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THERMAL EXPANSION OF THE CRYOPROTECTIVE AGENT COCKTAIL DP6 IN COMBINATION WITH VARIOUS SYNTHETIC ICE MODULATORS

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INTRODUCTION

Differential thermal expansion is the driving mechanism of thermo-mechanical stress during cryopreservation by vitrification (*vitreous* means *glassy* in Latin). Thermo-mechanical stress can be harmful in cryopreservation, causing structural damage such as fractures and plastic deformations. Its effect only intensifies with the size of the specimen, with wide implications on organ banking and transplant medicine. Unfortunately, the correlation between structural damage and the development of thermo-mechanical stress is largely unexplored in the context of cryopreservation.

Cryoprotective agents (CPAs) are commonly used in order to suppress crystallization in cryopreservation processes, where ice formation is the cornerstone of cryoinjury. Unfortunately, relatively high cooling rates are required in order to facilitate vitrification, which may also intensify the resulting thermo-mechanical stress. One alternative to reduce the cooling rate while controlling the rate of ice growth is by introducing synthetic ice modulators (SIMs) into the CPA cocktail [1]. Recent studies on thermal expansion of the CPA cocktail DP6 demonstrated that SIMs indeed hamper crystal growth without affecting the thermal expansion coefficient of the mixture [2].

As part of an ongoing effort to investigate thermo-mechanical effects in cryopreservation, the current study expands on previous efforts [2,3] to study thermal expansion of SIM+CPA cocktails. The unique contribution of this study is in measuring the thermal expansion of M22 [4], which has shown very promising results in kidney cryopreservation [5], in measuring various ingredients of M22 when mixed with DP6, which has been studied as a base solution [2,3,6], and in measuring the thermal expansion of DP6 mixed with sucrose, which has recently shown promising results in blood vessels cryopreservation in large samples [7].

METHODS

Method of Analysis: The experimental apparatus used in the current study has been reported previously [3,8]. In brief for the completeness of presentation, it consists of a passively controlled closed system, where expansion of the specimen and its holding

Table 1: Coefficients of best-fit polynomial approximation for thermal strain, $\varepsilon = a_2T^2+a_1T+a_0$, and the coefficient of thermal expansion, $\beta=2a_2T+a_1$, where n is the number of data sets for corresponding material and R_ε is the thermal strain ratio measured over the temperature range of -80°C and 20°C with reference to DP6+UCV

Material	Temperature Range, °C	$a_0 \times 10^3$	$a_1 \times 10^4$	$a_2 \times 10^7$	n	$\sigma \times 10^4$	R_ε
DP6	20…-41.9	-3.62	2.14	9.50	7	1.45	N/A
DP6+UCV	20...-87.8	-7.44	2.28	6.84	5	3.67	1
DP6+UCV+1% X1000	20...-84.4	-7.33	2.32	6.57	7	1.15	1.037
DP6+UCV+1% Z1000	20…-84.3	-7.37	2.49	6.55	5	1.87	1.134
DP6+UCV+1% X1000+1%Z1000	20…-84.4	-5.75	2.43	6.72	6	2.68	1.094
DP6+UCV+0.5M Sucrose	20…-81.7	-5.07	2.27	5.16	7	2.82	1.075
M22	20…-91.1	-4.71	2.52	6.24	6	5.61	1.167

chamber drive pressure changes, which is the measured parameter. Simultaneous measurements of temperature by means of thermocouples, facilitate the correlation between pressure and temperature. First, pressure calibration provides the relationship between pressure and displacement in the system. Next, the linear thermal strain in the system is calculated by:

$$\varepsilon = \frac{1}{3}\frac{\Delta V_{\mathrm{CPA}} + \Delta V_{chamber}}{V_0} \quad (1)$$

where ΔV_{CPA} is the volume change in the specimen, $\Delta V_{chamber}$ is the calculated volume change of the CPA vessel, and V_0 is the initial volume of the cooling chamber containing the specimen. The volume change in the cooling chamber is calculated by:

$$\Delta V_{chamber} = 3V_0 \int_{T_0}^{T_i} \beta_{chamber}\, dT \quad (2)$$

where T_0 and T_i are the initial and final temperatures of the cooling chamber, respectively, $\beta_{chamber}$ is the thermal expansion coefficient of the chamber (brass). Finally, the thermal expansion of the sample is calculated by:

$$\beta = \frac{d\varepsilon}{dT} \quad (3)$$

The uncertainty in the thermal strain is within 4%, when calculated over a temperature range of -90°C and 20˚C [8].

Materials: The primary ingredients in DP6 are 3M dimethyl sulfoxide (DMSO) and 3M propylene glycol in Unisol (UCV) as a vehicle solution in the current study. M22 is a more complex cocktail containing 5 permeating cryoprotectants (pCPAs) and 3 non-permeating cryoprotectants (npCPAs). The pCPAs in M22 are DMSO (22.3%), formamide (12.9%), ethylene glycol (16.8%), N-methylformamide (3%), and 3-methoxy-1,2-propanediol (4%), while the npCPAs are polyvinyl pyrrolidone K12 (2.8%), X-1000 ice blocker (1%) and Z-1000 ice blocker (2%). Table 1 lists the tested materials in the current study, where some of the ingredients of M22 where further mixed with DP6 in effort to improve its vitrification qualities. Additionally, DP6 mixed with sucrose has also been tested, following recent promising results of blood vessels vitrification in large volumes [7].

Thermal Protocol: When coated with a layer of a thermal insulator, the cooling chamber is immersed in liquid nitrogen. The boiling of liquid nitrogen on the surface of thermal insulator facilitates cooling. The typical cooling rate ranges from 10˚C/min at 0°C and 5°C/min at -94°C, with an average value of 7.3°C/min. These rates are above the critical cooling rate for vitrification of the respective solutions, which range below 3.5°C/min. The solutions become so viscous around -95°C that thermal expansion measurements become impractical at lower temperatures. Passive rewarming is performed by free heat convection to room temperature. The typical rewarming rates range between 10°C/min at -92°C to 2°C/min at 0˚C, with an average value of 4.9°C/min. The thermal strain measurements reported here were taken during cooling. In order to study crystallization events during rewarming, differences between thermal strain measurements during rewarming and cooling were further compared [3].

RESULTS AND DISCUSSISON

Table 1 lists the coefficients of polynomial approximation for the measured thermal strain, while Fig. 1 displays the results graphically. One can see from Fig. 1 that the vehicle solution Unisol enables thermal expansion measurements of DP6 down to a lower temperature than that for pure DP6 in water. This is due to the fact that Unisol contains 0.194M glucose, which is a glass promoting agent—it creates more favorable conditions for vitrification. Note that the lower temperature limit for measurements in the current system is around -95°C, when the vitrified material becomes too viscous to flow freely.

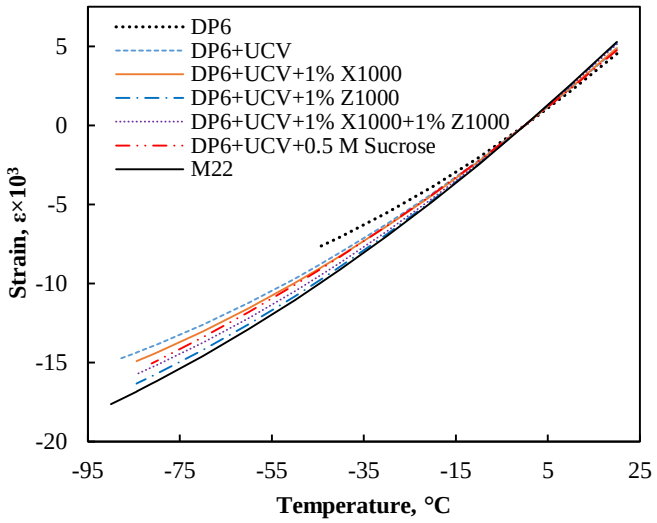

Figure 1 Polynomial approximations of thermal Strain for all the materials used in the study.

DP6 in water begins to crystallize around -35°C at the cooling rates achieved in this study. The addition of SIMs like X1000 and Z1000 to DP6 further suppresses crystallization and, hence, permits thermal expansion measurements down to lower temperatures when compared with DP6 in water.

The addition of SIMs showed an increase in the magnitude of thermal strain, where R_ε in Table 1 is the ratio of thermal strain for a particular DP6+SIM cocktail with respect to that of DP6 in Unisol, when calculated over the temperature range of -80°C and 20°C.

In conclusion, the current study shows that CPA cocktails of DP6 mixed with selected SIMs promote vitrification, which is evident when the SIM cocktail behaves as liquid down to lower temperatures. Interestingly, Unisol also displays vitrification promotion qualities, although it is more generally considered for its biocompatible vehicle solution characteristics. With DP6 in Unisol solution as a reference, linear thermal strain may increase by up to 17% over the temperature range of -80°C and 20°C for the other cocktails tested, which may increase thermo-mechanical stresses when the liquid-like CPA is trapped in a solid-like surroundings. Out of all the cocktails tested, M22 displayed the highest thermal strain, which is consistent with its highest solute concentration.

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