

Biomechanical Analysis of Decellularized Human Umbilical Veins Following Sodium Dodecyl Sulphate Treatment

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ABSTRACT

Background: The decellularization procedure aims to make tissue engineering-vessel grafts (TEVG) made of biologic material, such as xenograft and allograft materials, less immunogenic to prevent graft rejection. Human umbilical veins are known as a potential scaffold material for making TEVG because they already have a basic blood vessel structure and are easy to obtain. Sodium dodecyl sulphate (SDS) is an effective decellularization agent and can maintain the extracellular matrix well.

Objective: This study aim to assess whether the biomechanical properties are maintained after decellularization using SDS to determine whether the graft is predicted to be functional.

Materials and Methods: This is an in-vitro experimental study involving the decellularization process of umbilical vein scaffolds using SDS at concentrations of 0.5% and 1% with a duration of 12 hours and 24 hours. The biomechanical features tested were tensile strength, Young's modulus, burst pressure, and suture holding capacity.

Results: The biomechanical results after decellularization with SDS proved quite effective in preserving the umbilical vein's biomechanics. Immersion in 0.5% SDS for 24 hours showed the biomechanical results closest to the control.

Conclusions: A low-concentration SDS agent with 24-hour immersion is effective in removing cellular material, as seen from histology results, and it is still able to maintain the biomechanics of the human umbilical vein scaffold.

Keywords: Human umbilical vein, sodium dodecyl sulphate, biomechanics features

1. INTRODUCTION

Autologous grafts are still the standard graft for various reconstruction and revascularization procedures of small-diameter blood vessels or less than 6 mm. Unfortunately, the high demand for these grafts is not matched by the amount of availability. This insufficiency necessitates exploring alternative options, including synthetic grafts, animal-derived grafts, and human umbilical vessels (Mallis et al., 2018). The rapid development of tissue engineering technology has also become an alternative method for making blood vessel grafts. The leading cause of graft failure is poor interaction between the host and the graft, which results in rejection reactions, hyperplasia, and thrombosis. The use of tissue engineering is expected to be able to support host-graft interactions by providing sufficient endothelial layers in the intraluminal and smooth muscle cells in the blood vessel walls, where the process of making these neo-blood vessels can be done in vitro before being implanted in the host. The human umbilical vein has previously been used as an alternative graft source by fixing the graft in glutaraldehyde solution. Still, this process compromises the biomechanics and functionality of the graft due to the difficulty of endothelial growth on the luminal surface of the graft. (Guidoin et al., 1986) It is associated with a high incidence of post-implantation thrombosis. Nevertheless, the umbilical vein remains a potential scaffold material due to its structure and compliance, which resemble native vessels, and the low risk of interspecies infection transmission that may occur when using xenograft materials. (Hoenicka et al., 2007) The foundation for making these blood vessel grafts begins with determining the scaffold

material that can provide an environment for new living cells, has biomechanics that are close to autologous graft, and can maintain cellular functionality. (Li et al., 2023) One strategy to overcome these limitations is to decellularize the human umbilical vein, thereby removing all cellular material while retaining the native extracellular matrix (Mallis et al., 2018).

Decellularization is a pivotal process in tissue engineering, aiming to eliminate cellular components from a tissue or organ while preserving the extracellular matrix (Ahlmann et al., 2021). The resultant decellularized scaffold offers a three-dimensional framework that can support cell adhesion, proliferation, and differentiation, making it an ideal substrate for tissue regeneration and vascular graft development (Boccafroschi et al., 2015). The selection of a decellularization method hinges on several factors, including tissue type, cellularity, matrix composition, and the intended application of the scaffold. Sodium dodecyl sulphate (SDS) is an anionic surfactant commonly employed in decellularization protocols due to its efficacy in solubilizing cell membranes and disrupting cell-cell interactions. While decellularized umbilical arteries have been studied for their potential as vascular grafts, research on decellularized umbilical veins, particularly concerning their biomechanical properties after SDS treatment, remains limited (Mallis et al., 2020).

This study aims to investigate the biomechanical properties of human umbilical veins following decellularization using SDS. It is essential to assess how SDS treatment affects the structural integrity and mechanical behaviour of the umbilical vein, as these factors directly influence the graft's ability to withstand physiological stresses and maintain patency after implantation. The availability of vascular substitutes with consistent diameter and mechanical properties that closely mimic native human vessels would significantly advance the field of vascular tissue engineering. The objective of this study was to compare the effectiveness of decellularization with SDS 0.5% vs. 1% immersed for 12 hours vs. 24 hours in the umbilical vein to establish which one has greater potential.

2. MATERIALS AND METHODS

Procurement

The inclusion criteria used were umbilical cords with normal morphology from term pregnancies with normal evolution and no complications in the baby. The exclusion criteria were umbilical cords obtained from pregnancies with complications such as preeclampsia, gestational diabetes, or infection. This study has received ethical approval from the Ethics Committee of Dr. Soetomo Hospital Surabaya. The approval number is 1111/KEPK/X/2024. The written consent was obtained from pregnant women undergoing sectio-caesaria at the Surgery Unit of Dr. Soetomo Hospital Surabaya. Umbilical cord samples were cleaned from residual blood and stored in a closed container at 4° C in a saline solution to maintain their integrity.

Umbilical vein dissection

The clean umbilical cord was cut 10 cm and a 6 mm stainless steel mandrel 20 cm long was inserted into the lumen of the vein while straightening the cord. The umbilical cord was then frozen until it was stiff enough to be subjected to an automated dissection process using a Lathe machine with a knife speed of 3000 rpm with a target thickness of 0.75 mm.

Table 1. Sampel Description

Maternal	Pregnancy	Comorbid	Gestasional age (week)	SC indication	Baby weight (gram)	Cord length (cm)
PLO/ 30 y.o/ 68 kg/ 150 cm	GIIP1010	Tidak ada	35	Placenta accreta	2200	52
UAS/30 y.o/ 102 kg/ 159 cm	GIIP2002	Tidak ada	39	Placenta accreta	2900	60
FMW/ 34 y.o/ 43 kg/ 147 cm	GVP3011	Tidak ada	39	Placenta accreta	2800	60
LAH/42 y.o/ 63.5 kg/ 155 cm	GVIIP2132	Tidak ada	37	Placenta accreta	2600	54
NRV/35 y.o/ 64 kg/ 156 cm	GIIP2002	Tidak ada	38	Placenta accreta	2600	58

Decellularization

Samples were divided into control and treatment groups (0.5% SDS 12 hours, 0.5% SDS 24 hours, 1% SDS 12 hours, and 1% SDS 24 hours). The treatment group was immersed in 0.5% and 1% SDS solution for 12 hours and 24 hours in an

incubator at room temperature. The samples were then rinsed with PBS solution for the same duration. Rinsing can be repeated until the tissue pH is 7.0.

Biomechanics evaluation

This test includes tensile strength, Young's modulus, burst pressure, and suture holding capacity. Tensile strength and Young's modulus tests were carried out using an Instron 3365 universal testing machine. The sample was cut to a standard size and placed between the clamps of the testing machine and given a load of 5 N with a pulling speed of 5 mm/min until the tissue was damaged. Data were recorded for the creation of stress-strain curves and the calculation of tensile strength and Young's modulus values. The burst pressure test was carried out by applying hydrostatic pressure to the lumen of the vein and measuring the maximum pressure that the vein wall could withstand before it ruptured. The suture retention strength test was carried out by sewing the sample with 3.0 silk thread and measuring the force required to pull the suture until the tissue or suture broke.

Table 2. Biomechanics features after Decellularization

Variables	Control (n=5) (Mean \pm SD)	Group I : 0,5%-12h (n=5) (Mean \pm SD)	Group II : SDS 0,5%- 24h (n=5) (Mean \pm SD)	Group III : SDS 1%- 12h (n=5) (Mean \pm SD)	Group IV : SDS 1%- 24h (n=5) (Mean \pm SD)	p value
Tensile strength	1.38 \pm 0.81	1.15 \pm 1.21	2.17 \pm 0.41	1.73 \pm 0.56	1.42 \pm 0.27	0.287
Modulus Young	2.67 \pm 1.87	1.04 \pm 0.68	2.38 \pm 0.66	2.23 \pm 1.11	1.24 \pm 0.12	0.136
Suture Holding capacity	1.85 \pm 0.62	1.20 \pm 0.23	1.41 \pm 0.27	1.19 \pm 0.29	1.20 \pm 0.23	0.071
Burst Pressure	85.52 \pm 44.32	73.22 \pm 45.10	78.50 \pm 57.19	182.02 \pm 69.33	25.76 \pm 21.09	0.003

Statistical Analysis

Data were analyzed using SPSS 26 (Chicago, IL) statistical software. Data normality was performed using Saphiro-wilk, and the homogeneity of variance was tested using the Levene statistic. ANOVA was used to compare mean differences between groups, followed by post-hoc tests to identify specific significant differences. A p-value < 0.05 was considered statistically significant.

3. RESULTS

A total umbilical cord length of 284 cm was obtained in good condition from 5 term pregnant women. After decellularization, SDS 0.5% immerse for 24 hours shows the closest biomechanics features compare to control group.

4. DISCUSSION

Based on those results, decellularization using SDS can disrupt the mechanical integrity of the human umbilical vein, leading to a reduction in tensile strength, Young's modulus, burst pressure, and suture retention strength. Determining the optimal concentration and duration of SDS is essential for preserving the biomechanical properties of the extracellular matrix. Tensile strength and Young's Modulus are two indicators of graft resistance to the dynamics of changes in pressure and tension of blood vessels after implantation (Badylak et al., 2001). Modification of blood vessel wall thickness can be an alternative to control blood vessel compliance (Rodriguez et al., 2012). Abouleisman in a previous study compared HUVs that were manually dissected and decellularized with 1% SDS for 24 hours with different thicknesses of 0.4 mm and 0.8 mm, it was found that wall thickness had a significant effect on tensile strength output (Aboulesiman et al., 2008). Although there was a decrease in tensile strength after decellularization, the biomechanical condition

improved after culturing with mesenchymal stem cells (MSCs) for 2 weeks (Aboulesiman et al., 2008).

Suture retention is important to maintain the integrity of the vascular anastomosis after surgery and is influenced by several factors, such as wall thickness, thread size, number of stitches, puncture distance from the edge, and suture technique. In a study conducted by Meng Xin (2019), the thicker the graft wall, the greater the suture-holding capacity of the graft. The number of stitches also determines the strength of the anastomosis; the closer the distance between the stitches is, the more evenly the load will be distributed on the edge of the graft. But again, the more stitches, the more holes are made and the risk

of damaging the graft itself. Stitches can also cause immune reactions because they are foreign objects (Meng X et al., 2019).

Burst pressure describes the ability of blood vessels to withstand pressure from within without rupturing. Decreased burst pressure can increase the risk of aneurysm or leakage in blood vessel grafts. This underscores the importance of considering these biomechanical parameters when evaluating the effectiveness of the decellularization process for vascular tissue engineering applications. In Daniel's study (2005), the burst pressure value of the scaffold with the automatic dissection method was higher compared to the manual dissection method ($1,082 \pm 113.4$ mm Hg and 699.2 ± 399.1 mmHg, $p = 0.001$). However, when compared to post-decellularization, burst pressure experienced an insignificant decrease.

In addition to the decellularization process, the umbilical vein dissection method also contributes to structural changes. The advantage of the automatic dissection method using a Lathe machine is that it can produce a more uniform and controlled wall thickness compared to the manual method. However, this method can also potentially damage collagen and elastin fibers in the extracellular matrix due to extreme temperatures and the use of intraluminal mandrels. Daniel reported that a temperature of -20°C was not enough to freeze the umbilical cord, so the dissection resulted in an inhomogeneous size, and cooling too quickly with nitrogen gas to -80°C could result in micro fracture of the scaffold. The size of the mandrel is expected to be large enough to stretch the inner diameter of the umbilical vein so that a similar diameter is obtained, thereby reducing or eliminating the gap between the mandrel and the scaffold. This small gap between the mandrel and the scaffold tends to get neater automatic dissection results due to less variation in cutting depth and minimal fracture of the scaffold. The umbilical vein in the umbilical cord anatomically runs in a spiral, so it is essential to straighten this vein when inserting the mandrel so that the tension can be evenly distributed longitudinally. Daniel, in his study, reported that one of the factors that caused the large variation in biomechanical outcomes could be caused by some vein segments still in a spiral condition when the automated dissection process was carried out (Daniel et al., 2005).

However, this study has several limitations. The small number of samples limits the statistical power of the analysis. Further studies with larger sample sizes are needed to confirm these findings and to investigate the effects of different decellularization parameters on the biomechanical properties of blood vessels. In addition, this study did not include histomorphological evaluation of the decellularized umbilical vein. Further evaluation is needed to assess the effectiveness of decellularization and to ensure that the extracellular matrix is maintained.

5. CONCLUSION

In conclusion, decellularizing the human umbilical vein using SDS can impact its inherent biomechanical properties, warranting careful optimization of the decellularization protocol to preserve the structural integrity required for tissue engineering applications. The concentration and duration of SDS exposure are critical factors influencing the mechanical strength, elasticity, and burst pressure of the decellularized vein. Group SDS 0.5% immerse for 24 hours become the most promising group. But for further research regarding TEVG in the future, this decellularization could be considering as the base line method.

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