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RESEARCH ARTICLE

Contact-free monitoring of vessel graft stiffness – proof of concept as a tool for vascular tissue engineering

Markus Hoenicka[1,2]*, Marcel Kaspar[3], Christof Schmid[2], Andreas Liebold[1] and Siegfried Schrammel[3]
[1]*Department of Cardiothoracic and Vascular Surgery, University of Ulm Medical Centre, Ulm, Germany*
[2]*Department of Cardiothoracic Surgery, University of Regensburg Medical Centre, Regensburg, Germany*
[3]*Ostbayerische Technische Hochschule (OTH) Regensburg, FB Maschinenbau, Regensburg, Germany*

Abstract

Tissue-engineered vessel grafts have to mimic the biomechanical properties of native blood vessels. Manufacturing processes often condition grafts to adapt them to the target flow conditions. Graft stiffness is influenced by material properties and dimensions and determines graft compliance. This proof-of-concept study evaluated a contact-free method to monitor biomechanical properties without compromising sterility. Forced vibration response analysis was performed on human umbilical vein (HUV) segments mounted in a buffer-filled tubing system. A linear motor and a dynamic signal analyser were used to excite the fluid by white noise (0–200 Hz). Vein responses were read out by laser triangulation and analysed by fast Fourier transformation. Modal analysis was performed by monitoring multiple positions of the vessel surface. As an inverse model of graft stiffening during conditioning, HUV were digested proteolytically, and the course of natural frequencies (NFs) was monitored over 120 min. Human umbilical vein showed up to five modes with NFs in the range of 5–100 Hz. The first natural frequencies of HUV did not alter over time while incubated in buffer ($p = 0.555$), whereas both collagenase (-35%, $p = 0.0061$) and elastase (-45%, $p < 0.001$) treatments caused significant decreases of NF within 120 min. Decellularized HUV showed similar results, indicating that changes of the extracellular matrix were responsible for the observed shift in NF. Performing vibration response analysis on vessel grafts is feasible without compromising sterility or integrity of the samples. This technique allows direct measurement of stiffness as an important biomechanical property, obviating the need to monitor surrogate parameters. Copyright © 2016 John Wiley & Sons, Ltd.

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1. Introduction

Coronary artery disease and peripheral arterial disease are two common maladies that often require revascularization. Autologous vessels such as internal mammary artery and saphenous vein are commonly used as bypass grafts and yield good long-term results. However, some patients lack suitable vessels because of comorbidities, previous removal or limb loss. Vessel grafts made from synthetic polymers such as ePTFE (expanded polytetrafluoroethylene) or Dacron have not met clinical acceptance in heart surgery, although there is considerable use of these prostheses in lower limb revascularization (Desai et al., 2011). Vascular tissue engineering may provide superior prostheses, as scaffolds can be engineered to match the biomechanical properties of native vessels, and a cell coating, with endothelial cells lining the luminal side, can provide an antithrombotic and bioactive surface to the blood.

Although some types of tissue engineered vascular grafts (TEVGs) have entered clinical testing (Cittadella et al., 2013), general acceptance is still lacking, especially for their use as coronary artery bypass grafts. One key problem to be solved is a potential compliance mismatch between graft and stenosed vessel, which acts as the starting point of intimal hyperplasia and thus of restenosis (Salacinski et al., 2001). Manufacturing processes of TEVGs often utilize perfusion bioreactors both for cell seeding and for conditioning (Bilodeau and Mantovani, 2006). The latter is required to adapt the nascent vessels to the pressure and flow conditions of the arterial system. Unless the process uses non-degradable scaffolds with appropriate biomechanical properties to start with, conditioning uses one or more of luminal pressure, shear forces and pulsation to elicit extracellular matrix production and remodelling by the seeded cells until biomechanical properties match those of the target vascular bed. This usually requires collagen and elastin synthesis to provide the extracellular matrix with strength and elasticity (Patel et al., 2006; Kothapalli and Ramamurthi, 2009).

Mechanically induced matrix synthesis and remodelling can be evaluated easily, such as by histochemical techniques and tensile testing, once the manufacturing process has terminated. However, monitoring the maturing process while the graft is still being conditioned under sterile conditions would facilitate better control of the properties of the finished products. This requires the integration of sensors into the bioreactor, or the application of contact-free techniques that do not compromise sterility. Systems to record uniaxial force–displacement relationships have been

*Correspondence to: Markus Hoenicka, University of Ulm Medical Centre, Department of Cardiothoracic and Vascular Surgery, Albert-Einstein-Allee 23, 89081 Ulm, Germany. E-mail: markus.hoenicka@uniklinik-ulm.de
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implemented in bioreactors (Paten et al., 2011) to monitor tissue-engineered tendons. However, these are designed to withstand one primary stress direction along their main axis. Blood vessels, however, can be approximated by tubes that are distended by luminal pressure. According to the cylinder stress theory, hoop (circumferential) stress is up to twice as large as axial stress. Therefore, assessing only longitudinal force–displacement relationships may not suffice in vessel grafts. It was hypothesized that forced vibration response analysis, a structural testing technique commonly used in engine construction, might provide sufficient data to monitor progress during conditioning. The present proof-of-concept study developed such a system and demonstrated its utility to monitor proteolytic changes in vessel wall integrity. Human umbilical veins (HUV) are a readily available source of human blood vessels (Hoenicka et al., 2012). Their use as endothelium-denuded scaffold in vascular tissue engineering has been shown in a previous study (Hoenicka et al., 2013). Methods were also established to decellularize these vessels (Mangold et al., 2015) in order to obtain mock vessels that consist mostly of extracellular matrix. Therefore, the system was tested using umbilical veins.

2. Methods

2.1. Study design

As vascular tissue engineering, with its intended increase in graft stiffness over time, is too time-consuming to establish the planned analytical technique, we applied an inverse approach by enzymatically modifying vessel walls to weaken them noticeably within a reasonable timeframe (Dobrin et al., 1984).

First, proteolytic conditions were optimized to achieve noticeable weakening of the vessel walls, as judged by strain measurements within 120 min. Then the experimental setup was optimized to allow recording of forced vibration responses. Modal analyses provided insight into the vibrational patterns and allowed selection of a frequency range that allowed the determination of natural frequencies. Finally, changes in natural frequencies of HUV were recorded during proteolysis.

2.2. Harvesting of umbilical veins

All experiments were approved by the ethics committee of University of Regensburg. Human umbilical cords were collected from term or near-term pregnancies (gestational age at least 35 weeks) after obtaining written informed consent from the pregnant women. Donors with known infectious diseases such as human immunodeficiency virus (HIV) or hepatitis were excluded. Cords were stored in Krebs–Henseleit buffer (NaCl 118 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, NaH_2PO_4 1.2 mM, $NaHCO_3$ 16.7 mM, dextrose 5.5 mM, $CaCl_2$ 1.2 mM) which contained penicillin (100 U/ml) and streptomycin (100 μg/ml; PAA, Pasching, Austria) as preservatives and hydroxyethyl piperazineethanesulfonic acid (HEPES) (25 mM) to stabilize pH at 7.4. Umbilical veins were dissected free from Wharton's jelly and stored in Krebs–Henseleit buffer for further processing.

2.3. Decellularization procedures

Human umbilical veins were decellularized using previously published methods (Mangold et al., 2015). Decellularization was verified histochemically by haematoxylin-eosin staining of thin sections. Samples were stored in 70% ethanol at 4°C and were equilibrated in phosphate-buffered saline (PBS) before analysis.

2.4. Measurement of vessel wall movements

Figure 1 shows a schematic and an overview image of the experimental setup. Segments of native or decellularized HUV (length 4–7 cm) were mounted in a transparent vessel chamber using glass tubes of 3 mm outer diameter at the mounting sites. One tube was connected to a piece of silicone tubing bearing a stopcock for venting purposes. The other tube was connected to a three-way valve. One of the inlets was connected to a syringe which allowed filling of the system with appropriate solutions. The other inlet was connected to a 2.5 ml glass/Teflon syringe (TII-XII; Hamilton, Bonaduz, Switzerland) connected to a linear motor (LinMot, Spreitenbach, Switzerland), which provided the excitation of the samples. The motor was driven by the source output of a dynamic signal analyser (HP 35670 A; Hewlett-Packard, Böblingen, Germany). The chamber containing the HUV segments was filled with PBS to avoid dehydration. An external reservoir exerted hydrostatic pressure amounting to 80 mmHg onto the chamber to mimic the in vivo situation.

Sample movements were read out using two alternative techniques. Pilot experiments and modal analyses employed a laser-Doppler vibrometer (OFV-3001 and OFV-353; Polytec, Waldbronn, Germany). The laser was focused on the abluminal surface of the samples in order to track velocity and position of the vessel wall with resolutions of 0.5 μm/s and 0.08 μm, respectively. Subsequent experiments used a smaller and simpler triangulation-based laser distance sensor (M5 L/10; MEL, Eching, Germany) to track the position only with a resolution of 3 μm. In addition to the samples, the linear motor was monitored with another laser distance sensor to ensure that the input signal was transformed to the expected deflection. The output signals of both sensors were processed by the dynamic signal analyser mentioned above. The resulting data were imported and further processed in Matlab (MathWorks, Ismaning, Germany).

2.5. Modal analysis

After mounting decellularized HUV in the chambers as described above, a baseline measurement was performed in PBS. Signal amplitude was adjusted to obtain a barely visible oscillation of the samples, which resulted in a

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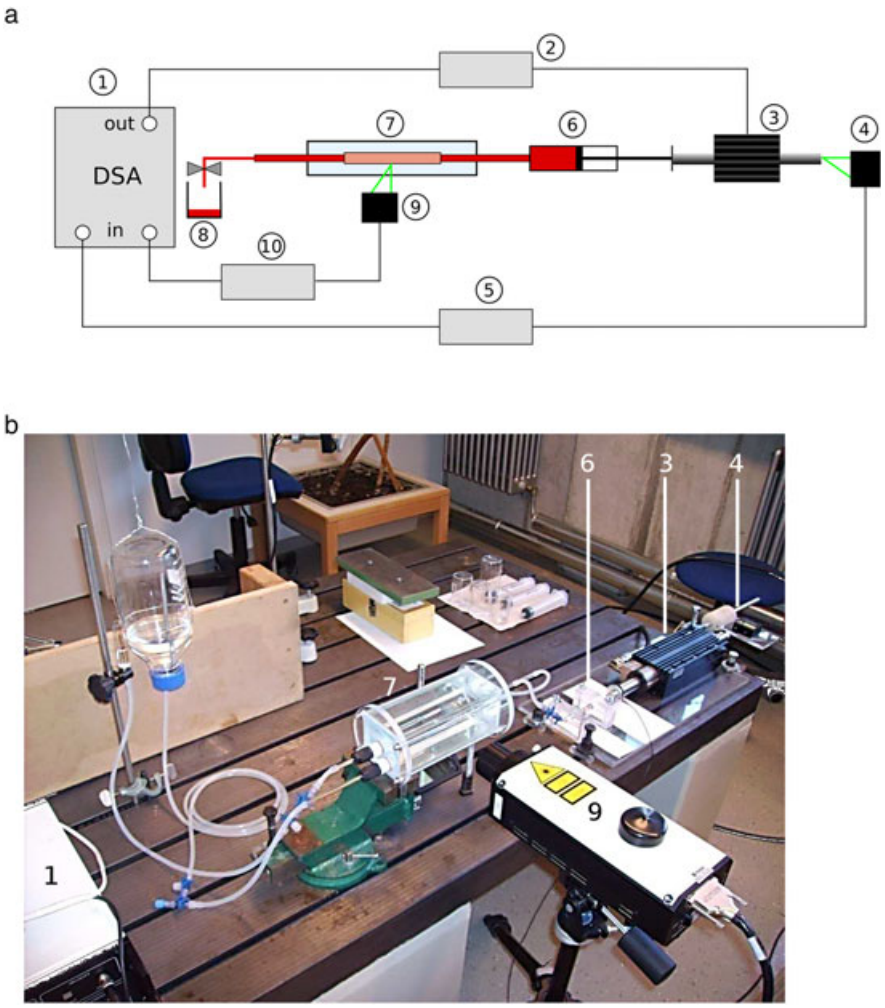

Figure 1. Experimental setup. (a) Schematic; the liquid system is shown in red. (b) Overview of main components. 1, dynamic signal analyser; 2, controller; 3, linear motor; 4, triangulation-based laser sensor monitoring motor movements; 5, controller; 6, syringe; 7, vessel chamber with mounted human umbilical vein; 8, overflow container; 9, triangulation-based laser sensor monitoring the vein sample (b shows the laser vibrometer used for modal analyses; forced vibration response measurements used a laser sensor here, identical to the one in position 4); 10, controller. [Colour figure can be viewed at wileyonlinelibrary.com]

maximum displacement of 0.15 ml by the syringe plunger. Sample movements were recorded every 2.5 mm along the longitudinal axis and every 90° around the circumference of the vessels. In this way, for example 80 readings could be recorded from a 5 cm vessel segment. The data were analysed to model the vessel wall oscillations in two perpendicular planes.

Data were recorded at a frequency of 1280 Hz and processed by fast Fourier transformation using a multichannel multi-analyser system, Portable Pulse 3560, including a Frontend Type 2827, a LAN Interface Module Type 7533, a 4/2 Channel Input/Output Module Type 3109 and the LabShop V10.1 software (all from Brüel & Kjær, Nærum, Denmark). Modal analysis was performed with the STAR Modal software V5.22 (Spectral Dynamics, Haar, Germany).

2.6. Proteolysis of HUV

In order to weaken the structure of the vessel wall, the samples were treated by proteolytic enzymes. Stock solutions of collagenase and elastase were prepared in PBS and were further diluted in PBS to obtain working solutions.

To establish suitable digestion conditions, weakening of decellularized HUV was monitored over time by strain measurements. HUV rings of 2 mm segment length were incubated in 24-well plates containing 1 ml of enzyme working solution or PBS as control. The plates were incubated either at room temperature or at 37°C. The strains of each ring at a force corresponding to physiological pressure were measured at 30, 60, 90 and 120 min after starting the incubation, as described below.

For the measurement of forced vibration responses, the vessels were mounted in chambers as described above. A baseline measurement was performed in PBS. The solution was replaced with either collagenase (300 U/ml in PBS) or elastase (3 U/ml in PBS). Responses were read every 10 min for a total time of 120 min.

2.7. Strain measurement

Changes of material properties of HUV during proteolytic digestion were measured isotonically in an organ bath system OA-5300 (FMI GmbH, Seeheim/Ober-Beerbach, Germany). Rings of 2 mm segment length were mounted

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between two cylindrical supports, one of which was connected to a force transducer. The supports were moved apart at a constant speed of 0.5 mm/s, and ring dimensions were determined via the clearance of the supports as soon as the ring exerted 40 mN of tensile force. According to Laplace's law, 40 mN is equivalent to the expected wall tension at a blood pressure of 120 mmHg using the given average dimensions of HUV (Hoenicka et al., 2013). An increase in ring dimensions to achieve the target tensile force indicated a weakening of the vessel wall during proteolytic digestion. At least four rings were tested per subject and treatment.

2.8. Drugs, chemicals and reagents

Collagenase (type 1) and elastase were purchased from Worthington (Lakewood, NJ, USA). All other chemicals were of analytical grade and were obtained from Sigma (Taufkirchen, Germany) or from Merck (Darmstadt, Germany) unless noted otherwise.

2.9. Data analysis and statistical procedures

Data are presented as mean ± standard deviation; n refers to the number of subjects. Time-courses were normalized to 100% at 0 min. They were compared by mixed-model analysis followed by Tukey post-tests using R (R Core Team 2014). Pearson's correlation coefficients were calculated to assess linear dependence between two parameters. Differences were assumed to be significant if the error probability p was less than 0.05.

Natural frequencies are immediately available from the frequency spectra and are sufficient as a readout parameter for this study. If needed, these data can be easily converted to stiffness data using the following steps:

1. Calculate the damping ratio D:

$$D = \frac{f_{upper} - f_{lower}}{f_0}$$

where f_{upper} and f_{lower} denote the frequencies next to the natural frequency f_0 with an amplitude of 70.7% (−3 dB) of the maximum.

2. Assuming a damped spring-mass system, calculate the stiffness c as:

$$c = m \bullet \left(\frac{\omega_D}{\sqrt{1 - D^2}} \right)^2$$

where m is the mass and ω_D is the natural frequency of the graft.

3. Results

3.1. Optimization of proteolytic digestion

Human umbilical vein rings were incubated in proteolytic enzymes and strain at the equivalent of physiological blood pressure was recorded every 30 min for a total of 120 min. In the presence of 100 U/ml of collagenase working solution, the circumference required to arrive at a tensile force of 40 mN increased 1.46 ± 0.84-fold at 37°C but only 1.26 ± 0.36-fold at room temperature, which was close to the time controls (1.10 ± 0.55-fold, n = 4). Collagenase at 300 U/ml resulted in sufficient changes of 2.89 ± 0.64-fold at 37°C and of 2.23 ± 0.35-fold at room temperature (n = 5, Figure 2a), although 9.9% of the rings incubated at 37°C eventually failed to withstand the target force at this enzyme concentration. Elastase at 3 U/ml induced comparable changes of strain (2.90 ± 0.30-fold at 37°C and 2.80 ± 0.87-fold at room temperature, n = 6, Figure 2b). All forced vibration response experiments were conducted at room temperature to simplify the instrument setup.

3.2. Modal analysis

Three modes of excitation were compared in preliminary tests. Applying pulses manually was not sufficiently reproducible, but induced transversal oscillations visible on high-speed camera recordings (not shown). Sweep sine caused mechanical strain in case of resonance (data not shown). Therefore white noise in a frequency band from 0 to 200 Hz was employed in the measurements. Data from two HUV were recorded and analysed. Analysis of the first sample indicated pulsating ballooning in addition to longitudinal oscillations of the vessel wall. This behaviour was attributed to the short free length of approximately 30 mm. The second HUV with a free length of approximately 40 mm displayed longitudinal oscillations and oscillations combined with pulsation which allowed one to discern five modes and their associated NFs (Table 1). Modes 1 and 2 are depicted in Figure 3. See the Supplementary material online, Movies S1 and S2, for an animated depiction of mode 1.

3.3. Forced vibration response analysis during enzymatic digestion

Modal analysis had shown that the modes most easily accessible by laser distance sensing (i.e. the ones resulting in simple longitudinal sine oscillations) occurred below 50 Hz. Therefore the range of excitation frequencies was limited to 0–50 Hz in all subsequent experiments.

A total of 9 native and 11 decellularized HUV were analysed while being digested with elastase, and 6 native and 6 decellularized HUV were treated with collagenase. Five native vessels were used as controls which were incubated in the absence of proteolytic enzymes. All native samples showed at least two NFs, although one native vessel lost the second frequency approximately 20 min into the proteolysis. In one case, one of the frequencies split in two during proteolysis. A representative time-course is shown in Figure 4. In most decellularized HUV three NFs were found. Two decellularized HUV had only two detectable NFs, and one had four.

The NFs of native vessels incubated in PBS alone did not change over time (p = 0.555, n = 5, Figure 5). Time

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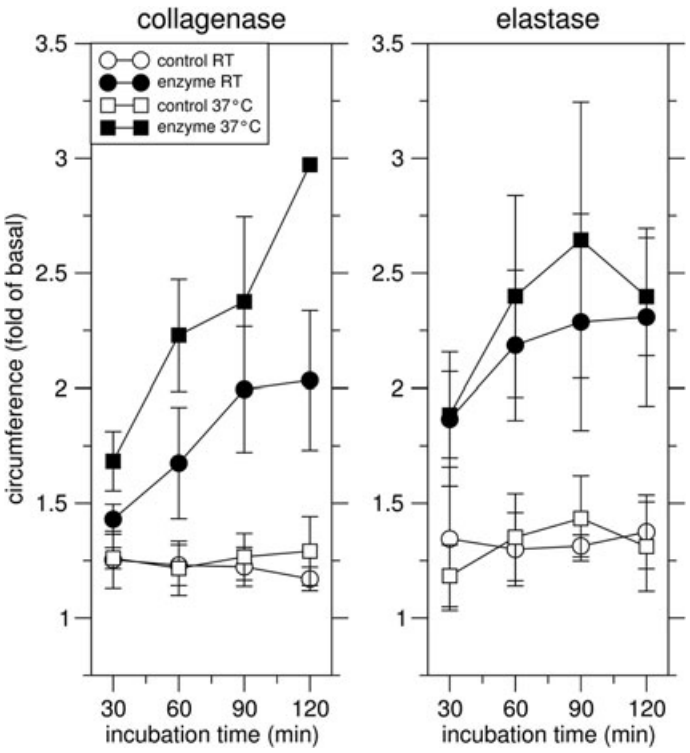

Figure 2. Time-courses of isotonic force measurements (the circumference required to achieve a load of 40 mN) taken from human umbilical vein rings incubated in proteolytic enzymes (filled symbols) or buffer (open symbols). Left panel: collagenase 300 U/ml (n = 5). Right panel: elastase 3 U/ml (n = 6). Circles and squares indicate incubations at room temperature (RT) and at 37°C, respectively

Table 1. Modes of a human umbilical vein

Mode	Natural frequency (Hz)	Description
1	11.5	0.5 sine
2	21.3	sine
3	42.4	1.5 sine
4	64.1	pulsation
5	82.0	2.5 sine + pulsation

courses of enzyme-treated vessels differed from time courses of PBS controls (collagenase: $p = 0.0061$, $n = 6$; elastase: $p < 0.001$, $n = 9$). Although elastase appeared to reduce NFs slightly larger than collagenase, there was no significant difference between these treatments ($p = 0.370$, Fig. 5a). In decellularized HUV, both enzymes showed almost identical time courses ($p = 0.967$, collagenase $n = 6$, elastase $n = 11$; Figure 5b).

The decrease in NFs correlated with the increase in vessel circumference at 40 mN target force during collagenase treatment ($r = -0.985$, $p = 0.015$). A similar correlation was found during elastase treatment, although this did not reach significance ($r = -0.893$, $p = 0.108$).

4. Discussion

Vascular tissue engineering must consider two main properties of blood vessels. First, the luminal surface has to mimic the properties of an intact endothelium in terms of its interaction with blood cells. Second, the compliance should match that of the upstream and downstream vessels to avoid early graft failure (Salacinski et al., 2001). The former may be achieved by seeding patient-derived endothelial cells on the luminal surface (Bordenave et al., 2005), or by providing a surface which allows blood-borne progenitor cells to create an endothelium in situ (Talacua et al., 2015). The latter requires either a stable matrix with the desired properties, or a cell-seeded scaffold which is conditioned appropriately under flow conditions (Seliktar et al., 2000; Baguneid et al., 2004; Yazdani et al., 2009). Flow, pressure, and shear forces may influence the mechanical properties of the resulting grafts. Optimizing the mechanical properties of grafts is a daunting task given the elaborate biphasic properties of native blood vessels (Shadwick, 1999).

During method development, biomechanical properties of vessel grafts can be tested easily by tensile testing and burst pressure measurements (Rossmann, 2010; Sarkar et al., 2006; ISO 7189:1998/2001). These methods usually compromise sterility and are destructive, leading to an at least partial loss of the graft, and are therefore not suitable for continuous monitoring. To circumvent this limitation for graft production, various methods were devised to probe tissue-engineered constructs during the conditioning phase. One approach is to monitor changes in extracellular matrix components as signs of matrix remodelling. Ultrasound signals were shown to correlate with the hydroxyproline content of vessel grafts as a marker of collagen formation (Kreitz et al., 2011). Zaucha et al., described a bioreactor vessel chamber that is accessible for confocal imaging to quantify collagen (Zaucha et al., 2009). In addition, biochemical markers of extracellular matrix synthesis in the culture medium have been

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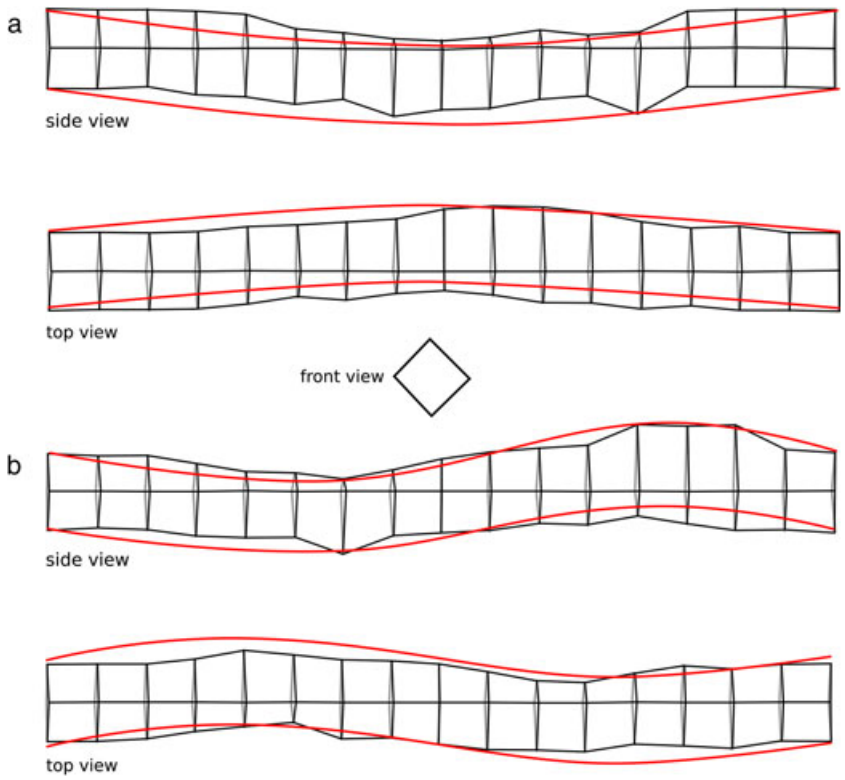

Figure 3. Representative examples of modes 1 (a) and 2 (b) of a human umbilical vein (see also Table 1). The vessels display a half (mode 1) and a full (mode 2) sine wave in both top and side view. Node distance was 2.5 mm longitudinally and 90° circumferentially. Red lines indicate an interpolated best-fit sine wave representing the measured data. See the Supplementary material online, Movie S1, for an animated version of mode 1. [Colour figure can be viewed at wileyonlinelibrary.com]

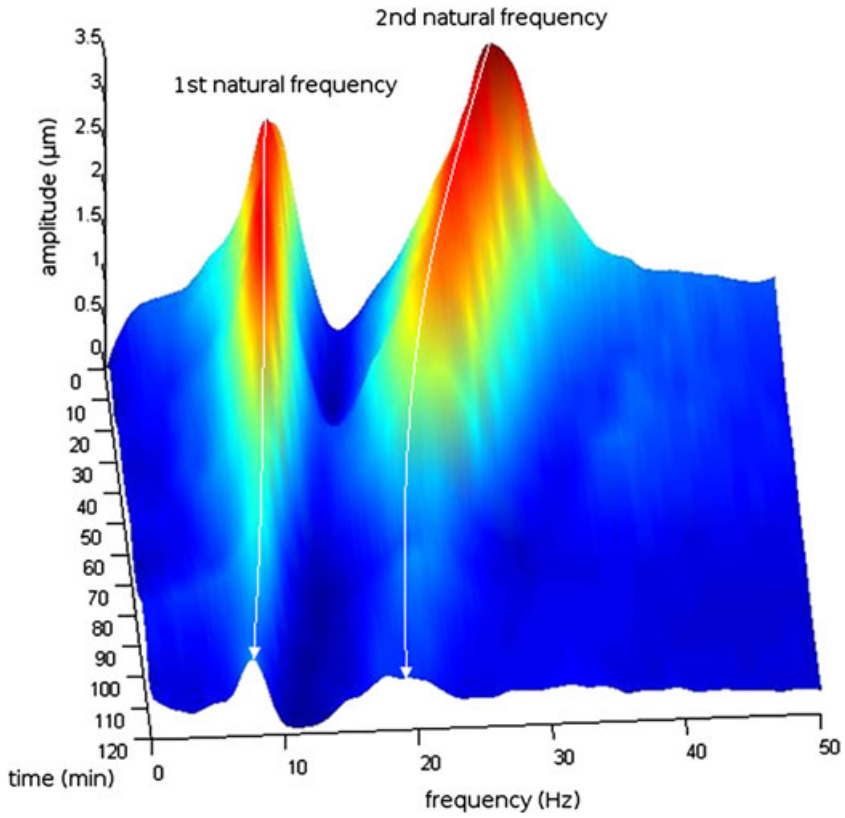

Figure 4. Representative example of the time courses of first and second natural frequencies of a human umbilical vein during proteolysis by 3 U/ml elastase. Colour indicates amplitude. [Colour figure can be viewed at wileyonlinelibrary.com]

shown to correlate with collagen and elastin synthesis during vascular tissue engineering (Tuemen et al., 2013). However, the amount of extracellular matrix or its synthesis rate are but surrogate markers of mechanical graft properties. Therefore bioreactors were developed which provide run-time biomechanical measurements. A

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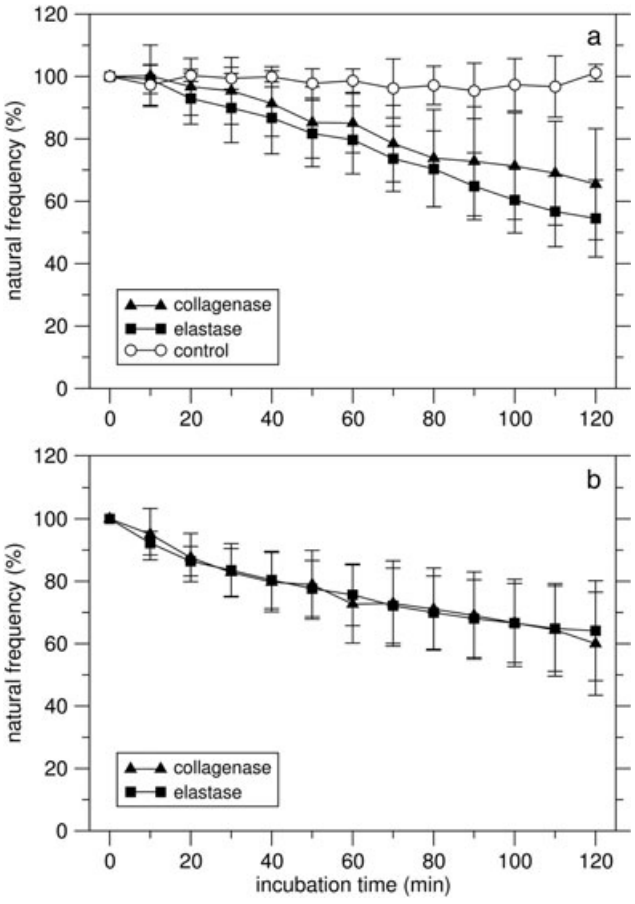

Figure 5. Time-courses of first natural frequencies of human umbilical veins. (a) Native veins; (b) decellularized veins. Triangles and squares denote collagenase (300 U/ml) and elastase (3 U/ml) treatments, respectively. Open circles denote control incubations in enzyme-free buffer. n = 5–11 per condition

bioreactor designed for heart valve engineering calculated compliance from flow and pressure data (Vismara et al., 2010). Another bioreactor was equipped with an ultrasonic probe to measure wall thickness, and a digital camera with image processing to determine the outer dimensions of the graft (Zaucha et al., 2009). Combined with pressure measurements, this setup allowed the reading of graft compliance within the limitations of the optical size determinations.

The present study attempted to transfer a structural testing technique from mechanical engineering into the realm of vascular tissue engineering. Forced vibration response analysis is commonly used to identify and minimize vibrations and the resulting noise, for example, from automobile parts and from production plants (Inman, 2013). The testing procedure usually involves an energy source (e.g. an electrodynamic shaker) to induce vibrations, and readout systems, such as vibrometers, which determine the response of the object in one or more positions. The resulting data are then subjected to Fourier analysis to determine transfer functions which, among others, indicate natural frequencies of the object. This technique has been used to probe blood vessels before. Rossmann (2010) glued a microphone and an accelerometer onto a bovine vein in order to determine its elastic modulus. In the case of vascular tissue engineering, this technique can be employed to obtain an estimate of graft stiffness and to follow its changes over time as long as sterility can be maintained. Therefore it was important to avoid an energy source that required direct contact with the sample. Instead, energy was transferred through the medium by means of a syringe attached to a linear motor. Along the same lines, readout of the response used a triangulation-based laser sensor as a contact-free method that does not compromise sterility. A HUV of sufficient length showed five modes with known NFs.

To demonstrate the utility of this technique for monitoring the conditioning of tissue-engineered blood vessels, conditions that led to noticeable changes in the wall structure within a far shorter time frame compared with tissue engineering processes were sought. Limited proteolysis of blood vessels has been used to study the mechanical properties of blood vessels as early as 1957 (Roach and Burton, 1957). The formation and progress of aortic aneurysms has also been studied by enzymatic weakening of the aortic wall, either to initiate the disease in animal models (Dobrin et al., 1984; Anidjar et al., 1990), or to study tissue stretch ex vivo (Kratzberg et al., 2009). Trypsin, a serine protease not specific for extracellular matrix components, and elastolytic and collagenolytic enzymes were employed successfully in these above-mentioned studies. The digest conditions were optimized to achieve noticeable weakening of the vessel wall within 2 h. In order to measure up to eight samples in parallel with a limited supply of umbilical cords, we chose to digest individual rings instead of vessel segments. Here, the enzymes attack the abluminal side and the cut surface in addition to the luminal side, leading to a faster matrix degradation compared with the later experiments which applied the enzyme to the lumen of the mounted vessel segments only. A small number of vessel rings had already failed mechanically after 2 h during collagenase treatment, therefore the stretch required to achieve the target force of 40 mN may be underestimated. Although these conditions gave good results in the later experiments on larger vessel segments, these different digest conditions make correlations between organ bath data and NF data difficult, in addition to the limited number of time-points in organ bath data. Whereas both collagenase and elastase resulted in a strong negative correlation between NFs and circumferences in tensile testing, only the former turned out to be significant. The time-course of tensile testing suggests that elastase digestion reached a maximum after 120 min incubation, whereas this did not seem to be the case during vibrational testing.

The technique allowed one to follow the enzyme-induced decrease of NFs of at least one mode over time in each of the samples. The inverse process (i.e. the stiffening of a vessel wall over time) is conceptually the same but has yet to be shown experimentally. Preliminary experiments using formaldehyde or glutardialdehyde to crosslink matrix proteins were unsuccessful because of the rapid progress of the chemical reactions which exceeded the speed of the analysis (data not shown).

Although the experimental setup used in the present study was quite elaborate and bulky, the technique itself can be downsized considerably. Preliminary experiments had

shown that a laser vibrometer was not required and could be replaced by compact triangulation-based laser sensors. Further, the use of a dynamic signal analyser is convenient, but not mandatory, as the subset of functions required for these analyses can be emulated in software. The force and displacement required to induce vibrations can be provided by compact linear motors with a small footprint. Therefore we consider this technique useful for integration into bioreactors.

The measurements presented in the present paper were not designed to distinguish between different types of stiffness. As mentioned above, transversal waves occur with particular modes of excitation but they cannot be detected easily by laser distance sensors. However, excitation by single pulses was not further explored in this study although high-speed camera data (not shown) indicated that the pulse propagated longitudinally. The propagation speed could be read out using two or more sensors. Longitudinal waves were detectable by laser distance sensors in all samples, but the contribution of circumferential stiffness to the overall stiffness indicated by the measurements is likely to be low. Pulsations occurred in several but not all vessels, although these might provide the closest approximation of circumferential stiffness measurements. Therefore, further refinements of both excitation and readout might improve the specificity for circumferential stiffness of these measurements.

In conclusion, vibration response analysis can be implemented in a contact-free fashion for tissue-engineering purposes. The technique developed in the present study is suitable for monitoring tissue-engineered constructs without compromising sterility or integrity of the samples. Stiffness is a biomechanical readout parameter that reflects changes of the composition and the dimension of vessel walls over time. The method allows further improvements to make it more selective for circumferential stiffness.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting information on the internet

The following supporting information may be found in the online version of this article:

Movie S1. Representative example of mode 1 of human umbilical veins (top view).

Movie S2. Representative example of mode 1 of human umbilical veins (front view).