A novel dual responsive nanocomposite double network hydrogel with good mechanical property

Yang Chen, Shiya Qiao, Junrong Yu*, Yan Wang, Jing Zhu and Zuming Hu
State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, Shanghai 201620, China
Address: No.2999 North Renmin Road, Songjiang District, Shanghai, China.
Correspondence to: Junrong Yu (E-mail: yjr@dhu.edu.cn)

Abstract. Mechanical strong intelligent hydrogels have drawn the increasing attention in recent decades. We have designed a novel mechanical strong biocompatible dual pH- and temperature- responsive carboxymethyl chitosan (CMCTs)/graphene oxide (GO)/poly (N-isopropylacrylamide) (PNIPAM) nanocomposite double network hydrogel by a simple, one-pot in situ free radical polymerization initiated by the ultraviolet light, with the GO nanosheets as the crosslink centers instead of toxic common organic crosslinkers. CMCTs, EDC/NHS, or GO nanosheets are the pivotal factors for fabricating the mechanical strong dual responsive hydrogel. By adjusting the above factors, the optimal mechanical strong hydrogel displayed a high compressive strength of 0.792 MPa at the breaking strain of 0.836. Besides, we also investigated the relationship between the swelling and deswelling behaviors of the synthesized hydrogel and the above mentioned factors. Owing to this outstanding performance, they may have the potential application in drug delivery system and chemical valves in the future.

1. Introduction
Hydrogels, cross-linked three dimensional polymer networks consisting of a great amount of water, have been extensively investigated in recent years in various biomedical areas including drug delivery systems, biosensors and chemical valves actuators. Since the intrinsic structural heterogeneity and/or lack of efficient energy dissipation mechanism [1], most of the synthesized hydrogels are usually brittle and weak, which greatly restrict their high-end applications, such as tendon, muscle, ligament and cartilage. According to the dissipation-induced toughening theory [2], enormous efforts has been made to construct tough and mechanical strong hydrogels in recent decades, such as hydrophobically associated hydrogels [3], dual cross-linked single network hydrogels [4], double network (DN) hydrogels [5] and nanocomposite (NC) hydrogels [6]. Generally speaking, the developing of NC and DN hydrogels are an effective way to enhance the mechanical strength of the synthesized hydrogels.

Graphene oxide (GO) has drawn increasing attention in the past decade owing to the extremely large surface area, large amount of polar functional groups, excellent mechanical strength and low costs for mass production [7]. Apart from acting as the reinforcing fillers, GO nanosheets can also be used as the crosslink centers instead of the common used organic molecules, which will enhance the biocompatibility of the prepared hydrogels. Fan et al. [8] have synthesized the mechanical strong polyacrylamide (PAM)/sodium alginate DN hydrogels with GO as the reinforcing fillers. Ruan et al. [9] have synthesized the carboxymethyl chitosan (CMCTs)/GO nanocomposite hydrogels with the GO
nанослоя, действующих как центры кросслигирования, используя N-(3-диметиламино пропил)-N-этилкарбодимиддиэтилхлорид (EDC) и N-гидрокси суцинимид (NHS) как био-кросслигатор, связывающий карбоксил и амин между CMCTs и графитными нанослоями.

CMCTs, derived from chitosan, have plenty of active pendant groups on its polymer chains, such as hydroxyl, amino and carboxyl, which are good for preparing the biocompatible pH- and temperature- responsive hydrogels, drug delivery systems and biomedical scaffold. Cheng et al.\cite{10} have synthesized the pH- and temperature- responsive poly(N-isopropylacrylamide) (PNIPAM)-g-CMCTs hydrogels, which has a good biocompatibility and can be used as the drug carrier of cisplatin. Gorgieva et al.\cite{11} have prepared the chitosan-gelatin hydrogels, which have a good biocompatibility and can be used as the scaffolds. However, the above fabricated hydrogels usually are brittle and weak.

Taking the above factors into consideration, we proposed a facile, one-pot in situ free radical polymerization to synthesize a novel pH- and temperature- responsive CMCTs/GO/PNIPAM NC DN hydrogels, which use GO as the crosslink centers instead of the organic cross-linker to improve the biocompatibility and mechanical properties of the hydrogels. We got the optimum conditions for preparing the mechanical strong CMCTs/GO/PNIPAM hydrogels through changing the crucial factors, the concentration of CMCTs, EDC/NHS and GO nanosheets. Besides, the fabricated NC DN hydrogels also have the obvious pH and temperature responsive properties. These new hydrogels will have the potential use in tissue engineering and drug delivery systems in future.

2. Materials and methods

2.1 Materials

Carboxymethyl chitosan (CMCTs) and graphene oxide were prepared as described in our previous work\cite{5}. The chemicals below were all analytical grade and used as received.

2-hydroxy-4’-(2-hydroxyethoxy)-2-methylpropophenone (I2959) were supplied by the Tokyo Chemical Industry Co., Ltd., Japan. N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich Co., Ltd., America. 4-Morpholineethanesulfonic acid (MES) and N-isopropylacrylamide (NIPAM) was supplied by Sinopharm Chemical Reagent Co., Ltd., China.

2.2 Synthesis of the CMCTs/GO/PNIPAM hydrogel

The synthesis process of CMCTs/GO/PNIPAM NC DN hydrogel is schematically shown in Fig. 1. First of all, a predetermined amount of GO aqueous solution was diluted to a certain concentration with 0.65 M MES aqueous solution in a breaker and a quantitative amount of CMCTs was added in. Subsequently, the mixture was stirred for 24 h to obtain the uniformly dispersed solution. Secondly, a calculated amount of NIPAM and I 2959 was added in and stirred for another 2 h. Then, a given EDC/NHS (with the molar ratio 4:1) was added in and stirred for the last 0.5 h to achieve the homogenous solution. Following by the solution was bubbled with nitrogen for 15 min and then it was put into the 20 °C water bath for 24 h to get the cross-linked CMCTs/GO/PNIPAM hydrogel. At last, the cross-linked hydrogel was irradiated under the ultraviolet (UV) light (365nm, 6W) for 2 h.

The obtained hydrogels are designed as CmGnPxH in this paper, where C, G, P and H represent CMCTs, GO, PNIPAM and hydrogels, respectively; m stands for the concentration of CMCTs (g/ml), n stands for the feeding amount of GO to the weight of NIPAM (%) and x stands for the added weight amount of EDC (mg); the totally water content is 3 mL and the weight of NIPAM is fixed at 0.3 g.
2.3 Characterization
Fourier transform infrared (FTIR) spectra of the dried hydrogel samples were measured with a Nicolet 8700 spectrometer (U.S.A). The cross-section images of the hydrogels were achieved by a Quanta 250 (FEI Company, U.S.A) environment scanning electron microscope (ESEM) at the voltage of 10 kV. X-ray diffraction (XRD) measurements were performed on a SAXS instrument (Japan) using Cu kαradiation (λ=0.154 nm) in a step of 0.02ºs⁻¹ with the angle range from 5º to 60º. Raman spectra were carried out on an Invia-Reflex spectrometer (U.S.A) with 532 nm laser excitation.

2.4 Compressive test of the hydrogels
For the compressive test, hydrogels were synthesized in the glass tubes with the diameter of 8.47 mm and the length of 30 mm. After the hydrogel samples were pushed out from the tubes, they were immediately cut into the length about 6 mm and tested by an INSTRON 5969 instrument (U.S.A). The compressive test conditions are listed as below: initial gauge length of 6 mm, crosshead speed of 10 mm min⁻¹ and the temperature of 20 ºC. For the tests in 2.4-2.7, each sample was tested at least three times and the average value was taken.

2.5 Swelling behavior of the hydrogels
The equilibrium swelling ratios (ESR) of different dried hydrogel samples were measured in different pH value phosphate buffers. A given amount of dried hydrogels were immersed in different pH value PBS solutions at 25 ºC for 24 h. After taken out from the PBS solutions, the hydrogels’ excess surface water was removed with filter paper before weighted. The ESR value of the hydrogel samples were obtained from the following equation[12]:

\[
ESR = \frac{(W_e - W_d)}{W_d}
\]

Where, \(W_e\) and \(W_d\) stand for the weight of the dried and swollen hydrogels, respectively.

2.6 Deswelling behavior of the hydrogels
The deswelling behavior of the hydrogel samples was investigated by monitoring the water content in hydrogels. The water retention (WR) related to the deswelling ratio was defined as:

\[
WR = \frac{(W_t - W_d)}{(W_e - W_d)}
\]

Where, \(W_t\) represents the weight of swollen hydrogels at different time intervals after the equilibrium hydrogels at 25ºC were quickly transferred to the deionized water at 45ºC.

3. Results and discussion

3.1 FTIR analysis
Fig. 2 (a) The FTIR spectra of GO, CMCTs, CPH and CGPH. (b) The XRD spectra of GO, CMCTs, CPH and CGPH. (c) The Raman spectra of GO, CGPH, CMCTs and CPH.

As illustrated in Fig. 2a, it is clearly to see the spectra of GO, CMCTs, CPH and CGPH. The spectrum of GO displays characteristic peaks at 1725, 1624, 1390, 1221 and 1064 cm\(^{-1}\), which stands for C=O carbonyl stretching, aromatic C=C deformation vibration, aromatic O-H deformation vibration, asymmetric and symmetric C-O stretching in the C-O-C group, respectively\[13\]. In the FTIR curve of CMCTs, the broad peak around 3400-3200 cm\(^{-1}\) is refer to both the N-H and O-H stretching vibrations, and the peak at 2900 cm\(^{-1}\) is belong to the C-H stretching vibrations. The peaks near 1416 and 1601 cm\(^{-1}\) are associated with the symmetric and asymmetric stretching vibration of COO- group respectively\[14\]. In the spectrum of CPH, the characteristic peaks at 3295, 2973, 1641, 1543, 1459 and 1387 cm\(^{-1}\) are attributed to O-H stretching vibration, C-H and N-H stretching vibration, C=O stretching of amide group, N-H deformation vibration, -CH\(_3\) and –CH(\(\text{CH}_3\))\(_2\), respectively \[15\]. By comparing the FTIR spectra of CPH and CGPH, the N-H stretching vibrations in CPH located at 3295 cm\(^{-1}\) shifts to 3304 cm\(^{-1}\), and the peak of C=O stretching of the amide group moves from 1641 to 1644 cm\(^{-1}\), which infers that with the addition of GO, there exists a strong hydrogen bonding interaction in the CGPH. By contrasting the spectra of CPH and CGPH, it is easily to see that the characteristic peak at 3297 cm\(^{-1}\) in CPH shifts to 3306 cm\(^{-1}\) in CGPH. The predominated reason might be that with the addition of more GO nanosheets, the number of the hydrogen bonds increases a lot in CGPH, which making the peak at 3297 cm\(^{-1}\) change a little.

3.2 XRD spectrum
The XRD spectra of GO, CMCTs, CPH and CGPH are shown in Fig. 2b. A lower characteristic diffraction peak of GO is located at 2\(\theta\) = 10.18\(^\circ\), related with an interlayer distance of 0.877 nm, which is in line with the literature reported before\[16\]. From the curve of CGPH, it is obvious to see that there is no peak appear at 2\(\theta\) = 10.18\(^\circ\), which demonstrates that GO have a good dispersability in hydrogels. The representative peak of CMCTs is located at 2\(\theta\) = 20.5\(^\circ\). With the incorporation of PNIPAM, a new typical peak appears at 2\(\theta\) = 8.6\(^\circ\) in CPH, which refers to the diffraction peak of PNIPAM; with the addition of GO, this peak shifts from 2\(\theta\) = 8.6\(^\circ\) to 6.8\(^\circ\), which means that the added GO increase the distance between the polymer chains (CMCTs and PNIPAM).

3.3 Raman spectrum
There are usually two evidently characteristic bands in the Raman spectra of carbon materials, D (~1350 cm\(^{-1}\)) and G band (~1590 cm\(^{-1}\))\[17\]. As illustrated in Fig. 2c, the GO nanosheets show two typical characteristic peaks at 1350 and 1590 cm\(^{-1}\). In general, the intensity ratio of ID/IG of GO is about 0.8, however, the ID/IG ratio in CGPH is about 1.05. The mainly reason for this phenomenon is that part of the carboxyl groups on GO have already reacted with amino on CMCTs, which results in more defects on the surface of the GO, and simultaneously the G band of GO shifts to 1600 cm\(^{-1}\) in CGPH. Although the defects on the surface of GO nanosheets increase, the Raman pattern remains, which demonstrates that the overall graphitic structure is reserved\[18\].

3.4 Mechanical properties
As shown in Fig. 3, with the addition of more CMCTs, EDC/NHS or GO, the breaking compressive
strength at first increases and then decreases; the compressive modulus increases while the breaking strain decreases. The predominant reason for this phenomenon is that as the content of CMCTs, EDC/NHS or GO increases, the reaction speed increases gradually, resulting in more crosslink points, shorter crosslink chains in the hydrogels (lower breaking compressive strain) and higher compressive modulus ($\varepsilon_c = 0.05-0.1$). However, if the reaction speed is too quickly, the crosslink density in the hydrogels would be too high, leading to more irregular inter-crossing polymer chains, lower compressive strength, and shorter polymer chains. It is easily to infer that the content of CMCTs, EDC/NHS or GO has predominant effect on the compressive properties of the designed hydrogels and the optimal prepared hydrogel has the highest compressive strength of 0.792 MPa at the breaking strain of 0.836.

3.5 SEM images

From Fig.4, it is evidently to see that the porous structure of the NC DN hydrogels is generally at the micrometer level. With the introduction of more CMCTs, the interaction between the polymer chains increases, resulting in shorter pore size (Fig 4a-d). As the cross-linker (EDC/NHS) increases, the crosslink density also increases, leading to smaller pores in the hydrogels. Similarly, with the incorporation of more GO nanosheets, the interaction between the polymer chains also increases, results in more crosslink points and smaller pores in the hydrogels. Comparing with the results of the mechanical property, we can concluded that when the content of CMCTs, EDC/NHS or GO nanosheets is higer, the pores in the hydrogels would be denser and smaller, resulting in higher mechanical strength; however, when the pore size is too dense and small, there would be more stress concentration points in the hydrogels, leading to lower compressive strength.

3.6 Swelling behaviors

From Fig.5, it exhibits the swelling behaviors of CGPH in different pH values at the temperature of 25 °C. Generally speaking, it is obvious to see that as the pH value increases, ESR of the synthesized hydrogels decreases (pH = 2-6), and subsequently increases (pH = 7-12). When pH =2, although large
amount of hydrogen bonds are existed in the hydrogels, there are also exist plenty of –NH$_3^+$ ions. At this time, the repulsive force between –NH$_3^+$ plays the major role in swelling, so the ESR value is high. As the pH value increase from 2-6, the hydrogen bonds decrease greatly while the –NH$_3^+$ ions vanish slowly, so the ESR value of the hydrogels decreases gradually. When the pH value is higher (7-12), there are lots of –COO- ions on the CMCTs polymer chains, the repulsive force between them making the ESR value increase.

At the same pH value, as the content of CMCTs or EDC/NHS increase, the corresponding ESR values decreases; the mainly reason for this regular might be that there are more crosslink points in the hydrogels, which keeps step with SEM results. However, with the incorporation of more GO nanosheets, ESR value increases at first and then decreases. The interpretation for this phenomenon might be that when the additive amount of GO nanosheets is little, these nanosheets mainly make the distance between the polymer chains increases but can’t form the effective crosslink points between them, hence the corresponding ESR value increases; as the content of GO nanosheets continue increases, the effective crosslink would be formed, which leads to more crosslink density and lower ESR value of the hydrogels.

3.7 Deswelling behaviors
The deswelling behavior of the fabricated hydrogels was shown in Fig. 6. It is easily to see that with the addition of more CMCTs or EDC/NHS, the deswelling rate of the hydrogels decreases dramatically and the final water content increases. The main reason for this phenomenon perhaps is that the crosslink density increases greatly as the content of CMCTs or EDC/NHS increases, making the water diffuse out from the gels more difficult, which can be supported by the images in Fig. 4. However, as the content of GO nanosheets increases, the deswelling rate of the gels increases and the final water content decreases at first and then increases. The mainly reason probably is that the addition of GO nanosheets benefits the motion of water molecule but whether the GO nanosheets could form the effective crosslink may have different results on the final water content(see the interpretation in Section 3.6).

4. Conclusion
In conclusion, we synthesized a novel mechanical strong nanocomposite double network CMCTs/GO/PNIPAM hydrogel through a facile, one-pot in situ free radical polymerization irradiated by the UV light, using the GO nanosheets as the crosslink centers instead of the organic molecules to enhance the biocompatibility and mechanical property of the hydrogels. Under the optimal conditions,
the designed hydrogel exhibited the highest compressive strength of 0.792 MPa at the failure strain of 0.836. Furthermore, the hydrogel also had obvious dual pH- and temperature-responsive properties, and their swelling and deswelling behaviors was intimately associated with content of CMCTs, EDC/NHS or GO nanosheets. This valuable property makes the fabricated hydrogels potential as the drug delivery carriers.

Acknowledgement
This work was financially supported by Shanghai International S&T Cooperation Fund (16160731302), Natural Science Foundation of China (No.51473031) and Nature Science Foundation of Shanghai (Grant No. 17ZR1401100).

References
[1] Sun J-Y, Zhao X, Illeperuma WRK, Chaudhuri O, Oh KH, Mooney DJ, et al. Highly stretchable and tough hydrogels. Nature. 2012;489:133.
[2] Zhao X. Multi-scale multi-mechanism design of tough hydrogels: building dissipation into stretchy networks. Soft Matter. 2014;10(5):672-87.
[3] Cui Z, Cheng R, Liu J, Wu Y, Deng Y. Hydrophobic association hydrogels based on N-acryloyl-alanine and stearyl acrylate using gelatin as emulsifier. RSC Adv. 2016;6(45):38957-63.
[4] Fan H, Wang J, Jin Z. Tough, Swelling-Resistant, Self-Healing, and Adhesive Dual-Cross-Linked Hydrogels Based on Polymer–Tannic Acid Multiple Hydrogen Bonds. Macromolecules. 2018;51(5):1696-705.
[5] Chen Y, Song G, Yu J, Wang Y, Zhu J, Hu Z. Mechanically strong dual responsive nanocomposite double network hydrogel for controlled drug release of aspirin. J Mech Behav Biomed 2018;82:61-9.
[6] Chen Y, Wang H, Yu J, Wang Y, Zhu J, Hu Z. Mechanically strong and pH-responsive carboxymethyl chitosan/graphene oxide/polyacrylamide nanocomposite hydrogels with fast recoverability. J Biomat Sci-Polym 2017;28(16):1899-917.
[7] Avouris P, Chen Z, Perebeinos V. Carbon-based electronics. Nat Nanotechnol. 2007;2:605.
[8] Fan J, Shi Z, Lian M, Li H, Yin J. Mechanically strong graphene oxide/sodium alginate/polyacrylamide nanocomposite hydrogel with improved dye adsorption capacity. J Mater Chem A. 2013;1(25):7433-43.
[9] Ruan J, Wang X, Yu Z, Wang Z, Xie Q, Zhang D, et al. Enhanced Physiochemical and Mechanical Performance of Chitosan-Grafted Graphene Oxide for Superior Osteoinductivity. Adv Funct Mater. 2016;26(7):1085-97.
[10] Cheng C, Xia D, Zhang X, Chen L, Zhang Q. Biocompatible poly(N-isopropylacrylamide)-g-carboxymethyl chitosan hydrogels as carriers for sustained release of cisplatin. J Mater Sci. 2015;50(14):4914-25.
[11] Gorgieva S, Kokol V. Preparation, characterization, and in vitro enzymatic degradation of chitosan-gelatine hydrogel scaffolds as potential biomaterials. J Biomed Mater Res A. 2012;100 (7):1655-67.
[12] Huang T, Xu HG, Jiao KX, Zhu LP, Brown HR, Wang HL. A Novel Hydrogel with High Mechanical Strength: A Macromolecular Microsphere Composite Hydrogel. Adv Mater. 2007;19(12):1622-6.
[13] Seol YG, Trung TQ, Yoon O-J, Sohn I-Y, Lee N-E. Nanocomposites of reduced graphene oxide nanosheets and conducting polymer for stretchable transparent conducting electrodes. J Mater Chem. 2012;22(45):23759-66.
[14] Selvakumaran S, Muhamad II, Abd Razak SI. Evaluation of kappa carrageenan as potential carrier for floating drug delivery system: Effect of pore forming agents. Carbohydr Polym. 2016;135:207-14.
[15] Kono H, Teshirogi T. Cyclodextrin-grafted chitosan hydrogels for controlled drug delivery. Inter J
Biol Macromol. 2015;72:299-308.

[16] Justin R, Chen B. Characterisation and drug release performance of biodegradable chitosan–graphene oxide nanocomposites. Carbohyd Polym. 2014;103:70-80.

[17] Zhong M, Liu Y-T, Xie X-M. Self-healable, super tough graphene oxide-poly(acrylic acid) nanocomposite hydrogels facilitated by dual cross-linking effects through dynamic ionic interactions. J Mater Chem B. 2015;3(19):4001-8.

[18] Park S, Lee K-S, Bozoklu G, Cai W, Nguyen ST, Ruoff RS. Graphene Oxide Papers Modified by Divalent Ions—Enhancing Mechanical Properties via Chemical Cross-Linking. ACS Nano. 2008;2(3):572-8.