Ice Crystal Dynamics in the Nanocomposite Networks

Ahn S^{1,*}, Lee SJ²

¹Berkeley Laboratory, USA

²Department of Mechanical Engineering, Pohang University of Science and Technology, Korea

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***Corresponding Author:** Sungsook Ahn, Berkeley Laboratory, USA.

E-Mail: sungsookahn@yahoo.com

Abstract

Water is abundant in every day's life and critically useful in many biological systems and in water-based mechanical devices. Freeze-thaw process is one of the inevitable dynamics especially for the materials working at sub-zero conditions where ice crystal changes the physical property of the whole crystal-embedded composite systems. However, still many phenomena have not been explained in terms of crystal control methodology in conjunction with mechanical properties. In this study, ice crystal dynamics occurring in network systems has been discussed. Small size of network structure contributes to crystal growth inhibition especially time-dependent recrystallization. This could be explained by nano-scale confinement effect at the initial nucleation/growth stage, controlling size and shape of ice crystallites. The physical property of crystal embedded-nanocomposite is dominated by ice crystal behaviors over the network. This includes freezing point depression, frequency-dependent and temperature-dependent storage modulus changes and cooling rate-dependent dynamics. This study sheds light on ice crystal control methodology which would be useful in various materials and machines working under freeze-thaw dynamics.

Keywords: Hydrogel; Rheology; Nano-scale confinement; Ice crystal; Recrystallization

Introduction

Freezing of a liquid leads to the crystal growth whose size and nature are related to the processing parameters, in particular the freezing rate and temperature [1]. Freeze-thaw involves repetitive growth and melting of ice crystals in cracks and pores of the materials [2]. Due to volume expansion during the freezing and recrystallization (growing and/or merging) during the thawing, cryopreservation inevitably takes disruption of live cells and organs [3, 4]. Hydrogels are three-dimensional polymeric materials where the bulk matrix contains water [5,6]. Water ingress into the network structure swelling into a gel [7,8] while the matrices are

mechanically compliant [9,10]. This allows the hydrogel components to mimic biotissues and organs as well as design actuators and sensors for various biomedical applications [11]. Cell encapsulation with such hydrogels provide a three-dimensional environment similar to the in vivo conditions. In this point, a polymeric cryogel has been developed with many crosslinking [12]. In addition to aforementioned cryopreservation of live systems, ice crystal control ability has broad futuristic usefulness such as soft robotics working at low temperature condition, polymeric materials applied in aerospace environments, and many another industrial

applications related with freeze-thaw condition. However, freeze-thaw mechanics of hydrogel has not been fully understood especially in ice crystal control methodology related with dynamic mechanical properties of the whole crystal embedded-composite systems.

In terms of the ice formation and growth processes, three key macroscopic effects associated with ice growth (nucleation is a separate property) are followings [13]; First, dynamic ice shaping (DIS) is the morphology modification of a growing ice crystal. Second, thermal hysteresis (TH) is a difference between the freezing and melting points. The non-equilibrium depression of the freezing point is greater than what is expected on a colligative basis. The resulting freezing point depression is lower than melting point, expressed as TH. Third, ice recrystallization inhibition (IRI) is the kinetically suppressed crystal growth (Ostwald ripening). IRI property operates independently with little correlation to the DIS and magnitude of TH activity, indicating relation with multiple molecular mechanisms. In addition to the solution property of water itself, environmental factors can affect on the IRI behavior. This leads to mechanical property changes of the whole composite systems where the crystals work as embedded crystallites in the continuum.

Crystals of paraffins have been reported to be inhibited at the hydrophilic-hydrophobic interface in the controlled emulsion systems [14,15]. On the other hand, the size and shape of gold crystal have been controlled by self-assembled amphiphilic polymeric templates [16,17]. Some reports have discussed freezing point depression in the network system [18]. However, water crystal dynamics has not been discussed in the network confinement point of view. In this study crosslinked network model system has been developed with different mesh size (correlation length, ζ) to observe ice crystal control in the

aspects of crystal size and their growth. The rheological properties (especially storage modulus, G') of ice-embedded networks are investigated in terms of ice crystal mechanisms related with nanoscale confinement phenomena.

Results and Discussion

Nanoscale network model design

Binary and quaternary thiol end-capped polyethylene glycol (PEG) molecules are incorporated for AuNP interconnection where the multi-linked AuNP surface works as a junction point in the network [19]. Molecular weight between the junction point (M_p) and structures of PEGs are controlled. A bi-functional 2PEG 10000 and tetra-functional 4PEG 10000 with $M_p = 10,000$, have been selected. In addition, the number of incorporated PEG molecules per a AuNP is controlled as times ($\times 5$), ten times ($\times 10$) and twenty times ($\times 20$), considering the multi-reactive sites on a single AuNP. The designed nanocomposite systems are hydrogels where 38 to 45% of water.

The designed nanocomposite networks exhibit characteristic structure investigated by small angle X-ray scattering (SAXS) (Figure 1). Beyond the critical q^* value the systems are responsive to temperature, while below q^* value all the systems are stable, indicating a presence of responsive frame. Under the given synthetic condition, 2PEG 10000 and 4PEG 10000 exhibit detectable network structures. To get broad q range, two sample-to-detector distance conditions in different q regions are combined. The observed q region is broad from a single crystal size region to polymer structure region. The focus of this study is in the size region of polymer structures. By the fundamental relation of scattering, $q = 4\pi\sin\theta/\lambda$ (where, θ is scattering angle and λ is the wavelength of the X-ray) and the size relation, $n\lambda = 2d\sin\theta$ (n is a integer and d is the size of the target object), the size and q values are in inverse relations, $d \sim 2\pi/q$.

Figure (1): Nanocomposite model systems with varying network size measured by small angle X-ray scattering (SAXS). SAXS profiles of 2PEG10000 linked nanocomposite.

Figure (1A): And 4PEG10000 linked nanocomposite system

Figure (1B): The q values decrease along with temperature increase from 20 to 80°C, reflecting ζ value decrease. The number of PEGs per AuNP is diversified $\times 5$, $\times 10$ and $\times 20$. The detected q values for each temperature and PEG/AuNP condition are summarized in the inset Tables.

Figure (1C): The size of each system is evaluated which is inversely proportional to q .

Figure (1D): The size is represented by the correlation length (ζ) of network system. This is schematically illustrated for two cases where the size of state I is smaller than state II, $\zeta_I < \zeta_{II}$.

According to the observed q values summarized in the tables in (Figure 1) and aforementioned size vs. q value relation, the size of nanocomposite is graphically evaluated for each system (Figure 1C). Increased temperature leads to decreased size and 4PEG system display smaller size than 2PEG due to lower critical solution temperature (LCST) property of PEG [19]. More prominently, the large number of PEGs linked to a single AuNP significantly decreases the network size due to high crosslink density. The evaluated size corresponds to correlation length (ζ) in the

network system (Figure 1D). In this scheme the ζ in state I (ζ_I) is smaller than that of state II (ζ_{II}).

Ice crystal dynamics in the designed nanocomposite

IRI assay involves the formation of a polynucleated ice wafer by dropping a small volume of sample onto a pre-cooled surface. The ice crystals are then annealed at a sub-zero temperature, above the eutectic phase transition, and the growth of the ice crystals are monitored. The time length of the annealing affects the outcome, because this is only a kinetic procedure with slow

growth still occurring. IRI is one of the most popular methods to evaluate the efficacy of the crystal inhibiting ability of the designed materials. In this study, compared with the crystal size of pure DI water, ice crystal size of each designed systems are compared by splat experiments and microscopic observation using polarized light microscopy (Methods). As a standard system, ice crystal size of pure DI water is compared. After ice crystal wafer formation onto a dry ice condition (-78°C) for microscopic observation, the samples are stabilized at -8°C followed by annealing. Pure DI water shows prominent recrystallization during

the 30 min annealing indicated by significant ice crystal size growth (Figure 2). In addition, ice crystals of designed nanocomposites are evaluated. One of the representative images with 2PEG10000 ($\times 5$) are shown in (Figure 2) (below). From these optical images, IRI evaluations are based on mean largest grain size (MLGS) averaged by 20 grains. Obtained values at -8°C after 30 min annealing (D_t) are compared. Using the DI water crystal size as a standard ($D_{s,t}$), the normalized grain size is evaluated,

$$R = (D_t) / (D_{s,t}) \quad (1)$$

Figure (2): Polarized light microscopy images of the ice crystal of pure DI water (upper) and water in 2PEG10000 ($\times 5$) system (lower). After ice crystal wafer formation onto a dry ice condition (-78°C), the samples are stabilized at -8°C (left) followed by 30 min annealing at the same temperature (right). Scale bar is 0.1 mm.

At a fixed temperature as of -80°C , the images are obtained after 30 min annealing (Figure 2A). In addition, time-dependent recrystallization phenomena at the same temperature (-8°C) is observed with further 60 min and 120 min annealing (Figure 3, left). DI water displays effective recrystallization indicated by the fast size increase as of 1.5 times at 60 min and 2.5 times at 120 min compared with the size at 30 min annealing. For each designed

nanocomposite system with varying PEG number per AuNP as of $\times 5$, $\times 10$ and $\times 20$, normalized crystal size is evaluated (Figure 3, right). Considering the ζ observed in Fig 1C, each system shows different network mesh size, which might affect on the initial stage of ice crystallization and on the annealed ice crystal size. Considering the temperature responsiveness of the designed nanocomposites, the expected mesh size is in several nanometers at sub-

zero condition. As summarized in (Figure 3), highly crosslinked system ($\times 20$) thus with smaller ζ , effectively inhibits crystal

growth, importantly the time-dependent recrystallization.

Figure (3): Relative crystal grain size, $R = (D_t) / (D_{s,t})$. The size at a time t (D_t) is normalized by the DI water crystal size at -8°C after 30 min annealing ($D_{s,t}$) as a standard.

In terms of ice recrystallization inhibition mechanism, anti-freeze protein is known to selectively attach on one side of ice crystal by which elongated ice crystal shape is observed after annealing [13]. In this point, antifreeze protein type III displays both ice crystal growth inhibitor and simultaneously promoter depending on the concentration [20]. The crystal shape of anti-freeze protein type III (1% aqueous solution) and designed 4PEG10000 ($\times 20$) after 30 min annealing is compared (Figure 4). Due to selective z-axis attachment of protein on the ice crystal, needle-like structure is prominent. Meanwhile, the designed 4PEG10000 nanocomposite ($\times 20$) displays

tiny round shape also different from the hexagonal shape of pure DI water (Figure 2 upper). This indicates that the designed nanocomposites hinders natural ice crystal growth kinetics in isotropic direction, resulting in rounded crystal shape. The nano-scale network contributes to ice crystal inhibition in characteristic ways. One suggestion is nano-scale confinement of initial nucleation/growth stage of ice crystallites. The nucleated ice crystallites confined in the nanoscale space grow to the crystallites relatively small in size and characteristic structure inhibited against time-dependent recrystallization.

Figure (4): Crystal shape of 1% aqueous solution of anti-freeze protein type III (left) and $\times 20$ nanocomposite of 4PEG10000 (right) after 30 min annealing

Rheological investigation of nanocomposite system with embedded ice crystals

The storage modulus (G') and loss (G'') are obtained by frequency sweep test from 0.1 to 10 Hz, with a stress value within the linear range. At low temperature, the rheological behavior of nanocomposite hydrogel is mainly influenced by the ice fraction and the structure. Ice crystal size measured by microscopy correlated the with the modulus where small crystals contribute to low G' [21]. The G' is correlated with the solid-body like behavior thus the hardness/stiffness property of the nanocomposite hydrogel. On the other hand, the value of G'' at low temperatures can be correlated with the flow ability and scoop ability of the nano composite hydrogel. The temperature effect of the designed nanocomposite is significantly expressed in terms of G' , thus discussed in detail.

The solid-like property indicated by storage modulus (G') of the designed nanocomposites are arranged in (Figure 5). The nanocomposite hydrogels at 20 °C are evaluated before ice crystal formation (Figure 5A). The G' is high for highly cross linked system ($\times 20$) for each 2PEG and 4PEG nanocomposite. As illustrated in Figure 1A and 1B, the 2PEG nanocomposite is only composed of AuNP junctions while 4PEG has mixture of AuNP and polymer junctions. This AuNP-only junctioned 2PEG exhibits slightly higher G' than 4PEG. In terms of the slope of frequency sweep where lower slope indicates harder texture, 2PEG is slightly harder than 4PEG systems at each degree of crosslinking.

The frequency sweep of the designed nanocomposites are performed at -8 °C from 0.01 to 10 Hz (Figure 5B). The time-dependent coarsening (Ostwald ripening) of ice crystals in the designed nanocomposite is measured by -1 °C/min cooling rate from 0 to -8 °C. Compared with nanocomposites above zero temperature

without ice crystal formation, the G' is hundreds times higher. In addition, the tendency is opposite where the lower crosslinking ($\times 5$) shows higher values. This result indicates that the G' is dominated by the ice crystal in the nanocomposites not by the nanocomposite property without ice crystal. Considering that the correlation length (ζ) of the network (Figure 1C) and the ice crystal size measured at instant freezing on the dry ice (Figure 3), the observed high G' is induced by large ζ and bigger ice crystal. Nonetheless, the AuNP-only junction effect in 2PEG system still contribute to higher values over 4PEG. Prominently both 2PEG and 4PEG, highly cross linked ($\times 20$) system shows significantly far lower G' than other systems. This indicates that there is a critical condition where the G' values are effectively lowered.

The G' values of the nanocomposites are evaluated by temperature sweep during the cooling from 1 to -10 °C at -1 °C/min cooling rate (Figure 5C). In addition to the G' variation, there is a prominent freezing point depression and this is proportional to the degree of crosslinking. There is some intermediate region where the G' value increases during the temperature changes. The highly cross linked system ($\times 20$) exhibits relatively sharp increase than other systems. This might be caused from the smaller ice crystal size for which it takes relatively short time to get to the equilibrium. For large crystal size, more steps including recrystallization are needed to get to a final size.

The G' values of the selected two 4PEG nanocomposites of ($\times 5$) and ($\times 20$) are evaluated by different cooling rate during at the -0.5, -1 and -2 °C/min rate (Figure 5D). Overall, the G' values are not differentiated by the cooling rate changes. However, for less cross-linked system ($\times 5$) the intermediate region shows a tendency for which the fast cooling shows more abrupt G' value increase along with temperature decrease.

Figure (5): Storage modulus (G') of the designed systems.

Figure (5A) Frequency sweep of the designed nanocomposites at 20 °C .

Figure (5B) Frequency sweep of the designed nanocomposites at –8 °C.

Figure (5C) Temperature sweep of the designed nanocomposites at from 1 to –10 °C by –1 °C/min cooling rate

Figure (5D) Those of the selected nanocomposites by –0.5, –1 and –2 °C/min cooling rate.

Methods

Small-angle X-ray scattering (SAXS)

Synchrotron small angle X-ray scattering (SAXS) measurements are obtained at the 4C beamline at Pohang Accelerator Laboratory (PAL, Pohang, South Korea) equipped with a position-sensitive two-dimensional (2D) detector. Two energy settings are employed for wavelength modulation: 10 (0.0675 nm^{–1}) and 18 keV (0.1217 nm^{–1}). A W/B₄C double multilayer monochromator is installed to deliver monochromatic X-rays with a wavelength of 6.75 nm (18360 keV) and spread of $\Delta\lambda/\lambda = 0.01$. The 2D scattered X-rays are recorded by a CCD camera (Mar CCD, Mar USA, Inc., and CCD165). The collected SAXS data are corrected by subtracting the background and empty cell scattering. 1 mm-thick samples are used by using quartz tube. The sample-to-detector distance (SDD) of 4m covers the q range of $0.0679 \text{ nm}^{-1} < q < 1.64094 \text{ nm}^{-1}$, where $q = (4\pi/\lambda)\sin(\theta/2)$ is the magnitude of the scattering vector and θ

is the scattering angle. The q range is calibrated using polystyrene-*block*-poly(ethylene-*ran*-butylene)-*block*-polystyrene (SEBS) ($q = 0.19165 \text{ nm}^{-1}$). On the other hand, the 1m SDD covers the q range of $0.346 \text{ nm}^{-1} < q < 7.68039 \text{ nm}^{-1}$. This q range is calibrated using silver behenate ($q = 1.052 \text{ nm}^{-1}$). During the measurements, the temperature is precisely controlled at an isothermal condition of 20 °C if no heating plan is involved. To increase the sample temperature to 40 °C and 60 °C, programmed heating coil is employed connecting to the copper-based sample holder.

IRI evaluation

A 10 μL droplet of sample is dropped from approximately 1.5 m height onto a microscope coverslip (VWR cat#48404-453, 24 $\times$ 50 mm) placed on an aluminum plate (10 cm in thickness) cooled to –78 °C using dry ice in a styrofoam box. Upon impact with the microscope coverslip

the sample droplet spreads out, instantly freezes and forms a disk shape wafer ice. This iced wafer is then placed on the temperature-controlled thermoelectric refrigerated system (Space-Ray, Charlotte, NC, USA) under the polarizing light microscope (OPTIPHOT-POL, Nikon, Tokyo, Japan). The images are obtained by camera (Canon EOS 5DSR, Canon USA, Melville, NY, USA) under the live view shot mode and instant images are obtained.

After the iced wafer formation on the dry ice condition (-78°C), the ice wafer is stabilized at -40°C for 2 min and then heated to -8°C for 5 min ($6.4^{\circ}\text{C}/\text{min}$ heating rate). The photographs of the iced wafers are taken in every 5 min at -8°C and the time-dependent crystal size change is observed. From these optical images, mean largest grain size (MLGS) is averaged by 20 grains. Obtained MLGS values at -8°C after a certain annealing time (D_t) are normalized by that of DI water as a standard ($D_{s,t}$); $R = (D_t) / (D_{s,t})$. In this study, the annealing time is controlled to be 30, 60 and 120 min.

Rheology

Rheology measurements at above-zero and sub-zero condition are determined by oscillatory test by using MARS Rheometer, Haake, cone-plate sensor (C35/2Ti). A Peltier plate controls the temperature of the samples during the analyses. Rheology measurements of the samples produced at the same conditions are replicated thrice. Prior to the frequency test, a deformation amplitude test, from 0.1 to 1000 Pa, at a constant frequency of 0.5 Hz, is carried out at -8°C to determine the linear viscoelastic regime. The constant deformation amplitude of 30 Pa is chosen for all oscillatory measurements. Such deformation amplitude is established within the linear range, the oscillation frequency is varied between 0.1 to 10 Hz and the temperature is kept constant at -8°C . The elastic (G') and viscous (G'') modulus are evaluated as a function of frequency. In the oscillatory thermorheometry conducted within the range of linear viscoelasticity, the temperature is continuously decreased from 1 to -10°C by $-1^{\circ}\text{C}/\text{min}$ cooling rate. And for selected

nanocomposites of 4PEG nanocomposites of ($\times 5$) and ($\times 20$), -0.5 , -1 and $-2^{\circ}\text{C}/\text{min}$ cooling rate is applied at a constant frequency of 1 Hz and the mechanical spectra are obtained.

Conclusion

Ice crystal dynamics occurring in model network systems have been investigated in this study, which is significantly affected by network structure in nano-scale. Especially time-dependent recrystallization is significantly inhibited. Both size and shape of ice crystal are changed by nano-scale confinement indicating effective crystal growth inhibition at the initial stage of crystal formation. Correlation length (ζ) of the network is significantly related with the ice crystal size obtained by instant cooling (by Splat assay). In addition, rheology result also demonstrates that the suppressed ice crystal generates lower modulus of the whole crystal-embedded composite systems.

Supporting Information

Further experimental details and additional experimental data are available *via* the authors.

Notes

The authors declare no competing financial interest.

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