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Biomechanical properties and microstructure of neonatal porcine ventricles

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ABSTRACT

Neonatal heart disorders represent a major clinical challenge, with congenital heart disease alone affecting 36,000 new-borns annually within the European Union. Surgical intervention to restore normal function includes the implantation of synthetic and biological materials; however, a lack of experimental data describing the mechanical behaviour of neonatal cardiac tissue is likely to contribute to the relatively poor short- and long-term outcome of these implants. This study focused on characterising the mechanical behaviour of neonatal cardiac tissue using a porcine model, to enhance the understanding of how this differs to the equivalent mature tissue. The biomechanical properties of neonatal porcine cardiac tissue were characterised by uniaxial tensile, biaxial tensile, and simple shear loading modes, using samples collected from the anterior and posterior walls of the right and left ventricles. Histological images were prepared using Masson's trichrome staining, to enable assessment of the microstructure and correlation with tissue behaviour. The mechanical tests demonstrated that the neonatal cardiac tissue is non–linear, anisotropic, viscoelastic and heterogeneous. Our data provide a baseline describing the biomechanical behaviour of immature porcine cardiac tissue. Comparison with published data also indicated that the neonatal porcine cardiac tissue exhibits one-half the stiffness of mature porcine tissue in uniaxial extension testing, one-third in biaxial extension testing, and one-fourth stiffness in simple shear testing; hence, it provides an indication as to the relative change in characteristics associated with tissue maturation. These data may prove valuable to researchers investigating neonatal cardiac mechanics.

1. Introduction

Congenital heart disease (CHD) annually affects approximately 36,000 new-borns within the European Union (Dolk et al., 2010, 2011), and describes a series of structural cardiac disorders, including ventricular and atrial septal defects. Multi-physics modelling and the development of new synthetic materials are innovative approaches seeking to positively influence the clinical outcomes; however, an acute lack of data describing the biomechanical behaviour of neonatal cardiac tissue, twinned with the structural changes to the tissue during maturating, is potentially limiting the effectiveness of these novel techniques.

The adult cardiac tissue is known to exhibit highly complex behaviour, including non-linearity and anisotropy (Demer and Yin, 1983; Humphrey et al., 1990; Novak et al., 1994; Sacks and Chuong, 1993; Yin et al., 1987; Hill et al., 2014; Dokos et al., 2002; Sommer et al., 2015), as a consequence of its intricate structure (Carapella et al., 2014; Helm et al., 2005; Karlon et al., 2000; Palit et al., 2015; Streeter et al., 1969). Critically, ventricular wall functionality differs between the adult and neonate, as the latter can only increase the cardiac output by increasing the heart rate (although only limited), whereas the adult heart can also increase stroke volume (Cote, 1993; Cox, 2011). The neonate heart also has a greater fraction of fibrous tissue to contractile tissue, than an adult (Cote, 1993; Cox, 2011), whilst there is variation in the collagen fibril density, and mono-nucleated and bi-nucleated cell concentrations (Anversa and Capasso, 1991; Gazoti Debessa et al.,

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2001; Nguyen et al., 2001). Increasing age is also associated with changes in the cardiac matrix, and an increase in collagen fibril cross-linking and assembly (Lindsey et al., 2005). No experimental data exists to quantify neonate tissue, however, meaning simulations adopt and/or scale adult data, incorporating an unknown level of error (Lindsey et al., 2005; Giannico et al., 2006; Petrossian et al., 1999; Shinoka and Breuer, 2008).

Computational modelling is increasingly used in adult cardiology to understand the behaviour of structural components, enabling the simulation of normal and pathophysiological conditions and leading to new interventions (Dokos et al., 2000; Eriksson et al., 2013a, 2013b; Nash and Panfilov, 2004; Niederer et al., 2011; Usyk et al., 2001; Wall et al., 2006). A lack of appropriate data to describe neonatal tissue limits the widespread use and effectiveness of sophisticated techniques, to investigate neonatal-based disorders. Surgical intervention, aiming to restore normal function, includes the implantation of synthetic materials to mimic natural tissue behaviour (Petrossian et al., 2006; Twine and McLain, 2010; Wang et al., 2007); however, the relatively poor short- and long-term outcomes may be partly associated with the scant literature describing neonatal cardiac biomechanics. This lack of knowledge may also be contributing to the relatively limited success of biological scaffolds (Wang et al., 2010; Kurobe et al., 2012); hence, enhanced knowledge of tissue behaviour to achieve more effective designs has the potential to positively influence CHD mortality and morbidity (Kurobe et al., 2012).

We thus aim to systematically quantify the biomechanical properties of neonatal right and left ventricles, using an accepted neonatal porcine animal model (Bassols et al., 2014; Vodicka et al., 2005; Aigner et al., 2010; Almond, 1996; Book and Bustad, 1974; Cooper et al., 1991; Douglas, 1972; Luo et al., 2012). These biomechanical data are consolidated with histological imaging, to provide a comprehensive analysis of tissue from the right and left ventricle free walls (RVFW, LVFW). This study will provide baseline data describing the behaviour of neonatal porcine tissue and, through comparison to equivalent mature data, will also be able to provide an insight into the effect of maturation on tissue behaviour. Such data may then prove useful to those researchers investigating immature cardiac tissue mechanics and the clinicians/bioengineers exploring new intervention techniques in congenital heart diseases.

2. Materials and methods

2.1. Materials

Forty-three, one-day-old neonatal porcine hearts (Yorkshire) were acquired from a local abattoir house in Mississippi, from donor piglets mass: 2.0 – 2.2 kg, length: 0.35 – 0.48 m. The deceased piglets all appeared fully developed, meaning that they were most likely to have died from hypoxia either during or immediately after, farrowing (i.e. birth). All donor's hearts were presumed to be healthy, pending subsequent inspection. The piglets were collected within hours of their death and transported to the Tissue Bioengineering Laboratory at the Mississippi State University, stored in ice-cooled boxes at 4°C. The hearts were then promptly dissected out and visually examined for any macroscopic damage or disruption, with any that failed this assessment being excluded from further investigation. The anterior and posterior aspects of the LVFW and RVFW were then identified (Fig. 1), before defining the FSN-coordinate system as the fibre axis (F), defined as the mean-fibre direction as observed by the external surface texture; the sheet axis (S), defined as the direction transverse to the fibre axis within the layer; the sheet-normal axis (N), defined as the direction perpendicular to both the fibres and layers (Dokos et al., 2002; Sommer et al., 2015; Holzapfel and Ogden, 2009). In this study, the fibre axis (F) is described as the 'mean-fibre direction' (MFD), and sheet-normal axis (N) as the 'cross fibre direction' (CFD) (Sommer et al., 2015). Such a method is inherently subjective, though was performed in a manner consistent with previous studies (Dokos et al., 2002; Sommer et al., 2015).

Uniaxial extension testing samples were dissected from twenty hearts. Ten randomly selected hearts had samples of dimensions 20 mm (l) x 10 mm (w) x 3 mm (t) dissected, with the longest dimension aligned to the MFD. The remaining 10 hearts were used to harvest samples in the CFD (Fig. 2(a), (b) & (c)). All 20 samples were then trimmed using a cutting punch, achieving a traditional dog-bone shape and a 5 mm minimum width. For biaxial extension analysis, five samples ($15 \times 15 \times 3$ mm) were dissected using a square-shaped cutting punch from the LVFW, and a further five hearts used for RVFW samples (Fig. 2(d) & (e)). Each sample was dissected such that the presumed MFD and CFD were consistent with the x- and y-axes of the cutter. A similar approach, though smaller cutter (3×3 mm), was adopted to dissect tissue for shear analysis. These were collected from the anterior and posterior aspects of the LVFW and RVFW, dissected from the equatorial regions of 5 hearts (Fig. 2(f) & (g)). The final eight hearts were used for histological analysis (Fig. 1). Thirty-two cubic samples ($5 \times 5 \times 5$ mm) were dissected using a square-shaped cutter from the equatorial regions of the anterior and posterior aspects of LVFW and RVFW (Fig. 1). In this instance, a coordinate system was established that ensured consistent orientation of the cutter, as this analysis served to quantify the relative fibre alignment. Hence, the cutter was always aligned with the vertical axis of the heart, defined as passing through the apex and base.

2.2. Methods

The directional anisotropy of the LVFW and RVFW tissue was investigated at different deformation states, via four independent protocols. Biomechanical testing was completed within 12 h of birth, to produce data from fresh tissue.

2.2.1. Microstructural analysis

All 32 samples were fixed in 4% paraformaldehyde for 48 h and then in 70% glutaraldehyde. Samples were then processed through a standard histological preparation protocol, including being dehydrated in graded alcohol, cleared with xylene and then embedded within paraffin wax. Each block was cut perpendicular to the transmural direction, with a 2 mm deep section selected for analysis (Fig. 1). A standard Masson's trichrome staining protocol was performed, with the sections finally mounted with Permount. The Masson's trichrome stain identified muscle fibres in red, and collagen fibres light blue.

2.2.2. Uniaxial extension test

Both ends of the dog-bone-shaped samples were wrapped in emery paper and clamped into the stainless steel grips of a uniaxial testing machine (Mach-1; Biosyntech, MN). This produced samples with a dimension of approximately 15 mm (l) x 5 mm (w) x 3 mm (t), with five measurements taken with digital callipers. Each sample was preconditioned with 10 cycles at 10% strain, before being loaded to failure at 1.5 mms^{-1} ramp speed. Engineering stress was computed by normalising the applied force to the initial cross-sectional area, and engineering strain calculated by normalising the displacement to the initial gauge length. Mean peak stress, which describes the stress of failure, was then calculated from these data for the MFD and CFD samples. The last preconditioning cycle was used to quantify the myocardial hysteresis (to account for energy dissipation due to the viscoelastic behaviour), dividing the area enclosed by the loading and unloading curves (energy dissipation) by the area beneath the loading curve (energy input).

2.2.3. Biaxial extension test

Biaxial mechanical properties were investigated using a biaxial testing system, described in detail elsewhere (Grashow et al., 2006a, 2006b). The square samples, which had been dissected with the observed MFD and CFD aligned with the x- and y-axes were then mounted