Preparation of Bacterial Cellulose Based Hydrogels and Their Viscoelastic Behavior

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Abstract. Bacterial cellulose (BC) based hydrogels have been prepared in blended with carboxymethylcellulose and polyvinyl pyrrolidone by using heat treatment. The properties of BC-CMC and BC-PVP hydrogels were compared with pure BC, CMC and PVP hydrogels. These hydrogels were investigated by measuring their structural, morphological and viscoelastic properties. Through the morphological images, alignment of the porous flake like structures could be seen clearly within the inter-polymeric network of the hydrogels. Also, the detail structure analysis of the polymers blended during the hydrogel formation confirms their interactions with each other were studied. Further, the viscoelastic behavior of all the hydrogels in terms of elastic and viscous property was studied. It is observed that at 1% strain, including CMC and PVP hydrogels, all the BC based hydrogels exhibited the linear trend throughout. Also the elastic nature of the material remains high compared to viscous nature. Moreover, the changes could be noticed in case of blended polymer based hydrogels. The values of complex viscosity (η^*) decreases with increase in angular frequency within the range of ω = 0.1-100 rad.s^{-1}.

INTRODUCTION

The materials obtained from the renewable sources are in focus in the today's strategic research area [1]. There seems to be chances for the fossil fuels to be exhausted in the coming future so interest has been focused on the development of biodegradable materials with the concern of environment and healthcare of living organisms [1]. Concern with this regard, cellulose which is one of the polysaccharides widely available in nature has attracted the attention of researchers as it is renewable and biodegradable biopolymer [2]. The cellulose in structure is a linear polymer made up of glucose molecules linked by β (1–4) glyosidic linkages [3]. Moreover the abundant source of cellulose is through plants and next by different micro-organisms [3]. The pure cellulose is obtained through solvent extraction process [2]. However, there are chances for the degradation of the crystalline structure within the cellulose so cellulose obtained from bacterial source is preferred [2]. Bacterial cellulose (BC) is produced from the strain of *Gluconacetobacter xylinus*, which is a Gram-negative, rod shaped and aerobic bacterium [3]. BC differs from plant cellulose in many aspects like high crystallinity, ultrafine network structure, high water absorption capacity, high mechanical strength and biocompatibility [3, 4]. Because of such valuable and useful properties persisted by BC, it has huge demand in several biomedical fields like artificial skin for burn, blood vessels, wound

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healing material, drug delivery etc. [2, 3]. Along with this, BC has already been utilized in paper making, as separation membrane, electro conductive carbon film and so on.

A new generation in the research field dealing with the increase in utilization of polymer is the preparation of hydrogels which is the 3-dimensional network of the polymeric structure. This hydrogel has porous structure, can expand and have greater water absorption capacity and elasticity [5, 6]. For designing any polymeric material to have its application in biomedical fields, it's necessary to improve its functional properties. So, blending of any natural or synthetic molecules can result in production of tailored polymeric material [7]. In recent years, BC became the subject of intensive studies and has been used in synthesis of hydrogels with various other combinations. In the present study, BC based hydrogels have been prepared with the use of carboxymethylcellulose (CMC) and also polyvinyl pyrrolidone (PVP). CMC is one of the water soluble derivatives of cellulose ether utilized in the fields of food, cosmetics, and paint as a viscosity modifier, thickener, emulsion stabilizer, and water-retention agent [8]. CMC can absorb large amount of water, can swell in form of hydrogel and has good physical property inform of viscoelasticity [8]. PVP is also one of the synthetic biodegradable polymer which has already recorded its application in the area of pharmaceutical and also in medicines because of its high absorption capacity, complex forming abilities and high mechanical property [6, 7, 9, 10].

Inspired by such interesting properties of CMC and PVP, different hydrogels have been prepared in blending with the bacterial cellulose (BC) and designated as BC-CMC and BC-PVP hydrogel. Further, as a control set BC, CMC and PVP hydrogels were also prepared, Moreover, there is no report yet about the hydrogels prepared in combination of BC with CMC and PVP. This paper mainly reports about the synthesis of BC based hydrogels and their characteristics property have been evaluated in the form of morphology, structural analysis and also its rheological property.

EXPERIMENTAL

For the preparation of bacterial cellulose based hydrogels, firstly bacterial cellulose was synthesized using basal synthetic H-S nutritive medium. The bacterial strain *Gluconacetobacter xylinus CCM 3611*^{T} (*syn. Acetobacter xylinum*) was used to obtain the bacterial cellulose in form of membrane. This bacterial cellulose membrane obtained was lyophilized and then grinded into pieces. Several bacterial cellulose based hydrogels were prepared using the different combinations of bacterial cellulose with carboxymethylcellulose (CMC) and polyvinylpyrrolidone (PVP). The detailed composition of the polymeric solution used to prepare the hydrogel is shown in the Tab. 1. However, these polymer solutions were prepared using solvent casting technique under 15 lbs pressure and 120°C temperature for 20 minutes. Finally, the round shaped, smooth and off-white colors with 6 – 7 mm thickness hydrogel sample were obtained. Five different hydrogel samples were prepared and termed as BC, CMC, PVP, BC-CMC and BC-PVP hydrogel. Some of the hydrogel samples were kept under the freeze drying process (temperature = –80°) for 48hrs. The freeze dried samples were used for the characterization purpose. Further some of the fresh hydrogel samples were utilized in the study of viscoelastic behavior.

Table 1. Polymer composition for hydrogel preparation.

Sample Name	PVP (%)	CMC (%)	BC (%)	PEG (%)	Agar (%)	Glycerine (%)	Water (%)
BC	0	0	1	1	2	1	95
CMC	0	1	0	1	2	1	95
PVP	1	0	0	1	2	1	95
BC-CMC	0	0.5	0.5	1	2	1	95
BC-PVP	0.5	0	0.5	1	2	1	95

All the hydrogels prepared were characterized in the form of structure analysis, morphological analysis as well as their viscoelastic property under 1% strain.

The structural analyses of the BC, PVP, CMC, BC-CMC, BC-PVP, hydrogels were confirmed using Fourier transform infrared spectroscopy (FT-IR). The ATR-FTIR spectroscopic analysis was conducted by using NICOLET 320 FTIR Spectrophotometer with "Omnic" software package over 4000-600 cm^{-1} at room temperature. A uniform resolution of 2 cm^{-1} was maintained throughout in all cases.

The morphological behavior of all the hydrogels were determined by scanning electron microscopy (SEM). The SEM analysis was carried out on VEGA II LMU (TESCAN) operating in the high-vacuum/secondary electron

imaging mode at an accelerating voltage of 5-20 kV. The samples were sputter coated with a thin layer of palladium/gold alloy to improve the surface conductivity and tilted 30° for better observation. The images were taken at magnification of 100x-10kx.

The viscoelastic properties of bacterial cellulose based hydrogel were investigated by using a parallel plate rheometer (ARES; Rhemetrics Scientifics, USA) testing with a "TA Orchestrator" software package. A 25 mm diameter parallel plate measuring geometry with a gap of about 2 – 3 mm was used, employed at strain amplitude (1%) to maintain the measurement range within the linear viscoelastic region. Dynamic frequency sweep test were carried out at 28^{o}C temperature to observe the storage (G') and loss (G") moduli, and complex viscosity (η^{*}) as a function of a wide range of angular frequency (ω: 0.1-100 rad.s^{-1}).

RESULTS AND DISCUSSION

Morphology of BC Based Hydrogels

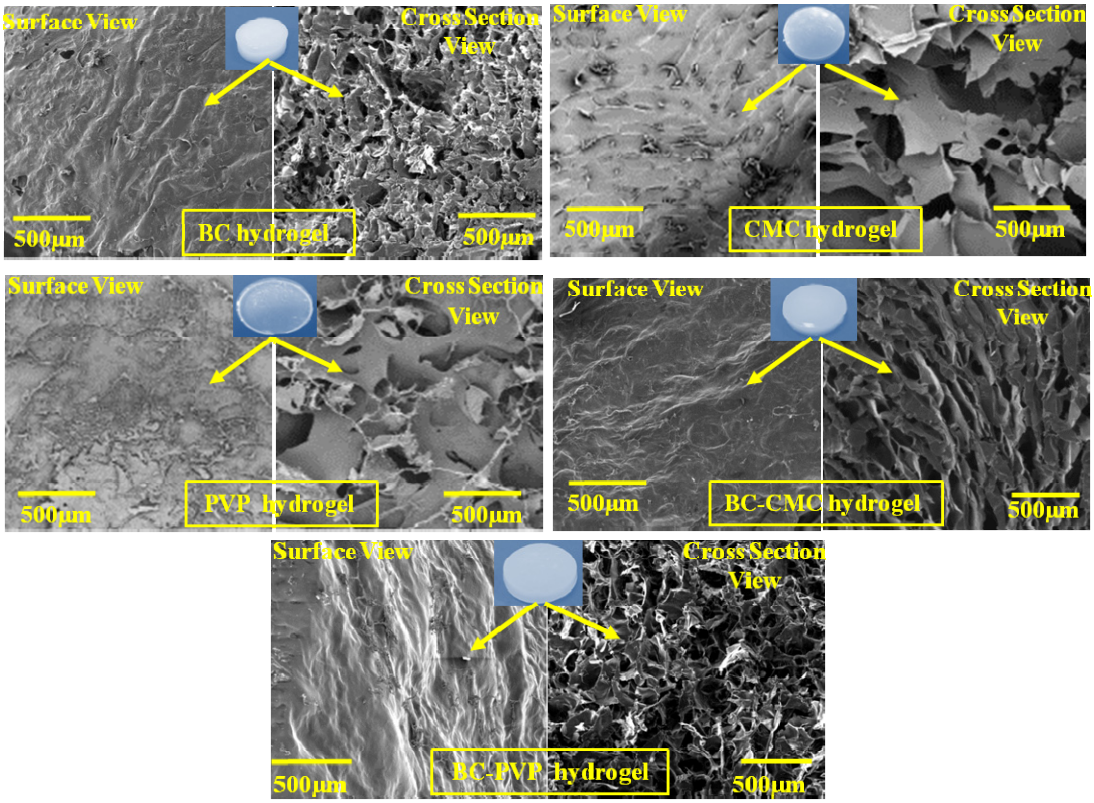

FIGURE 1. SEM Micrographs: BC, CMC, PVP, BC-CMC and BC-PVP hydrogel.

The optical and morphological views (surface and cross-sectional) of BC, CMC, PVP, BC-CMC and BC-PVP hydrogels are depicted in Fig. 1. It can be seen clearly through the surface images that the spaces gets filled between cellulosic structures of the matrices in form of small white dots spread throughout the matrix. However, due to aggregation of these cellulose particles smoother surface is seen in all the types of hydrogel matrices. On the other hand, in the cross sectional images of these hydrogels the alignment if the porous flakes like structure could be seen. However, it can be said that the increase in number of inter-polymeric networks creates dense structure as well as small pore size. These types of hydrogels having porous structures are capable enough to enhance the diffusion of water/salts/electrolytes in all the directions making them suitable for drug loading and controlled release of drugs through drug delivery process.

Structural Analysis of Bacterial Cellulose Based Hydrogels

The FTIR spectra of BC, CMC, BC-CMC and BC-PVP and PVP are shown in Fig. 2. A strong band seen between 3340-3350 cm^{-1} corresponds to hydroxyl (–OH) group stretching. However the characteristics peak seen in BC hydrogel is little shifted as compared to other blended hydrogel. Moreover the peak present between the range of 1050-1160 cm^{-1} shows the presence of –C-O group whose intensity increases in case of BC-CMC and BC-PVP type of blended hydrogel as compared to only BC and CMC hydrogel. This clearly indicates that the polymer interaction and H-H bonding has taken place between the polymers. Also the peak found at 1643 cm^{-1} and 1427 cm^{-1} corresponds to the amide group which confirms the interaction of PVP with BC.

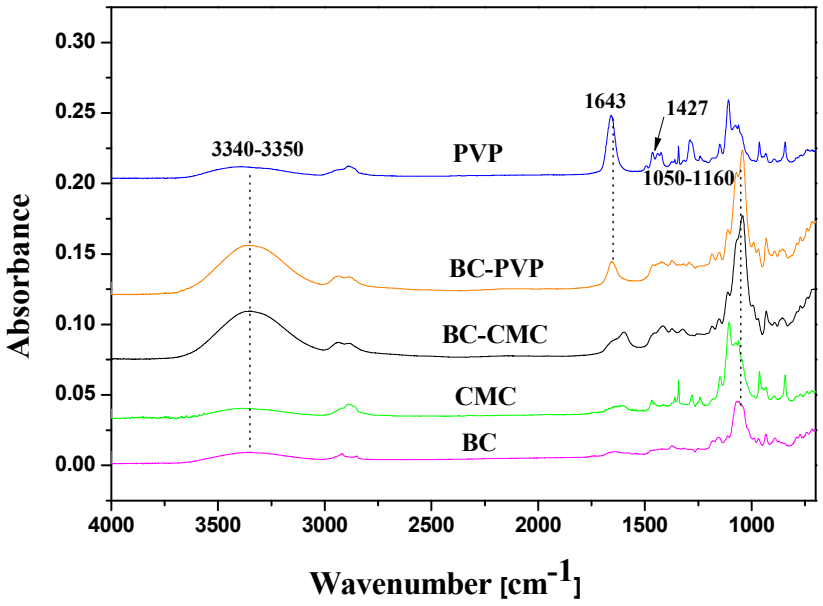

FIGURE 2. FTIR Spectra of BC, CMC, BC-CMC, BC-PVP and PVP hydrogel.

Viscoelastic Properties of BC Based Hydrogels

The dynamic viscoelastic properties of all the BC based hydrogels in the form of storage modulus (*G'*), loss modulus (*G"*) and complex viscosity (η^*) were studied at 1% strain and at room temperature. It can be seen through the Fig. 3 and 4, that the *G'*and *G"* of all the hydrogels are plotted as function of angular frequency at 1% strain. In Fig. 3, BC (1%) and CMC (1%) as single candidate of base polymer along with their blended form of BC-CMC (0.5%) together during the preparation of hydrogel is shown. Whereas in Fig. 4, BC (1%) and PVP (1%) as single candidate of base polymer along with their blended form of BC-PVP together is shown. As seen from the Fig. 3 and 4, the values with the storage modulus (*G'*) with all the hydrogels is higher as compared to loss modulus (*G"*) in the frequency range of (0.1 – 100 $rad.s^{-1}$). This confirms the general characteristics of polymers cross-linked to form hydrogels. In case of BC based hydrogel in Fig. 3, initially there is slight decrease in the values of storage modulus and then gradually the values rises after certain value of angular frequency (ω = 10$rad.s^{-1}$). Whereas in case of CMC based hydrogel (Fig. 3) the storage modulus gradually starts rising from ω = 0.1 up to ω =100$rad.s^{-1}$. However, when there is a mixture of BC and CMC together as base polymers, the trend of *G'* obtained are in between the values of the *G'* of BC and CMC individually. This shows that the true interaction has occurred between the polymers. Also, the blending of BC-CMC is confirmed through the IR-spectra as shown in Fig. 2. It can be said that the trend of linearity as well as plateau behavior is maintained in all the cases of hydrogels in Fig. 3 which confirms that the material is strictly/rigid cross-linked gel or solid-like material.

Through Fig. 4, it is observed that in case of BC and PVP based hydrogel, there is slight decrease and then increase in the values of storage modulus with increase in angular frequency from ω = 0.1 up to ω =100 $rad.s^{-1}$. The same trend is noticed in the hydrogel prepared using the mixture of BC and PVP but even though being blended the

values of *G*' gets lower in comparison to both BC and CMC based hydrogel. From this observation it can be predicted that there is not much compatibility seen during the blending of BC and PVP as observed in BC and CMC in Fig. 3.

Overall, through all the observation noted from Fig. 3 and 4, it can be said that the hydrogels maintain their elastic behavior throughout and viscous nature remains in the lower stage always.

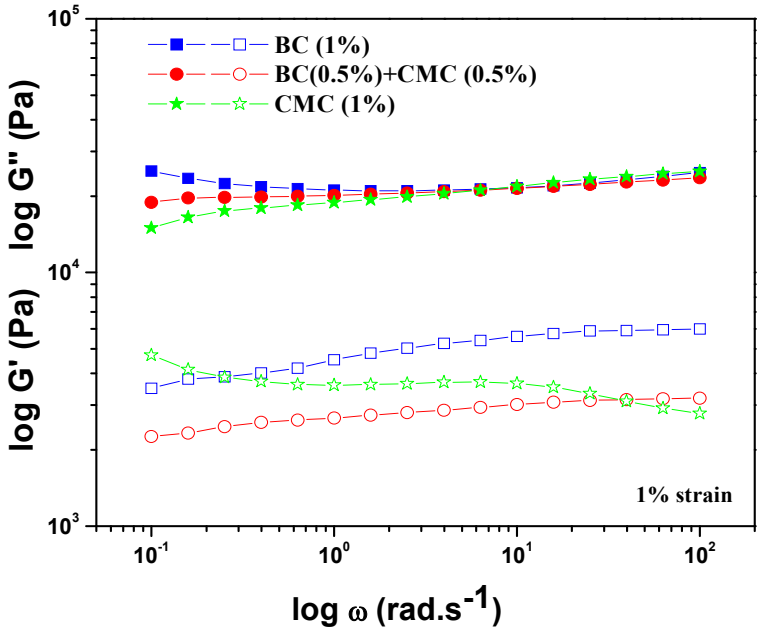

FIGURE 3. Effect of angular frequency (ω) at 1% strain on storage modulus (*G*', filled symbol) and loss modulus (*G*", non-filled symbol) for BC, CMC and BC-CMC hydrogel.

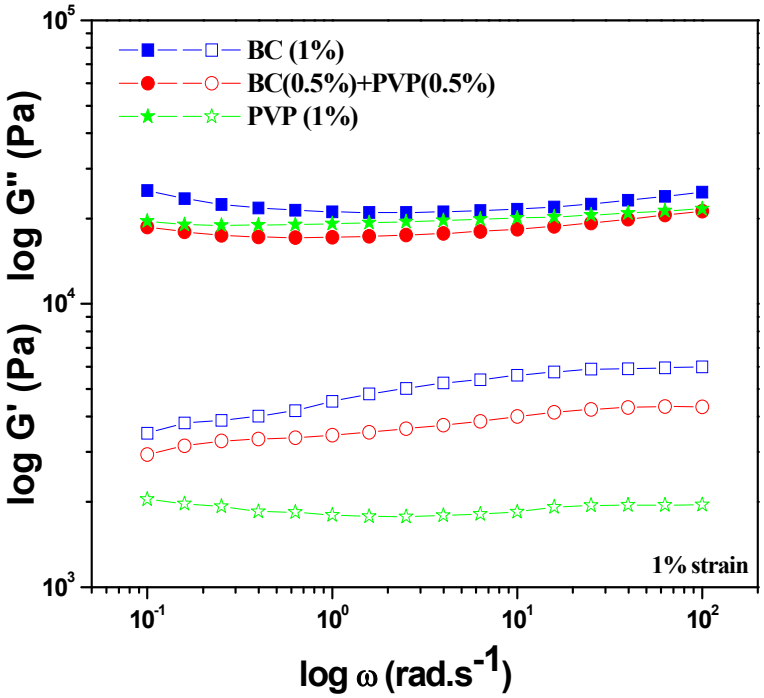

FIGURE 4. Effect of angular frequency (ω) at 1% strain on storage modulus (*G*', filled symbol) and loss modulus (*G*", non-filled symbol) for BC, PVP and BC-PVP hydrogel.

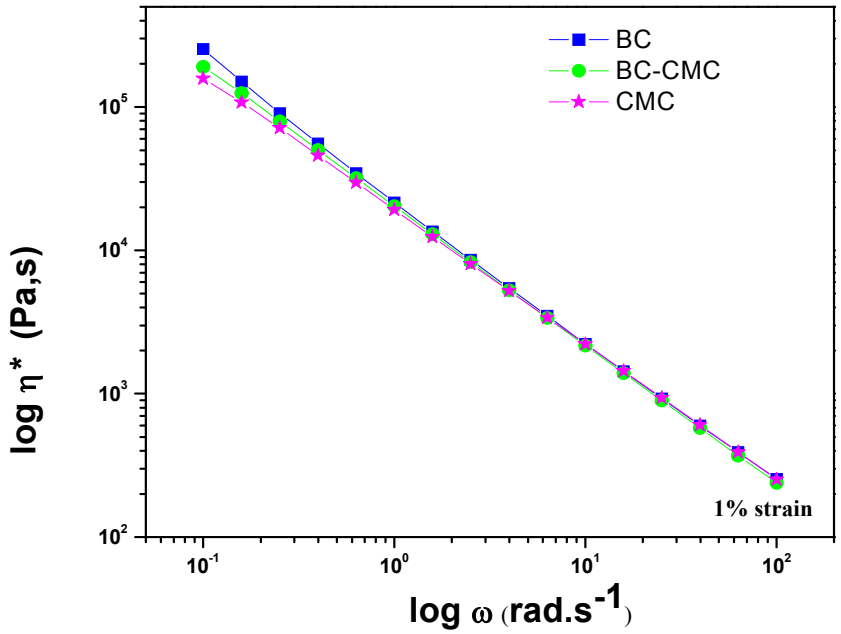

FIGURE 5. Effect of angular frequency (ω) at 1% strain on complex viscosity (η^*) for BC, CMC and BC-CMC hydrogel.

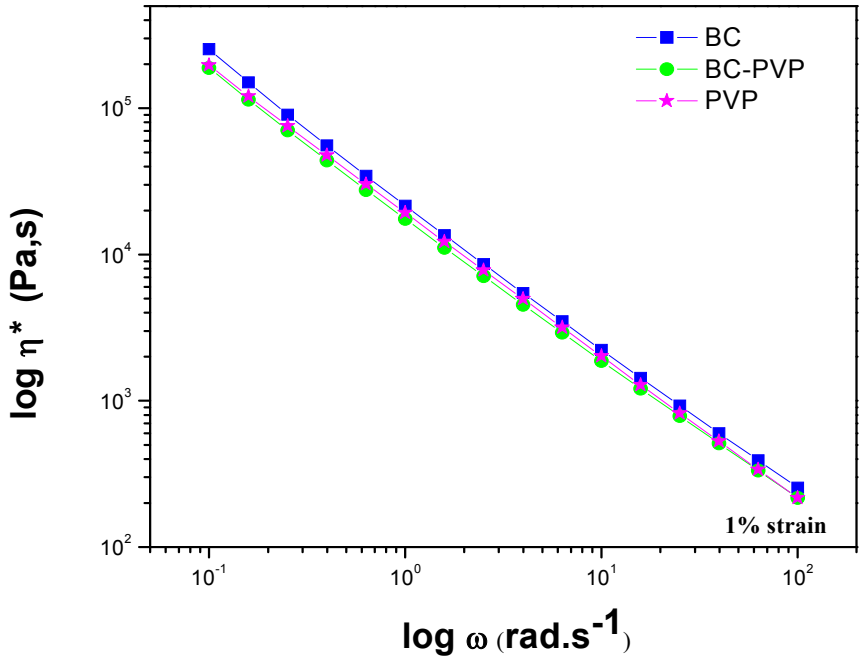

FIGURE 6. Effect of angular frequency (ω) at 1% strain on complex viscosity (η^*) for BC, PVP and BC-PVP hydrogel.

Complex viscosity under 1 % strain versus angular frequency ω plots in double - logarithmic coordinates for all the hydrogels are shown in Fig. 5 and 6. It can be seen that the values of complex viscosity at 1% strain decreases linearly with the increase of ω. However, this same trend throughout remains the same which reflects the true nature of polymeric based material.

CONCLUSION

In this work, BC and BC based hydrogels including PVP and CMC hydrogel have been prepared using the heat treatment technique. CMC and PVP were also used as a form of synthetic based polymers along with BC, a natural biopolymer. The structural analyses of the hydrogels were confirmed using FT-IR which showed that BC is nicely blended with CMC as well as PVP. The changes could be seen in the morphological image of the hydrogels which were observed through scanning electron microscopy and both the surfaces as well as cross-section. Hydrogels having porous structures (as shown in Fig. 1) are capable enough to enhance the diffusion of water/salts/electrolytes in all the directions making them suitable for drug loading and controlled release of drugs through drug delivery process. Further, considering the data of rheological measurement, it can be mentioned that both the BC based blend hydrogels ("BC-CMC" and "BC-PVP") are reasonably maintaining their elastic behavior throughout as comparable to BC, CMC and PVP hydrogels. Moreover, viscous nature of these hydrogels remains in the lower stage always. Due to such excellent viscoelastic properties and internal cross linking structure, all these BC based biomaterials/bio-based material may find suitable application in the tissue engineering area, particularly in soft tissue replacement (e.g. cartilage, tendon, cardiovascular tissue or ligament) and as wound management.

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