Ligament-like tough double-network hydrogel based on bacterial cellulose

by Ananda Putra

Ligament-like tough double-network hydrogel based on bacterial cellulose

Yui Hagiwara · Ananda Putra · Akira Kakugo ·
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Abstract Using the double-network (DN) method, bacterial cellulose/polyacrylamide (BC/PAAm) DN gels able to sustain not only high elongation but also high compression have been synthesized by combining BC gel as the first network with PAAm as the second network in the presence of N,N'-methylene bisacrylamide (MBAA) as a cross-linker. This DN gel was obtained by modifying the monomer concentration of the second network, acrylamide monomer (AAm) and MBAA, and by controlling the water content of the first network, BC gel. The mechanical properties are discussed in term of the swelling degree (q), which is independent of the concentration of AAm and MBAA. It was found that, for BC/PAAm DN gels with the first network formed from BC gel with high q ($BC_{q=120}$), the tensile and compressive modulus (E) scales with q as $E \propto q^{-2}$.

The tensile fracture stress, σ_F, of this DN gel was almost independent of q, that is $\sigma_F \propto q^0$, but the compressive fracture stress, σ_F, scaled with q as $E \propto q^{-2}$. Meanwhile, the tensile and compressive fracture strain (ε_F) of the gel is almost independent of q, which is caused by AAm concentration change, but linearly increased with q, which is caused by MBAA concentration change. Furthermore, by decreasing the water content of the BC gel prior to polymerization of the second (PAAm) network, a ligament-like tough BC/PAAm DN gel could be obtained with tensile strength of 40 MPa.

Keywords Bacterial cellulose · Polyacrylamide · N,N'-methylene bisacrylamide · Tensile strength · Compressive strength

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Introduction

Design and fabrication of materials suitable for soft-tissue replacement are important aspects of this biomedical application. There is a need to develop a material that will not only display similar mechanical properties to the tissue it replaces, but that also shows improved lifespan, biocompatibility, and durability, with low degree of calcification. One promising material that could be suitable for this purpose is hydrogel. Hydrogels are three-dimensional hydrophilic polymer

networks produced by reactions of one or more monomers or by the association of bonds between chains; they can absorb from 20% up to thousands of times their dry weight of water (Hoffman 2002; Park and Park 1994). Due to their unique properties, a wide range of medical, pharmaceutical, and prosthetic applications have been proposed such as contact lenses, synthetic wound coverings, drug delivery systems, and organ and tissue replacement (Tarchevsky and Marchenko 1991; Klemm et al. 1988; Vandamme et al. 1998; Jonas and Farah 1998; Kobayashi et al. 1992; Wan et al. 2002; Ratner et al. 2004; Stammen et al. 2001; Paradossi et al. 2003; Xu et al. 2004).

Bacterial cellulose (BC) is one such hydrogel: a form of extracellular cellulose produced by bacteria of the genus *Acetobacter* such as *Acetobacter xylinum* (*A. xylinum*) and consisting of a hydrophilic ultrafine fibril network stack in a stratified structure. It has high tensile strength along the fibril layer direction, but low compressive modulus properties perpendicular to the stratified direction. Water in a BC gel is easily squeezed out of the gel after slight compression, such as finger pressure, after which the swelling property does not recover due to hydrogen-bond formation between cellulose fibrils. We have developed biocompatible hydrogels with high, reversible swelling ability after repeated compression by combining BC with natural polymers such as gelatin using the semi-interpenetrating network (semi-IPN) technique known as the double network (DN; Kong et al. 2003; Nakayama et al. 2004; Yasuda et al. 2005). These gels exhibit high compressive fracture; however, their tensile strength was still not high enough to compete with ligament or tendon (Ambrosio et al. 1998).

Polyacrylamide (PAAm) gel is an acrylate polymer (–CH$_2$CHCONH$_2$–) formed from acrylamide subunits; it is readily cross-linked and highly water absorbent. PAAm gel is one of the most popular synthetic polymer gels in the field of protein separation and medical applications. It can be used as an electrophoresis gel, thickener or suspending agent. Recently it has been used as a subdermal filler for esthetic facial surgery. Considering the composite structure of soft load-bearing tissue, we hypothesize that the combination of BC and PAAm would mimic the role of natural soft tissues such as ligament or tendon.

In this study, we report a great improvement of the mechanical properties of a BC gel which sustains not only high compression but also high elongation by combining BC with PAAm (BC/PAAm) using the DN technique. By controlling the water content of the BC gel prior to polymerization of the second (PAAm) network, we could obtain a ligament-like tough BC/PAAm DN gel with tensile strength of 40 MPa.

Experimental

Materials

The microorganism used was *A. xylinum*, American Type Culture Collection (ATCC) 53582. We used a culture medium based on Hestrin and Schramm's medium (Hestrin and Schramm 1954), the constituents of which were as follows (wt.%): 5 g bacto-peptone (Difco Bacto-Peptone, Becton–Dickinson and Co., USA), 5 g yeast extracts (Difco Becton–Dickinson and Co.), 2.7 g disodium hydrogen phosphate (Na$_2$HPO$_4$: Junsei Chemical Co. Ltd.), 1.15 g citric acid (Wako Pure Chemical Industries, Ltd.), and 20 g D(+)glucose (Wako Pure Chemical Industries, Ltd.). An aqueous solution of NaOH (Kantou Chemical Co. Ltd) was used for purification of the BC gel. Acrylamide (AAm, Junsei Chemical Co. Ltd.), N,N'-methylene bisacrylamide (MBAA, Wako Pure Chemical Industries, Ltd.), and potassium peroxydisulfate (KPS, Wako Pure Chemical Industries, Ltd.) were used for synthesizing PAAm and BC/PAAm gels.

Synthesis of BC as the first network gel

Hestrin–Schramm's medium (pH 6.0) and cell suspension prepared from an *A. xylinum* culture (ATCC 53582) at volume ratio of 10:1 were poured in sterile culture glass. These samples were incubated at 28°C for 3 or 4 days under static conditions. The gel-like, disc-shaped BC about 2–10 mm in thickness and 80 mm in diameter thus obtained was purified by soaking in a large amount of distilled water for 1 day (the water being changed three times), followed by autoclaving in a 1% (w/v) aqueous solution of NaOH at 121°C for 20 min to remove the bacterial debris and alkali-soluble components. It was then cooled to room temperature (25 ± 2°C) and washed several times with distilled water, followed by: (1) soaking in distilled water for a long period of time, so that *it* attained a pH of 7, and (2) storage in distilled water at 25 ± 2°C prior to use.

Synthesis of BC/PAAm double-network (DN) gel

Three sets of experiment were carried out to produce BC/PAAm DN gel. The first set of BC/PAAm DN gels was prepared with various AAm concentrations and constant MBAA concentration. The purified disc-shaped BC gel (as-prepared) of 10 mm thickness and 80 mm diameter was immersed in an aqueous solution of AAm with various concentrations ranging from 0 to 4 M containing 0.1 mol% MBAA and 0.1 mol% potassium peroxydisulfate (KPS) for 2 days until reaching equilibrium. The second set of BC/PAAm DN gels was prepared by immersing as-prepared BC (size similar to the first one) in 4 M AAm solution with various concentrations of MBAA ranging from 0 to 5 mol% and 0.1 mol% KPS for 2 days until reaching equilibrium. The third set of BC/PAAm DN gels was prepared as follow: prior to immersing the BC gel into 4 M AAm solution containing 2.5 mol% MBAA and 0.1 mol% KPS, the water content of the BC was controlled by removing water from the BC gel by controlled pressing (Tensilon RTC-1310A, Orientec Co., Tokyo, Japan) of the BC gel (size similar to the first one) in the range of 2–50% of the initial thickness at 25 ± 2°C.

The second network of PAAm was subsequently polymerized in the presence of the BC gel at 60°C for 6 h between two glass plates. After this polymerization process, the obtained BC/PAAm DN gel was immersed in distilled water for 1 week, the water being changed twice every day to remove any unreacted materials.

Measurement

Swelling degree, q (water content)

The water content of the BC/PAAm DN gels was determined from the swelling degree (q), defined as the ratio of the weight of the as-prepared swollen sample (W_s) to that of the dry sample (W_d).

$$q = \frac{W_s}{W_d} \tag{1}$$

The dry gels were obtained by drying the samples at 105°C to constant weight. Each reported q value is an average value of at least three parallel measurements.

Material properties of BC/PAAm DN gel (compressive and tensile tests)

The basic mechanical properties of gels were determined with a tensile-compressive tester (Tensilon RTC-1310A, Orientec Co., Tokyo, Japan) at 25 ± 2°C. For the compression test, samples were cut into a disk shape (diameter 10 mm) and compressed perpendicularly to the BC/PAAm DN gel layers by two parallel metal platens that were connected to a load cell at a strain rate of 10% min^{-1} at 25 ± 2°C. For the tensile test, samples were cut into a dumbbell shape (dowel: width 2 mm, length 12 mm; bell: width 10 mm, length 7 mm). It was then stretched parallel to the BC/PAAm DN gel layers at strain rate of 10% min^{-1}. The strain rate was determined according to the initial length of the specimens. The failure points of the compressive and tensile tests were determined from the peak of the stress–strain curve. Tensile and compressive modulus, E, was determined by the average slope over the strain ratio of 0–10% of the strain ratio from the stress–strain curve. Both compressive and tensile test were repeated at least three times.

Results and discussion

BC/PAAm DN gel using as-prepared BC gels with various AAm concentrations

To obtain the BC/PAAm DN gel using as-prepared BC gels, the as-prepared BC gel (first network) was polymerized with the second (PAAm) network by immersing it in various concentrations (0–4 M) of AAm solution with 0.1 mol% MBAA as a cross-linker and 0.1 mol% KPS as an initiator. The effect of AAm concentration on the mechanical properties of BC/PAAm DN gel is shown in Figs. 1 and 2. For comparison, the swelling degree, q, and mechanical properties of ligament are also shown in Fig. 1 (Woo et al. 2004). To measure the mechanical properties of BC/PAAm DN gel, compressive and tensile tests were carried out samples in directions perpendicular and parallel to the stratified structure of the BC gel. BC gel, having an ultrafine fibril network stacked in a stratified structure, shows mechanically anisotropic properties, with a high tensile modulus (E = 2.9 MPa) along the fibril layer direction but a
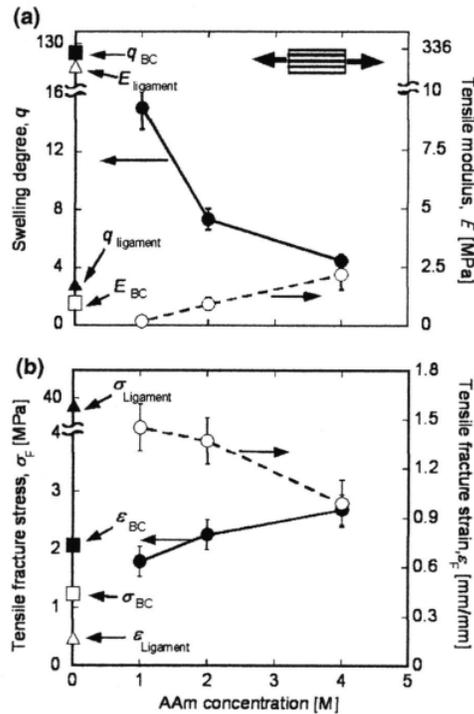
Fig. 1 Effect of AAm concentration on the tensile strength performance of BC/PAAm gel under the conditions of 0.1 mol% MBAA and 0.1 mol% KPS: **a** swelling degree, q, and tensile modulus, E, as functions of the concentration of AAm (M) of the BC/PAAm gel. **b** Tensile fracture stress, σ_F, and tensile fracture strain, ε_F, as functions of the concentration of AAm (M) of BC/PAAm gel. The symbols are as follows: **a** (●) $q_{BC/PAAm}$; (■) q_{BC}; (▲) $q_{ligament}$; (○) $E_{BC/PAAm}$; (□) E_{BC}; (△) $E_{ligament}$; **b** (●) σ_F BC/PAAm; (■) σ_F BC; (▲) σ_F ligament; (○) ε_F BC/PAAm; (□) ε_F BC; (△) ε_F ligament
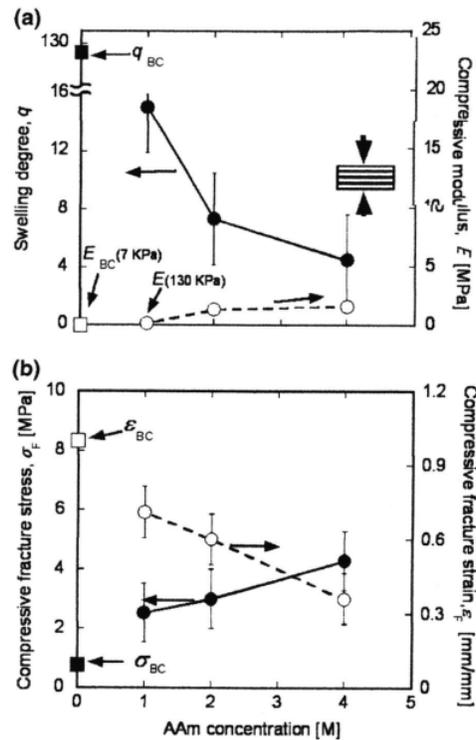
Fig. 2 Effect of AAm concentration on the compressive strength performance of BC/PAAm gel under the conditions of 0.1 mol% MBAA and 0.1 mol% KPS: **a** swelling degree, q, and compressive modulus, E, as functions of the concentration of AAm (M) of BC/PAAm gel. **b** Compressive fracture stress, σ_F, and compressive fracture strain, ε_F, as functions of the concentration of AAm (M) of BC/PAAm gel. The symbols are as follows: **a** (●) $q_{BC/PAAm}$; (■) q_{BC}; (○) $E_{BC/PAAm}$; (□) E_{BC}; **b** (●) σ_F BC/PAAm; (■) σ_F BC; (○) ε_F BC/PAAm; (□) ε_F BC

low compressive modulus ($E = 0.007$ MPa) perpendicular to the stratified direction (Nakayama et al. 2004).

Figure 1 shows the effect of the concentration of AAm on the tensile strength of BC/PAAm DN gels. The swelling of the BC gel as a single network drastically decreased (by about ten times) after the BC gel was combined with AAm (1 M) to produce BC/PAAm DN gels. This indicates that the water in the BC gel network was replaced by the PAAm gel. The swelling of the BC/PAAm DN gel continued to decrease on increasing the concentration of AAm (Fig. 1a). Up to an AAm concentration of 4 M, the

swelling of BC/PAAm DN gel was 4.5. This swelling was still higher than that of ligament ($q = 2.86$). Meanwhile, the tensile modulus, E, of BC/PAAm DN gel increased linearly with increasing AAm concentration, but was still much lower (more than 100 times) than the tensile modulus of ligament ($E = 332$ MPa). The tensile fracture stress, σ_F of BC/PAAm DN gel tended to increase with increasing AAm concentration and was more than 20 times lower than that of ligament ($\sigma_F = 38.6$ MPa; Fig. 1b). However, the tensile fracture strain of BC/PAAm DN gel decreased with increasing AAm concentration and was more than ten times higher

than that of ligament ($\varepsilon_F = 0.17$ mm mm^{-1}). The tendency for the compressive strength was similar to that for the tensile strength for the BC/PAAm DN gel (Fig. 2).

These results indicate that the concentration of AAm affected the mechanical performance in terms of both the tensile and compressive strengths. Higher concentration of AAm (4 M) led to high tensile and compressive strength of the BC/PAAm DN gels.

BC/PAAm DN gel using as-prepared BC gels with various MBAA concentrations

Since the mechanical properties of the gel also depend on the cross-linker concentration of the second network, we investigated the effect of the degree of cross-linking on the tensile and compressive strength by changing the MBAA concentration while keeping the in feed AAm concentration constant at 4 M. The concentration of KPS was also kept constant at 0.1 mol%.

Figures 3 and 4 show the effect of MBAA concentration on the tensile and compressive strength properties of the BC/PAAm DN gels. Both tensile and compressive moduli of BC/PAAm DN gels increased with increasing MBAA concentration (Figs. 3a, 4a). Meanwhile, as shown in Fig. 3b, drastic increase of the tensile fracture stress of the BC/PAAm DN gel was observed for MBAA concentration below 1 mol%. In contrast, the tensile fracture strain of the BC/PAAm gels drastically decreased up to MBAA concentration of 1 mol%. Above 1 mol% MBAA concentration, there was no significant improvement of either tensile fracture stress and strain. This indicates that 1 mol% was an effective MBAA concentration for the cross-linker to improve the tensile strength performance of the BC/PAAm DN gel. This value is in agreement with the concentration of cross-linker for any DN gel synthesized by the DN method (Gong et al. 2003).

In Fig. 4b, substantial increase in the compressive fracture stress, σ_F, of the BC/PAAm DN gels was observed with increasing MBAA concentration. Above 2.5 mol% MBAA concentration, the compressive fracture stresses were almost constant with MBAA concentration. The compressive fracture strain, ε_F, of BC/PAAm gels drastically decreased up to 1 mol% MBAA concentration and was almost constant above this value. The maximum tensile and
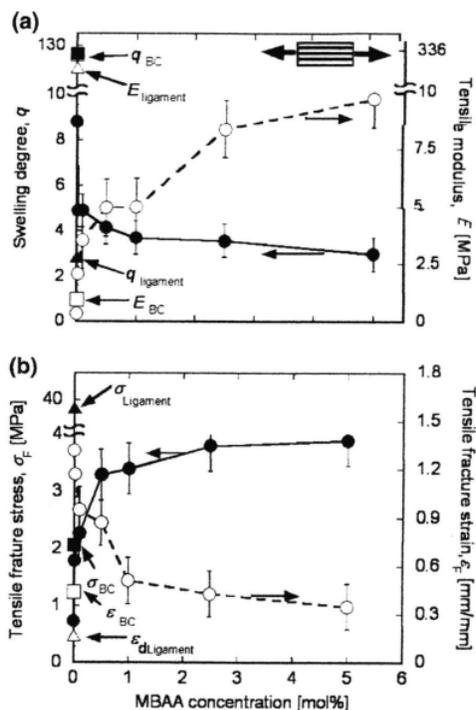

Fig. 3 The effect of MBAA cross-linker concentration on the tensile strength performance of BC/PAAm gel under the conditions of 4 M AAm and 0.1 mol% KPS: **a** swelling degree, q, and tensile modulus, E, as functions of the concentration of MBAA (mol%) in the BC/PAAm gel. **b** Tensile fracture stress, σ_F, and tensile fracture strain, ε_F, as functions of the concentration of MBAA (mol%) in the BC/PAAm gel. The symbols are as follows: **a** (●) $q_{BC/PAAm}$, (■) q_{BC}; (▲) $q_{ligament}$; (○) $E_{BC/PAAm}$; (□) E_{BC}, (△) $E_{ligament}$; **b** (●) σ_F BC/PAAm; (■) σ_F BC, (▲) σ_F ligament; (○) ε_F BC/PAAm, (□) ε_F BC; (△) ε_F ligament

compressive fracture stress was observed to be about 3.5 and 6.5 MPa at 5 mol% MBAA concentration, similar to the compressive and tensile fracture stress of the BC/gelatin DN gel system reported previously (Nakayama et al. 2004). It should be noted that these values are still lower than the tensile fracture stress of ligament ($\sigma_F = 39$ MPa; Woo et al. 2004) and the compressive fracture stress of articular cartilage (McCutchen 1978).

In order to investigate whether swelling is a universal parameter, we investigated the relationship between swelling and the mechanical properties of BC/PAAm DN gels prepared at different
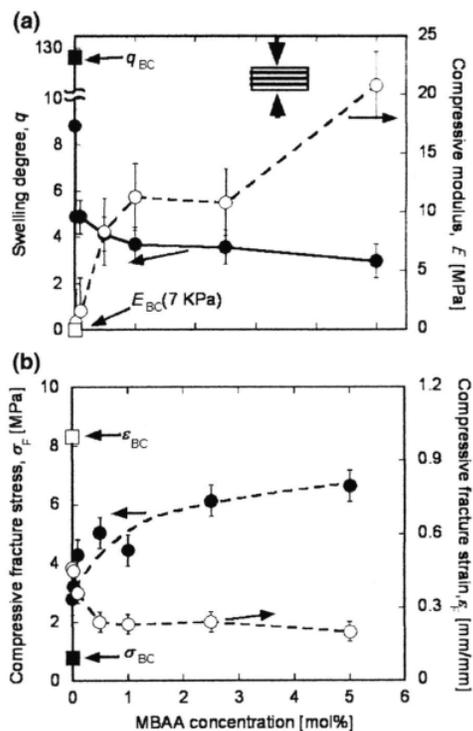
Fig. 4 The effect of MBAA cross-linker concentration on the compressive strength performance of BC/PAAm gel under the conditions of 4 M AAm and 0.1 mol% KPS: **a** swelling degree, q, and compressive modulus, E, as functions of the concentration of MBAA (mol%) in the BC/PAAm gel. **b**, compressive fracture stress, σ_F, and compressive fracture strain, ε_F, as functions of the concentration of MBAA (mol%) in the BC/PAAm gel. The symbols are as follows: **a** (●) $q_{BC/PAAm}$; (■) q_{BC}; (○) $E_{BC/PAAm}$; (□) E_{BC}; **b** (●) σ_F BC/PAAm; (■) σ_F BC; (▲) σ_F ligament; (○) ε_F BC/PAAm; (□) ε_F BC

concentrations of AAm (1–4 M) and MBAA (0–5 mol%). We plotted the relationships of tensile and compressive modulus, and tensile and compressive fracture stress and strain against swelling on a logarithmic graph, which is useful to elucidate the correlation over a wide range of these parameters (Figs. 5, 6).

In Figs. 5a and 6a, we can see that the tensile and compressive modulus of the DN gel increased with decreasing swelling, following a power law of
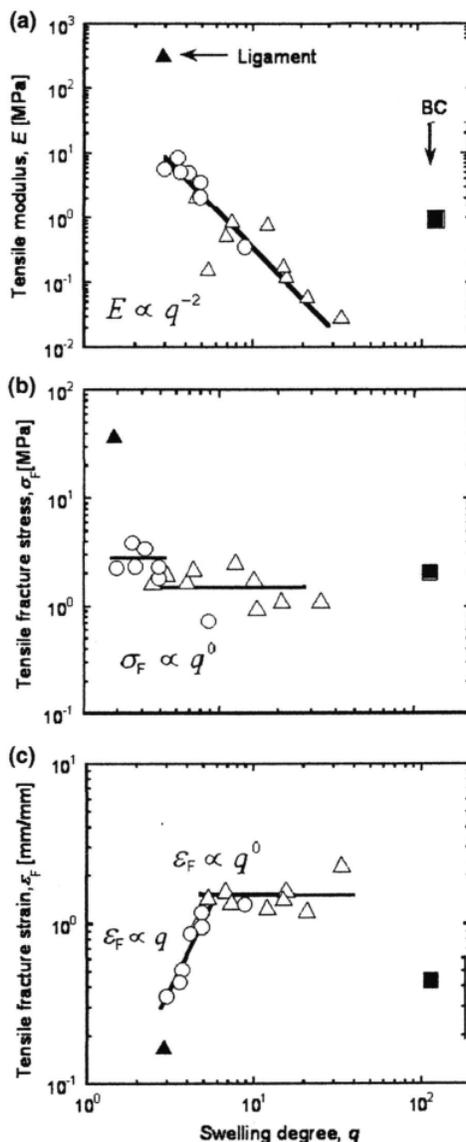

Fig. 5 Dependence on swelling degree, q, of: **a** tensile modulus, E **b** tensile fracture stress, σ_F, and **c** tensile fracture strain, ε_F. The symbols are as follows: (■) BC; (▲) ligament; and BC/PAAm DN gel with various concentration of (△) AAm, and (○) MBAA
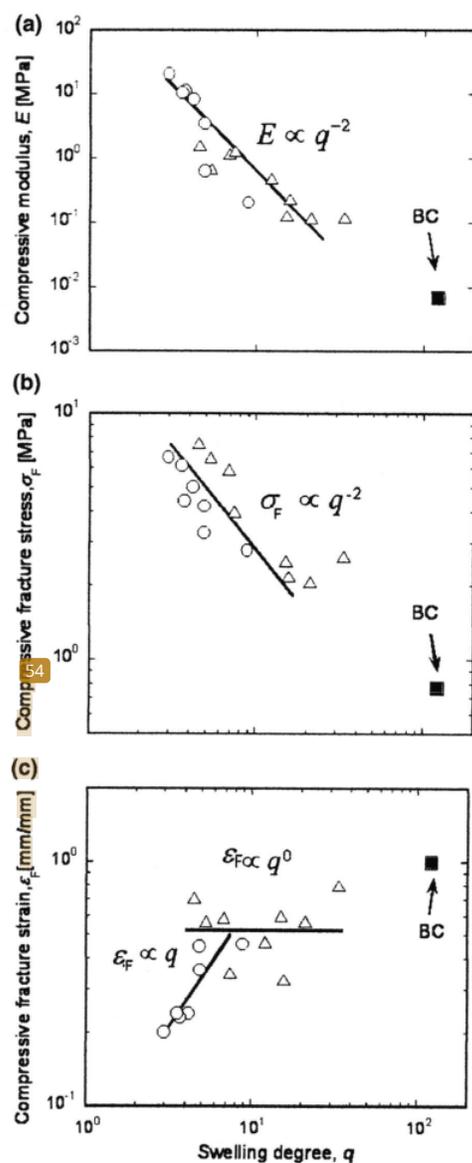
Fig. 6 Dependence on swelling degree, q, of: **a** compressive modulus, E **b** compressive fracture stress, σ_F, and **c** compressive fracture strain, ε_F. The symbols are as follows: (■) BC; and BC/PAAm DN gel with various concentration of (△) AAm, and (○) MBAA

$E \propto q^{-2}$, regardless of the structural parameter changes of either AAm or MBAA concentration. This relation is close to that of a hydrogel swollen in good solvent ($E \propto q^{-2.25}$) (Obukhov et al. 1994), indicating that the contribution to the initial modulus from the fine stratified structure of the BC gel was negligible. Tensile fracture stress of gel prepared at various concentrations of AAm and MBAA did not change with swelling ($\sigma_F \propto q^0$), with a value similar to that of BC itself (Fig. 5b). However, the compressive fracture stress increased with decreasing swelling, following a power law of $E \propto q^{-2}$ (Fig. 6b). This indicates that the fracture stress is dominated by the BC in elongation but by AAm and MBAA concentrations in compression. Tensile and compressive fracture strains were almost independent of AAm concentration in feed which caused change in swelling ($\varepsilon_F \propto q^0$) and showed a value close to that of BC. However, fracture strain was dependent on MBAA concentration, which caused fracture strain to increase linearly with q ($\varepsilon_F \propto q$) (Figs. 5c, 6c). These results suggested to us how to improve the first network gels: the BC gel should network prior to polymerization of the second (PAAm) network, as described below.

BC/PAAm DN gel using BC with controlled water content

In order to create a BC/PAAm DN gel using BC with controlled water content, the network density of the BC gel was increased by removing water from the BC gel, which was performed by compressing the (as-prepared) BC gel appropriately. The BCs were compressed in the range of 2–50% of initial thickness at $25 \pm 2°C$ and reswelled in distilled water for 6 h to create BC with controlled water content. The water content of BC was calculated as swelling degree, q, denoted as $BC_{q=x}$, where x is the swelling of the BC with controlled water content. The swelling of uncompressed (as-prepared) BC was 120 ($BC_{q=120}$). When the as-prepared BC gel was compressed by up to 50% of its initial thickness and reswelled in distilled water for 6 h, it showed swelling of 40.7 ($BC_{q=40.7}$). As-prepared BC compressed by up to 2% of its initial thickness and reswelled would obtain swelling of 1.9 ($BC_{q=1.9}$).

Double-network gel consisting of BC with controlled water content and PAAm ($BC_{q=x}$/PAAm DN gel) was obtained by immersing the BC with controlled water content in 4 M AAm solution containing 2.5 mol% MBAA and 0.1 mol% KPS, which was then heated at 60°C for 6 h for polymerization. Typical compressive and tensile strength curves of as-prepared BC (single-network) gel
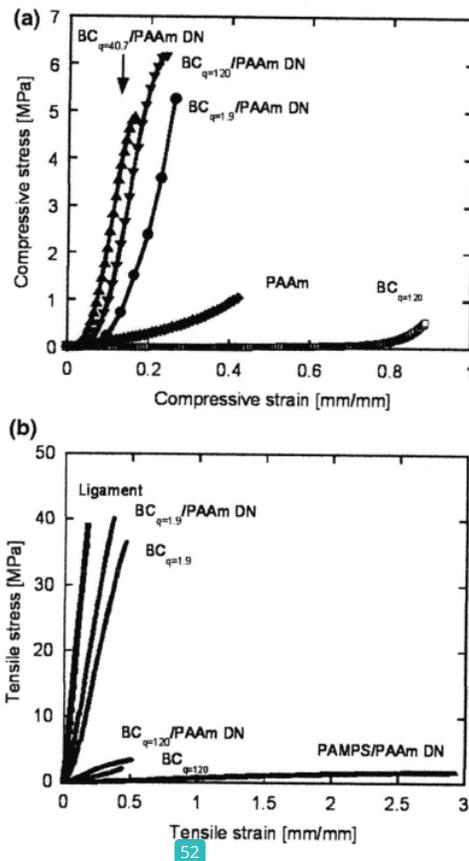

Fig. 7 **a** Compressive stress–strain curves of BC, PAAm, and BC/PAAm DN gel with controlled BC water content. Compression was performed perpendicular to the stratified direction of the BC and DN gels. **b** Tensile stress–strain curves of ligament, BC, PAMPS/PAAm DN gel, and BC/PAAm DN gel with controlled BC water content. Elongation was performed parallel to the stratified direction of BC and DN gels. BC/PAAm DN gel was obtained by immersing the BC with controlled water content into 4 M AAm solution containing 2.5 mol% MBAA and 0.1 mol% KPS

without compression ($BC_{q=120}$), PAAm gel, and $BC_{q=x}$/PAAm DN gel are shown in Fig. 7.

No significant difference in the compressive test was found between as-prepared BC/PAAm ($BC_{q=120}$/PAAm) and that with controlled water content ($BC_{q=1.9}$/PAAm and $BC_{q=40.7}$/PAAm) in the DN gels, even though their compressive strength was much higher than that of the BC (single-network) and PAAm gels (Fig. 7a). This indicates that the compressive strength properties of BC/PAAm DN gel were not affected by the water content of BC as the first network.

However, the water content of BC affected the tensile strength properties of the BC/PAAm DN gel, as shown in the tensile stress–strain curves in Fig. 7b. The tensile fracture stress of $BC_{q=1.9}$/PAAm was about 40 ± 10 MPa, which is about ten times higher than that of $BC_{q=120}$ (single-network) gel, four times that of $BC_{q=120}$/PAAm gel, and slightly higher than that of $BC_{q=1.9}$ (36 ± 10 MPa). This indicates that the tensile strength properties of the BC/PAAm DN gels are predominantly determined by the water content/network density of the BC gel. We predicted that the role of the PAAm network in the BC/PAAm DN gel is to hold the water, which is similar to the BC/gelatin system (Nakayama et al. 2004). Decreasing the water content of BC in the BC/PAAm DN gel would improved the tensile strength of the BC/PAAm DN gel. It was observed that the tensile fracture stress of $BC_{q=1.9}$/PAAm DN gel with low-water-content BC was higher than that of ligament (38 ± 10 MPa). Furthermore, the tensile strength of BC/PAAm DN gel is much higher than that of other DN gels that also use PAAm as the second network, such as the combination of poly (2-acrylamide-2-methyl-1′-propanesulfonate) and polyacrylamide (PAMPS/PAAm; Gong et al. 2003). This excellent tensile strength of BC/PAAm DN gel might be applied in the medical and pharmaceutical field (Klem et al. 2006).

Conclusions

BC/PAAm DN gel was obtained by modifying the monomer concentration of the second network, AAm, and cross-linker, MBAA, and by controlling the water content of the first network, BC gel. The BC/PAAm DN gels with their first-network BC gels with high swelling ($BC_{q=120}$) exhibited tensile and

compressive moduli that scaled with swelling as $E \propto q^{-2}$. The tensile fracture stress of these DN gels was almost independent of swelling ($\sigma_F \propto q^0$), but the compressive fracture stress scaled with q as ($\sigma_F \propto q^{-2}$). Meanwhile, the tensile and compressive fracture strains of the gels were almost independent of swelling ($\varepsilon_F \propto q^0$), which was caused by AAm concentration change, but linearly increased with swelling ($\varepsilon_F \propto q$), which was caused by MBAA concentration change. Furthermore, excellent improvement of tensile strength of BC/PAAm DN gel could be obtained by decreasing the water content of BC *prior to* polymerization of the second (PAAm) network. The tensile fracture stress of the BC$_{q=1.9}$/PAAm gel reached as high as 40 ± 10 MPa, which is similar to that of the tensile fracture stress of ligament, 38 ± 10 MPa ($q = 2.86$). To the best of our knowledge, this is the first report of BC combined with synthetic polymer that exhibits such a high tensile strength. This gel might be applied in the medical and pharmaceutical field.

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