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Enhancement of bacterial cellulose rehydration via BTCA cross-linking

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Low rehydration ability of bacterial cellulose (BC) after its first drying is an unfavourable effect especially for biomedical applications. In this study, it was aimed to overcome this defect through BC cross-linking. So, 1,2,3,4-butanetetracarboxylic acid (BTCA), as a common cross-linker of cotton, was used in different concentrations (5, 10 and 20w/v %) for this purpose. BC samples were cross-linked after synthesis in their wet state. Cross-linking of BC didn't show any significant change in XRD patterns in compare with uncross-linked (raw) samples. Meanwhile, BET surface area testing showed that the surface volume of the cross-linked sample with BTCA 20% has increased 12 times more than uncross-linked BC. Surface and cross section FESEM images showed also thepreserved structure of cross-linked BC. In addition, water absorption of cross-linked sample (BTCA 20%) was about 300% more than uncross-linked sample. Comparing them indicated that cross-linked sample had more water swelling rate and lower water release rate and finally it was concluded that cross-linked BC show a high potent for medical applications.

Keywords: Bacterial cellulose (BC), rehydration, 1,2,3,4-Butanetetracarboxylic acid (BTCA), cross-linking, porosity

INTRODUCTION

Bacterial cellulose (BC), synthesized by some bacteria, is a significant biopolymer which discovered two centuries ago but only in last decades becomes an attractive natural nanomaterial in academic research and industrial application [1-4]. BC is composed of several millions β-1,4-glucopyranosyl units which made at first subfibrils, then fibrils and finally nanoribons. The width of nanoribons are between 40-70 nm which they formed nanoporous 3D network pellicle on the surface of static culture medium [5-10]. Due to simple purification and modification, BC is an interesting material for biomedical purpose [11].

During synthesis the empty spaces between nanoribbons would be occupied with water molecules, bacteria and media components which provide a nanofibrous hydrogel [12]. During drying process, trapped water inside BC hydrogel would be removed and a light and stiff film would be produced which has not good rehydration ability [13-16].

There are few efforts to overcome the above mentioned defect. Adding some hydrophilic and water soluble materials such as alginate, carboxymethyl cellulose and chitosan to culture medium or post synthesis treatments like producing interpenetrating polymer network with gelatin [17-24].

In this research, a novel method is proposed for preserving 3D structure of BC after drying. It is based on BC cross-linking with an appropriate cross linking agent. Here, 1,2,3,4-Butanetetracarboxylic acid (BTCA) as a common cellulose cross-linker was used. BTCA was used in textile, paper and wood industry to crosslink cellulosic materials and improved some properties like wrinkle recovery and reinforcement [25-30]. Therefore, it has high potential in preventing collapse of BC 3D structure. Synthesized samples were characterized with ATR-FTIR, XRD and FESEM. Their wettability, water swelling rate and water release rate were also determined. All discussion was based on comparing cross-linked and uncross linked samples and the effect of cross linker agent was also investigated.

MATERIAL AND METHOD

Culturing and purification

Acetobacter xylinum ssp. sucrofermentans BPR2001 (provided by Tarbiat Modares University) was introduced into Histrin-Scharmm (HS) medium containing 2 w/v% glucose, 0.5 w/v% peptone, 0.5 w/v% yeast extract and 0.27 w/v%, Na_2HPO_4 (All chemicals were from Sigma Aldrich, Germany). Culture medium was sterilized by autoclaving and the pellicles were prepared by static condition at 30°C and pH=5 in Erlenmeyer flask. After 7 days of cultivation, pellicles of BC were collected and purified by sodium hydroxide 0.1 N for 90 min at boiling temperature. After purification, pellicles were neutralized and rinsed with distilled water.

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Treatment of BC with BTCA

Base on a cross linking procedure for cotton fabric (As a cellulosic material), bacterial cellulose samples were cross-linked [31]. Specimens were immersed in different concentrations of BTCA (5, 10 and 20 W/V %) for 24 hours at 30°C. Sodium hypophosphate (SHP) was also added to BTCA solutions as catalyst (1:2 SHP to BTCA). After impregnation, samples were dried and cured at 180°C for formation of chemical bonds or cross-linking [25, 32]. Then, they were washed with distilled water for removing any chemical residual and left at room temperature for 24 hr. The chemical structure of specimens was investigated by ATR-FTIR (Perkin Elmer model Frontier; USA) spectroscopy. The spectra were recorded with resolution of 4cm^{-1} and an accumulation of 40 scans in range of 400 to 4000cm^{-1}.

Micrometer instrument (ACCUD Co.; Measuring range: 0-25mm) was applied for measuring the thickness of samples. 10 measurements were made for each sample and the mean average was reported. XRD (Model: 3003PTS, SEIFER, Germany) was used for assessing the crystallinity of samples. The crystallinity index (CI) was calculated from diffracted intensity data using the peak area of the crystalline (I_{002}) and amorphous regions (I_{am}) [12, 13].

Surface area and porosity of specimens were measured, at 77 K using Barrett–Emmett–Teller (BET).

Wettability

Wettability of samples was determined by static immersion test. Static immersion test was carried out based on BS 3449:1990. Briefly, dried samples were weighted and immersed in distilled water for 2 min and then taken out from water and shacked to remove extra water in surface and reweighted. Finally water absorption was calculated as follow:

$((W_2-W_1)/W_1) \times 100$ (1)

where W1 and W2 are weight of dried and wet samples, respectively. The test was repeated 5 times and the mean value for each sample was reported [21].

Swelling rate (SR)

Samples were cut into 1 cm^2 and dried at 60°C for 6 hr. After that they were weighed and immersed in distilled water at 21°C.The specimens were taken out every 1min and after draining they were reweighed. The test was continued until the absorption became constant and then %SR was calculated by the below equation:

$$\%SR = [(W_t - W_d)/ W_d] \times 100\% \quad (2)$$

W_t= weight of wet sample

W_d= weight of dry sample

Eventually %SR of the specimens at different times were plotted against time [33].

Water release rate (WRR)

To evaluate the drying rate (Water release rate), samples were steeped in distilled water for 24 hr. Then, they were took out and excess water of BC surface was removed by a filter paper. Samples were weighed and placed in room temperature and weighed repeatedly in specified times until there was no further changes. Finally, the curve of losing weight against time was drawn [34-36].

FESEM observation

FESEM microscope (Carl Zeiss, SIGMA VP) was applied to observe structure, surface morphology and cross section of samples.

RESULTS AND DISCUSSION

Many researches have studied the mechanism of polycarboxylics and hydroxyl group of cellulose reaction for conventional cellulosic materials such as cotton. BTCA as a polycarboxylic acid in presence of heating attempts to provide a five-member cyclic anhydride intermediate and joints to a cellulose chain and in the second step through repeating of the process, cross-linked two cellulose chains with esterification[25,35].

ATR-FTIR

The ATR-FTIR was used to analyze the effect of BTCA cross-linking on functional groups and bonds in bacterial cellulose (Fig.1). The peaks in the region 3200-3600 cm^{-1} related to O-H cellulose stretching frequencies. In addition peaks around 1100-1200cm^{-1} and 1060cm^{-1} are related to C-O-C bond in β-glucose molecules and cyclohexane ring in cellulose chain respectively [3,33,36]. Extra peak in cross-linked sample in the 1710-1740cm^{-1} region confirmed COO bond in cross-linked samples [31]. According to specimen preparation method, after cross-linking all samples washed with distilled water to remove uncross-linked BTCA and other by-products of cross-linking process to specify esterification peak to "Carboxyl Bridge" which bonded in BC nanofibrils.

XRD

XRD patterns of cross-linked and uncross-linked samples were shown in Fig 2. These diffractograms showed two main peaks, 2Θ = 22.7°and 14.7°in cross-linked and uncross-linked BC profiles which represented the (002) and (110) lattice diffraction of polymorph respectively, which proved previous reported data [3,12,17]. According to these patterns and Segal et al. method [13], the crystallinity index of all samples were around 63% which is obvious

that the cross-linking process has not adversely effect on BC crystals and it happened in amorphous region of this biopolymer.

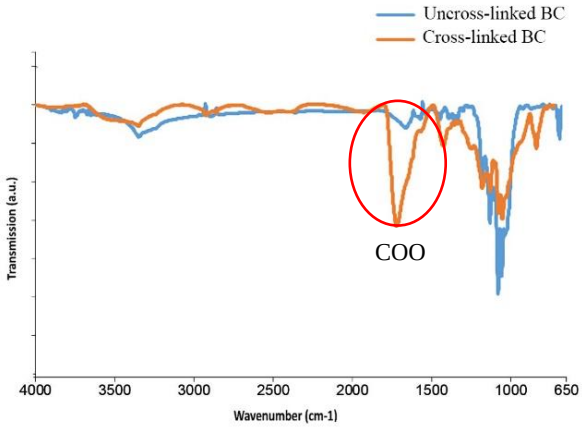

Fig. 1. ATR-FTIR spectrum of uncross-linked and cross-linked BC with BTCA 20 w/v %.

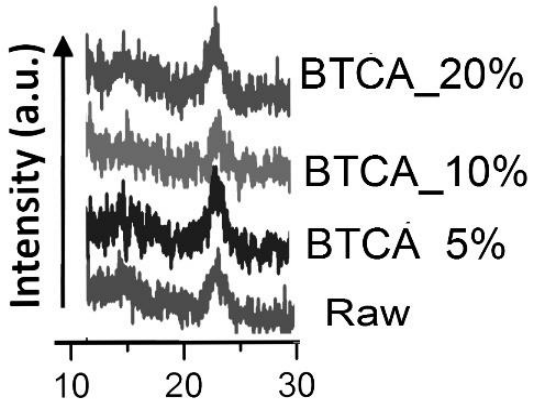

Fig. 2. XRD patterns of cross-linked BC with BTCA in 5, 10 and 20 w/v % and purified BC.

BET

Specific surface area of uncross-linked and cross-linked sample with BTCA 20% were measured through BET. Total Surface area for raw BC was 0.36 m^2g^{-1} whereas the cross-linked one showed 1.16 m^2g^{-1} which indicated that cross-linking process preventing collapse of bacterial cellulose 3D structure and via esterification of nanofibrils in pellicles, retained more porosity. In fact, chemical reaction between hydroxyl groups of cellulose chain and carboxyl groups of BTCA limited nanofiber entanglements during drying process. Obtained result from BET proved that cross-linking reaction is able to provide more porous pellicle rather than pure dried BC.

FESEM

Surface and cross section of dried purified and cross-linked BC with BTCA 20% were shown in Fig. 3. Fig. 3a indicated 3D structure and multilayer contain collapsed BC which occurred during drying process and the nanofibers involved in entanglement points. Regarding to bacteria and pollutant were removed from pellicle with alkaline treatment, porosity is available in purified BC which is obvious in Fig. 3a and b. In addition many water molecules trapped inside this 3D network which made of very thin layers and nanofibers during cultivation time. After purification and cross-linking process the porosity of BC increased. Due to carboxyl bridges inside of nanofibers, these bonds retained the porous area in comparison to raw dried BC. Porous surface and cross section of cross-linked sample in Fig. 3c and d showed the pores which are able to absorb more liquid, increase water holding capacity and water release rate of cross-linked samples.

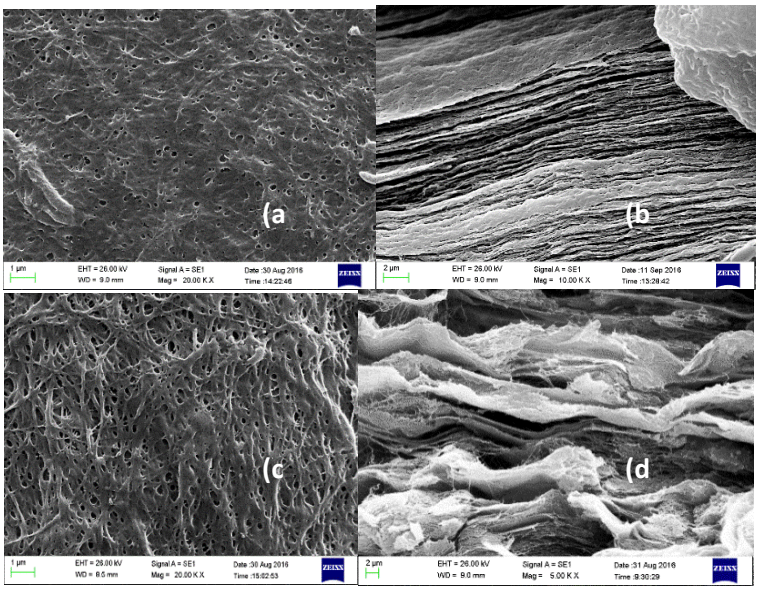

Fig. 3. FE-SEM images of purified (a, b) and cross-linked BC (c, d) with BTCA 20 w/v%.

Thickness

Thickness of pellicles is a one of the most important factors which showed the difference between cross-linked and uncross-linked samples (Fig. 4). According to obtained results, increasing of acid concentration enhanced more thickness of specimens. The thickness of cross-linked sample with BTCA 20% is about 12 times more than purified pellicle.

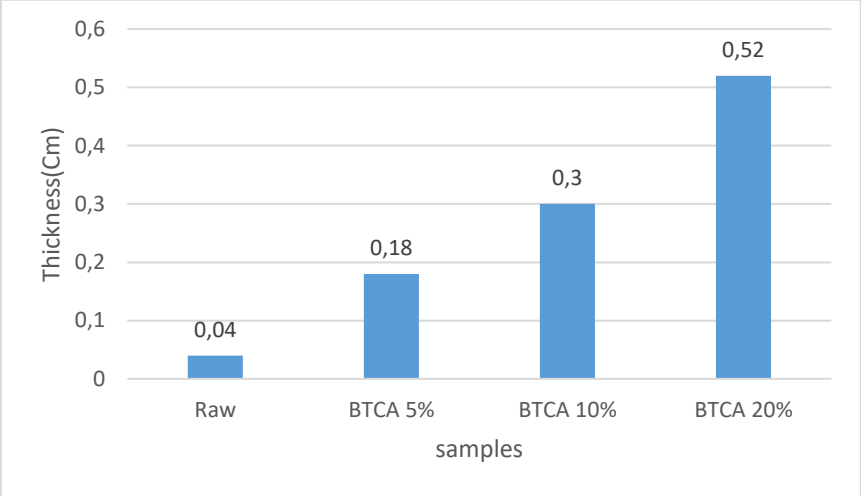

Fig. 4. Comparing thickness of dried uncross-linked and cross-linked BC with BTCA in 5, 10 and 20 w/v%.

Wettability

Static immersion test was used to measure water absorption capacity of samples. This study evaluated the effect of cross-linking process on water absorption. Results from Fig. 5 confirmed the porosity of samples through water absorption. After cross-linking cellulosic nanofibril of BC with BTCA, movement of BC nanofibers would be difficult and therefore collapsing of 3D network decreased. Thus this issue provided good opportunity to remain vacant regions inside the structure which are suitable for liquid absorption. The wettability of cross-linked sample with 20% acid is around 300% more than dried purified BC which prove more porosity in cross-linked BC.

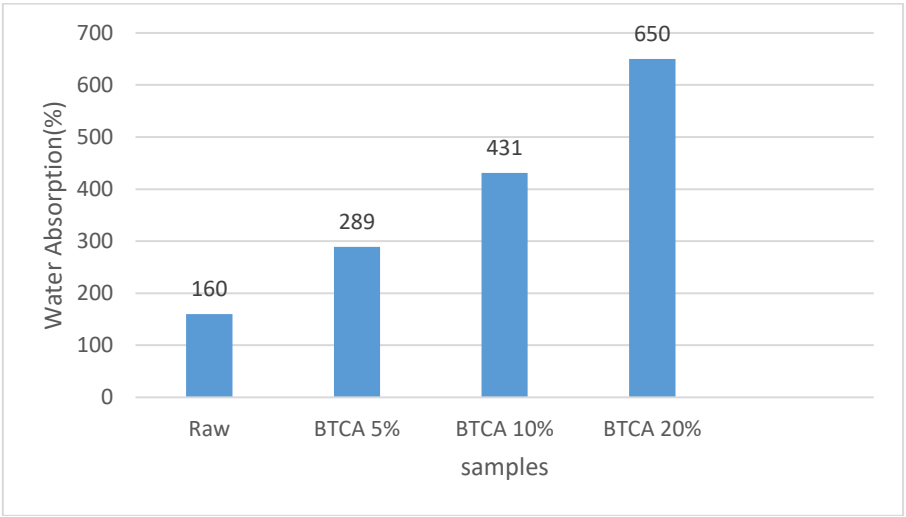

Fig. 5. Wettability of dried uncross-linked and cross-linked BC with BTCA in 5, 10 and 20 w/v%.

Water swelling rate

Saturation time of wound dressing has significant role in modern wound dressing. Physical and chemical water absorption can support this feature. BC based on its 3D structure has capacity to absorb water 100 times more than its weight [12] but drying process decreases water absorption property and limits the usage of BC. To accomplish this matter, cross-linking was used to increase saturation time of cross-linked BC. Fig. 6 showed an improvement of the water swelling rate with increasing acid concentration which has agreement with other results. Cross-linking treatment of BC made new structure with special porosity which is able to absorb more than 450% water during 18 min. comparing cross-linked BTCA samples and purified BC confirmed the high amount of pores inside of cross-linked specimens.

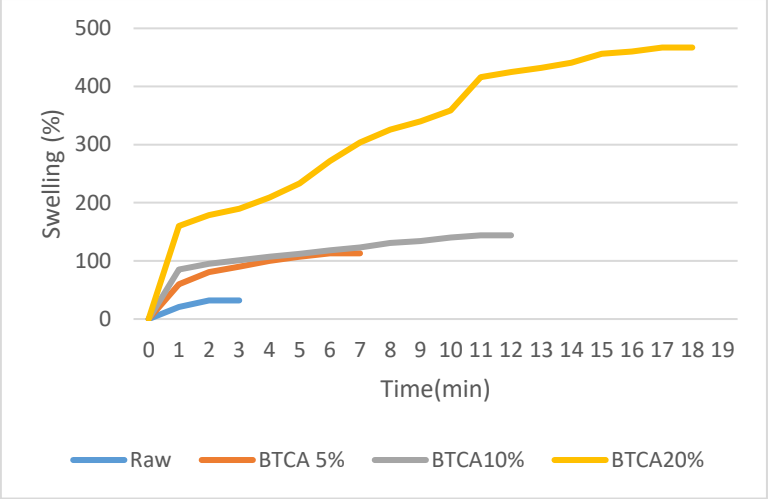

Fig. 6. Water Swelling Rate of BC cross-linked with BTCA in 5, 10 and 20 w/v %.

Water release rate

Moisture in wound healing management has specific role and it has prevented pain and accelerate healing. According to this issue, the water release rate of cross-linked and uncross-linked sample investigated. Based on results represented in Fig. 7, the raw BC was dried after 1 hour but water inside the structure of other cross-linked samples retained at least 6h. By chemical esterification, the hydrophilicity of BC decreased due to replacing hydroxyl group with carboxyl one but regarding to increasing the porosity of BC pellicles, retaining water increased and this property has major role in advanced wound dressing.

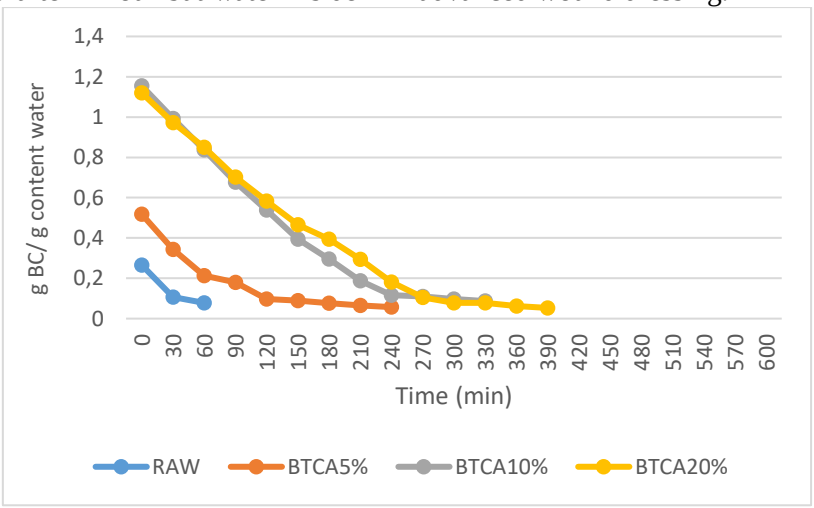

Fig. 7. Water release rate of BC cross-linked with BTCA in 5, 10 and 20 w/v %.

CONCLUSION

Collapsing of bacterial cellulose during first drying causing a significant decrease in rehydration ability. In this study, a new method based on bacterial cellulose cross-linking presented. A conventional cross linker, BTCA was used for this purpose. According to the results, cross-linked BC provided higher porosity, water holding capacity, swelling rate and drying time which are significant in biomedical usage.

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