Experimental evidence of the compressibility of arteries

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Abstract

A definitive answer to the question whether artery walls are incompressible is to our opinion not yet categorically provided. Experimental-based evidence on the level of compressibility in artery walls is not easily achieved because of the difficulties associated with measurement of very small differences in volumes under physiological pressure in these biological tissues. Past experiments aimed at addressing the question considered different species, different arteries, the experimental devices were not accurate enough and a statistical analysis of the results was missing.

A precise experimental device together with a thorough testing protocol, a careful selection of arteries and a statistical analysis is presented for a definitive evaluation of the artery wall compressibility. We provide experimental evidence that in saphenous and femoral porcine arteries under physiological pressure range a relative compressibility of 2 - 6% is observed. The pre-assumption of incompressibility in many phenomenological constitutive models of artery walls should probably be re-evaluated.

Keywords: Artery, compressibility, experimental observations

[1]Equal contribution.

1. Introduction

The biomechanical response of the artery tissue is a topic of major importance and intensive research, and several phenomenological constitutive models have been proposed for the prediction of their passive response. The common models are hyperelastic (uniquely determined by a strain energy density function, SEDF, Ψ) pre-assuming the incompressibility of the artery tissue under physiological conditions, see e.g. the recent review [13]. This assumption is based on the argumentation that the artery wall is comprised mostly of water, which is considered incompressible.

In this case, the SEDF contains only an isochoric (volume preserving) part associated with the elastic matrix, and a part associated with the two families of the collagen fibers. For example in [12] the following decomposition of the SEDF was suggested:

$$\Psi = \frac{\mu}{2}(\mathrm{I}_C \mathrm{III}_C^{-1/3} - 3) + \Psi_{fibers}, \tag{1}$$

where I_C and III_C are the first and third invariants of the right Cauchy-Green tensor C. The material parameter μ is associated with the shear modulus at infinitesimal strains (or ground-state).

However, if in reality the artery is compressible or even slightly compressible, the SEDF has to be enriched by another volumetric term that must account for it. One such option is [20]:

$$\Psi = \frac{\mu}{2}(\mathrm{I}_C \mathrm{III}_C^{-1/3} - 3) + \frac{\kappa}{2}(\mathrm{III}_C^{1/2} - 1)^2 + \Psi_{fibers}, \tag{2}$$

with the material parameter κ being associated with the bulk modulus at infinitesimal strains (or ground-state).

The stresses resulting from SEDFs under the assumption of incompressibility are significantly different compared to those obtained under the slightly compressible assumption (see e.g. [18]). As an example, slight compressibility (volume-change of up to roughly 3%) resulted in circumferential stresses in the arterial wall up to 100% higher compared to the incompressible case for the physiological pressure of 100 mmHg as documented in [21, 8]. For almost-incompressible materials $\varepsilon = \mu/\kappa$ is usually a small parameter, but not zero, and the interested reader is referenced to a detailed analysis of such cases for $\varepsilon \ll 1$ in [14, 15].

A definitive experimental-based answer on the level of compressibility in artery walls is therefore of biomechanical interest but not easily answered because of difficulties to measure accurately very small differences in volume under physiological pressure.

Numerous publications in the past three decades report on experiments aimed at measuring the "incompressibility" in arterial walls. The reported results, however, differ significantly due to the variation in methods, number and quality of examined arteries and lack of a systematic experimental protocol and statistical evaluation of the experimental observation.

We first provide a critical review of these past experiments: Lawton [17] experimented on dog aorta, investigating the thermo-elastic behaviour under uniaxial loads. In part of his study he used a dilatometer to determine the volume-change of the aorta during uniaxial tension. The apparatus (shown in Fig. 1) included a brass tube in which the specimen was submerged in saline solution while measuring the change in liquid level during extension. Lawton implemented a thermodynamic theory to calculate the tension force taking the change in volume into account. A small change in volume (less than 1%) was reported being within the range

of experimental error, resulting in the conclusion that the artery wall is incompressible. Carew et al. [3] combined analytical and experimental approaches to

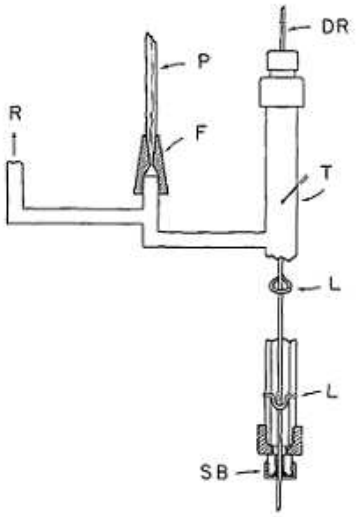

Figure 1: Schematic drawing of the dilatometer [17]. The specimen was connected to the loops (L) and the change in volume was measured by the liquid extruded into the pipet (P).

assess the compressibility of the artery in terms of the bulk modulus κ. Cylindrical segments of various dog arteries submersed in a control volume filled with physiological solution were tested. The arteries were stretched to $3-10\%$ of their initial length, and inflated to pressures of $167-197$ mmHg. Then the saline solution within the specimen was allowed to transfer to the glass flask, allowing the inner and outer pressures to equilibrate. The change in water level was a direct representation of the change in tissue's volume. Bulk modulus was calculated using the axial, circumferential and radial stresses, measured by the inner pressure and axial load. The testing device is shown in Fig. 2. It was concluded that the volume-change in dog aortas is negligible, and generally under 1%. Tickner and

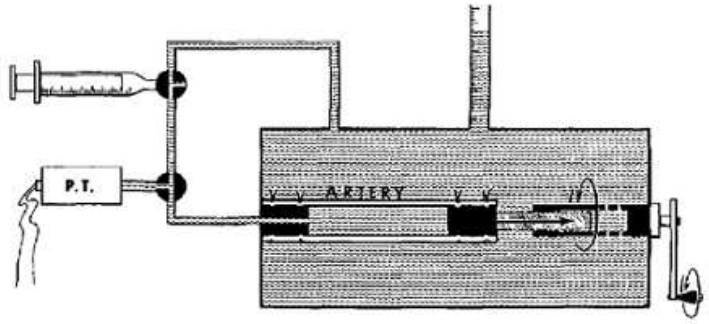

Figure 2: Schematic drawing of the dilatometer as shown in [3]. Saline was pumped into the artery, and stretched to $3-10\%$ of its initial length. The capillary tube shown at the top was directed horizontally, thus the water leaving the glass flask through the tube did not affect the outer hydrostatic pressure.

Sacks [19] reported the highest volume-change in comparison with other studies, $20-35\%$ for various human arteries. They placed the tested specimens vertically, sealed with a small weight at the bottom, designed to stretch the artery and seal it (see Fig. 3). The arteries were inflated by air and wall thickness determined by X-rays so the change in volume was computed for different inner pressures and axial loads. A decrease in volume of up to 35% for an inflation pressure of 300 mmHg was reported. The dry environment and air used for inflating the artery may had resulted in an extreme volume change. Chuong and Fung [5] experimented on rabbit aortas, cutting the artery open into a rectangular segment and compressing it (Fig. 4) while measuring the amount of liquid extruded from it (assumed to be the volume-change of the tissue). The compressibility for four aorta specimens was estimated in the range of $0.5-1.26\%$ per 10 kPa of compression stress, in the range of $0-30$ kPa. The maximum change in volume in the results

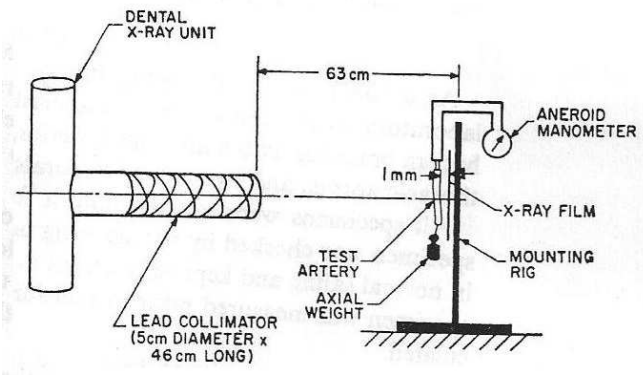

Figure 3: Schematic drawing of the test system shown in [19].

presented was about 3%, for 30 kPa of compression stress, (equivalend to 225 mmHg). The effect of the arterial volume-change was reported to be negligible and unaffect the computation of stresses. It was not mentioned, however, if the evaporation of water from the glass slip was taken into account, a parameter which may influence the results, even in small quantities. In addition the small number of specimens presented is problematic for definitive conclusions. Furthermore, the volume-change not associated with water extrusion was not discussed, making the assumption that the change in volume was solely represented by the liquid extruded from the tissue during uniaxial compression. It is also possible that due to the slicing of the artery a non representative amount of liquid was extruded.

Girerd et. al [10] used ultrasonic techniques to measure volume-changed on samples of human mammary and radial arteries under physiological pressure (175 mmHg). The inner diameter and wall thickness were measured, showing inconsistent results. Although the results imply a change of 2-15% in the artery wall area, the authors concluded that no significant change during pressurization occurred and the artery volume is conserved. The small amount of tested specimens

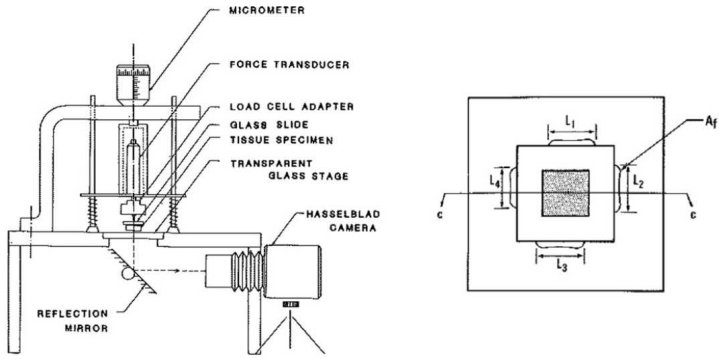

Figure 4: Schematic drawing of the system presented in [5]. The system for compressing the arteries (left) and a magnification of the glass slip during experiment (right). Tissue was loaded by a compressive force applied on the glass cover slip.

and the large diversity in the results shown makes it difficult in our opinion to conclusively reach any conclusion. Ultrasound methods were also utilized in a later study by Boutouyrie et al. [2]. The researchers performed in-vivo measurements of the cross-section area of human carotid artery walls. They reported a decrease of 4.7 % in the cross-sectional area of healthy carotids.

Two similar studies by Faury et al. [9] and Chesler et al. [4] used transillumination, measuring the wall thickness change in mice arteries in-vivo and in-vitro. Both studies reported a change of roughly $15-20\%$ in tissue volume in the physiological pressure spectrum. It is likely that the high compressibility levels measured were partly due to the transillumination techniques, and the large measurement-error reported at low pressures in [9].

Recently, Di Puccio et al. [6] presented a test system based on the principle shown in [3], in an attempt to recreate physiological conditions for an accurate determination of arterial compressibility, see Fig.5. The test rig was comprised of

a transparent PMMA tube filled with saline containing the examined specimen, which was connected at both ends to sealed tubes. The inner volume of the artery and tubing was separated entirely from the rest of the PMMA tube - defining the outer volume. The inner volume was pressurized with an angioplasty syringe to a pressure of up to $0.1MPa$, causing the tested specimen to inflate and thus pushing the saline in the outer volume through a horizontal capillary. The degree of volume-change in the tissue was measured by comparing the amount of removed saline to the amount of saline pumped into the artery. A drop of mercury was inserted to the tubing from the syringe to the inner volume, serving as a marker to indicate the inner volume-change. In [6] only two experiments on porcine arteries were reported: one specimen showed a change of up to 6% in volume while the other $\sim 20\%$.

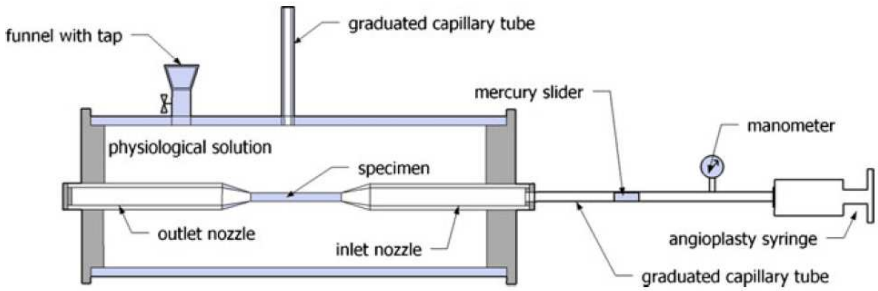

Figure 5: Schematic drawing of the system in [6]. The specimen is inside the PMMA tube filled with saline, surrounded by saline.

A summary of tested arteries and compressibility range reported is provided in Table 1. One may observe that different species and different arteries were considered, that it is unclear whether several specimens were taken from the same subject and that statistical means of analysis of the results is missing. In addition

in most cases the experimental protocol and experimental errors are not provided. These drawbacks suggest that any conclusions on the compressibility of arterial walls may be misleading, and a precise device together with a thorough testing protocol, a careful selection of arteries and statistical analysis is required for a definitive evaluation of the artery's compressibility. We provide therefore a detailed description of the experimental system and all measures undertaken to assure the precise measurements as well as the detailed experimental protocol used to assess precisely the relative volume change.

2. Methods

A new experimental device was designed and constructed based on [6]. The artery wall's volume-change is of orders of micro-liters therefore the experimental device had to be sensitive and well calibrated. The lumen of an artery was water filled at increased pressure that resulted in deformation of the artery and the extrusion of water surrounding it through a small-diameter tube. The difference in the volume of the extruded water and the volume inserted into the artery's lumen was the change of the artery's wall volume.

2.1. The experimental device

Typically arteries having an outer diameter of $3-6$ mm and a length of about 20 mm were considered. The initial tissue volume of a specimen of this size was roughly 200μL (micro liter). To obtain an internal pressure of about 300 mmHg it was required to pump approximately 300μL of liquid into the artery's lumen. The *test chamber* (see Fig. 6) was a PMMA tube, with an internal diameter of 32 mm, sealed at both ends by rigid plastic caps. System's components were manufactured from materials that do not deform under minor pressures experienced in

Table 1: Summary of experimental data from past studies.

Publication	Year	Animal	Artery	# Spec.	V_0 (μL)	$\Delta V/V_0$ %	Method	Notes
Lawton [17]	1954	Canine	Aorta	NS	1000	Negligible	Extension tests	
Carew et al. [3]	1968	Canine	Various	31	426 – 2933	Negligible	Static inflation test	
Tickner & Sacks [19]	1967	Human	Various	9	NS	Up to 35%	X-ray measurements	Usage of air
		Canine	Thoratic aorta	2				
			Femoral artery	2				
Dobrin & Rovick [7]	1967	Canine	Carotid	NS	NS	Negligible	X-ray measurements	
Chuong & Fung [5]	1984	Rabbit	Thoratic aorta	4	41.9 – 53.9	Negligible	Strip compression	
Girerd et al. [10]	1992	Human	Mammary, Radial	6	$\sim$ 32.3 (for 1 cm length)	Negligible	US measurements	inconsistent
Faury et al. [9]	1999	Mouse	Various	14	NS	15 – 18%	Transillumination	
Boutouyrie et al. [2]	2001	Human	Carotid	15	103.5 (for 1 cm length)	$4.7 \pm 2.7\%$	US measurements	
Chesler et al. [4]	2004	Mouse	Left pulmonary	12	~ 0.3	15 – 20%	Transillumination	
Di Puccio et al. [6]	2012	Swine	Renal artery	2	78.5 – 251.3	6 – 20%	Dynamic inflation test	

NS - not specified. US - ultrasound

experiments, and did not react with any physiologic liquid. Through the center of each cap a hollow small diameter metallic tube was inserted, enclosing the main part of the pressurized volume V_p. One cap was perforated at two locations by medical needles, that were connected to plastic tubes. These needles were used to fill the test chamber with water and to allow the exit of air bubbles trapped inside the test chamber.

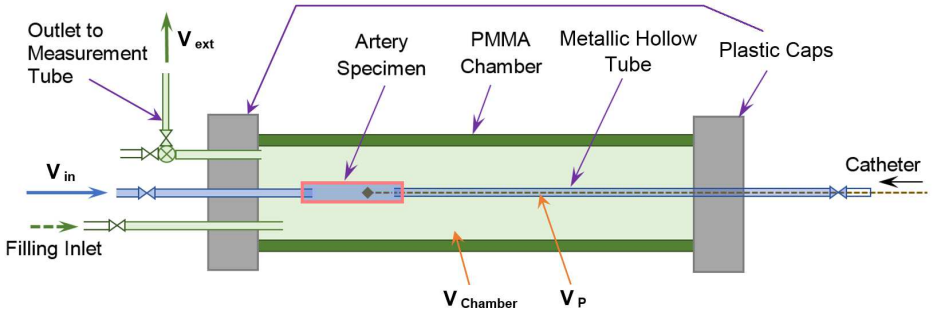

Figure 6: A schematic figure of the testing device.

The artery specimen was tied to the metal hollow tubes inserted from either side of the test chamber, using surgical thread, and was checked for any leakage prior to the experiment (by inflating it with blue medical pigment (Methylene blue) so to make sure no leakage is visible) as shown in Figure 7(Top). The long metallic tubes (extruded from the un-perforated cap) could had been moved, so that the specimen was easily secured to both tubes before it was sealed in the test chamber and the outer volume isolated. After the specimen was securely tied it was ready so that pressure in V_p could had been monitored (comprising of the artery lumen and inner volumes of the metal tubes). A pressure sensor was inserted by a catheter through the long tube into the tied artery. To the short metallic tube a syringe pump was connected that inserted colored water V_{in}, in-

flating the artery. One of the needles perforating the cap, after filling the test chamber, was used as the exit for the extruded water from the PMMA tube (see Figure 7(Bottom). The extruded water went into a horizontal thin transparent tube having an inner diameter of 0.84*mm*, so that the volume of the extruded water V_{ext} could had been measured by monitoring the movement of the end surface of the water. This allowed for a clear sensitive observation and did not add errors by capillary forces. The open end and horizontal placement of the thin tube maintained a constant pressure in the test chamber, so hydrostatic pressure could not had been accumulated. It is important to emphasize that the pressure in the

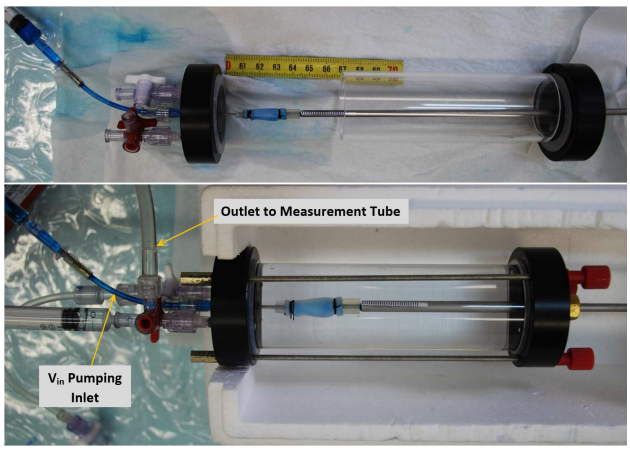

Figure 7: The experimental system while the artery specimen is attached (Top), and after the chamber is filled with water and volume-pressure experiment is performed (Bottom).

chamber surrounding the artery is unchanged as a result of the increased pressure in artery's lumen (the chamber is open through the measurement tube). The transparent chamber allowed one to monitor the specimen during experiment, to ensure there were no leaks from the artery lumen and that no air bubbles formed in the chamber $V_{chamber}$. To release any dissolved gases from the water, boiled

water at room temperature was used. The colored water was pumped into the inner volume to allow the identification of any leaks. A NE-1000 programmable single syringe pump by New Era Pump Systems, Inc. together with a 1 milliliter glass syringe were used to pump the colored water into the inner volume. This allowed to pump a minimum amount of 1μL at a time. The accuracy of the amount of liquid inserted by the syringe pump was checked by weighing distilled water extruded from the syringe using analytical scales, with a measurement error of 0.1 milligram (0.1μL). The volume of water extruded from the chamber's volume was measured. Each μL was equivalent to 1.795 mm of liquid in the transparent tube (factor found experimentally by using the device without a specimen). The inner pressure was measured by a catheter pressure sensor and a SmartMap monitor made by VOLCANO, with a 0.38*mm* wire, providing a pressure measurement in the range of -39 – 399 mmHg and an accuracy of $\pm$3%. The setup of the testing device and all its components are shown in Fig. 8.

2.2. Calibration of the experimental device

Several verification procedures were performed to ensure the accuracy of the experimental observations:

- Leakage in the experimental device was checked by inserting water into the experimental chamber at a pressure of 250 mmHg without an artery. No leaks were present.

- The inserted fluid volume (by syringe pump with a glass syringe) was verified by an analytical scale. The measurement error was less than 0.1μL, (less than 0.5% of the volume for a pressure of 100 mmHg in the smallest specimens). No influence of pressure on the accuracy of the inserted fluid

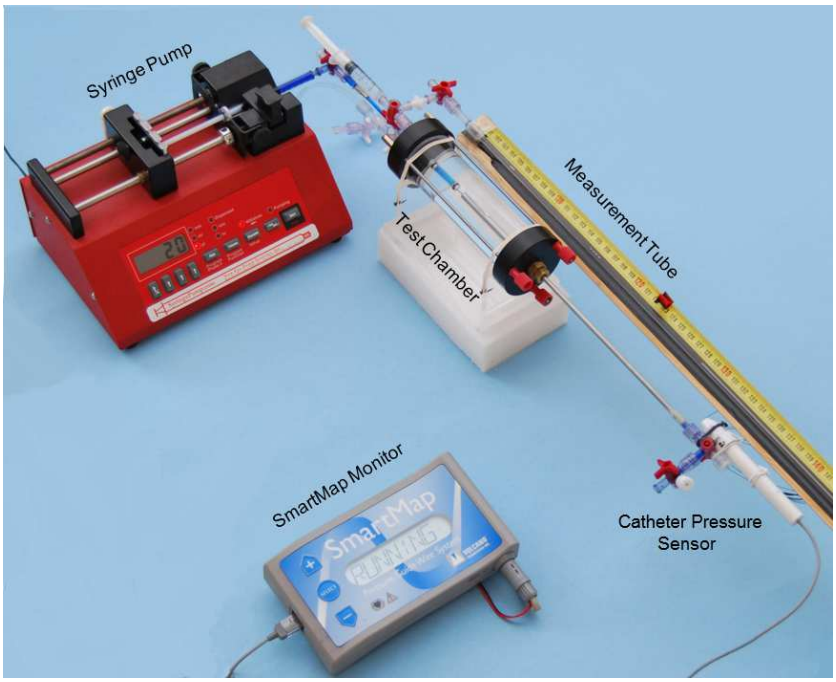

Figure 8: Photograph of the testing system components.

was observed. Also increasing the outer pressure (connecting the outlet to a tube filled with water at different heights) had no effect on the results.

- The measurement accuracy of the volume of water driven by the syringe into the artery was determined by connecting the syringe directly to the measurement tube, bypassing the test chamber. A relation of 1.795 mm per 1 μL pumped by the syringe was found. Thus the error of the output volume is 1 mm or $\frac{1}{1.795}\mu$L, since we use a millimetric scale in experiments.

- The pressure measurement by the catheter sensor was verified by connecting the closed system to a long tube, filled with water and opened to the air. The pressure was reset to zero and the tube was then elevated to different heights.

Given the height, hydrostatic pressure in the closed system was calculated and compared to the sensor measurement having a ± 1 mmHg error.

- The experimental device may be susceptible to temperature change resulting in volume change. To prevent this problem, the device was isolated from the environment by styrofoam to maintain a constant temperature, and experiments were performed within a very short time-frame. So to be further on the safe-side, during this time-frame the temperature in the lab was unchanged.

- Artery volume was measured at the end of each experiment, assuming no irreversible change during the experiment. Average wall thickness was measured under a glass slide by a height indicator, and the surface area was computed by photograph analysis after the artery was flattened on a mm-scale. The error of the height indicator was 0.01 mm, and the error in the area measurement was less than 2%. These errors accumulated to a total error of up to $\approx 5\%$ in the tissue volume calculation.

- Control experiments were performed on incompressible latex rubber tubes of several sizes to verify volume change. These were performed exactly as the experiments on the arteries. Since rubber volume's change is known to be negligible at these pressures, any measured change in volume was contributed as inaccuracies of the testing device. Results were used as a calibration for the volume-change measured in the artery wall, see elaboration in section 3.

- It was noticed that immediately after each volume dose was inserted, the pressure had a peak value and thereafter slowly and continuously decreased.

This phenomenon did not occur in rubber specimens. Therefore, the phenomena was attributed to the relaxation of the arterial wall. To avoid errors, the pressure was measured immediately after each dose was introduced, measuring the peak pressure value.

2.3. Experiment protocol

Porcine femoral, saphenous and one carotid arteries were extracted from female pigs sacrificed for medical research not associated with the vascular system. Prior to excision, heparan sulfate was given to the sedated animal to prevent blood clots in the arteries. The excised specimens were kept in saline solution at $2-4^{\circ}$ C for at most 24 hours. The arteries were skeletonized (connective tissue removed around the arteries), cut to an appropriate length without bifurcating branches and attached to the metallic tubes by surgical thread. Colored water was then inserted into the lumen to remove trapped air and to check for leaks (by increasing the inner pressure to a value of ≈ 200 mmHg). If leakage was detected the artery was discarded, otherwise the test chamber was carefully sealed and filled with clear water, while allowing air bubbles to be pushed out.

A preconditioning protocol was then followed by determining the amount of inlet water ($V_{in,300mmHg}$) that produced 300 mmHg pressure within the lumen, and repeatedly pumping it in and out until peak pressure in consecutive cycles remained constant. Following preconditioning, increments of a tenth of $V_{in,300mmHg}$ were inserted at a pumping rate of $400\frac{\mu L}{min}$. Immediately after each dose, the pressure and the water level in the measurement tube were recorded. Denoting water volume pumped into the artery by V_{in} and the volume extruded from the test chamber by V_{ext}, the tissue volume-change calculated was $\Delta V = V_{in} - V_{ext}$ and relative volume-change was $\frac{\Delta V}{V_0}[\%]$ where V_0 is the initial volume of the examined spec-

imen. Each experiment was repeated 3-4 times. Photographs of a typical artery during experiment is shown in Fig. 9. Following the experiment each artery was

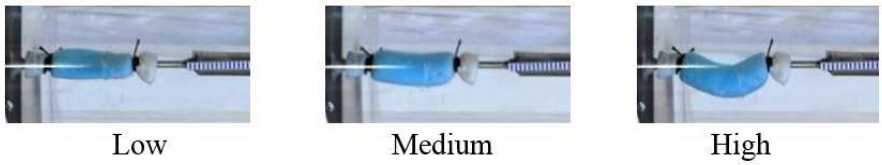

Figure 9: A typical artery during experiment under low, medium and high inflation pressures.

placed on a a millimetric grid, and measured by photograph analysis. Edges of the artery outside the securing strings were trimmed, and the specimen was cut along its length. A micro slide was placed on the flattened artery and photographed. Wall thickness was measured by a height indicator (the force exerted by the height indicator is minor and applied over the entire artery's surface, so it did not influence the measured thickness). Volume was determined by area and wall thickness. An example of the photographs is presented in Fig. 10.

Pressure measurements started at 0 mmHg. However analysis of results was performed starting at 50 mmHg which is considered the lower limit of a physiological value (normal porcine blood pressure is $\approx 80/130$ mmHg [11, 1].). Because the femoral and saphenous arteries are considered relatively large blood vessels, and since the normal blood pressure in these large vessels is $\approx 80/130$ mmHg, then the physiological pressures we consider varies between ≈ 50 mmHg and $\approx 250-300$ mmHg representing an intense activity. These are the two limits considered in our experiments. Relative volume-change was calculated in relation to the volume at a pressure of ≈ 50 mmHg (the exact value varied between experiments).

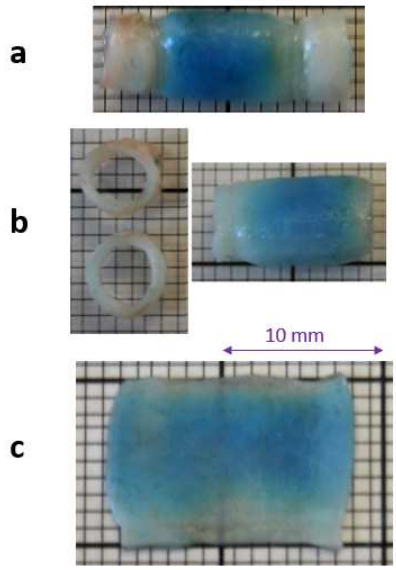

Figure 10: Photos for V_0 determination of a typical artery. a - The entire specimen. b - With the edges cut (right), and a cross-sectional picture of the removed edges (left). c - Spread out to measure the area. The blue color of the tissue is a result of the methylene blue used in our experiments.

2.4. Specimens

Twelve specimens were collected, mostly harvested from a long segment starting at the external iliac, through the femoral bifurcation and ending at the saphenous, see Table 2.

Rubber specimens made of two different materials, different lengths and several diameters were considered as summarized in Table 3. These are of dimensions that were as close as possible to the artery segments and were used to identify the "system's overall bias from incompressibility". Since rubber is stiffer than arteries, smaller volume doses were used for same levels of internal pressure. Therefore rubber tubes longer than the typical lengths of artery specimens were tested,

Table 2: Summary of the arteries (all female porcine).

No.	Artery	L mm	D_0 mm	WT mm	V_0 mm^3	Weight Kg	Age Mo.
1	Femoral	11	3.0	0.36	35.6	32	3
2	Saphenous	19.5	2.0	0.36	45.6	84	6
3	Femoral	18	3.7	0.43	89.4	30	3
4	Saphenous	14.5	2.5	0.62	58.4	65	6
5	Saphenous	11	1.9	0.76	54.3	65	6
6	Saphenous	27	2.5	0.51	75.7	80	8
7	Femoral	10	5.0	0.52	72.8	60	6
8	Femoral	22.5	5.5	0.61	219.6	75	8
9	Femoral	9	5.1	0.5	67.5	N/A	N/A
10	Saphenous	11	1.9	0.41	26.7	N/A	N/A
11	Carotid	18	4.0	0.59	116.8	47	4
12	Femoral	16	6.0	0.69	209.8	169	N/A

resulting in larger values of initial volume.

2.5. Data analysis

All data was statistically analyzed by SPSS version 21 (SPSS IBM, New york, USA). The influence of pressure on relative change in volume was analyzed by a multivariate analysis (linear regression) with logarithmic pressure and logarithmic relative change in volume, with a restricted maximum likelihood (REML) estimation. We considered artery initial volume, length, wall thickness and outer diameter as influencing parameters. The model accounted for clusters created by an artery. The clustered structure of the data had to be accounted for due to cor-

Table 3: Summary of the control specimens.

Spec. No.	Material	Length mm	D_0 mm	WT mm	Volume mm^3
i	Silicone-Rubber	52	5.0	0.9	602.8
ii	Latex Rubber	169	3.7	0.995	1402.6
iii	Latex Rubber	338	3.7	0.995	2805.1
iv	Latex Rubber	670	3.7	0.995	5560.5
v	Latex Rubber	20.5	5.6	0.97	291.7
vi	Latex Rubber	41	5.6	0.97	583.5
vii	Latex Rubber	94	5.6	0.97	1337.7

relation assumed between observations belonging to one artery. Specification of a clustered structure in the regression model yielded an unbiased statistical estimation. The model used in this analysis was linear with robust standard errors. A 95% confidence interval level was set for all tests, with a p-value< 0.05 considered significant.

3. Results

Two of the arteries (arteries 1 and 10) were excluded from our results and analysis due to several problems detailed in Appendix B. In addition, all data points over 300 mmHg were omitted so to maintain data only in the physiological range. The excluded data is nevertheless presented in same appendix. The results from the remaining artery experiments are shown in Fig. 11. The points in the graph are the averages for each specimen whereas the raw results are given in Appendix B. Arteries of larger initial volume show less volume-change. For

the physiological normal pressure range $\approx 50-200$ mmHg the relative volume-change is $\approx 2-6\%$.

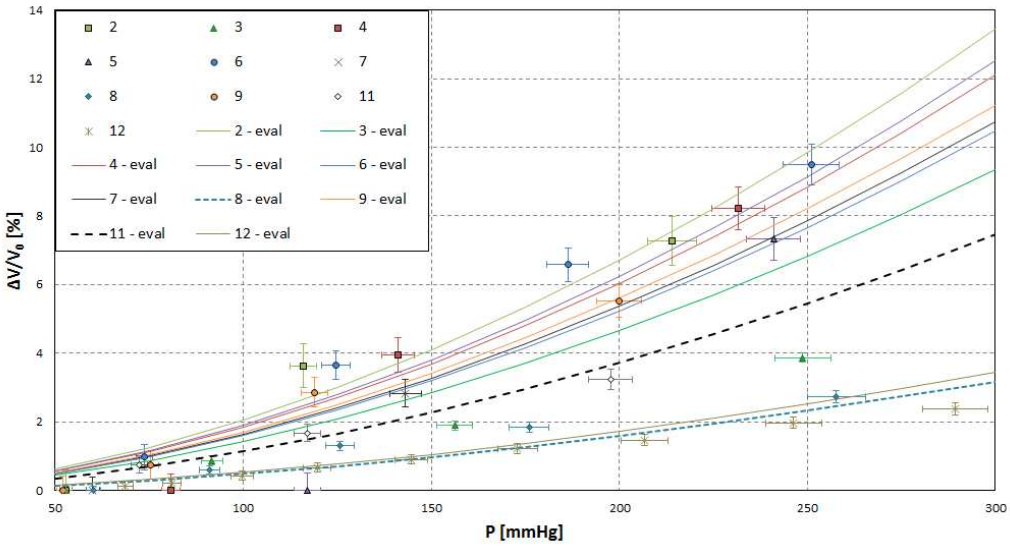

Figure 11: Relative volume-change for arteries. Data points are averages for each artery. The solid lines are evaluated by (3).

Experimental data for rubber specimens is shown in Fig. 12 (note the different scale compared to Fig. 11).

3.1. Statistical analysis

Among all relations that were analyzed statistically a multi-linear dependance was found between the logarithm of the relative volume-change $ln\frac{\Delta V}{V_0}$ to the logarithm of the pressure lnP and volume of the specimens V_0. The following relationship was determined:

$$\ln\frac{\Delta V}{V_0} = a\cdot\ln P + b\cdot V_0 + c \quad \Rightarrow \quad \frac{\Delta V}{V_0} = P^a\cdot\exp(b\cdot V_0)\cdot\exp(c) \tag{3}$$

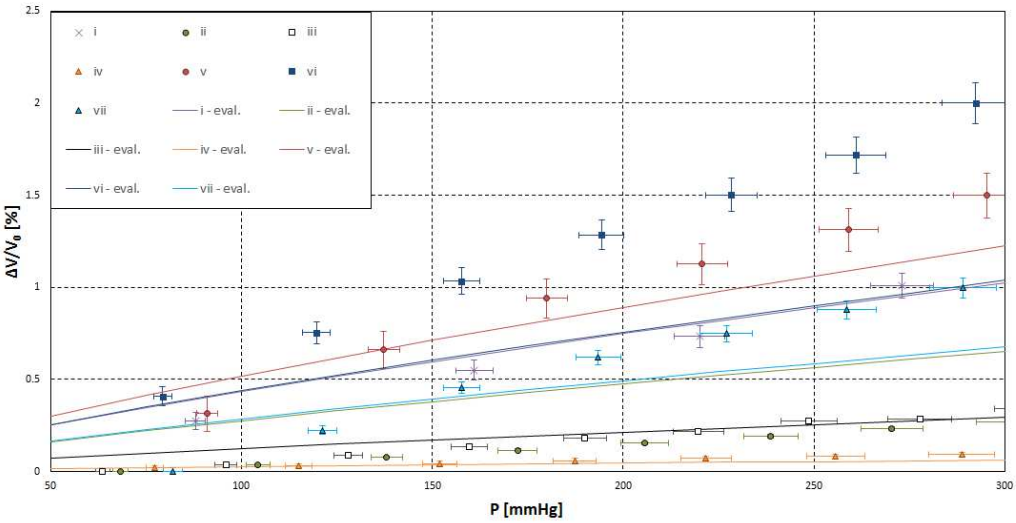

Figure 12: Relative volume-change for rubber specimens. Data points are averages for each rubber specimen. The solid lines are evaluated by (3).

Table 4: Parameter estimates from statistical analyses. A - arteries, R - rubber specimens.

Parameter	Estimate	Std. Err.	p <
c_A	-6.798	0.464	0.000
b_A	-0.008	0.002	0.001
a_A	1.714	0.086	0.000
c_R	-4.086	0.954	0.000
b_R	-5.66×10^{-4}	1.18×10^{-4}	0.005
a_R	0.781	0.178	0.000

where a, b, c are the determined constants by statistical analysis, see Table 4. The experimental data together with $\Delta V/V_0$ vis P relations by (3) are presented in Figs. 11 and 12).

The dependency of the relative volume-change on outer diameter, artery wall-thickness and length was also investigated statistically. It was found that these parameters have no influence on the relative volume-change (with $p < 0.05$).

Since rubber tubes are assumed to be incompressible, then any compressibility detected by the experimental device for them is associated to experimental errors. Thus, we may subtract the measured volume-change of the rubber tubes from the volume change of the arteries, so to eliminate any sources of false volume change:

$$\frac{\Delta V}{V_0} = P^{a_A} \cdot \exp(b_A \cdot V_0) \cdot \exp(c_A) - P^{a_R} \cdot \exp(b_R \cdot V_0) \cdot \exp(c_R) \qquad (4)$$

We plot $\frac{\Delta V}{V_0}$ vis P according to (4) in Fig. 13. Notice that the difference between arteries is because of the total volume of the tissue. Some of the larger arteries show a negative volume-change in the low pressure regime. This is an artifact of (4) that will be addressed in Section 4.

Of course that in reality, one does not have access to the volume of the artery but to its diameter. In Appendix A we provide a similar statistical analysis where we consider D^2 as one of the independent variables instead of the initial volume.

4. Discussion

A high-precision experimental system has been developed to measure the compressibility of arteries at physiological pressures. Preliminary experiments on nine porcine arteries were performed until all precision issues and problems were addressed and corrected - these were not accurate enough and have been discarded (relative volume change in these experiments was relatively high and similar to the values reported in [6]). After the experimental system has been finalized and calibrated, twelve more porcine arteries were tested, out of which two

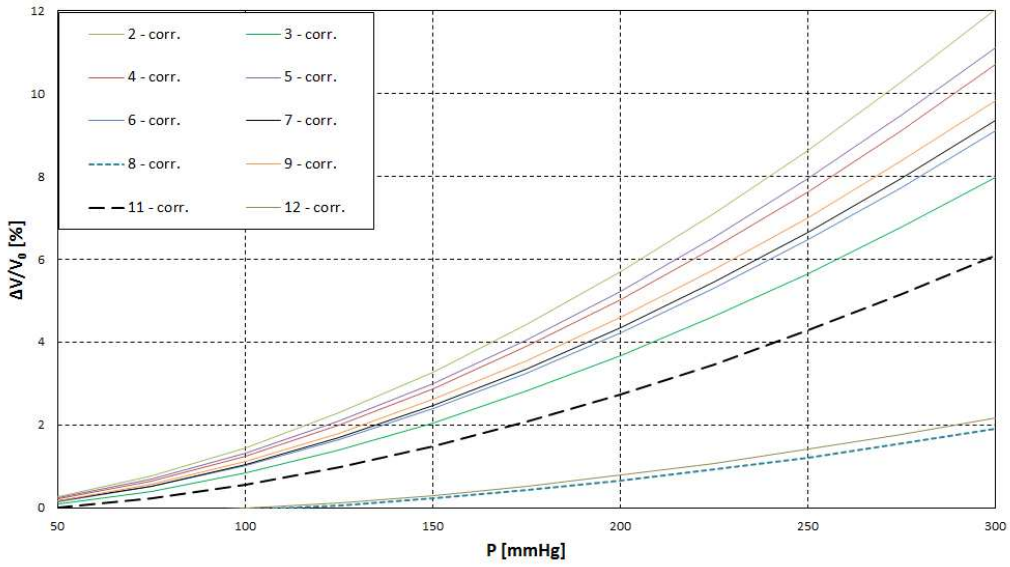

Figure 13: Corrected relative volume-change in arteries calculated by (4).

were discarded and the other ten analyzed.

Statistical analysis of the test measurements conclude that arteries *are compressible*. A relative compressibility of 2 - 6% in the physiological range ($\approx$ 50 – 200 mmHg) was evident. Phenomenological and semi-phenomenological constitutive models that aim at representing the arterial wall have to address the compressibility, because a slight relative volume-change at the reported range can cause large changes in the circumferential stresses ($\sigma_{\theta\theta}$) in the arterial wall. The assumption of incompressibility in artery simulations is therefore invalid and should be re-evaluated. Extracting the bulk modulus accurately for porcine arteries from the results obtained from our work was impossible because the internal diameter of the artery in the inflated state could not have been measured to a satisfactory accuracy. Advanced ultrasound techniques will be introduced in future

experiments to allow the measurement of the artery's internal diameter with sufficient accuracy to allow the computation of the bulk modulus and its relation to the shear modulus.

The statistical analysis indicated that artery's volume (V_0) is a significant parameter correlated to the relative volume change (besides of course the pressure which is the most significant parameter). Other parameters (initially considered in our statistical analysis) showed a less significant connection to the relative volume change. A statistical model with more that one parameter resulted in non significant results probably because V_0 is related already to D_0, artery wall thickness and L.

Figures 11 and 13 show that smaller arteries (with a smaller V_0) demonstrate a significant higher relative volume-change compared to the larger arteries. This phenomena may be related to the tissue properties (probably affected by the artery's dimension). It was anticipated that in larger diameter arteries, higher hoop (or circumferential) stresses would be obtained for the same internal pressure. As a result, deformation is larger and the volume-change was anticipated to be larger. This effect was noticed in rubber tubes but not in artery specimens. A more thorough analysis taking into consideration wall thickness and internal diameter is planned to further investigate this issue.

Rubber is considered incompressible since its bulk modulus κ is significantly larger than the shear modulus. For the relatively low pressures of our experiments it is reasonable to assume that no measurable volume-change of the rubber is obtained. We therefore attribute any measured relative volume-change in the rubber specimens to experimental errors resulting from inaccuracies not considered (indeed the "relative volume changes" in these tubes was significantly lower com-

pared to the arteries). We thus subtracted the predicted relative volume-change in rubber tubes from the relative volume change of the arteries (equation (4)). As a result, for two of the arteries a very small negative volume-change was obtained at the low pressure range, which is clearly an artifact that may be discarded.

Compared to past studies on the topic, the experimental device reported here and the followed protocol allowed an accurate determination of the relative volume change in arteries. The statistical analysis substantiated the evidence of small, but not negligible relative volume-change in arteries at physiological pressures. Hyperelastic constitutive models that are frequently used in the literature assuming incompressibility of arteries should probably be revisited and predicted stresses re-examined.

Several limitations are associated with the current study: a) The arteries were immersed in water and inflated by water instead of a physiological liquid as Krebs solution [16]. Nevertheless, one additional experiment was performed using Krebs solution that showed same results as with water. b) Any volume change in the artery wall as a result of diffusion of liquids in and out of the artery wall could not have been measured by the presented experimental device, c) The inner diameter of the arteries could not have been measured during inflation by our experimental device. Thus κ/μ could not be quantified. d) Peak pressures are observed immediately after the quantum process in which we introduce V_{in} into the artery. Because we only control V_{in} we could not control the rate of change of pressure. The rate of pressure change in most experiments was about 20 $mmHg/sec$ which is a bit slower than the physiological one which is about 40 $mmHg/sec$. Future improvements of the experimental system will consider different pressure rates.

Having demonstrated that the experimental device is precise and small changes (but yet not negligible) in volume do occur in arteries, a large number of experiments is planned using the device to enable the computation of the material properties including bulk modulus of a variety of arteries, thus to better estimate the stress state that develops in artery walls.

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Conflict of interest None of the authors have any conflict of interest to declare that could bias the presented work.

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Appendix A. Statistical analysis with the diameter as the dependent variable

In addition to the analysis presented in Section 3, we examined the dependence of the specimens' volume change on the internal pressure *and the initial diameter D*, instead of the volume. This is because in realistic situation the outer diameter is available but not the volume of the artery. Several statistical analyses were performed. Significant statistical measures were obtained by considering the square of the initial diameter (D^2):

$$\ln\frac{\Delta V}{V_0} = d\cdot\ln P + e\cdot D^2 + f \;\;\Rightarrow\;\; \frac{\Delta V}{V_0} = P^d\cdot\exp(e\cdot D^2)\cdot\exp(f) \qquad \text{(A.1)}$$

where d, e, f were determined by statistical analysis, see Table A.5. A plot of

Table A.5: Parameter estimated by statistical analyses - dependency on D^2. A - arteries, R - rubber specimens.

Parameter	Estimate	Std. Err.	p <
f_A	-6.973	0.493	0.000
e_A	-0.035	0.011	0.014
d_A	1.695	0.086	0.000
f_R	-7.688	1.046	0.000
e_R	0.115	0.021	0.003
d_R	0.769	0.178	0.000

the test results according to the current estimation is presented in Fig. A.14. Fig. A.15 shows the rubber results with same estimation. Notice that in this case the difference between specimens dependes of the initial diameter.

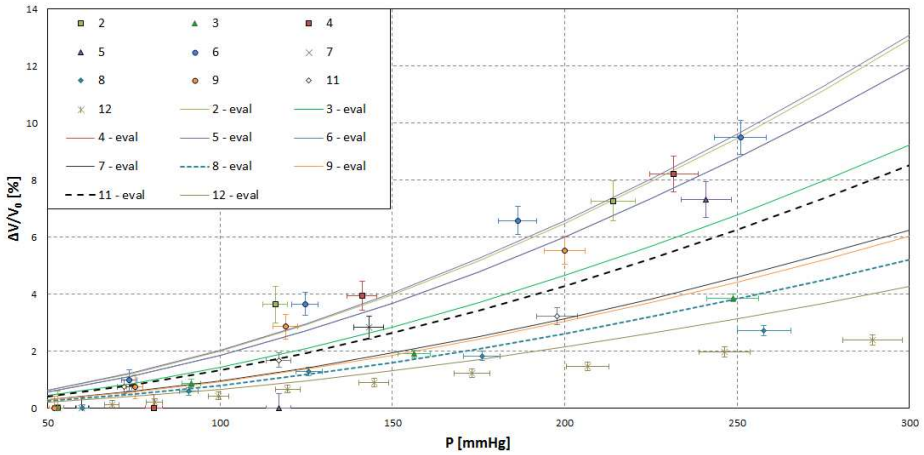

Figure A.14: Artery experimental data with the alternative estimation calculated by (A.1). The difference between approximations shown in solid lines is based on the initial diameter of each specimen.

Similarly to Section 3 we subtract the volume change of the rubber tubes from that of the arteries:

$$\frac{\Delta V}{V_0} = P^{d_A} \cdot \exp(e_A \cdot V_0) \cdot \exp(f_A) - P^{d_R} \cdot \exp(e_R \cdot V_0) \cdot \exp(f_R) \tag{A.2}$$

We plot $\frac{\Delta V}{V_0}$ vis P according to (A.2) in Fig. A.16.

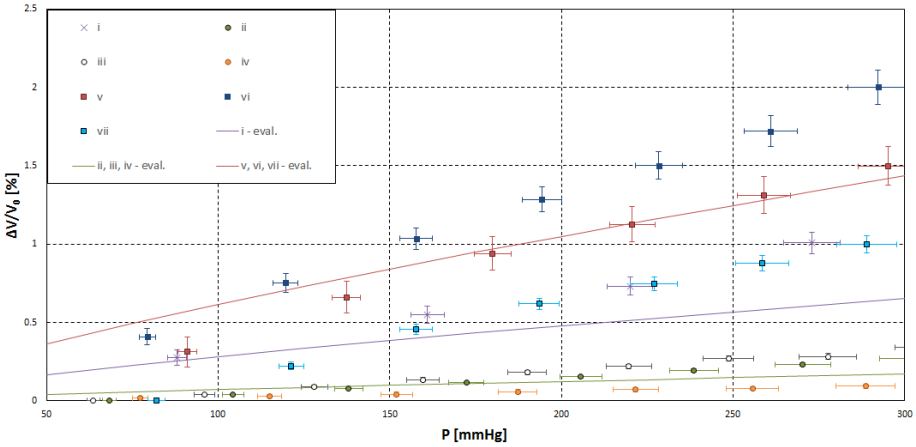

Figure A.15: Rubber experimental data with the alternative volume change estimation calculated by (A.1). The solid lines represent the approximations, notice that some solid lines are correct for more than one estimation - this is because some rubber specimens were of the same diameter but had different lengths.

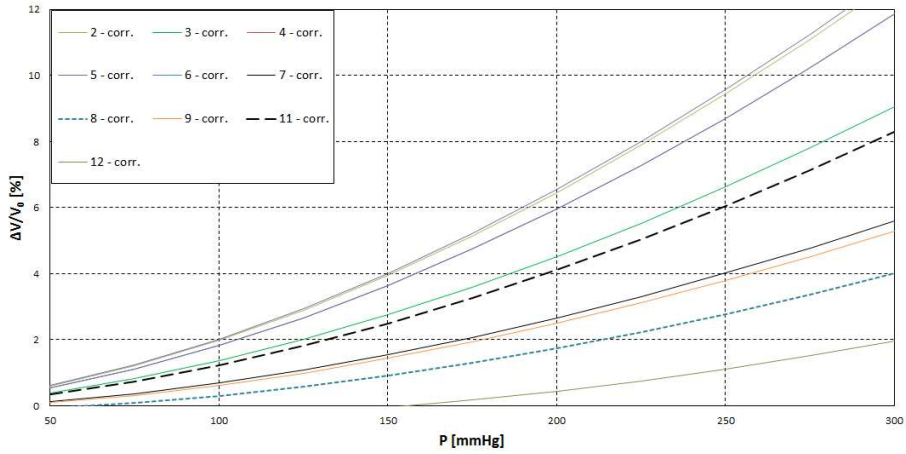

Figure A.16: Alternative corrected relative volume-change in arteries calculated by (A.2).

Appendix B. Experiment Data

Appendix B.1. Excluded arteries

Experiments were conducted on twelve arteries after the experimental device was thoroughly checked for accuracy. Two arteries were problematic and were excluded from the statistical analysis. The first excluded artery (spec. no. 1) was the first experiment we performed in the improved testing device, and the test protocol was not finalized. The experiment was not repeated more than once and there is a possibility that a leak was present and was not detected. The second excluded artery (spec. no. 10) was strangely deformed, leading to difficulty in determining the accurate initial volume of this specimen. As a result the volume-change calculated was extreme. In Fig. B.17 we show a photo of the the excluded artery (spec. no. 10). The results from both of excluded experiments are shown in the next subsection, along with the raw data of all experiments.

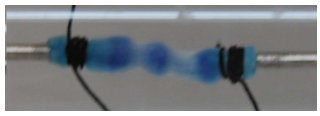

Figure B.17: Photo of spec. no. 10 during experiment. The shape of the outer surface is suspicious and led us to exclude the measurements of this specimen.

Appendix B.2. Raw data

In the following section we present the original calculated volume-change as measured during performed experiments. Tables B.6–B.17 detail the numerical data for all experiments.

Table B.6: Artery results for spec. no. 1

Test #	V_{in} μL	V_{ext} μL	P_{in} mmHg	$\frac{\Delta V}{V_0}$ %
1	0	0	36	0
	20	17.27	134	7.66
	40	32.31	382	21.57

Table B.7: Artery results for spec. no. 2

Test #	V_{in} μL	V_{ext} μL	P_{in} mmHg	$\frac{\Delta V}{V_0}$ %
1	0	0	53	0
	5	3.34	116	3.63
	10	6.69	214	7.26
	15	10.03	346	10.90

Table B.8: Artery results for spec. no. 3

Test #	V_{in} μL	V_{ext} μL	P_{in} mmHg	$\frac{\Delta V}{V_0}$ %
1	0	0	52	0
	20	18.94	91	1.18
	40	37.88	159	2.37
	60	55.71	250	4.80
2	0	0	49	0
	20	18.94	87	1.18
	40	37.88	146	2.37
	60	56.27	246	4.17
3	0	0	52	0
	20	19.50	90	0.56
	40	39.00	157	1.12
	60	57.38	253	2.93
4	0	0	59	0
	20	19.50	99	0.56
	40	38.44	163	1.74
	60	56.82	246	3.55
	80	75.77	383	4.73
Average			53	0
			91.75	0.87
			156.25	1.90
			248.75	3.86
			383	4.73

Table B.9: Artery results for spec. no. 4

Test #	V_{in} μL	V_{ext} μL	P_{in} mmHg	$\frac{\Delta V}{V_0}$ %
1	0	0	95	0
	5	1.67	176	5.70
	10	3.34	264	11.39
	15	6.13	342	15.18
2	0	0	68	0
	5	1.67	143	5.70
	10	3.90	233	10.44
	15	6.13	324	15.18
3	0	0	96	0
	5	2.23	134	4.74
	10	5.57	226	7.58
	15	8.36	334	11.37
4	0	0	63	0
	5	3.90	126	1.88
	10	6.13	210	6.63
	15	8.64	318	10.89
5	0	0	79	0
	5	3.34	126	2.84
	10	6.13	232	6.63
	15	8.91	329	10.42
6	0	0	84	0
	5	3.34	142	2.84
	10	6.13	225	6.63
	15	8.91	318	10.42
Average		37	80.83	0
			141.17	3.95
			231.67	8.22
			327.50	12.24

Table B.10: Artery results for spec. no. 5

Test #	V_{in} μL	V_{ext} μL	P_{in} mmHg	$\frac{\Delta V}{V_0}$ %
1	0	0	112	0
	5	0.56	240	8.18
	10	2.51	371	13.79
2	0	0	127	0
	5	0.56	248	8.18
	10	2.23	383	14.30
3	0	0	112	0
	5	1.95	235	5.61
	10	3.62	361	11.74
Average			117	0
			241	7.32
			371.67	13.28

Table B.11: Artery results for spec. no. 6

Test #	V_{in}	V_{ext}	P_{in}	$\frac{\Delta V}{V_0}$
	μL	μL	mmHg	%
1	0	0	44	0
	5	2.79	88	2.92
	10	6.13	142	5.11
	15	8.36	203	8.77
	20	11.14	266	11.70
	25	15.04	324	13.15
	30	18.94	359	14.60
2	0	0	66	0
	5	2.79	117	2.92
	10	5.57	182	5.85
	15	8.36	239	8.77
	20	11.14	302	11.70
	25	14.48	349	13.88
3	0	0	67	0
	5	2.79	115	2.92
	10	6.13	174	5.11
	15	8.91	248	8.04
	20	12.26	321	10.22
	25	15.04	365	13.15
Average			44	0
			73.67	0.97
			124.67	3.65
			186.33	6.58
			251	9.50
			315.67	11.69
			357.67	13.88

Table B.12: Artery results for spec. no. 7

Test #	V_{in}	V_{ext}	P_{in}	$\frac{\Delta V}{V_0}$
	μL	μL	mmHg	%
1	0	0	72	0
	20	17.83	172	2.98
	40	35.10	351	6.73
2	0	0	59	0
	20	18.38	139	2.22
	40	35.10	313	6.73
3	0	0	53	0
	20	17.83	126	2.98
	40	35.10	290	6.73
4	0	0	49	0
	20	17.83	117	2.98
	40	35.10	272	6.73
5	0	0	67	0
	20	17.83	162	2.98
	40	34.54	357	7.50
Average			60	0
			143.20	2.83
			371.60	6.89

Table B.13: Artery results for spec. no. 8

Test #	V_{in} μL	V_{ext} μL	P_{in} mmHg	$\frac{\Delta V}{V_0}$ %
1	0	0	63	0
	50	49.03	92	0.44
	100	97.49	127	1.14
	150	145.40	175	2.09
	200	193.31	253	3.04
	250	240.67	345	4.25
2	0	0	70	0
	50	49.03	101	0.44
	100	96.94	139	1.40
	150	144.85	197	2.35
	200	192.20	282	3.55
3	0	0	56	0
	50	47.91	85	0.95
	100	95.26	117	2.16
4	0	0	49	0
	50	48.47	82	0.70
	100	97.49	116	1.14
	150	146.52	156	1.59
	200	195.54	229	2.03
	250	243.45	333	2.98
5	0	0	63	0
	50	49.03	95	0.44
	100	98.61	130	0.63
	150	147.08	176	1.33
	200	194.99	267	2.28
	250	242.34	391	3.49
Average			60.20	0
			91	0.60
			125.80	1.29
			176	1.84
			257.75	2.73
			356.33	3.57

Table B.14: Artery results for spec. no. 9

Test #	V_{in}	V_{ext}	P_{in}	$\frac{\Delta V}{V_0}$
	μL	μL	mmHg	%
1	0	0	52	0
	20	19.50	75	0.74
	40	37.88	117	3.14
	60	56.27	193	5.53
	80	74.09	335	8.75
2	0	0	52	0
	20	18.94	76	1.57
	40	37.88	121	3.14
	60	55.71	205	6.36
	80	72.42	348	11.22
3	0	0	52	0
	20	20.06	75	-0.08
	40	38.44	119	2.31
	60	56.82	202	4.70
	80	74.09	344	8.75
Average			52	0
			75.33	0.74
			119	2.86
			200	5.53
			342.33	9.57

Table B.15: Artery results for spec. no. 10

Test #	V_{in} μL	V_{ext} μL	P_{in} mmHg	$\frac{\Delta V}{V_0}$ %
1	0	0	88	0
	3	1.11	140	7.05
	6	2.79	200	12.02
	9	3.90	259	19.07
	12	5.01	328	26.12
2	0	0	92	0
	3	1.95	146	3.93
	6	3.62	204	8.90
	9	5.29	208	13.86
	12	7.52	339	16.75
	15	9.19	389	21.72
3	0	0	87	0
	3	1.95	139	3.93
	6	4.46	200	5.77
	9	4.74	266	15.95
	12	5.85	348	23.00
	15	7.52	400	27.97
Average			89	0
			141.67	4.97
			203	10.14
			262.5	17.51
			338.33	20.96
			394.5	24.84

Table B.16: Artery results for spec. no. 11

Test #	V_{in}	V_{ext}	P_{in}	$\frac{\Delta V}{V_0}$
	μL	μL	mmHg	%
1	0	0	49	0
	15	13.93	76	0.92
	30	27.30	121	2.31
	45	40.11	202	4.19
	60	53.48	334	5.58
2	0	0	44	0
	15	13.93	69	0.92
	30	27.86	111	1.84
	45	41.23	187	3.23
	60	54.04	308	5.10
3	0	0	46	0
	15	14.48	72	0.44
	30	28.97	119	0.88
	45	42.34	204	2.28
	60	54.60	338	4.63
Average			46.33	0
			72.33	0.76
			117	1.68
			197.67	3.23
			326.67	5.10

Table B.17: Artery results for spec. no. 12

Test #	V_{in} μL	V_{ext} μL	P_{in} mmHg	$\frac{\Delta V}{V_0}$ %
1	0	0	48	0
	50	49.58	69	0.20
	100	99.16	104	0.40
	120	118.66	126	0.64
	140	138.16	148	0.88
	160	157.10	178	1.38
	180	176.60	212	1.62
	200	195.54	252	2.12
	220	214.48	290	2.63
	240	233.98	337	2.87
2	0	0	58	0
	20	20.06	66	-0.03
	40	39.55	78	0.21
	60	59.05	94	0.45
	80	78.55	111	0.69
	100	98.05	138	0.93
	120	117.55	164	1.17
	140	137.05	196	1.41
	160	155.99	235	1.91
	180	175.49	275	2.15
	200	194.43	322	2.66
3	0	0	53	0
	20	19.50	61	0.24
	40	39.55	71	0.21
	60	59.61	84	0.19
	80	79.11	101	0.43
	100	98.61	122	0.66
	120	118.11	148	0.90
	140	137.60	177	1.14
	160	157.10	212	1.38
	180	176.04	252	1.89
	200	194.99	303	2.39
	220	214.49	350	2.63
Average			53	0
			68.67	0.13
			81	0.20
			99.67	0.42
			119.67	0.66
			144.67	0.90
			173	1.23
			206.67	1.47
			246.33	1.97
			289.33	2.39
			336.33	2.72