Biomechanical *in vitro* culture platform for biological tubular structure

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***Abstract*— The use of bioreactors for the application of strictly monitored physical stimuli to biological constructs is of major importance in the field of tissue engineering.**

In this work we describe the design of a novel biomechanoreactor for the *in vitro* culture and biomechanical characterization of tubular biological structures. The biomechanoreactor consists of a culture chamber, for the vessel housing, hydraulic circuit and a programmable monitoring and control system, able both to apply mechanical stimulation and to evaluate biomechanical properties of the sample hosted within the bioreactor. Functional experiments were performed for testing the performances of the bioreactor as both testing unit and culture system.

***Keywords*—bioreactor, dynamic conditioning, biomechanical characterization, vessels.**

I. Introduction

TISSUE engineered blood vessel (TEBVs) represents as a promising alternative to native veins or arteries for replacement therapy. In the latest years several efforts focused on developing vascular engineered have been addressed by combining vascular cells (endothelial and smooth muscle cells, fibroblasts) with various type of scaffolds (hydrogels biopolymeric scaffolds, degradable synthetic scaffolds, decellularized matrix). In this context, the use of bioreactor have an important role in the development of living and functional tissue engineered grafts, providing controlled environments for reproducible and accurate application of specific regimes of mechanical force to 3D constructs.

Accordingly, in this work we describe the development of a novel biomechanoreactor able to integrate a testing unit able to carry out tissue biomechanical characterization within a culture system where cellular constructs grow. The device, improvement of an existing bioreactor previously developed in our laboratory [1], is designed to allow the *in vitro* culture of tubular biological structures, such as native and tissue-engineered blood vessels (TEBVs), in a controlled and strictly reproducible dynamic environment; in addition, the biomechanoreactor permits to perform biomechanical characterization tests on biological tubular structure hosted within.

II. Materials and Methods

A. Architecture of the biomechanoreactor

The biomechanoreactor consists of: a culture chamber; hydraulic circuit (silicon tubing) and actuators (pump and solenoid pinch-valve); a monitoring and control system (M/C) for the application of mechanical stimulation to the hosted samples.

The culture chamber includes a vessel housing, allowing to host tubular structures of different diameters and lengths, inserted into a 50 ml Falcon tube, which acts as a reservoir (Fig 1). The M/C system is able to apply the dynamic stimulation to the hosted samples, in the form of shear stress, and pressure-related wall stress. Moreover, the automated control system permits to evaluate biomechanical properties of the hosted samples, e.g. the compliance, and to perform rupture tests to determine their burst pressure. The M/C system involves a custom LabView software, which controls the hydraulic actuators via an I/O board running on a PC, and operates via a pressure-based feedback loop.

B. Functional assessment of the biomechanoreactor

Functional experiments were carried out for testing the performances of the biomechanoreactor.

To test the robustness and reliability of the system as a testing unit, compliance evaluation and burst pressure measurements were carried out on native vessels. Porcine coronary arteries (n=6) were isolated from pigs, side branched were ligated, and immediately stored at 4°C in PBS supplemented with 1% penicillin–streptomycin for up 24 h before use. Vessel segments (30-mm-length) were mounted within the culture chamber connected to the hydraulic circuit, submersed in saline buffer and pressurized at a flow rate of 4 ml/min until failure (Fig 1, B). During the loading phase, the M/C system allowed the acquisition of the volume infused over time and the instantaneous intraluminal pressure, simultaneously, and used the available data to measure burst pressure (defined as the highest pressure values attained prior to rupture.) and to estimate the vessel's compliance in the physiological range (80-120 mmHg). This parameter is computed by normalizing the volumetric compliance with respect to the volume at the 80 mmHg [2].

The biomechanoreactor was also tested as a culture system with tissue engineered constructs. Small-caliber TEBVs (3.5-mm inner diameter, 25-mm length) were built up by embedding porcine aortic smooth muscle cells (SMCs) in rat tail-derived collagen gel scaffolds [3]. Following 24-h incubation period in which this mixture was let jellify around a mandrel, the cell-seeded constructs were cultured statically in a Petri dish for one week. At the end of maturation period in static conditions, SMCs-seeded collagen gel-based scaffolds was mounted within the bioreactor, the system was filled with culture medium (DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin) and placed inside an incubator at 37° (Fig. 2, A). Preliminary dynamic conditioning involved the application of arterial-like shear stress (1 dyne/cm^2) by perfusing the TEBVs at 10 ml/min for 3 days. Static cultured

TEBVs were used as control. After culture period, TEBVs were processed for the biomechanical and biological characterization.

B.1 Biological and biomechanical characterization of TEBVs

To evaluate the vialibility of the construct cultured within the bioreactor, MTT assay was performed. Rings of cultured TEBVs (2-4 mm) were incubated at 37°C for 3 h in a solution with culture medium (100 µL/mL) and methylthiazol tetrazolium (MTT) (5mg/mL in water). After incubation, culture medium was removed, and 200 µl of 0.05M HCl in isopropyl alcohol were added to dissolve formazan salts formed. The optical density at 570 nm was determined using a UV-VIS spectroscopy (V-630 UV-Vis Spectrophotometer, Jasco, USA). The absorbance was directly proportional to number of viable cell.

Preliminary biomechanical characterization of TEBVs was assesses through uniaxial tensile test and unconfined compression tests. Uniaxial tensile tests were carried out at room temperature using Instron 5564 rig with a 2.5 N load cell (Instron Corporation, Norwood, MA, USA). Tissue samples (3-mm-ring specimens) were mounted onto stainless steel L-shaped hooks and elongated to failure at a constant rate of deformation of 1 mm/min. Force and displacement data were recorded over time, simultaneously, through load cell signal acquisition. To calculate tensile stress, the measure force was divided for the cross-sectional area (approximated as two rectangles with sides equal to the width and wall thickness of the ring). Stress-strain data was used to obtain the tensile strength and percentage of elongation at break. An estimation of the construct burst pressure (BP) was calculated from maximum load reached (UTS, ultimate tensile stress) by rearranging the Laplace's law for a pressurized thin-walled cylinder [4], [5]:

$$BP_{estimated} = 2 \times \frac{UTS \times t}{ID} \quad (1)$$

where t is the wall thickness and ID is the unpressurized internal diameter of the construct.

Unconfined compression tests were performed using the ElectroForce BioDynamic Test Instruments (Bose Corp., ElectroForce Systems Group, MI, USA). Disk-shaped samples (ø = 3 mm) were compressed by a circular plate at 1 mm/min. Compression tests were performed at room temperature with a single compression run [6].

III. RESULTS

Functional tests were performed in order to check the reliability M/C system during the compliance and burst pressure measurements. Rupture tests performed on six porcine coronary arteries reveal an average burst pressure of 1986±372 mmHg. Furthermore, native vessels show a compliance of 0.0045± 0.0003 $mmHg^{-1}$. These results are comparable to the data reported in literature [2], [7], thus confirming the system reliability.

In vitro cultures of collagen-based TEBVs were carried out for the validation of the biomechanoreactor as culture system. For preliminary biological test, colorimetric MTT assay was performed. Results reported in Fig 2, B show the viability of TEBVs cultured in both static and dynamic conditions within the bioreactor. Preliminary biomechanical characterization tests of TEBVs cultured within the bioreactor (in both static and dynamic condition) are still ongoing. Testing of burst pressure and biomechanical properties is required to prevent catastrophic failure of the TEBVs and tolerate hydrodynamic and mechanical force exerted *in vivo*. [8].

IV. CONCLUSION

In this study a multi-purpose, flexible, and versatile culture system is presented. The device was designed with aim of applying different physical stimuli, such as shear stress, and pulsatile pressure stimulation to hosted biological tubular structures. The bioreactor also allows the evaluation of the biomechanical properties of the hosted samples, involved in compliance and burst pressure measurements. These tasks are achieved thanks to a robust and reliable M/C system.

Preliminary MTT analysis reveals the viability of the constructs cultured within the bioreactor up to 3 days. This demonstrates the system is a promising laboratory-oriented tool for stimulating the 3D regeneration of engineered vascular tissue.

Future challenges include the application of cyclic mechanical strain to TEBVs during the culture to better mimic the hemodynamic forces experienced by vasculature *in vivo*. This biomechanical stimulus, together with an appropriate biochemical conditioning, could represent a successful methodology to engineer biological-derived living and functional vascular grafts.

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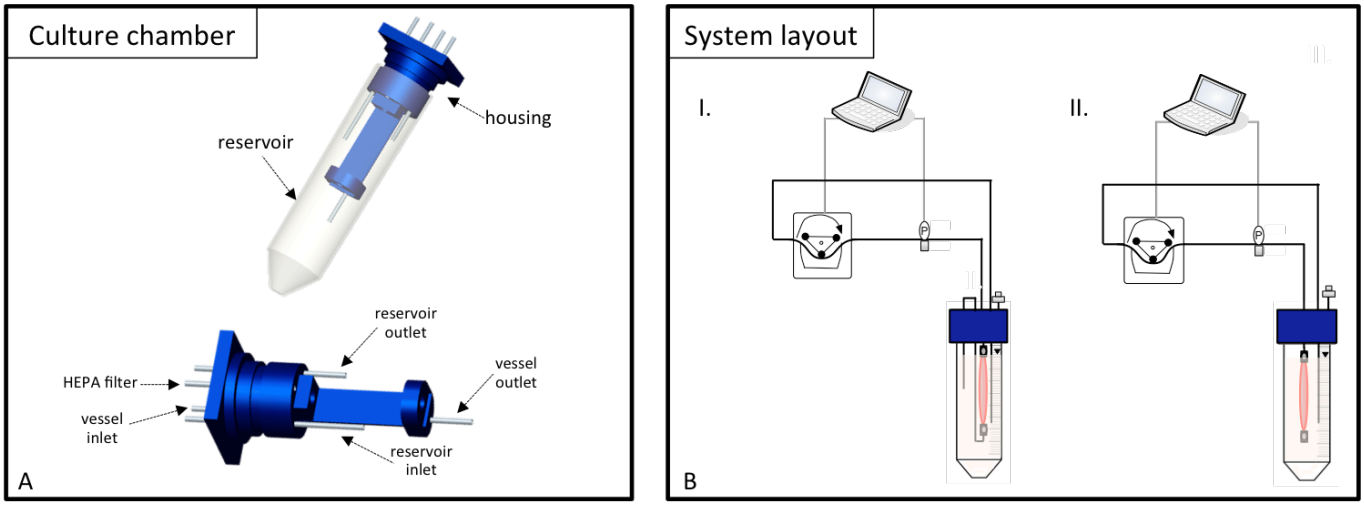

Fig.1: A) Sketch of the EVCS. (A) Three-dimensional (3D) CAD model of the SV culture chamber. B) Hydraulic configuration of the biomechanoreactor during dynamical culture (I) and biomechanical characterization (II): thick lines, the hydraulic circuit; thin lines, the monitoring and control system (M/C) signals. In particular, the M/C system manages the pump, and a pressure sensor registers the pressure signal.

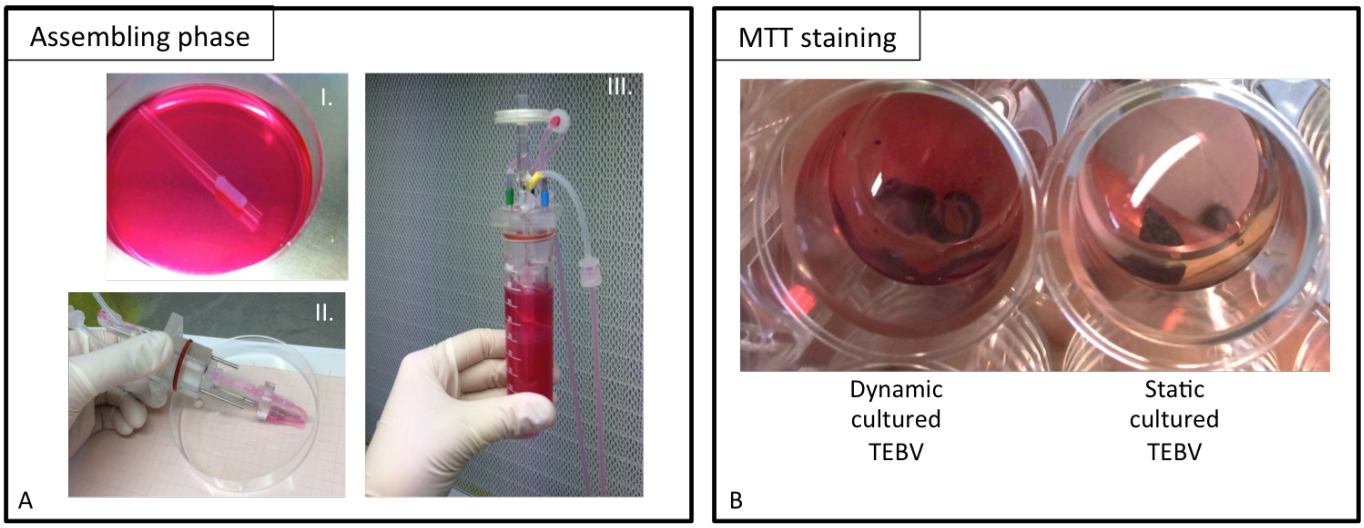

Fig.2: A) Assembling phases of the culture system under laminar flow hood: (I-II) the TEBV sample is mounted within the culture chamber and secured via loops. (III) The culture chamber is then connected to the hydraulic circuit, filled with culture medium and then incubated for 7 days at 37°C. B) Images of TEBV samples stained with MTT after 3 days of culture within the biomechanoreactor under dynamic and static conditions.