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Research paper

Mechanical properties, anisotropic swelling behaviours and structures of jellyfish mesogloea

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ARTICLE INFO

Article history:
Received 15 September 2011
Received in revised form
21 October 2011
Accepted 22 October 2011
Published online 31 October 2011

Keywords:
Jellyfish mesogloea
Mechanical properties
Anisotropic swelling
Hydrogels

ABSTRACT

Learning from nature is a promising way for designing and fabricating new materials with special properties. As the first step, we need to understand the structures and properties of the natural materials. In this work, we paid attention to the mesogloea of an edible jellyfish (*Rhopilema esculenta Kishinouye*) and mainly focused on its structure, mechanical and swelling properties. Scanning electron microscope (SEM) investigations show that jellyfish mesogloea has a well-developed anisotropic microstructure, which consists of nano-sized membranes connected with many fibres. The tensile and compressive properties of swollen and dried jellyfish mesogloea samples are measured. The jellyfish mesogloea displays very high tensile strength (0.17 MPa) and compressive strength (1.43 MPa) even with 99 wt % water. The mechanical properties of jellyfish mesogloea exceed most synthetic hydrogels with similar or even lower water contents. Swelling in acidic and basic buffer solutions weakens the mechanical properties of jellyfish mesogloea. The dried jellyfish mesogloea has very high tensile strength and modulus, which are very similar to those of synthetic plastics. The swelling properties of jellyfish mesogloea in solutions with different pH values were studied. The jellyfish mesogloea exhibits pH-sensitive and anisotropic swelling properties. The jellyfish mesogloea swells (expands) in height but deswells (shrinks) in length and width, without significant change in the volume. This phenomenon has never been reported for synthetic hydrogels. This study may provide gel scientists new ideas in designing and fabricating hydrogels with well-defined microstructures and unique mechanical and swelling properties.

1. Introduction

Biological materials constitute most of the body of plants and animals. They usually show extreme, unexpected functional properties, since long history of evolution has imparted them complex, often hierarchical, and effective structures to resolve many challenges of nature. Learning from nature is a promising way for designing and fabricating new materials with special properties. As the first step, we need to understand the structures and properties of the natural materials (Aizenberg and Fratzl, 2009; Bar-Cohen, 2006; Fratzl, 2007).

Jellyfish are primitive invertebrates belonging to the *Scyphozoan* class. It is widely distributed in the oceans all over the world. Research about jellyfish mainly focuses on

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doi:10.1016/j.jmbbm.2011.10.005

their components (Shaposhnikova et al., 2005), organism morphologies and tissue structures (Nordström et al., 2003; Scemes and Mcnamara, 1991; Szollosi, 1964), and toxins (Mariottini and Pane, 2010; Nagai, 2003). The umbrella of jellyfish is composed of inner skin (or exumbrella), mesogloea and outer skin (or subumbrella) (Nagai et al., 1999). It is mainly extracellular matrix (ECM) made up of Type II collagen (Hsieh, 2005), as well different polyunsaturated fatty acids (White and Hager, 1977) and about 96–97 wt% (with outer skin) water (Hsieh et al., 2001; Lowndes, 1942) when swollen to equilibrium. Mesogloea is a kind of non-living jelly-like substance containing a gastrovascular system (Hsieh and Rudloe, 1994). The edible jellyfish has a well-developed mesogloea in which a large amount of water can be retained. Fresh jellyfish quickly spoils at ambient temperature out of water (Hsieh and Rudloe, 1994). The fast degradation of fresh jellyfish (Tinta et al., 2010) limits its direct utilization. Thus, fresh jellyfish must be processed with table salt–alum mixture. Table salt decreases the water content of jellyfish mesogloea and help to maintain the microbial stability. Alum acts as a disinfectant as well as a hardening agent to produce and maintain a firm texture by precipitating the collagen of jellyfish mesogloea (Hsieh and Rudloe, 1994). When the salts in the processed jellyfish are extracted, as was done here, the water content can be as high as 99 wt% (Hsieh et al., 1996).

Hydrogels are three-dimensional hydrophilic polymeric networks that can absorb and retain a considerable amount of water. Synthetic hydrogels have many biomedical and pharmaceutical applications, however, the mechanically weak nature of most synthetic hydrogels strongly impeded their practical applications in load bearing areas (Anseth et al., 1996). Several kinds of hydrogels with high mechanical strengths have been reported in recent years, *e.g.* topological gel (TP gel) (Okumura and Ito, 2001), nanocomposites hydrogel (NC gel) (Haraguchi and Takehisa, 2002), double-network gel (DN gel) (Gong et al., 2003), macromolecular microsphere composite hydrogel (MMC gel) by our group (Huang et al., 2007), and some other ones (Malkoch et al., 2006; Tan et al., 2010; Tang et al., 2010; Wu et al., 2010). These tough hydrogels usually have well-defined network structures. The mechanical properties of normal synthetic hydrogels and tough hydrogels have been well reviewed by Naficy et al. (2011).

Most tissues (tendons, ligaments etc.) of animals are mainly composed of biological gels which are usually elastic and tough at a high water content and anisotropic in mechanical and other properties (Anssari-Benam et al., 2011; Jeng et al., 2011). And in some living beings like jellyfish, hydrogel composes almost their whole body. From the viewpoint of mechanical properties, jellyfish is an interesting living organism, since it possesses relatively high mechanical strength even with such a high water content. For example, when jellyfish cruise or hover in the ocean, their bodies cannot be damaged by water pressure and water shear force. To the best of our knowledge, less attention has been paid to the mechanical properties of jellyfish umbrella mesogloea, and the relationship between its structure and mechanical properties so far.

We have fabricated a kind of hybrid hydrogel by incorporating jellyfish mesogloea with a synthetic hydrogel. The hybrid hydrogel exhibits strikingly high mechanical strengths (Wang et al., 2011). In this work, we characterized the microstructures of the mesogloea of an edible jellyfish (*Rhopilema esculenta Kishinouye*) with SEM, and tested the mechanical properties of jellyfish mesogloea samples equilibrium swollen in water and an acetic acid aqueous solution (pH = 4.00) and dried jellyfish mesogloea. In addition, we also investigated the tensile properties and the swelling properties of jellyfish mesogloea in buffer solutions with different pH values. This study aims to provide a fundamental understanding of the structure and properties of jellyfish mesogloea, it may provide gel scientists new ideas in designing and fabricating hydrogels with well-defined microstructures and unique mechanical and swelling properties.

2. Experimental

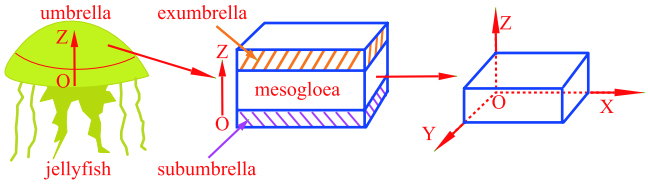
2.1. Materials

All of the jellyfish material used in this study was from preserved animals. The edible jellyfish (*Rhopilema esculenta Kishinouye*) umbrellas were bought from fish market, which were preserved with a mixture of table salt and alum. They were firstly washed with tap-water and then thoroughly washed with deionized water for 72 h to remove the salts in them as far as possible, changing deionized water once a day to avoid the autolysis of jellyfish. The jellyfish umbrellas had a diameter of 30–50 cm, the central part with a diameter of 15–20 cm was cut out for this study. The outer skin of the swollen jellyfish umbrellas was removed carefully, leaving only the mesogloea as the raw materials. The sample preparation process is shown in Scheme 1. The anatomical axes are also indicated, the direction perpendicular to the jellyfish umbrella surface is designated as OZ and the parallel direction is designated as OX or OY.

Acetic acid (AR grade), hydrochloric acid, sodium hydroxide and potassium chloride were all AR grade and purchased from Beijing Chemical Reagents Company (Beijing, China). Standard buffer reagents, potassium hydrogen phthalate, mixed phosphate and sodium tetraborate, were purchased from Shanghai REX Co-perfect Instrument Co. Ltd (Shanghai, China). Acidic solutions of pH = 1.00 and 2.00 were prepared with hydrochloric acid and potassium chloride, buffer solutions of pH = 4.00, 6.86 and 9.18 were prepared with the standard buffer reagents, and an alkaline solution of pH = 13.00 was prepared with sodium hydroxide and potassium chloride. All pH values were calibrated with a pH meter (PHS-3E, Shanghai REX Instruments Factory, Shanghai, China).

2.2. Scanning electron microscope (SEM) investigations

The samples for morphology inspection by SEM were cut from the inner part of swollen jellyfish mesogloea, and then they were plunged into liquid nitrogen for about 5 min. The rapid freezing in liquid nitrogen avoids the formation of large ice crystals which may damage the microstructures of jellyfish mesogloea. The frozen samples were subsequently freeze-dried in the FD-1B-50 vacuum freeze dryer (Beijing Boyikang Laboratory Apparatus Co., Ltd.) until all water was removed. The dried samples were cracked in the directions perpendicular and parallel to the jellyfish umbrella surface. After being sputter-coated with gold for 15 min, the morphologies of these fractured surfaces were observed



Scheme 1 – The sample preparation process and the anatomical axes. Left: jellyfish umbrella, the central part was cut out; Middle: the structure of jellyfish umbrella, the outer skin of the swollen jellyfish umbrella was removed to leave only the mesogloea; Right: jellyfish mesogloea and the anatomical axes, the direction perpendicular to the jellyfish umbrella surface is designated as OZ and the parallel direction is designated as OX and OY.

with a Hitachi S-4800 cold field emission scanning electron microscope (Tokyo, Japan) with an accelerating voltage of 5 kV.

2.3. *Mechanical testing*

The jellyfish mesogloea equilibrium swollen in water or acetic acid aqueous solution (pH = 4.00) was cut into dumbbell shaped specimens standardized as DIN-53504 S2 (overall length: 75 mm; width: 10 mm; inner width: 4 mm, gauge length: 20 mm, thickness: 3 mm) for tensile testing and cylindrical shaped specimens (20 mm in diameter and about 10 mm in height) for compression testing. In addition, the tensile properties of jellyfish mesogloea swollen in buffer solutions with different pH values (pH = 1.00, 2.00, 4.00, 6.86, 9.18 and 13.00) were also measured. All tests were performed by using an Instron 3366 electronic universal testing machine (Instron Corporation, MA, USA). 100 N and 1 kN load cells were used for tensile and compression tests, respectively; and crosshead speeds of 25 mm min^{-1} and 10 mm min^{-1} were applied for tensile and compression tests, respectively.

Some jellyfish mesogloea samples were dried in air at ambient temperature for 72 h, and then they were cut into dumbbell shaped specimens (inner width: 4 mm, gauge length: 20 mm, thickness: about 0.06 mm) for tensile testing. The testing conditions were the same as those for the swollen specimens.

The compressive stress, σ_c, was calculated by σ_c = Load/πr^2, where r is the original radius of the specimen. The strain (ε_c) under compression was defined as the change in the thickness (h) relative to the original thickness (h_0) of the freestanding specimen, $\varepsilon_c = (h_0 - h)/h_0$. The tensile stress σ_t was calculated as follows: σ_t = Load/tw (t and w were the initial thickness and width of the dumbbell shaped gel sample, respectively). The tensile strain ε_t is defined as the change in the length relative to the gauge length of the freestanding specimen, and the fracture tensile strain or elongation λ is the tensile strain of a sample when it breaks. Stress–strain ranges used for calculating initial elastic modulus (E_t) are indicated in the text. At least five specimens per experimental point were tested in all mechanical properties measurements to obtain reliable values.

2.4. *Water content measurements*

Thermogravimetric analysis of an equilibrium swollen jellyfish mesogloea was carried out with TG 50 (Mettler, Switzerland) at a heating rate of 10 °C min^{-1} in a nitrogen atmosphere with a flow rate of 10 mL min^{-1}.

Different to normal synthetic hydrogels, the jellyfish mesogloea lose water under an applied force. To evaluate the change of water content during the mechanical tests, the masses of the specimens before and after testing were gravimetrically measured. Freeze drying was used to remove water and hence obtain the dry weights of the jellyfish specimens. These samples were frozen by liquid nitrogen quickly and then freeze-dried in a FD-1B-50 vacuum freeze dryer (Beijing Boyikang Laboratory Apparatus Co., Ltd.).

2.5. *Swelling properties*

The swelling behaviours of jellyfish mesogloea in buffer solutions with different pH values were examined. The equilibrium swollen jellyfish mesogloea was cut into cubic samples (about 20 mm in length, 15 mm in width and 5 mm in height), then they were immersed into the buffer solutions with different pH values, the change of their dimensions (length, width and height) with time was recorded.

3. Results and discussion

3.1. *Microstructures*

Equilibrium swollen jellyfish mesogloea was freeze-dried with a vacuum freeze dryer or dried in air at room temperature. Since biomaterials usually have an anisotropic microstructure, the freeze-dried samples were fractured in the directions perpendicular or parallel to the jellyfish umbrella surface, and then the morphologies of the fractured surfaces were observed with SEM (Fig. 1(a)–(d)). Insets are included in Fig. 1 to show the anatomical axes and the surfaces observed.

Viewed from the direction perpendicular to the jellyfish umbrella surface, the jellyfish mesogloea has a layered porous network structure (Fig. 1(a)), which consist of dense thin membranes and many nano-sized fibres connected to the membranes (Fig. 1(b)). The dense membranes are arranged in the direction parallel to the umbrella surface, and the distance between any two membranes is about several to ten micrometres. Viewed from the direction parallel to the umbrella surface, a structure consisting of coils or meshes is shown (Fig. 1(c)), and the meshes are also formed by fibres. The thicknesses of the thin membranes and the diameters of the fibres are mostly about 20–50 nm, with a mean and standard deviation of 35 ± 11 nm. The air-dried sample does not show the porous structure as in the freeze-dried sample, but it also shows a layered structure (Fig. 1(e)), and the layers (Fig. 1(f)) have the similar sizes as those of the membranes and fibres found in freeze-dried samples, this may suggest

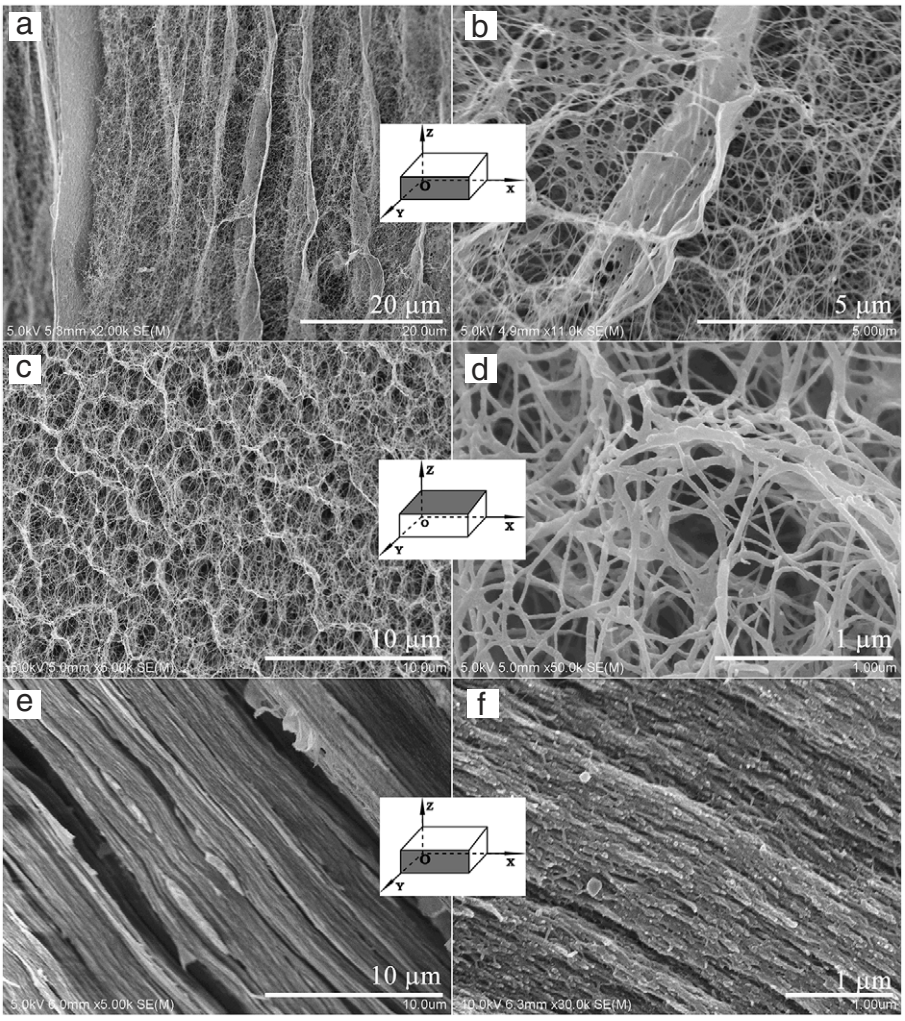


Fig. 1 – SEM micrographs of the jellyfish mesogloea prepared with the freeze-drying method (a)–(d) and drying in air at room temperature (e, f). The insets in the micrographs show the directions observed with SEM, indicated by the dark surfaces perpendicular (a, b, e, f) or parallel (c, d) to the jellyfish umbrella surface.

that the layered structure is formed by the collapse of the network structure of jellyfish mesogloea during drying. These SEM investigations prove that the jellyfish mesogloea has a well-developed anisotropic microstructure.

Since collagen is the main material in the jellyfish mesogloea (Hsieh, 2005), the nano-sized thin membranes and fibres might be formed by the aggregation of collagen. Collagen helix is a major secondary structure of collagen, and it consists of a triple helix made of polypeptides having the typical sequence $(Gly-X-Y)_n$. The X residues are frequently proline while the Y residues are frequently hydroxyproline or hydroxylysine (Leikin et al., 1994). The three chains are bonded to each other through the hydrogen bonding between the peptide NH groups of glycine residues and the CO groups of residues on the other chains. Some covalent cross-links are present within the triple helices, and a variable amount of covalent crosslinking between tropocollagen helices leads to the formation of well-organized aggregates (such as fibrils) (Meyers et al., 2011; Morinaga et al., 2010). Larger collagen fibres are bundles of fibrils. Membranes are also found in the microstructure of jellyfish mesogloea. However, we cannot find any information on the chemical and physical structures of the membranes. Membrane proteins may take a major part in the formation of membranes. Structural information is available for only a relatively small number of these proteins, since membrane proteins are present in a heterogeneous environment that poses major obstacles for existing structural methodologies (Miura and imura, 1985). When dried in air at ambient temperature, the network structure of jellyfish mesogloea collapsed to form a compact layered structure.

From the SEM investigations and the discussion above, it is reasonable to conclude that jellyfish mesogloea has a hierarchical, anisotropic microstructure.

3.2. *Mechanical properties*

Tensile and compression tests were performed on swollen and dried jellyfish mesogloea samples. In our previous study, we have measured the tensile and compressive properties of the equilibrium swollen jellyfish mesogloea (Wang et al., 2011). However, we used cubic specimens for the tensile tests and found that the cubic specimens usually broke at the

gripped part, since the load applied to fix the specimens to the clamping apparatus might damage the samples. Therefore the obtained fracture tensile stresses and strains must be lower than the true values. In this study, we prepared gel specimens with a standardized dumbbell shape to ensure the breakage will occur in the gauge length. In addition, for the fabrication of hybrid hydrogel, in our previous study we swelled jellyfish mesogloea in an acidic monomer–acrylic acid (AA) aqueous solution (Wang et al., 2011). The swelling process may affect the mechanical properties of jellyfish mesogloea, so the tensile and compressive properties of jellyfish mesogloea swollen in an acetic acid aqueous solution (pH = 4.00) were also studied in this work.

The samples equilibrium swollen in water are designated as SW and those swollen in the acetic acid aqueous solution (pH = 4.00) are designated as SA. The typical tensile stress–strain (σ_t–ε_t) and compression stress–strain (σ_c–ε_c) curves of the swollen and dried samples are shown in Fig. 2. The σ_t–ε_t curves of SW and SA are shown in Fig. 2(a). The fracture tensile stress (σ_t) and elongation at break (ε_t) of SW are about 0.17 MPa and 100%, respectively, both are higher than those (0.1 MPa and 80%) of SA. The σ_c–ε_c curves of SW and SA are shown in Fig. 2(b). The fracture compressive stress (σ_c) and strain (ε_c) of SW are about 1.4 MPa and 86%, respectively, they are also higher than those (0.78 MPa and 80%) of SA. It is necessary to point out that both SW and SA did not break into many small pieces under compression, instead only a big crack formed. The fracture of SW and SA is indicated by a small drop in σ_c as shown in Fig. 2(b). Both tensile and compression tests indicate that when swollen in an acidic solution, the tensile and compressive properties of jellyfish mesogloea are weakened.

During our work, we found that the jellyfish mesogloea formed a rigid thin film after being dried in air at ambient temperature. We measured the tensile properties of the dried SW and SA samples, and their σ_t–ε_t curves are shown in Fig. 2(c). The fracture tensile stresses of the dried jellyfish mesogloea films are much higher than those of the swollen samples; they are about 47 MPa and 25 MPa for dried SW and SA, respectively; but their elongations at break decrease dramatically to be only about 2.5% and 3.4%.

The fracture tensile stress (σ_t) and elongations at break (ε_t), fracture compressive stress (σ_c) and strain (ε_c), and tensile elastic modulus (E_t) are summarized in Table 1.

Tough synthetic hydrogels usually have S-shaped tensile stress–strain (σ_t–ε_t) curves, *i.e.* σ_t firstly increases with ε_t rapidly with ε_t, and then at a lower rate, at last increases quicker again till fracture (Civerchia-Perez et al., 1980; Yannas and Tobolsky, 1967). The elastic modulus (E) of a hydrogel is usually calculated in the low strain range (*e.g.* 10%–30%). However, the SW and SA jellyfish mesogloea showed J-shaped σ_t–ε_t curves, the σ_t firstly increases with ε_t linearly but very slowly, and then at a faster rate to an almost linear range; at last decreases till fracture (Fig. 2(a)). This kind of σ_t–ε_t curve has been reported for some other crosslinked collagen films (Weadock et al., 1983). The elastic moduli (E_t) of the SW and SA jellyfish mesogloea calculated in the low strain range (0%–10%) are quite low (Table 1). We have also calculated the E_t of the jellyfish mesogloea in the second linear range (40%–60%), and the calculated values (Table 1) are about two orders of magnitude higher than those calculated in the low strain ranges.

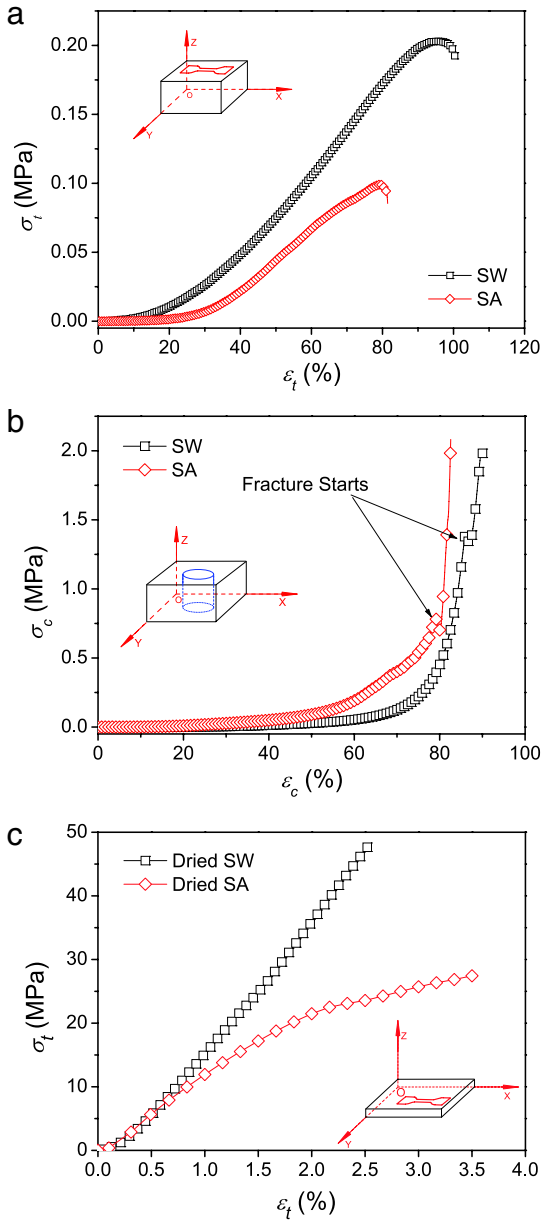


Fig. 2 – Typical tensile (a) and compressive (b) stress–strain curves of swollen jellyfish mesogloea, and tensile stress–strain curves of jellyfish mesogloea dried in air at ambient temperature for 72 h (c). SW: equilibrium swollen in water; SA: swollen in the acetic acid aqueous solution (pH = 4.00). The insets show the directions from which the specimens were cut.

Conventional synthetic hydrogels generally have very low σ_t(~10 kPa), low ε_t (<50%), low σ_c (<0.4 MPa) and low E_t (<100 kPa) (Naficy et al., 2011; Gong et al., 2003). On the contrary, the recently developed tough hydrogels exhibit largely improved mechanical properties. For example, NC gels exhibit very high ε_t (usually >1000%), low to medium σ_t (10–1000 kPa) and low E_t (1–50 kPa) (Haraguchi and Takehisa, 2002; Haraguchi et al., 2002; Xiong et al., 2008). The MMC gels (Huang et al., 2007), peroxidized micelles initiated and crosslinked (pMIC) hydrogels (He et al., 2011)

Table 1 – Mechanical properties of swollen and dried jellyfish mesogloea.

Sample	σ_t (MPa)	ε_t (%)	σ_c (MPa)	ε_c (%)	E_t (MPa)
SW	0.17 ± 0.05	102.7 ± 22.3	1.43 ± 0.41	81.2 ± 6.0	0.026 ± 0.001[a]; 0.30 ± 0.02[b]
SA	0.10 ± 0.07	80.3 ± 7.1	0.76 ± 0.35	76.1 ± 5.7	0.003 ± 0.0008[a]; 0.19 ± 0.05[b]
Dried SW	47.6 ± 1.6	2.5 ± 0.2	–	–	1600 ± 58[c]
Dried SA	27.4 ± 0.9	3.6 ± 0.3	–	–	1300 ± 24[c]

The strain ranges used for E_t calculation are:
[a]0%–10%
[b]40%–60%
[c]0.25%–1.0%.

and the hybrid gels (Wang et al., 2011) developed by our group show extremely high σ_c (up to tens of MPa), low to medium σ_t (50–230 kPa) and E (10–690 kPa). DN gels have very high E (sub-MPa to several MPa), high σ_c (up to 20 MPa) and very high σ_t (sub-MPa to several MPa) (Gong et al., 2003; Nakayama et al., 2004).

From the mechanical test results, we can conclude that the jellyfish mesogloea has excellent mechanical properties. The σ_t and σ_c of swollen jellyfish mesogloea are much higher than those of conventional synthetic hydrogels. The absolute values of the mechanical properties of jellyfish mesogloea are similar to or lower than those of the tough synthetic hydrogels. However, we must notice that the water content of jellyfish mesogloea is ultrahigh (about 99 wt%), whereas the tough synthetic hydrogels are usually measured with a wide water content range of 50–95 wt%. No reports on the mechanical properties of the hydrogels with a water content of 99 wt% can be found. As one can image, the mechanical properties of the tough hydrogels must become much worse if they can swell to such a high water content as in jellyfish mesogloea, since the mechanical properties dramatically decrease with increasing water content. As proven by the fact that the modulus of a pMIC gel decreases from 0.51 MPa to only 0.07 MPa when the water content decreases from 57.6 wt% to 80 wt% (He et al., 2011). The dried jellyfish mesogloea has very high σ_t and E_t, which are very similar to those of synthetic plastics. Not conservatively speaking, the mechanical strengths of jellyfish mesogloea exceed most synthetic hydrogels with similar or even lower water contents.

To understand the origin of the mechanical properties of jellyfish mesogloea, we need to know its chemical and physical structures. The chemical compositions of jellyfish mesogloea has been well understood, it is mainly made up of Type II collagen (Hsieh, 2005), and polyunsaturated fatty acids (White and Hager, 1977). Collagen has been applied to the fabrications of polymeric materials (Banwell et al., 2009; Cross et al., 2011; Deng et al., 2010), however, none of them can achieve the mechanical properties of jellyfish mesogloea. For example, the self-assembling hydrogels based on standard linear peptides with purely α-helical structures (Banwell et al., 2009) have a water content of about 99.1% (a value similar to that of jellyfish mesogloea), but their moduli (1 kPa or less) is far less than that of jellyfish mesogloea (26 kPa, Table 1). Therefore, we believe that the excellent mechanical properties of jellyfish mesogloea are attributed to its special physical structures.

The diameters of the fibres found in jellyfish mesogloea are mostly about 20–50 nm (Fig. 1). The collagen triple helix is about 1.5 nm in diameter (Buehler, 2006). So each fibre in jellyfish mesogloea is composed of about 100–1000 triple helices and hence a tripled number of polypeptide chains if they are closely packed. In normal synthetic hydrogels, two or more single polymer chains are crosslinked by a multifunctional cross-linking agent to form a network. It is reasonable to predict that the big fibres in jellyfish mesogloea are much stronger than those single polymer chains in normal synthetic hydrogels. In addition, the membranes and fibres in jellyfish mesogloea are distributed very evenly, and thus the applied force can be evenly shared by the whole structure. This energy dissipation mechanism makes crack initiation and propagation is difficult to occur in jellyfish mesogloea. Whereas the cross-linking points and the chain lengths between cross-linking points in normal synthetic hydrogels are distributed heterogeneously, hence the applied load concentrates on the weakest regions (the shortest chains) and leads to their fracture. The microscopic fracture can be expanded to a macroscopic fracture in the gel due to the successive breakage of the next weakest regions. Therefore, the big fibres and their even distribution are possibly the main reasons for the high mechanical strengths of the jellyfish mesogloea.

The fibres in jellyfish mesogloea are connected with the membranes and with each other to form coils and meshes. When the applied strain is small, the stress can be dispersed by the deformation of the network through the bending and stretching of the coils and meshes, therefore the increasing rate of σ_t with ε_t and hence E_t is slow. With the increase of the applied strain, the bending of the coils and meshes reaches the maximum and then the applied stress has to be sustained by the stretching of the fibres. Due to the strong interactions (hydrogen bonding and covalent bonds) among the triple helices in the fibres, the fibres are not easy to be stretched. Therefore, σ_t increases with ε_t at a much faster rate. Because the polypeptides in the triple helices are in the stretched conformation, consequently the elongation of the fibres is small. The breaking of hydrogen bonding and covalent bonds among the triple helices might account for the fracture of the jellyfish mesogloea.

When the jellyfish mesogloea is dried in air at ambient temperature, the loss of water makes the membranes and the fibres collapse to form a compact layered structure. The dehydration may produce more hydrogen bonds among collagen fibres in close contact, and it may also provide a driving force for the occurrence of some chemical reactions to introduce more covalent bonding among the fibres (Eyre, 2003). The stronger interactions lead to the high tensile stresses and the high moduli, and the dramatic decrease in tensile strains is due to the lack of the deformation mechanism by bending of the coils and meshes in the swollen

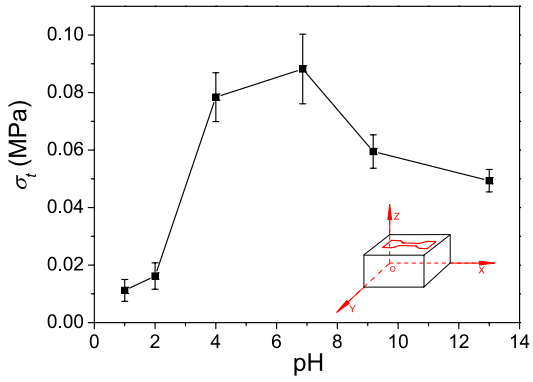


Fig. 3 – Tensile strength of the jellyfish mesogloea swollen in buffer solutions with different pH values for 1 h. The inset shows the direction from which the specimens were cut.

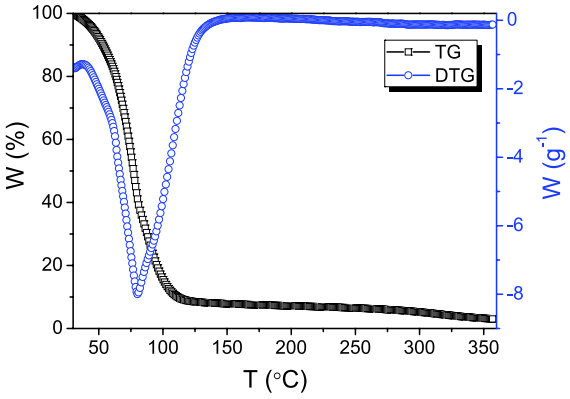


Fig. 4 – TG–DTG curves of the jellyfish mesogloea equilibrium swollen in water.

state. The moduli of the dried jellyfish mesogloea are about four to six orders of magnitude higher than those of swollen samples. This fact may serve as an evidence for the existence of stronger interactions in the dried sample, since if there are no more interactions among the fibres the dried samples should have moduli only two orders of magnitude higher than those of the corresponding swollen ones with a water content of 99 wt%.

Fig. 2 shows that the mechanical properties of the jellyfish mesogloea swollen in an acetic acid solution (pH = 4.00) become weaker. To know the effect of pH on the mechanical properties of the jellyfish mesogloea in a wider pH value range, we carried out the tensile tests of the jellyfish mesogloea swollen in buffer solutions with different pH values (1.00, 2.00, 4.00, 6.86, 9.18 and 13.00). The variation of fracture tensile stress (σ_t) with pH value is shown in Fig. 3.

The σ_t of jellyfish mesogloea is very small in buffer solutions with pH values of 1 and 2, less than 0.02 MPa. When pH value is increased from 2 to 4, the σ_t increases rapidly and it is almost quadrupled. The σ_t reaches the maximum at pH = 6.86, after then it decreases with increasing pH value. It is necessary to mention that the σ_t of the jellyfish mesogloea decreases significantly with swelling time in the buffer solutions in the first half hour, no obvious decrease could be observed after 1 h swelling, therefore, the tensile tests of the jellyfish mesogloea were carried out after being swelled in the buffer solutions for 1 h. It is also need to note that the σ_t of jellyfish mesogloea swollen in the buffer solutions is lower than that swollen in water, even when the pH value (6.86) is very close to that of pure water (pH = 7.00). The main difference between the pH = 6.86 buffer solution and pure water should be the ionic strength, and hence ionic strength may also have a significant effect on the mechanical properties of jellyfish mesogloea.

The weakening of jellyfish mesogloea in both acidic and basic buffer solutions is in agreement with the results on the articular cartilage and corneal stroma (Loret and Simões, 2010). Variation of pH has strong implications on the electric charge of these tissues, and on their mechanical properties. The possible reason is that part of the interactions among the triple helices in the fibres is broken in the acidic solution. This will be discussed in more details in the swelling part.

3.3. *Water content*

Jellyfish mesogloea contains about 99 wt% water, but thermogravimetric analysis proved that the majority of water (>95 wt%) is free water (Fig. 4). The water in jellyfish mesogloea could be easily squeezed out under an applied force, and the squeezed jellyfish mesogloea could not restore its original shape by absorbing water. We measured the water losses of the equilibrium swollen jellyfish mesogloea during compressive and tensile tests, and they were about 57 wt% and 52 wt%, respectively. The water loss in the compressive tests was higher than that in the tensile tests. The apparent reason is that in tensile tests water loss mainly occurred in the both ends of dumbbell-shaped samples which were gripped with clamping apparatus; whereas during compressive tests water loss occurred in the whole sample.

There is an interesting comparison between the water loss of jellyfish mesogloea and normal synthetic hydrogels. Normal synthetic hydrogels can lose water through evaporation slowly, but water in them cannot be squeezed out during the mechanical tests. This is partially due to the hydrophilic nature of the polymers in normal synthetic hydrogels and hence more bound water in them. In addition, the polymer chains are randomly distributed in the gels and hence there are not real “pores” in the hydrogels in the swollen state, though pores can be observed with SEM when the gels are freeze-dried and the polymer chains collapse to form the pores. On the contrary, most of the water in jellyfish mesogloea is free water. In addition, jellyfish mesogloea shows a layered macroporous structure with pore sizes in micrometres, and the pores are composed of inter-connected collagen fibres (Fig. 1(a)). This structure is very possibly the same as that in the swollen state, since there is not a mechanism for the formation of the fibres and membranes via freeze-drying. This penetrating macroporous structure provides the channels for the free migration of water. Therefore, the water in jellyfish mesogloea can be easily squeezed out. These results and discussion suggest that water in jellyfish mesogloea is mechanically held by the stiff network of jellyfish mesogloea.

For normal hydrogels, dehydration during test can have a very significant effect on their mechanical properties, since stronger interactions (hydrogen bonding, physical entanglement) can be formed among the polymer chains which become closer to each other with the loss of water. However,

the distance between the fibres and membranes (hundreds nanometres to micrometres) in the jellyfish mesogloea is much bigger than the distance between the polymer chains in normal synthetic gels, so no strong interactions can be formed among the separated nano-sized fibres or membranes in the presence of water. Therefore, we do not think that water loss during the tests would have significant effect on the mechanical strengths of jellyfish mesogloea. Only when the jellyfish mesogloea has been fully dried, the fibres and membranes are in close contact, strong interactions can be formed and hence its mechanical strength increases dramatically. An evidence for the formation of strong interactions is that dried jellyfish mesogloea became nearly insoluble (Eyre, 2003).

3.4. *Swelling properties*

Mature crosslinked collagen is water insoluble but it swells to some extents. Swelling/deswelling property is an important aspect of hydrogels. There is no report on the swelling properties of jellyfish mesogloea yet. Therefore, we included them in this study. The swelling/deswelling of normal synthetic hydrogel is generally isotropic, which means that the physical changes occur equally in three dimensions. Only a few hydrogels with anisotropic swelling properties have been reported (Aizenberg and Fratzl, 2009; Buyanov et al., 2010). We found that when the jellyfish mesogloea was immersed in solutions with different pH values, it displayed a very interesting anisotropic swelling property.

The jellyfish mesogloea used was desalted and equilibrium swollen in deionized water. Its water content was about 99 wt%. The cubic jellyfish mesogloea samples were put into the buffer solutions with different pH values (1.00, 2.00, 4.00, 6.86, 9.18 and 13.00), and then the change of their dimensions (length, width and height) with time was recorded. The height of a sample is the length on the direction perpendicular to the umbrella surface of jellyfish, and the length and width are the lengths on the direction parallel to the umbrella surface of jellyfish (see the inset in Fig. 5(a)). The ratio of the length, width and height (l) to the original values (l_0), i.e. l/l_0, is plotted as a function of swelling time (t), and l/l_0–t curves for jellyfish mesogloea in the pH = 2.00 solution are shown in Fig. 5(a).

In an acidic solution (pH = 2.00), the jellyfish mesogloea expanded in height but shrank in length and width in the first 30 min, and then reached a new equilibrium. The new equilibrium height increased about 85%, but the width and length decreased about 25%. The change of width and length with swelling time was almost the same, indicating there is no difference in swelling on these two directions. Calculating from the dimensions before and after swelling, it can be found that there was almost no change in the volume of the jellyfish mesogloea specimen. So the term "swelling" here has a meaning different from the common concept.

The relative changes in the height and length $[(l - l_0)/l_0]$ of jellyfish mesogloea in solutions with different pH values are included in Fig. 5(b), the heights and lengths are taken at the swelling time of 1 h, a time ensuring the swelling reaches the new equilibrium. There is a small increase in the absolute value of $(l - l_0)/l_0$ when pH value was increased from 1.00 to 2.00, but after then the value decreases with increasing pH value, and there is almost no change in the solution with a pH

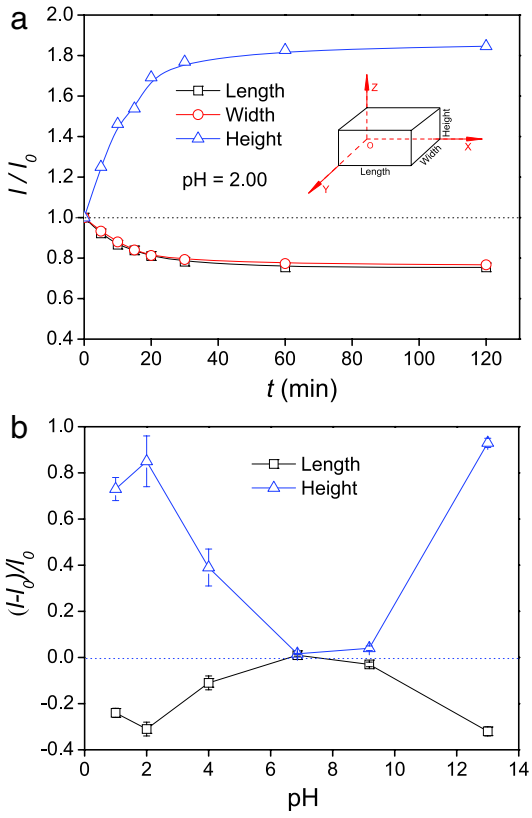


Fig. 5 – Anisotropic swelling phenomenon of jellyfish mesogloea samples in aqueous solutions with different pH values. (a) size change ratio (l/l_0) in length, width and height with time (pH = 2.00), (b) the size change ratio in length and height at a swelling time of 1 h with pH value. The inset in (a) shows the definitions of length, width and height according to the anatomical axes.

value of 6.86. Anisotropic swelling of jellyfish mesogloea was also observed in alkaline solutions. The anisotropic swelling behaviour was not significant in the pH = 9.18 buffer solution, whereas it was obvious in the pH = 13.00 buffer solution. In the full pH range tested, the change of swelling with pH in length (width) is totally opposite to that in height.

The swelling of collagen in both acidic and alkaline solutions has been reported (Bowes and Kenten, 1950a; Deng et al., 2010; Fratzl, 2007). Bowes and Kenten (1950a,b) studied the swelling of collagen *D* in hydrochloric acid/sodium chloride and sodium hydroxide/sodium chloride systems, they found that the swelling firstly increases sharply with rise in pH to about 2, then decreases with pH to about 5, the swelling keeps constant or increases slowly in the pH range of 5–10, and then increases again very rapidly with further rise in pH. The swelling of jellyfish mesogloea with pH in the direction perpendicular to the umbrella surface (Fig. 5(b)) is almost the same as that reported by Bowes and Kenten (1950a,b).

Jellyfish mesogloea is a porous network structured by collagen fibres, and its major component is Type II collagen. So there are probably some similarities between the swelling behaviours of jellyfish mesogloea and collagen. The amino acids in collagen contain an amino group NH_3^+, a carboxyl

group COO^-, a hydrogen atom and a side chain. Stable peptide bonds form between the amino group and carboxyl group of two successive amino acids. Due to the presence of the amino- and carboxyl groups on these side chains which can be electrically charged, the collagen molecule becomes positively charged at low pH, and negatively charged at high pH. Therefore, the presence of the hydrogen ion H^+, hydroxyl ion OH^-, and metallic ions would affect the mechanical and swelling properties, since the electrical charge on collagen varies with pH (Winkler, 1973). There is an interesting parallel between the swelling (Fig. 5(b)) and tensile strength (Fig. 3) of jellyfish mesogloea in buffer solutions with different pH values, *i.e.* the tensile strength is weaker when the directional swelling/deswelling ratio is higher at either a low or a high pH value. So the change of tensile and swelling properties with pH value may have the same reasons.

The swelling of collagen can be divided into osmotic and lyotropic ones. Osmotic swelling (Donnan swelling) comes from the increase of charge bound at protein surface. It takes place when pH of the solution is off the isoelectric point (IEP), which corresponds to the balance between the negatively charged groups and positively charged groups. The IEP of the collagen in jellyfish mesogloea cannot be found, but a wide range from about 3 to 9 has been reported for other collagens (Bar-Cohen, 2006; Bowes and Kenten, 1950b). The high swelling ratio of jellyfish mesogloea in low and high pH solutions should be due to the osmotic swelling, since the existence of excess ions having opposite charges inside the gel initiates action of osmotic forces. When pH decreases to be less than 2, the increase of ionic strength suppresses collagen swelling. Lyotropic swelling occurs at every pH if only salt concentration is high enough (over 0.5 molar) or in solutions having lower salt concentration, and a neutral pH, it decreases the cohesion of the fibres and is not completely reversible. For the jellyfish mesogloea samples swollen in aqueous solutions with different pH values, it seems that the osmotic swelling is the main driving force and lyotropic ions play an additional role.

For the reported hydrogels with anisotropic swelling properties (Aizenberg and Fratzl, 2009; Buyanov et al., 2010), they swell only in one direction or swell in all directions but with different swelling rates. The jellyfish mesogloea swells (expands) in height but deswells (shrinks) in length and width. To the best of our knowledge, this phenomenon has never been reported for synthetic hydrogels. This kind of anisotropic swelling may have important implications in designing hydrogels for some special applications.

The anisotropic swelling of jellyfish mesogloea might be due to its layered microstructure. Due to the lack of the detailed chemical and physical structures of the membranes and the fibres, we cannot provide a clear explanation for the anisotropic swelling of jellyfish mesogloea. However, from the experiment results, *i.e.* the total opposite changing tendency of swelling with pH in height and length (width), we may conclude that the membranes should have a structure different to that of the fibres. In an acidic or alkaline environment, the membranes shrink whereas the fibres swell. We hope that the advances in microbiology and related disciplines and the improvements in characterizations will provide better understanding of multi-level structures of jellyfish mesogloea and hence the mechanical and swelling properties of jellyfish mesogloea.

4. Conclusions

We studied the structure, mechanical and swelling properties of the mesogloea of an edible jellyfish (*Rhopilema esculenta Kishinouye*). The jellyfish mesogloea has a well-developed layered porous structure, which consists of evenly distributed nano-sized membranes and fibres. The membranes and fibres might be formed by the aggregation of collagen triple helices. The jellyfish mesogloea exhibits excellent mechanical properties even with a very high water content (≈99 wt%), and the dried jellyfish mesogloea has mechanical properties similar to those of synthetic plastics. The strong fibres and their even distribution are considered as the main reasons for the high mechanical strengths of the jellyfish mesogloea. The mechanical properties of jellyfish mesogloea vary with pH values, since the electrical charge on collagen varies with pH due to the change of ionic strength. In addition, the jellyfish mesogloea exhibits pH sensitivity and an interesting anisotropic swelling property in aqueous solutions with different pH values, *i.e.* the jellyfish mesogloea swells (expands) in height but deswells (shrinks) in length and width. The pH sensitivity of jellyfish mesogloea is mainly due to the osmotic swelling induced by the increase of charge in the fibres. The reason for the anisotropic swelling property of jellyfish mesogloea is not clear yet, however, it should be related to its layered microstructure.

Learning from natural materials and understanding their structure–function relationships can provide us new ideas for designing and fabricating new materials with well-defined microstructures and special properties. Jellyfish mesogloea mechanically holds a large amount of water by its stiff network. This is different to the water-absorbing mechanism of normal synthetic hydrogels. The mechanical strength of jellyfish mesogloea comes from the evenly distributed strong fibres which consist of hundreds of collagen triple helices. This structure has not been reported in synthetic hydrogels yet. At last, the special anisotropic swelling property found in jellyfish mesogloea, *i.e.* swelling in one direction but deswelling in another direction simultaneously, has never been reported for synthetic hydrogels. We believe that the unique structure, extraordinary mechanical and swelling properties of jellyfish mesogloea can inspire gel scientists with new ideas in designing and fabricating novel hydrogels or other materials to meet the requirements of some special applications.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 50673013, 21074014), China–Australia Fund for S&T Cooperation (Grant No. 50811120112) and Beijing Municipal Commission of Education.

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