 7.9 mm, 6.5 mm, 7.1 mm and 7.4 mm for BC/Ch, BC/Col, BC/Ch/Col, BC/Co/Ch respectively.

4 CONCLUSIONS

In summary, bacterial cellulose based biocomposites for scaffold material had been prepared and the antimicrobial activity had been investigated. The results showed that BC/Ch has the highest antimicrobial activity against E. coli and S. aureus with the inhibition zone of 10.15 mm and 7.9 mm, respectively. As it have antimicrobial activity and did not contain hazardous chemical contamination, it can be concluded that the BC, BC/Ch, BC/Ch/Co and BC/Co/Ch was the potential scaffold material for skin tissue engineering.

ACKNOWLEDGEMENTS

This work is completely financed under PMDSU 2019 research scheme by Ministry of Research, Technology, and Higher Education (KEMENRISTEK DIKTI), Republic of Indonesia.

REFERENCES

Ataide, J. A. et al. (2017) ‘Bacterial Nanocellulose Loaded with Bromelain: Assessment of Antimicrobial, Antioxidant and Physical-Chemical Properties’, Scientific Reports, 7(1), pp. 2–10. doi: 10.1038/s41598-017-18271-4.

Esa, F., Tasirin, S. M. and Rahman, N. A. (2014) ‘Overview of Bacterial Cellulose Production and Application’, Agriculture and Agricultural Science Procedia. Elsevier Srl, 2, pp. 113–119. doi: 10.1016/j.aaspro.2014.11.017.

Gea, S. et al. (2018) ‘Enhancing the quality of nata de coco starter by channeling the oxygen into the bioreactor through agitation method’, in AIP Conference Proceedings. doi: 10.1063/1.5082469.

Kucińska-Lipka, J., Carayon, I. and Janik, H. (2015) ‘Bacterial cellulose in the field of wound healing and regenerative medicine of skin: recent trends and future prospectives’, POLYMER BULLETIN, 72(9), pp. 2399–2419.

Ozdil, D. and Aydin, H. M. (2014) ‘Polymers for medical and tissue engineering applications’, Journal of Chemical Technology and Biotechnology, 89(12), pp. 1793–1810. doi: 10.1002/jctb.4505.

Pal, S. et al. (2017) ‘Silver-Functionalized Bacterial Cellulose as Antibacterial Membrane for Wound-Healing Applications’, ACS Omega, 2(7), pp. 3632–3639. doi: 10.1021/acsomega.7b00442.

Stumpf, T. R. et al. (2018) ‘In situ and ex situ modifications of bacterial cellulose for applications in tissue engineering’, Materials Science and Engineering C. Elsevier B.V, 82, pp. 372–383. doi: 10.1016/j.msec.2016.11.121.

Tsai, T. T. et al. (2017) ‘Antibacterial cellulose paper made with silver-coated gold nanoparticles’, Scientific Reports. Springer US, 7(1), pp. 1–10. doi: 10.1038/s41598-017-03357-w.