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Analysis of the effect of partial vitrification on stress development in cryopreserved blood vessels

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Abstract

Thermal stress development in blood vessels, during processes associated with vitrification (vitreous means glassy in Latin), is studied. This paper addresses the limiting case where the specimen completely crystallizes, while the cryoprotectant medium (CPA) completely vitrifies. This case is expected to provide upper boundary estimates for stresses for the more common problem of a partially vitrified sample. The CPA is modeled as a linear viscoelastic medium, with viscosity increasing exponentially with decreasing temperature; given the assumption of complete crystallization, the blood vessel is modeled as linear elastic below the freezing temperature. Consistent with previous observations, the CPA is found to behave linear elastically below a set-temperature, at which point the viscosity rises sufficiently quickly with further cooling. This observation reduces computational efforts and allows for parametric studies based on suitably chosen wholly elastic models. Both 2D concentric cylinder models of the blood vessel in a straight configuration and a 3D model of the vessel curled in a vial of CPA are studied; 2D models are shown to bound the results of the more general 3D problem. It is found that stress in the CPA decreases with increase in CPA volume, at least under conditions where the temperature can be viewed as uniform. Planar cracks are predicted to form transverse to the vessel axis, and to propagate right up to the blood vessel wall. Should such cracks propagate into the vessel, even over only a few μm, the mechanical damage to the lumen, or to endothelial cells, may cause the blood vessel to completely loose its functionality at the end of the cryopreservation protocol.

Keywords: Vitrification; Solidification; Solid mechanics; Thermal stress; Blood vessels; Set-temperature

1. Introduction

In the present era of arterial replacement, at least 345,000–485,000 autologous coronary grafts [1,2] (either arteries or veins) and over 200,000 autogenous vein grafts into peripheral arteries are performed each year [3]. A recent marketing report indicated that at least 300,000 coronary artery bypass procedures are performed annually in the USA, involving in excess of 1 million vascular grafts [4]. Many of these patients do not have autologous veins suitable for grafts due to pre-existing vascular disease, vein stripping, or use in prior vascular procedures. It has been estimated that as many as 30% of the patients who require arterial bypass procedures will have saphenous veins unsuitable for use in vascular reconstruction [5].

In recent years, an increasing and sometimes urgent need has emerged for alternative blood vessel substitutes, when autologous blood vessels are not available and in circumstances where synthetic graft materials are usually unsatisfactory. Tissue engineered blood vessels (TEBV) offer potential alternative vascular grafts for autologous transplantation. However, the long culture period required for vessel construction represents a potential limitation for use of this technology (typically, 8–10 weeks). If engineered vessels could be constructed ahead of their anticipated clinical need and stored for later use, the clinical utility of this new technology might be greatly enhanced. TEBV may provide an alternative approach for the estimated 30% of patients requiring arterial bypass procedures for whom no suitable autologous graft materials

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are available. Tissue engineering technology is believed to have the potential to produce the "next generation" of biological grafts for use in both vascular and cardiac surgery.

Since the advent of transplantation science, cryopreservation has been recognized as a highly desirable method for facilitating availability of the highest quality material. This goal has been achieved in some cellular systems, but extrapolation to organized tissues and organs is fraught with additional problems that have only recently been addressed. Application of the principles of cryobiology to the growing needs of the emerging tissue engineering field – with TEBV as an example – has emphasized the need for greater understanding of the fundamentals of low temperature preservation as they apply to three-dimensional multi-cellular tissues. An understanding of the thermo-mechanical stresses inherent to cryopreservation of complex biological systems is crucial for the development of improved methods of storage. This important aspect of cryobiology has thus far received little attention, whereas the basic tools necessary for the study of these fundamental problems have not been available. The current study represents another step in an ongoing effort to analyze and quantify thermal stress effects in cryopreservation, with application to blood vessel storage.

Classical cryopreservation using low concentrations of cryoprotectants (CPAs) has been shown to conserve many important properties of vascular allografts. However, the techniques developed for freezing vascular allografts are not reliable. Fractures have been observed in cryopreserved arteries [6,7]. Nataf et al. [8] found that the contractile responses of both cryopreserved sheep carotid arteries and human internal mammary arteries were abolished. Studies on the endothelial and smooth muscle function in canine internal mammary arteries, using an eight-step protocol, demonstrated that smooth muscle functions were poorly preserved after cooling below $-12\,^{\circ}C$ [9]. Similar observations have been made in the recent studies of human arteries. Cryopreserved human internal mammary arteries and femoral arteries had both poorly preserved smooth muscle functions and endothelial functions [10,11]. Freeze substitution of cryopreserved blood vessels demonstrates high levels of extracellular ice formation [12,13]. There have been several hypotheses on mechanisms of freezing-induced injury based upon a variety of factors [14,15], but it has been suggested that the disadvantages of traditional cryopreservation revolve primarily around ice formation [16,17].

Vitrification (glass formation) is an alternative to conventional freezing of biological materials with ubiquitous applications in cell, tissue, and organ storage. Here, the effect of increased viscosity of the cryoprotectant with decreasing temperature is utilized with the application of high cooling rates. If the typical time scale to form crystals exceeds the typical time scale of cooling to cryogenic temperatures, the cryoprotectant solution may reach an "arrested liquid state" known as glass. No appreciable degradation occurs over time in living matter trapped within a vitreous matrix, and vitrification is potentially applicable to all biological systems. However, vitrification is associated with higher concentration of cryoprotectants, which are potentially very toxic, and cryoprotective cocktails are currently studied by various research groups to overcome this obstacle. Other difficulties with vitrification are known to be associated with uniform loading of the tissue with the cryoprotectant solution; these difficulties are more relevant to bulky specimens, and are related to scale up studies of vitrification.

2. Modeling of stress and relevance of current study

The current study is part of a long-term effort to develop engineering tools for predicting the propensity for cracking to occur in contemplated cryopreservation protocols. Predictions of cracking rely on calculations of mechanical stress, which is dependent upon a host of factors, including the cooling and warming protocols, the geometry of the specimen, the geometry of the surrounding CPA, and on the physical properties of the specimen and CPA. The properties of the specimen are further coupled with the properties of the pure CPA through the amount of CPA that permeates into the tissue. The concentration of the permeated CPA plays a key role in determining the tendency of the specimen-CPA system to vitrify, crystallize, or a combination thereof.

In contrast to the approach typical of biology studies, the common engineering approach attempts to identify, and possibly isolate, the key mechanisms dominating the phenomenon. Once identified, each mechanism is investigated independently, often with the goal of developing a mathematical model to predict its effect. As appropriate, models for multiple mechanisms may be combined into a more comprehensive model. Stresses during cryopreservation can be attributed to two distinct mechanisms associated with thermal expansion: temperature gradients, brought about by high cooling and/or warming rates, in a domain with homogeneous thermal expansion coefficient, and differences in thermal expansion coefficient between tissues and pure CPA at uniform temperature. The current paper addresses the second effect, which produces stresses regardless of the cooling rate; thus, the effect of cooling rate does not emerge from this study. As shown in previous work with CPA vitrifying on substrates and in vials [18–20], the change in viscosity plays a significant role. Stresses due to thermal expansion difference between CPA and the substrate or vial only begin to rise at temperatures approaching glass transition, when the viscosity is very high. These stresses can rise because the plate or vial themselves are solid and stiff throughout the cooling protocol.

By analogy with the case of CPA and a substrate, stresses can arise due to difference in thermal expansion between CPA and a blood vessel, particularly when the blood vessel crystallizes; such stresses are addressed in the current paper. In particular, the current study explores the special case in which the blood vessel fully crystallizes, while the CPA vitrifies. Cryomacroscopy studies of blood vessels cooled in a vial of