Scaling Effects on the Residual Thermomechanical Stress During Ice-Free Cooling to Storage Temperature

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Cryopreservation via vitrification (glass formation) is a promising approach for long-term preservation of large-size tissues and organs. Unfortunately, thermomechanical stress, which is driven by the tendency of materials to change size with temperature, may lead to structural failure. This study focuses on analysis of thermomechanical stress in a realistic, pillow-like shape cryobag as it is cooled to cryogenic storage, subject to sufficiently high cooling rates to facilitate vitrification. Contrary to common perception, it is demonstrated in this study that the maximum stress in the specimen does not necessarily increase with increasing size of the specimen. In fact, the maximum stress is affected by the combination of two competing effects, associated with the extent of the temperature gradients within the specimen and its overall volume. On one hand, the increase in specimen size gives rise to more prominent temperature gradients, which can intensify the thermomechanical stress. On the other hand, the temperature distribution at the core of larger specimens is more uniform, which leads to a larger portion of the specimen transitioning from fluid to a glassy material almost instantaneously, which carries a moderating effect on the overall mechanical stress at the glassy state (i.e., lower residual stress). In conclusion, this study demonstrates the role of container shape optimization in reducing the thermomechanical stress during cooling. [DOI: 10.1115/1.4047420]

Keywords: cryopreservation, vitrification, thermomechanical stress, cryobag, computational mechanics, heat transfer, stress analysis

1 Introduction

The World Health Organization (Geneva) has recognized the global shortage of organs for transplantations as a major public health challenge [1]. Nonetheless, only 10% of the worldwide need for organ transplantation are being met. This growing shortage of donor organs is a major hurdle limiting the effectiveness of organ transplantation in spite of incredible scientific, medical, and surgical development in the field [2]. In fact, a recent paper in *Nature Biotechnology* has argued that the ability to replace organs and tissues on demand could save or improve millions of lives each year globally and create public health benefits on par with curing cancer [3]. Cryopreservation by means of vitrification (*vitreous* in Latin means *glassy*) is well appreciated as a promising means for long-term storage of large-size specimen, with direct implications on the future of organ banking and transplant medicine [3]. The long-term preservation of organs and tissues outside the human body will not only help the 120,000 patients on the official transplant wait-list but also millions other suffering from end-stage organ disease [3]. Additional areas of medical research which benefit from tissue banking include cancer treatment, trauma care, disaster preparedness, tissue engineering, drug screening, disease monitoring, regenerative medicine, and many more [4]. The current paper addresses engineering contributions from the field of applied mechanics in order to improve tissue preservation and organ banking.

Successful vitrification of small tissue samples, in the scale of μm to mm, such as stem cells [5,6], corneas [7,8], and pancreatic islets [9,10], has been extensively reported. However, vitrification of larger-sized specimens and organs represents a more complex task, which is yet to be accomplished with high fidelity [11]. It calls for the solution of a multiparameter optimization problem, such as those drawn from the underlying principles of cryobiology—to maintain cell viability and achieve functional recovery, from the field of thermal engineering—to create the conditions preferable for vitrification, and from the field of solid mechanics—to preserve structure integrity of the biological material.

Vitrification involves relatively high cooling and rewarming rates in a specimen permeated with cryoprotective agent (CPA) in order to avoid ice formation [12,13]. While those high cooling and rewarming rates are favorable to facilitate vitrification, they may give rise to the hazardous effects associated with thermomechanical stress [14] (interchangeable with *thermal stress* in this study). Thermal stress may lead to structural damage in various ways, essentially leading to cryopreservation failure [15–20]. Thermal stress in the specimen is driven by differential thermal expansion [20], which may be the result of temperature gradients [14], phase change effects [21], material inhomogeneity [22,23], interaction between the specimen and its surrounding solution [22,24], and interaction with the container walls [16,18].

Thermal stress in cryopreservation is path-dependent [25,26], which means that its accumulated effect depends upon the sequence of events that take place along a specific thermal protocol for a specific specimen and container setup. Contrary to common perception in the field, it has been shown that the rewarming phase of cryopreservation is not the inverse of the cooling phase in the sense that rewarming does not relieve (or undo) stresses developed during cooling [25,26]. In fact, it has been demonstrated that the level of

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thermal stress only intensifies at the early stage of rewarming [15,20,25,27]. With this complex picture in mind, the onset of rewarming at the end of cryogenic storage has been identified as being most susceptible to structural damage and fracturing based on experimental observations [28,29] and theoretical analyses [15,20,25,27].

Volumetric heating methods to overcome harmful effects during the rewarming phase of the cryoprotocol have been presented [20,30,31], primarily aiming at achieving high enough rewarming rates throughout the specimen to prevent crystallization (related terms are *devitrification* and *recrystallization*) [31,32] and moderating the devastating effects associated with steep temperature gradients [14,33]. Volumetric heating may be achieved by exciting nanoparticles dispersed in the specimen with an electromagnetic field [31,34] or as the direct outcome of exposing the specimen to an electromagnetic field in various frequencies [35,36]. Either way, volumetric heating can alleviate the hazardous effects of thermal stress during rewarming. Unfortunately, the specimen cannot be exposed to a similar volumetric effect during cooling and, hence, alleviating thermal stress during cooling remains a main challenge, which is the subject matter of this study.

Analytical [14,16] and numerical [15,20,30] studies have been presented in order to predict the thermal stress history in specimens undergoing vitrification. The current study adds another layer of knowledge by using a previously established numerical solution approach, which have been further validated against experimental data [37], to study the effect of specimen size on the developed thermal stress. In particular, using a first-order analysis, the current study explains the counterintuitive outcome that a higher thermal stress level may develop in a smaller specimen subject to identical cooling conditions. This observation has implications on the design of thermal protocols in order to improve cryopreservation for the benefit of tissue and organ banking.

2 Mathematical Formulation

2.1 Model Geometry and Parameters. This study considers a pillow-shaped cryobag filled with CPA, as illustrated in Fig. 1(a) [20]. In essence, this study presents a first-order analysis, where the CPA-loaded biological specimen and the surrounding CPA have the same physical properties [38–41]. Taking advantage of geometrical symmetry, only one-eighth of the cryobag is analyzed (Fig. 1(b)). Table 1 lists the range of cryobag dimensions considered in this study, where the curved surface of the cryobag is modeled by [20]:

$$z = d + c \left[1 - \left(\frac{x}{a} \right)^2 \right] \left[1 - \left(\frac{y}{b} \right)^2 \right] \quad (1)$$

A cryobag is assembled by soldering two sheets of thin and flexible material such as fluorinated ethylene propylene (FEP) (KryoVue Cryopreservation Peel Pouch, American Fluoroseal Corp., Gaithersburg, MD). The welded region of the cryobag is approximated by adding a base thickness of 1 mm to the geometric model ($2d$ in Fig. 1(b)), and it may have an effect on stress concentration and rate of convergence of the numerical simulation. The current study only models the CPA contained in the cryobag, while the thin and compliant cryobag material is omitted from calculations. Such approximation helps in reducing computation costs with no compromise on the quality of results [20]. Figure 1(c) displays an example of the finite element mesh used to model the cryobag and three representative points which are used herein for discussion of results, referring to the volumetric center of the cryobag (P_a), the center of the longest edge (P_b), and the center of the curved surface of the cryobag (P_c).

Consistent with previous studies, the thermal conditions of a controlled rate cooling chamber are assumed in the current study

Fig. 1 Illustration of the pillow-shaped cryobag geometry: (a) a rendering of the cryobag model, where only 1/8th of it is simulated due to symmetry; (b) geometric parameters used for scaling analysis; and (c) FEA mesh of the pillow-shaped cryobag. Points of interest: P_a is the volumetric center of the cryobag, P_b is the center of the longest edge, and point P_c is the center of the curved surface of the cryobag.

Table 1 Geometrical parameters of a pillow-shape cryobag (Fig. 1) used for parametric investigation, where c/a represents the width ratio and b/a represent the filling ratio

a (mm)	r_0 (mm)	d (mm)	Width ratio, b/a	Filling ratio, c/a (Cryobag volume, V , cc)
61.0	2.0	0.5	0.2	0 (4.24), $\frac{1}{6}$ (29.0), $\frac{1}{3}$ (53.8), $\frac{1}{2}$ (78.6), $\frac{2}{3}$ (103), $\frac{5}{6}$ (128), 1 (153)
			0.4	0 (8.4), $\frac{1}{6}$ (58), $\frac{1}{3}$ (108), $\frac{1}{2}$ (158), $\frac{2}{3}$ (207), $\frac{5}{6}$ (257), $\frac{9}{10}$ (280), 1 (306)
			0.5	$\frac{2}{3}$ (258), $\frac{18}{25}$ (280), $\frac{5}{6}$ (321), 1 (382)
			0.6	0 (12.64), $\frac{1}{6}$ (86.4), $\frac{1}{3}$ (162), $\frac{1}{2}$ (235), $\frac{3}{5}$ (280), $\frac{2}{3}$ (310), $\frac{5}{6}$ (385), 1 (459)
			0.7	$\frac{1}{2}$ (280), $\frac{2}{3}$ (362), $\frac{5}{6}$ (425), 1 (536)
			0.8	0 (16.2), $\frac{1}{6}$ (116), $\frac{1}{3}$ (215), $\frac{9}{20}$ (280), $\frac{1}{2}$ (314), $\frac{2}{3}$ (414), $\frac{5}{6}$ (513), 1 (612)
			1.0	0 (21.0), $\frac{1}{6}$ (145), $\frac{1}{3}$ (269), $\frac{9}{25}$ (280), $\frac{1}{2}$ (394), $\frac{2}{3}$ (518), $\frac{5}{6}$ (642), 1 (766)

[37,42], characterized by an average heat transfer coefficient of $346 \text{ W/m}^2 \text{ K}$ for a Kryo-10 model. Previous studies [38–41] have demonstrated that the thermomechanical properties of a CPA-loaded tissue specimen are dominated by the CPA used, where the current study uses the cocktail VS55 as a representative CPA [17,30,31,43] (Table 2).

The numerical problem is solved using the commercial finite element analysis (FEA) code ABAQUS (Dassault Systems, Inc.), where an inbuilt meshing module is used to prepare the geometric model. The study utilizes eight-noded biquadratic quadrilateral elements (DCAX8 for heat transfer analysis and CAX8R for stress analysis), with about 5×10^4 to 6×10^4 elements for each model, based on a mesh convergence study. Automatic time stepping is used in ABAQUS, with the following constraints for determining the size of the time-steps: maximum temperature change of up to 1° C between consecutive time-steps, maximum inelastic strain rate of 1% within the time-step, and a global time-step cap of 25 s.

2.2 Heat Transfer Model. The problems of heat transfer and solid mechanics are assumed to be one-directionally coupled, where the heat generation due to viscous dissipation is negligible. Hence, the heat transfer problem is solved independently first [15,20,30,42], and the results of the heat transfer problem are used as input for the solid mechanics problem. Due to its high viscosity in the temperature range of interest, heat transfer within the CPA domain is assumed by conduction only

$$\rho C_p \dot{T} = \nabla \cdot (k \nabla T) \quad (2)$$

where ρ is the density, C_p is the specific heat, T is the temperature, and k thermal conductivity.

A convective boundary condition is used to describe the heat transfer between the outer surface of the specimen and the cooling chamber environment [42]

$$-k \frac{dT}{d\hat{n}} = h_{\text{eff}} (T_w - T_\infty) \quad (3)$$

where $\hat{n}$ is the direction normal to the wall, h_{eff} is the effective heat transfer coefficient, accounting for convection and radiation heat transfer, and the subscripts w and ∞ refer to the outer surface of the specimen and the cooling chamber air temperature, respectively.

2.3 Solid Mechanics Model. The CPA is modeled as a Maxwell fluid [15,20,30], for which the total strain rate is calculated as [16,40,44]

$$\dot{\epsilon}_{\text{total}} = \dot{\epsilon}_{\text{creep}} + \dot{\epsilon}_{\text{elastic}} + \dot{\epsilon}_{\text{thermal}} \quad (4)$$

where the creep, elastic, and thermal strain rates are calculated by

$$\dot{\epsilon}_{\text{creep}} = \frac{S}{2\eta} \quad (5)$$

$$\dot{\epsilon}_{\text{elastic}} = \frac{1}{E} [(1 + \nu)\dot{\sigma} - \nu \mathbf{I} \cdot \text{tr}(\dot{\sigma})] \quad (6)$$

$$\dot{\epsilon}_{\text{thermal}} = \beta \dot{T} \mathbf{I} \quad (7)$$

where S is the deviatoric stress tensor, η is the viscosity, E is the elastic modulus, ν is the Poisson's ratio, $\mathbf{I}$ is the identity matrix, tr is the trace of a matrix, and β is the linear thermal expansion coefficient.

Table 2 Material properties of the CPA (VS55) used in this study

Property	Value
Viscosity, Pa·s	1.21×10^4
	$4.2783 \times 10^{-23} e^{-0.6091 T}$
	4.63×10^{14}
Glass transition temperature, $^\circ \text{C}$	−123
Density, kg/m^3	1100
Thermal conductivity, $\text{W/m } ^\circ \text{C}$	0.3
Specific heat, $\text{J/kg } ^\circ \text{C}$	2400
Thermal expansion coefficient, $1/^\circ \text{C}$	1.1×10^{-4}
Young's modulus, Pa	0.8
Poisson's ratio	0.25

Fig. 2 Typical cooling thermal history in the controlled rate cooling chamber. Section C_1 is fast cooling at a rate of $5^\circ\text{C}/\text{min}$, H_1 is temperature hold above glass transition temperature for stress annealing, C_2 is slow cooling at a rate of $1^\circ\text{C}/\text{min}$, and H_2 is temperature hold at storage temperature. The glass transition temperature of the CPA is displayed using a dashed line.

2.4 Thermal Protocol. The representative vitrification cooling protocol analyzed in this study consists of four segments, as illustrated in Fig. 2: (C_1) a constant cooling rate of $5^\circ\text{C}/\text{min}$ from an initial temperature of $T_0 = 0^\circ\text{C}$ to an intermediate hold temperature of $T_h = -122^\circ\text{C}$, which is 1°C above the glass transition temperature for VS55 [39]; (H_1) temperature hold at T_h until the domain reaches thermal equilibrium; (C_2) a constant cooling rate of $1^\circ\text{C}/\text{min}$ down to the storage temperature, T_s ; and (H_2) temperature hold at T_s until the specimen thermally equilibrate at the storage temperature. The initial fast cooling in section C_1 is above the critical cooling rate (CCR) for VS55, while the slow cooling rate in section C_2 is set to reduce mechanical stress development as the CPA gradually turns into an elastic solid [15,20]. The hold temperature T_h is selected to facilitate stress relaxation before the material is further cooled in the solid state. The duration of the temperature holds in H_1 and H_2 was varied according to the size of the specimen, to ensure thermal equilibrium before advancing to the next step of the thermal protocol.

3 Results and Discussion

A set of 47 representative cases have been investigated in this study, representing variable cryobag width ratio, b/a , and filling ratio, c/a , as listed in Table 1. While the thermal and stress histories at key locations in a pillow-shaped cryobag have been reported previously [20], they are described here in brief for completeness of presentation for a special case: $a = 61\text{ mm}$, $b/a = 0.6$, $c/a = 1$. For this case, Fig. 3 displays the thermal history and the maximum principal stress histories at the three points of interest (Fig. 1(c)). Time lag in the order of minutes and temperature differences of up to 108°C (for example, at $t = 26\text{ min}$, time period C_1) can be observed between the center temperature, T_a , and the outer surface temperature, T_c . This significant temperature gradient within the specimen gives rise to differential thermal contraction, which is the driving force for thermal stress [20]. The viscosity of the CPA is low enough at higher temperatures, such that flow within the CPA prevents stress development. Significant stress develops in the specimen only when the temperature is cooled to lower temperatures,

Fig. 3 Maximum principal stresses and temperature history at locations specified in Fig. 1(c) for cryobag with geometric parameters $a = 61\text{ mm}$, $b/a = 0.6$, $c/a = 1$

starting several degrees above the glass transition temperature, with the onset of stress development depending on the cooling rate [16].

As the outer surface of the specimen is initially cooled faster than the center, tensile stress dominates at the outer surface while the center of the specimen experiences compressive stress. The maximum tensile stress in the cryobag during time period C_2 can be either at point P_b or P_c , depending on the specific geometric parameters. For the case presented in Fig. 3, the overall maximum tensile stress is observed at point P_b (2.5 MPa), while P_c experiences a maximum tensile stress of 0.51 MPa. Eventually, when the specimen thermally equilibrates at the storage temperature, the stress at the center of the specimen rises to an all times maximum tensile value, while the outer surface of the specimen experiences negligible stress at that stage. For the particular set of parameters used for the case presented in Fig. 3, the maximum stress at P_a is 0.78 MPa during section H_2 . The maximum tensile stress for the case presented in Fig. 3 is less than the 3.2 MPa stress-to-fracture threshold suggested for a closely related reference solution of 7.05M DMSO [16,18].

Figure 4 displays a parametric study for the maximum principal stress results, for the cases listed in Table 1. It can be observed from Fig. 4 that the cryobag with largest volume ($c/a = 1.0$, $b/a = 1.0$, and volume of $V = 766\text{ cc}$ according to Table 1) does not result with the maximum stress from all the cases studied. The maximum stress at points P_a , P_b , and P_c is observed for the cryobag with parameters ($c/a = 1.0$, $b/a = 0.6$, $V = 459\text{ cc}$), ($c/a = 1.0$, $b/a = 0.7$, $V = 536\text{ cc}$), and ($c/a = 2/3$, $b/a = 1$, $V = 518\text{ cc}$), respectively. This means that the stress in the cryobag does not necessarily increase with its size, which stands in contrast to the commonly held perceptions in the community of cryobiology. In a broader view, Fig. 4 displays how packaging shape and dimensions can affect the thermomechanical stress that the specimen may experience. In order to further investigate the effect of the cryobag volume on the maximum principle stress, Fig. 5 displays results at point P_a for selected width and filling ratios. For example, the stress at P_a increases monotonically with the increasing size of the cryobag for a filling ratio of $c/a = 1/2$, as could be expected. However, this is not true for the other filling ratios ($c/a = 1/6$, $c/a = 1/3$, $c/a = 2/3$, $c/a = 5/6$, and $c/a = 1$) displayed in Fig. 5. In fact, the highest value of the stress is found at different combinations of the width and filling ratios.

This counterintuitive behavior of thermal stress with the change in cryobag size is the result of two opposing-trend effects as

Fig. 4 Maximum principal stress during cooling at three locations based on the set of the 47 cases listed in Table 1: (a) volumetric center, P_a ; (b) center of longest edge, P_b ; and (c) center of curved surface, P_c

Fig. 5 Parametric study results for maximum principal stress at the center of the cryobag, P_a , Fig. 1, for varying filling and width ratios

follows. The first effect is derived from the tendency of the material to contract with the decreasing temperature, resulting from the physical phenomenon of thermal expansion (in practice, contraction during cooling). Since different adjacent layers of the bag tend to contract at different rates, mechanical stress builds up to make the contraction of those layers compatible. The steeper the temperature gradient is, the higher the resulting stress gets, a phenomenon which would only intensify with the increasing size of the cryobag if the entire material would be solid from the onset of cooling [14]. For a vitrifying material however, the entire domain becomes solid only at lower temperatures and the contribution of this effect is discussed below. The first effect is defined as the temperature gradient effect herein for simplicity.

The second effect is defined as the volume effect herein. In order to explain it, consider an imaginary line connecting points P_a and P_c in Fig. 1. Further consider the instant at which the bag surface temperature reaches -130°C along the process of constant cooling rate on the bag surface (see Fig. 2). The instantaneous temperature distribution along that imaginary line when the bag surface (P_c) reaches -130°C is displayed in Fig. 6 for various filling ratios. Also displayed in Fig. 6 is the glass transition temperature (T_g) of -123°C for VS55, below which the viscosity of the material is so high that the material behaves like a linear-elastic solid in any practical times scale [14,44].

In Fig. 6, only the portion of the temperature distribution curve between T_g and -130°C represents material which behaves like solid. Note that the temperature gradients relating to that portion

Fig. 6 Temperature distribution along a line connecting points P_a and P_c (Fig. 1(c)) when the surface temperature (P_c) reaches -130°C , for cryobags with filling ratio $c/a = 1$.

of the domain are higher for a larger volume bag (a higher b/a ratio while c/a is kept constant), where higher stress may develop due to the temperature gradient effect. At the same time however, a larger portion of the cryobag transitions from fluid to solid-like material at a more uniform temperature distribution in the remaining portion of the domain (the temperature distribution above -123°C is more moderate). It follows that a large portion of the cryobag transitions behave like solid under lower differential thermal expansion when the cryobag size increases. Consequently, when the temperature distribution equilibrates at the storage temperature, a lower residual stress develops, which is the stress built into the system due to the fact that different areas in the domain turned into solid-like material at different times [22]. In summary, the volume effect is associated with lower residual stresses in larger specimens.

In practice both the temperature gradient effect and the volume effect participate in the vitrification process as the cryobag is cooled to cryogenic temperatures. The temperature gradient effect is more pronounced in relatively small volumes (or filling ratios), when the cryobag transitions to a solid-like material relatively quickly. Conversely, the volume effect is more pronounced in relatively large volumes, when the material transitions to a solid-like material under a wide temperature range. For intermediate volumes, both effects may be significant, giving rise to the counterintuitive observation discussed in the context of Figs. 4 and 5, namely, that the stress does not necessarily increase with the increasing size of the cryobag. The fact that the cryobag under investigation has a more complex geometry of a pillow shape with variable filling ratios, rather than a sphere for example, makes the identification of the geometry leading to the peak stress more involved.

In order to provide further insight into the above combined effects, a special case study was investigated where the volume of the cryobag is preserved, while the key cryobag dimensions are varied. For this case, Fig. 7 displays the maximum principle stress at the three points of interest as a function of width and filling ratios, for a representative cryobag volume of 280 cc. Note that the filling ratio is a function of the width ratio in this case. Figure 7 also displays the maximum stress, σ_{max} , among the three points of interest (Fig. 1(c)).

It can be observed that σ_{max} coincides with the stress either at P_b or P_c for the geometric parameter combinations considered in Fig. 7, while the stress at the center of the specimen P_a is much

Fig. 7 Parametric study results for variable geometric parameters when the cryobag volume is maintained constant, where the maximum principal stresses correspond to the locations displayed in Fig. 1(c)). σ_{max} refers to the maximum stress in the cryobag among the locations displayed in Fig. 1(c).

lower. The minimum σ_{max} of 1.15 MPa is observed for the cryobag with geometrical parameters of $c/a = 0.91$ and $b/a = 0.4$. The next local minima of σ_{max} at about 1.25 MPa are observed for the geometrical parameters of $c/a = 0.53$ and $b/a = 0.69$, and again for the geometrical parameters of $c/a = 0.36$ and $b/a = 1.0$. For those two local minima, σ_{max} is found at P_c for the geometrical parameters of $c/a = 0.36$ and $b/a = 1.0$, and again for the geometrical parameters of $c/a = 0.53$ and $b/a = 0.69$, where the stresses at P_b and P_c are equal. Note that, while the maximum stress between the above three minima is within less than 10% difference, these points correlate with three significantly different cryobag geometries. Furthermore, the point at which the maximum stress is found is different, which may have further implications on the progression of fractures, once the strength-to-fracture of the brittle material has been reached. Finally, two maxima for σ_{max} at about 1.47 MPa can be observed from Fig. 7, but for significantly different cryobag proportions ($b/a = 0.8$ and $b/a = 0.60$), where the maximum stress is found at different locations for those maxima.

From the above discussions in Fig. 7, a few key takeaways for selecting cryobag geometric parameters stand out. First, since the stress at the center of the specimen is significantly below the stress at the outer surface and the edges, it is advantageous to place the biological material at the center of the cryobag. However, in practice, the stress at the outer surface might play a larger role in selecting the cryobag parameters, since the specimen is likely to fall close to the surface under the effect of gravity. Second, the maximum stress in the cryobag specimen can be reduced by 22% by varying the geometric parameters of the cryobag. Note that while these discussions are specific to the geometric parameters considered for a 280 cc cryobag, the numerical framework presented in this current study can be incorporated for similar analysis of differently sized cryobags. In essence, this is a demonstration on how to optimize a cryobag dimensions while maintaining shape similarity, in order to preserve structural integrity.

Recall that the goal in the current study is to explain the counterintuitive observation that a higher thermal stress may develop in a smaller specimen subject to identical cooling conditions. While this study utilizes a pillow-shape cryobag geometry and a representative vitrification thermal protocol to analyze the problem, the observations of this study are quite general and not necessarily limited to that geometry. For example, a similar

challenge has been recently reported in a study comparing stress in rabbit kidney and human kidney models, when enclosed in a cylindrical CPA container and subject to identical cooling conditions. There, a higher stress was observed in the smaller rabbit kidney model, but a plausible explanation for that effect was not available [50]. While that previous study reported on counterintuitive observations in the thermal stress behavior between two kidneys of specific sizes, the current study addresses a range of specimen sizes in order to provide insight into the corresponding scaling effect.

The thermal protocol applied in the current study and the one applied in the earlier kidney model study [50] are also different, which means that the above counterintuitive scaling effect on thermal stress should also be suspected in other specimens of practical sizes and subject to practical protocols. Unfortunately, no single dimensionless number or a key parameter that signifies this effect has been identified, which necessitates the use of a similar numerical approach when tailoring and optimizing a specific cryoprotocol for a specific application.

Another effect which may intensify thermal stress is excessive surface deformation, which may occur due to differential thermal contraction in the glass forming specimen under extreme conditions [37,42]. Such surface deformation may not only affect similarities between small and large specimens but may also give rise to stress concentration and the likelihood to structural failure. However, that effect of surface deformation is considered a secondary effect and it is still the subject matter of a parallel investigation.

4 Summary and Conclusions

Volumetric rewarming as a means of ice-free recovery from cryogenic storage has been presented as an effective way to reduce the thermal stress in cryopreserved specimen. This has shifted the focus on investigating the thermal stress generated while cooling the specimen to cryogenic storage, and developing means to reduce it. Toward this end, the current study uses numerical methods to analyze the effects of geometric parameters on the thermal stress developed in a cryopreservation specimen undergoing vitrification cooling protocol with the ultimate goal of specimen container shape optimization.

From a parametric study for the maximum thermomechanical stress in variable cryobag geometrical ratios, a counterintuitive relationship is observed between the maximum stress and the overall bag size. The maximum stress does not monotonically increase with increasing cryobag size, but instead the trend is dominated by two competing effects, referred herein for convenience as the temperature gradient effect and the volume effect. The temperature gradient effect is associated with the increase in stress with the increasing thermal gradients, which in turn increase with the cryobag size. The volume effect, on the other hand, is associated with a larger portion of the cryobag transitioning from fluid to a solid-like material under more uniform temperature distribution with increasing cryobag size, and thus resulting in lower stresses. These competing effects can explain results of a previous work, suggesting higher stresses in cooling a rabbit kidney as opposed to a human kidney [50].

To demonstrate the applicability of the computational model in the service of cryopreservation optimization, thermomechanical stress was analyzed for a variable cryobag geometry while the overall volume is preserved, with 280 cc as a representative volume. It was demonstrated that various geometrical parameters may lead to similar stress maxima, while the location of those maxima may depend on the specific case. The latter observation has implications not only on the likelihood to fracture as the developing stress reaches the strength of the material but also on the progression of fractures. However, the analysis of fracture dynamics is not included in the current study.

For the application of cryopreservation by vitrification, the current study shows that the thermal stress during cooling can be

substantially reduced by selecting appropriate specimen shape parameters and cooling protocol. More broadly, the current study emphasizes the uncompromised need to integrate applied mechanics concepts and tools in the design and optimization of cryopreservation protocols for any specimen size.

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Conflict of Interest

There are no conflicts of interest.

Data Availability Statement

The datasets generated and supporting the findings of this article are obtainable from the corresponding author upon reasonable request. The authors attest that all data for this study are included in the paper.

Nomenclature

k	= thermal conductivity, W/m °C
t	= time, s
E	= Young's modulus, Pa
T	= temperature, °C
V	= volume, cc
I	= identity matrix
S	= deviatoric stress tensor
$\dot{T}$	= temperature rate, °C/min
h_{eff}	= effective heat transfer coefficient, W/m ² °C
r_0	= radius of cryobag corner, mm
C_1	= fast cooling segment of thermal protocol
C_2	= slow cooling segment of thermal protocol
C_p	= specific heat, J/kg °C
H_1	= temperature hold above T_g
H_2	= temperature hold at storage temperature
T_0	= initial temperature, °C
T_g	= glass transition temperature, °C
T_h	= hold temperature, °C
T_s	= storage temperature, °C
T_w	= specimen surface temperature, °C
T_∞	= cooler environment temperature, °C
$2a$	= length of cryobag, mm
$2b$	= width of cryobag, mm
b/a	= width ratio
$2c$	= height of cryobag, mm
c/a	= filling ratio
$2d$	= height of welded region of cryobag, mm
tr	= trace of a matrix
x, y, z	= coordinates, mm
CCR	= critical cooling rate, °C/min
P_a, P_b, P_c	= points of interest (Fig. 1(c))
T_a, T_b, T_c	= temperature at corresponding point of interest (Fig. 1(c)), °C
β	= thermal expansion coefficient, 1/°C
$\dot{\epsilon}$	= strain rate, 1/s
$\dot{\epsilon}_{creep}$	= creep strain rate, 1/s
$\dot{\epsilon}_{elastic}$	= elastic strain rate, 1/s
$\dot{\epsilon}_{thermal}$	= thermal strain rate, 1/s
$\hat{n}$	= normal direction
η	= viscosity, Pa·s
ρ	= density, kg/m ³

σ = stress, MPa
 $\dot{\sigma}$ = stress rate, Pa/s
 σ_a , σ_b , σ_c = stress at the corresponding point of interest (Fig. 1(c)), MPa
 σ_{max} = maximum stress, MPa
 ν = Poisson's ratio

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