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Research Paper

The impact of long term freezing on the mechanical properties of porcine aortic tissue

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ABSTRACT

Background: Preservation of the native artery's functionality can be important in both clinical and experimental applications. Although, simple cryopreservation techniques offer an attractive solution to this problem, the extent to which freezing affects the tissue's properties is widely debated. Earlier assessments of the mechanical properties post-freezing have been limited by one or more of the following: small sample numbers, uncontrolled inter-specimen/animal variability, failure to account for the impact of potential errors in thickness measurements, short storage times and uniaxial test methods.

Material and methods: Biaxial mechanical tests were performed on porcine aortic samples ($n=89$) extracted from superior, middle and inferior regions of five aortas, stored in isotonic saline at -20°C for 1 day, 1 week, 1, 6 and 12 months, thawed and retested. The sample's weight and thickness were also measured pre and post-freezing. A total of 178 tests were performed and elastic modulus was assessed by calculating the slope of the Cauchy stress–stretch curve at the low and high stretch regions in both the circumferential (θ) and longitudinal (L) directions.

Results: The weight of the samples increased post-freezing. However, in general, no significant difference was found between the elastic modulus of porcine aortic tissue before and after freezing at -20°C and was unaffected by storage time. Although more accurate measuring instruments are warranted to confirm this finding, minor changes to the elastic modulus as a result of freezing were negatively correlated with regional variances i.e. changes in the elastic modulus decreased from the superior to the inferior region.

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Conclusions: These results indicate that for applications which require preservation of the gross mechanical properties, storing the tissue at −20 °C in isotonic saline, for an extended period of time, is acceptable.

1. Introduction

Evaluating the mechanical properties of biological tissue is becoming increasingly important in improving our standard of healthcare. In particular, accurate determination of the native artery's mechanical properties plays a key role in assessing its usefulness as an allograft or autograft, the engineering of replacement arteries (Hoenig et al., 2005; Dahl et al., 2007), perfecting surgical techniques (Kühnapfel et al., 2000) and ensuring accurate diagnosis of tissue pathologies (Doyle et al., 2012; Mulvihill et al., 2013). Unfortunately, studies reporting the mechanical data of human tissue are sparse owing partly to the challenges involved. To avoid any adverse effects of tissue degradation, the optimum time to perform mechanical testing is immediately after surgical excision. However, in a surgical environment where opportunities to harvest such tissues are often unpredictable, coupled with logistical constraints, this can prove challenging. In reality, it is often necessary for the tissue to be stored for extended periods of time prior to the commencement of tests (Okamoto et al., 2002; Hawkins et al., 2003; van Andel et al., 2003; Lally et al., 2004; Gasser et al., 2008).

Simple cryopreservation techniques, including freezing the tissue at a temperature of −20°C, are the most widely accessible methods of preservation in both a clinical and non-clinical setting. However, it has been shown that freezing, regardless of temperature, induces the formation of ice crystals in the extracellular matrix (ECM) which can lead to intracellular ice formation and cellular dehydration (Karlsson and Toner, 1996). Studies have also recorded reductions in the dimensions and weight of tissues post-freezing which suggests there may be a '*bulk water movement phenomena*' occurring during the freezing process (Venkatasubramanian et al., 2006; Hemmasizadeh et al., 2012). These changes may affect the key elements responsible for maintaining the mechanics of the tissue for example, the loss of smooth muscle cell (SMC) viability (Venkatasubramanian et al., 2006) and alterations to the collagen and elastin fibres (Venkatasubramanian et al., 2006; Chow and Zhang, 2011). Although, these post-freezing changes have been reported at a microstructural level, there is little agreement regarding the magnitude of change to the gross mechanical properties responsible for maintaining functionality. Some authors have conducted studies detailing its effects on the mechanical properties of animal aorta however; collectively the results have been inconclusive.

A study by Stemper et al. (2007) analysed the mechanical properties of porcine aortic tissues ($n\sim26$) after freezing at −20 °C for three months and reported no significant alteration to the properties when compared to the control group. This study was limited to uniaxial test methods which cannot fully characterise the anisotropic nature of arterial tissue (Sacks 2000). Despite this, a similar result was reported by Virues Delgadillo et al. (2010) who employed biaxial test methods to assess the effect of this preservation method on the mechanical properties of porcine aorta ($n=3$) after two months. In contrast to these findings, though using similar test procedures, Chow and Zhang (2011) observed a significant increase in the high stress linear elastic modulus of bovine aortic tissue ($n\sim5$) as a result of freezing at −20 °C for three weeks. Other studies investigating the effects of cold storage at various temperatures and cooling rates have also reported conflicting results (Venkatasubramanian et al., 2006; Adham et al., 1996; Wang et al., 2006; Venkatasubramanian et al., 2010; Hemmasizadeh et al., 2012).

Determining the effect of freezing on the gross mechanical properties has both experimental and clinical applications. However, despite numerous studies there is still little agreement on what this effect is. Research to date have been limited by one or more of the following: small sample numbers, uncontrolled inter-specimen/animal variability, failure to account for potential errors in thickness measurements, short storage times and uniaxial test methods and therefore it is difficult to compare results or determine the true effect of freezing on the tissue's mechanical properties. The aim of this study is to employ biaxial test methods to identify and quantify the effect of a common simplified cryopreservation technique on the characteristics of porcine aorta when the effect of tissue variability is controlled. In particular, we aim to investigate if freezing in isotonic saline at −20 °C effects (a) thickness and bulk weight and (b) mechanical properties. Furthermore, we aim to investigate if changes to the mechanical properties are dependent on (a) storage time and (b) aortic region.

2. Materials and methods

2.1. Tissue specimens

The descending thoracic aorta was excised from five pigs at the local abattoir. The pigs were approximately six months old and weighed between 40 and 60 kg. All descending thoracic aortas were removed within 20 min post-mortem, placed in isotonic saline and transported to the laboratory immediately. No pigs were sacrificed for the sole purpose of this project.

2.2. Tissue preparation

To prepare the aortas for testing, the loose connective tissue was carefully removed and the aortas were rinsed several times in isotonic saline. The tubular aortas were cut