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1 **Internal Damage Evolution in Double-Network Hydrogels Studied by**
2 **Microelectrode Technique**

3

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24

25 **Abstract**

26 Double-network (DN) hydrogels have attracted considerable attention owing to
27 their unique mechanism to show extraordinary mechanical strength and toughness.
28 Although the toughening mechanism of the DN gels, breaking of the relatively stiff and
29 brittle first network as sacrificial bonds, is widely accepted, the microstructure and
30 morphology evolution of the internal damage has hardly been revealed. In this study,
31 we study the internal structures of the first network in partially damaged DN gels by
32 using the microelectrode technique (MET), based on the Donnan effect of the
33 polyelectrolyte first network. We measure the spatial distribution of the electric
34 potential of the pre-stretched and then re-swelled DN gels. From the anisotropic depth
35 profiles of potential and re-swelling ratio, the microstructures of DN gels at a scale
36 larger than the microelectrode probe size (~ 200 nm) are revealed at the pre-yielding,
37 yielding, and strain-hardening regimes.

38

39 Introduction

40 Hydrogels are promising biomaterials for artificial organs owing to the high water
41 content and soft properties, similar to natural tissues.¹ To mimic load-bearing bio-
42 tissues such as cartilage, one would have to improve the strength and toughness of
43 hydrogels. Double network (DN) hydrogels, initially developed by Gong's group in
44 2003, have excellent mechanical properties comparable to those of human cartilage.^{2,3}
45 A DN hydrogel consists of two interpenetrated polymer networks with contrasting
46 structural and mechanical characteristics: the first network is stiff but brittle, while the
47 second is compliant and stretchable. Such DN hydrogels were initially developed by
48 using the abundantly cross-linked polyelectrolytes as the first network and the loosely
49 cross-linked neutral polymers as the second network.² The polyelectrolyte hydrogel
50 swells extensively in water owing to the counter-ions-induced osmotic pressure,
51 forming a highly stretched, brittle network and leaving ample space for the polymer
52 chains of the second network.

53 Despite the high water-content (~ 90 wt%), DN gels exhibit unprecedented
54 mechanical properties. For example, the DN gels consisting of poly(2-acrylamido-2-
55 methylpropanesulfonic acid) (PAMPS) as the first network and polyacrylamide (PAAm)
56 as the second network are relatively stiff (elastic modulus of 0.1-1.0 MPa), very strong
57 (tensile strength of 1-10 MPa), stretchable (tensile failure strain of 1000-2000 %), and
58 extremely tough (tearing fracture energy of 10^2 - 10^3 J/m²).⁴

59 It has been shown that such a strengthening/toughening effect is universal,
60 independent of the specific polymer chemistry. For example, tough DN gels from

61 diverse pre-stretched neutral polymer networks as the first network have been
62 developed using the molecular stent method.⁵ Additionally, tough double-network and
63 triple-network elastomers have also been developed.^{6,7}

64 Previous studies^{4,8,9} have related the extraordinary toughness of DN gels to the
65 distributed damage of the first network, which dissipates a large amount of energy. Thus,
66 the covalent bonds of the first network serve as sacrificial bonds, providing toughness
67 for the DN gels. Tough DN gels exhibit large hysteresis on the stress–strain curves and
68 a strain–softening behavior similar to the Mullins effect^{10,11} of rubbers owing to the
69 extensive internal fracture of the brittle first network.^{8,9}

70 For the DN gels, the typical tensile behavior of the samples shows three regimes
71 during loading: the pre-necking regime, in which the stress increases with the strain;
72 the necking regime, in which the stress plateaus at a constant value, independent of the
73 strain, and a narrow necking zone appears and gradually devours the entire sample with
74 the increase of strain; and the hardening regime, in which the stress increases with the
75 strain again.⁸ Upon unloading, a sample may swell further in water because of the
76 partial damage in the brittle first network locally releases the constrained second
77 network, and a new osmotic balance may be reached by absorbing more water. Based
78 on the mechanical hysteresis and re-swelling behavior of PAMPS/PAAm DN gels, an
79 internal damage mechanism was proposed.¹² In the pre-necking regime, the load was
80 mainly carried by the first network. Although some short strands of the first network
81 break, the damaged regions are far from percolating. In the necking regime, the cracks
82 percolate, fragmenting the first network into discontinuous islands. As the region

83 between the fragments are only connected by the soft and stretchable second network,
84 the material softens significantly, leading to the necking phenomenon. In the hardening
85 regime, the fragments of the first network further break into smaller islands due to strain
86 hardening of the second network at large stretching.

87 Some structural analyses have been performed to examine the microstructures of
88 partially damaged DN gels. When the heterogeneous model of DN gel was firstly
89 proposed, the presence of “voids” in the first network PAMPS was deduced according
90 to the result of dynamic light scattering (DLS)¹³, varying the molecular weight of the
91 polymer PAAm¹⁴, and mechanical experiment^{15,16}. Using small-angle neutron
92 scattering (SANS) technique, it was revealed that the heterogeneity in a PAMPS/PAAm
93 DN gel, after a small compression (~ 50% strain), had a characteristic length of about
94 1.5 μm .¹⁷ According to the result of wrinkled morphology of ultra-thin DN gel¹⁸ and
95 anisotropic re-swelling behavior,¹² those fragments were considered as rectangular or
96 plate-like structure. Assuming that the dissipated energy is used for the fragments
97 formation through breakage of the covalent bonds, a characteristic size of about 100
98 nm has been estimated, at a tensile strain of 1,100%, for typical DN gels.⁴ However,
99 there are few direct evidences to verify the assumed microstructure changes during the
100 tensile deformation. How the size changes with respect to the tensile strain,
101 corresponding to the heterogeneous fragmentation of PAMPS networks, is yet unclear.
102 A method to directly probe the microstructures of partially damaged DN gels is required.

103 A polyelectrolyte gel has macro-ions fixed on the polymer network and small
104 counter-ions localized inside the network frame. This concentration difference of the

105 counter-ions between inside and outside of the hydrogel leads to an electric potential
106 difference, known as the Donnan potential, between the polyelectrolyte gel and the bath
107 solution. Therefore, by measuring the spatial distribution of the electric potential in a
108 polyelectrolyte hydrogel, one can determine the local counter-ion density, which
109 directly reveals the local structure of the hydrogel. The microelectrode technique (MET)
110 is widely used for detecting the electric potentials in living cells, in neurons reacting to
111 stimuli, and in live animals.¹⁹ Recently, some co-authors succeeded in applying the
112 MET for detecting the electric potential of brittle polyelectrolyte hydrogels by using an
113 extremely thin capillary electrode.²⁰ This technique allows the accurate probing of the
114 spatial distribution of polyelectrolyte density from the depth profile of the electric
115 potential inside the hydrogels with a spatial resolution in the order of probe size.

116 In this study, we study the microstructural evolution of partially damaged DN
117 hydrogels by measure the electric potential distribution of the re-swelled samples. As
118 shown by previous works, the internal fracture of the polyelectrolyte first network
119 results in anisotropic re-swelling of the DN gels,¹² which indicates a decreased
120 concentration of the first network in the fractured region and enhanced structure
121 heterogeneity. By measuring the evolution of the electric potential distribution with the
122 pre-stretched strains that have been experienced by the samples, we can elucidate the
123 structure change of the first network induced by internal fracture. Firstly, the DN gels
124 are uniaxially stretched to various strains, and then the samples are re-swelled in water.
125 The anisotropic re-swelling behavior of damaged DN hydrogels in different stretch
126 regimes is characterized. Then, the depth profiles of the electric potential of the pre-

127 stretched DN gels in the re-swollen state are measured using the MET from the three
128 directions of the samples. From the potential variation, the anisotropic microstructure
129 of the fractured PAMPS network is elucidated. Furthermore, the structure at the
130 boundary of the necked and un-necked zones is also studied using the MET to reveal
131 the origin of the necking phenomenon.

132

133 EXPERIMENTAL

134 *Materials*

135 2-Acrylamido-2-methylpropanesulfonic acid (AMPS) was provided as a courtesy
136 from Toa Gosei Co., Ltd and used as received. Acrylamide (AAm) purchased from
137 Junsei Chemical Co., Ltd was recrystallized from chloroform. *N,N'*-
138 methylenebisacrylamide (MBAA), 2-oxoglutaric acid (OA), and potassium chloride
139 (KCl) were purchased from Wako Pure Chemical Industries, Ltd and used as received.

140 *Synthesis of DN hydrogels*

141 DN hydrogels were synthesized according to the previous report². Briefly, the first
142 network of PAMPS gels, coded as 1-4-0.6, was prepared via radical polymerization,
143 from an aqueous solution containing 1 M AMPS monomer, 40 mM MBAA as a cross-
144 linker, and 6 mM OA as an initiator. The precursor solution was poured into a reaction
145 cell consisting of two glass plates spaced with 1 mm silicone rubber. PAMPS hydrogels
146 were obtained after ultraviolet (UV) irradiation for 8 h in an argon atmosphere. Then,
147 the PAMPS gels were immersed into an aqueous solution containing 2 M AAm
148 monomer and 0.2 mM OA for 1 d. The swollen gels were sandwiched between two

149 glass plates and UV irradiated for 8 h in an argon atmosphere for the second
150 polymerization of PAAm. Subsequently, DN gels were obtained and immersed into
151 pure water for about one week until equilibrium was reached. No chemical cross-linker
152 was added in the polymerization of the second network and the PAAm chains were
153 chemically connected to the PAMPS network via the residual cross-linkers of the
154 PAMPS network.²¹

155

156 *Mechanical tensile tests*

157 A tensile test was performed using a universal mechanical test machine (Tensilon
158 RTC-1150A, Orientec Co.). The swollen DN gels, of 2.51 mm-thick, were cut into
159 dumbbell-shaped specimens with standardized JIS K 6251-7 sizes (length of 35 mm,
160 width of 2 mm, and gauge length of 12 mm) with a gel cutting machine (DumbBell Co.,
161 Ltd.). The DN specimens were stretched to different strains at a constant velocity of
162 100 mm/min at room temperature. In this work, the stress σ refers to the nominal stress,
163 defined as the tensile force divided by the cross-section area of the un-deformed sample,
164 and the engineering strain, ε , is defined as the change in the gauge length divided by
165 the gauge length of the un-deformed sample.

166

167 *Re-swelling ratio measurements*

168 To measure the re-swelling ratios at different positions, the dumbbell-shaped DN
169 gel with a gauge length of 12 mm was divided into 11 sections by markers (0.1mm in
170 width, 1 mm in distance) using a plastic mask before stretching (**Figure 2a**). The spaced

171 markers were printed onto the gel surface by using hydrophobic white ink with the aid
172 of a mask that is made by a computer-controlled laser cutter. Then the DN gel was
173 stretched uniaxially to a prescribed strain and then unloaded. The stretched sample was
174 re-swelled in pure water for 2 d until equilibrium was reached. After that, the width,
175 thickness, and length of each different section was measured according to the markers.
176 The linear re-swelling ratios between the re-swollen state and the virgin state prior to
177 stretching, denoted as λ_w , λ_t , and λ_l for the width, thickness, and length directions, were
178 calculated. The volume re-swelling ratio, q , was estimated as $q = \lambda_w \lambda_t \lambda_l$. For the necking
179 samples with coexisting necked and un-necked zones, λ and q values were obtained
180 from sections of the two types of regions. After that the size measurement, the re-
181 swollen samples were transferred into the reference KCl solution for more than 2 d until
182 equilibrium was reached.

183

184 ***Potential measurement***

185 The electrical potential ϕ of the samples in relative to the bath solution was
186 measured by using the MET, according to our recent report.²⁰ Briefly, the
187 microelectrodes were prepared by placing a reversible silver/silver chloride electrode
188 into a glass capillary. Depending on the experiments, the tip diameter of the capillary
189 was varied between 100 and 1500 nm. The glass capillaries were prepared by pulling a
190 borosilicate glass tube (outer diameter of 1.00 mm, inner diameter of 0.78 mm) using a
191 horizontal automatic micropipette puller (P-2000, Sutter) and then filled with a 3 M
192 KCl solution and connected to the inputs of a high-impedance intracellular preamplifier

193 (Model 8700 Cell Explorer, Dagan). A carbon electrode, which was placed in the bath
194 solution, was used as the reference electrode. The re-swelled DN gels, which had
195 thickness of 2.5–5.0 mm depending on the pre-stretch ratio, was immersed in a 10^{-5} M
196 KCl solution for equilibrium prior to the measurement. A glass microelectrode
197 controlled using a micromanipulator (DMA-1511, Narishige) was inserted into the
198 hydrogel at a constant speed of 0.4 $\mu\text{m/s}$. The output signals were recorded using an
199 oscilloscope (Iwatsu, DS-4264) in the real-time mode. The electrical potential signals
200 were collected at 100 sampling per second. All the measurements were performed at
201 25 °C. The spatial resolution from the micromanipulator in the depth profile of the
202 insertion direction was 5 nm. Unless otherwise specified, the potential measurements
203 were performed by using a microelectrode with tip diameter 170–200 nm, and the
204 obtained potential values were the average over this length scale.

205 It should be noted that the residual chemicals due to the uncompleted reaction in
206 the DN synthesis should be carefully removed before the potential measurement. The
207 leakage of the residual ionic monomers from the DN gels would increase the ionic
208 strength of the reference solution, leading to a decrease in the measured potential value.
209 We have confirmed that to reduce the leakage of the chemical residuals to a level that
210 causes negligible change in the ionic strength of the reference solution ($\text{KCl} = 10^{-5}$ M),
211 the DN gels should be repeatedly washed in a large amount of deionized water for more
212 than one month. In this paper, unless specifically mentioned, the DN gels were washed
213 in a large amount of deionized water for approximately one week before stretching, and
214 then further washed in deionized water for another one week prior to the MET

215 measurements. Such samples still contained residual chemicals, resulting in an increase
216 in the ion concentration in the reference solution. From the potential difference (~ 38
217 mV) measured after one-month washing and after two-weeks washing, the ionic
218 strength of the reference solution was estimated as 4.4×10^{-5} M, which is several times
219 higher than the pre-set reference solution ($C_{\text{KCl}} = 10^{-5}$ M) due to leakage of residual
220 chemicals from the short washed DN gels. Accordingly, the potentials measured in this
221 study were relative to the reference solution with an effective ionic strength of 4.4×10^{-5}
222 M, and were approximately ~ 38 mV lower in absolute value than those measured
223 with the pre-set reference solution of 10^{-5} M KCl.

224

225 Results and discussion

226 *Re-swelling ratio of pre-stretched DN gel*

227 The stress–strain curve of the DN gels used in this study, as shown in **Figure 1**,
228 well reproduces the typical stress–strain curve of a conventional DN gel, showing the
229 three deformation regimes. The re-swelling behavior of the DN gels after different
230 degrees of pre-stretching is shown in **Figure 2**. During re-swelling, a DN sample
231 increases its size in all directions, but exhibits strong anisotropy. The linear re-swelling
232 ratios in the length direction are much larger than those in the width and thickness
233 directions, as shown in **Figure 2b**. In the necking regime, where the softened zone (the
234 narrow necked part) and unsoftened zone (the thick unnecked part) coexist as shown in
235 **Figure 1**, two different degrees of re-swelling are observed as illustrated in **Figure 2a**.
236 The re-swelling of the softened zone is much larger than the unsoftened zone in all the

237 three directions, and all the data show a slight increase with the increase of the pre-
238 strain. This is consistent with the slight increase of the stress in the necking regime,
239 indicating that the internal structure of the PAMPS network is further damaged with the
240 increase of the strain, both in the unsoftened and softened zones. In the hardening
241 regime, where the unsoftened zone disappears, and the sample deforms homogeneously
242 (**Figure 1**), the whole sample shows a homogeneous re-swelling. The volume re-
243 swelling ratios q , which is the product of the re-swelling ratio of the three directions, is
244 shown in **Figure 2c**. q increases with the pre-stretch level and become significantly
245 large. The result is consistent with the previous results on the anisotropic internal
246 damage of uniaxially stretched DN gels.¹²

247

248 *Electrical potential distribution of pre-stretched DN gel*

249 Here, we use the MET to examine the microstructure evolution of the DN gels
250 after stretching and unloading. From the equilibrium condition of electrochemistry, the
251 Donnan potential, ϕ , i.e. the electrical potential difference between the gel and the
252 solution, is related to the ratios of mobile-ion concentration C and activity coefficient γ
253 between deep in the gel (g) and the bath solution (s):²²

$$254 \quad \phi = \frac{2.3RT}{zF} \log \frac{\gamma_s C_s}{\gamma_g C_g}. \quad (1)$$

255 Here, z is the valence of the mobile ion in consideration, R is the gas constant, T
256 is the absolute temperature, and F is the Faraday constant. The subscripts g and s denote
257 the parameters in the gel and in the solution, respectively. When the salt concentration
258 in the bath solution is significantly lower than the macro-ion concentration of the

259 hydrogels, the average concentrations of macro-ions and the mobile counter-ions over
260 a length scale significantly larger than the mesh size of the network (on the order of
261 nanometers) are equal, as required by the charge-neutrality condition. Thus, by using a
262 microelectrode probe with a diameter significantly larger than the mesh size, we can
263 measure the local average concentrations of mobile counter-ions and the macroions
264 with a spatial resolution determined by the microelectrode probe size. Since the PAMPS
265 network in a DN gel has a strong concentration fluctuation at length scales much larger
266 than the mesh size of the network due to inhomogeneous chemical crosslinking,²³ this
267 MET method can probe the spatial charge density variation at a scale larger than the
268 probe size. To select a proper probe size, first, we measured the potential of a virgin DN
269 gel by using microelectrodes of different tip diameters in the range of 100 ~ 1000 nm.
270 This size range is significantly larger than the mesh size of the PAMPS network²³. As
271 shown in **Figure S1(a)**, the potential measured by microelectrodes with thick tips ($d >$
272 200 nm) is independent of the probe size, showing a value well in agreement with the
273 theoretical value estimated from the bulk average concentration of PAMPS, while that
274 measured by thin microelectrodes ($d < 200$ nm) decreases with the probe size.
275 Furthermore, a thin microelectrode shows a spatial fluctuation of potential (**Figure**
276 **S1(b)**) while a thick microelectrode does not (**Figure S1(c)**). These results clearly show
277 that thin probe measurements are affected by the spatial variation of charge density
278 while thick electrode measurements reflect the averaged potential in a large-scale. The
279 deviation of the potential measured by thin electrodes towards smaller values indicates
280 that the soft regions with low PAMPS concentration is sampled more often due to less

281 volume exclusion effect of the thin electrodes. In this study, we use a probe of tip
 282 diameter $d = 170 - 200$ nm, large enough to measure the average structure of the virgin
 283 DN gels but small enough to probe the microstructure induced by internal damage of
 284 the DN hydrogels.

285 For a pre-stretched and then re-swelled DN gel with a volume re-swelling ratio q
 286 relative to its virgin gel, the potential ϕ of the gel relative to the potential of its virgin
 287 DN gel, ϕ_0 , is theoretically related to q as

$$288 \quad \phi = \frac{2.3RT}{zF} \log \frac{\gamma_s C_s}{\gamma_g C'_g} = \frac{2.3RT}{zF} \log \frac{\gamma_s C_s}{\gamma_g C_g / q} = \phi_0 + \frac{2.3RT}{zF} \log q, \quad (2)$$

289 where C'_g is the PAMPS counterions concentration of the re-swollen sample, and C_g
 290 is the counterions concentration of the virgin DN gel. In **Eq. (2)**, we assume that the
 291 activity coefficient of counterions in the gel, γ_g , does not vary with the re-swelling ratio.
 292 The theoretical value of $2.3RT/zF$ for the PAMPS network with positive mono-valent
 293 counter-ions ($z = +1$) at 298 K is 59 mV. **Eq. (2)** has been experimentally confirmed by
 294 using MET for PAMPS gels.²⁰ So we can quantitatively estimate the local swelling ratio
 295 of the damaged DN gel from the potential profiles using **Eq. (2)**.

296 **Figure 3a** schematically illustrates the shape of re-swelled sample after being pre-
 297 stretched to the necking regime. It should be noticed that the sample surfaces
 298 perpendicular or parallel to the tensile direction, in the y,z-plane or x,z-plane,
 299 respectively, were cut surfaces, and the surface parallel to the tensile direction in the
 300 x,y-plane was un-cut surface. **Figure 3b** shows the depth profiles of the electrical
 301 potential for the DN gel measured from the x,y-plane of the re-swollen sample
 302 subjected to different pre-stretching strains. The electric potential drastically decreased

303 from zero at the gel-solution interface, and then plateaued at negative values, except for
304 some spatial fluctuations. The virgin DN gel ($\varepsilon = 0$), showed the largest potential drop.
305 The pre-stretched samples showed smaller potential drops, and the values decreases
306 with the increased strains.

307 The average bulk electrical potentials of re-swollen DN gels pre-stretched at
308 different strains are shown in **Figure 3c**. In the pre-necking regime, the potential values
309 were amplitude decrease than that of virgin DN gel, indicating that a decreased charge
310 density of the PAMPS networks in the DN gel. In the necking regime, where the
311 softened zone and unsoftened zones co-existed, the electrical potential in the unsoftened
312 zone showed only a small decrease in relative to that in the pre-necking regime, while
313 the potential in the softened zone exhibited a very large change towards the amplitude
314 decrease direction. Moreover, the absolute values of the potential in the necking regime
315 slightly decreased with the further increase of the strain, both for the softened and
316 unsoftened zones. This phenomenon is in consistent with the slight increase of the stress
317 in the stress-strain curve (**Figure 3c**, upper figure), the increase of the re-swelling ratio
318 (**Figure 2b, c**), and the varied retardation under circular polarized optical observation
319 (supporting information **Figure S2**). These results indicate that even in the necking
320 regime, the PAMPS networks in the co-existed softened and unsoftened zones were
321 further fractured slightly with the increase of the strain. In the hardening regime, the
322 potential values became amplitude decrease, clearly indicating that the PAMPS network
323 is further diluted by internal-fracture-induced re-swelling.

324 To see the correlations between the pre-stretch-induced potential changes and the

325 pre-stretch-induced re-swelling ratio changes of the sample, we plot the average
326 potential drop ϕ against the logarithmic volume re-swelling ratio $\log q$ in **Figure 3d**.

327 All experimental data points fall on a straight line, approximately,

$$328 \quad \phi/\text{mV} = -155 + 110 \log q. \quad (3)$$

329 The slope is obtained, from linear regression by using the data on **Figure 3d**, to be 110
330 mV, which is about two times of the theoretical value of 59 mV from **Eq.2**, and the
331 measured potential drops are lower than the values estimated from the average re-
332 swelling ratio q by using **Eq. (2)**. The larger the re-swelling ratio q , corresponding to a
333 more heterogeneous microstructure, the more the potential deviation. This increase in
334 the potential deviation indicates the enhanced heterogeneous microstructure for
335 samples with increased pre-stretching. This is because a decrease in the electrode size
336 relative to the spatial heterogeneity leads to a decrease in the measured potential due to
337 the same reason of the electrode size dependence as seen for a non-stretched virgin DN
338 gel (**Figure S1**). For samples experienced large pre-stretching strains, the inter-
339 fragment regions of PAMPS network, which is relatively soft with low chain density of
340 the PAMPS network, are more likely to be probed by the electrode, leading to more
341 pronounced deviation from the average values of potential.

342

343 ***Structure anisotropy and heterogeneity induced by internal fracture***

344 To reveal the structure anisotropy induced by tensile deformation, we further
345 measured the potential of the samples from the two cut surfaces (y,z- and x,z-planes)
346 and compared the results with those for the virgin surface (x,y-plane), as shown in

347 **Figure 4.** For comparison, the results measured from the x,y-plane are also shown in
348 the figure. Thicker gradient layers of 25–70 μm were observed in the potential curves
349 measured from the cut surfaces on virgin samples ($\varepsilon=0$), and in the unsoftened zone of
350 necking ($\varepsilon=5.53$) samples, compared to those from the non-cut surface. These gradient
351 layers imply the existence of damaged zones caused by cutting. No gradient layer was
352 observed in the softened zone ($\varepsilon = 5.53$ and 8.25) of the necking samples and the
353 hardening sample ($\varepsilon=15.34$), owing to the bulk internal damage of the samples under
354 large pre-stretching.

355 The average potential values in the bulk of the samples measured from the y,z- and
356 x,z-planes were quite close to those measured from the x,y-plane, as shown in **Figure**
357 **4.** These bulk results measured from the y,z- and x,z-planes are also added in **Figures**
358 **3c, d** for comparison. However, the fluctuation of the potential profiles, which reveals
359 the concentration fluctuation of the PAMPS network, is distinctly anisotropic for the
360 pre-stretched samples. For clarity, the enlarged potential profiles in the bulk regions
361 measured from the three surfaces for the virgin ($\varepsilon = 0$) and pre-stretched ($\varepsilon = 8.25$,
362 softened zone) samples are shown in **Figures 5a** and **5b**, respectively. No potential
363 anisotropy is observed for the virgin sample, while the pre-stretched samples shows
364 distinct anisotropy in the potentials. A large spatial fluctuation of $\zeta = 4.66 \pm 3.26 \mu\text{m}$
365 is clearly observed for the profile from the y,z-plane, along the direction of pre-
366 stretching. On the other hand, the profiles from the x,y-plane and x, z-plane, in parallel
367 to the pre-stretching direction, are much smoother. These results clearly show the
368 anisotropy in the internal fracture of the PAMPS network.

369 Next, we observe how the potential fluctuation changes with respect to the pre-
370 stretch strain. **Figure 5c** shows the potential profiles in the stretching direction
371 measured from the y,z-plane at different pre-stretched strains. For the virgin sample,
372 the potential fluctuation is small (in the range of -145 to -170 mV). The fluctuation level
373 first increases and then decreases with the pre-stretched strain. The potential fluctuation
374 in the softened zone of necking sample pre-stretched at strain $\varepsilon = 5.53$ ranges from -80
375 to -120 mV. The average distance between neighboring valleys at this pre-stretched
376 strain is approximately $\zeta = 4.46 \pm 2.52 \mu\text{m}$. With further damage in the gel, both the
377 fluctuation and the average distance increase; e.g. the average distance in the softened
378 zone increases to $\zeta = 4.66 \pm 3.26 \mu\text{m}$ at a pre-stretch strain of $\varepsilon = 8.25$. When the sample
379 is in the strain-hardening regime after the stress plateau in the stress-strain curve, i.e.,
380 when the entire sample is softened, the potential fluctuation reduces, while the average
381 distance between potential valleys further increases (e.g., $\zeta = 5.66 \pm 4.45 \mu\text{m}$ at a pre-
382 stretch strain of $\varepsilon = 15.34$).

383 If two damaged PAMPS fragments are only connected by the second network
384 PAAm with inter-fragments distance comparable to or larger than the electrode diameter,
385 the minimum potential values of re-swelled DN gels should be very close to that of the
386 second PAAm networks (which is approximately -10 ~ -30 mV, slightly more negative
387 to the potential of the pure PAAm gel due to co-polymerization of the residual AMPS
388 monomer into the second network). In fact, much large negative potentials were
389 measured at softened zone and hardening zone, as shown in **Figure 5b**. This suggests
390 that the fracture occurs at various length scales. To characterize the multi-scale structure

391 change induced by internal fracture of the PAMPS network, we performed a statistical
392 analysis on the potential fluctuation profiles obtained along the pre-stretch direction
393 (y,z-plane) for each pre-stretch strain. The measured potentials are analyzed according
394 to the probability density, as shown by the histograms with a sampling step of 1.5 mV
395 (**Figure 6**), at various pre-stretch strains. The statistical data are taken from the potential
396 profiles of bulk regions in an observation window of $\sim 27 \mu\text{m}$ (**Fig. 5c**). Prior to any
397 deformation or damage, at $\varepsilon = 0$, the potential exhibits a unimodal distribution over a
398 relatively narrow range (-165 to -148 mV) (**Figure 6a**), which indicates a relatively
399 homogeneous structure. Upon damage, the potential distribution widens, and turns into
400 bimodal (**Figures 6b-f**), especially for the softened zone on a necked sample, as shown
401 in **Figures 6c** and **6e**.

402 By fitting the histograms of the measured potential data with unimodal or bimodal
403 Gaussian distributions, we summarize the peak potentials, full width at half maximum
404 (FWHM), integral area fraction of high potential peak (peak 1) in **Table 1**. Obviously,
405 with increasing the pre-stretched strains, the peaks shift to lower potential values, which
406 indicates that the densities of PAMPS networks decrease by internal fracture-induced
407 swelling. FWHM increases with the pre-stretch strains in the necking regime, both in
408 unsoftened zone and in softened zone. The widening of the potential distribution,
409 especially the splitting from the unimodal to the bimodal distribution, reflects the
410 increase in the structural heterogeneity caused by internal damage of the PAMPS
411 networks: the PAMPS networks fractures to form parts with relatively high charge
412 density (the PAMPS fragments) and low charge density (the inter-fragment regions).

413 With the increase of the pre-stretch strains, the intensity of the high-potential peak
414 decreases while that of the low-potential peak increases, indicating that the high-density
415 parts gradually break, giving way to low-density parts. When the sample is further
416 stretched to the hardening regime, the high-density parts almost disappear, and potential
417 distribution becomes almost unimodal again, and the corresponding FWHM values
418 decrease, as shown in **Figure 6f**. This suggests that the high-density parts of the PAMPS
419 network fracture and the corresponding PAMPS fragments become homogeneous again
420 in the hardening regime. The evolution of the high- and low-density distributions can
421 be characterized quantitatively by a parameter, f_A , defined as the volume fraction of
422 the high-density parts of PAMPS networks, corresponding to the integral area fraction
423 of high potential peak in **Figure 6**. With the fracture of the DN gel, f_A decreased from
424 1 to 0.80~0.88 in unsoftened zone, and to 0.42 ~ 0.50 in softened zone of yielding
425 regime, and further to 0.26 in hardening regime (**Table 1**).

426

427 ***Morphology evolution of internal damage in DN gel***

428 From the potential distribution transformation from unimodal to bimodal, the
429 PAMPS network fractures, separating the homogeneous DN gel into parts of high
430 charge density and low charge density. The average size of bimodal structure is several
431 μm for re-swelled samples, as seen from the analysis in **Figure 5**. Both potential peaks
432 shift to lower values, indicating that both parts experience further damage. The high-
433 charge-density parts and low-charge-density parts experience minor and major damage,
434 respectively. Both parts will swell further when immersed in water. The parts of minor

435 damage, with connected PAMPS networks, swell less; while the parts of major damage
 436 swell more owing to the pullout of the PAAm chains from the fragmented PAMPS
 437 network. As a result, the minor damaged parts show a relatively higher potential than
 438 the major damaged parts. From the values of the two peak potentials (**Figure 6**), the
 439 average charge concentration in the parts of minor damage in the softened zone, for
 440 example, is estimated to be 4.2~5.2 times that in the parts of major damage.

441 Let q_1 and q_2 be the re-swelling ratios of the parts with minor and major
 442 damage, respectively, and q be the average re-swelling ratio of the gel. The volume
 443 fraction of the parts with minor damage after re-swelling, α , is related to the same
 444 volume fraction prior to re-swelling α_0 by

$$445 \quad q\alpha = \alpha_0 q_1. \quad (4)$$

446 Similarly, the re-swelling ratios of different parts are also related as

$$447 \quad \frac{1}{q} = \frac{\alpha}{q_1} + \frac{1-\alpha}{q_2}. \quad (5)$$

448 Estimating the volume fraction α from the probability density distribution
 449 (**Figure 6**), and the re-swelling ratios q_1 and q_2 from the potential peaks by using **Eq.**
 450 **(2)**, we list the results in **Table 2**, together with those directly measured. The average
 451 re-swelling ratio q calculated from **Eq. (5)** agrees reasonably well with that
 452 determined from the volume change of the samples.

453 It can be found that the re-swelling ratios gradually increase with the pre-stretch
 454 strain. The volume fractions, α and α_0 , of the parts with minor damage after and
 455 before re-swelling, respectively, gradually decreases with the increase of the pre-stretch
 456 strain. The volume fraction α of the re-swollen state is slightly smaller than that before

457 re-swelling, α_0 , due to the relatively small swelling ratio of the parts with minor
458 damage. In the hardening regime, α_0 decreases to 0.28 at the pre-stretch strain of
459 15.53, which means that about 72% of the total volume before re-swelling is taken by
460 the parts with major damage.

461 T

462 h

463 e

464 *Necking occurring from surface to bulk*

465 c Finally, we reveal where the necking initiates. As mentioned previously, the pre-
466 stretched gel sample in the necking regime exhibited two co-existed zones: softened
467 zone and un-softened zone. Using polarized optical microscopy, we observed a small
468 transition zone between these two zones, of thickness ~ 1.7 mm in the unloaded state
469 (Figure S3, supporting information). During the tensile test, this transition zone in the
470 necking region became slightly opaque, indicating the formation of a heterogeneous
471 structure. Here, we examine the structure in this transition zone using the MET. The re-
472 swollen shape of the DN gel around this transition zone (after a stretch of $\varepsilon = 6.93$) is
473 illustrated in Figure 7a. Figure 7b shows the depth profile of the electric potential in
474 the transition zone measured from a non-cut surface. For comparison, the results for the
475 softened zone and unsoftened zone are also shown. In contrast to the electric potential
476 in the softened and unsoftened zones, the electric potential in the transition zone shows
477 a much thicker gradient layer (~ 75 μm), connecting the skin with the same electric
478 potential of potential in the softened zone and the bulk with electric potential in the unsoftened zone.

479 T

480 h

481 e

926 that in the un-softened zone. This result implies that the damage initiates from the
927 surface, and then propagates through the depth of the gels. Moreover, the potential
928 fluctuation is also found to be more significant in this gradient layer than in the depth,
929 showing the fragmentation of the PAMPS networks.

930

931 Conclusions

932 We employ the MET to study the internal fracture behavior of the pre-stretched
933 DN gel under uniaxial deformation. This method gives structure information at a scale
934 larger than the probe size of ~ 200 nm in this study. The measured electric potential
935 distributions in re-swollen DN gels reveal the stretch-induced anisotropy: the material
936 is softened more significantly along the tensile direction, possibly related to the
937 coalescence of the internal cracks on the first network. With the increase of pre-stretch,
938 the statistical distribution of potential values changes from unimodal to bimodal,
939 indicating the coexistence of minor damaged parts and the major damaged parts. The
940 volume fraction, α , of the minor damaged part is predicted to decrease with the increase
941 of pre-stretch strain, while the volume fraction of the major damaged part increases
942 with stretch and reaching 0.72 in the hardening regime. The average distance between
943 neighboring major (or minor) damaged parts along the tensile direction are almost
944 constant at ~ 2 μm for the un-swollen sample, independent of the pre-stretch strain.
945 Additionally, the microstructure of the transition region between the necked and un-
946 necked zones suggests that the damage initiates from the gel surface and then
947 propagates into the depth.

948

949 ASSOCIATED CONTENT

950 The Supporting Information is available free of charge on the ACS Publications
951 website at DOI: @@@.

952 The measured electric potential of virgin DN gel with microelectrodes of different
953 tip diameters, **Figure S1**. Image of the pre-stretched DN gel under polarized optical
954 observation by tensile test, **Figure S2**; Image of the transition zone under polarized
955 optical microscope, **Figure S3**. (PDF)

956

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970

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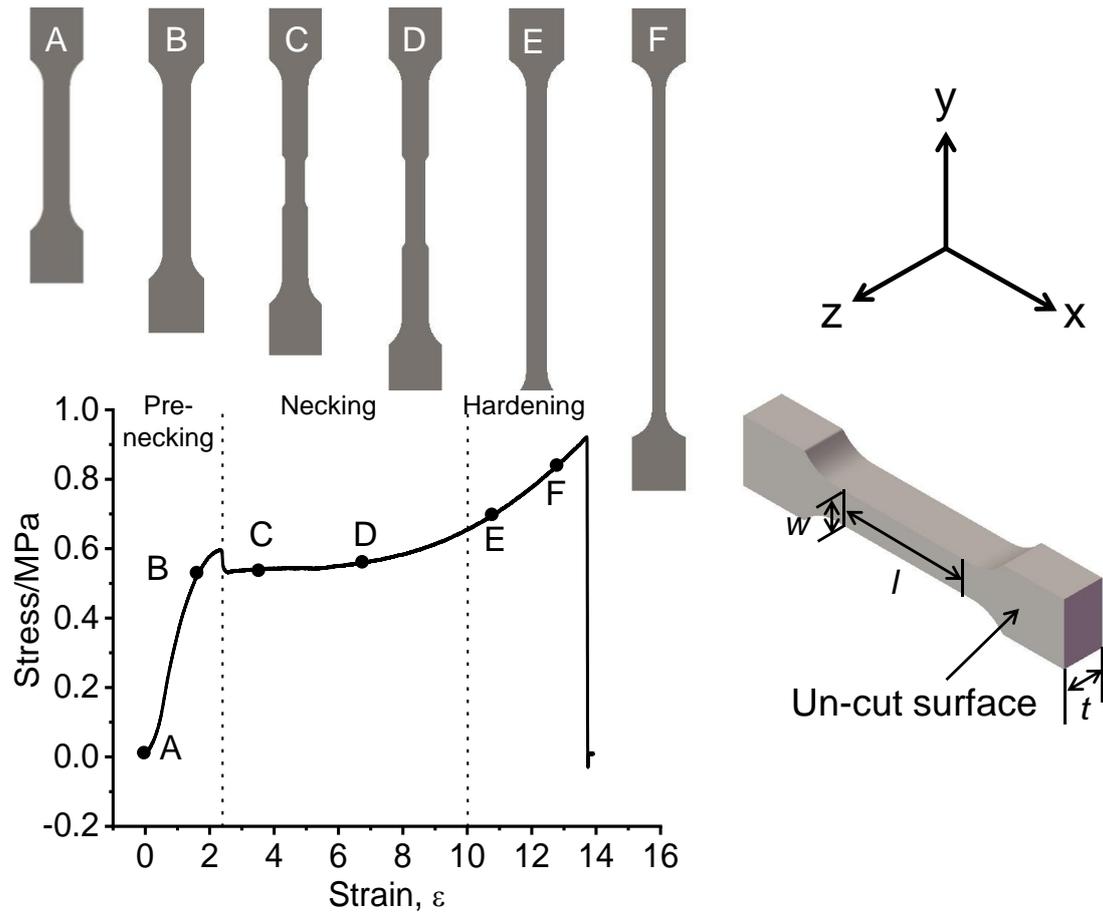


Figure 1. Loading curve of a typical DN gel under uniaxial elongation. The curve has three characteristic regimes of deformation: pre-necking, necking, and hardening, as shown by the vertical dotted lines. The schematic illustrations above the loading curve show the shapes of the sample in these three regimes. The right figure shows the 3D illustration of the dumbbell shape sample and the coordinates used in this study. The w , l and t represent the width, length and thickness of the DN sample, respectively. The tensile direction is defined as parallel to the x-axis, the width direction and the thickness direction of the sample are defined as parallel to y-axis and z-axis, respectively. The sample surface in x,y-plane is the un-cut surface, and those in y,z- and x,z-planes are cut surfaces. The tensile test was performed at 100 mm/min.

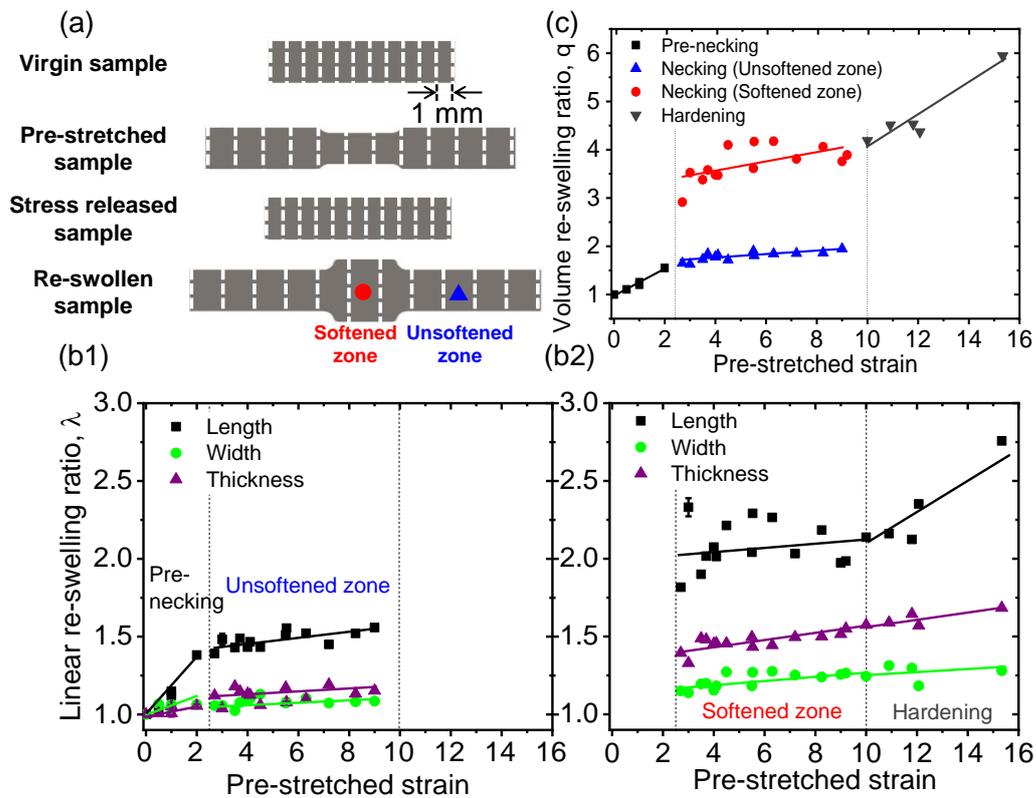


Figure 2. (a) Schematic illustrations showing the shape change of the sample. The red dot and blue triangle represent the softened (necked) zone and the unsoftened (un-necked) zone, respectively in the necking regime. The vertical white dash lines indicate the markers with 1 mm intervals used to measure the local re-swelling ratios at various positions. (b) Linear re-swelling ratios λ in length, width, and thickness of the pre-stretched DN gel at pre-necking regime and unsoftened zones of necking regime (b1), and at softened zone of necking regime and hardening regime (b2). (c) Volume re-swelling ratio q by volume of the samples after being pre-stretched at different maximum strains. Owing to the internal fracture of the first polyelectrolyte network, the pre-stretched sample, especially in the softened zone, re-swells remarkably in water.

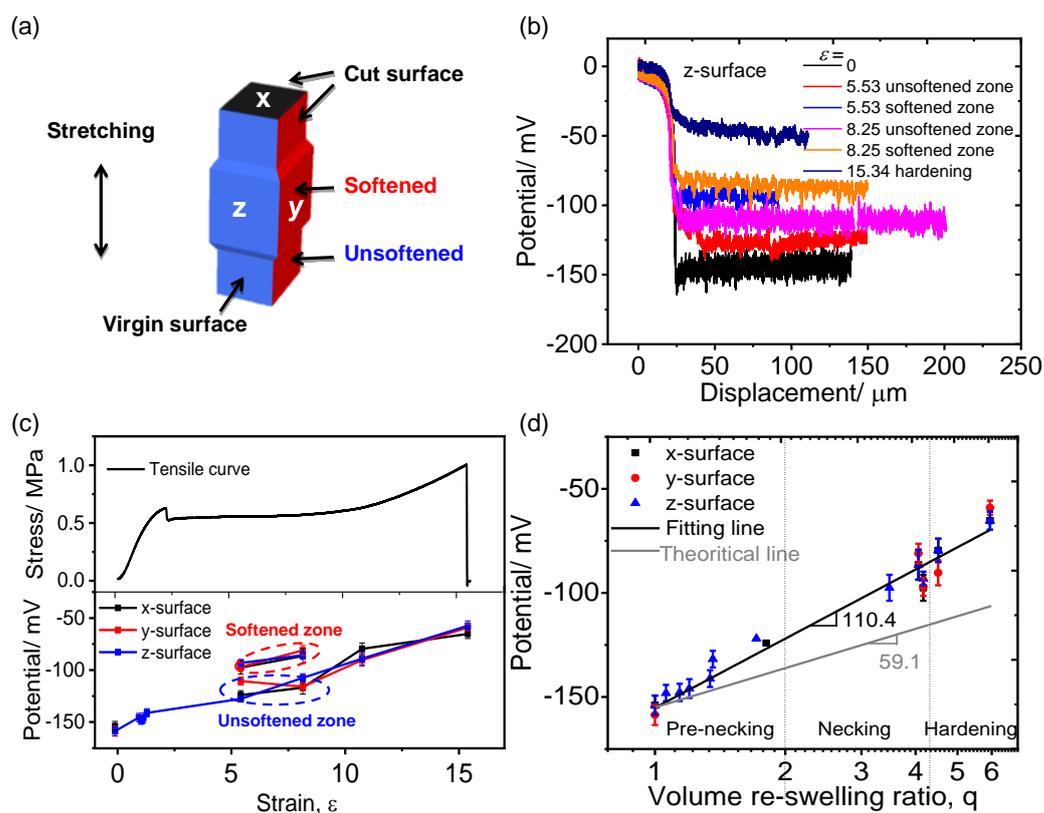


Figure 3. (a) Schematic illustration of three different surfaces of a pre-stretched DN gel used to measure the electric potential. For a necking sample, the softening and the unsoftened zone co-exist, and the softened zone underwent a larger swelling than the unsoftened zone in the reference solution. (b) Depth profiles of the electric potential measured by the microelectrode technique from the un-cut surface (x,y-plane) into the bulk of the DN hydrogel after unloaded from different strains ε . (c) Tensile stress and strain curve (top) and average bulk potentials of re-swelled samples pre-stretched at different strains detected from the three different surfaces (bottom). In the necking regime, the measurements were performed on both the softened zone and the unsoftened zone. (d) Correlation between the bulk re-swelling ratio q and the electric potential for pre-stretched DN gels re-swelled in the reference solution. The electric

potentials in necking regime were measured at the softened zone. The fitting line in the semi-log plot has a slope of 110 mV. The grey straight line is the theoretical curve from **Eq.2**. The effective salt concentration of reference solution was 4.4×10^{-5} M.

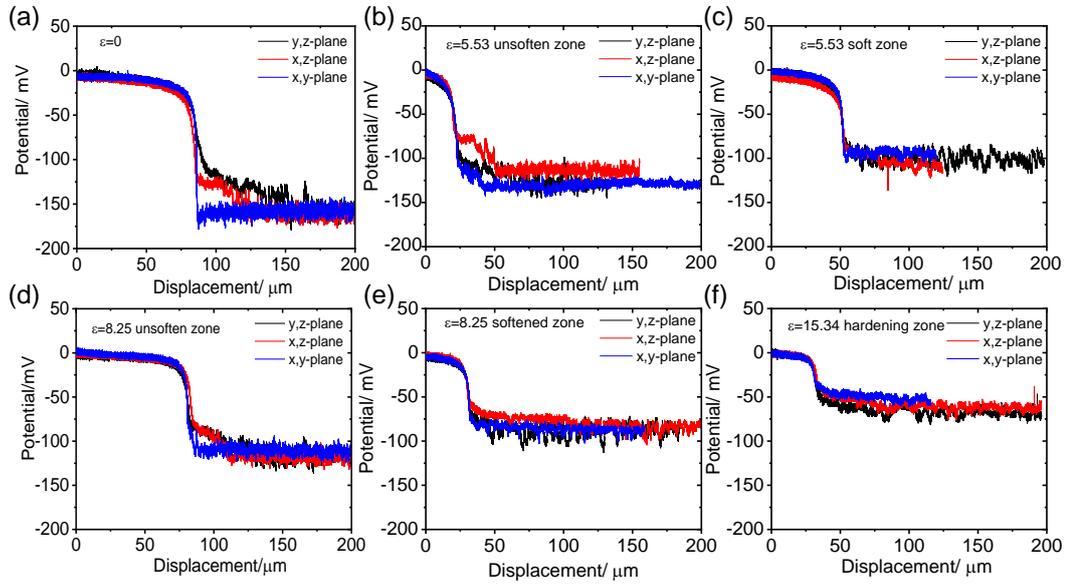


Figure 4. Depth profiles of the electrical potential measured from the y,z-, x,z- and x,y-planes of re-swollen DN gels at different pre-stretched regimes: (a) virgin sample ($\epsilon = 0$); (b, c) necking regime ($\epsilon = 5.53$), unsoftened zone (c) and softened zone (d); (d, e) necking regime ($\epsilon = 8.25$), unsoftened zone (d) and softened zone (e); and (f) hardening regime ($\epsilon = 15.34$). The y,z-, x,z- and x,y-planes are defined in **Figure 1**.

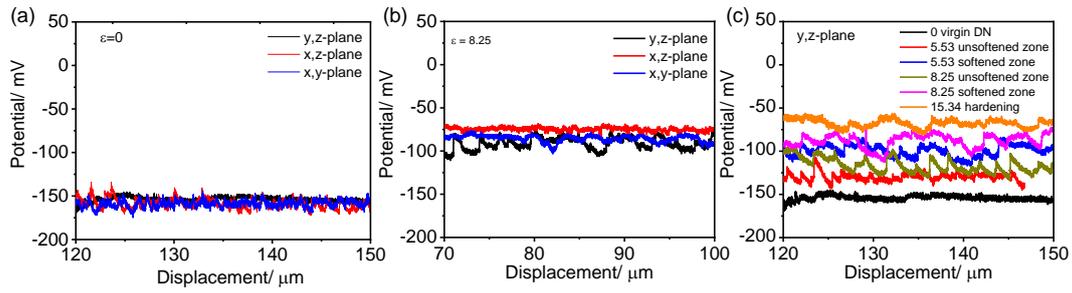


Figure 5. Potential fluctuation profiles for bulk DN gels, measured from the y,z-, x,z- and x,y-planes for (a) virgin samples and (b) in the softened zone of a sample pre-stretched to necking regime ($\epsilon = 8.25$), as well as (c) from the y,z-plane at different pre-stretch strains. The horizontal axis is shifted for some data to allow comparison in the same observation window.

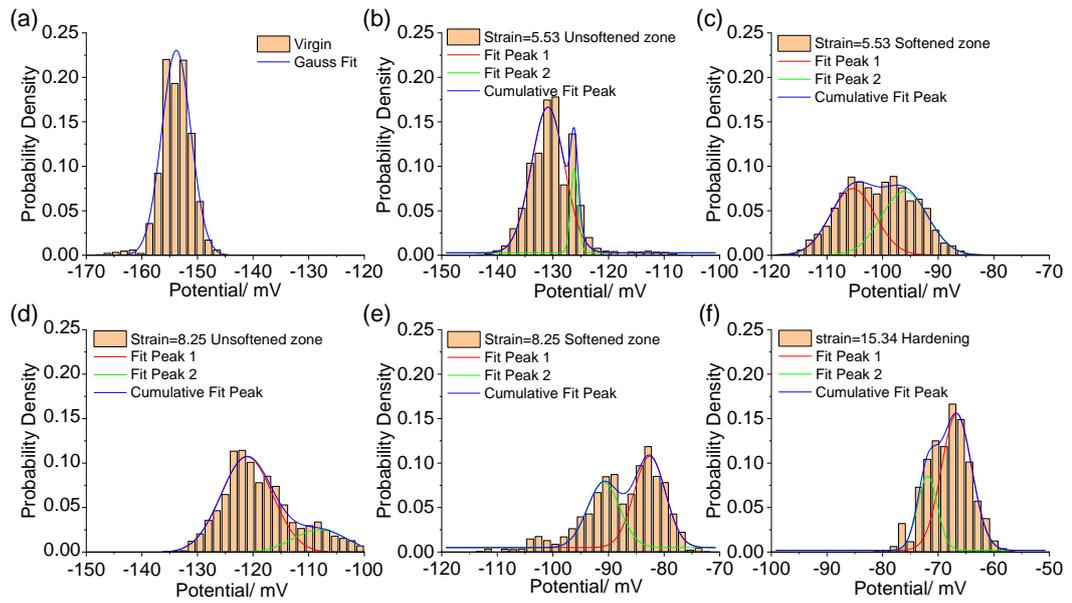


Figure 6. Statistical analysis of the electrical potential values measured from the y,z- plane of DN gel subjected to different pre-stretch strains. The probability densities are fitted to unimodal or bimodal Gaussian distributions. The sampling step for the histograms is 1.5 mV. The statistical data are summarized from the raw data shown in **Figure 5c**.

Table 1. Peak positions, FWHM (w), and integral area fraction of peak 1, α , obtained from **Figure 6** with the Gaussian function at different pre-stretched strains. $f_A = A_1/(A_1 + A_2)$, where A_1 and A_2 are the integral area of high potential peak (peak 1) and low potential peak (peak 2), respectively.

Sample	virgin	necking				hardening
	$\varepsilon=0$	$\varepsilon=5.53$ unsoftened zone	$\varepsilon=5.53$ softened zone	$\varepsilon=8.25$ unsoftened zone	$\varepsilon=8.25$ softened zone	$\varepsilon=15.34$
Peak 1/ mV	-153.8	-130.9	-105.4	-121.1	-90.9	-71.9
Peak 2/ mV	-	-126.1	-96.0	-108.0	-82.6	-66.7
w_1 / mV	5.26	5.94	8.04	9.27	5.88	3.58
w_2 / mV	-	1.35	8.37	9.67	5.65	5.44
f_A	1.00	0.88	0.50	0.80	0.42	0.26

Table 2. Structure parameters calculated from potential data using a simplified bimodal model.

Strain	necking				hardening
	5.53 (unsoftened)	5.53 (softened)	8.25 (unsoftened)	8.25 (softened)	15.53
q_1 (cal.)	1.61	2.75	1.98	3.85	5.73
q_2 (cal.)	1.78	3.35	2.61	4.52	6.32
q (cal.)	1.63	3.02	2.08	4.21	6.16
q (exp.) ¹⁾	1.80	3.88	1.86	4.06	5.94
α (cal.)	0.88	0.50	0.80	0.42	0.26
α_0 (cal.)	0.89	0.55	0.84	0.46	0.28
ζ (exp.) (μm) ²⁾	-	4.46 \pm 2.52	-	4.66 \pm 3.26	5.66 \pm 4.45
ζ_0 (cal.) (μm) ³⁾	-	1.95	-	2.14	2.06

¹⁾: Measured from bulk size change of the DN gels; ²⁾: The potential fluctuation length in stretch direction; ³⁾: $\zeta_0 = \zeta/\lambda_1$ where λ_1 is the linear re-swelling ratio in the stretching direction.

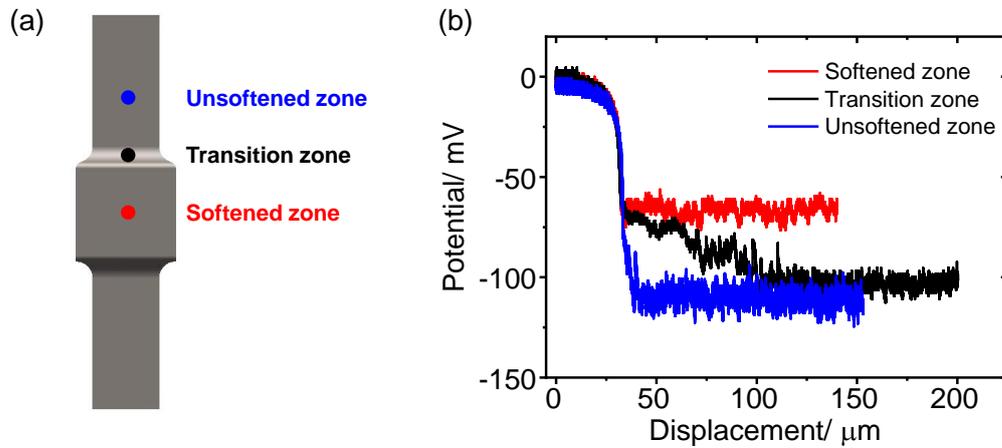


Figure 7. (a) Schematic illustration of a necked sample in the re-swollen state. After re-swelling, the unsoftened zone, transition zone, and softened zone of the sample could be distinguished according to the swelling degree. The dots show the positions where the potentials were measured. (b) Electric potential profiles measured from the non-cut surface of the sample, in the softened zone, transition zone and unsoftened zone. The potential profile of the transition zone suggests that the internal damage initiates from the surface to the depth.

Supporting information

Internal Damage Evolution in Double-Network Hydrogels Studied by Microelectrode Technique

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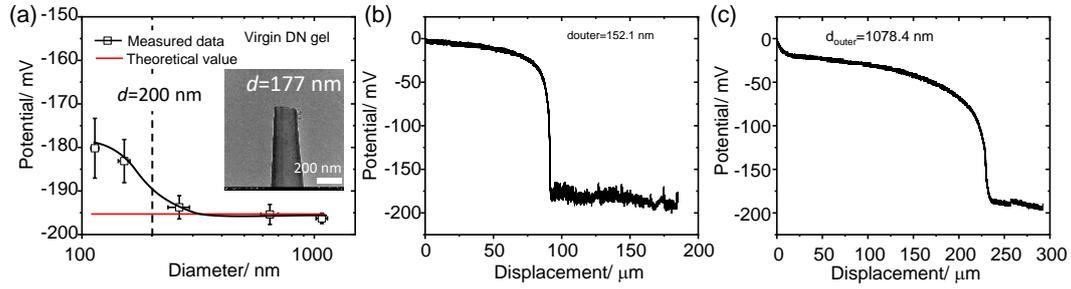


Figure S1. (a) The measured electric potential of the virgin DN gel by using microelectrodes of different tip diameters. The horizontal red line indicates the theoretical potential estimated from Eq. (1) by using the bulk concentration of PAMPS in the virgin DN gel ($C_g = 1/19.7$ M), the KCl concentration ($C_s = 10^{-5}$ M), the activity coefficient ($\gamma_g = 0.35$, $\gamma_s = 1.0$). A typical TEM image of the glass capillary ($d_{\text{outer}} = 177$ nm) is shown in it. (b) The measured potential curve of the virgin DN gel by using a thin microelectrode ($d_{\text{outer}}=152.1$ nm). (c) The measured potential curve of the virgin DN gel by using a thick microelectrode ($d_{\text{outer}}=1078.4$ nm). In this experiment, the residual chemicals are carefully removed to a level less than 10^{-5} M by washing the samples in deionized water for more than one month before the potential measurement.

In situ optical observation of necking process

To observe the change in the orientation degree of the network strands during necking, two pieces of circular polarized films were fixed on the front of a video and a white lamp.⁵¹ The video camera recorded the shape and isochromatic images of the samples during test. The optical retardation change of the samples during the tensile test was estimated by comparing the birefringence colors using a Michel-Levy chart.⁵²

This simple method roughly indicated the stress distribution of the samples during deformation but provided no information regarding the stress direction.

Circular polarized optical observation in necking region

To understand why the re-swelling ratio in the softened zone increases with the pre-stretching strain, we observed the optical polarization of the sample during a tensile test with a pair of circular polarizing films. Under the circularly polarized light, the birefringence of the anisotropic DN gel can be estimated during stretching. **Figure S2** shows the polarized optical images of the DN gel at various strains. With the increase of the stretching, the retardation in the necked zone, as shown by the blue arrows, increases, reflecting the increase of the stress concentration. Furthermore, the retardation in the necked zone is not homogeneous, even at a high strain ($\epsilon = 8.33$). Because the retardation is directly proportional to the true stress, the results indicate that the true stress applied in the necked zone varies with respect to the strain. That is, the increase of the true stress in the necked zone further breaks the PAMPS fragments into smaller sizes, increasing the re-swelling ratio in the softened zone. This result further explains the previous result that the initial elastic moduli of pre-stretched DN gels slightly decreases at a large pre-strain in necking stage.¹²

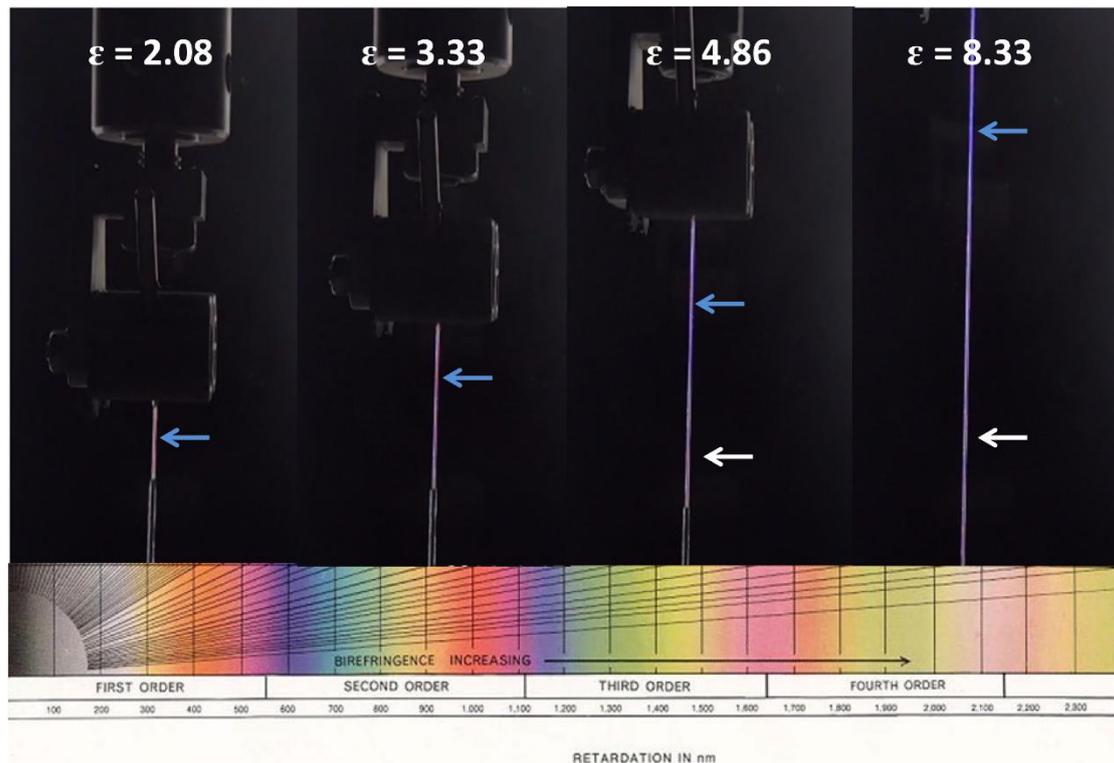


Figure S2. Birefringence images of the DN gel during the uniaxial elongation. The necking zone propagates with the increase of the strain. The birefringence color in the necking zone slightly changes with respect to the strain (blue arrows) and varies with respect to the position (white arrows), indicating that the orientation degree increases with the increase of the stretching and that the stretching is not homogeneous even in the necking region. The images were obtained via *in situ* observed by circular polarized optical observation during the uniaxial elongation.

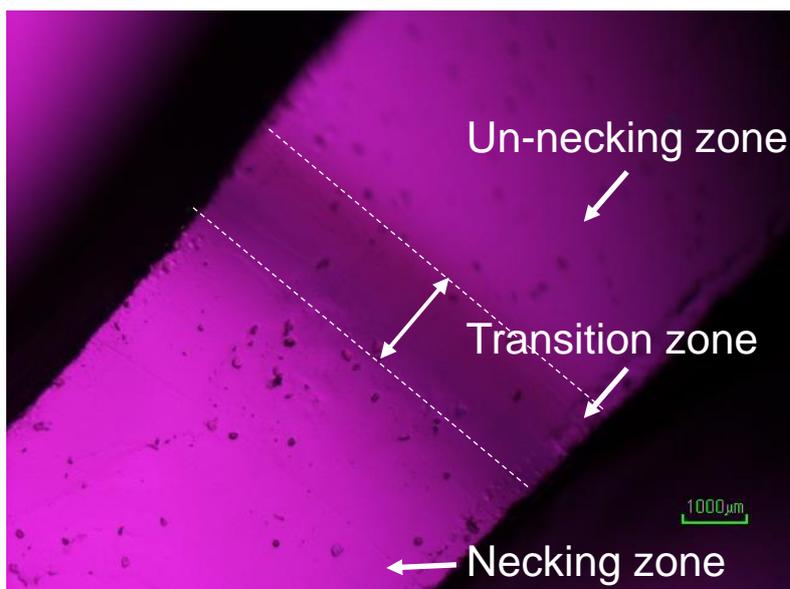


Figure S3. Image of the pre-stretched DN gel at $\varepsilon = 3.8$, obtained using a linear polarized optical microscope. A weakly ordered structure is observed in the transition zone. The observation was performed in the relaxed state of the sample without re-swelling.

References

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- (S2) Robert Hoffman and Michael W. Davidson, Michel-Levy Birefringence Chart.
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