JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 57 (2016) 321–333

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jmbbm


Research Paper

# The effects of decellularization and cross-linking techniques on the fatigue life and calcification of mitral valve chordae tendineae

*Gillian M. Gunning*[a,b,1], *Bruce P. Murphy*[a,b,c,*]

[a]*Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, 152–160 Pearse Street, Dublin 2, Ireland*

[b]*Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Dublin 2, Ireland*

[c]*Advanced Materials and Bioengineering Research Centre (AMBER), Trinity College Dublin, Dublin 2, Ireland*

ARTICLE INFO

*Article history:*
Received 3 November 2015
Received in revised form
5 January 2016
Accepted 14 January 2016
Available online 22 January 2016

*Keywords:*
Mitral
Chordae tendineae
Fatigue
Calcification
Decellularization
Cross-linking

ABSTRACT

In cases of severely diseased mitral valves (MV), the required treatment is often valve replacement. Bioprosthetic and stentless replacement valves are usually either fully or partially composed of animal derived tissue treated with a decellularization process, a cross-linking process, or both. In this study, we analysed the effects of these treatments on the fatigue properties of porcine MV chordae tendineae (CT), as well as on the calcification of the CT using an *in vitro* technique. CT were tested in 4 groups; (1) native, (2) decellularized (DC), (3) decellularized and cross-linked with glutaraldehyde (DC-GTH), and (4) decellularized and cross-linked with 1-ehtyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)(DC-EDC). CT were tested in both uniaxial tension, and in fatigue at 10 MPa peak stress (1 Hz). The cycles to failure (mean ± SD) for the four groups are as follows; Native- 53,397 ± 55,798, DC- 28,013 ± 30,634, DC-GTH- 97,665 ± 133,556, DC-EDC- 318,601 ± 322,358. DC-EDC CT were found to have a slightly longer fatigue life than the native and DC groups. The DC-EDC group also had a marginally lower dynamic creep rate, meaning those CT elongate more slowly. After *in vitro* calcification, X-ray microtomography was used to determine relative levels of calcification. The DC-EDC and DC-GTH groups had the lowest volume of calcific deposits. Under uniaxial testing, the ultimate tensile strength (UTS) of the DC-GTH CT was statistically significantly reduced after calcification, while the UTS was relatively unchanged for the DC-EDC group. Overall, these results indicate that a treatment of decellularization plus cross-linking with EDC may improve the fatigue life of porcine CT, reduce the rate of elongation, and help the CT resist the negative effects of calcification. This may be a preferable treatment in the preparation of porcine MVs for the replacement of diseased MVs.



*Corresponding author at: Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, 152–160 Pearse Street, Dublin 2, Ireland. Tel.: +353 1 896 8503; fax: +353 1 679 5554.
E-mail addresses: ggunning@tcd.ie (G.M. Gunning), bruce.murphy@tcd.ie (B.P. Murphy).
[1]Tel.: +353 1 896 3678; fax: +353 1 679 5554.

http://dx.doi.org/10.1016/j.jmbbm.2016.01.009
1751-6161/

## 1. Introduction

The mitral valve (MV) is a complex and finely tuned system, composed of four individual parts working in unison to achieve good valve function. These components are the leaflets, annulus, papillary muscles (PMs) and chordae tendineae (CT). The CT act as tethering components, preventing the leaflets from inverting or flailing during systole. When one or more CT ruptures, or elongates, the leaflets can flail and the performance of the valve is compromised, resulting in mitral regurgitation (MR). The mitral valve is also highly prone to calcification, in which calcium deposits often form on the annulus, and may also form on the leaflets and CT (Pachon and Zamorano, 2008; David et al., 1998). This may lead to thickened, shortened chordae, as well as chordal fusion, which in turn can lead to valve insufficiency (David et al., 1998).

When a mitral valve becomes severely diseased, the valve must be either repaired or replaced. Options for valvular replacement include homografts, mechanical valves, and bioprosthetic valves. Bioprosthetic valves are generally constructed from animal tissue (often porcine valves or bovine pericardium) either mounted in a stent, or stentless. While mechanical valves have excellent durability, they pose thrombotic complications which require anticoagulation medication (Grunkemeier et al., 2000; Chiang et al., 2014; Hoffmann et al., 2008; Tillquist and Maddox, 2011). The thrombotic risk is reduced with homografts and bioprosthetic valves, and furthermore homografts and stentless bioprosthetic valves have improved haemodynamics (Grunkemeier et al., 2000; Chiang et al., 2014; Hoffmann et al., 2008; Tillquist and Maddox, 2011). However, tissue valves and patches are prone to mineralization, or calcification, which may eventually lead to their failure (Fukunaga et al., 2015; Chauvaud et al., 2003; Olivito et al., 2012; Schoen and Levy, 2005). This is more particularly the case in children, in whom calcification and degeneration of prostheses is especially acute (Saleeb et al., 2014; Manji et al., 2012; Siddiqui et al., 2009). Potentially in the future the use of non-calcifying decellularized crosslinked xenogeneic grafts/material may have a role to play in surgical or catheter based replacement of diseased mitral valves.

A common treatment of animal tissue being prepared for implantation is cross-linking with glutaraldehyde. In the past, glutaraldehyde fixation has been used to decrease immunogenicity and mask antigens, while also improving durability (Manji et al., 2006). While cross-linking with glutaraldehyde has been the most common preparation technique for many years, it is often reported that it is associated with calcification of the valve and does not allow for tissue remodelling (Everaerts et al., 2004, 2006; Tedder et al., 2009; Golomb et al., 1987).

The principal mechanisms of calcification are both varied and disputed. High levels of calcification may be associated with young patients, treatment of the valve with glutaraldehyde, and high mechanical stress (Schoen and Levy, 2005). According to Schoen and Levy (2005), the most predominant nucleation sites for calcific deposits are cells. Collagen and elastin may also serve as nucleation sites for calcification, but the effects are less pronounced. As a result, decellularization of tissue has been investigated as a method of reducing both calcification and immune response. Furthermore, glutaraldehyde may not be a satisfactory method of cross-linking, as it has been reported to induce calcification *in vivo* (Everaerts et al., 2004, 2006; Tedder et al., 2009; Golomb et al., 1987). Tamura et al. (1995) implanted glutaraldehyde treated mitral valve grafts in sheep in order to determine the performance of the valves, and their failure modes. The valves became highly calcified, and failed through fracture of calcified chordae tendineae.

Several groups have begun more recently to investigate other cross-linking techniques (carbodiimide based methods, such as *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC)) as an alternative to glutaraldehyde fixation. Contrary to the evidence suggesting that glutaraldehyde causes calcification, Everaerts et al. (2004) demonstrated that cross-linking reduces the calcification of heart valve leaflet and mural tissue compared to fresh valves. It was also demonstrated that EDC based methods of cross-linking reduce calcification compared to the standard glutaraldehyde techniques.

The mechanical properties of calcified human CT have been investigated in the past by Casado et al. (2012). In this study calcified human CT were subjected to uniaxial tensile tests, and were observed under scanning electron microscope. It was found that the calcified CT had reduced stiffness and reduced resistance to fracture (lower ultimate tensile stress). However the effect of decellularization and cross-linking on the mechanical properties of the CT is unknown. Furthermore, the effect of these procedures on the fatigue properties has not previously been investigated. Similarly, the fatigue behaviour of calcified CT has yet to be investigated. Previously, we have studied the fatigue properties of the CT, hypothesising that fatigue could be a cause of spontaneous rupture (Gunning and Murphy, 2015). In this study, we aim to investigate the effects of decellularization and cross-linking techniques on the fatigue properties of the CT and their propensity to calcify.

## 2. Methods and materials

### 2.1. *Isolation of chordae tendineae*

Marginal CT were dissected from fresh porcine hearts and frozen in PBS for preservation until testing (the technique has been described in detail by us previously (Gunning and Murphy, 2015)). Briefly, the marginal chordae were dissected in such a way that a portion of leaflet and a portion of PM remained attached, in order to enable gripping.

CT were divided into three treatment groups: 1) Decellularized (DC), 2) Decellularized and cross-linked with glutaraldehyde (DC-GTH), 3) Decellularized and cross-linked with 1-ehtyl-3-(3-dimethylaminopropyl) carbodiimide (DC-EDC). In each of these groups, 8 CT were prepared for tensile testing, and 6 were prepared for fatigue testing. Native (control) CT have been tested in tensile and fatigue modes previously (Gunning and Murphy, 2015).

Following this a further set of CT were prepared for calcification treatments. CT were divided into four treatment groups: 1) Native, 2) DC, 3) DC-GTH, 4) DC-EDC. Each of these groups was immersed in a calcifying bath at 37 °C for 42 days. The resultant calcified CT are denoted by the post-script "-calc"; Native-calc, DC-calc, DC-GTH-calc, DC-EDC-calc.