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Fracture Behaviour of Bacterial Cellulose Hydrogel: Microstructural Effect

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Abstract

A growing interest in fibrous biomaterials, especially hydrogels, is due to a fact that they promise a good potential in biomedical applications thanks to their attractive biological properties and similar microstructure that mimics its *in vivo* environment. Since they are usually employed as a main load-bearing-component when introduced into body environment, a comprehensive understanding of their application-relevant mechanical behaviour, such as deformation and fracture, as well as structure-function relationships is essential. To date, deformation behaviour and mechanisms of hydrogels were well documented; still, a lack of understanding of their fracture behaviour, especially structure-function relationships, could complicate an evaluation of their applicability. Hence, this work carried out four types of test – uniaxial tension, single-notch, double-notch and central-notch fracture testing – to investigate fracture behaviour of fully-hydrated and freeze-dried bacterial cellulose (BC) hydrogel. Our results support a significant role of interstitial water – free and bonded water – played in fracture behaviour of the studied BC hydrogel.

Keywords: fracture behaviour, bacterial cellulose hydrogel, microstructural effect, high water content, porosity

1. Introduction

Some hydrogel biomaterials are considered as potential replacements for soft tissues acting as a main load-bearing component. Thanks to their good biocompatibility and microstructure similar to that of real tissues (Shi et al., 2014),

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they found numerous potential applications as components for wound dressing (Fu et al., 2012; Fu et al., 2013), drug-delivery systems (Huang et al., 2013), direct implants (e.g. ear cartilage (Nimeskern et al., 2013), cornea (Wang et al., 2010), bloody vessels (Malm et al., 2012; Zang et al., 2015), etc.) and scaffold materials for both BC-based biomaterials (e.g. artificial heart-valve leaflets (Millon et al., 2006), etc.) and *in-vitro* tissue regeneration (e.g. muscle (Bäckdahl et al., 2006), peripheral nerves (Kowalska-Ludwicka et al., 2013), etc.).

Recently, a growing interest to assess their potential applications was focused on characterization of their application-relevant mechanical behaviour (Zhao et al., 2014, 2015). Gao et al. (2015) performed an *in aqua* cyclic tensile and compressive tests at 37°C to study inelastic behaviour of a bacterial cellulose (BC) hydrogel, suggesting that its non-elastic (viscoplastic) deformation was accomplished with elastic deformation mainly resulting from formation of entanglements and a fibre-reorientation process. Hydrogels mostly consists of a fibrous network embedded into a high content of interstitial water. Due to a viscous contribution of water and fibre-water interaction, they demonstrate typical creep (Gao et al., 2016a) and stress-relaxation behaviours (Gao et al., 2016b) with stress dependence. In particular, an anomalous strain-rate-dependent behaviour, with transitions between various behaviours – insensitive to strain rate, strain-rate hardening and strain-rate softening – was documented (Gao et al., 2016c).

In fibrous biomaterials, fibres with stiffness that is higher than that of water dominate a load-bearing process when undergoing deformation; also, their arrangement and properties play a significant role in toughness of fibrous network, which is of vital importance to biomedical practice. A first understanding of individual components – fracture behaviour of fibrous network – was largely achieved by performing fracture testing using notched specimens accompanied with structural observations during experiments (Koh et al., 2013; Yang et al., 2015; Ridruejo et al., 2015). It was demonstrated that fibre reorientation in the vicinity of the notch tip dominates fracture behaviour of the fibrous network; still, fracture behaviour of a more complex system, i.e. involving aqueous environment as in hydrogels, was rarely investigated mainly due to the challenges to observe microstructural changes.

BC hydrogels consist of high-crystalline cellulose fibres surrounded with bound water that form hydrogen bonds. Fibres are naturally interweaved and randomly distributed in a fibrous layer. Some fibres acting as cross-links interconnect layers to construct a multi-layer nonwoven-like structure with a high porosity. Two groups of BC specimen were prepared – fully hydrated and freeze-dried BC hydrogels – to study network behaviour *in aqua*. Four types of test – uniaxial tension, single-notch, double-notch and central-notch fracture testing – were performed to quantify fracture behaviour of each group of specimens. Micro-morphological observations with SEM were used to study network behaviour in the vicinity of the notch tip in a process of deformation. Our results evidenced the significant role of interstitial water played in fracture behaviour of the studied BC hydrogel.

2. Materials and Method

2.1. Synthesis of bacterial cellulose hydrogel

Gluconacetobacter xylinum (ATCC53582) was used for bio-synthesis of the studied BC hydrogel. The bacterium was cultured in a Hestrin and Schramm (HS) medium, which was composed of 2% (weight) glucose, 0.5% (weight) yeast extract, 0.5% (weight) peptone, 0.27% (weight) disodium phosphate and 0.15% (weight) citric acid. After incubating statically for 7 days at 30°C and achieving the thickness of BC hydrogel in the range approximately from 3 mm to 5 mm, its samples were dipped into deionized (DI) water for 2 days, and then steamed by boiling in a 1% (weight) NaOH solution for 30 mins to eliminate bacteria and proteins. Afterwards, the BC hydrogels were purified by washing in DI water until its pH value approached 7, and then were stored in DI water at 4°C. In a natural state, the BC hydrogel demonstrates a randomly distributed fibrous layers with some cross-links to interconnect them, forming a multi-layered structure with a large space between layers to hold water.

2.2. Sample preparation

Two states of BC specimens – fully-hydrated (Fig. 1b) and freeze-dried (Fig. 1c) – were employed in this work. Wet BC hydrogel sheets were first cut into specimens for uniaxial tension, single-notch, double-notch and central-notch fracture testing with dimensions shown in Fig. 1a. It is worthwhile noting that the lowest cross-section width was the same in all the specimens – 4 mm. Second, in order to minimize the effect of a cutting procedure, all the specimens were immersed into DI water for 2 hrs. Third, a half of the specimens for a fully hydrated state were still stored in DI water at 4°C while the rest – used for the freeze-drying state – were placed into a freeze-drier for 24 hrs to remove interstitial water. Finally, the Congo red was used to make a system of marks on the surface of specimens for a further study using digital image correlation.

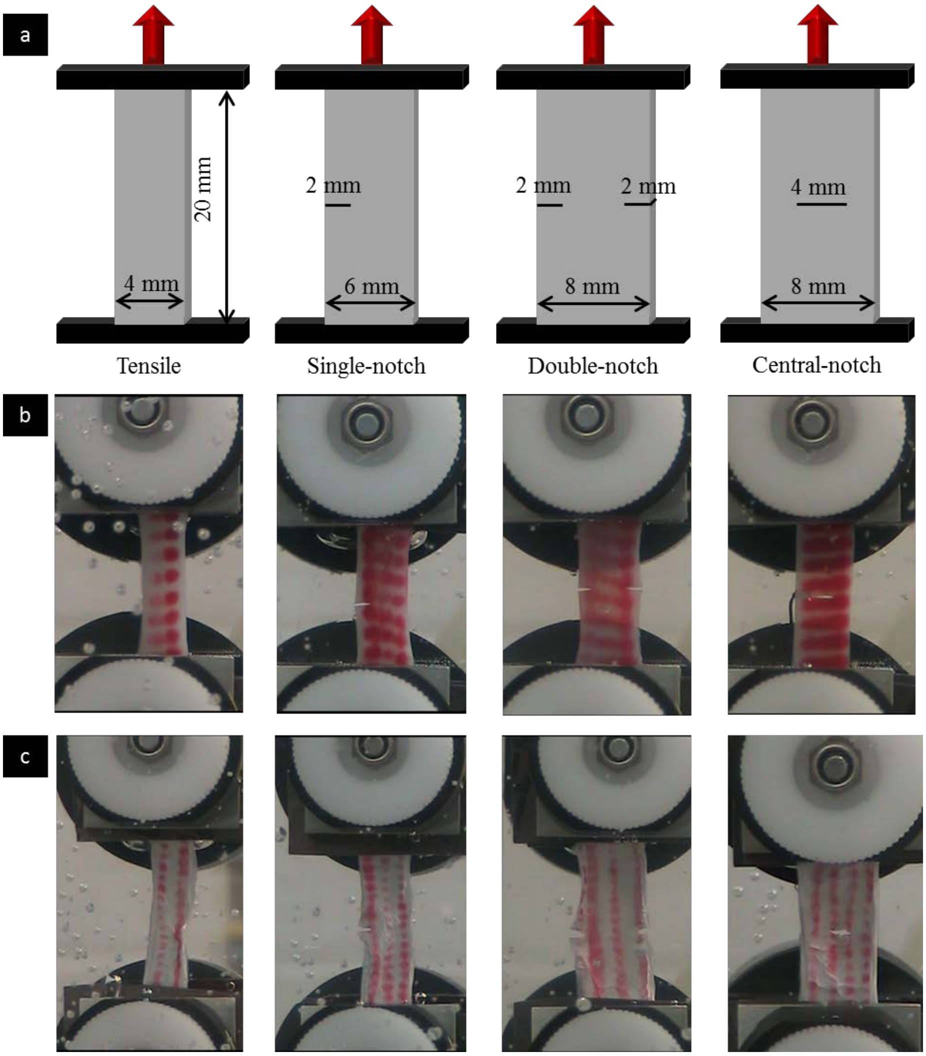

Fig. 1. Dimensions of specimens used in uniaxial tension, single-notch, double notch and central notch fracture testing (a) for fully-hydrated (b) and freeze-dried (c) specimens of BC hydrogels

2.3. Experimental procedure

A universal commercial testing system with a Bio-Puls Bath (Instron 3130-100 BioPuls Bath, Instron, USA) provided precise displacement-controlled load with an aqueous environment at constant temperature of 37.0 ±1.0°C. Specimens were clamped with pneumatic grips together with water-proof sand papers. A force magnitude was measured with a 100-N load cell (2530 Series Low-profile Static Load Cell, Instron, USA), and the level of deformation was recorded by crosshead displacement. Considering the floppy character of the BC hydrogel, a pre-load of 0.05 N was applied before the start of each test. Specimens ($n=7$) for each testing were subjected to a quasi-static loading regime under loading rate of 1 mm/min until failure. A high-resolution camera was placed in front of a tested specimen perpendicular to the loading direction.

2.4. Micro-morphological observation

After some deformation, specimens of three types of fracture testing were fixed with a custom-made fixture (see Gao et al., 2015 for details). After freeze-drying and gold coating, a field-emission gun scanning electron microscope (FEG-SEM) was used to observe fibre arrangement in the vicinity of the notch tip to study the effect of microstructure on fracture behaviour of the BC hydrogel.

3. Results

Averaged stress-strain curves (with error bars demonstrating the extent of scatter in experimental results) for uniaxial tension, single-notch, double-notch and central-notch fracture testing for fully-hydrated and freeze-dried BC hydrogel are shown in Figs. 3a and b, respectively. A character of evolution of the tangent modulus E, representing the level of instantaneous stiffness of specimens at a certain strain, of each curve for the fully-hydrated and freeze-dried specimens of the BC hydrogel are shown in Figs. 3c and d, respectively. Thus, some observations could be presented as following:

- The fully hydrated BC hydrogel demonstrated non-linear stress-strain behaviour with a material stiffening process both in presence and absence of the notches.
- The freeze-dried BC hydrogel showed a relatively linear behaviour for each testing regime, with a material softening-stiffening process occurring in the range of strain from ~5% and ~15%.
- The freeze-dried BC hydrogel was much stiffer than the fully-hydrated one.
- Generally, the BC hydrogel with a single notch was stiffer than that without a notch and softer than double-notched and central-notched ones for both fully-hydrated and freeze-dried states.
- For both states of the BC hydrogels, stiffness of the double-notched specimen was almost the same.

From the micro-morphological observations, in the fully-hydrated BC hydrogel shown in Fig. 3a, the area away from the notch tip showed a response to external loading, and fibres aggregated to rearrange towards the loading direction, while, in the vicinity of the notch tip, the surface remained smooth. At magnification of 500×, the aggregation of fibres could be observed near the notch tip (Fig. 3c). In the freeze-dried specimens of the studied BC hydrogel, the aggregation of fibres was not observed both away and near the notch tip (Figs. 3b and d).

4. Discussion

From the acquired experimental data it is evident that the fully-hydrated BC hydrogel had nonlinear stress-strain behaviour with a material-stiffening process, mainly caused by fibre reorientation (Gao et al., 2015), while the freeze-dried BC hydrogel demonstrated a relatively linear behaviour with an anomalous region at strain between ~5% and ~15% where a material's softening-stiffening process occurred. It is worth noting that the tangent modulus at the beginning and the end of this region was almost on the same magnitude (as shown with a dashed line in Fig. 2d); thus, a feasible assumption can be suggested that the behaviour in this region was caused mainly by a water effect – absorption of external water in aqueous environment when undergoing deformation. From micro-morphological observations it was clear that the process of fibre aggregation to reorient along the loading direction was not present; thus, in absence of interstitial water, interactions between fibres would be too strong to prevent fibre reorientation, as a result, increasing global stiffness and showing quasi-linear stress-strain behaviour.

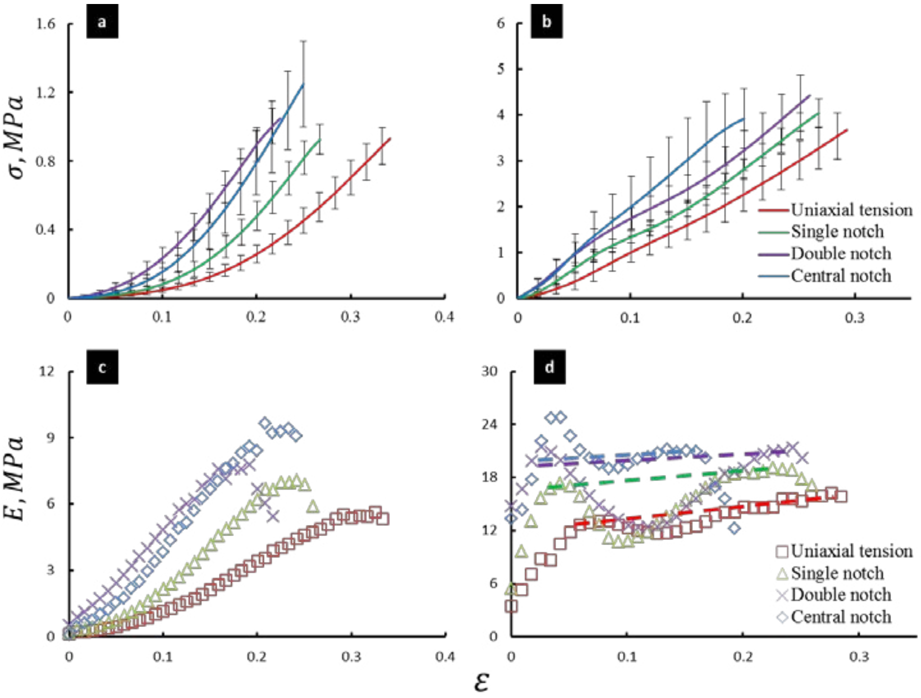

Fig. 2. Stress-strain curves and evolution of tangent modulus in four types of testing for fully-hydrated (a and c) and freeze-dried (b and d) specimens of BC hydrogels

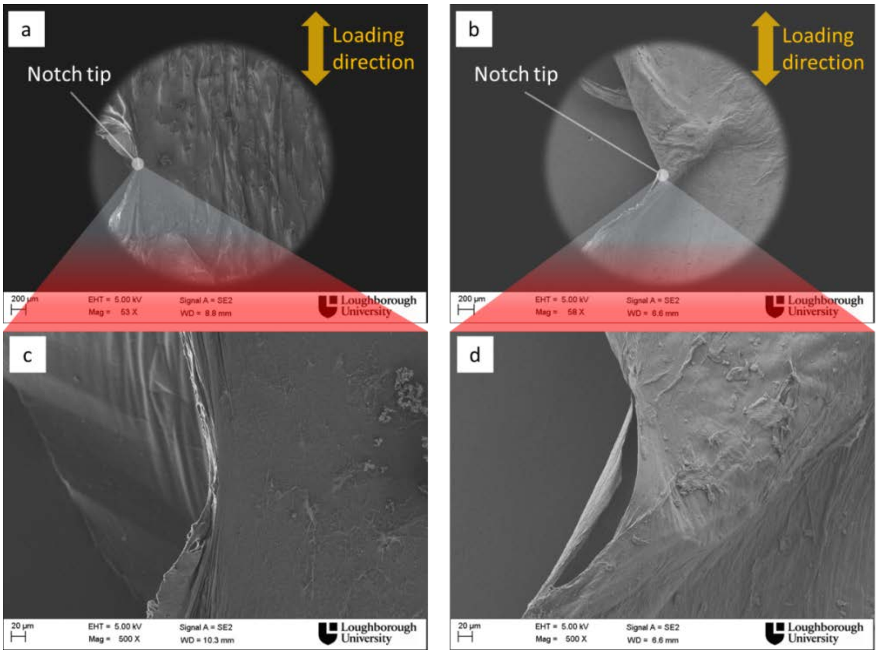

Fig. 3. Microstructure in vicinity of notch tip of deformed notch specimens at magnification of 50× and 500× for fully-hydrated (a and c), and freeze-dried (b and d) specimens of BC hydrogel

Since fibres in the vicinity of the notch tip would reorient along the loading direction, the material appears to be stiffer and tougher (Koh et al., 2013), coinciding with results obtained in this study. This explains the fact that the double-notch and central-notch specimens demonstrated a stiffer response than the single-notch one – a larger number of tips causing reorientation of a larger fraction of fibres along loading direction. Still, this assumption could not perfectly explain the case for the freeze-dried BC hydrogel. According to our observations, the fibre-reorientation process was not fully completed, suggesting a quasi-linear elastic response, while the stiffening trend in these four types of tests was similar to that of the fully-hydrated BC hydrogel, indicating some unexplored fracture mechanisms in the notched nano-fibrous systems.

5. Conclusions

This work carried out four types of mechanical tests – uniaxial tension, single-notch, double-notch and central-notch fracture testing – with micro-morphological observations of accompanying structural changes to investigate fracture behaviour of fully-hydrated and freeze-dried specimens of the BC hydrogel. The obtained experimental data suggested that both states of the BC hydrogel a stiffer response in presence of notches. Micro-morphological observations supported the assumption that the fibre-reorientation process in the vicinity of the notch tip was a feasible mechanism explaining such stiffening behaviour. In the absence of interstitial water, the freeze-dried specimens of the BC hydrogel were much stiffer than the fully-hydrated ones since the interaction between fibres would be much stronger and, as a result, the fibre-reorientation process would be less accomplished comparing with that in the fully-hydrated state.

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Reference

Bäckdahl, H., Helenius, G., Bodin, A., Nannmark, U., Johansson, B. R., Risberg, B., Gatenholm, P. (2006). Mechanical properties of bacterial cellulose and interactions with smooth muscle cells. *Biomater., 27*(9), 2141-2149.

Fu, L., Zhang, Y., Li, C., Wu, Z., Zhuo, Q., Huang, X., Qiu, G., Zhou, P., Yang, G. (2012). Skin tissue repair materials from bacterial celluose by a multiayer fementation method. *J. Mater. Chem., 22*(24), 12349-12357.

Fu, L., Zhou, P., Zhang, S., Yang, G. (2013). Evaluation of bacterial nanocellulose-based uniform wound dressing for large area skin transplantation. *Mater. Sci. Eng. C, 33*, 2995-3000.

Gao, X., Kusmierczyk, P., Shi, Z., Liu, C., Yang, G., Sevostianov, I., Silberschmidt, V.V. (2016a). Through-thickness stress relaxation in bacterial cellulose hydrogel. *J. Mech. Behav. Biomed. Mater., 59*, 90-98.

Gao, X., Shi, Z., Kuśmierczyk, P., Liu, C., Yang, G., Sevostianov, I., Silberschmidt, V.V. (2016b). Time-dependent rheological behaviour of bacterial cellulose hydrogel. *Mater. Sci. Eng. C, 58*, 153-159.

Gao, X., Shi, Z., Lau, A., Liu, C., Yang, G., Silberschmidt, V.V. (2016c). Effect of microstructure on anomalous strain-rate-dependent behaviour of bacterial cellulose hydrogel. *Mater. Sci. Eng. C, 62*, 130-136.

Gao, X., Shi, Z., Liu, C., Yang, G., Sevostianov, I., Silberschmidt, V.V. (2015). Inelastic behaviour of bacterial cellulose hydrogel:In aqua cyclic tests. *Polym. Test., 44*, 82-92.

Huang, L., Chen, X., Xuan, N.T., Tang, H., Zhang, L., Yang, G. (2013). Nano-cellulose 3D-networks as controlled-release drug carriers. *J. Mater. Chem. B, 1*, 2976-2984.

Koh, C.T., Strange, D.G.T., Tonsomboon, K., Oyen, M.L. (2013). Failure mechanisms in fibrous scaffolds. *Acta Biomater., 9*, 7326–7334.

Kowalska-Ludwicka, K., Grobelski, B., Cala, J., Grobelski, B., Sygut, D., Jesionek-Kupnicka, D., Kolodziejczyk, M., Bielecki, S., Pasieka, Z. (2013). New methods Modified bacterial cellulose tubes for regeneration of damaged peripheral nerves. *Arch. Med. Sci., 9*(3), 527-534.

Malm, C.J., Risberg, B., Bodin, A., Backdahl, H., Johansson, B.R., Gatenholm, P., Jeppsson, A. (2012). Small calibre biosynthetic bacterial cellulose blood vessels: 13-months patency in a sheep model. *Scand. Cardiovasc. J., 46*(1), 57-62.

Millon, L.E., Wan, W.K. (2006). The polyvinyl alcohol-bacterial cellulose system as a new nanocomposite for biomedical applications. *Biomed. Mater. Res. B, 79*, 245-253.

Nimeskern, L., Avila, H.M., Sundberg, J., Gatenholm, P., Muller, R. and Stok, K.S. (2013). Mechanical evaluation of bacterial nanocellulose as an implant material for ear cartilage replacement. *J. Mech. Behav. Biomed. Mater., 22*, 12-21.

Ridruejo, A., Jubera, R., González, C., LLorca, J. (2015). Inverse notch sensitivity: Cracks can make nonwoven fabrics stronger. *J. Mech. Phys. Solids, 77*, 61-69.

Shi, Z., Zhang, Y., Phillips, G. O., Yang, G. (2014). Utilization of bacterial cellulose in food. *Food Hydrocolloids, 35*, 539-545.

Wang, J., Gao, C., Zhang, Y., Wan, Y. (2010). Preparation and in vitro characterization of BC/PVA hydrogel composite for its potential use as artificial cornea biomaterial. *Mater. Sci. Eng. C, 30*, 214-218.

Yang, W., Sherman, V.R., Gludovatz, B., Schaible, E., Stewart, P., Ritchie, R.O., Meyers, M.A. (2015). On the tear resistance of skin. *Nat. Commun., 6*, 6649.

Zang, S., Zhang, R., Chen, H., Lu, Y., Zhou, J., Chang, X., Liu, G., Wu, Z., Yang, G. (2015). Investigation on artificial blood vessels prepared from bacterial cellulose. *Mater. Sci. Eng. C, 46*, 111-117.

Zhao, W., Liu, C., Wu, F., Lenardi, C. (2014). Investigation on the mechanical behaviour of poly(hydroxyethyl methacrylate) hydrogel membrane under compression in the assembly process of microfluidic system. *J. Polym. Sci. Part B, 52*, 485–495.

Zhao, W., Shi, Z., Chen, X., Yang, G., Lenardi, C., Liu, C. (2015). Microstructural and mechanical characteristics of PHEMA-based nanofibre-reinforced hydrogel under compression. *Compos. Part B Eng., 76*, 292-299.