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Influence of hydroxyapatite nanoparticles on the viscosity of dimethyl sulfoxide–H_2O–NaCl and glycerol–H_2O–NaCl ternary systems at subzero temperatures ☆

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ABSTRACT

The viscosity, at subzero temperatures, of ternary solutions commonly used in cryopreservation is tremendously important for understanding ice formation and molecular diffusion in biopreservation. However, this information is scarce in the literature. In addition, to the best of our knowledge, the effect of nanoparticles on the viscosity of these solutions has not previously been reported. The objectives of this study were thus: (i) to systematically measure the subzero viscosity of two such systems, dimethyl sulfoxide (Me_2SO)–H_2O–NaCl and glycerol–H_2O–NaCl; (ii) to explore the effect of hydroxyapatite (HA) nanoparticles on the viscosity; and (iii) to provide models that precisely predict viscosity at multiple concentrations of cryoprotective agent (CPA) in saline solutions at subzero temperatures. Our experiments were performed in two parts. We first measured the viscosity at multiple CPA concentrations [0.3–0.75 (w/w)] in saline solution with and without nanoparticles at subzero temperatures (0 to −30 °C). The data exhibited a good fit to the Williams–Landel–Ferry (WLF) equation. We then measured the viscosity of residual unfrozen ternary solutions with and without nanoparticles during equilibrium freezing. HA nanoparticles made the solution more viscous, suggesting applications for these nanoparticles in preventing cell dehydration, ice nucleation, and ice growth during freezing and thawing in cryopreservation.

Introduction

In cryopreservation, CPAs such as glycerol, Me_2SO and other hydrophilic non-electrolytes, can protect suspended cells from freezing injuries [2,22]. The toxicity of CPAs at high concentration is restrained at low temperatures [26]. However, conventional cryopreservation methods require loading of CPA(s) before freezing, which causes significant osmotic and metabolic damage to the preserved cells [10,19,24].

Programmed slow-freezing and vitrification are two commonly used conventional cryopreservation methods associated with CPAs. Compared with programmed slow-freezing, vitrification is more effective for several cell types [22,23] because it entirely eliminates lethal ice formation. However, the use of vitrification is still hindered by its requirement for high CPA concentrations and rapid cooling/warming rates.

Recently, a liquidus-tracking method has been developed for vitrifying articular cartilage [19]. The cornerstone of this method can be traced back to 1965 [2], when Farrant proposed a method to vitrify smooth muscle tissue by progressively increasing the Me_2SO concentration in isotonic saline solution. The liquidus-tracking method is similar to Farrant's original work, but relies on adjusting the CPA concentration of the real tissue to track the liquidus curve. This requires that the cooling steps should be chosen to guarantee that the freezing point of the tissue is slightly lower than the current temperature. The method thus avoids ice formation, and does not require high CPA concentrations at above-zero temperatures. Furthermore, rapid warming is not required [19].

Aside from the liquidus-tracking method, researchers have attempted to employ new techniques such as nanotechnology to overcome the technical challenges in cryopreservation.

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Nanoparticles have been found to change the thermal conductivities, crystallization and ice growth of cryopreserved solutions [7,8,14]. Therefore, nanoparticles show promise for modulating the freezing conditions experienced by cells or tissues during cryopreservation.

The viscosity of the cryopreservation solution plays an important role in the outside environment of the tissue or cells, owing to its relationship to ice formation and diffusion [12,16,26]. The liquidus tracking method could especially benefit from a model that elaborates viscosities at multiple CPA concentrations in isotonic solutions at subzero temperatures. Viscosities of ternary CPA solutions are scarce in the literature, whereas those of binary CPA solutions have been abundantly published [4,13,15,16,20,25]. Furthermore, the influence of nanoparticles on the viscosity of CPA solution has not yet been reported. Hence, it is necessary to develop viscosity models of ternary CPA solutions at subzero temperatures with and without nanoparticles.

In this study, we measured and quantitatively analyzed the subzero viscosities of both glycerol–H_2O–NaCl and Me_2SO–H_2O–NaCl solutions with and without nanoparticles. Hydroxyapatite (HA) nanoparticles were used because of their excellent biocompatibility [14,27]. Our experiments were divided into two parts. We first modeled the viscosity to elaborate its dependence on temperature and CPA concentration. The Williams–Landel–Ferry (WLF) model [26] was used to obtain the estimated model parameters. We then measured and analyzed the viscosity of residual unfrozen CPA–H_2O–NaCl solutions during equilibrium freezing.

Materials and methods

Preparation of solutions

Me_2SO, glycerol, and NaCl were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Hydroxyapatite (HA) nanoparticles, short rod-like (20 nm in diameter, 100 nm long), were purchased from Nanjing Emperor Nano Material Co. Ltd (Nanjing, China). All solutes were used directly without further purification.

For the viscosity modeling experiment, CPA–H_2O–NaCl solutions, *i.e.* Me_2SO–H_2O–NaCl and glycerol–H_2O–NaCl, were prepared by mixing the CPA with saline solutions. The saline solution contained 0.9% (w/w) NaCl and 99.1% (w/w) deionized water. The mass fraction of CPA in the ternary solution varied from 0.3 to 0.75 (w/w).

For the viscosity measurements of the simulated residual unfrozen solutions, CPA–H_2O–NaCl solutions were prepared at 10% (v/v) CPA, a concentration widely used in general cell cryopreservation. The mass ratio of CPA to NaCl was kept constant during freezing. The mass percent of CPA and NaCl at each temperature was calculated according to the ternary phase diagram equations [17,18]:

$$T_{m1} = (-0.6 + 0.17\tan^{-1}(R_1)) \cdot W_1 + \left(\frac{1}{132}\tan^{-1}\left(\frac{R_1}{2}\right) - 0.001\right) \cdot W_1^2 - 0.00045 \cdot W_1^3 \quad (1)$$

$$T_{m2} = (-1.6 - 1.27R_2 - 0.25R_2^2)^{-1} \cdot W_2 - 0.010W_2^2 \quad (2)$$

where the subscripts 1 and 2 refer to the variables of the Me_2SO and glycerol based solutions, respectively. T_m is the freezing point of the ternary solution. R is the weight ratio of CPA to NaCl. W is the total solute concentration in g/100 g.

CPA–H_2O–NaCl solutions with HA nanoparticles were prepared by mixing 0.1% (w/w) HA nanoparticles with the previously prepared CPA–H_2O–NaCl solutions. To disperse the nanoparticles, the solutions were oscillated in an ultrasonic cleaner (Shanghai KUDOS Ultrasonic Instrument Co., Ltd, China) for 7 min before each experiment; the nanoparticles then remained dispersed for the entire duration of the measurements (<5 h).

Viscosity measurement system

The viscosity measurement system contains three parts: a rotary viscometer (Shanghai Jingtian Electronic Instrument Co. Ltd, China) which is coupled with a cryostat (custom-made in our laboratory), a low temperature bath (NCB-2400, Eyela Tokyo Rikakikai Co. Ltd, Japan) and a data acquisition instrument (34970A, Agilent Technologies, Santa Clara, CA, USA). The control accuracy of the viscometer is ± 1% and that of the low temperature bath is ± 0.1 °C.

The viscometer, the core of the system, measures the sample's dynamic viscosity. The cryostat around the sample is connected to the low temperature bath by two pipes that are coated with heat insulating materials to minimize heat loss. The inner space of the cryostat is filled with circulated solution from the low temperature bath to stabilize the sample's temperature. The data acquisition instrument contains a computerized interface and data logger, and is used for real-time temperature monitoring and recording.

System validation

To validate the reliability of the viscosity measurement system, the viscosity of a Me_2SO–H_2O binary mixture was measured at 25 °C at different Me_2SO mass fractions, and the results were compared with values obtained from the literature [11] (Table 1). The values obtained in this study are slightly higher than those from the literature. However, the relative error is below 4%, suggesting that the viscosities measured from our system are reliable.

Williams–Landel–Ferry (WLF) model

The ternary viscosity data were characterized with the WLF model [26]:

$$\eta = \eta_g \exp\left[-\frac{C_1(T - T_g)}{C_2 + T - T_g}\right], \quad (3)$$

$$\ln \eta_g = aw_1^3 + bw_1^2 + cw_1 + d, \quad (4)$$

$$\ln T_g = \frac{\sum_{i=1}^{3} x_i \Delta C_{pi} \ln T_{gi}}{\sum_{i=1}^{3} x_i \Delta C_{pi}}, \quad (5)$$

where T_g is the glass transition temperature of ternary solutions, T_{gi} is the glass transition temperature of the pure component, ΔC_{pi} is the change in heat capacity at the glass transition of the pure component, and x_i is the molar fraction of the pure component. ΔC_{pi} and T_{gi} are listed in Table 2, where each component is denoted by a different value of i. η_g is the viscosity of the ternary solution at T_g, w_1 is the mass fraction of CPA. C_1, C_2, a, b, c, d are empirical parameters

Table 1
Comparison of measured viscosity values (mPa·s, means ± SD, $n = 5$) with those from the literature for Me_2SO–H_2O binary solutions at 25 °C.

Me_2SO mass fraction	Experiment	Literature[a]	Relative error
0.39	2.1480 ± 0.0879	2.1919	0.020
0.52	3.1200 ± 0.0500	3.0140	0.035
0.65	3.6740 ± 0.0462	3.6708	0.001
0.72	3.8620 ± 0.0415	3.7200	0.038
0.81	3.4260 ± 0.0321	3.3669	0.017
0.91	2.6160 ± 0.0241	2.5501	0.026
1	1.9960 ± 0.0378	1.9960	0.000

[a] From Ref. [11].