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# Fabricated Bacterial Cellulose-Based Composites for Skin Tissue Engineering

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**Abstract:** Bacterial Cellulose (BC) has the ability as tissue scaffolding, since, this material has excellence properties including high water holding capacity, high crystallinity as well as elasticity. This research focused on investigating the fabrication of bacterial cellulose-based compisites for skin tissue engineering. BC was produced via agitated culture medium while its adding with chitosan and collagen were obtained through ex-situ method which was done by immersing BC pellicles to chitosan or collagen solution for 24 h. Based on the Thermal Gravimetric Analysis (TGA), the scaffold material BC-Chitosan-Collagen gave a rising value of temperature which was 35.43°C for 1% mass loss, implying the improvement of thermal properties of materials. From Differential Thermal Analysis (DTGA), it is recorded that $T_{max}$ of BC-collagen is 330°C. Scanning Electron Microscope (SEM) shows that the presence of chitosan and collagen into BC fibers resulted in a well-interconnected network structure. These results imply that BC-based composites with collagen or chitosan can serve for skin tissue engineering.

**Key words:** BC, chitosan, collagen, composite, ex-situ

## INTRODUCTION

Skin-burnt damages irreplaceable skins regeneration due to random injuries. Medical treatments have been applied extensively, however such autografts, allografts, xenografts and amnion treatments were not capable to substitute the skins (Alexis *et al*., 2017). These drawbacks occur due to the limited skin areas that need to be replaced, donor incompatibility, low antibacterial properties which emerges some difficulties in regenerating the skins cartilage (Picheth *et al*., 2017). An alternative method is required to overcome this injury and one of it is engineered tissue scaffolding (Sheikh *et al*., 2015). An ideal scaffolding material comprises average pores between 100-200 μm, high mechanical properties as well as high biocompatibilities and biodegradabilities (Akter, 2016).

One of the materials that meet the explained criteria is bacterial cellulose. The advantages of bacterial cellulose are its biocompatibility and functionality, served as a non-toxic material, its capability to absorb water having a high degree of crystallinity having good mechanical and thermal properties and its characteristic to be easily sterilized (Loh *et al*., 2018; Shukla *et al*., 2015). As known, bacterial cellulose is widely used as membrane filtration, wound dressing, scaffolding and artificial skin-making materials (Nandgaonkar, 2014). Pristine bacterial cellulose, however is not ideal enough to be used as scaffold application because of the tight surface of the fiber making it difficult for cells to enter through the fiber (Lv *et al*., 2015). Based on that, bacterial cellulose needs to be modified such as making bacterial cellulose in its biocomposite form.

Regarding the needs of fabricating bacterial cellulose-based scaffold, two of the compatible materials are chitosan and collagen. It is known that collagen served as a promising material to be composed of bacterial cellulose because of its excellent biocompatibility. A composite of BC/collagen (BC/Col) was proved to have a preferable porous structure, thus, providing better nutrients transport and metabolic wastes. Besides, the porous structure served as channels for the growth of cell and tissue (Noh *et al*., 2019). Chitosan also served as a preferable material to be employed in engineering applications of skin tissue because of its superiority in wound healing such as hemostasis, accelerating tissue regeneration and collagen synthesis fibroblasts (Ma *et al*., 2001). A study by Cai *et al*. found that the composite of Bacterial Cellulose/Chitosan (BC/Ch) resulted in a well-interconnected porous network structure and improved thermal stability (Zhijiang *et al*., 2011).

Based on the above explanation, the research of fabricating bacterial cellulose-based scaffolf materials with chitosan and collagen needs to be studied further. This study presents the investigation of BC-chitosan, BC-collagen, BC-chitosan-collagen and BC-collagen-chitosan scaffold materials. To our best knowledge, this is the first study of the later materials, providing the latest

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research of the use of bacterial cellulose for skin tissue engineering. The method used to yield bacterial cellulose was static culture because it results in bacterial cellulose with high Young's modulus and tensile strength (Krystynowicz *et al*., 2002), high Degree of Polymerization (DP) and crystallinity, compared to bacterial cellulose produced by agitation culture (Watanabe *et al*., 1998). In the formation of bacterial cellulose-based composites with collagen or chitosan, the technique used was ex-situ approach. The resulted composite was then characterized by Thermal Gravimetric Analysis (TGA), Differential Thermal Analysis (DTGA) and Scanning Electron Microscope (SEM).

## MATERIALS AND METHODS

All materials used in this research are purely graded. Coconut water was purchased from traditional market, glacial acetic acid $(CH_3COOH)_{(l)}$, ethanol, sodium hydroxide $(NaOH)_{(s)}$ and sodium hypochlorite $(NaOCl)_{(l)}$, glucose, urea were purchased from Sigma Aldrich. *Acetobacter xylinum* starter as bacterial strain was obtained from material and polymer laboratory of Universitas Sumatera Utara. Lastly, chitosan was purchased from PUI Chitosan and Advanced Material, Universitas Sumatera Utara.

**The preparation of bacterial cellulose:** Aerobic gram-negative bacteria is actively fermented at pH 4.5 and at a temperature between 25 and 30ºC using carbohydrate as carbon resources (Esa *et al*., 2014). BC productionwas performed in coconut water medium, containing glucose (20 g/L) andurea (5 g/L). The culture medium pH was adjusted to 4.5 by involving glacial acetic acid (1 ml/L). The culture medium was then sterilized in autoclave to eliminate possible contaminants and then allowed to reach room temperature. *Acetobacter xylinum* starter was added and inoculated in incubator at 28ºC for 7 days in static culture medium. During this inoculated time, cellulose fiberwas secreted by *Acetobacter xylinum* in the form of gel. The gel obtained was immersed in 2.5% NaOH for 24 h to purify the BC from the bacteria and culture medium. The BC gel was then bleached overnight in NaOCl. Finally, the BC gel was washed by using distilled water until the neutral pH was reached.

**Preparation of BC-chitosan:** The amount of purified BC gel was immersed in 20 mL of aqueous chitosan (0.2 g/L) in 1% acetic acid solution for 24 h. Then, the immersed samplewas dried in freeze dryer for 24 h. The final sample was labeled as BC-chitosan.

**The preparation of BC-collagen:** The amount of purified BC gel was collected to be immersed inside of 20 mL aqueous of collagen (0.2 g/L) for 24 h. Then immersed BC-collagen gel was dried in freeze dryer to remove the water content for 24 h. The final samples were labeled as BC-collagen.

**Characterization:** All samples were investigated with Thermal Gravimetric Analysis (TGA) and Scanning Electron Microscope (SEM) to analyze thermal properties and morphological surface of samples, respectively.

## RESULTS AND DISCUSSION

By adding chitosan and collagen into bacterial cellulose, a high thermal stability composite was resulted. The TGA curves indicate that the thermal stability of composites is higher than that of bacterial cellulose and collagen. Based on the curves, evaporation temperature of water and the loss of organic compounds correspondents from 50-110°C while a decomposition of carbonic-based compounds are indicated between 100-350°C. Subsequently, carbonization process of samples takes place in between 350-600°C.

The addition of chitosan and collagen is expected to improve the physical characteristic. As many as 1% mass loss of Bacterial Cellulose (BC) occurred at 31.80°C (Fig. 1), the composite BC-chitosan showed an increasing value of temperature which was 35.43°C for 1% mass loss. This implies to the improvement of thermal properties of material due to the presence of fillers. Based on the TGA analysis, the best thermal stability is demonstrated by BC-chitosan composite, suggesting highly physical interaction among the constituents.

Whilst, a half percentage of mass loss of samples occurred at for 331°C BC-chitosan, 316°C for BC-collagen, 400°C for bacterial cellulose. The maximum temperature at 612°C was applied to obtain the highest degree of degradation for every sample.

The thermal decomposition temperature of samples known as the maximum degradation weight loss rate ($T_{max}$) was determined from DTGA curves. Based on DTGA curve produced, it can be seen that the maximum degradation temperature of bacterial celulose is recorded at 324.6°C, followed by BC-chitosan, BC-collagen at temperature of 304.5°C, 330.4°C, respectively with chitosan possessing the lowest decomposition peak at 304°C (Fig. 2).

Figure 3 displays the typical SEM images of all samples. Particularly, Fig. 3a illustrates that fiberin BC is ranged commonly between 20-100 nm. BC was known to be able to interact with matrices/resins and bioactive components such as antimicrobials, regeneration enhancer or drugs to develop materials for medical devices and drug delivery systems (Albu *et al*., 2014). This advantage lead to the vast amount of research about employing BC as scaffold based material (Shukla *et al*., 2015).

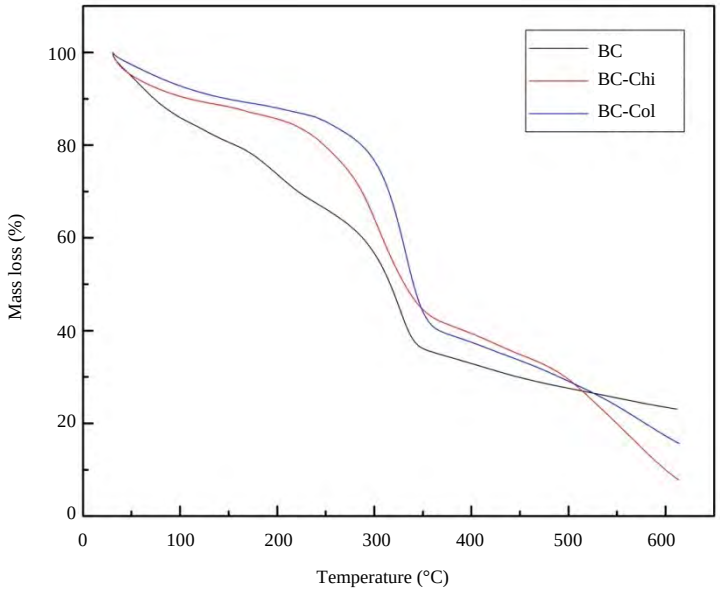


Fig. 1: TGA spectra of BC, BC-chitosan, BC-collagen

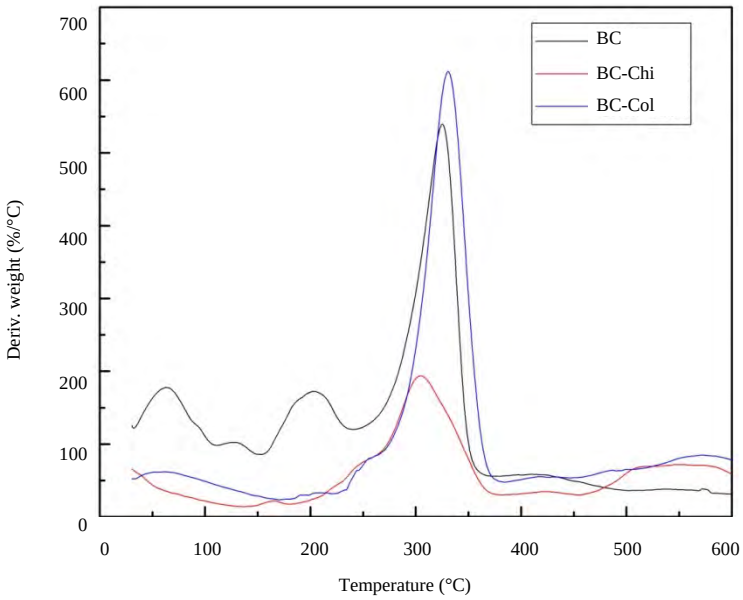


Fig. 2: DTGA curves of bacterial cellulose, BC-chitosan, BC-collagen

After being treated with chitosan (Fig. 3b), the surface morphology shows some changes. Moreover, the BC nano fibrils cannot be observed for the coverage by a thick layer of chitosan. This results the same porous structure but with bigger pore size. This is consistent to (Zhijiang *et al*., 2011) studied that show the cross sectional image. It can be observed that chitosan molecules can penetrate BC and forms layers of BC-chitosan scaffolding material. The BC nanofibrils can be observed between layers, noted that the present nanofibrous BC and BC-chitosan composite has well interconnected pore network structure and large surface area for cellular attachment and vascularization. In other words, when the BC-Chitosan was used as tissue engineering scaffolds, it can promote cellular ingrowths.

On the other hand, different results are observed on BC-collagen scaffold material. In Fig. 3c, the BC fiber can still be seen; this is because collagen does not cover the entire BC surface like chitosan. So that, the SEM image indicates that BC-chitosan and BC-collagen composites surface still shows BC fiber content.

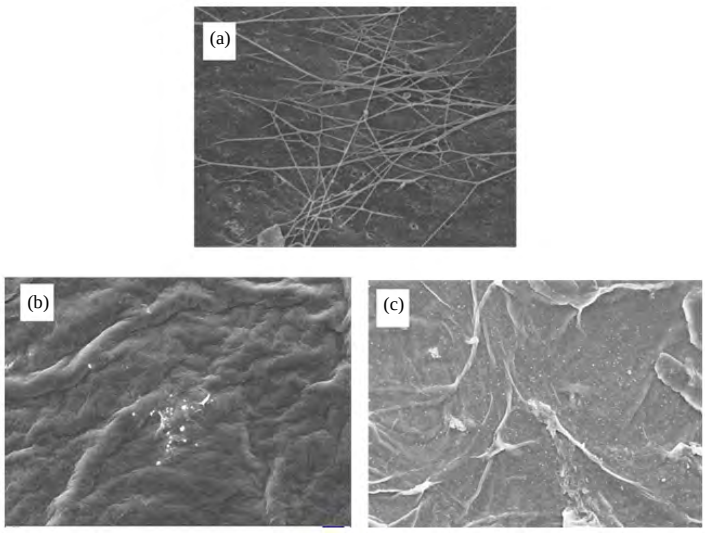


Fig. 3: SEM images of (a) Bacterial cellulose, (b) BC-chitosan and (c) BC-collagen

## CONCLUSION

BC-chitosan, BC-collagen composites have been successfully prepared by immersing wet BC pellicle into chitosan solution or collagen solution. SEM images show that both chitosan and collagen molecules can penetrate into BC forming multilayer structure of composites, giving well-interconnected porous network structure which is a crucial issue for cells to be grown. The thermal stability also shows certain improvement when BC adding by chitosan and collagen. Thus, the prepared composites have capacity to be applied for skin tissue engineering.

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