Experimental studies on hemodialysis access innovations

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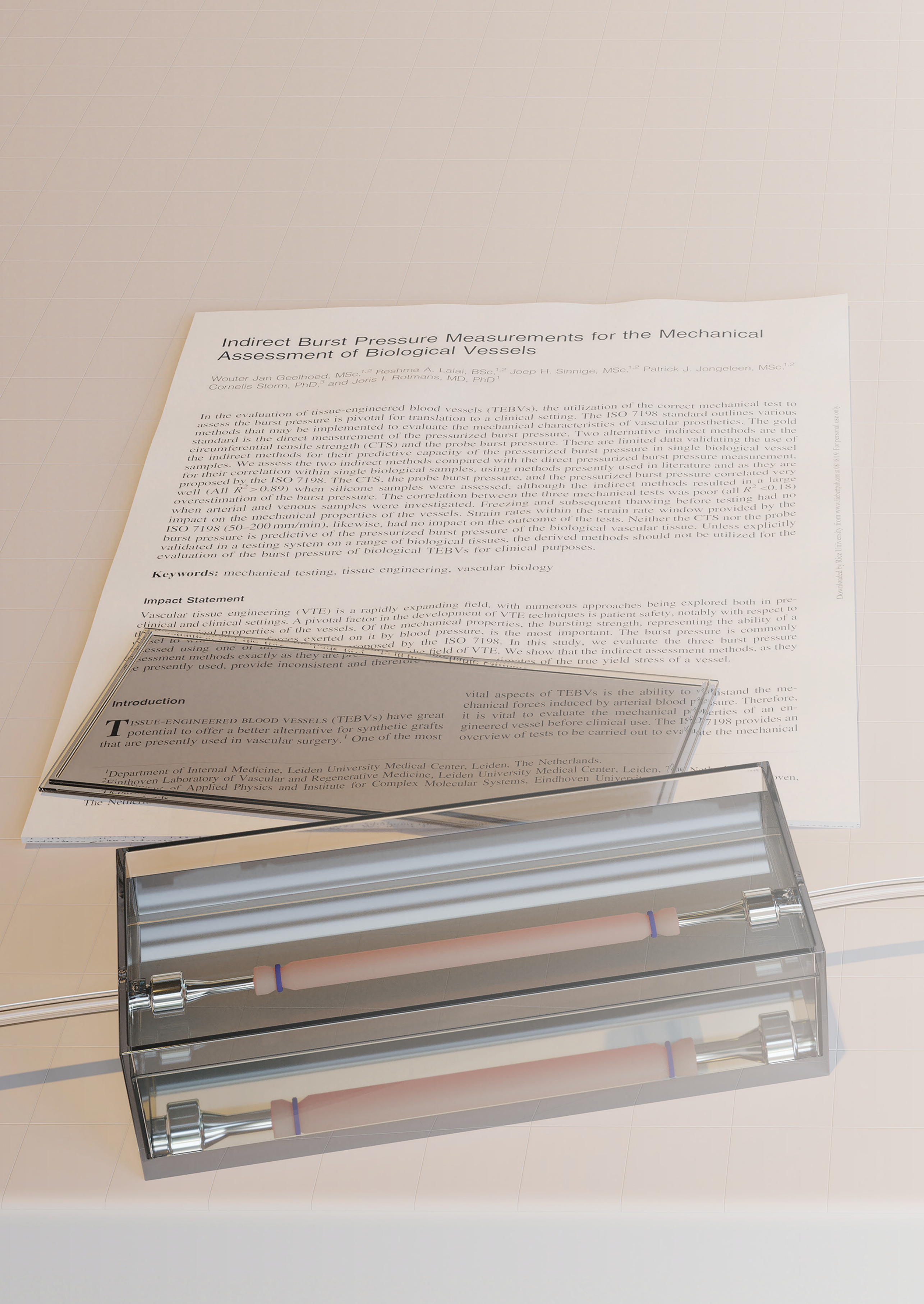
Citation

Geelhoed, W. J. (2020, October 6). *Experimental studies on hemodialysis access innovations*. Retrieved from https://hdl.handle.net/1887/137442

Author: Geelhoed, W.J.
Title: Experimental studies on hemodialysis access innovations
Issue Date: 2020-10-06



Indirect Burst Pressure Measurements for the Mechanical Assessment of Biological Vessels

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In the evaluation of tissue-engineered blood vessels (TEBVs), the utilization of the correct mechanical test to assess the burst pressure is pivotal for translation to a clinical setting. The ISO 7198 standard outlines various methods that may be implemented to evaluate the mechanical characteristics of vascular prosthetics. The gold standard is the direct measurement of the pressurized burst pressure. Two alternative indirect methods are the circumferential tensile strength (CTS) and the probe burst pressure. There are limited data validating the use of the indirect methods for their predictive capacity of the pressurized burst pressure in single biological vessel samples. We assess the two indirect methods compared with the direct pressurized burst pressure measurement, for their correlation within single biological samples, using methods presently used in literature and as they are proposed by the ISO 7198. The CTS, the probe burst pressure, and the pressurized burst pressure correlated very well (All $R^2 > 0.89$) when silicone samples were assessed, although the indirect methods resulted in a large overestimation of the burst pressure. The correlation between the three mechanical tests was poor (all $R^2 < 0.18$) when arterial and venous samples were investigated. Freezing and subsequent thawing before testing had no impact on the mechanical properties of the vessels. Strain rates within the strain rate window provided by the ISO 7198 (50–200 mm/min), likewise, had no impact on the outcome of the tests. Neither the CTS nor the probe burst pressure is predictive of the pressurized burst pressure of the biological vascular tissue. Unless explicitly validated in a testing system on a range of biological tissues, the derived methods should not be utilized for the evaluation of the burst pressure of biological TEBVs for clinical purposes.

Keywords: mechanical testing, tissue engineering, vascular biology

Impact Statement

Vascular tissue engineering (VTE) is a rapidly expanding field, with numerous approaches being explored both in preclinical and clinical settings. A pivotal factor in the development of VTE techniques is patient safety, notably with respect to th[illegible]ical properties of the vessels. Of the mechanical properties, the bursting strength, representing the ability of a [illegible]sel to wi[illegible] forces exerted on it by blood pressure, is the most important. The burst pressure is commonly [illegible]essed using one of [illegible] proposed by the ISO 7198. In this study, we evaluate the three burst pressure [illegible]sessment methods exactly as they are [illegible] field of VTE. We show that the indirect assessment methods, as they [illegible]e presently used, provide inconsistent and therefor[illegible] [illegible] of the true yield stress of a vessel.

Introduction

Tissue-engineered blood vessels (TEBVs) have great potential to offer a better alternative for synthetic grafts that are presently used in vascular surgery.[1] One of the most vital aspects of TEBVs is the ability to withstand the mechanical forces induced by arterial blood pressure. Therefore, it is vital to evaluate the mechanical properties of an engineered vessel before clinical use. The ISO 7198 provides an overview of tests to be carried out to evaluate the mechanical

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Chapter 3

Indirect burst pressure measurements for the mechanical assessment of biological vessels

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Tissue Engineering Part C: Methods 2019 Vol. 25, No. 8

Abstract

In the evaluation of tissue engineered blood vessels, the utilization of the correct mechanical test to assess the burst pressure is pivotal for translation to a clinical setting. The ISO 7198 standard outlines various methods that may be implemented to evaluate the mechanical characteristics of vascular prosthetics. The gold standard is the direct measurement of the pressurized burst pressure. Two alternative, indirect methods are the circumferential tensile strength and the probe burst pressure. There is limited data validating the use of the indirect methods for their predictive capacity of the pressurized burst pressure in single biological vessel samples. We assess the two indirect methods as compared to the direct pressurized burst pressure measurement, for their correlation within single biological samples, using methods currently used in literature and as they are proposed by the ISO 7198. The circumferential tensile strength, the probe burst pressure and the pressurized burst pressure correlated very well (All $R^2 > 0.89$) when silicone samples were assessed, although the indirect methods resulted in a large overestimation of the burst pressure. The correlation between the three mechanical tests was poor (all $R^2 < 0.18$) when arterial and venous samples were investigated. Freezing and subsequent thawing prior to testing had no impact on the mechanical properties of the vessels. Strain rates within the strain rate window provided by the ISO 7198 (50-200mm/min), likewise, had no impact on the outcome of the tests. The circumferential tensile strength nor the probe burst pressure are predictive of the pressurized burst pressure of biological vascular tissue. Unless explicitly validated in a testing system on a range of biological tissues, the derived methods should not be utilized for the evaluation of the burst pressure of biological tissue engineered blood vessels for clinical purposes.

Introduction

Tissue engineered blood vessels (TEBVs) have great potential to offer a better alternative for synthetic grafts that are currently used in vascular surgery [1]. One of the most vital aspects of TEBVs is the ability to withstand the mechanical forces induced by arterial blood pressure. Therefore, it is vital to evaluate the mechanical properties of an engineered vessel prior to clinical use. The ISO 7198 provides an overview of tests to be carried out to evaluate the mechanical properties of vascular prosthesis [2]. There are three methods listed in the ISO 7198 that could be utilized to assess the mechanical strength of a vessel; one direct method; the pressurized burst pressure, and two indirect methods; the circumferential tensile strength (CTS), and the probe burst pressure. The latter two methods require only a small piece of tissue (5mm, and 15mm respectively), which is a major advantage for in situ engineered vascular grafts, as the tissue size that can be excised for mechanical analysis is limited. The pressurized burst pressure is considered the gold standard, but this test requires a much longer tissue segment (>40mm). In testing of vascular prosthetics, the pressurized burst pressure, CTS, and probe methods are used interchangeably, and numerous studies provide mechanical testing data carried out according to the ISO 7198 [3-12]. However, the ISO 7198 was primarily developed for vascular prosthesis, and it is unclear to what extent its testing modalities carry over to biological tissue, due to the large variation to be inherent to such tissues.

It has previously been reported that the CTS is likely to overestimate the burst pressure of a given sample [3-5]. Previous studies have shown that with an adaptation of Barlow's formula (**Eq. 1**), the circumferential tensile strength of a grouped sample could be used to estimate of the burst pressure of a vessel [5]. Barlow's formula states, that the burst pressure of a cylindrical pipe may be computed from its wall thickness (t), its outside diameter (D_0) and its yield stress (σ_Y) as

$$P = \frac{2\sigma_Y t}{D_0}. \tag{1}$$

However, it is still not known if there is an accurate correlation between the three testing modalities if a biological single sample were to be assessed. For applications such as mandrel based *in situ* TEBV, the burst pressure value must be evaluated precisely per patient using a small sample segment, as only a single sample is available [13-15].

The burst pressure may be influenced by the rate at which the sample is strained [16]. The ISO 7198 provides a window of strain rates that may be implemented. In this study, the impact of the strain rate on the measured burst pressure was evaluated. In addition, the impact of short-term freezing on the mechanical properties of the vessels was assessed.

The main objective of this study is to evaluate if the CTS and probe burst pressure methodologies currently used in literature provide an accurate and consistent estimation of the pressurized burst pressure. Moreover, the validity of these methods for the assessment of biological samples is evaluated.

Methods

Study design and sample preparation

A custom system was built to analyze the pressurized burst pressure, CTS, and probe burst pressure according to the ISO 7198 (**Fig. 1**). Initially, the system was assessed using silicone tubing (J. Lindemann GmbH, Helmstedt, Germany), to determine the accuracy of the system. Silicone tubing with a wall thickness of 0.5, 1, 1.5, 2, and 3 mm were tested to assess the variance of the system. Different strain rates, within the window provided by the ISO 7198 guidelines, were used to assess the impact of strain rate on the measurement outcome. Following validation of the testing set-up using silicone tubing, bovine arteries and veins were evaluated. Carotid arteries and jugular veins of 8-month-old bovine calves were collected at the abattoir. Samples were at least 9cm in length and were cut using a custom-made cutting device (**suppl. Fig. 1**) to obtain standardized segments of 40 mm, 15 mm and 5 mm long, for the pressurized burst pressure, probe burst pressure and circumferential tensile strength test, respectively. Care was taken to remove only the peri-adventitial tissue from the samples in a uniform manner by a single technician. The samples were transported in DMEM medium supplemented with 1x penicillin/streptomycin. The samples were frozen at -20°C for a maximum of 7 days prior to mechanical testing. Two and a half hours before mechanical testing the samples were placed at 7°C for 2 hours, then at 37°C for 30 minutes as described by O'Leary et, al [17]. A subset of samples was not frozen to assess the impact of freezing on the mechanical tests. Care was taken to exclude any vessels with branches on the test location from the experiment. The number of samples used for each test can be seen in **figure 1**. During all tests the samples were kept at 37°C using a custom-made perfusion system. The samples were cleaned and tensioned to 60g before being cut using a custom designed device to allow all samples to be cut exactly equal. A 40mm segment was taken for the pressurized burst pressure, a segment with a length of 15 mm was taken for the probe burst pressure, and remaining 5mm segments were used for the CTS. Silicone control samples had an internal diameter of 6mm and a wall thickness of 0.5, 1, 1.5, 2, and 3mm. Finally, to verify freezing has no impact on the measurement outcome, segments of 8 samples were tested when unfrozen and frozen for 1, and 7 days.

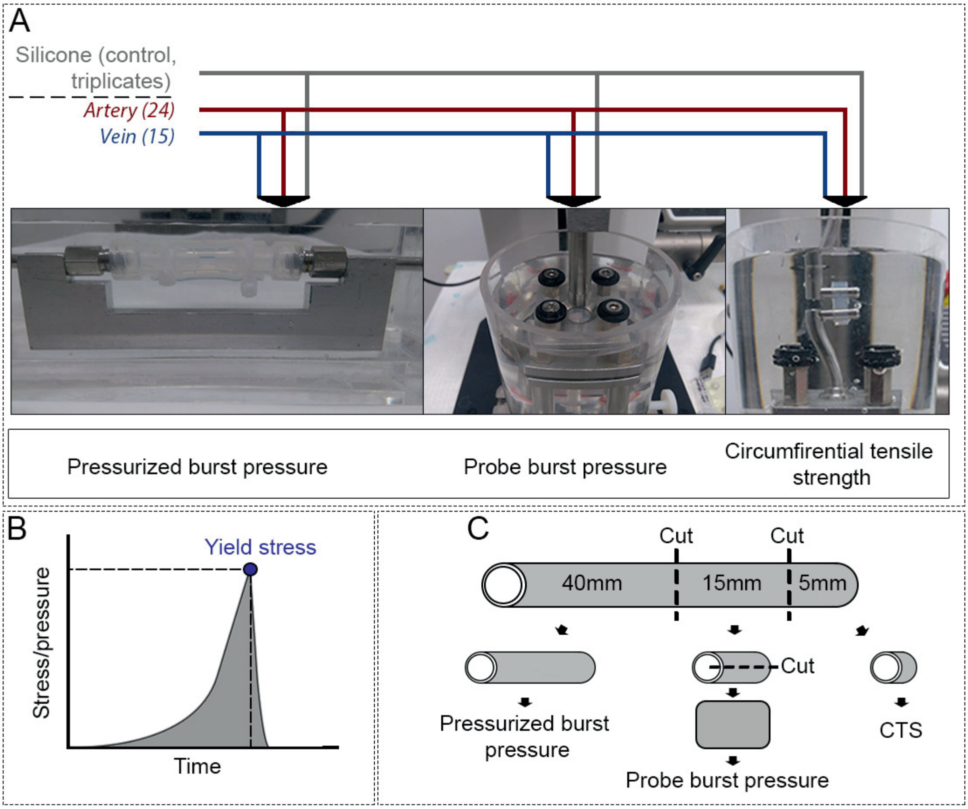

Figure 1. A) An overview of the samples and the tests done on the samples. B) graphical representation of the yield stress. C) The segmentation of the sample vessel used for the analysis.

Pressurized burst pressure

For the pressurized burst pressure, the vessel segment was placed between two Luer connectors and fastened using surgical vessel-loops and placed in a pressurized perfusion system (**Fig. 1a**). Phosphate buffered saline (PBS) was used to pressurize the vessel. Both the PBS surrounding the vessel and the PBS used for perfusion was kept at 37ºC at all times. The internal pressure was measured using a pressure gauge (Fluke, Washington, USA), and pressure could be induced using a 50ml syringe pump connected to a tensile tester (Mark-10, Washington, USA). The vessel length was tensioned to 60g during the tests, whereafter the pressure was increased in a steady state until vessel rupture. Care was taken to assure the rupture occurred away from the Luer connectors.

Circumferential tensile strength

The CTS was measured by inserting a small ring segment of tissue between two pins in a custom-made testing set-up (**Fig. 1b**). One of the pins is then moved away until sample failure. The ring segment was submerged in 37°C PBS during the entirety of the tests. The system was connected to a tensile tester, which could control pin movement and register force using a load cell (Series-5, Mark-10, Washington, USA) capable of registering at 7000 Hz. The CTS is a means to determine the yield stress σ_y (in units of Pa), which is related to the load at failure (F_{CTS}) as **Eq. (2)**. **Eq. (1)** and **Eq. (2)** are then combined to give **Eq. (3)**, which can be used to estimate the yield stress. In this equation, P is the measured internal pressure in units of Pa, F_{CTS} is the force at failure, in units of N, during the test, L_0 is the initial length (m) of the sample and D_0 (m) is the initial diameter. , previously defined, is the wall thickness in m.

$$\sigma_y = \frac{F_{CTS}}{2L_0 t}, \tag{2}$$

$$P = \frac{F_{CTS}}{L_0 D_0}. \tag{3}$$

To investigate the impact of the initial elastic modulus on the strength of a vessel, the stress-strain curves, the Young's modulus (**Eq.4**) was calculated, whereupon the correlation between the 3 mechanical tests where adjusted for the Young's modulus.

$$E = \frac{FL_0}{A\Delta L}. \tag{4}$$

Probe burst pressure

The probe burst pressure was measured by cutting open a vessel segment and wedging it between two plates in a custom-made testing system (**Fig. 1a**). The plates have a central cavity through which a pin can protrude, exerting a strain on the vessel. The system was connected to a tensile tester which controlled the pin and could record the measured force. The central pin was pressed into the vessel until rupture. The yield stress was recorded, and the burst pressure was estimated using **Eq. (5)**, in which A_{sample} (m^2) is the initial surface area of the sample.

$$P = \frac{F}{A_{sample}} \tag{5}$$

Statistical analysis

The data are presented as mean ± standard deviation (SD). Correlation was assessed using linear regression analysis. Comparison of the burst pressure assessment modalities for arteries and veins were analysed using a repeated measured one-way ANOVA with Tukey post-hoc analysis using GraphPad Prism 7.02. P-values < 0.05 were considered statistically significant.

Results

Method validation using silicone control samples

To validate the system, various thicknesses of silicone tubing were used to compare the testing modalities at different strain rates. The accuracy of the tests is evidenced by the low variation within the tests (**Fig. 2**). All three tests correlated well with each other as illustrated in **figure 3**. Although the probe burst pressure correlated well with the CTS and the pressurized burst pressure, both the probe burst pressure and CTS overestimated the burst pressure substantially when compared to the pressurized burst pressure. Using **Eq. (3)** and **Eq. (4)** to transform CTS and probe burst pressure respectively gives an overestimation of the pressurized burst pressure for both tests. A force of 100N would translate into a pressure of 2.5·10^4 and 4.9·10^3 mmHg for the CTS and probe burst pressure respectively.

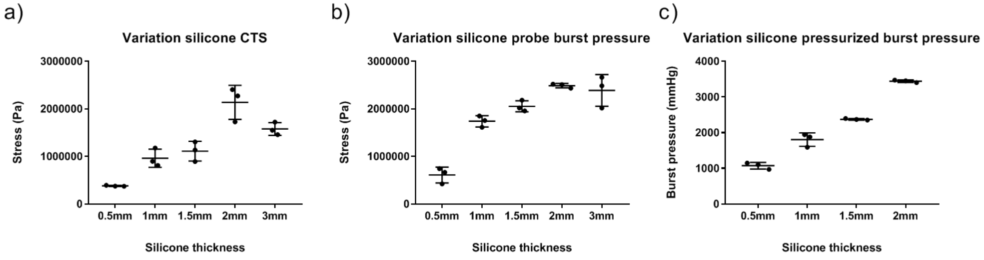

Figure 2. Variation in burst pressure of silicone tubing of different thickness using 3 testing modalities; a) CTS, b) Probe burst pressure, c) pressurized burst pressure. Only the 100mm/min strain speed is shown.

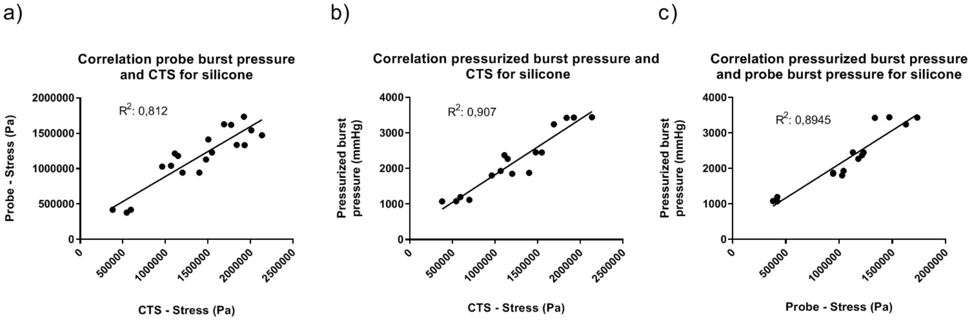

Figure 3. Correlation between the burst pressures of silicone tubing using the 3 testing modalities.

To assess if the strain rate within the window provided by the ISO 7198 (50-200mm/min) impacts the burst pressure, different rates were assessed and compared. We find, that the strain rate did not impact the measurements, and that the pressurized burst pressure measurements were particularly insensitive to changes in strain rate (**Fig. 4**).

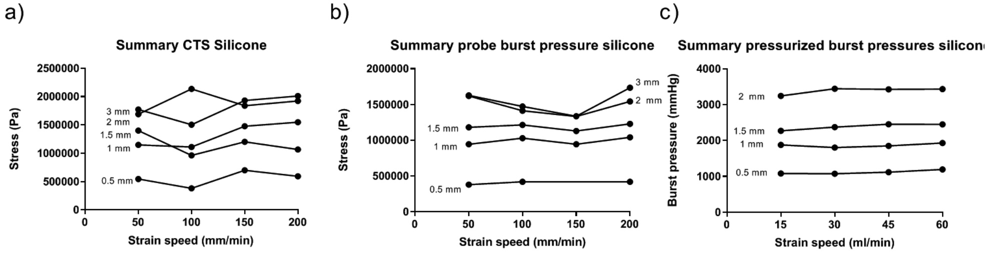

Figure 4. Impact of strain rate on burst pressure obtained by a) the CTS, b) the probe bust pressure, and c) the pressurized burst pressure test. The strain rate for the pressurized burst pressure has a different scale as the strain rate needed to be adjusted to the speed at which fluid was compressed.

Comparison of the tests using biological samples

To assess if these mechanical tests are also applicable for the assessment of biological tissue, bovine arterial and venous samples were tested using all three testing modalities (**Fig. 5**). The within-sample variation for the CTS and probe burst pressure for arterial samples was low (**Fig. 6**). The within-sample variation of the pressurized burst pressure could not be assessed due to insufficient sample length. There was no significant correlation between the tests for the arterial and venous samples (**Fig. 7**). For the arterial samples, the CTS overestimate the pressurized burst pressure. For the venous samples, both the CTS and probe burst pressure overestimate the directly determined pressurized burst pressure. Adjusting the data for the initial elastic modulus had no impact on the correlative capacity of the data (**suppl. Fig. 2**), suggesting that the elastic modulus of the does not explain the variability between tests.

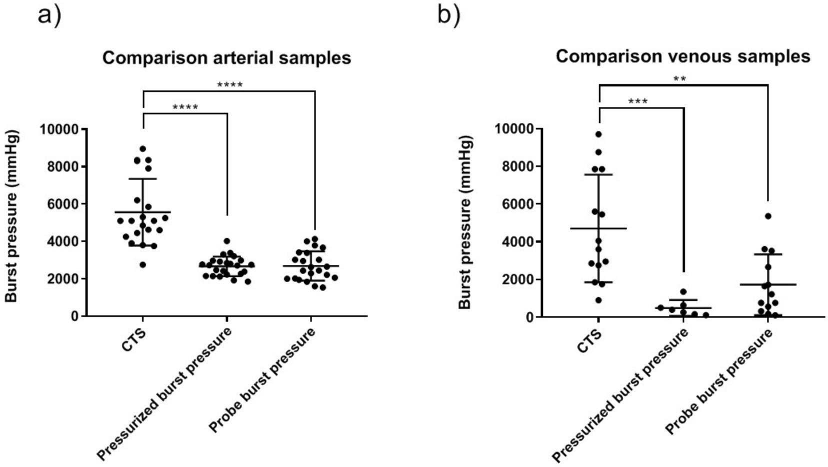

Figure 5. Burst pressure of a) arterial and b) venous samples using the three testing modalities. P < 0.005: **, P < 0.0005: ***, P < 0.0001: ****.

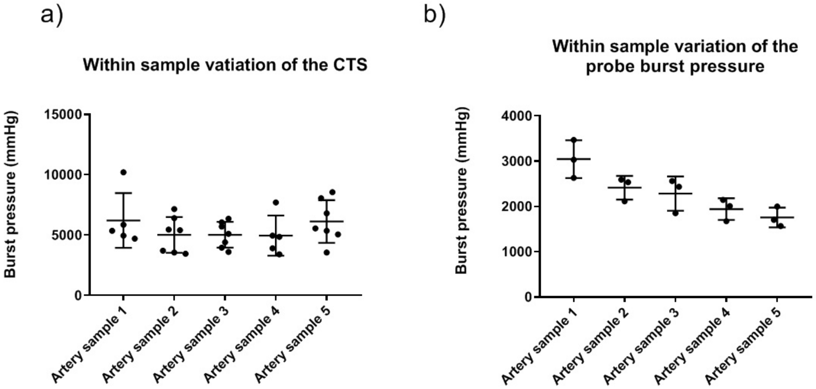

Figure 6. Variation in the CTS (a) and probe burst pressure (b) measurements using arterial samples. The variation of the pressurized burst pressure measurement could not be attained due to insufficient sample length.

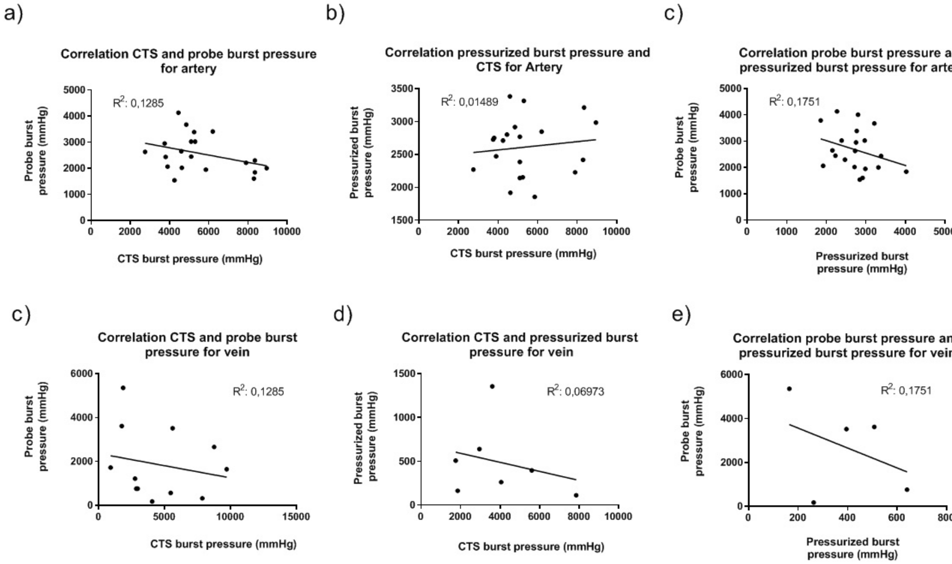

Figure 7. Correlation between testing modalities for arterial (a-c) and venous (d-f) samples. The correlation between the three mechanical tests was very poor.

The impact of freezing on biological samples

To confirm that short term freezing did not impact the results, various samples were assessed for the impact of freezing on the test outcome. The CTS was chosen to assess the impact of short term freezing as this would allow the most measurements to be done on a single sample. No difference between the groups was observed (**Suppl. Fig. 3**).

Discussion

The present study revealed that the CTS and probe burst pressure provide an overestimation of the pressurized burst pressure in both biological and synthetic material. In addition, the outcomes of the three test modalities evaluating the mechanical properties of different types of biological blood vessels correlate poorly with each other. The experiments using silicone tubing validated the experimental set-up with regard to all three tests as seen from the high correlation between the tests for all measurements and very low within sample variations. A low within sample variation is seen for all three tests, especially for the pressurized burst pressure, suggesting this is the most accurate of the tests.

For biological samples, the CTS significantly overestimated the pressurized burst pressure. When evaluating arterial and venous samples, CTS results resulted in a 2-fold and 10-fold higher estimated burst pressure when compared to the pressurized burst pressure respectively. Moreover, the burst pressures obtained with these two tests modalities did not correlate with each other, despite the low variation within the individual biological samples. The question arises why the different tests have such a poor correlation, whereas previous studies by Laterreur et al [5] revealed that the CTS was predictive of the pressurized burst pressure when using a grouped analysis, which is already not predictive for single sample comparison. Differences in collagen alignment between samples might explain the observed variation between samples. However, such difference does not necessarily explain the poor correlation between the 3 tests. In this respect, it is importance to mention that the pressurized burst pressure identifies the weakest spot of a vessel segment whereas both the CTS and probe burst strength measure the average yield stress of a vessel segment. Consequently, the poor correlation between the 3 tests might be explained by the fact that as they measure different aspects of the mechanical characteristics of blood vessels, the tests would be likely not to correlate with one and other. For the pressurized burst pressure, the forces are equally distributed along the vessel, yet a single week spot may incur higher forces. Barlow's formula assumes uniform circumferential stress in the vessel wall, yet soft cylinders often fail in a non-uniform manner, at a localized bulge that may experience far higher local stresses. Thus, the yield strength of the material is exceeded locally long before it would do so globally if stresses were distributed homogenously. Thus, the pressurized burst pressure is mainly a measure of the yield stress of a vessel at its weakest point. The yield stress of the probe burst pressure, on the other hand, assumes a flat sample and evenly distributed load, while neither assumption remains valid when the sample is close to failure. This effect, likewise, yields an inaccurate estimate of the burst pressure of a sample. Like the probe burst strength, the CTS assumes an evenly distributed load while not taking into account the weakest point in a vessel. Where the pressurized burst pressure measures the weakest point of a vessel, the CTS disregards this and measures the average yield stress. Thus, although the literature suggests that an estimation of the burst pressure can be made using the derived methods, both the CTS and probe burst strength are inherently likely to overestimate the yield stress of a vessel compared to the pressurized burst pressure if a sample is not perfectly homogeneous.

As seen in the silicone control, a perfectly homogeneous vessel provides a high correlation between all tests, and the indirect methods can indeed be used to predict the pressurized burst pressure. Biological samples are inherently inhomogeneous, and the lack of correlation between the tests confirms this. Thus, a first conclusion that can be made is that the three tests correlate well and could be predictive with each other so long as perfect homogeneity can be assumed. This excludes the use of the indirect methods for biological tissue assumed to have any slight inhomogeneity.

Moreover, estimating to the pressurized burst pressure using the CTS or probe burst pressure with **Eq. (3)** and **Eq. (5)** respectively gave high overestimations for both silicone and biological samples. Therefore, despite homogeneity of the vessel, as in the silicone control, the indirect methods cannot be accurately recalculated to estimate the pressurized burst pressure using these formulas.

If an indirect method is implemented for the mechanical assessment of biological tissues, the correlative capacity between the indirect method and the direct pressurized burst pressure must first be assessed. When a high correlation is observed, subsequent measurements can be estimated. If no correlation is observed for the biological tissue, as in this study, the direct pressurized burst pressure must be used.

The ISO 7198 was meant for the mechanical assessment of vascular prosthetics. Often, two indirect methods of burst pressure assessment, the CTS and probe burst pressure, are implemented for the assessment of TEBVs. Vascular prosthesis are capable of exhibiting far greater homogeneity than biological vessels due to their composition. Most vascular grafts materials such as ePTFE are mainly composed of a single material. Being composed of numerous cell types, and extracellular components, biological vessels are inherently heterogeneous. Collagen is mainly responsible for the strength of the vessel[18]. It is known that the alignment of collagen is vital in determining the mechanical properties it elicits. In aneurysm formation, for example, the incorrect alignment of collagen leads to the integrity of the vessel wall being compromised[19]. Although TEBVs theoretically have the ability to be more homogeneous than biological tissues, biological variation will remain an issue.

The variation of the pressurized burst pressure for the arteries and veins is unknown due to a lack of sample length. Although the silicone data may indicate that the pressurized measurements have a low variation, this cannot be said for certain for the biological vessels.

Conclusion

The indirect methods can be used to estimate the pressurized burst pressure as long as perfect homogeneity of the sample can be assumed, and high correlation is validated. Transforming the CTS and probe burst pressure values to mmHg directly should not be done as this provides potentially large overestimations of the pressurized burst pressure, partly as these indirect methods are inherently prone to providing a higher yield stresses than the direct pressurized burst pressure. Indirect methods of burst pressure assessment should not be used for the clinical evaluation of TEBVs unless perfect homogeneity is assumed, and a high correlation is validated.

Acknowledgements

This research was funded by a personal VIDI-grant (917.15.328) of JI Rotmans from ZonMW. We thank the Department of Instrumental Affairs at Leiden University Medical Center for the technical assistance with respect to the materials used in this study. We thank T.Boer&Zn for supplying the samples used in this study.

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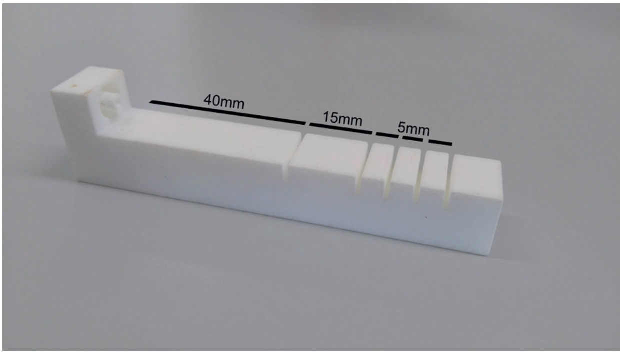
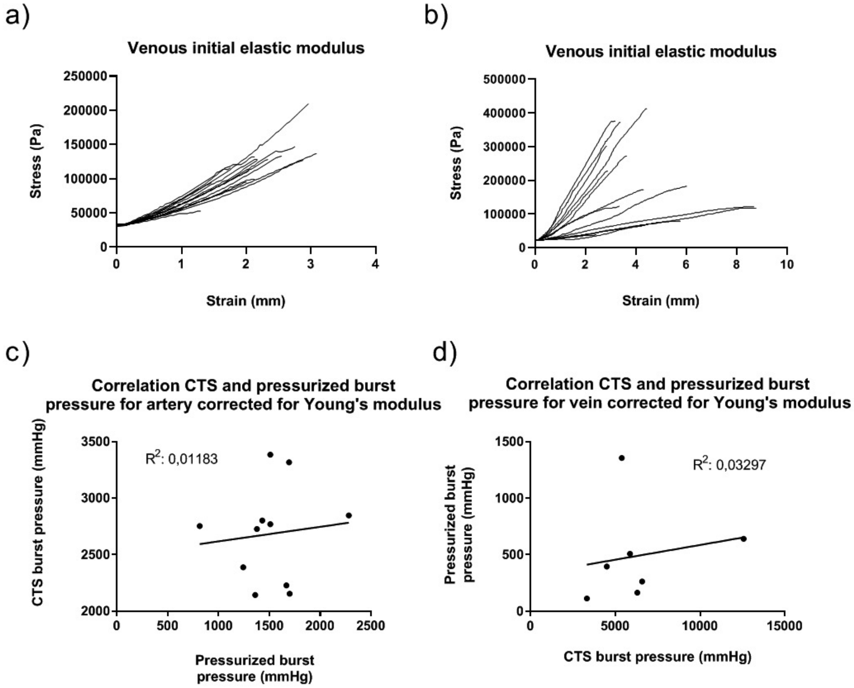
Supplementary materials



Supplemental figure 1. A custom made cutting device to assure each piece is cut precisely to the correct length



Supplemental figure 2. a + b) the initial elastic modulus of the stress-strain curves of the arterial and venous samples. c + d) the adapted correlation between the CTS and pressurized burst pressure for arterial and venous samples.