Citric acid cross-linked regenerated bacterial cellulose as biodegradable and biocompatible film for food packaging

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Research Article

Keywords: Regenerated cellulose, citric acid, food packaging, bioplastics

Posted Date: March 21st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2704167/v1

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Version of Record: A version of this preprint was published at Cellulose on September 19th, 2023. See the published version at https://doi.org/10.1007/s10570-023-05500-5.
Abstract

The global depletion of petrochemical resources, coupled with the environmental problems caused by the widespread use of traditional plastics, have brought more attention to exploring biodegradable materials. However, the high preparation cost and complex manufacturing processes leave us few choices of raw materials of biodegradability. Herein, regenerated bacterial cellulose (RBC) was used to prepare a kind of environmentally-friendly material that degrades rapidly. Further addition of citric acid (CA) enhances its mechanical properties and degradability, resulting in a CA-cross-linked regenerated cellulose (CA-RC) film with a fracture strength of 93.40 MPa and Young's modulus of 4.2 GPa, which behaves better than commercial plastic wrap in food preservation. In addition, the film could be completely degraded in soil within two weeks, of which the biocompatibility is verified by both cell proliferation and hemolysis experiments. The results show that the CA-RC films have great application prospects in food packaging and biomedical materials.

Introduction

The global productivity of plastic products increases by about 5% every year, and the annual use of plastic bags in China have exceeded 4 million tons (Geyer et al.). Traditional plastics made from petroleum are difficult to degrade, which have brought serious environmental issues. To reduce the pollution, researchers are developing degradable replacements for petroleum-based materials (Lal et al., 2020; Sharma et al., 2021; Sheth et al., 2019). For instance, degradable plastic eventually decomposes into CO₂, water, and minerals under natural conditions, causing little environmental damage (Shah et al., 2008). Biodegradable plastics have become a research hotspot due to its abundant resources, renewability, and excellent biocompatibility (Rai et al., 2021). Current biodegradable plastics are mostly made from cellulose, polylactic acid (PLA), polyhydroxyalkanoates (PHA) (Altman, 2021), protein (Rojas-Lema et al., 2021), and starch (Zhang et al., 2022). However, there still exist several problems in the development of degradable plastics, including high production cost, complex preparation process, limited supply of raw materials, and critical degradation conditions (Chiellini et al., 2004; Kim et al., 2021). For example, PLA, PHA and protein-based plastics require complex preparation processes and high cost (Chen et al., 2016; Fei et al., 2015; Qi et al., 2017; Suriyamongkol et al., 2007). Starch-based biodegradable materials are derived from grain, which leads to unfavorable food waste in the current food shortage (Bangar et al., 2022; Chavan et al., 2022). Therefore, it is important to develop environmentally friendly, cheap, and high-quality degradable plastics.

Cellulose comes from a wide range of sources such as plants and bacteria, so bioplastic derived from cellulose is considered to be of commercial potentials (Zhou et al., 2022). Heidarian et al. reported a degradable composite made of cellulose extracted from sugarcane and PLA with the Young's modulus of 716.5 MPa, fracture strength of 32.6 MPa, and elongation at break of 4.5%, which degrades in more than two months (Heidarian et al., 2018). However, the addition of PLA in the two-component biodegradable plastic inevitably increases production cost.
As a star material in the cellulose family, bacterial cellulose (BC) is produced by microbial fermentation. Compared with plant fibers, BC possesses a unique nanoscale fiberous structure of three-dimensional network, high crystallinity (about 90%), high Young's modulus (10 MPa), and high purity (about 95%) (Choi et al., 2022). Rich in oxygen-containing functional groups such as hydroxyls, BC surfaces can be easily modified and developed as food packaging materials, textile materials, biomedical materials and many other applications (Choi et al., 2022; Meftahi et al., 2018; Nurlidar et al., 2015; Xie et al., 2020; Ye et al., 2019). Since pristine BC films are non-transparent, non-antibacterial, and usually thicker than plastic wrap, modification of BC or incorporation of other components like silver or chitosan are required for to achieve the requirements as food packaging (Abral et al., 2021; Salari et al., 2018), including transparency, anti-bacterial properties and formability. However, the preparation of composite materials usually requires complicated process and high cost, using sole BC as biodegradable plastic is therefore of great interest.

Herein, we demonstrate a simple method for preparing food packing material solely from BC. By casting the regenerated BC solution and crosslinking with citric acid (CA), we obtained CA-crosslinked regenerated cellulose (CA-RC) films. The obtained CA-RC films exhibited excellent mechanical and degradable properties, with a breaking strength of 93.40 MPa and elongation at break of 7%, which could be completely degraded within two weeks. As food packaging material, the CA-RC film prevented the growth of microorganisms and showed improved food preservation compared to commercial wrapping material. Combined with its good biocompatibility, CA-RC has great application prospects in food packaging and biomedical materials.

**Experiment and Characterizations**

**Materials**

BC hydrogels were obtained by static incubation of *Acetobacter xylinum* NUST 4.2 preserved in our laboratory at 37°C for 72 hours. The detailed culture procedures can be referred to our previous work (Sun et al., 2007). Sodium hydroxide (NaOH), hydrogen peroxide (H₂O₂), citric acid (CA), and crystal violet were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China) (analytical grade, ≥99%). N, N-dimethylacetamide (DMAC) (analytical grade, ≥99%) was purchased from damas-beta company (shanghai, China). Lithium chloride (LiCl) (analytical grade, ≥99% was purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Mouse embryonic fibroblasts NIH/3T3 cell was purchased from Shanghai Chinese Academy of Sciences (Shanghai, China). Paraformaldehyde and TritonX-100 were purchased from Aladdin Biochemical Technology Co., LTD (Shanghai, China). All experiments were performed with deionized (D.I.) water and chemicals without further purification.

**Preparation of bacterial cellulose solution**

The BC hydrogel was first subjected to alkali boiling with 0.3% NaOH and 0.3% H₂O₂ at 80°C for two hours. After washing with D.I. water to remove the impurities, BC hydrogel was freeze-dried to obtain the
BC aerogel, and further pulverized by a wall breaker to obtain BC powder. One gram of BC powder was subsequently added into 100 mL of LiCl in DMAC (LiCl: DMAC=8:100, w/v), and stirred at 100°C for 2 hours, followed by stirring at room temperature for 24 hours. The obtained BC solution was centrifuged at 10,000 rpm for min to remove undissolved BC, and concentrated to 3 wt% by rotary evaporation for further use.

Preparation of citric acid cross-linked regenerated cellulose film (CA-RC)

The CA solution (1 wt% in DMAC) was first mixed with the BC solution and stirred thoroughly to obtain a uniformly dispersed mixed solution, which was shortly vacuumed to remove air bubbles, poured into a mold, and dried at 80°C for 3 hours. In the drying process, BC was crosslinked with CA to achieve a transparent film. After the reaction, the obtained composite film was immersed in water for 48 hours to remove the solvent and residual CA that did not react. During this process, water was replaced every eight hours. Finally, the biodegradable CA-RC film was obtained by drying the composite film at 80°C for 12 hours. In our study, four CA-RC films with different CA contents, 5 wt%, 10 wt%, 15 wt%, and 20 wt% were prepared, which were named as CA_{5}\%-RC, CA_{10}\%-RC, CA_{15}\%-RC and CA_{20}\%-RC, respectively. Regenerated cellulose (RC) film was prepared using a similar process as a control.

Characterizations

The chemical structure of the sample was analyzed by Fourier transform infrared spectrometer (Thermo Scientific NICOLET IS-20, USA), with the wavelength range of 4000-500 cm\(^{-1}\) and the resolution of 2 cm\(^{-1}\). XRD patterns were obtained by the X-ray diffractometer D8 Advance (Bruker, Germany) with Cu-K\(\alpha\) radiation (\(\lambda=0.1548\) nm) at a scanning rate of 4° min\(^{-1}\), working voltage at 40 kV, and current at 35 mA. Thermogravimetric analyzer (NETZSCH TGA 209F 1E-0090-L) was used to analyze the thermal properties of the materials in N\(_2\) atmosphere at the heating rate of 10°C min\(^{-1}\) and temperature range of 30-800°C. The transmittance of the sample in the wavelength range of 200-800 nm was collected by a UV-visible spectrophotometer (UV-1800, Japan). The surface morphology and microstructure of the sample were observed by scanning electron microscope (Hitachi S-4800) under 25 kV voltage after spraying gold on the surface. The X-ray photoelectron spectroscopy of the samples were obtained by the RBD-upgraded PHI-5000C ESCA system (Perkin Elmer) with Mg K\(\alpha\) radiation (\(h=1253.6\) eV). The mechanical properties of the materials were measured by an electronic universal testing machine (TY8000-A) with the tensile speed set at 1 mm min\(^{-1}\). The size of each tensile sample was 30 mm\(\times\)10 mm and the thickness of the material was measured with a spiral micrometer. For each sample five measurements were performed and the result was expressed as mean ± standard deviation (S.D.). The water and oil contact angles of the materials were measured by Data Physics OCA25L (Germany). The O\(_2\) permeability rate were measured by Oxygen permeator GTR-701R.

Water absorption test and oil absorption test
Dried samples were put into excessive D.I. water at room temperature. After incubation for 0.5, 1 and 24 hours, each sample was taken out, dried with filter paper by removing the water from surface, and weighed for three times. Using the same method, the dried samples were put into edible vegetable oil and weighed after 0.5, 1 and 24 hours of incubation. The weight changes in water absorption and oil absorption over time can be calculated by the following formula (eq.1):

\[ W(\%) = \frac{W_2 - W_1}{W_1} \quad \text{(eq.1)} \]

Where \( W_1 \) is the mass of the dried sample and \( W_2 \) is the real-time mass of the sample after water or oil absorption.

Degradation test

The samples were cut into the same size (20 mm × 20 mm), stained with crystal violet, and placed in soil at a depth of 20 mm from the surface (temperature 24-28°C, relative humidity 40-50 %). They were watered every two days, and regularly photographed to check the degradation degree.

Food packaging simulation experiment

Freshly peeled banana was cut into evenly sized cubes and placed into small glass jars, sealed with clingfilm commercially available or CA_{15%}-RC. Each treatment was further separated into two groups, one of which was placed in a 4°C refrigerator, and the other was placed at room temperature for regular observation and photo recording.

Cell culture and proliferation test

NIH/3T3 cells purchased from ATCC were cultured in Dulbecco's modified Eagle's medium (DMEM) (HyClone, Logan, USA) containing 10% fetal bovine serum (FBS), 1% penicillin and streptomycin (PS) in an incubator with 5% CO_2 at 37°C, and sub-cultured regularly.

Cell proliferation on the CA-RC films was studied by the Cell Counting Kit-8 (CCK-8) in 96-well plates. The as-synthesized materials were sterilized by soaking in 75% ethanol under UV irradiation, then washed with phosphate buffer (PBS) before seeding the cells. In each well of the plate, 100 μL of cell suspensions (5×10^4 ml^{-1}) was inoculated containing the CA-RC films, while cells growing on the plate well were used as control group. The plates were incubated for 72 hours before cell culture medium containing 10% CCK-8 was added. After further incubation for 2 hours in the incubator, the absorbance was measured at 450 nm with a microplate reader (Infinite F50). Cell viability was calculated using the following formula (eq.2):
Cell viability = \frac{OD_{test} - OD_{blank}}{OD_{control} - OD_{blank}} \quad (eq.2)

Cell morphology and structure

The cell morphology and structure were observed after culturing on the CA-RC film. Specifically, CA-RC films were seeded onto a 24-well plate, and soaked with 75% ethanol for sterilization. After washing with PBS, \(5 \times 10^4\) of NIH/3T3 cells were seeded onto the film put in each well and cultured in a 5% \(CO_2\) incubator at 37°C. Three days later, the cells were washed with PBS, immobilized with 4% paraformaldehyde in a dark environment for 10 min, then Hoechst 33342 (Invitrogen, USA) and Tritc Phalloidin (Soloarbio, China) were added and incubated for 5-10 min under darkness to stain the nucleus and actin, respectively. Finally, the cells were washed with PBS and observed by the confocal laser microscope.

Hemolysis test

Human blood was collected from vein, treated with anticoagulant EDTA, washed with sterile saline and centrifuged to remove the white blood cells in the supernatant. The lower red blood cells were taken for subsequent treatments. For each treatment, 2 mg of sample was mixed with 1 mL of saline and 100 \(\mu\)L of red blood cells, and incubated in a shaker at 100 r at 37°C for 1 h. Positive group contains 1 mL of saline, 100 \(\mu\)L of red blood cells and 10 \(\mu\)L of Triton X-100. Negative group contains 1 mL of saline and 100 \(\mu\)L of red blood cells. After incubation, the solution was centrifuged at 10,000 rpm for 10 min and the supernatant was transferred into a 96-well plate, of which the absorbance was measured at 540 nm with a microplate reader. The hemolysis (%) was calculated using the following formula (eq.3):

\[\text{Hemolysis (\%)} = \frac{OD_{sample} - OD_{veControl}}{OD_{veControl} - OD_{veControl}} \quad (eq.3)\]

The experiments involving human blood samples were approved by Department of Blood Transfusion, Jinling Hospital and were all performed in accordance to relevant laws and national guidelines.

Results And Discussion

The pristine BC exhibited as a white foamy aerogel after freeze drying (Fig. 1a), which contains over 95% cellulose without further purification. It was dissolved in the N,N-dimethylacetamide (DMAc)/LiCl co-solvent to yield regenerated cellulose (RC) solution. To prepare biodegradable CA-RC films, hydrogel-like RC solution was cast into a box, mixed with CA as a cross-linking agent, followed by high-temperature drying to obtain transparent CA-RC films (Fig. 1b). CA is an environmentally friendly cross-linking agent that can form intramolecular linkages between cellulose chains through an esterification reactions (Fig.
which replaces the original intramolecular hydrogen bonds and forms a stable three-dimensional network structure.

FTIR spectra confirm the occurrence of the esterification reaction (Fig. 2a). Before reaction, RC film has a strong broad absorption band at around 3600-3000 cm\(^{-1}\), which can be attributed to the stretching vibration of the -OH. The band at 2880 cm\(^{-1}\) is due to the stretching vibration of the C-H bond. C-O stretching vibrations of C-O-H and C-O-C groups generate bands those are located at 1150 cm\(^{-1}\) and 890 cm\(^{-1}\), respectively. The band of CA-RC at 3600-3000 cm\(^{-1}\) is more intense than that of RC, which is attributed to the hydrogen bond interactions (Uliniuc et al., 2013). The characteristic band at 1700 cm\(^{-1}\) is attributed to the stretching vibration of the ester group. Therefore, FTIR results confirmed the successful crosslinking of cellulose by CA (Adewuyi et al., 2017; Awada et al., 2014; Reddy et al., 2010).

XRD patterns of BC exhibit two characteristic peaks at \(2\theta = 14.48^\circ\) and 22.85° (Fig. 2b), corresponding to (110) and (002) planes, respectively, which are typical peaks of type cellulose structure (Salihu et al., 2021). The characteristic peak of RC film at \(2\theta = 20.4^\circ\), can be attributed to the transformation of cellulose to cellulose, indicating successful generation of regenerated cellulose (Kassem et al., 2020). The characteristic diffraction peaks of CA locate at \(2\theta = 10.8^\circ\), 16.6°, 22.1° and 29.0°, which are absent in the composite film, suggest that CA has been dispersed into cellulose and did not affect the crystal structure of cellulose (Pereira et al., 2022).

The thermal decomposition of RC and CA-RC shows two stages (Fig. 2c). In the first stage, the masses are reduced by nearly 10% due to the evaporation of water at about 40-100°C. In the second stage, the films begin to decompose at about 250°C, and the initial degradation temperatures decrease slightly with the increase of CA content, which may be attributed to the reduction of the cross-linking extent within the polymers (Shao et al., 2019). Furthermore, the final residual amount of CA-RC is higher than that of RC, which means the char yields increase with the addition of CA, indicating that the addition of CA promotes the carbonization of cellulose (Shi et al., 2007).

Fig. 2(d) shows the transmittance spectra of all samples measured in the wavelength range of 200-800 nm, which are all larger than 75% under visible light. Compared to RC, the transmittance is slightly reduced (<5%) with the addition of CA, due to the cross-linking between RC and CA that densifies the RC film. The high transparency of all samples comes from the uniform distribution of CA in the RC substrate, which results in strong chemical affinity and interactions between CA and RC that prevent cellulose from crystallization or separation, therefore preventing phase separation.

Surface morphology and mechanical properties

The pristine BC exhibits a three-dimensional interconnected network-like microstructure with fiber diameters of 30-80 nm as observed by SEM (Fig. 3a). However, no obvious fibers, holes or gaps can be observed on the surfaces (Fig. S2) and fracture surfaces (Fig. 3b-f) of the RC and CA-RC films, implying that the BC nanofibers had been completely dissolved. The above analysis is consistent with the test
results of XRD, that cellulose I had been successfully converted into cellulose II. Furthermore, as the CA concentration increases, the film surface becomes smoother and denser, implying that the CA has uniformly cross-linked the RC. CA enables stronger intramolecular bonding between the cellulose chains inside RC through both covalent and hydrogen bonds, which renders the film excellent mechanical properties.

The tensile strength of RC was measured to be 57.75 MPa, and increases with the addition of CA (Fig. 3g). When the CA concentration is 15 wt%, the tensile strength reaches the maximum value of 93.40 MPa. However, the tensile strength decreases to 58.22 MPa with further increase of CA concentration (20 wt%). Compared with other regenerated cellulose based films, the tensile strength of CA$_{15\%}$-RC is 45.3 MPa higher than that regenerated cellulose membrane doped with 15% CA (Soheilmoghaddam et al., 2021). The elongation at break of RC is 13.2%, which decreases after adding CA (Fig. 3h). This is attributed to two reasons. On the one hand, crosslinking between CA and RC interconnects the polymer molecules, resulting in increased tensile strength. However, further increase of CA concentration leads to excessive crosslinking and hinders the movement between polymer chains, resulting in a decrease in tensile strength and elongation at break. On the other hand, the high concentration of CA results in acid hydrolysis of glycosidic bonds on cellulose, resulting in reduced tensile strength and elongation at break (Olivato et al., 2012; Reddy et al., 2009; Shi et al., 2007). Young’s modulus is in the same trend as tensile strength, for the CA$_{15\%}$-RC is 4.22 GPa (Fig. 3i), 54% higher than that of RC. Apparently, the toughness only slightly decreases in the presence of CA, for CA integrated into RC matrix gaps to form relatively strong networks. The results above together show that CA$_{15\%}$-RC has excellent mechanical properties.

Hydrophilic and lipophilic properties

Considering RC and CA-RC films are to be used in practical applications, we also tested their hydrophilic and lipophilic properties. The water and oil contact angles of RC were measured to be 68.75° and 38.92°, respectively, indicating its hydrophilic nature due to abundant hydroxyl groups existing. With the increase of CA concentration, the water contact angle slightly increases, while the oil contact angle decreases significantly (Fig. 4a), indicating that CA-RC has better lipophilicity than RC. The water absorption of all films after 24 h exceeds 120%, and almost reaches equilibrium within 0.5 h in contact with water (Fig. 4b), indicating that the cellulose films absorb water quickly. The results indicate that as the concentration of CA increases, the free movement of polymer chains is hindered due to crosslinking reactions, resulting in lower diffusion of water within the CA-RC films. The oil absorption histograms (Fig. 4c) show that the oil absorptions of all cellulose films at 24 h are below 55%, implying poor oil absorption. The slightly higher oil absorption of CA-RC films than that of RC films may be attributed to the fact that the macromolecules in vegetable oil are more likely to form hydrogen bonds with few unreacted carboxyl groups in CA, as previously reported (Adewuyi & Pereira, 2017; Guan et al., 2021; Guzman-Puyol et al., 2022; Pereira et al., 2022).

Food preservation with CA-RC films
Owing to the excellent mechanical property, high transparency and natural origin, CA-RC films are great candidates for biodegradable packaging materials. Additionally, since they have fast rate of hydrophilicity and poor oil adsorption as mentioned above, we wonder if CA-RC films could be used as food packaging to prevent water loss or lipid oxidation. To this end, we first measured the water vapor permeabilities (WVP) and the oxygen permeabilities (OP) of RC and CA-RC films, to assess their performances on blocking the evaporation of water and diffusion of oxygen (Table S1). No significant changes in water vapor permeabilities were observed among the samples tested, varying from 1624 to 1698 g m\(^{-2}\) day\(^{-1}\). More, O\(_2\) permeability of CA\(_{15\%}\)-RC is 2.13E-17, 25% and 65% higher than those of CA\(_{5\%}\)-RC and CA\(_{10\%}\)-RC, respectively. We did not collect data of OP for RC and CA\(_{20\%}\)-RC for it cracked during the measurement, probably due to its smallest tensile strain among all samples.

To check if the cellulose films also have food preservation abilities, we used them as wrapping papers on freshly peeled banana at both room temperature and 4°C and observed the appearances of fruit over time. CA\(_{15\%}\)-RC was selected as the representative among all samples for its mechanical performance. Specifically, the breaking strength of the wet CA\(_{15\%}\)-RC film is 16.47 Mpa and the elongation at break is 17.83% (Fig. S5). At room temperature (Fig. 6), banana flesh sealed with commercial PVC plastic wrap developed observable mold on the surface on day 6 and were almost completely covered with mold on day 9. In contrast, only a small amount of mold appeared on the surface of banana sealed with CA\(_{15\%}\)-RC on day 9. When stored at 4°C, the peeled banana sealed with commercial PVC plastic wrap started to rot on day 9, while the one sealed with CA\(_{15\%}\)-RC showed little change on appearance. On day 23, the banana flesh sealed with commercial PVC plastic wrap started to develop mold on the surface, while CA\(_{15\%}\)-RC wrapping obviously slowed down the rotting of bananas. No obvious mold was observed but darkened flesh due to oxidation. Although RC film with a CA concentration of 15% has the largest OP value among the the CA-RC samples, it still has better preservation effect than commercial plastic wrap. Moreover, its excellent mechanical strength makes it possible to be manufactured as edible water balloons (Fig. S6), which could replace traditional PET plastic bottles as water containers to alleviate the increasingly environmental pressure. Taken together, the results show that CA-RC films, as functional materials with excellent degradable properties, have potential application prospects in food packaging, which is of great significance in reducing plastic pollution and protecting the environment.

Degradation properties

We then seek the direct evidence to prove the fast degradability of CA-RC samples. By studying the degradation of the cellulose films in soil, we found that CA addition accelerates the degradation of RC films. In soil, microorganisms attack the molecular chains of cellulose under water and light, causing the cellulose film to become loose and porous, which gradually broken down and degraded. Buried in soil, the CA\(_{15\%}\)-RC film became shattered on day 8 and completely degraded after 11 days (Fig. 5). In contrast, the RC film fragmented on day 11 and did not fully degrade until about 18 days later. This is because higher CA concentration accelerates the hydrolyzation of the glycosidic bond by acid, so the material decomposes faster. Compared with other degradable materials (PLA need 70 days (Wang et al., 2022),
and PHA need 3 months (Pérez-Arauz et al., 2019), CA-RC films has obvious advantages (Fig. S3). In addition, the material does not produce black smoke or pungent odor during incineration (Fig. S4), and it is inferred that the residual products are only CO₂ and H₂O, so RC and CA-RC films can also be directly degraded by combustion. The above two degradation approaches can significantly reduce the cost and time of waste disposal with little impact on the environment.

Biocompatibility tests

Finally, we performed both cytocompatibility and hemolysis tests on the films to investigate the biocompatibility of RC and CA-RC. First, NIH/3T3 cell proliferation on the films was evaluated by CCK-8 assay and the result is shown in Fig. 7(a), higher than 70% in all treatment groups. According to ISO 10993-5, RC and CA-RC composite films could be judged as non-cytotoxic materials. The viability decreases with the addition of CA content, which may be attributed to the enhanced acidic environment in the film. In addition, NIH/3T3 cells cultured for 3 days on different materials were fixed and stained and observed by confocal microscopy for cell structure and morphology. Fig. 7(b)-(f) show that the cells generally attach and spread well on the material, indicating that the material is suitable to cell growth. The cell density on CA₁₅%-RC film is the lowest, consistent with the CCK-8 result, suggesting CA concentration needs to be optimized in biomedical applications. In addition, the films were incubated with human erythrocytes, and the results are shown in Fig. S7. The hemolysis rates are all less than 1% in all treatments, far below the allowable limit of biological materials (5%), which can be reflected by direct observation from the sample photo in Fig. 7(g), suggesting the contact between red blood cells and the film dose not affect cell survival. As RC and CA-RC films meet blood compatibility standards and show cytocompatibilities, they can be used as biomaterials for development and application.

Conclusion

In conclusion, CA-crosslinked regenerated cellulose films were successfully prepared using CA as a crosslinking agent. When the concentration of CA added is 15%, the mechanical properties of the CA-RC film were significantly improved, of which fracture strength is 93.40 MPa, and the toughness is 4.10 MJ m⁻³. CA₁₅%-RC also has excellent degradation performance compared to the most commonly used degradable material, such PLA, which takes 3.5 folds longer to degrade. In terms of protecting freshly peeled banana from rotting, CA₁₅%-RC shows better preservative effect than commercial PVC plastic wrap. In addition, RC and CA-RC samples both show biocompatibilities in cell culture and erythrocyte contact. These results show that CA-RC bioplastic film is an excellent substitute for traditional fossil plastics, which is of great significance for reducing plastic pollution and environmental protection. It also has potential value and development prospects in the field of biomedical materials.

declarations

Ethics approval and consent to participate
The blood comes from the Department of Blood Transfusion, Jinling Hospital, Nanjing University School of Medicine, Nanjing 210002, China. There are no ethical issues involved in this experiment.

Declaration

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

the National Natural Science Foundation of China (51873087 and 81801839), the Fundamental Research Funds for the Central Universities (30920130121001), Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD, China), and General project of Jiangsu Provincial Health Commission H2019110.

Credit authorship contribution statement

Qingqing Yu: Data curation, Writing- Original draft preparation. Luyu Yang: Conceptualization, Methodology and Editing. Shujun Wang: Reviewing. Lei Zhang: Reviewing and Editing. Dongping Sun: Supervision, Reviewing

Acknowledgements

We thank the financial support from the National Natural Science Foundation of China (51873087 and 81801839), the Fundamental Research Funds for the Central Universities (30920130121001), Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD, China), and General project of Jiangsu Provincial Health Commission H2019110.

References


**Figures**

![Figure 1](image)

**Figure 1**

The preparation of materials, (a) the dissolution and regeneration process of BC, inset is an optical photograph of BC aerogel standing on a rose, (b) preparation of the highly transparent CA-RC film by solution casting, (c) the chemical structures of cellulose before and after reactions with citric acid as the crosslinking agent. Characterizations on the chemical structure of CA-RC film.
Figure 2

Basic characterization test of materials, (a) FTIR spectra, (b) XRD patterns, (c) TGA curves, and (d) UV-vis spectra of RC, CA$_{5\%}$-RC, CA$_{10\%}$-RC, CA$_{15\%}$-RC, and CA$_{20\%}$-RC.
Figure 3

(a) SEM image of BC, (b)-(f) the fracture surface SEM images of RC, CA$_5$%-RC, CA$_{10}$%-RC, CA$_{15}$%-RC, and CA$_{20}$%-RC, (g) stress-strain curves of RC and CA-RC with different CA contents, (h) diagram of the change in tensile strength and elongation at break, (i) Young's modulus and toughness of RC and CA-RC with different CA contents.

Hydrophilic and lipophilic properties
Figure 4

(a) Water and oil contact angle histogram of different samples, (b) changes of water absorption at 0.5, 1, and 24 h after incubating in water, (c) changes of oil absorption at 0.5, 1 and 24 h after incubating in vegetable oil.
Figure 5

Photos showing the food preservation of commercial PVC plasticwrap and CA$_{15\%}$-RC film at room temperature and 4°C.
Figure 6

(a)-(e) Digital photos showing the degradation of RC and CA-RC films in soil (at room temperature) over time. The films were stained with crystal violet for clarification.
Figure 7

Biocompatibility testing of materials, (a) the cytotoxicity of NIH/3T3 cells on RC film were assessed by CCK-8 after seeding for three days, (b)-(f) the confocal microscopic images showing cell density and morphology on RC, CA$_{5\%}$-RC, CA$_{10\%}$-RC, CA$_{15\%}$-RC, and CA$_{20\%}$-RC films after incubation for three days, (g) optical view of hemolysis on blood erythrocytes incubated with RC, CA$_{5\%}$-RC, CA$_{10\%}$-RC, CA$_{15\%}$-RC, and CA$_{20\%}$-RC. Positive control contains Triton X-100 (1% v/v), while negative control is saline.

Supplementary Files

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