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The effect of a thermal renal denervation cycle on the mechanical properties of the arterial wall

Alan A. Hopkins [a,b,1], William S. Sheridan [a,b,1], Faisal Sharif [a,c], Bruce P. Murphy [a,b,d,*,2]

[a] Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, 152-160 Pearse Street, Trinity College Dublin, Dublin 2, Ireland
[b] Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Dublin 2, Ireland
[c] Regenerative Medicine Institute, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland
[d] Advanced Materials and Bioengineering Research (AMBER) Centre, Trinity College Dublin, Dublin, Ireland

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ABSTRACT

The aim of this study was to determine the effect that a thermal renal denervation cycle has on the mechanical properties of the arterial wall. Porcine arterial tissue specimens were tested in three groups: native tissue, decellularized tissue, decellularized with collagen digestion (e.g. elastin only). One arterial specimen was used as an unheated control specimen while another paired specimen was subjected to a thermal cycle of 70 °C for 120 s ($n=10$). The specimens were subjected to tensile loading and a shrinkage analysis. We observed two key results: The mechanical properties associated with the elastin extracellular matrix (ECM) were not affected by the thermal cycle. The effect of the thermal cycle on the collagen (ECM) was significant, in both the native and decellularized groups the thermal cycle caused a statistically significant decrease in stiffness, and failure strength, moreover the native tissue demonstrated a 27% reduction in lumen area post exposure to the thermal cycle. We have demonstrated that a renal denervation thermal cycle can significantly affect the mechanical properties of an arterial wall, and these changes in stiffness and failure strength were associated with alterations to the collagen rather than the elastin extracellular matrix component.

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1. Introduction

Catheter based renal denervation (RDN) has recently been developed, and successfully adopted, as a procedure to control high blood pressure in patients that are resistant to lifestyle changes and antihypertensive medical therapy. The procedure has demonstrated efficacy and good mid-term safety data in a resistant hypertensive patient cohort. Whereby initial clinical trials demonstrate that office based systolic blood pressure can be substantially reduced and adverse procedure-related events minimised (Krum et al., 2009; Investigators, 2011; Esler et al., 2012). The reduction in office based blood pressure has been demonstrated to be durable, with patients sustaining a reduction in blood pressure out to three years (Krum et al., 2013). The devices employed in this procedure use thermal energy as the method to obliterate the sympathetic renal nerves. In some instances a single radio frequency (RF) electrode is utilised at multiple sites (Krum et al., 2009), or multiple electrodes are used simultaneously (Worthley et al., 2013), or one larger spiral electrode can be employed (Ormiston et al., 2013), or catheter-based high-frequency ultrasound transducers can be used (Mabin et al., 2012). One feature common to all of the devices/protocols is the application of a thermal cycle to the area surrounding the renal artery, commonly involving temperatures of approximately 70 °C for a time period of 1–2 min.

As a consequence of the thermal cycle the arterial wall is exposed to an elevated temperature; however, limited pre-clinical documentation of alteration to the arterial wall's structure post thermal ablation is available in the literature. In a study by Rippy et al. (2011) porcine histology results at six months post ablation are depicted, the histology images detail areas of medial fibrosis and medial proteoglycan deposition, demonstrating that some form of thermal injury or remodelling persists within a healthy vessel six months post thermal ablation. Clinical findings demonstrate that significant vasospasm is present post catheter based renal denervation (Templin et al., 2013), in a study by Templin and co-workers Optical Coherence Tomography (OCT) was utilised to identify: vasospasm induced by the EnligHTN™ catheter (St. Jude Medical),

* Corresponding author at: Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, 152-160 Pearse Street, Trinity College Dublin, Dublin 2, Ireland
E-mail addresses: ahopkin@tcd.ie (A.A. Hopkins), sheridaw@tcd.ie (W.S. Sheridan), Faisal.Sharif@nuigalway.ie (F. Sharif), bruce.murphy@tcd.ie (B.P. Murphy).
[1] Tel.: +3531 896 3678; fax: +3531 679 5554.
[2] Tel.: +353 1 896 8503.

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and vessel wall oedemas that were caused by the Simplicity catheter (Medtronic). Additionally, there have been cases of stenosis occurring post RDN, in one patient two stenotic sites were observed in a renal artery five months post the denervation procedure (Kaltenbach et al., 2012), separately a second patient presented with a hemodynamically relevant stenosis near the ostium six months post catheter based renal denervation (Vonend et al., 2012). Potentially these findings suggest that the wall of the artery may be detrimentally modified post thermal catheter based renal denervation.

The effect of elevated temperatures on the arterial wall's extracellular matrix may play a role in altering the functionality of a healthy vessel. The medial layer of the arterial wall is exposed to elevated temperatures, this in turn can affect the major structural proteins (elastin and collagen) or/and the smooth muscle cells. Previously it has been shown that a significant level of medial necrosis has been observed when radio-frequency energy was applied to heat a porcine arterial wall to 70 °C for 60 s (Fram et al., 1993). The depth of heat necrosis in this study was measured as 1031 µm; demonstrating that substantial medial layer smooth muscle cell death may occur during renal denervation procedures that use temperatures in the range of 70 °C. Additionally, there is a fine balance associated with the smooth muscle cells and the extracellular matrix components of the vessel wall, whereby small collagen fibres and large collagen bundles as well as fenestrated elastin sheets all interact with the smooth muscle cells to form a functioning arterial wall (O'Connell et al., 2008). Any disruption to this extracellular matrix architecture may cause vessel wall dysfunction. The denaturation temperature of collagen has been reported in a number of studies as being in the range of 50–60 °C (Gross, 1964; Venkatasubramanian et al., 2010). These results would imply that some form of reduction in mechanical stability of the arterial wall would occur post a renal denervation thermal cycle, as it contains significant amounts of collagen—the main structural element. Elastin denaturation on the other hand does not seem to be affected by the temperature range associated with thermal renal denervation procedures (Lillie et al., 1994). In these prior studies the overall effect on the arterial wall structural integrity has not been documented, and only isolated aspects of the mechanics or denaturation of the ECM components have been investigated with respect to increases in temperature.

We hypothesised that heating an arterial wall to a temperature level associated with thermal renal denervation procedures will affect the structural integrity of the arterial wall. To test this hypothesis we designed and conducted a series of experiments that heated and subsequently mechanically tested the arterial tissue post heating to determine the effect on the mechanical properties.

2. Methods

2.1. Tissue harvest

Porcine carotid arteries were freshly harvested from 70 to 90 kg pigs in a local abattoir (Lislin Meats Ltd, Mullagh, Co. Cavan, Ireland). Common carotid arteries in the 5–7 mm outer diameter range were excised. All arteries were returned to the laboratory and stored in phosphate buffered saline (PBS) on ice. Samples were additionally prepared by removing excess connective and adventitial tissue and cut into approximately 30 mm long segments. All specimens were then frozen in PBS at −20 °C for later use. Specimens were divided into three groups: native, decellularized, and decellularized with collagen digestion. This allowed us to examine the effects of the thermal cycle on the native tissue, the isolated extracellular matrix, and a scaffold that closely resembles an elastin-only scaffold.

2.2. Decellularization

To investigate the effect of the temperature rise on the isolated extracellular matrix components, we removed the cells from the tissue with a decellularization protocol. Decellularization was carried out as previously described (Sheridan et al., 2012). In brief, the protocol consists of an enzymatic digestion and detergent extraction. Constructs were immersed in de-ionized water for 24 h at 4 °C under rotational agitation and subsequently incubated in 0.05% Trypsin with 0.02% EDTA (Sigma-Aldrich, Ireland) for 1 h at 37 °C. After a short rinse in PBS to remove excess trypsin, the samples were placed in a solution of 2% Triton X-100 and 0.8% ammonium hydroxide (Sigma-Aldrich, Ireland) in de-ionized water for 72 h at 4 °C under rotational agitation. The solution was changed daily. A final wash sequence of 48 h in de-ionized water was undertaken to remove any residual chemicals from the constructs.

2.3. Collagen digested tissue

To investigate the effect of the temperature rise on arterial elastin we removed the collagen from the tissue in a secondary process that occurred after decellularization. In this process sodium hydroxide (NaOH) was used to digest the collagen. The additional step involved subjecting the samples to sonication in 1.5 M NaOH for 180 min prior to a final wash sequence of 48 h in de-ionized water under constant agitation, to remove excess chemicals; the de-ionized water was changed after 24 h.

2.4. Specimen preparation and thermal cycle

Specimens were prepared by firstly thawing the retrieved arteries and subsequently isolating a 4–6 mm diameter, and 30 mm length segment of an artery. This segment was then cut into two equal 4 mm length specimens, these specimens were cut from adjacent sections in the centre of the segment length and used as paired controls in the mechanical testing phase. A specially designed tool was used to ensure every specimen was always cut to 4 mm in length. Subsequently one of the paired specimens was subjected to a thermal cycle. The thermal cycle consisted of immersion in a temperature controlled water bath at 70 °C for 120 s. This thermal cycle mimics a thermal renal denervation cycle.

2.5. Mechanical testing

Uniaxial tensile tests were carried out to determine the tensile mechanical response of the native, decellularized and collagen digested tissues using a Zwick tensile testing machine (Zwick Z005, Roell, Germany). The following groups ($n=10$ each) were tested: (i) native arterial tissue, (ii) decellularized tissue, (iii) collagen digested tissue. Each of the three groups consisted of 10 unheated specimens and 10 specimens exposed to the thermal cycle. Each specimen was tensile tested to failure and the force was recorded using a 100 N load cell. Custom made grips, a PBS bath and video extensometry was used to determine the specimen's displacement, see Sheridan et al. for a more detailed description of the tensile test experimental set-up (Sheridan et al., 2012). True stress (σ) and true strain (ε) were utilised as the investigated parameters.

2.6. Shrinkage

Specimen shrinkage was recorded by capturing an image of the specimen's inner lumen dimensions prior to mechanical testing. All samples were mounted on the grips and subjected to a small force of 0.5 N and an image was subsequently acquired of the cross section of the specimen. The inner circumference was determined from the image, and consequently the area was calculated, this was conducted for all the unheated and heated paired specimens.

2.7. Histology and scanning electron microscopy (SEM)

The specimens that were investigated by histological analysis were embedded in paraffin wax in an automatic tissue processor (ASP300, Leica, Germany). All samples were sectioned longitudinally using a rotary microtome (Leica microtome, Leica, Germany). 7 µm sections were cut and collected on glass slides and subsequently washed through a graded series of ethanol from 100 to 70% (v/v). Samples of native, decellularized tissue, and decellularized with collagen digestion were stained using Picrosirius Red. To further verify cell removal, sections were stained with diamidino-2-phenylindole (DAPI). Sections were dehydrated and cleaned in ascending concentrations of ethanol and xylene before coverslips were mounted (DPX mountant, BDH). Specimens viewed in the SEM were fixed in 2.5% buffered glutaraldehyde for 24 h. These specimens maintained their cylindrical configuration. Each specimen was then subjected to sequential washes of 30%, 50%, 70%, 90% and 100% ethanol for 15 min with the final wash performed three times. The dried samples were mounted on an aluminium stub and sputter coated with gold. An SEM (Zeiss Ultra Plus, Germany) was used to view the ultrastructure of the medial layer of the control specimens and specimens subjected to a thermal cycle.

2.8. Statistical analysis

All comparisons were conducted utilising paired tests on the normally distributed date, whereby each control unheated specimen was associated with a

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