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Research Article

An intelligent bioreactor system for the cultivation of a bioartificial vascular graft

Cardiovascular disease is the most common cause of death, accounting for 31% of deaths worldwide. As purely synthetic grafts implicate concomitant anticoagulation and autologous veins are rare, tissue-engineered vascular grafts are urgently needed. For successful in vitro cultivation of a bioartificial vascular graft, the suitable bioreactor should provide conditions comparable to vasculogenesis in the body. Such a system has been developed and characterized under continuous and pulsatile flow, and a variety of sensors has been integrated into the bioreactor to control parameters such as temperature, pressure up to 500 mbar, glucose up to 4.5 g/L, lactate, oxygen up to 150 mbar, and flow rate. Wireless data transfer (using the ZigBee specification based on the IEEE 802.15.4 standard) and multiple corresponding sensor signal processing platforms have been implemented as well. Ultrasound is used for touchless monitoring of the growing vascular structure as a quality control before implantation (maximally achieved ultrasound resolution 65 μm at 15 MHz). To withstand the harsh conditions of steam sterilization (120°C for 20 min), all electronics were encapsulated. With such a comprehensive physiologically conditioning, sensing, and imaging bioreactor system, all the requirements for a successful cultivation of vascular grafts are available now.

Keywords: Bioreactor / Bioartificial vascular graft / Sensors for physiological parameters / Tissue engineering / Wireless data transfer

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1 Introduction

In a senescent society, organ diseases that are associated with irreversible damage of organs are frequent and an increasing percentage of older patients requires bioartificial tissue and organ substitutes. In 2012 the leading individual cause of death annually was cardiovascular disease at 31% of deaths worldwide, according to the World Health Organization [1], and arteriosclerotic lesions lead to loss of functioning arteries. Surgeries such as angioplasty or a coronary or peripheral artery bypass graft are nowadays based upon the use of autologous veins and arteries. Unfortunately, the availability of these in older people is limited. Synthetic grafts of materials such as expanded polytetrafluoroethylene, polyethylene terephthalate, and polyurethane are capable of replacing large-diameter arteries, although they

require concomitant anticoagulant treatment with the risk of severe side effects. In addition, small-diameter synthetic vascular grafts (<6 mm) have a high probability of failure due to thrombus formation and compliance mismatch [2,3].

Tissue-engineered products (TEP) are currently developed as biohybrids combining the best aspects of autologous and synthetic grafts and they are discussed as substitutes for purely synthetic implants due to lower side effects, especially occurring in bioartificial vascular grafts with smaller diameters. The bioprocesses underlying TEPs require high quality under controlled conditions. The vision of autologous vascular grafts is the production on the basis of fibrin scaffolds, combined with autologous progenitor cells to form a bioartificial vascular graft [4]. Successful tissue-engineered small-caliber vascular grafts have also been developed using novel biodegradable composite fibrin-poly(lactide) scaffolds, arterial endothelial cells, smooth muscle cells, and fibroblasts out of an ovine source [5]. Grown from a patient's own stem cells, the cellular lining of the grafts should reduce the chance of rejection and thrombosis while removing the need for an autologous artery source. However, tissue-engineered grafts have long incubation periods and should offer both elasticity and strength while being biologically

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Abbreviations: B-Mode, brightness-mode; FIA, flow injection analyser; RTD, residence time distribution; SoC, system-on-chip; TEP, tissue-engineered products; XG, xanthan gum

compatible [6] according to criteria enabling the pass through international ATMP (advanced therapeutic medicinal products) guidelines before they enter the clinic.

In detail, cultivation of the graft in a perfusion bioreactor before implantation into the patient has to be strongly controlled in terms of biochemical and physical parameters as well as sterility and cell vitality. Both thickness and homogeneity of the vessel walls and the perfusion during the cultivation process has to be monitored by a contact-free measurement system (i.e. ultrasound detection).

Bioreactors for vascular grafts commonly represent the type of a pulsatile perfusion bioreactor [7] since the pulse wave is the crucial factor for the formation of a resistible vascular structure. These pressure monitored bioreactor systems were used for different cultivation approaches (xenogenous grafts, fibrin scaffolds with fibroblasts) with the purpose to create vascular structures with high tear strength [6]. Monitoring comprising cultivation factors such as pH, glucose, and lactate over weeks was developed for cultivation of more complex structures (porous scaffolds [8–10] with mammalian cells) and sensors were coupled to the growing structure via fiber connections. Noninvasive visualization of the growing structure used light [5] or fast sequenced ultrasound images [10]. In our project, we aim to develop a pulsatile perfusion bioreactor combining pressure sensing with sensing of further relevant cultivation factors with a wireless transfer mode to avoid any contamination risk imposed by fiber connections. Pressure sensing will be fine-tuned by an ongoing shear stress simulation on each wall section, since pressure is not only used to meet stability criteria, but also as differentiation factor for stem cells or progenitor cells. In contrast to the rather vague noninvasive visualization of the growing structure using light [10], we use high resolution ultrasound for exact description of the vascular graft and for quality control. Altogether, we develop a multifunctional bioreactor system featuring various sensors and controllers, integration of ultrasound imaging, and wireless data communication.

2 Materials and methods

2.1 Bioreactor system

A schematic of the newly developed bioreactor system is presented in Fig. 1A and the realized hardware setup is depicted in Fig. 1B. The size was designed according to the suggestions of the cooperating surgeons to be suitable to cultivate a TEP for an artificial peripheral bypass with a diameter of about 8 to 10 mm and a length of about 110 mm. Therefore, the cylindrical reactor housing was dimensioned with a radius of 5 cm and a length of 14 cm. The side parts of this bioreactor tube can be removed to enable the harmless placement of the endothelialized scaffold tube into the reactor. This tube is connected to a circulation system made of silicone pipes via the side parts. There are two separate circulation circuits containing medium. The inner circuit leads through the vascular graft and the outer circuit leads to the surrounding space, providing an environment comparable to the interstitial room inside the body. The 4 mm silicone pipe is connected to a media reservoir and a pulse chamber modulated by a linear voice coil actuator; sensors are either integrated into

the bioreactor tube or attached to several sensor nodes within the external pipe.

The bioreactor housing is constructed of polyetherimide (Thyssen Krupp Plastics, Garbsen, Germany), a thermo-resistant high-performance plastic material resistant to temperatures above 130°C, therefore suitable for heat sterilization. Furthermore, the material is ultrasound-permeable and allows the application of ultrasound imaging.

Besides the custom-built, cylindrical bioreactor housing with inner and outer fluid circuits; there is a motorized cradle for rotation of the bioreactor. The rotational motion is important to enable a homogenous nutrient flow and avoid gravitational effects on the graft leading to asymmetric cell growth during cultivation. The rotation is performed by the stepper motor ST4209 (Nanotec Electronic GmbH and Co. KG, Munich, Germany). The motor itself has a step width of 0.9° (360° ÷ 400 steps). The gear ratio between the cradle and bioreactor is 3.2:1. This enables rotation with a step size of ~0.3°. The stepper motor is controlled by the SMC135 motor controller, which can receive commands via a common RS232-serial interface, allowing the bioreactors' rotation to be regulated via a common computer with an USB-Serial-Adapter.

2.1.1 Gas reservoir

By the means of MFCs (mass-flow-controllers, Fa. Bronkhorst, Netherlands) gases in a preset mixing ratio can be provided for the required nutrient. These could be compressed air, CO₂, O₂, and N₂. The MFCs are temperature compensated. For the cultivation process, a range of pO₂ values between 10 and 100 mbar (equivalent to 1.5% or 0.015 mM and 21% or 0.22 mM) is necessary and can be applied depending on the current phase of the cultivation process. CO₂ saturation is held constant at 5%.

2.1.2 Pressure

Physiological pressure is simulated by combining a pulse-free base pressure created by two continuously running pumps for each circulation system (Multiflow, GAMPT mbH, Merseburg, Germany) and pressure pulses generated by a linear voice-coil actuator (LAH 16–23, BEI Kimco Magnetics Division, Vista, CA, USA) in the inner circulation. Maximal and minimal pressure can be regulated and shall prospectively be adapted to physiological conditions during vasculogenesis, i.e. in the fetal circulation, where a maximal pressure of 70 to 80 mbar would be sufficient. Higher pressure values can be exerted to test for durability of the graft.

The following formula describes the form of four Gaussian distribution functions combined to create an input signal, mediated by software located on the monitoring and controlling laptop.

$$x = \sum_{i=1}^4 f_i(t) = \sum_{i=1}^4 a_i e^{-\frac{(t-b_i)^2}{c_i}} \quad (1)$$

The parameters a , b , and c determine the amplitude, position, and width of the curve, respectively, and were used to reproduce a physiological pressure profile (Table 1). In the outer circuit, only a minor pressure has to be exerted according to physiological circumstances with a tissue pressure of 2–4 mbar.

Figure 1. (A and B) Block diagram and photo of the Bioreactor System (ultrasound device not shown). (A) The bioreactor has an interior circuit, where media flows through the graft creating the proper shear stress and pressure conditions as well as maintaining the cells, and an outer circuit providing an interstitial environment with low flow for perfusion. Automated sensor control is exerted by a flow injection analyzer (FIA). Sensor signals are detected wirelessly by a coordinator node and recorded in a computer system. (B) The continuous pump for the external circulation is located on the left side, the bioreactor with the ultrasound transducer on top, the two bottles serve as medium reservoirs, the voice coil actuator with cooling, the other continuous pump for the internal circulation on the right side, the electronic control board in front for receiving the pressure signals and for regulating the voice coil.

Table 1. The parameters of the four functions comprising the input signal for the voice coil

	<i>a</i>	<i>b</i>	<i>c</i>
f_1	26	1.5	0.05
f_2	28	1.7	0.2
f_3	8	2.1	0.03
f_4	12	2.6	1

2.2 System modeling

To assure a homogeneous medium distribution inside the bioreactor housing, which is crucial for the growth process, tracer

test were performed and the fluid dynamics during the pulsatile mode were described and adjusted to physiological conditions.

2.2.1 Residence time

Residence time distribution was determined using an approximation of a Dirac pulse. The Dirac pulse was approximated adding 100 μ L of the tracer substance methylene blue into the flow immediately upstream of the bioreactor on the inner circuit. The concentration gradient of the tracer was measured at a rate of 1 Hz and wavelength of 664 nm with a spectrophotometer (Biochrom Libra S50, Cambridge, UK). The Dirac measurements were used to determine whether different functions of the

bioreactor (i.e. rotation and pulse rate) had any effect on the residence time of the graft.

2.2.2 Computational fluid dynamics

As wall shear stress is a parameter of interest for the differentiation of stem cells and endothelial progenitor cells, care has been taken to predict and target its reliability. Initial calculations were made assuming fully developed, steady, and laminar flow of a Newtonian fluid through a tubular scaffold inside the bioreactor. The wall shear stress resulting from a given flow rate, fluid viscosity, and graft radius [11] can be estimated with Eq. (2),

$$\tau = \frac{4\mu Q}{\pi r^3} \quad (2)$$

where Q represents volumetric flow rate, r radius, μ dynamic viscosity, and τ represents shear stress. Estimates using this equation required high flow rates to achieve shear stress comparable to the in vivo situation in a human artery when using Newtonian fluids such as watery medium. Therefore, it is advantageous to add viscous substances to the medium to approximate the viscosity of blood as a non-Newtonian fluid and to enable more-easily obtainable flow rates.

For non-Newtonian fluids comparable to blood another computational method had to be applied for estimation of shear stress and flow rates subject to the exerting pressure levels of the pulse wave in the bioreactor. Here, the COMSOL Multiphysics software was chosen in order to provide a more accurate model, taking into account not only the non-Newtonian character of the fluid but also the pulsatile flow patterns using the laminar flow physics interface, using the base program and the microfluidics module. COMSOL can be further applied in the future to include the cause and effect of stretching of the graft walls by the pressure pulses subject to varying scaffold-materials to be tested. This can be performed using the fluid-structure-interaction interface, provided by an additional COMSOL module. Here, the Carreau-model was used with the viscosity-shear rate relation:

$$\eta_{eff}(\dot{\gamma}) = \eta_{\infty} + (\eta_0 - \eta_{\infty})(1 + (\lambda\dot{\gamma})^2)^{\frac{n-1}{2}} \quad (3)$$

Where $\dot{\gamma}$ is the shear rate, $\eta_0 = 0.03131 \text{ Pa}\cdot\text{s}$ is the zero shear rate viscosity, $\eta_{\infty} = 0.00025 \text{ Pa}\cdot\text{s}$ is the infinite shear rate viscosity, $\lambda = 0.061601 \text{ s}$ is the relaxation time (s), and $n = 0.55483$ is a dimensionless power rate [12].

2.2.3 Media viscosity and xanthan gum (XG)

Human blood is a viscous fluid with non-Newtonian properties with a viscosity of about $4 \text{ mPa}\cdot\text{s}$, or 4 cP [13]. It functions as a shear-thinning or pseudoplastic fluid. In contrast, medium used in bioreactors is a water-like, Newtonian fluid. This has a great impact on the fluid dynamics, because the level of wall shear stress depends on the fluid viscosity (see Eq. (2)). In order to match shear stress in real blood in an artery, the viscosity must be matched or compensated for by additional factors. Since the radius is set by the growth process, the shear stress can only be adjusted by altering the flow rate. As human blood is approximately four times as viscous as water and cell-culture medium lacks any viscosity-modifying component, the flow rate of a Newtonian fluid used for the culturing process would have to be quadrupled. This would have undesirable side effects impeding

the perpetuation of a desirable pressure. In contrast, elevating the viscosity of the fluid permits a lower and more physiological comparable flow rate. Therefore XG was added to the medium that resembles a polysaccharide produced by the bacterium *Xanthomonas campestris*. This stable thickener was not expected to affect the osmolality of the culture medium due to its high molar mass (approximately $2 \times 10^6 \text{ g/mol}$) [14] and represents a safe food additive without adverse effects [15] and has been proved biocompatible for cultures not only of bacteria and fungi [16], but also for vessel culture and cells used in tissue engineering [13]. A range of $0.2\text{--}0.4 \text{ g/L}$ XG (Sigma-Aldrich, St. Louis, MO, USA) was dissolved overnight in tap water [17] at 37°C using a rotary shaker and the viscosity was measured using a rheometer (MCR 302, Anton Paar, Graz, Austria) and a 40 mm diameter plate-plate test with a 0.2 mm gap width and $280 \mu\text{L}$ fluid. The samples were tested at shear rates logarithmically from 1 to 1000 s^{-1} that included the point of greatest physiological interest at 200 s^{-1} [17].

2.3 System monitoring

The bioreactor system provides cultivation conditions for the proposed scaffold and cells that need to be constantly monitored and regulated. In addition to this, control of the cultivation conditions, possible weak points in the wall structure of the vascular graft must be detected and recorded. Since the vascular graft represents an advanced therapy medicinal product (ATMP), that has to pass through a complicated and challenging certification process before being implanted into a patient, a complete screening concerning the medium supply, the quality of the graft, and the cultivation procedure of the vascular graft must be established. For reuse after steam sterilization, electrical components were selected as being temperature-resistive. The monitoring system is built up of two separate parts:

- Autonomous sensor nodes for the control of physical and biochemical cultivation conditions for the proposed growth process (temperature, position during rotation, pressure and corresponding shear stress, glucose and lactate, pO_2 , pH).
- Ultrasound imaging for observation of the vascular graft (wall strength, homogeneity of the vessel walls, detection of weak points, resistance, local distinctive features such as thrombi, cell detritus)

2.3.1 Autonomous sensor nodes

For the control of physical and biochemical parameters within the bioreactor system, autonomous sensor nodes have been developed. Each sensor node is placed at certain location in the bioreactor system, to measure a parameter as close as possible to the vascular graft being cultivated. To enable a high degree of flexibility and avoid cabling each sensor node contains the system-on-chip (SoC) CC2538 by Texas Instruments. This SoC incorporates a 32-bit ARM Cortex M3 microcontroller and additional hardware modules such as a dedicated 2.4 GHz IEEE 802.15.4 compliant RF transceiver for wireless data transfer according to the ZigBee-specification. Additionally, each sensor node can be powered by a common 9V battery.

2.3.2 Sensing physical parameters

Monitoring of physical parameters comprises continuous pressure, temperature, and bioreactor rotation readings of which the latter two are monitored by a sensor node within the bioreactor housing. The components of this sensor node are placed on a printed circuit board (PCB). Its design was adjusted into one of the end covers of the bioreactor adapted to the medium connectors to exclude overlapping with the ultrasound detector on top of the bioreactor housing. For this sensor node, wireless inductive energy supply is envisioned but currently a battery is used.

Since the bioreactor is filled with watery medium, this PCB had to be sealed with a temperature-resistive, solid material suitable for steam sterilization at $>120^{\circ}\text{C}$, which is bioinert and does not deteriorate the wireless data transmission. For encapsulation, the material EPO-TEK 301–2 by Epoxy Technology as a two component epoxy resin for medical products was used. Its initially liquid component materials were mixed according to the manufacturer's instruction and cured for 3 hours at 80°C .

Pressure is monitored both at the inlet and outlet of the inner and outer liquid cycle. The respective sensor nodes also feature the SoC stated above for wireless data communication.

2.3.3 Electrochemical sensors

For detection of nutrients and metabolites (glucose and lactate) and for a regulated measurement of pO_2 , electrochemical sensors, partially with enzyme layers, are used. The electroanalytical readings require an electric hardware (potentiostat) located on the sensor nodes. Flow injection analysis (FIA) has gained high relevance for exact sample delivery [18]. The FIA in this bioreactor system uses buffer as flow carrier by removing less than 1 mL volume from either the inner or outer liquid cycle per sensor measurement. Measurements are performed ongoing during cultivation in a frequency of every 5 minutes. The FIA comprises an injector and selector valve (Knauer, Berlin, Germany) and a peristaltic pump with four channels (Ismatec, Wertheim, Germany) in order to address the different sensors and the mandatory waste reservoir. An electronic control element with suitable software regulates the different elements of the FIA and coordinates the refilling of the bioreactor circulation system with medium supply.

Glucose concentrations for cultivation range between 0 (cell starvation approach) and 4.5 g/L. According to cultivation experience with the cell systems used, lactate concentrations between 0.7 g/L and 2.5 g/L are to be expected. pO_2 has to be regulated between 10 and 150 mbar (resulting in an atmospheric oxygen saturation of 1.5–21%), since cultivation of for example mesenchymal stem cells requires a hypoxic milieu at the beginning of the cultivation process [19], which is stepwise raised to a normoxic milieu.

CO_2 saturation is determined at a constant bicarbonate content of the medium, where the pH equals $\log \text{pCO}_2$ and vice versa. The pH is to be kept constant at 7.4 ± 0.2 , unstable pH-values may indicate possible contamination caused by bacterial or fungal infection or acidification by unwanted metabolic processes.

2.3.4 Ultrasound imaging

Using the Brightness-Mode (B-Mode) of an Ultrasound Device, slice images of the growing vascular graft can be taken noninvasively. The rotation of the bioreactor enables to take slice images from 360° angles in order to enable a later 3D visualization of the growing vascular graft.

A LOGIQ S7 from GE Healthcare is used as ultrasound device. The connected ultrasound probe is an ML6-15-D containing a total of 1008 piezo elements. At the maximal frequency of the probe, 15 MHz, the ultrasound device has a resolution of $\sim 65 \mu\text{m}$.

Furthermore, the perfusion velocity as well as the vascular resistance (R) can be monitored using the Doppler-mode applied on the artificial pulse wave generated by the pump. The resistance can be calculated according to the estimation formula (4) for pulse wave analysis [20]:

$$R = V_{\max} \times V_{\text{enddiastolic}} / V_{\max} \quad \text{with} \\ V_{\max} = \text{maximal pulse wave velocity} \quad (4)$$

3 Results

3.1 Modeled system

3.1.1 Residence time is independent from rotation and pulsation

The residence time of the medium within the bioreactor, in both circuits, is an important parameter providing information about the mixing and homogeneity of the media filling the bioreactor. Both the residence time distribution (RTD) and average residence time were considered. They were determined by conducting a series of Dirac pulse experiments (washout experiments). It could be shown, that there is no impact of the rotation of the bioreactor and the pulsation of flow on the residence time in the inner circuit and as well as of the rotation of the bioreactor on the outer circuit, which does not experience pulsatile flow. The volume of the inner circuit of the reactor is approximately 3.0 mL. Data obtained were compared to the average residence time ($\bar{t}$) indicating perfectly mixed phase, for an idealized laminar tubular reactor:

$$\bar{t} = \frac{L}{\bar{u}} \quad (5)$$

where L is the length of the bioreactor and $\bar{u}$ is the average fluid element velocity [21].

The RTD ($E(t)$) is obtained from these experimental data by normalization,

$$E(t) = \frac{C_t - C_0}{\int_0^{\infty} [C_t - C_0] dt} \quad (6)$$

where $E(t)$ represents the exit age distribution, C_t represents the concentration of the tracer, C_0 the initial concentration of the tracer, and t the time.

The $E(t)$ was measured subject to the current pulse rates in a range from 0.5 to 2 Hz and subject to the rotation rate of the cylindrical reactor tube containing the vascular graft in a range from 0.5 to 4 rpm (Fig. 3A and B).

Figure 2. (A and B) Pressure pulses generated by the pulse chamber over time, here using a silicone pipe instead of a natural graft material. (A) Pulse curve before the vascular graft. (B) Pulse curve behind the vascular graft. Peak pressures are generated by the voice coil, a baseline pressure at approximately 115 mbar is generated by the continuous pump.

Figure 3. Residence time distribution (RTD) in vascular graft bioreactor depending on the (A) pulse rate of the fluid (0.5, 0.75, 1.0, 1.5, 2.0 Hz) measured at 0.10 L/min and depending on (B) rotation speed of the reactor (0.5, 1, 2, 3, 4 RPM) measured at 0.225 L/min. The vertical lines represent the average residence time of each RTD. Neither the RTD nor the average residence time varies significantly as a function of pulse rate or rotation speed.

The average residence time ($\bar{t}$) was calculated by integrating the RTD as follows:

$$\bar{t} = \frac{\int_0^{\infty} t E(t) dt}{\int_0^{\infty} E(t) dt} \quad (7)$$

3.1.2 Distinctive curve determinants generate a physiological pulse curve

The pulse generated by the voice coil actuator and the continuous pump was designed to mimic a physiological pulse wave inside the human circulation. It was detected before the vascular graft and behind it in the inner circle of the bioreactor system (Fig. 2A, B).

Table 2. Flow rates necessary to hit shear stress targets, calculated with Eq. (2) and via COMSOL simulation

Shear stress (dyne/cm ²)	Flow rate–Eq. (3) (L/min)	Flow rate–COMSOL (L/min)
0.5	0.037	0.0079
1.0	0.074	0.032
3.5	0.26	0.16
7.5	0.55	0.52
15	1.1	1.5

For the measurement of these pressure curves, the pressure sensor node described in the method section has been used. During the experiments, a silicon tube with an integrated 3D-printed tube for mimicking a rough surface was used as vessel replacement. Polylactide served as printing material. For this measurement, the sensor has been read with a frequency of 20 Hz.

3.1.3 Fluid dynamics were estimated

For shear stress estimation, first Eq. (2) and secondly the mentioned COMSOL software is used (see Table 2, rows 2 and 3). Here, rigid cylinders of 8 mm diameter are assumed, that means that the COMSOL value is the average shear stress along a wall of 80 mm length. The velocity values derived by the COMSOL simulations (see Fig. 4A) range up to 0.4 m/s assuming a density of 1 g/mL and a fluid viscosity defined by the Carreau-Model [12] and estimates shear stress values along the arc length of the tubular scaffold (Fig. 4B) at different time points of the pulse wave (legend), with the highest shear stress of 0.9 Pa at the minimum pressure level of the respective pulse wave (12 000 Pa). Furthermore, we could show that according to the experimental setup of previous studies [22], it is possible to create a blood analogue fluid for culturing a vascular graft in vitro, e. g. inside a bioreactor (Fig. 5).

3.2 Monitored system

3.2.1 Encapsulated electronics are sustainably functional after steam sterilization

The epoxy resin used for encapsulation of the electronics on the PCB depicted in Fig. 6A turned solid and colorless during the hardening process, and resisted temperatures up to 360°C. Figure 6B shows the sensor node right after encapsulation on a bioreactor end cover. The thickness of the capsule layer is about 2 mm and 1 mm above the sensors.

For verification of temperature sensor data, the encapsulated temperature sensor was compared to the data of a reference sensor. This reference experiment included heating and cooling phases and was performed over one hour. During this time, the temperature difference stayed below 0.8 K. This is acceptable within the terms of the target application for a physiologically cultivated ATMP where extreme temperature alterations will not occur [23].

The bioreactors' rotation is measured by an accelerometer, which is mounted on the same PCB as the temperature sensor.

This type of sensor is not affected by the encapsulation. The measurement of the bioreactors' rotation is possible with an accuracy of 0.5°.

The pressure in the internal and external pipe could be measured with an accuracy of 1 mbar. A calibration of each sensor separately is mandatory, due to slight variation in sensibility and offset value of each sensor. A frequent measurement of the pressure on both pipes is shown in Fig. 2.

The developed sensor node for the electrochemical measurement of the nutrient parameters features a detectable current range between <1 nA and 1 A, allowing for the usage of a wide variety of sensors. To verify the functionality of one electrochemical sensor system, an exemplary measurement with defined glucose solutions has been performed using a macroelectrode sensor design and PBS reference solutions with glucose concentrations ranging up to 2 g/L. The obtained current responses revealed a resolution of <0.1 g/L. The standard deviation of the measured current is 0.02 μ A.

To test the sterilization process on the electronic elements placed inside the bioreactor, a steam sterilization process was performed at 120°C for 30 min in an autoclave. All surfaces were completely sterilized. Some areas within the encapsulation showed slight cracks in the epoxy resin, caused by encapsulated air pockets, expanded by the heat during the sterilization process. After the test, the encapsulation was still intact and the monitoring system was fully functional. Any detrimental effects on the electronics were not apparent [24, 25]. Further sterilization processes, however could further damage the encapsulation. To avoid encapsulated air pockets, the epoxy resin should be applied in a vacuum chamber.

3.2.2 Wireless data transmission achieves a coverage of up to 30 cm

All sensors described in the method section communicate their recorded data wirelessly to software running on a monitoring laptop. The used SoC CC2538 allows for wireless data transmission according to the ZigBee-specification with reliable data transmission and low-power usage. The ZigBee network is either organized in star or mesh topology, resulting in great scalability to allow for both the usage of a single monitoring and controlling laptop with a single network coordinator node connected, while also providing enough capacity for future sensor or controller node developments.

The maximum coverage of the sensor nodes was measured by evaluating the received signal strength indication (RSSI) value at different distances. Sensor nodes placed in the external pipe achieve coverage of up to 60 m without package loss or a drop of the RSSI below -90 dBm. Although the bioreactor, the PCB encapsulation and the medium have a strong dampening effect on the signal strength (measured by the RSSI) of the sensor node inside the bioreactor, a coverage of 30 cm was achieved (Table 3). With a close placement of the network coordinator in star topology or a router in a mesh network, a fully connected wireless network can be established around the bioreactor system. This allows easy data transport without any cabling and a flexible integration of further sensor nodes to be developed.

Figure 4. (A) Using Newtonian fluid, the fluid velocity was simulated. The length of the arrows indicates the corresponding fluid velocity across the diameter of the vascular graft. In addition, the velocity over the displayed locations is given color coded with the corresponding scale in m/s (y-axis on the right hand side). (B) The lower depicted diagram shows the wall shear stress (y axis, in Pascal) over the length of the vascular graft (x-axis, arc length in mm) at different time points of the pulse curve. The pulse curve is shown in the upper diagram (y-axis: pulse wave pressure values in Pascal, x-axis: time in s).

3.2.3 Ultrasound enables 3D-visualization for quality control of the graft

Ultrasound imaging enables a noninvasive and “touchless” monitoring of the vascular structure during cultivation. Since the bioreactor system does not have to be opened, the risk of infectious contamination is minimized. The used ultrasound probe ML6-15-D captured slice images along the whole width of the vascular graft at once using the B-Mode. Figure 7 shows the ultrasound imaging of a decellularized equine artery as possible scaffold for the later bioartificial graft. The imaging was performed within the rotating bioreactor. This allows a virtual

3D-visualization of the vascular graft providing detailed information on the wall strength and homogeneity of the vessel walls, on possible weak points, resistance, and local distinctive features such as thrombi. Additionally, the 3D-visualization presents the geometry for the dynamic fluid estimation as part of the system modeling process using COMSOL. The whole image acquisition and image processing tool chain is described in [25]. Since the acquisition of one slice image affords about one second, the capture of the whole circumference of the graft (360° in 1° steps) takes 6 min followed by a computational time (using MATLAB) for image composition of 20 min. The performance of this

Figure 5. Rheometrical determination of viscosity as a function of shear rate for XG solutions at 37°C ($N = 1$, $n = 3$). Higher concentration solutions have greater viscosity at the same shear rate, and higher temperatures lower the solution's viscosity at a constant XG concentration (not shown). For comparison, the idealized curve according to the Carreau-model was depicted at XG = 0.3g/L and a measurement was also performed with human blood.

Figure 6. (A and B) Monitoring system printed circuit board (PCB) front view before (A) and after (B) bioinert encapsulation.

Table 3. RSSI over distance depending on the bioreactor content

Distance [cm]	RSSI [dBm]	
	Air	Nutrient
0	−65	−77
5	−66	−81
10	−66	−88
15	−68	−80
20	−70	−88
25	−70	−86
30	−70	−81

3D-visualization is planned in 2-day-intervals providing continued and detailed information about the growth process of the vascular graft. For 3D-visualization, the usage of a Hamming-Filter and a threshold of 15% of the dynamic range matched the average wall thickness of the graft at 101% ($100\% \pm \text{optimal}$). The offset of the graft radius amounted to 0.35 mm ($0 \text{ mm} \pm \text{optimal}$). An exemplary 3D-visualization of a male bovine vein could successfully be performed [24].

Furthermore, the perfusion velocity as well as the vascular resistance can be monitored using Doppler-mode applied on an artificial pulse-wave generated by the voice-coil-actuator [20].

Figure 7. Brightness mode. Ultrasound imaging of a decellularized equine artery as possible scaffold for the later bioartificial graft. The imaging was performed within the rotating bioreactor as described before.

4 Discussion

In the present time, the key technology for reproducible and efficient cultivation of TEPs such as vascular grafts is the application of a suitable bioreactor. The presented bioreactor is built specifically for extra corporal cultivation of vascular grafts and can be used at a higher pressure profile to elevate the durability of the produced bioartificial graft. But in contrast to previous models solely focused on durability of a graft [7–10], this bioreactor system is also designed to regulate and monitor the cultivation process of a physiological and biocompatible seeded scaffold. This is achieved by conditioning the engineered tissue by precise modulation of a pulse wave and the resulting shear stress. Physiologically modeled pump dynamics have been shown to be advantageous for in vitro endothelialized small diameter vascular grafts, since constructs exposed to pulsatile flow expressed more functional transcriptional profiles and lower metabolic activity comprising a nonactivated phenotype. Besides, the pulsatile flow evoked a higher production of nitric oxide as one of the main vasodilative and anti-inflammatory factors for the maintenance of vasculature [11].

A further possibility to narrow the conditions inside the reactor to a physiological state is the alteration of the cultivation media. XG is suitable for use with cell seeded scaffolds since it had no effect on cell growth [26]. Several substances with higher viscosity and pseudoplastic qualities are available to match the media's characteristics with the rheology of blood: polyacrylamide [17,27], glycerol [26,28], and xanthan gum (XG) [17,26,28,29] all provide this effect. Apart from its viscous fashion, and in contrast to polyacrylamide [30], we favor XG as medium thickener since it has no sustaining viscoelastic properties impacting the unsteady flow of a blood analog fluid through a bioreactor that makes the process unpredictable [28]. As blood has a viscosity of 4 mPa·s at a shear rate of 200 s⁻¹, it should be adequate to add Xanthan gum in a concentration of 0.3 g/L to the nutrient or medium at 37°C to achieve physiological conditions during cul-

tivation of the vascular graft inside of the bioreactor. According to the results of van den Broek [17], Xanthan gum was not associated to any unwanted side effects such as contamination or others during the cultivation process. Nevertheless, its use in cultivating a vascular graft inside of a suitable bioreactor is still to be evaluated in further studies. According to a simulation using XG solutions in an abdominal aneurysm model, the shear thinning fluid has a decelerating effect on scalar shear rates but leads to comparably higher maximal shear stress values [22] at a constant system pressure as compared to a Newtonian fluid. This is advantageous, since high shear stress promotes endothelial cell survival and quiescence, alignment in the direction of flow, and a secretome comprising predominantly vasodilative and anticoagulant factors whereas low shear stress increases the cellular secretion of coagulant factors and vasoconstrictive factors and might therefore lead to a preactivation of the cells on the scaffold before implantation into a living system favoring thrombotic complications [13].

The monitoring of the cultivation conditions based upon the single sensors is planned to be performed permanently throughout the total growth process of several weeks. Due to energy-efficient circuit designs, each different sensor node features a runtime between several days and several weeks, depending on the measurement frequency. It is envisioned to utilize sensors with significantly smaller dimensions of the reactive electrode areas in the future, since both the measurable range and the stability of the readings are expected to increase. A more sustainable alternative to the consumptive electrochemical sensors used here is given by optical sensors based upon “infrared spectroscopy” (IR) [31] and will also be tested.

Using an encapsulated monitoring system inside the bioreactor, rotation angle, and temperature can be measured and the processed values are transmitted wirelessly out of the bioreactor up to a distance of 30 cm. An epoxy resin was used as an autoclavable encapsulation material. Even if the temperature sensor is encapsulated by the resin, an accurate measurement is possible. Further pressure and electrochemical sensors (glucose, lactate,

and oxygen) have been integrated into the inner and outer liquid circuit. Corresponding signal recording and processing PCBs have been designed.

Altogether, the combination of miniaturized sensor nodes and wireless data transfer enables a flexible monitoring system for the measurement of physical and biochemical cultivation conditions for the proposed growth process. The achieved transmission range is fully acceptable as it is possible to position a receiver close to the bioreactor. A further increase of the transmission range would afford an increased transmission power and consequently also an increased corresponding energy consumption that would result in an unacceptable battery size.

Applying ultrasound technology, it is possible to monitor the cell growth of the graft. A further enhanced quality control of the structure inside the bioreactor is provided by consistently taking a sequence of ultrasound images inside the bioreactor from different angles that can be combined to a 3D-visualization of the growing vascular graft showing differentiated structures. Therefore, ultrasound technology is suggested to be superior as compared to the simple use of light that only allows a rather vague measurement of the grafts' dimensions [7]. It is, however, not suitable to monitor specific processes, e.g. an implant-associated inflammation. Here, MR-imaging using iron oxide nanoparticle and labeling textile-fibers made of polyvinylidene-fluoride has proven more valuable [32] and this method could also be tested in the here presented bioreactor system. Furthermore, other technologies such as Scanning Laser Optical Technology (SLOT) have been applied on inflamed cultivated structures. The current problem of this technology, however, is the relatively small penetration depth [33] and the very high technical effort.

With the designed bioreactor described here, a fully functional system is available for cultivating TEPs enabling the variation and monitoring of all relevant parameters. For further development of the monitoring system, it is planned to extend the number of sensors for different parameters (e.g. pH, and further metabolites) and to integrate a wireless power supply into the bioreactor.

Practical application

This work is dedicated to the establishment of a suitable bioreactor for containment and cultivation of a tissue-engineered bioartificial vascular graft. The bioreactor shall be located next to its envisioned usage site in the operating room. To avoid contamination via fiber connections into the bioreactor, wireless data transfer was implemented. The bioreactor is transparent for ultrasound to allow for noninvasive control of the growing structure and is autoclavable to fulfill existing standing operation procedures concerning hygiene before implantation of the graft into patients. The system includes a variety of suitable sensor systems for quality control including temperature, pressure, position, glucose, lactate, oxygen. The bioreactor provides a pulsatile mode, based upon the frequent stimulation of a voice coil actuator as well as a basic pressure pump. Using a state-of-the-art flow dynamics simulation tool, shear stress rates can be estimated all through the cultivation process.

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5 References

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