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The gelation process and protein absorption property of injectable SA-CMBC hydrogel used for procoagulant material

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Carboxymethylated bacterial cellulose (CMBC) was composited with sodium alginate (SA) to obtain SA-CMBC hydrogel, cross-linked by calcium ion, which was generated from the hydrolyzing of calcium carbonate with the addition of glucono-delta-lactone (GDL). The addition of CMBC could regulate the properties of SA-CMBC hydrogel, such as gelation time, mechanical property, protein absorption and procoagulant property. The addition of CMBC shortened the gelation time, the shortest of which reached to 4.9 min, and increased the mechanical property, the best of which reached to 0.118MPa. The hydrogel with 25% weight fraction of CMBC showed the best protein absorption property. The procoagulant activity of the SA-CMBC hydrogel was investigated by activated partial thromboplastin time (APTT) and prothrombin time (PT), and hydrogel with 20% weight fraction of CMBC showed the best procoagulant property.

Introduction

Injectable hydrogel has attracted more and more attention in recent years. Hydrogel could be injected into specific positions with minimal trauma; drugs, bioactive molecules and cells or stem cells can be delivered to human body by simply mixing with hydrogel before injection. Hydrogel could conform into specific shapes in correspondence with the irregular positions where injected. Due to these advantages mentioned above, different kinds of injectable hydrogels could be used in various medical fields including drug controlled release[1, 2], tissue engineering scaffold[3], tissue repair[4, 5] and intraoperative hemostasis[6]. In addition, by controlling the gelation process and other properties of hydrogel, the injectable hydrogel could be used in wound dressing, tissue repair and intraoperative hemostasis fields.

Sodium alginate (SA), a well-known natural polysaccharide that carries a negative charge, has several favourable properties, including biocompatibility, non-toxicity and ease of gelation[7]. Due to these properties, SA has numerous applications on biomedical fields such as wound dressing[8, 9], cell encapsulation[10, 11], drug delivery[12, 13] and tissue engineering[14]. However, its applications are limited by the low mechanical performance and uncontrolled crosslinking speed of pure alginate hydrogel. To solve this problem, different components, such as chitosan[15], hyaluronate[16], calcium silicate[17], bioactive glass[18], have been composited with SA to improve its performance.

Bacterial cellulose (BC), a pure cellulose nanofiber produced by bacterial, is remarkable for its high mechanical strength and significant physical properties. Its high water content and purity enable the material biocompatible for multiple medical applications[19]. Furthermore, researches on different derivatives of BC, such as acetylation[20], sulfation[21], phosphorylation[22] and succinylation[23], have been directed toward special applications in the field of biomaterials. Carboxymethylated bacterial cellulose (CMBC) is a derivative of cellulose, which is obtained by reacting cellulose with sodium monochloroacetate in the presence of NaOH. CMBC has a wide range of applications, such as sewage purification[24], drug release[25], wound dressing[26], etc. Referring to the previous study in our laboratory, we have confirmed the protein absorption property of CMBC membrane[27]. Our group has also composited CMBC with SA and discussed the basic performances of the hydrogel preliminarily, like chemical structure, swelling property and mechanical property[28], whereas there is still no research reported about the potential biomedical applications of this composite hydrogel, such as its use in protein absorption and procoagualnt property.

In this study, we focused on exploring the possibilities of SA-CMBC hydrogel as an injectable protein absorption and procoagulant material, and we focused specifically on the influence of CMBC on the properties of injectable hydrogel. The hydrogel was injected before its crosslinking, at which time the fluidity of the hydrogel was suitable for injection. CMBC, acting as a new crosslinking agent, could adjust the gelation process, mechanical property and other properties of hydrogels. The components of the system were varied so that the gelation process of hydrogel could be adjusted to satisfy its injectable property. The protein absorption of hydrogel was investigated by ultraviolet spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR) and scanning electron microscopy (SEM). Furthermore, the procoagulant activity of the SA-CMBC hydrogel was investigated by the classical

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coagulation assays, activated partial thromboplastin time (APTT) and prothrombin time (PT).

Materials and methods

Materials

BC used in this study was supplied by the Hainan Yida Food Co, Ltd. BC was the gel-like cellulose membrane formed by A. xylinum AGR 60. As a pre-treatment to remove the bacterial cell debris, BC membranes were immersed in 0.1mol/L sodium hydroxide solution for 60 min at 90°C water bath, and then thoroughly washed to neutral by de-ionized water. The bovine serum albumin (BSA) for the adsorption experiments was used as received from Wako Pure Chemical Industries, Osaka, Japan. APTT and PT assay reagents were provided by Tianjin MD Pacific Technology Co, Ltd. The rats supplying the blood were bought from the Laboratory Animal Center of Chinese PLA General Hospital. All other reagents were reagent grade and used as received.

Preparation of the SA/CMBC hydrogel

CMBC was prepared from BC membranes according to our recent report[28]. After pre-treatment, BC membranes were broken up into fine fibers using a high-speed disperser. BC fibers were soaked in 10% sodium hydroxide solution, in which the BC/NaOH mass ratio was 1:9 and the proportion of water and ethanol was 3:4. The BC fibers were then stirred in a low speed at room temperature for 1 hour. Sodium chloroacetate (the molar weight of which was equal to NaOH) was then dissolved in a suitable amount of deionized water, and the solution was added into the mixture and stored at 90°C water bath for 4 hours. Finally, the entire mixture was centrifuged and neutralized with 10% HCl, and then thoroughly washed by deionized water to generate CMBC.

Sodium alginate was slowly added into deionized water to get the ratio of 2% (w/v) and the mixture was stirred using a magnetic stirrer. After complete dissolution of alginate, CMBC was added (using different weight fractions of CMBC from 10% to 30%) and the samples were stirred for more time. Then $CaCO_3$ (using various f-value, molar ratios of calcium ion and carboxyl) and glucono-delta-lactone (GDL, using various n-value, molar ratios of GDL and calcium ion) were added and stirred until evenly mixed. The solutions were allowed to stand for 2 hours to allow it fully form a gel. The samples with different weight fraction of CMBC were named as SA-CMBC10, SA-CMBC15, SA-CMBC20, SA-CMBC25 and SA-CMBC30, respectively.

Physical properties

To measure the gelation time, 5mL sol sample was put into the test tube and sloped every 30 seconds until the sample stopped flowing. The the gelation time was then recorded.

The hydrogel samples were kept still for 24 hours to fully gel. Then the compressive tests were performed by using a TA.XT Plus Texture Analyzer, equipped with two flat-surface compression stages. Before this test, the samples were cut into cuboids of 2 cm length, 2 cm width and 1cm height, and the sample size was measured using a standard caliper. The strain ramp rate was maintained at 1 mm per second for all of the tests.

Chemical structure information was recorded by FTIR (NICOLET 750) with the frequency ranging from 4000 cm^{-1} to 450 cm^{-1} and the resolution of 4 cm^{-1}. Before test, the hydrogels were freeze-dried using a freeze dryer (Labconco Corporation, USA).

Adsorption of BSA on the hydrogel

0.2 g BSA was dissolved in 100mL PBS (pH=3) solution in order to generate 2mg/mL BSA solution. The samples, which were cut into cuboids of 2 cm length, 2 cm width and 1cm height, were immersed in BSA solution and placed in constant temperature humidity chamber of 37°C and 60% humidity. At regular intervals, a certain volume of BSA solution was taken out and the ultraviolet absorption peak at 276 nm was measured. The standard curve of BSA with different concentrations was established in advance.

The hydrogels before and after protein absorption were freeze-dried and were tested by FTIR respectively. The morphologies and structures of hydrogels before and after protein absorption were examined by SEM (Apollo 300, 10 kV). The samples were freeze-dried and coated with gold in sputter coater under nitrogen atmosphere.

Procoagulant properties

The coagulant activity of the SA-CMBC hydrogel was investigated by the classical coagulation assays APTT and PT. Before the tests, different weight fractions of samples were prepared. Through the analysis of the value of APTT and PT, we could see the effect of weight fraction differences on coagulation activity. The assays were carried out according to the instructions of the manufactures.

The blood was collected from the jugular vein of rats. Then 0.109M (3.2%) trisodium citrate was well-mixed with whole blood in proportion of 1:9, and the mixture was centrifuged at 4°C and 3000r/min for 15 min. The upper plasma was taken out with a plastic pipette and used within 2 hours.

APTT assay summaries were as follows: 0.1 mL citrated normal rat plasma was added onto the surface of the hydrogel and incubated for 3 min at 37°C, then 0.1 mL APTT assay reagent, that was pre-incubated for 3 min at 37°C, was added and incubated for 5 min at 37°C. After that, 0.1 mL 0.025 mol/L $CaCl_2$ solution, which pre-incubated for 3 min at 37°C, was added and the clotting time was recorded. For the PT assay, 0.1 mL citrated normal rat plasma was added onto the surface of the hydrogel and incubated for 3 min at 37°C. Then 0.2 mL PT assay reagent, that was pre-incubated for 3 min at 37°C, was added and the clotting time was recorded.

Statistical analysis

All results were expressed as mean±standard deviation (SD) and statistical analysis of was performed by one-way analysis of variance (ANOVA). $P<0.05$ was considered as statistically significant.

Results and discussion

Gelation process

The gelation time was measured with the method of tilt test as shown in Fig. 1 A, B (A showed longer gelation time than B). With the passage of time, the cross-linking degrees of samples gradually increased whereas the mobility decreased continually until the steady gel was generated. Fig.1 C, D exhibited the gelation time reduced with the increasing of f-value and n-value. With the increasing of f-value, the total amount of calcium in the system raised; with the enhancement of n-value, GDL can be hydrolyzed to generate more gluconic acid, which decomposed calcium carbonate into more calcium ion in the same period. In view of the above-mentioned reasons, increasing of f-value and n-value could promote the cross-linking of sodium alginate and then shorten the gelation time. Fig. 1 E showed the change of gelation time over the weight fraction of CMBC. The gelation time shortened with the increasing of CMBC weight fraction at first, and the gelation time instead decreased when the weight fraction of CMBC continued increasing, which was explained in Fig. 2.

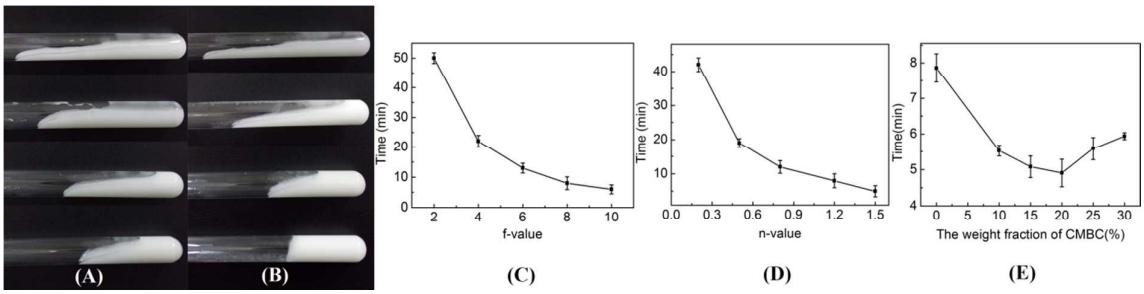

Fig. 1 (A, B) Image of gelation time measurement using tilt test;
Gelation time with different (C) f-value; (D) n-value; (E) weight fraction of CMBC

G-cells in alginate can combine with calcium ion to form stable "egg-shell" structure[29], making SA into steady gel (Fig. 2 A). With the addition of CMBC, calcium ions together with the carboxyl groups of CMBC could form the unstable flat grid structure[30], which enhanced the stability of gel (Fig. 2 B). Also, the CMBC fibers were fixed inside the hydrogel to enhance the stability. Therefore, the gelation time reduced with the increasing of the weight fraction of CMBC. However, when the weight fraction of CMBC was excessive, the reduction of SA, which was the principal part of cross-linking, would make the gel system unstable (Fig. 2 C), causing the gelation time to increase.

Chemical structure

The infrared spectroscopy curves of SA, CMBC and SA-CMBC were shown in Fig. 3 A. The SA-CMBC composites exhibited characteristic absorption bands at 3370 cm^{-1}(-OH stretching), 1595 cm^{-1} and 1412 cm^{-1}(-C=O stretching) and 1060 cm^{-1}(C-O-C stretching). There was no appearance or disappearance of any peaks associated with SA and CMBC, which proved that no bonding appeared between SA and CMBC within the composite hydrogel and that no chemical reaction occurred between SA and CMBC. Fig. 3 B showed the infrared spectroscopy curves of gels with different weight fractions of CMBC. From the figure, we can see that with the increase of weight fraction of CMBC, the absorption peak value of hydroxyl in 3370 cm^{-1}, carboxyl in 1412 cm^{-1} and 1595 cm^{-1}, and ether bond in 1060 cm^{-1} all improved. These changes of absorption peak were attributed to intermolecular interactions between the hydroxyl groups of CMBC and carboxyl groups of SA.

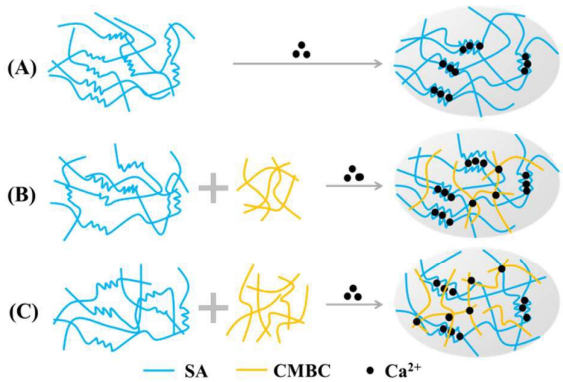

Fig. 2 Cross-linking mechanisms of (A) SA with no CMBC; (B) SA with moderate CMBC; (C) SA with excessive CMBC

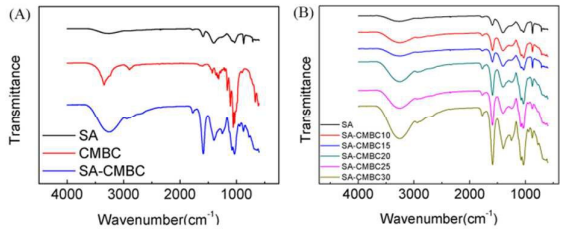

Fig. 3 (A) Infrared spectroscopy curves of SA, CMBC and SA-CMBC; (B) Infrared spectroscopy curves of gels with different weight fractions of CMBC

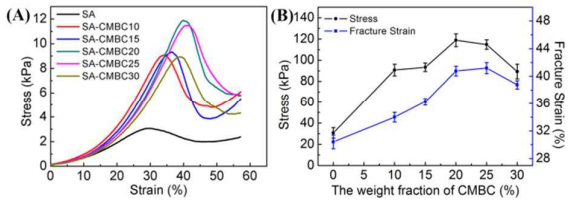

Figure 4 (A) Stress-strain curve of series of SA/CMBC composites; (B) Mechanical property with different weight fraction of CMBC

Mechanical property

Table 1 and Table 2 illustrated the changes of the maximum load over f-value and n-value when the gel system bear pressure stress. We can see that maximum load rose up with the increase of f-value and n-value at first, but it decreased while the f-value and n-value continued to increase. At the stage of load increasing, the system could generate more calcium ion with the increase of f-value and n-value, which could promote the cross-linking density of the gel and then increase the maximum load. However, when the f-value and n-value were too large, the reaction rate of calcium carbonate and gluconic acid hydrolyzed from GDL became so fast that the coproduct-carbon dioxide could not get away from the system and the residual carbon dioxide destroyed the gel structure, which lead to the reduction of gel strength.

Table 1 Mechanical property with different f-value

f-value	2	4	6	8	10
Stress/kPa	15.55	23.27	29.16	31.19	27.88

Table 2 Mechanical property with different n-value

f-value	2	4	6	8	10
Stress/kPa	15.55	23.27	29.16	31.19	27.88

Fig. 4 A displayed stress-strain curves of gels with different weight fractions of CMBC. The two curves in Fig. 4 B showed the mechanical properties of gels with different weight fraction of CMBC, which were the changes of maximum stress and fracture strain, respectively. The maximum stress and fracture strain increased with the addition of CMBC at first, and then decreased from a certain degree. With the addition of CMBC, its fibrous structure enhanced the strength of the gel, which increased the load that the gel could bear. While CMBC was overmuch, the decrease of SA would make the gel system tend to be unstable and reduced the gel strength. This kind of change could be explained by the gelation mechanism shown in Fig. 2.

Protein absorption

The SA-CMBC25 gel (the weight fraction of CMBC in gel was 25%) was placed in BSA solution of 2mg/mL and then the ultraviolet absorption spectrum of BSA solution was measured at regular intervals. The absorption quantity of BSA on different hydrogels over time was shown in Fig. 5 A. With the passage of time, the absorption quantity of BSA gradually increased, and the absorption rate reduced to zero after 40 hours, which meant the absorption of protein tended to be saturated. Fig. 5 B showed the final absorption quantity of BSA on hydrogels with different weight fractions. With the increase of CMBC weight fraction, the hydrogel absorbed more protein, and then the absorbing capacity instead dropped down when the weight fraction of CMBC added up to a certain degree. This outcome was due to the decrease of gelation degree and the unstable of the hydrogel structure. Comparing with our previous study[27], the maximum protein absorption capacity of CMBC membrane was about 20%, while which of SA-CMBC hydrogel reached to 80%. This outcome was because of the porous structure of SA-CMBC hydrogel, which allowed more protein entering inner material and improved protein absorption capacity.

The isoelectric point of BSA was 4.5-6.0. When the pH of solution was below the isoelectric point of BSA, BSA was positively charged, as at pH above the isoelectric point, BSA was negatively charged. Carboxyl in CMBC could be dissociated to be negatively charged in solution, so that at pH below the isoelectric point, BSA could be absorbed onto the hydrogel due to electrostatic interaction (Fig. 6). With the increasing of fraction weight of CMBC in composite hydrogel, the absorption capacity became higher.

Fig. 7 A, C showed surface morphology images (×5000) of hydrogels before and after absorbing protein, and Fig. 7 D was partial enlarged detail (×10000) of Fig. 7 C. It can be seen that contrasting with material before absorbing protein, particles with a few hundred nanometers size could be observed on the surface of material, which was considered as gather of protein absorbed on the material surface. The infrared spectroscopy curves in Fig. 7 B further approved the change of hydrogels before and after absorbing protein. Apparently, the characteristic absorption peak of Amide I in 1655cm^{-1}, which was characteristic absorption peak of protein, demonstrated the absorption of protein on the hydrogel. The cellular

functionality in terms of adhesion, proliferation and differentiation depends on protein adsorption on scaffold, which indicated the potential applications of injectable SA-CMBC hydrogel on tissue repair.

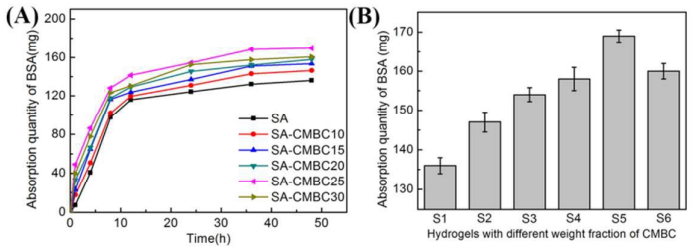

Figure 5 (A) Absorption quantity of BSA on different hydrogels over time; (B) Final absorption quantity of BSA on different hydrogels

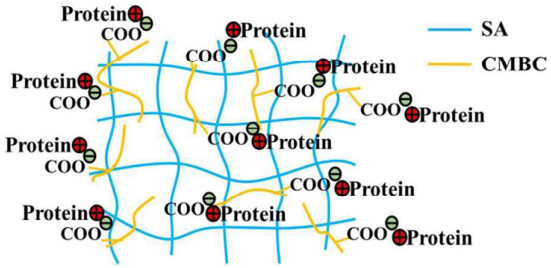

Figure 6 The protein absorption mechanism

Procoagulant properties

The APTT assay measures the coagulation factors in the intrinsic pathway, and the PT assay measures the activity of the extrinsic pathway[31]. Fig. 8 A, B were the results of APTT and PT tests, in which "Blank" was the control group and S1-S6 were hydrogels with different weight fractions of CMBC. The blank control group showed APTT as 24 s, while in test groups, the hydrogels showed APTT as less than 16 s. Moreover, the blank control group showed PT as 14 s, while in test groups, the hydrogels showed PT as less than 9 s. In general, the normal range of APTT and PT were 22-28s and 10-14s, respectively[32]. In fact, the clotting time of the plasma added with SA-CMBC hydrogel was conspicuously decreased. This outcome showed that the SA-CMBC hydrogel had a certain degree of procoagulant activities. We can see from the comparison between test groups that the sample of S4, in which the weight fraction of CMBC was 20%, showed superior promoting action in blood coagulation process. With the obvious procoagulant activity, the hydrogel could be a potential material for hemostasis.

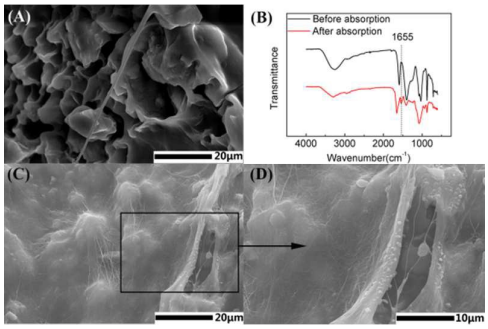

Figure 7 Surface morphology images (A) before and (C, D) after protein absorption; (B) FTIR spectrum of hydrogels

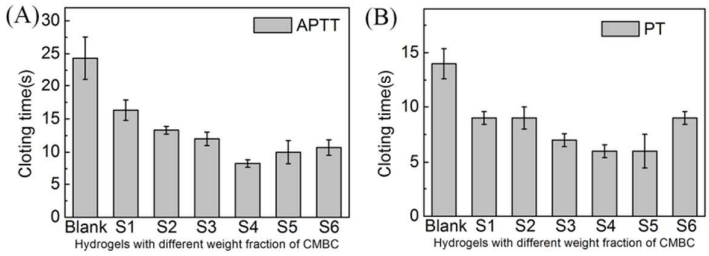

Figure 8 Procagulant activity of SA-CMBC hydrogel at different weight fraction of CMBC

Conclusions

In this study, bacterial cellulose was carboxymethylated to generate CMBC, which was composited with SA to obtain SA-CMBC hydrogel. CMBC existed obvious influence on properties of SA-CMBC hydrogel-gelation time, protein absorption and procoagulant properties. F-value of 8 and n-value of 0.5 gave rise to the best gelation process, which had shorter gelation time and better mechanical property. With different weight fractions of CMBC, the hydrogels showed different levels of promoting effects on protein absorption, and the hydrogel presents the best protein absorption property with 25% weight fraction of CMBC. The procoagulant property of SA-CMBC hydrogel reached to the best when the weight fraction of CMBC was 20%. Therefore, SA-CMBC hydrogel might be a desirable injectable protein absorption and procoagulant material that could be used in wound dressing, intraoperative hemostasis or other biomedical fields.

Acknowledgements

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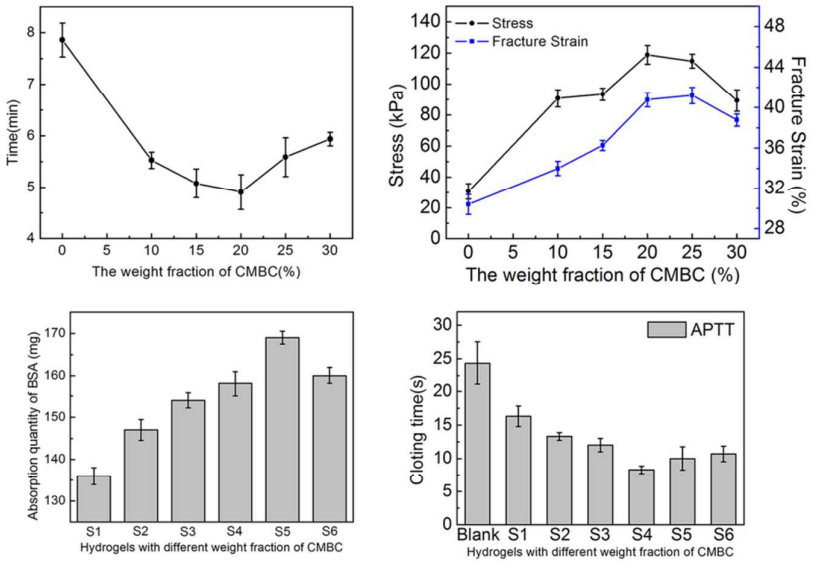
References

1. K. Li, L. Yu, X. Liu, C. Chen, Q. Chen and J. Ding, *Biomaterials*, 2013, **34**, 2834-2842.
2. Z. Lin, W. Gao, H. Hu, K. Ma, B. He, W. Dai, X. Wang, J. Wang, X. Zhang and Q. Zhang, *Journal of Controlled Release*, 2014, **174**, 161-170.
3. T. D. Johnson and K. L. Christman, *Expert opinion on drug delivery*, 2013, **10**, 59-72.
4. J. E. Frith, A. R. Cameron, D. J. Menzies, P. Ghosh, D. L. Whitehead, S. Gronthos, A. C. Zannettino and J. J. Cooper-White, *Biomaterials*, 2013, **34**, 9430-9440.
5. J. Wu, Q. Ding, A. Dutta, Y. Wang, Y. H. Huang, H. Weng, L. Tang and Y. Hong, *Acta biomaterialia*, 2015, **16**, 49-59.
6. R. Wang, B. Zhou, W. Liu, X.-h. Feng, S. Li, D.-f. Yu, J.-c. Chang, B. Chi and H. Xu, *Journal of biomaterials applications*, 2015, **29**, 1167-1179.
7. C. H. Goh, P. W. S. Heng and L. W. Chan, *Carbohydrate Polymers*, 2012, **88**, 1-12.
8. J. L. Shamshina, G. Gurau, L. E. Block, L. K. Hansen, C. Dingee, A. Walters and R. D. Rogers, *Journal of Materials Chemistry B*, 2014, **2**, 3924.
9. K. Y. Lee and D. J. Mooney, *Progress in polymer science*, 2012, **37**, 106-126.
10. I. Ghidoni, T. Chlapanidas, M. Bucco, F. Crovato, M. Marazzi, D. Vigo, M. L. Torre and M. Faustini, *Cytotechnology*, 2008, **58**, 49-56.
11. S. Utech, R. Prodanovic, A. S. Mao, R. Ostafe, D. J. Mooney and D. A. Weitz, *Advanced healthcare materials*, 2015, **4**, 1628-1633.
12. J. A. Chikar, J. L. Hendricks, S. M. Richardson-Burns, Y. Raphael, B. E. Pfingst and D. C. Martin, *Biomaterials*, 2012, **33**, 1982-1990.
13. W.-P. Voo, B.-B. Lee, A. Idris, A. Islam, B.-T. Tey and E.-S. Chan, *RSC Adv.*, 2015, **5**, 36687-36695.
14. Y. M. Kolambkar, K. M. Dupont, J. D. Boerckel, N. Huebsch, D. J. Mooney, D. W. Hutmacher and R. E. Guldberg, *Biomaterials*, 2011, **32**, 65-74.
15. L. Lacerda, A. L. Parize, V. Favere, M. C. Laranjeira and H. K. Stulzer, *Materials science & engineering. C, Materials for biological applications*, 2014, **39**, 161-167.
16. D. S. Morais, M. A. Rodrigues, T. I. Silva, M. A. Lopes, M. Santos, J. D. Santos and C. M. Botelho, *Carbohydrate Polymers*, 2013, **95**, 134-142.
17. Y. Han, Q. Zeng, H. Li and J. Chang, *Acta biomaterialia*, 2013, **9**, 9107-9117.
18. Y. Luo, C. Wu, A. Lode and M. Gelinsky, *Biofabrication*, 2013, **5**, 015005.
19. N. Petersen and P. Gatenholm, *Applied microbiology and biotechnology*, 2011, **91**, 1277-1286.
20. L. C. Tomé, R. J. B. Pinto, E. Trovatti, C. S. R. Freire, A. J. D. Silvestre, C. P. Neto and A. Gandini, *Green Chemistry*, 2011, **13**, 419.
21. Z. Qin, L. Ji, X. Yin, L. Zhu, Q. Lin and J. Qin, *Carbohydr Polym*, 2014, **101**, 947-953.
22. T. Oshima, S. Taguchi, K. Ohe and Y. Baba, *Carbohydrate Polymers*, 2011, **83**, 953-958.
23. X. Yin, C. Yu, X. Zhang, J. Yang, Q. Lin, J. Wang and Q. Zhu, *Polymer Bulletin*, 2010, **67**, 401-412.
24. S. Chen, Y. Zou, Z. Yan, W. Shen, S. Shi, X. Zhang and H. Wang, *Journal of hazardous materials*, 2009, **161**, 1355-1359.
25. X. Shi, Y. Zheng, G. Wang, Q. Lin and J. Fan, *RSC Adv.*, 2014, **4**, 47056-47065.
26. J. W. Yu, X. L. Liu, C. S. Liu and D. P. Sun, 2011, *Materials Science Forum* , 2011, **685**, 322-326.
27. Q. Lin, Y. Zheng, G. Wang, X. Shi, T. Zhang, J. Yu and J. Sun, *International journal of biological macromolecules*, 2015, **73**, 264-269.
28. Q. Lin, Y. Zheng, L. Ren, J. Wu, H. Wang, J. An and W. Fan, *Journal of Applied Polymer Science*, 2014, **131**, 3948–3957.
29. G. T. Grant, E. R. Morris, D. A. Rees, P. J. Smith and D. Thom, *FEBS letters*, 1973, **32**, 195-198.
30. M. Nara, H. Torii and M. Tasumi, *The Journal of Physical Chemistry*, 1996, **100**, 19812-19817.
31. K. Matsubara, Y. Matsuura, A. Bacic, M.-L. Liao, K. Hori and K. Miyazawa, *International journal of biological macromolecules*, 2001, **28**, 395-399.
32. L. Fan, X. Zhou, P. Wu, W. Xie, H. Zheng, W. Tan, S. Liu and Q. Li, *International journal of biological macromolecules*, 2014, **66**, 245-253.

Graphical abstract



CMBC showed obvious influence on properties of injectable SA-CMBC hydrogel, containing gelation time, mechanical property, protein absorption and procoagulant property.