

APPLIED SCIENCES AND ENGINEERING

Dehydration drives damage in the freezing of brittle hydrogels

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It is widely known that freezing breaks soft, wet materials. However, the mechanism underlying this damage is still not clear. To understand this process, we freeze model, brittle hydrogel samples, while observing the growth of ice-filled cracks that break these apart. We show that damage is not caused by the expansion of water upon freezing or the growth of ice-filled cavities in the hydrogel that exert pressure on the surrounding material. Instead, local ice growth dehydrates the adjacent hydrogel, leading to drying-induced fracture. This dehydration is driven by the process of cryosuction, whereby undercooled ice sucks nearby water toward itself, feeding ice growth. Our results highlight the strong analogy between freezing damage and desiccation cracking, which we anticipate being useful for developing an understanding of both topics. Our results should also give useful insights into a wide range of freezing processes, including cryopreservation, food science, and frost heave.

INTRODUCTION

Damage caused by the freezing of soft, wet materials is a widely important process. It hinders our ability to cryopreserve tissue (1) and has implications for plant and animal life at cold temperatures (2, 3). It is a key consideration in the cold storage of food and medicine (4–6), causes expensive deterioration to infrastructure in cold climates (7, 8), and shapes periglacial landscapes (9). Despite its widespread importance, and its long history of research, freezing damage is still poorly understood. For example, when freezing a particular soil type, it is not possible to predict how fast and in what form ice will grow (7, 9, 10), despite the availability of a wide range of frost-heave models (8, 11–15). Thus, our knowledge of freezing damage is largely empirical.

There are several key problems that make understanding the freezing process challenging. Freezing is affected by multiple factors, including temperature gradient, solute concentration, type of solute, and material porosity (16–18), and this makes it hard to isolate the key physics. Furthermore, most experiments are done on bulk, opaque samples [e.g., (11, 19)] and analyzed via approaches such as observing macroscopic sample changes (20), sectioning frozen samples (21), and tracking ice growth with techniques like differential scanning calorimetry (22) or magnetic resonance imaging (23). Although these methods give useful insights, they cannot visualize the crucial microscopic ice-growth processes that underlie damage. Last, commonly frozen materials like clays, colloidal suspensions, or tissue are often heterogeneous or have complex rheological properties, further obscuring the picture (24, 25).

To overcome these issues, we perform freezing experiments on model, transparent hydrogels. We observe samples with a confocal

microscope (16, 18, 26), allowing us to directly visualize three-dimensional (3D) ice crystal growth and the resulting hydrogel damage. Unexpectedly, this damage is caused by growing ice crystals dehydrating the surrounding hydrogel, leading to desiccation cracking.

RESULTS

Volumetric expansion versus cryosuction

It is important to emphasize that damage in soft materials is not typically caused by the ~9% volumetric expansion of water upon freezing. We demonstrate this by observing ice growth in a cavity in a water-impermeable silicone gel (Fig. 1, A and B). As water freezes in the cavity, there is a small expansion (see dashed line). However, this expansion, 2% in each dimension based on water's volumetric expansion upon freezing, is easily accommodated by the gel. Most soft materials can easily tolerate such strains.

Instead, damage is driven by cryosuction, whereby water is sucked toward ice at subzero temperatures, feeding ice growth (16). In Fig. 1 (C and D), we compare ice growth in a poly(ethylene glycol) diacrylate (PEGDA) hydrogel. This has a comparable shear modulus, μ , to the silicone gel. Now, ice growth in the gel is extensive, eventually ripping apart the hydrogel (movie S1). This growth is driven by cryosuction of water from the unfrozen hydrogel surrounding the ice. The water in the hydrogel does not freeze, as capillarity (the Gibbs-Thomson effect) prevents ice from growing into the hydrogel mesh until well below the bulk freezing temperature (9).

Freeze fracture in a temperature gradient

To understand how such freezing damage occurs, we freeze hydrogel-filled cells (Fig. 2A) and examine the resulting fractures. We use a freezing stage on a confocal microscope (Fig. 2A) (16, 26), allowing us to control temperature profiles. Here, we impose either isothermal conditions or fixed temperature gradients, g_T , along the x direction. In the latter case, ice grows into the hydrogel from the cold side of the cell, fracturing apart the top hydrogel/glass interface. A side view of a typical delamination, obtained via confocal microscopy, is shown in Fig. 2B; bright spots are fluorescent nanoparticles embedded in the

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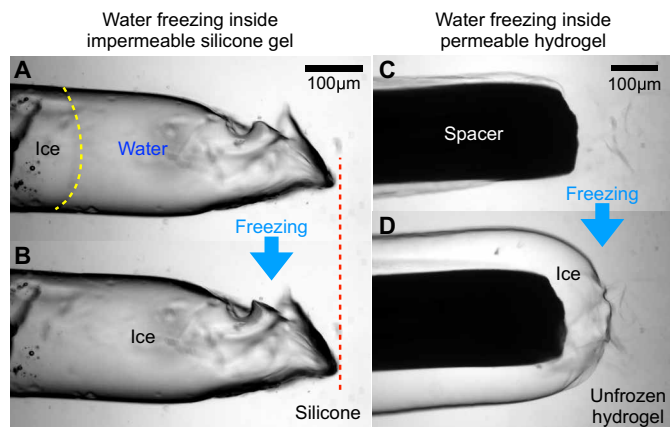


Fig. 1. Freezing damage in soft materials is not caused by the expansion of water upon freezing. (A and B) Freezing of water in a cavity in a silicone rubber ($\mu = 30$ kPa) causes negligible deformation to the rubber. The deformation is caused by the expansion of water upon freezing. (C and D) Freezing of a brittle, PEGDA hydrogel (90% water, $\mu = 37$ kPa) containing a metal spacer. Ice forms around the spacer and continues to grow by sucking in water from the surrounding hydrogel via cryosuction, leading to large stresses in the gel.

hydrogel. When g_T is not too large, this crack shape is essentially 2D, varying little in the y direction, and it reaches a steady state within 20 min (see the Supplementary Materials). Thus, such stationary interfacial cracks are very convenient for studying freezing damage. Interfacial crack behavior should also be representative of bulk freeze-fracture in PEGDA (these cracks have a similar toughness to that of bulk cracks; see the Supplementary Materials).

The overall shape of the delamination in Fig. 2B is reminiscent of a classical crack, with the large delamination opening suggesting that ice is pressurized and aggressively pushing apart the glass/hydrogel interface. However, as we will see, this is not correct: The ice is not exerting significant pressure on its surroundings, and the large delamination opening away from the crack tip is mainly caused by temperature-dependent hydrogel shrinkage. The shape is largely

independent of fracture parameters such as adhesion strength and thus does not immediately tell us about how the ice drives fracture. We demonstrate this by varying parameters such as the adhesion strength at the hydrogel/glass interface and the temperature gradient applied to the sample. We change hydrogel/glass adhesion using different silane pretreatments of the glass. PEG silane (3-[methoxy (polyethyleneoxy)9-12]propyltrimethoxysilane) is an unreactive, hydrophilic coating that should result in very low adhesion strength. Hexenyl silane (5-hexenyltrimethoxysilane) is a coupling agent containing a vinyl group that should chemically bind to the hydrogel, yielding a higher adhesion strength. We compare crack shapes for the different silanes (blue and purple data in Fig. 2C, both with $g_T = 1.1^\circ\text{C}/\text{mm}$). Here, dashed lines show the original sample thicknesses, and the crack tip is located at $x = 0$. Away from the crack tip, these shapes are very similar, suggesting that they are independent of adhesion strength. Only when we look closer to the crack tip do we see evidence of sensitivity to adhesion. By contrast, the shape away from the crack tip does depend on the temperature gradient in the sample. Figure 2D shows crack shapes for $g_T = 1.1^\circ, 1.6^\circ$, and $2.2^\circ\text{C}/\text{mm}$. These use a methacrylated silane (3-(trimethoxysilyl)propyl methacrylate, or “bind-silane”), which is another coupling agent that should give good glass/hydrogel adhesion. As we increase g_T , the overall crack shapes progressively open, except within about $50\ \mu\text{m}$ of the crack tip, where the shape appears to be fixed.

The large-scale crack shape is predominantly dependent on local temperature. In Fig. 2E, we replot the data from Fig. 2 (C and D) by scaling $h(x)$ by initial thickness, h_0 , and plotting this against the temperature, $T = g_T x$. There is an excellent collapse of all the data, excepting some small deviations near the crack tip (see inset). We see the same, temperature-dependent shape even when there is no crack at all: When we freeze a hydrogel layer that is simply submerged in water (Fig. 2C, see inset for schematic), we find a similar shape that also collapses onto the master curve. Thus, overall crack shape appears to be mostly set by the hydrogel just shrinking to a thickness that depends on the local temperature. The dashed curve in the figure is an empirical fit characterizing the collapsed data: $\frac{h}{h_0} = 0.18 + 0.24e^{2.25T} + 0.53e^{16.12T}$, where T is in Celsius.

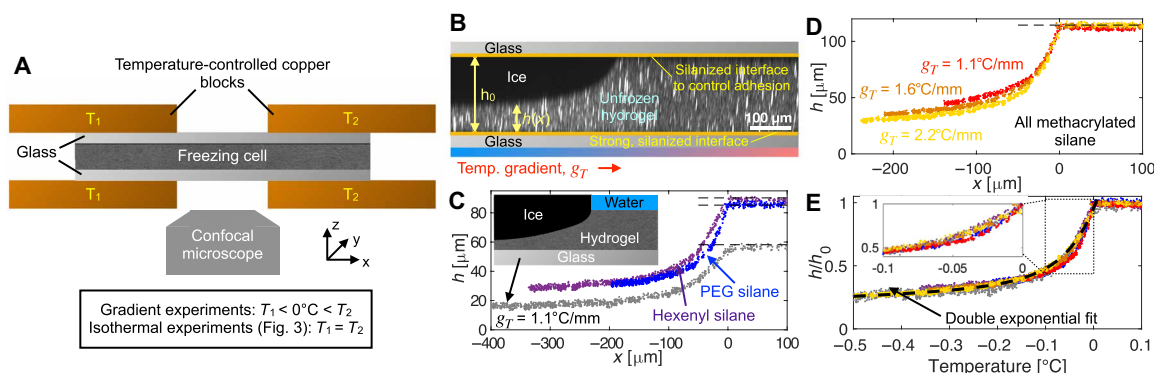


Fig. 2. Freezing of PEGDA hydrogels in a temperature gradient. (A) Schematic of the freezing apparatus. (B) A side view of a typical experiment, imaged with confocal microscopy. Bright spots are fluorescent tracer particles embedded in the hydrogel. Upon freezing, ice breaks the silanized, top interface between the hydrogel and the glass cell. (C) Hydrogel delamination profiles for three different interfacial strengths: strong, hexenyl silane (purple); weak, PEG silane (blue); and no interface (gray). The schematic shows the geometry for the latter case. (D) Hydrogel delamination profiles for a strong interface (methacrylated silane) at three different temperature gradients. In all experiments in (C) and (D), the hydrogel shape reaches a steady state after ~ 20 min, so we image samples after 40 min. (E) The data from (C) and (D) largely collapse when local thickness is scaled with initial hydrogel thickness and plotted against temperature. The dashed curve shows an empirical fit, described in the main text. The inset zooms in on the data near $T = 0$, showing small deviations from a perfect collapse.

Crack shape is largely set by cryosuction-induced dehydration

The fact that crack shapes largely depend on local temperature suggests that they are dominated by bulk-thermodynamic behavior. To explore this, we measure the equilibrium between ice and layers of hydrogel with a free, upper surface under isothermal conditions (see schematic in Fig. 3). Cryosuction sucks water out of the gel, until the suction is balanced by the dehydrated gel's osmotic pressure. We characterize this equilibrium by tracking fluorescent particles embedded in the gel layer (27) to measure how gel thickness, $h(T)$, changes with temperature (Fig. 3). At the bulk melting temperature, $T = 0^\circ\text{C}$, $h = h_0$. Upon cooling, $h(T)$ reduces monotonically with increasing undercooling, with the hydrogel rapidly losing more than 50% of its volume in the first degree of undercooling. Upon further cooling, the dehydration becomes more gradual, as the gel thickness approaches the fully dehydrated limit where only polymer remains (dotted line in Fig. 3, calculated using the thickness of the as-prepared hydrogel).

The isothermal shrinkage of the hydrogel with undercooling largely explains the shapes of the delamination profiles in Fig. 2. We add the empirical fit to the data from Figs. 2E and 3 as the red, dashed curve. These are rather similar, especially at low temperatures. A perfect match would indicate that hydrogels in temperature gradients just shrink uniaxially (vertically) until the local osmotic pressure of the gel balances the suction from the overlying ice. This is true at colder temperatures, where the hydrogel shrinks relatively uniformly. However, there is less agreement at warmer temperatures. Here, h changes rapidly with position for the samples in temperature gradients, indicating the presence of large shear strains (see later for confirmation). Thus, it is not unexpected to see that the data here do not match the isothermal results. However, overall, temperature-dependent shrinkage appears to explain the main features of freezing cracks.

Strain fields reveal that shear drives crack growth

To get insight into how ice actually breaks the hydrogel, we look closer to the crack tip. Here, displacements and strains are controlled

by interfacial fracture properties and reveal the specific form of the loading that drives fracture. They distinguish between mode I loadings acting to push crack faces apart, mode II loadings acting to shear crack faces past each other, and mixed mode loadings that combine the two behaviors (e.g., Fig. 4) (28). To determine hydrogel strains, we track the displacements of embedded nanoparticles in the hydrogel between the initial, stress-free state and the frozen, deformed state, using a large-strain tracking algorithm (27). We calculate the local deformation gradient, F , with a linear fit to the local displacement field. Then, the Green-Lagrange finite strain tensor is $E = (F^T F - I)/2$, where I is the identity matrix (see the Supplementary Materials for more details). Examples of the resulting (grid-interpolated) displacement fields, and the corresponding strains E_{zz} , E_{xx} and E_{xz} are shown in Fig. 5 (A and E), for a sample with a free upper surface (see schematic in Fig. 2C), and the hexenyl-silane sample from Fig. 2C. Corresponding plots of the trace of the strain matrix, E_{kk} , are also given in the Supplementary Materials.

The strain fields reveal an unexpectedly strong shear peeling the hydrogel off the overlying glass. Figure 5 shows the strain fields for the free-surface sample (B to D), and the adhered sample (F to H). Differences between these two datasets can be attributed to the fracture process that only occurs in the adhered sample. In the free-surface sample, Fig. 5B shows how the hydrogel contracts vertically under the ice ($x < 0$), while only slightly expanding under the water ($x > 0$). There is also a rather uniform horizontal contraction, likely driven by dehydration of the hydrogel (Fig. 5C), and a positive shear strain that is concentrated at the constrained bottom of the hydrogel layer (Fig. 5D). In the adhered sample, there is again vertical contraction of the hydrogel under the ice, this time accompanied by vertical stretching directly ahead of the crack tip (Fig. 5F). We also see horizontal contraction, qualitatively similar to what we see in the free-surface sample, but more concentrated near the crack tip (Fig. 5G). The largest qualitative difference is the appearance of a strong negative shear strain ahead of the crack tip, a signature of a “peeling” mode II crack loading (Fig. 5H). This shear can also be seen in the displacement field in Fig. 5E. In short, adhesion concentrates stresses and creates both vertical tension and shear at the crack tip.

We determine whether tension or shear drives fracture by measuring the magnitude of modes I and II loadings at the crack tip. We do this by fitting the displacement field around crack tips to the asymptotic field predicted by linear elastic fracture mechanics (LEFM) for interface cracks. We perform the fitting in a $30 \times 20 \mu\text{m}$ box directly to the right of the crack tip. This box is chosen so that it is small relative to the thickness of the layer and is positioned where the strains in the free-surface sample are relatively small (the results

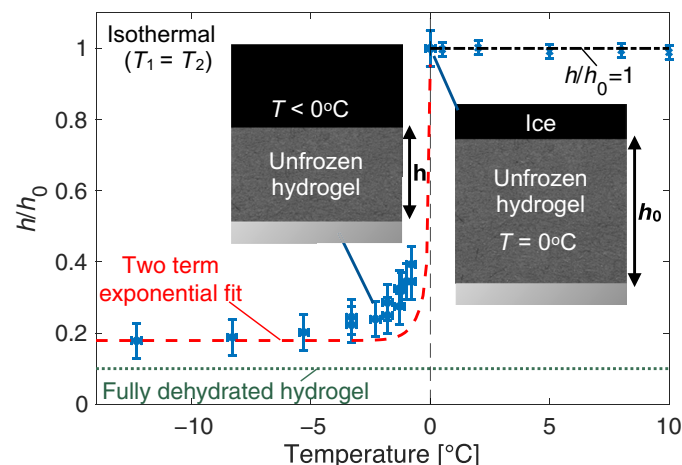


Fig. 3. Isothermal shrinkage of freezing hydrogels caused by cryosuction. A layer of PEGDA hydrogel shrinks with increasing undercooling. The gel is in contact with, and equilibrated with ice, and adhered on its bottom side to a rigid glass slide. The red, dashed curve shows the empirical fit to the data in Fig. 2E.

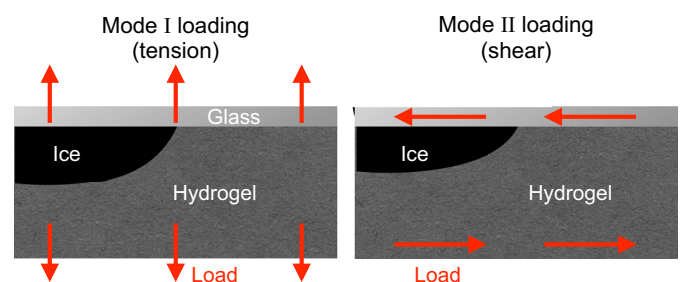


Fig. 4. Schematics showing the 2D modes of fracture.

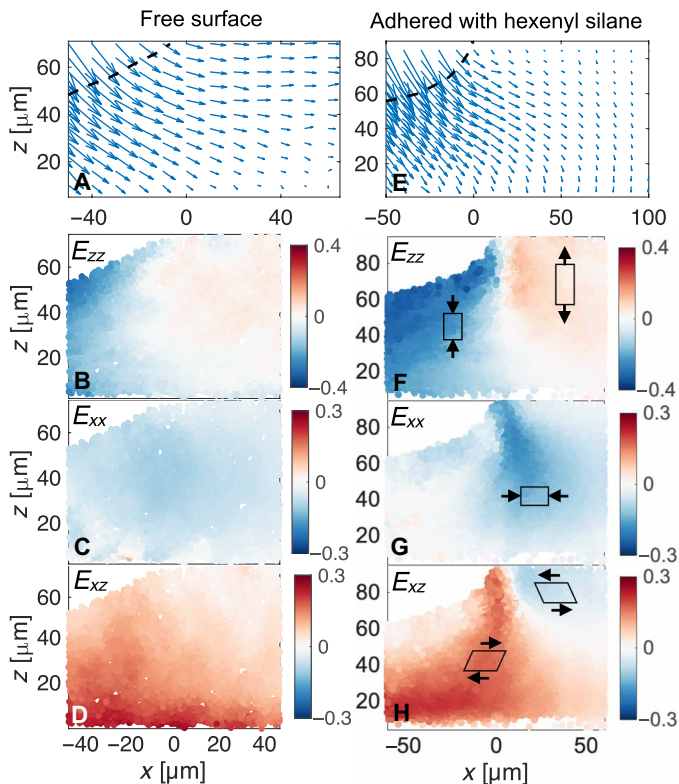


Fig. 5. Displacement and strain fields in freezing hydrogel samples. (A to D) A hydrogel with a free upper surface. (A) shows the displacement field near the crack tip (arrows are to scale with the figure axes) while (C) and (D) show the corresponding strain fields, E_{xx} , E_{zz} , E_{xz} . (E to H) The same data but for freezing of a hydrogel adhered to hexenyl-silane-treated glass.

are not sensitive to box size, as shown in the Supplementary Materials). The asymptotic, fitted field for interface cracks is more complex than the field for cracks in homogeneous materials, but we can still extract effective “stress intensity factors of classical type,” K_I and K_{II} , that characterize fracture, just like standard stress intensity factors (29). These represent the contribution of mode I and mode II loadings, respectively, at the scale at which the crack is loaded, here, $O(10\text{ }\mu\text{m})$ (see the Supplementary Materials for further details). The results are given in Table 1. We see that $|K_{II}| \gg |K_I|$, i.e., shear, not wedging open by pressurized ice, drives crack growth. Note that there is a potential source of error in our calculation of K_I and K_{II} , as strains in the fitted region are large enough ($\lesssim 20\%$) that LEFM could start to break down due to elastic nonlinearity. We estimate the magnitude of this error by noting that previous work on “weakly nonlinear” fracture in brittle hydrogels fitted LEFM predictions to

displacements ahead of crack tips at similar strains (30, 31). For slowly moving cracks, the extracted stress intensity factors had an error of $\sim 10\%$ when compared to more accurate measurements based on nonlinear fracture mechanics. Thus, we anticipate a similar error in our measurements (see the Supplementary Materials for further details).

Our measured values of K_I and K_{II} also yield fracture energies, Γ_f , of the hydrogel glass interface during freezing. From LEFM, $\Gamma_f = (K_I^2 + K_{II}^2) / [2\mu(1 + \nu)\cosh\pi\epsilon]$, where ν is the drained Poisson ratio of the gel and $\epsilon = -\log(3 - 4\nu)/(2\pi)$ (28). The extracted values (Table 1) match our expectation that PEG silane would have weaker adhesion than the two coupling agent silanes. The fracture energy of the PEG-silanized glass is equal to the interfacial energy of an ice/water interface [0.033 J/m^2 (32)], which we expect to be very similar to the ice/hydrogel interfacial energy. This relationship would suggest that this fracture energy may just come from the interfacial energy required to create fresh ice/hydrogel interface upon delamination. All the extracted fracture energies, Γ_f are somewhat smaller than fracture energies, Γ_p , measured by bulk peel tests at room temperature (see Table 1 and Materials and Methods). This difference could stem from several factors. First, errors in measuring K_I and K_{II} would lead to a compounded error in Γ_f : our estimate above of a 10% error for K_I and K_{II} would imply a potential error of up to $\approx 20\%$ in Γ_f . Furthermore, the freezing test is quasi-static and performed at cold temperatures, while the peeling test is dynamic and performed at room temperature. Both speed and temperature are known to affect gel properties [e.g., (33, 34)]. Last, in the dynamic peeling tests there are sources of dissipation due to processes like visco- and poro-elasticity in the bulk sample, friction, and slipping at the grips, which can all increase the measured fracture energy.

Dehydration, not pressurized ice growth, drives crack growth

The underlying mechanism for fracture is gel dehydration caused by cryosuction. A potential reason for the strong mode II component to fracture would be the ice directly exerting shear on the hydrogel. In this case, we would expect to see large shear strains directly under the ice crack in Fig. 5H. However, the shear there is rather small, ruling out this mechanism. Instead, we propose that shear arises due to cryosuction locally dehydrating the hydrogel around the ice. To the left of the crack tip, this dehydration can be accommodated by the hydrogel simply shrinking in the z direction. To the right of the crack tip, adhesion prevents shrinkage in the z direction, and the gel contracts laterally, yielding the rightward displacements in Fig. 5A. This resulting shear drives the mode II fracture. The mechanism is shown schematically for a simpler geometry in Fig. 6 (A and B).

Table 1. Measured fracture parameters. Fitted values of K_I and K_{II} for the three different silanes used, along with fracture energies from freezing (Γ_f) and bulk-peeling tests (Γ_p). A negative K_I is unexpected, as it indicates compression at the crack tip, but this is known to be possible in interfacial cracks (52).			
	K_I (Pa m ^{0.5})	K_{II} (Pa m ^{0.5})	Γ_f (J/m ²)
PEG silane	-19 ± 5	-55 ± 1	0.033 ± 0.003
Hexenyl silane	29 ± 6	-137 ± 3	0.19 ± 0.01
Methacrylated silane	150 ± 14	-305 ± 17	1.1 ± 0.14
			Γ_p (J/m ²)
PEG silane			0.49 ± 0.11
Hexenyl silane			1.25 ± 0.38
Methacrylated silane			1.82 ± 0.29

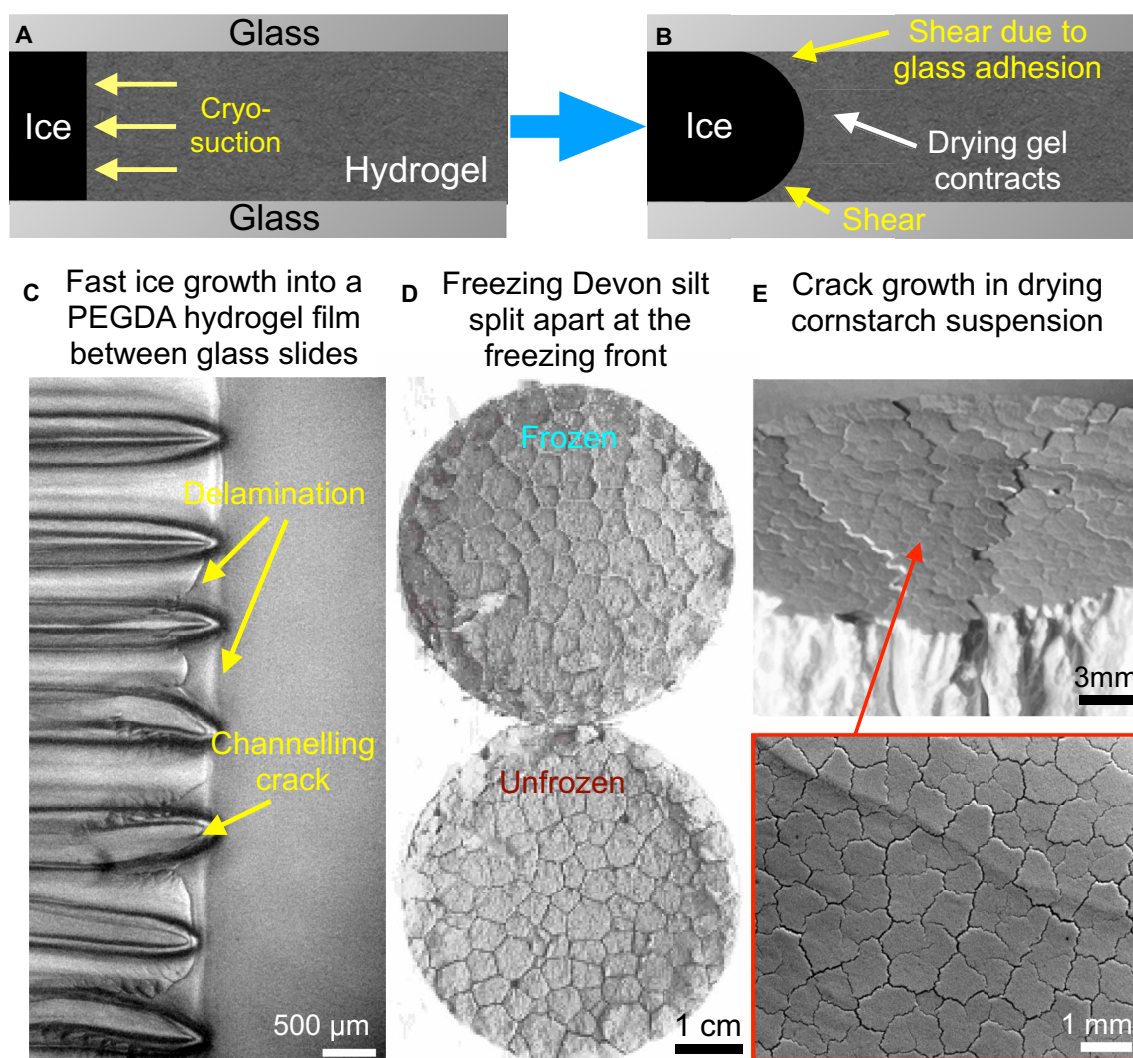


Fig. 6. The freezing/drying analogy. (A and B) Cryosuction drives suction of water out of a hydrogel toward cold ice. This dehydrates the hydrogel, leading to shear generation at the glass/hydrogel interface, which drives fracture. (C) When ice is grown quickly into the same hydrogel-filled cell used for experiments above, we see both delamination and bulk, channelling cracks. (D) A frozen plug of Devon silt, frozen from the top down, and split open at the freezing front [from (20) with permission]. The two images are the frozen and unfrozen halves of the sample. (E) A drying cornstarch suspension cracks in a similar pattern to the freezing silt. The suspension (50 wt % Xinliang cornstarch in water; thickness, 10 mm) was dried on a hot plate at 50°C for 24 hours.

We expect that this dehydration mechanism also drives bulk crack growth in freezing gels. An example of such crack growth is shown in Fig. 6C, resulting from fast ice growth into a 150- μm -thick, hydrogel-filled sample cell. Here, the ice is advancing into the hydrogel at 10 $\mu\text{m/s}$, in a temperature gradient $g_T = 13^\circ\text{C/mm}$. At these high rates of freezing, we see evenly spaced cracks that channel through the hydrogel (see also movie S2). For this type of crack, dehydration still occurs, as crack-ice growth dries out the nearby hydrogel. The dehydrated volume of hydrogel will try to shrink, but will be hindered from doing this by the presence of swollen hydrogel further away from the cracks, and will thus go into tension. Ultimately, the resulting tensile stresses should drive crack propagation.

Strong evidence for freeze-fracture as a dehydration process comes from comparing the behaviors of freezing-induced and

drying-induced fracture. For example, the evenly spaced, bulk cracks in Fig. 6C are very similar to channelling cracks that have been observed in drying films [e.g., (35, 36)]. Furthermore, drying our sample cells from one end induces very similar delamination and strain fields to those seen in Fig. 5 (see the Supplementary Materials). The more one looks into the structure of freezing cracks, the more one finds evidence of a strong freezing/drying analogy. For example, Fig. 6 (D and E) shows strong visual similarities between the crack patterns in freezing colloidal suspensions (silt and drying cornstarch, respectively). We do, however, anticipate some differences between freezing and drying. Most obviously, freezing is an almost incompressible process, while drying involves a large volume expansion as water turns into vapor. Thus, drying tends to be localized to the surfaces of bulk samples, so that the large volume of produced water vapor can evaporate

into the surroundings. By contrast, freezing can easily occur inside bulk materials, as there is no requirement to transport mass out of the material.

DISCUSSION

In conclusion, imaging ice growth into model hydrogels shows that it is not pressurized ice expansion upon freezing that drives damage. Instead, ice growth dehydrates the nearby hydrogel, leading to drying-induced stresses when this region of dried gel cannot freely shrink. These stresses ultimately cause damage; this mechanism is very similar to that underlying desiccation fracture in brittle, drying materials. While the microscopic freeze-fracture process is governed by classical fracture mechanics, macroscopically, the shape of the cracks is set by a simple balance between cryosuction and gel elasticity (Fig. 2).

Our results have important implications for understanding freezing. For example, we can apply insights from desiccation fracture to understand the freezing process: In desiccation, there is a strong rate dependence of fracture, with faster drying leading to large suction and differential drying, both of which cause fracture-inducing stresses (35, 37, 38). Our knowledge of this process should shed light on freezing damage, which is similarly known to be highly rate dependent (39, 40). As another example, drying films bonded to stiff substrates are much more likely to break than free-standing films, as they develop residual stresses as they dry out (35). By analogy, we expect that freezing damage will depend strongly on how the freezing sample is constrained or confined. The freezing/drying analogy may also help with investigating complex drying problems. It is hard to perform precise desiccation experiments due to difficulties in spatially controlling humidities and drying rates (41). Freezing offers an alternative approach to obtain precise control of drying experiments, simply by controlling temperature.

In the future, it will be important to understand the role of factors such as solutes and material properties upon the freezing process. Here, we have avoided additional solutes, to simplify our model system. However, these are generally present and can strongly affect freezing behavior (17). In particular, ice-active chemicals like ice-binding proteins, ice-nucleating agents, or cryopreservants will likely have dramatic effects on freezing damage (1, 42–45). Furthermore, gel properties could also affect freezing. For example, physical hydrogels and many biological materials can creep, reducing stress concentrations (46, 47), while tough hydrogels will be much less brittle than the gels we use here (46, 48). Polymer content should affect fracture, as this determines the degree of drying that a gel can undergo. Last, hydrogel mesh size should also play an important role, as this determines when ice can penetrate into the bulk of a hydrogel. Together, these considerations suggest that freezing fracture is a rich topic for future research.

MATERIALS AND METHODS

The silicone gel is made following the recipe in (49) and comprises a mixture of vinyl-terminated, silicone polymer chains (DMS-V31, Gelest) cross-linked with a methylhydrosiloxane-dimethylsiloxane copolymer (HMS-301, Gelest). Polymerization is catalyzed using Karstedt's catalyst (SIP6831.2, Gelest), and samples are cured at 60°C for several days to ensure complete reaction. The PEGDA hydrogel is fabricated by making a 10 vol % PEGDA (molecular

weight 700 Da, Sigma-Aldrich) solution in water. Then, we add 2-hydroxy-2-methylpropiophenone ultraviolet (UV) initiator as 0.0005 vol % of the solution and fluorescent nanoparticles (200-nm red, carboxylate-modified Fluospheres, Thermo Fisher Scientific) as 0.001 vol % of the solution. Last, we cross-link the gel in the glass cell under 365-nm UV light for 1 hour. We mechanically characterize gels by indenting bulk samples using a TA.XT Plus texture analyzer with a 500-g load cell (Stable Microsystems). The silicone gel shear modulus ($\mu = 30$ kPa) is measured with a 1-mm radius, cylindrical indenter. The hydrogel shear modulus ($\mu = 37$ kPa) and drained Poisson ratio ($\nu = 0.17$) are measured using a 1.6-mm radius, spherical indenter, following (50). The experiments are performed on a hydrogel submerged in water in an ice bath, to obtain properties of a gel at 0°C. From further notch tests, we measure the hydrogel's bulk fracture energy as $\Gamma = 0.28$ J/m² (see the Supplementary Materials).

The cell is made of two glass microscope slides, bonded together with spacers. To control the attachment of the hydrogel to the inside of the cell, we silanize the microscope slides before cell assembly. We clean the slides with ethanol and water, dry them, and then activate them in a UV ozone cleaner for 10 min (ProCleaner Plus, Bioforce Nanosciences). We mix together 900 μ l of ethanol, 50 μ l of deionized water, 50 μ l of glacial acetic acid, and lastly add 3 μ l of the appropriate silane. After 5 min of resting time, we apply 30 μ l of the mixture to each glass slide, and leave them for 3 min to allow full surface coverage. Next, we quench the reaction immersing the slides in ethanol, before drying the slides on a hot plate at 110°C for 10 min to complete the silanization reaction. Glass slides were stored in a dry box (humidity below 20%) until use.

The samples are imaged with a Nikon Ti2 Eclipse microscope with a 20 $\times$ air objective and a 3i Spinning Disk Confocal system. We use a 561-nm laser to image fluorescent tracer particles, and bright-field imaging for observing ice/water interface positions (e.g., Fig. 1).

We performed bulk, 90° peeling tests by peeling hydrogel films off glass surfaces silanized with different silanes. The fracture energy is then $\Gamma = (F_c/w)$, where F_c is the steady state peeling force and $w = 18$ mm is the width of the hydrogel contact line (51). The PEGDA gel (Sigma-Aldrich, Mn700) film was cured in the mold to a thickness of 300 μ m. One side of the gel was bonded to silanized glass, while the other side was adhered to a thin poly(vinyl chloride) backing layer (50 μ m thick) using cyanoacrylate (Deli 502 super glue). Peel tests were performed at room temperature on a Materials Test Systems stretch machine (E43.104-B) equipped with a BAB-100N load cell and 90° peeling fixtures, at a peeling rate of 10 mm/min. For each silanized glass-gel surface, Γ was calculated by measuring the force during the steady-state peeling stage, with a peel distance ranging from 2.5 to 5.5 mm. Tests were repeated at least five times.

Supplementary Materials

The PDF file includes:

Supplementary Text
Figs. S1 to S13
Legends for movies S1 and S2
References

Other Supplementary Material for this manuscript includes the following:

Movies S1 and S2

REFERENCES AND NOTES

- R. P. Tas, V. Sampaio-Pinto, T. Wennekes, L. W. van Laake, I. K. Voets, From the freezer to the clinic. *EMBO Rep.* **22**, e52162 (2021).
- E. E. Benson, *Plant Cryopreservation: A Practical Guide*, B. M. Reed, Ed., (Springer, New York, NY, 2008), pp. 15–32.
- C. A. Knight, A. Wierzbicki, Adsorption of biomolecules to ice and their effects upon ice growth. 2. A discussion of the basic mechanism of “antifreeze” phenomena. *Cryst. Growth Des.* **1**, 439–446 (2001).
- G. Petzold, J. M. Aguilera, Ice morphology: Fundamentals and technological applications in foods. *Food Biophys.* **4**, 378–396 (2009).
- S. Deville, Freezing Colloids: Observations, Principles, Control, and Use: Applications in Materials Science, Life Science, Earth Science, Food Science, and Engineering, in *Engineering Materials and Processes*, S. Deville, Ed., (Springer International Publishing, Cham, 2017), pp. 1–46.
- D. M. Matthias, J. Robertson, M. M. Garrison, S. Newland, C. Nelson, Freezing temperatures in the vaccine cold chain: A systematic literature review. *Vaccine* **25**, 3980–3986 (2007).
- S. S. L. Peppin, R. W. Style, The physics of frost heave and ice-lens growth. *Vadose Zone J.* **12**, 1–12 (2013).
- S. Kjelstrup, S. A. Ghoreishian Amiri, B. Loranger, H. Gao, G. Grimstad, Transport coefficients and pressure conditions for growth of ice lens in frozen soil. *Acta Geotech.* **16**, 2231–2239 (2021).
- J. Dash, A. Rempel, J. Wettlaufer, The physics of premelted ice and its geophysical consequences. *Rev. Mod. Phys.* **78**, 695–741 (2006).
- R. W. Style, S. S. L. Peppin, The kinetics of ice-lens growth in porous media. *J. Fluid Mech.* **692**, 482–498 (2012).
- J. M. Konrad, N. R. Morgenstern, A mechanistic theory of ice lens formation in fine-grained soils. *Can. Geotech. J.* **17**, 473–486 (1980).
- J. S. Wettlaufer, M. G. Worster, L. A. Wilen, J. G. Dash, A theory of premelting dynamics for all power law forces. *Phys. Rev. Lett.* **76**, 3602–3605 (1996).
- J. You, J. Wang, L. Wang, Z. Wang, J. Li, X. Lin, In situ observation of the unstable lens growth in freezing colloidal suspensions. *Colloids Surf. A Physicochem. Eng. Asp.* **553**, 681–688 (2018).
- J. Zhou, C. Wei, Ice lens induced interfacial hydraulic resistance in frost heave. *Cold Reg. Sci. Technol.* **171**, 102964 (2020).
- A. W. Rempel, Microscopic and environmental controls on the spacing and thickness of segregated ice lenses. *Quatern. Res.* **75**, 316–324 (2011).
- D. Gerber, L. A. Wilen, F. Poydenot, E. R. Dufresne, R. W. Style, Stress accumulation by confined ice in a temperature gradient. *Proc. Nat. Acad. Sci. U.S.A.* **119**, e2200748119 (2022).
- J. M. H. Schollick, R. W. Style, A. Curran, J. S. Wettlaufer, E. R. Dufresne, P. B. Warren, K. P. Velikov, R. P. A. Dullens, D. G. A. L. Aarts, Segregated ice growth in a suspension of colloidal particles. *J. Phys. Chem. B* **120**, 3941–3949 (2016).
- D. Dedovets, C. Monteux, S. Deville, Five-dimensional imaging of freezing emulsions with solute effects. *Science* **360**, 303–306 (2018).
- S. Taber, The mechanics of frost heaving. *J. Geol.* **38**, 303–317 (1930).
- D. L. Kane, K. Hinkel, A New Hypothesis on Ice Lens Formation in Frost-Susceptible Soils. *Proceedings of the Institute of Northern Engineering University of Alaska* (2008).
- T. Hagiwara, H. Wang, T. Suzuki, R. Takai, Fractal analysis of ice crystals in frozen food. *J. Agric. Food Chem.* **50**, 3085–3089 (2002).
- R. V. Devireddy, D. Raha, J. C. Bischof, Measurement of water transport during freezing in cell suspensions using a differential scanning calorimeter. *Cryobiology* **36**, 124–155 (1998).
- K. Watanabe, M. Mizoguchi, Amount of unfrozen water in frozen porous media saturated with solution. *Cold Reg. Sci. Technol.* **34**, 103–110 (2002).
- S. S. Peppin, M. G. Worster, J. S. Wettlaufer, Morphological instability in freezing colloidal suspensions. *Proc. Roy. Soc.* **463**, 723–733 (2007).
- S. Deville, E. Maire, A. Lasalle, A. Bogner, C. Gauthier, J. Leloup, C. Guizard, Influence of particle size on ice nucleation and growth during the ice-templating process. *J. Am. Ceram. Soc.* **93**, 2507–2510 (2010).
- D. Gerber, L. A. Wilen, E. R. Dufresne, R. W. Style, Polycrystallinity enhances stress buildup around ice. *Phys. Rev. Lett.* **131**, 208201 (2023).
- J. Y. Kim, S. Heyden, D. Gerber, N. Bain, E. R. Dufresne, R. W. Style, Measuring surface tensions of soft solids with huge contact-angle hysteresis. *Phys. Rev. X* **11**, 031004 (2021).
- J. W. Hutchinson, Z. Suo, Mixed mode cracking in layered materials. *Adv. Appl. Math. Mech.* **29**, 63–191 (1991).
- J. R. Rice, Elastic fracture mechanics concepts for interfacial cracks. *J. Appl. Mech.* **51**, 98–103 (1988).
- A. Livne, E. Bouchbinder, J. Fineberg, Breakdown of linear elastic fracture mechanics near the tip of a rapid crack. *Phys. Rev. Lett.* **101**, 264301 (2008).
- E. Bouchbinder, A. Livne, J. Fineberg, Weakly nonlinear theory of dynamic fracture. *Phys. Rev. Lett.* **101**, 264302 (2008).
- W. M. Ketcham, P. V. Hobbs, An experimental determination of the surface energies of ice. *Philos. Mag.* **19**, 1161–1173 (1969).
- N. Zhang, Z. Pan, J. Lei, Z. Liu, Effects of temperature on the fracture and fatigue damage of temperature sensitive hydrogels. *RSC Adv.* **8**, 31048–31054 (2018).
- I. Naassauui, O. Ronsin, T. Baumberger, A poroelastic signature of the dry/wet state of a crack tip propagating steadily in a physical hydrogel. *Extreme Mech. Lett.* **22**, 8–12 (2018).
- A. F. Routh, Drying of thin colloidal films. *Rep. Prog. Phys.* **76**, 046603 (2013).
- E. R. Dufresne, E. I. Corwin, N. Greenblatt, J. Ashmore, D. Wang, A. D. Dinsmore, J. Cheng, X. Xie, J. W. Hutchinson, D. A. Weitz, Flow and fracture in drying nanoparticle suspensions. *Phys. Rev. Lett.* **91**, 224501 (2003).
- R. W. Style, S. S. Peppin, A. C. Cocks, Mud peeling and horizontal crack formation in drying clays. *J. Geophys. Res. Earth Surf.* **116**, F01025 (2011).
- L. Goehring, L. Mahadevan, S. W. Morris, Nonequilibrium scale selection mechanism for columnar jointing. *Proc. Nat. Acad. Sci. U.S.A.* **106**, 387–392 (2009).
- E. Cabrera, L. C. Welch, M. R. Robinson, C. M. Sturgeon, M. M. Crow, V. A. Segarra, Cryopreservation and the freeze–thaw stress response in yeast. *Genes* **11**, 835 (2020).
- F. Engelmann, Plant cryopreservation: Progress and prospects. *In Vitro Cell. Dev. Biol. Plant* **40**, 427–433 (2004).
- L. Goehring, A. Nakahara, T. Dutta, S. Kitsunezaki, S. Tarafdar, *Desiccation cracks and their patterns: Formation and Modelling in Science and Nature* (John Wiley & Sons, 2015).
- R. C. Deller, M. Vatis, D. A. Mitchell, M. I. Gibson, Synthetic polymers enable non-vitreous cellular cryopreservation by reducing ice crystal growth during thawing. *Nat. Commun.* **5**, 3244 (2014).
- M. Fitzner, G. C. Soso, S. J. Cox, A. Michaelides, The many faces of heterogeneous ice nucleation: Interplay between surface morphology and hydrophobicity. *J. Am. Chem. Soc.* **137**, 13658–13669 (2015).
- M. Bar Dolev, I. Braslavsky, P. L. Davies, Ice-binding proteins and their function. *Annu. Rev. Biochem.* **85**, 515–542 (2016).
- Z. A. Kanji, L. A. Ladino, H. Wex, Y. Boose, M. Burkert-Kohn, D. J. Cziczko, M. Krämer, Overview of ice nucleating particles. *Meteorol. Monogr.* **58**, 1.1–1.33 (2017).
- R. Long, C.-Y. Hui, Fracture toughness of hydrogels: Measurement and interpretation. *Soft Matter* **12**, 8069–8086 (2016).
- T. Baumberger, C. Caroli, D. Martina, Fracture of a biopolymer gel as a viscoplastic disentanglement process. *Euro. Phys. J. E* **21**, 81–89 (2006).
- M. A. Haque, T. Kurokawa, J. P. Gong, Super tough double network hydrogels and their application as biomaterials. *Polymer* **53**, 1805–1822 (2012).
- J. Y. Kim, Z. Liu, B. M. Weon, T. Cohen, C.-Y. Hui, E. R. Dufresne, R. W. Style, Extreme cavity expansion in soft solids: Damage without fracture. *Sci. Adv.* **6**, eaaz0418 (2020).
- Y. Hu, X. Zhao, J. J. Vlassak, Z. Suo, Using indentation to characterize the poroelasticity of gels. *Appl. Phys. Lett.* **96**, 121904 (2010).
- M. D. Bartlett, S. W. Case, A. J. Kinloch, C.-Y. Hui, E. R. Dillard, Peel tests for quantifying adhesion and toughness: A review. *Prog. Mater. Sci.* **137**, 101086 (2023).
- M. Comninou, An overview of interface cracks. *Eng. Fract. Mech.* **37**, 197–208 (1990).
- R. W. Style, R. Boltysanski, G. K. German, C. Hyland, C. W. MacMinn, A. F. Mertz, L. A. Wilen, Y. Xu, E. R. Dufresne, Traction force microscopy in physics and biology. *Soft Matter* **10**, 4047 (2014).
- X. Deng, General crack-tip fields for stationary and steadily growing interface cracks in anisotropic bimaterials. *J. Appl. Mech.* **60**, 183–189 (1993).
- H. Tada, P. C. Paris, G. R. Irwin, *The Stress Analysis of Cracks Handbook*. (ASME Press, New York, 2000).

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