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LETTER TO THE EDITOR: ANALYSIS OF THERMO-MECHANICAL STRESS IN CRYOPRESERVATION

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Thermo-mechanical stress is known to be a significant mechanism of structural damage in the process of cryopreservation; its effect on blood vessels is one very relevant example. Zhao et al. (20) recently published a paper in *CryoLetters* which attempts to provide theoretical background for experimental studies on cryopreserved blood vessels reported by Pegg *et al.* (6), and Buján *et al.* (2). While the objective of Zhao et al. is highly commendable, the authors of this letter find the subject matter of that paper to be inconsistent with established modeling of similar problems, as well as with literature data on material properties.

One of the most significant underlying assumptions by Zhao *et al.* (20) is that the cryopreserved blood vessel behaves like a linear elastic material. A linear elastic model is characterized by a constant relationship between strain and stress, where, for example, the elongation of a blood vessel under axial load is proportional to the magnitude of the load. The material is further assumed to have thermophysical properties similar to those of pure water ice, but it possesses a phase transition temperature range of 0 to −20°C. The major weakness in that modeling originates from the fact that cryopreservation without cryoprotective agents (CPAs) is not feasible, and the addition of a CPA alters the material properties dramatically. For example, Pegg *et al.* (6) introduced 15% Me_2SO dissolved in CPTES to cryoprotect the specimen. If cooled fast enough, the CPA tends to form glass, in a process known as vitrification. For an ideal vitrified model, the material creeps under stress, according to which the blood vessel from the previous example would continue to elongate indefinitely under constant axial load. If cooled at a lower rate - as in the study reported by Pegg et al. (6) - only partial vitrification is expected. In the latter case, ice crystals first nucleate at about the solidification point for the specific CPA (significantly below the freezing temperature of pure water ice), while the concentration of the remaining solution elevates. The progress of crystal formation, and elevation of the concentration of the remaining solution, continues with cooling, until the remaining solution becomes so viscous as to form glass at the particular cooling rate. The volume fraction eventually occupied by ice crystals is affected by many factors, and the coexistence of crystallized and vitrified regions is an inevitable outcome of low cooling rate preservation. With a significant amount of highly viscous material, there is no reason to believe that the material behaves linear elastically, as suggested by Zhao *et al.* (20). On the other hand, with a significant amount of crystallized material, there is no reason to believe that the material behaves like an ideal vitrified material either.

While the mathematical model to describe the material cryopreserved by Pegg *et al.* is largely unknown, concepts from the general field of continuum mechanics are required in order to describe the continuous transformation from a liquid-like material to a solid-like material. In contrast, the model presented by Zhao *et al.* does not account for viscous effects at all.

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Even if one wished to approximate a cryopreserved material as linear elastic, the model presented by Zhao *et al.* in Eqs. (6)-(8) is not consistent with a proper accounting for solidification effects. The model presented by those equations was developed in the 1950s, as summarized in the classical textbook by B.A. Boley and J.H. Weiner *Theory of Thermal Stress* (1960) (this model did not originate in references 9 and 25 of Zhao *et al.* (20)) Equations (6)-(7) were copied from the classical source with a typo, where (1-ν) should be omitted from the denominator of both equations.

As early as 1963, Boley and Weiner suggested a technique to account for the moving front effect in a thermal stress process (1), but they neglected the thermal expansion upon solidification, which is significant in water ice formation. Following this early modeling, Rabin and Steif (9,11) developed a new modeling approach to account for volume changes at the freezing front. In broad terms, the new approach is based on the observation that the material cannot solidify with stress already built into it. Therefore, all stresses (except for hydrostatic pressure, which can be sustained even by low viscosity liquids) should be zero at an advancing freezing front. That is, material which has just solidified at the advancing freezing front must start with zero deviatoric stress. At a retracting freezing front, however, this constraint does not exist, and the material can sustain high stresses, bounded only by its strength. Based on this modeling, Rabin and Steif (9,11) demonstrated why the stress distribution during cooling is dependent on the thermal history of the specimen. Furthermore, due to the change in boundary condition from an advancing to a retracting freezing front, that model offers a plausible explanation for the reason that fractures frequently occur at the early stage of rewarming, rather than during cooling. By contrast, the model applied by Zhao *et al.* (20) is independent of the thermal history (it is dependent on the instantaneous temperature distribution only), and cannot explain the difference between cooling and rewarming.

The moving front effect on thermal stress problems has been overlooked by researchers in the area of cryobiology for a long time (3,5,8,15,16), while it was well appreciated by workers in the area of metal solidification and casting (4,17,18,19). However, the dramatic effect of the expansion upon freezing is not present in metal solidification and casting. The modeling suggested by Rabin and Steif (9,11) is applicable to a linear elastic material, as well as to an elastic-perfectly plastic material, but it does not incorporate any viscous effects.

Finally, the material properties used by Zhao *et al.* (20) are not consistent with data in the literature, in which thermal conductivity of ice is known to increase by four-fold when cooled to the liquid nitrogen boiling temperature, while the thermal conductivity of blood shows a similar trend (12). The specific heat decreases almost linearly with temperature in cryogenic temperatures, approaching a zero value at absolute zero temperature. The combined effect of increased thermal diffusivity (the ratio of thermal conductivity to specific heat) by an order of magnitude can be expected to dramatically affect the heat transfer simulation of a crystallized material in a linear elastic problem. Furthermore, the thermal expansion of pure water ice - as well as frozen biomaterials in the absence of cryoprotectants - is linearly dependent on temperature (10), and decreases by five-fold when cooled to the liquid nitrogen boiling temperature. In the presence of cryoprotectants, the thermal expansion of blood vessels can increase by several fold (7,13,14).

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