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Biomechanical and Structural Properties of Aortic Scaffolds Decellularized by Sonication Decellularization System

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Abstract

Extracellular Matrix (ECM) based bio scaffolds has increasingly emerged in tissue engineering application because it contains structural, biochemical, and biomechanical cues that have dramatic effects upon cell behaviors. Therefore, the aim of this study is to prepare aortic scaffolds based on ECM by sonication decellularization system with biomechanically and structurally preserve compared to native aortic tissues. Aortic tissues are sonicated at 170 kHz of ultrasound frequency in 0.1% and 2% SDS for 10 h. The effectiveness of sonication decellularization system to remove the cells is determined through Hematoxylin & Eosin staining and DNA quantification. Indentation test is used to evaluate the compression, stiffness and residual force for evaluating the biomechanical properties of aortic scaffolds. Structural properties of aortic scaffolds are evaluated by histological analysis using Picrosirius Red staining and Verhoeff-Van Gieson staining and biochemical testing using SIRCOL and FASTIN assay to measure the collagen and elastin of aortic scaffolds respectively. The aortic scaffolds prepared by sonication decellularization system shows the preservation of structural collagen and elastin and able to maintain the biomechanical properties of aortic scaffolds. The results of this study confirm the potential of sonication decellularization system as an alternative method to prepare aortic scaffolds for tissue engineering application.

Keywords: Bioscaffolds; Aortic; Decellularization; Sonication; Vascular

Introduction

End-stage of cardiovascular diseases such as myocardial infarction and coronary artery diseases often lead to demands for bypass surgery. The limitation of the availability of the appropriate autologous vessels due to the concomitant disease or previous use causes the artificial graft as a standard clinical alternative to autologous are preferred and commonly used [1]. Synthetic grafts that are composed of polyethylene terephthalate (Dacron) and polytetrafluoroethylene (Teflon) are frequently used and commercially available. However, these materials have proven inadequate and unsuitable for generating grafts with increased of thrombosis and infection, low compliance and poor elasticity [2,3]. Moreover, these synthetic materials have a lower rate of cell proliferation and ECM secretion when implanted long term [4].

The function of a vascular graft in the normal physiological response to prevent the thrombosis and inflammation has guided attempts to construct the graft that closely mimic the vascular native. The recent study has shown the use of xenogeneic tissues to construct the Extracellular Matrix (ECM) based bio scaffolds with the preservation of the ECM. The use of xenogeneic tissues is advantageous because providing the possibility of off-the-shelf bio scaffolds. Various xenogeneic tissues have been isolated to construct the bio scaffolds for a variety of tissue engineering application including small intestine reconstruction, skin reconstruction, and orthopedic application [5]. The availability of suitable xenogeneic tissues for bio scaffolds would greatly benefit the patient who needs to perform bypass surgery.

Numerous ECM based bio scaffolds have been constructed through a varied approach to mimic entirely the native tissues. These features include the significantly removal of the cells and the structural properties of collagen and elastin that are the main component of extracellular matrix in blood vessels which provides the viscoelasticity that further could determine the biomechanical properties of blood vessels [6,7]. The challenge to develop the ECM based scaffolds lies on decellularization process that required completely cellular components and preservation of biomechanical properties. Various techniques have been used for decellularization that involves any combination of physical, chemical and enzymatic methods with various solvent, detergent, and enzyme to lyse the cells while creating the free volume spaces upon which native host cells are able to proliferate [8]. Sodium Dodecyl Sulphate (SDS) is the most commonly used in the decellularization process as their effectiveness to remove the cells by dissolving the cell

membranes. Nevertheless, SDS also disrupts the integrity structure of ECM leading to biomechanically weak tissues [9].

In order to facilitate the penetration of SDS into the tissues, we have developed the sonication decellularization system to increase the effectiveness of the decellularization process. Our sonication decellularization system combines the use of ultrasound and SDS detergents in order to promote a more efficient and homogenous cellular removal during decellularization. Previous work using agitation method on vascular tissue needs to use more than one reagent, high concentration and longer treatment time to prepare significant removal of cells from bioscaffolds with different thickness of tissues [10]. To remove cells significantly from the deep structure of tissues, the tissues should excessively expose to the single reagent which resulting the ECM structure to disrupt, altering the collagen and elastin fibril network [11]. Sonication decellularization system has shown the effectiveness to removes cells significantly [12-14]. Sonication tends to disrupt the cell membranes and degrade the DNA by the cavitation and non-cavitation mechanism that involved acoustic streaming while SDS acts over the protein-protein bonds [15-18]. Thus, it is important to improve the permeability of the SDS which can decrease the treatment time and potential to destruct the ECM's structure.

Considering the decellularization treatment introduce some changes in biomechanical and structural properties of bio scaffolds, it is crucial to studies the effect of sonication decellularization on biomechanical and functionality of the bio scaffolds. The main goal of this study was to prepare the aortic scaffolds by sonication decellularization system with a significantly remove the cellular components, preserved structure, and content of collagen and elastic fibres, and maintenance of biomechanical properties of bio scaffolds. Thus, the novelty of this study is residing on the preparation the bio scaffolds using sonication decellularization system which virtually holds all the main aorta properties and characteristics.

Materials and Methods

Aortic tissue preparation

Fresh aortas from 7 months old porcine were obtained from a local slaughterhouse and the excess blood and fat surrounding the aorta were removed. Aorta was dissected using a scalpel by cutting into the size of 15×15×3 mm^3. An equal number of aortae were divided into the native (non-decellularized) and decellularized aorta groups. Native aortas were stored at -20°C while the remaining of aorta underwent decellularization.

Sonication decellularization treatment

The sonication decellularization system consists of the ultrasonic generator (Fx-500, Flexonic), roller pump (RP-1000, Eyela), water bath (LTB-250, As-One), temperature monitor (TR-71U, T&D), hydrophone (TC4013, Eastek), multiparameter meter (HI 9828, Hanna instruments) and the reactor. Aorta tissues were placed 10mm from the ultrasonic transducer and sonicated in 0.1% and 2% Sodium Dodecyl Sulphate (SDS) at constant temperature 36°C for 10 h. The ultrasound frequency was set at 170 kHz with the power output of 15 W. The tissue was washed in PBS for 5 days after decellularization to remove the residual SDS.

Immersion decellularization treatment

Aorta tissues were immersed in 0.1% and 2% SDS for 10 h at 36°C with 70 rpm agitation to ensure constant and homogenous condition throughout the treatment. The tissue was washed in PBS for 5 days after decellularization treatment to remove the residual SDS.

Histological analysis for observation of residual cells and structural preservation of collagen and elastin

To evaluate the removal of cells' efficiency, and the general histological characterization of aortic scaffolds, the H&E staining was performed. Native and decellularized aortic scaffolds were fixed in 4% paraformaldehyde for 24 h. Samples were dehydrated with the increased alcohol concentration of 70%, 80%, 90%, 100% for 1 h each, followed by washing with three times of Xylene and Xylene/paraffin (1:1) overnight. Then, the samples were embedded in paraffin and sectioned 3 µm thick for Hematoxylin & Eosin (H&E), Picrosirius Red (PSR) and Verhoeff-Van Gieson (VVG) staining.

DNA quantification for observation of residual DNA

Further tested to evaluate the quality of decellularization was performed by DNA quantification. Native and decellularized aortic scaffolds were incubated in 10% proteinase K at 4°C for overnight. The DNA was isolated using a DNeasy assay kit (Qiagen, USA) from native aortic tissue and decellularized aortic scaffolds (n=6) following manufacturers standard protocol. The total amount of DNA was quantified by spectrophotometer at 260 nm and normalized to the tissue weight of 25 mg.

Scanning electron microscopy for observation of ultrastructure's integrity

To characterize the ultrastructure of the ECM surface and fibre networks of aortic scaffolds, SEM images were used. Samples were fixed with McDowell-Trump fixative at 4°C for 24 h. Samples were then dehydrated with a series of increasing concentrations of ethanol 50%, 75% and two changes of 95% for 15 min respectively. Then continue to immerse in two changes of absolute ethanol for 20 min each. Dehydrated samples were immersed in Hexamethyldisilazane (HMDS) solution for 10 min and leave in a desiccator to air-dry at room temperature. Next, dried samples were mounted on SEM stub of metal and coated with Gold/Palladium using Sputter Coater (Leica Biosystem, US) and visualized with the SEM (Carl Zeiss, Germany).

Biochemical assay to evaluate collagen and elastin content

Biochemical assays were performed to quantify the protein of native tissues and aortic scaffolds such as collagen and elastin.

The samples were first freeze-dried to obtain the Nano size of samples.

Collagen content

Collagen contents were quantified using SIRCOL Soluble collagen assays kit following the manufacture recommendations (Biocolor, Belfast, Northern Ireland). Briefly, the collagen was extracted by acid pepsin at 4°C overnight. Then, the extracted collagen was incubated in colorimetric reagent (the dye Sirius red in picric acid) for 30 min. After that, the SR dye will release from the collagen-dye complex with alkali reagent (0.5 M NaOH). The absorbance was measured at a wavelength of 555 nm.

Elastin content

Elastin content was quantified using the FASTIN Elastin assay kit (Biocolor, Belfast, Northern Ireland). The samples were freeze-dried and weighed, and then the alpha elastin was isolated following the manufacturer's standard protocol. Aorta tissues were incubated in 0.25 M oxalic acid at 100°C for 1 h. Then the extracted alpha elastin was incubated with colorimetric reagent (TPPS in a citrate-phosphate buffer) for 90 min. Then, the elastin bound dye will release with Dye Dissociation Reagent. The absorbance was measured at a wavelength of 513 nm.

Indentation test to evaluate biomechanical preservation

Biomechanical testing was assessed and quantified for native and aortic scaffolds using indentation testing device as depicted previously 18. The sample size of 6 mm×6 mm×2 mm was placed into a custom-designed metallic plate with a circular cavity. The cylinder was punched out of the samples with a surface parallel to the base. The constraint compression-relaxation test was performed using a universal testing machine (MX-500N, IMADA) and a 3 mm steel ball at the tip of the indenter. Prior to each test, the indenter was placed at zero level base of the cavity to calibrate the indenter position. The test was analysed with a digital force analyser (FA PLUS, IMADA). Three phases in test cycles include: (1) dynamic compression with a constant load velocity of 10 mm/min until 3 N, (2) static compression of the sample for 60 s with a load of 3 N and (3) relaxation of the sample with a constant unload velocity of 10 mm/min until the load of 0 N. A total number of 4 cycles were performed with the interval of 30 s. Recorder software (F-S Recorder, IMADA) was used to display the load, indenter position, and time. The value can be calculated for stiffness that determined from the linear elastic slope of the loading curve between 0.5 N and 1.5 N as shown in Figure 1, relative sample compression that measured by indenter position in relation to absolute sample height and residual force that is load measured at the end of static compression as shown in Figure 2.

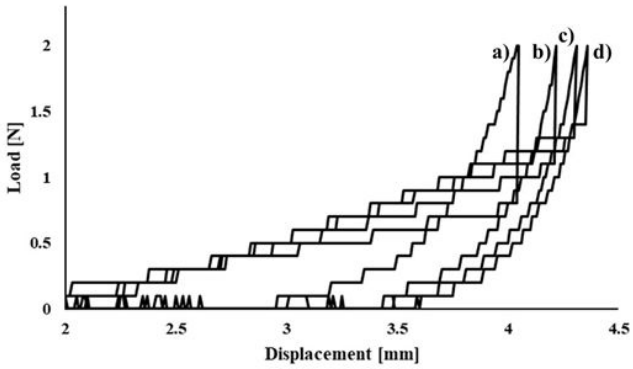

Figure 1: Schematic representation of indentation load-displacement graph showing the four repetitive cycles: (a) Preloading; (b) 1st cycle; (c) 2nd cycle; and (d) 3rd cycle. The slope represents the stiffness.

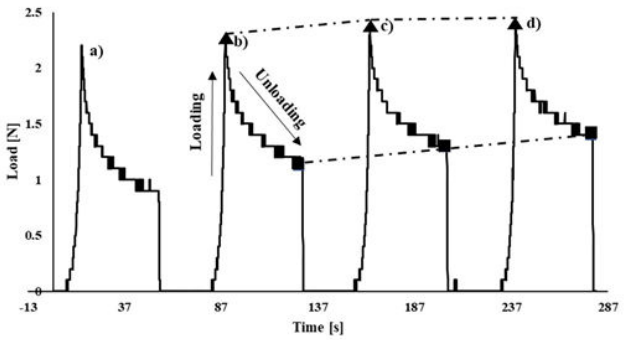

Figure 2: Schematic representation of indentation load-time graph showing the four repetitive cycles: (a) Preloading; (b) 1st cycle; (c) 2nd cycle; and (d) 3rd cycle. The triangle and square represent the compression and the residual force respectively.

Statistical analysis

Values in all data were expressed as mean ± Standard Deviation (SD). Significant differences between the native tissues and aortic scaffolds were analysed by One-way ANOVA using the SPSS for Windows version 10.0 (SPSS GmbH Software, Munchen, Germany). Statistical difference between the groups was determined using Turkey post-hoc test. P-values less than 0.05 were considered significant.

Results

Evaluation of cell removal

The efficacy of the sonication decellularization system to remove cells and matrix structure preservation were verified through the observation with H&E staining (Figure 3). The nuclei in the native sample show consistently spread and distributed with the typical histological structure of aortic tissues by the

basophilic dark purple staining. In the immersion treatment tissues, the nuclei were not completely removed. This is shown in figures b and c with the presented cell remnants by the basophilic dark purple staining. On the contrary, the effectiveness of sonication decellularization system was confirmed with the removal of all cells and preservation the matrix of the aortic scaffold with displayed no basophilic purple staining.

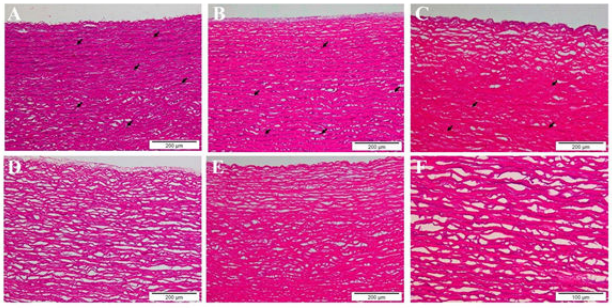

Figure 3: Hematoxylin-Eosin staining of: (a) Native tissue (b) 0.1% immersed aortic scaffolds (c) 2% immersed aortic scaffolds (d) and (f) 0.1% sonicated aortic scaffolds and (e) 2% sonicated aortic scaffolds.

Evaluation of DNA Residual

Further assessment to verify and strengthen the effectiveness of sonication decellularization system in decellularization, residual DNA contents were quantified (Figure 4). The average DNA content of the native aortic tissues was 3.9 ± 0.22 µg/mg. These values were significantly reduced to 0.6 ± 0.15 µg/mg ($p<0.01$) and 0.3 ± 0.07 µg/mg ($p<0.01$) resulting in an 87% and 92% decreased in 0.1% and 2% sonicated aortic scaffolds DNA content respectively. In contrast, the average content of the 0.1% and 2% immersed aortic scaffolds demonstrated only reduce about 74% and 64% compared to native tissues which showed residual DNA of 1.2 ± 0.20 µg/mg and 0.9 ± 0.08 µg/mg respectively.

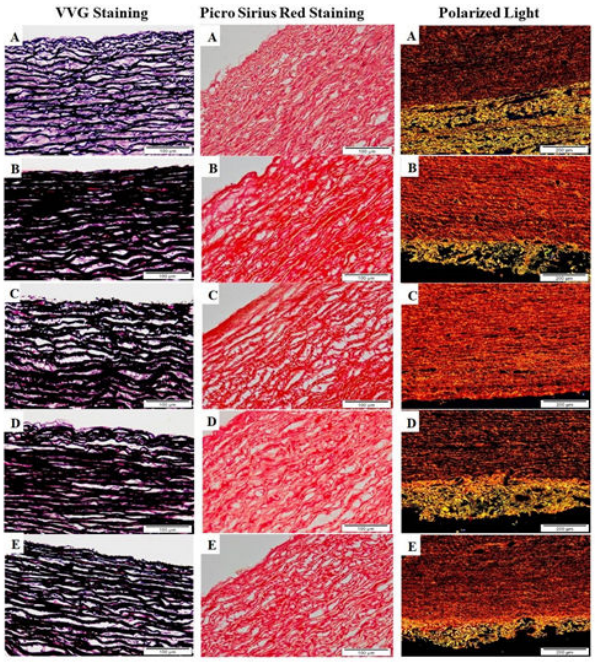

Figure 4: Verhoeff-van Gieson staining, Picro Sirius Red staining and polarized images of: (a) native aortic tissues; (b) 0.1% immersed aortic scaffolds; (c) 2% immersed aortic scaffolds; (d) 0.1% sonicated aortic scaffolds; and (e) 2% sonicated aortic scaffolds.

Evaluation of ECM's structure

For a more complete verification of ECM structure, collagen and elastin structure in native tissues and aortic scaffolds were identified by Picrosirius red and Verhoeff-Van Gieson staining respectively.

For elastic fibre detection, native, immersed and sonicated aortic samples were stained with Verhoeff-Van Gieson as shown in Figure 4. In this regard, tunica media were characterized by a high amount of elastic fibres, whereas tunica adventitia and intima had very few of elastic fibres. The staining revealed that sonication and immersion treatment able to maintain elastic fibres of aortic tissues, although some differences existed among the treatment. In native aortic tissues, lamellar elastic fibrils were crimped with the small wave-like shape that stained as dark blue while collagen fibres were stained as pink lie between the two fenestrated sheets of elastin. In contrast, the lamellar elastin fibrils of the decellularized aortic scaffolds were straight and had a large wavelike shape. The inter-lamellar spaces of the decellularized aortic scaffolds were enlarged due to the loss of inter-lamellar fine elastic fibrils and collagen fibres. The lamellar elastin fibrils at the tunica intima surface of the immersed aortic scaffolds with 2% SDS appeared to be fragmented.

For collagen fibre detection, native aortic tissues and decellularized aortic scaffolds were stained with Picrosirius red. In this regard, the major constituent of collagen fibrillar in tunica intima, media and adventitia is collagen type I and type III. In

native tissues, the staining under bright field light showed the collagen fibres were crimped with a wavy shape as a red color for type I and pink for type III. In contrast, the collagen fibres of the scaffolds treat with higher SDS concentration were straight with the enlargement of interfibrillar space due to the loss of fine collagen fibres type III.

Collagen fibres were evaluated under polarized light to observe the color and intensity of birefringence in each layer of aortic tissues. Two distinct layers could be observed from the polarized images. The inner layer which is the tunica intima and

Tunica media demonstrated the coarse bright red or yellow with thin green fibres and the peripheral layer which is the tunica adventitia contained the bright yellow. In native tissues, the staining under polarized light showed the collagen fibres as yellow to red color for type I and green for type III with low-intensity color. In contrast, the collagen fibres in immersed aortic scaffolds showed high-intensity color in yellow to red with less green colour appeared because of partial loss of fine collagen fibre type III. While in sonicated aortic scaffolds, the intense color of collagen fibres was similar to in native aortic tissue.

Evaluation of Ultrastructure Integrity

Upon decellularization, SEM was performed to determine the effect of sonication treatment on Extracellular Matrix (ECM) that focus on intimal surface and fibre network of aortic scaffolds (Figure 5). A confluent layer was demonstrated in native tissues (Figure 5a). On the contrary, all the aortic scaffolds demonstrated the absence of a confluent layer. The ECM fibres mesh was showed in immersed aortic scaffolds (Figures 5b and c) and sonicated aortic scaffolds (Figures 5d-f). In addition, the ECM surface of aortic tissue treated with sonication treatment showed packed loosely. However, the fibrous collagen network and elastin fibres were shown to still maintain on the ECM surface of 0.1% SDS and 2% SDS sonicated samples.

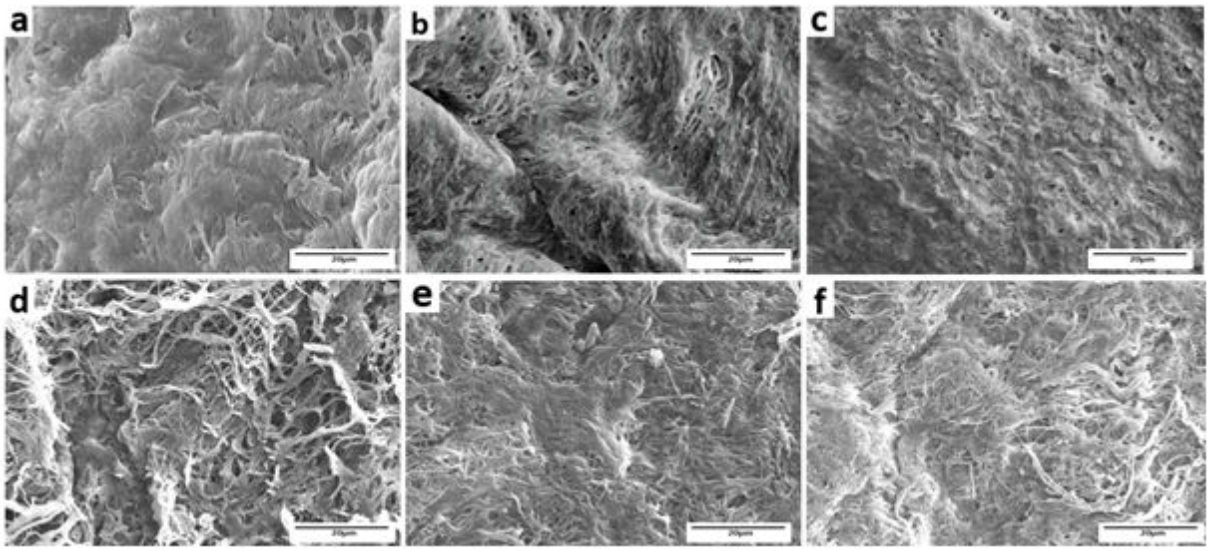

Figure 5: SEM images of: (a) Native aortic tissues; (b) 0.1% immersed aortic scaffolds (c) 2% immersed aortic scaffolds (d) and (f) 0.1% sonicated aortic scaffolds and (e) 2% sonicated aortic scaffolds.

Evaluation of collagen and elastin content

The effect of decellularization treatment on the extracellular matrix of collagen and elastin content of aorta was quantified biochemically using samples from native and decellularized aorta as shown in Figure 6.

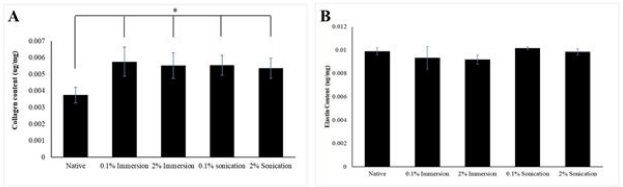

Figure 6: (A) Collagen and (B) Elastin content of the native aortic tissues and decellularized aortic scaffolds. *p<0.05 indicates native versus immersed and sonicated scaffolds.

Collagen preservation after decellularization

Average of soluble collagen content of native aortic tissues was 0.004 ± 0.0005 µg/mg. Soluble collagen content for

decellularized aortic tissues demonstrated significantly increased compared to native aortic tissue. Immersed aortic scaffolds with 0.1% (0.0057 ± 0.0009 µg/mg) and 2% (0.0055 ± 0.0008 µg/mg) SDS had higher elastin content than sonicated aortic scaffolds with 0.1% (0.0056 ± 0.0006 µg/mg) and 2% (0.0053 ± 0.0006 µg/mg) SDS.

Elastin preservation after decellularization

Average of elastin content of native tissues was 0.011 ± 0.0025 µg/mg. Elastin content in decellularized aortic scaffolds demonstrated insignificantly decreased compared to native aortic tissues. Immersed aortic scaffolds with 0.1% (0.0097 ± 0.0013 µg/mg) and 2% (0.0092 ± 0.0005 µg/mg) SDS had lower elastin content than sonicated aortic scaffolds with 0.1% (0.01 ± 0.0002 µg/mg) and 2% (0.0099 ± 0.0003 µg/mg) SDS. This indicates the sonication treatment cause a minor damage on the major elastin content.

Evaluation of biomechanical properties

This is important to evaluate the comparison of biomechanical properties between native tissues and aortic scaffolds to determine the long-term success of a vascular graft. Stiffness, compression and a residual force of native aortic tissues and decellularized aortic scaffolds were demonstrated in Figure 7. The stiffness of decellularized aortic scaffolds was measured to evaluate the ability of scaffolds to resist deformation that was characterized from the linear elastic slope from the loading curve between 0.5 N and 1 N (Figure 1). The stiffness showed an insignificant difference between native aortic tissues and decellularized aortic scaffolds during each cycle of testing. The stiffness of native aortic tissues, 0.1%, and 2% immersed aortic scaffolds and 0.1% and 2% sonicated aortic scaffolds from 2nd to 4th cycles were 4.69 ± 0.26 N/mm to 4.87 ± 0.3 N/mm, 4.52 ± 0.31 N/mm to 4.95 ± 0.21 N/mm and 4.8 ± 0.33 N/mm to 5.2 ± 0.49 N/mm, 4.88 ± 0.12 N/mm to 5.22 ± 0.08 N/mm and 5.11 ± 0.2 N/mm to 5.32 ± 0.2 N/mm respectively. Stiffness increased insignificantly throughout testing by 3.7% (stiffness of native aortic tissue), 9.5% (stiffness of 0.1% immersed aortic scaffolds), 8.4% (stiffness of 2% immersed aortic scaffolds), 6.9% (stiffness of 0.1% sonicated aortic scaffolds) and 4.3% (stiffness of 2% sonicated aortic scaffolds). The compression of native aortic tissue, 0.1% and 2% immersed aortic scaffolds and 0.1% and 2% sonicated aortic scaffolds were insignificantly increased throughout the testing from 2nd cycles to 4th cycles by 6.8%, 8.5%, 7.7%, 6.4%, and 7.8% respectively. During 2nd to 4th cycle, compression of native aortic tissue was 67.03 ± 3.2 % to 71.57 ± 2.1 %. Compression of 0.1% immersed and sonicated aortic scaffolds, had insignificantly decreased compared to native aortic tissues from 2nd to 4th cycles which was 62.4 ± 5.02 %, to 67.69 ± 4.95 % and 66.8 ± 9.1 % to 71.09 ± 8.6 %, respectively. In contrast, 2% immersed and sonicated aortic scaffolds had decreased significantly compared to native tissues from 2nd to 4th cycle swhich was 56.3 ± 6.8 % to 60.6 ± 7.3 % and 57.8 ± 7.1 % to 62.3 ± 7.7 % respectively.

The residual force of native aortic tissues, 0.1%, and 2% immersed aortic scaffolds, 0.1% and 2% sonicated aortic scaffolds were insignificantly increased throughout the testing by 17%, 27%, 18%, 30% and 26% respectively. The residual force of all aortic scaffolds was decreased insignificantly compared to native aortic tissues. The residual force of native tissue, 0.1% and 2% immersed aortic scaffolds, 0.1% and 2% sonicated aortic scaffolds from 2nd to 4th cycles were 1.4 ± 0.05 N to 1.6 ± 0.06 N, 1.1 ± 0.08 to 1.4 ± 0.06 N, 1.3 ± 0.06 to 1.5 ± 0.04 N, 1.2 ± 0.17 N to 1.5 ± 0.12 N and 1.2 ± 0.07 N to 1.5 ± 0.07 N respectively.

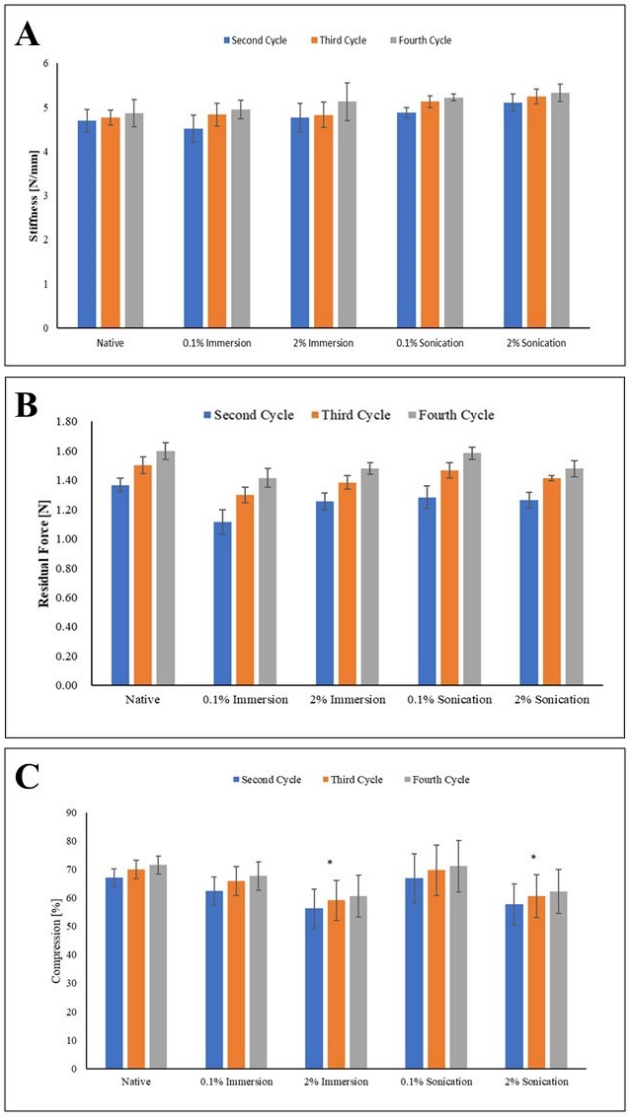

Figure 7: (A) Stiffness; (B) Residual force; and (C) Compression of native aortic tissues and decellularized aortic scaffolds. *p<0.05 indicates the significant difference between native tissues and aortic scaffolds.

Discussion

The main findings of the present study are structurally and biomechanically characterized the aortic scaffolds decellularized by sonication decellularization system that have the potential for tissue engineering application. The structural integrity of the aorta is important in the evaluation of decellularized samples as it affects the normal function and stability of the vessels. Elastic

fibres in the aorta have an important role to provide elastic distention during diastole and allow intrinsic recoiling to their original position during systole. While collagen limits the distention of the aorta to prevent the damage [19]. Therefore, both elastin and collagen structure are critical to the biomechanical behavior of the aorta in the cardiac cycle. In this regard, our analysis demonstrated that sonication treatment resulted in preservation of structural and biomechanical properties of aortic scaffolds compared to native tissue as determined by histological, biochemical and biomechanical analysis.

The histological analysis through H&E staining showed that the primary structure of sonicated aortic scaffolds was maintained on the microscopic scale with the intact of ECM fibres without the presence of any nuclei. Verhoeff-Van Gieson staining showed that elastin, a major ECM component of aortic tissues, was preserved. This was confirmed by elastin quantification by insignificantly reduced of the elastin content of sonicated aortic scaffolds. The reduction of elastin content was expected as the outcome of the decellularization process. In previous studies of the varying type of tissue, elastin has been shown significantly reduced after decellularization [20-23]. However, level of elastin and other cellular components can be restored *in vivo* after scaffolds reincorporate into the body as observed previously by Reimer et al. [20]. From Picrosirius staining, collagen fibres were preserved in sonicated aortic tissues. Biochemical quantification of collagen hydroxyproline has shown the increased level of collagen content in aortic scaffolds. Naturally, we expect the content of collagen in native tissue to be greater than in decellularized tissues. Since the collagen content is not be increased by decellularization. Because of the collagen is more resistant to SDS than other proteins, the large loss of total proteins and the retention of the collagen following decellularization constituted a higher percentage of total protein. This result is supported by the previous finding who made the same observation [24-26].

The ball indention test was used to identify the viscoelasticity parameters including stiffness, compressibility and residual force which can be derived from indentation load-displacement curve [27]. The ball indention test can measure the deformation of the materials based on the indentation depth of the ball penetrate the sample's surface with an applied load [28]. The use of the ball instead of the flat indenter is preferred to minimize the shear stress on the contact area of indenter and sample as flat indenter will produce the shear stress and tensile reactions [29]. Cyclic indentation testing was performed as an extension of automated ball indentation to provide the physiological loading condition to obtain viscoelastic properties of bio scaffolds [29-30]. The value of these viscoelasticity properties provides information regarding the biomechanical compliance of native aortic tissue and decellularized aortic scaffolds.

Briefly, the sample is loaded dynamically with constant velocity, then loaded statically and unload with constant velocity. The stiffness which was determined during the dynamic phase is important for the elastic characterization of scaffolds. The compression was measured during the static phase is important to indicate the viscosity of the scaffolds while the residual force was measured during the relaxation phase is important for the viscoelastic characterization of scaffolds which have an ability to return to its original shape (elasticity) and to expand under unconstrained compression (viscosity) [31]. Changes in biomechanical properties of aorta generally associated with modification of load-bearing structural components which alter the intrinsic and combined function of elastin, collagen, proteoglycan, and glycoprotein of ECM that involve complex interaction with cell signalling pathways [32].

The sonication treatment did not highly affect the biomechanical properties of the aorta tissues which insignificantly increased the stiffness and decreased the compression and the residual force. Stiffness is an important parameter of biomechanical properties of the aorta to determine the pulse pressure that depends on the distensibility of the aortic walls to eject the blood intermittently for efficient tissue perfusion [32]. According to Avolio et al. aortic stiffness is related to the "passive" mechanism that is related to material properties such as elastin, collagen, proteoglycan and glycoprotein and "active" mechanism that is related to cellular and molecular signalling [32]. Hence, the stiffening of aortic scaffolds might correlate to the increase of collagen concentration in aortic scaffolds due to the loss of other components in aortic scaffolds. According to Berillis et al. that study the age-related changes in the amount and concentration of collagen, the stiffness of the aorta are increased during aging with the increment of collagen concentration and decrement of the collagen amount [6]. Other study hypothesized that change in the interface between collagen, elastic and smooth muscle cells during aging lead to the change in stiffness of the vessel wall. Moreover, the previous study on the biomechanics of decellularized carotid arteries, which obtained the same results suggested that the stiffer of the decellularized scaffolds attribute to the loosening and uncrimping of collagen network [33]. In addition, the less compacted of aortic scaffolds lead to increasing of fibres motility that allows fibres to recruit and reorient easily towards the direction of applied load, resulting in increased stiffness of aortic scaffolds [31]. Meanwhile, native tissues had compacted of the structure with the fibre-fibre interaction and cell-fibre interaction that hinder the recruitment and reorientation towards the direction of the applied load which would lead to less stiffness.

Residual stress is an important parameter of mechanics walls because they counteract stresses from blood pressure to increase resistance to failure which is attributed to elastin and VSMC [34]. As the histological analysis demonstrated the preservation of elastic fibres in sonicated aortic scaffolds that similar to native aortic tissues, decreasing of residual force is most likely due to the removal of cells which lead to the increased porosity and disruption of cells fibre interaction, resulting in residual force to be released. The insignificantly decreased of residual force indicates more viscous properties in aortic scaffolds as suggested by the statement of Sandmann et al. [31].

Compressibility is an important parameter of mechanics walls over the pressure change during systolic and diastolic that is exerted by blood against the wall as indicated by thinning of the

aorta wall as pressure increase [35]. The alteration and disruption of the elastic fibres which contribute to the biomechanical might responsible for low compression of aortic scaffolds [36]. The insignificantly decreased of compression of aortic scaffolds indicates that the elastic fibres were not disrupted by decellularization treatment with a lower percentage of SDS. Based on this result, apparently, decellularization treatment has no effect on compression of aortic scaffolds but SDS concentration. Previous studies reported that the biomechanical properties of elastin could be dramatically affected by the addition of small molecular weight of various solutions including SDS [37,38]. For example, Kagan et al. studied the proteolysis of elastin-SDS complexes that reported it to stimulate the rate of digestion of elastin. The stimulation rate depends upon the SDS concentration and incubation time. The maximal stimulation to proteolyze the elastin is obtained when incubating with 1% SDS. In contrast, incubation with 0.03% SDS will inactivate the elastase to proteolyzed the elastin [39]. These suggest that 2% SDS used in both treatment study is dramatically stimulating the elastase by binding to the elastin yielding the substrate complex that more susceptible to proteolysis which affects the compression of aortic scaffold after decellularization treatment.

To the best of our knowledge this is the first report of biomechanical properties of aortic scaffolds decellularized by sonication decellularization system. Previous works have studied the factors that might influence the effectiveness of sonication in decellularization process, including solution parameter (pH, Dissolved Oxygen (DO), conductivity), sonication power and frequency, SDS concentration, and a distance of irradiation and treatment time [12]. The most effective cavitation intensity occurred during the 10 h of 170 kHz sonication when DO and pH in the lowest value while conductivity in the highest value due to the production of highly reactive free radical that responsible to destruct the cells. Surfactant concentration also contributes to the production of highly reactive free radical. Both 0.1% and 2% of the SDS concentration showed complete decellularization done by a previous study [13]. The acoustic intensity distribution of sonication is non-uniform with higher at the centre of irradiation. Increase the distance of the sample to the centre of irradiation resulting in the non-uniform decellularization which affects the cell removal effectiveness. Based on these previous studies, the sonication decellularization system enhances the SDS capacity to penetrate tissue, which will accordingly reduce the treatment time and improve the decellularization process. Overall, the aortic scaffolds decellularized by sonication decellularization system in this study considered as starting platform for further studies on other small-diameter blood vessels for tissue engineering blood vessels since it maintains the structure and biomechanical properties of native tissues.

Conclusion

In conclusion, the sonication decellularization system significantly removes the cells while preserving the structural integrity of collagen and elastin and biomechanical properties of aortic scaffolds in short treatment time. The sonication decellularization system has confirmed to cause minimal change in ECM structure and does no detrimental effect on the biomechanical properties of scaffolds. Therefore, this sonication decellularization system can be applied to obtain well-preserved bio scaffolds for the biomedical implant as promising decellularization protocol. Further investigation, it is necessary to evaluate the behavior of the cells and the interaction with the ECM structure to determine the cytotoxicity of the bio scaffolds.

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