An electro-mechanical bioreactor providing physiological cardiac stimuli

AN ELECTRO-MECHANICAL BIOREACTOR PROVIDING PHYSIOLOGICAL CARDIAC STIMULI

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Introduction

After myocardial infarction, the injury of the heart wall muscle is permanent because the myocardial tissue lacks significant intrinsic regenerative capability. In cardiac tissue engineering, the scaffold-based tissue replacement is one of the most studied approaches to restore and regenerate the functionality of the impaired cardiac muscle. Cells are seeded on scaffolds showing specific properties and are expected to proliferate and self-organize into a functional cardiac tissue for implantation. The role of physical stimuli in improving functional and mechanical properties of the engineered cardiac constructs has been widely demonstrated [1,2,3], and bioreactors can significantly contribute to this objective by providing a suitable environment for the maturation of the engineered cardiac tissue.

Starting from a previous prototype [4], we developed and implemented an electro-mechanical bioreactor, the Cardiac Patch Dynamic Culture Device (CPDCD), able to generate a biochemical and physical environment suitable for proliferation and cardiac differentiation of stem cells cultured on cardiac patches. In particular, by the delivery of tightly controlled uniaxial cyclic stretching and electrical stimulations mimicking the physiological physical stimuli of the cardiac tissue, this bioreactor allows to dynamically culture four biological constructs. Preliminary cellular tests demonstrated the suitability of the CPDCD.

Materials and Methods

The CPDCD was designed for controlling and delivering concurrent defined stretching and electrical patterns, according to the technical specifications reported in Table 1, collected from literature [2,3,5]. Cytocompatible, corrosion-resistant, and sterilizable materials were selected for bioreactor components in contact with culture medium and cardiac patches.

Stimulation parameters	Values
Strain (%)	0.5 – 20
Frequency of stretching stimulation (Hz)	1 – 2
Voltage (V/cm)	0.1 – 8
Frequency of electrical stimulation (Hz)	1 – 10
Pulse duration (ms)	1 – 2

Table 1. Technical specifications of stimulation parameters for mimicking the native physical stimuli of the cardiac tissue [2,3,5].

CPDCD set-up and components

The CPDCD (Fig.1) is composed of:

- a transparent, sealable and sterile *culture chamber* realized in polymethyl metacrylate (Fig.2) where cell-seeded cardiac patches are housed and submerged in culture medium (Fig.3b) during the entire duration of the experiments (100x97x55 mm^3; Working volume = 70 ml);
- a *mechanical stimulation system*, including holders, stepper motor, and controller (Fig.2), that provides defined uniaxial tensile cyclic stretching and is provided with control subsystem (Strain/elongation = 0.5 – 20%; Frequency = 1 – 2 Hz);
- an *electrical stimulation system*, including voltage source (Fig.3a) and five electrodes (Fig.2), that provides defined electrical stimulation to the biological constructs (Voltage = 6 – 8 V/cm; Square-wave pulse; Frequency = 1 – 10 Hz; Pulse duration = 0 – 10 ms);
- a *recirculation system*, constituted by oxygen-permeable tubes, peristaltic pump, fresh media reservoir, and waste receptacle (Fig. 3c), that allows automated medium replacement.

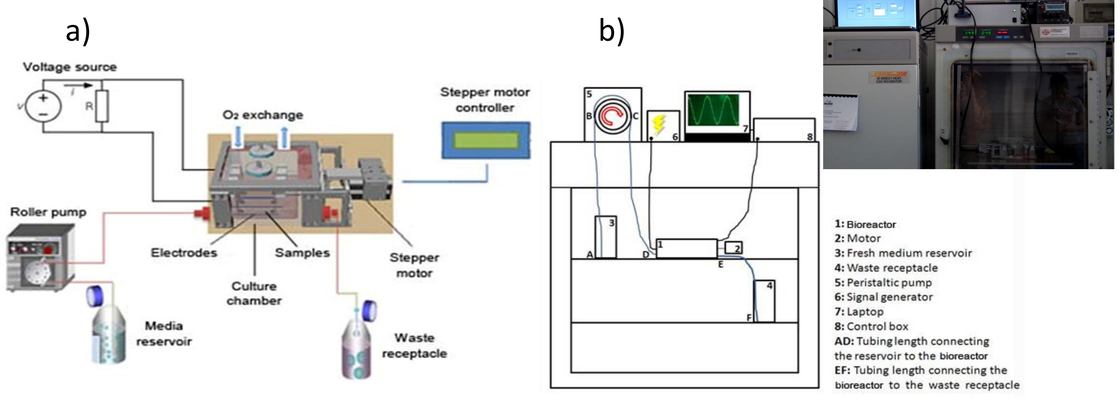

Figure 1. a) CPDCD set-up; **b)** Schematic representation and picture of its configuration within the incubator. The CPDCD is composed of: a culture chamber; a mechanical stimulation system; an electrical stimulation system; a recirculation system.

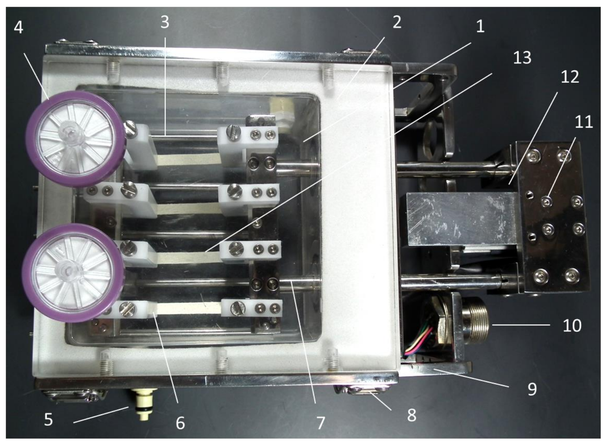

Figure 2. Bioreactor components: (1) Culture chamber, (2) Lid, (3) Electrode (4) Filter, (5) Quick-disconnect coupling, (6) Holder, (7) Shaft, (8) Latch, (9) Frame, (10) Cable connector, (11) Shaft holder, (12) Motor, (13) Patch

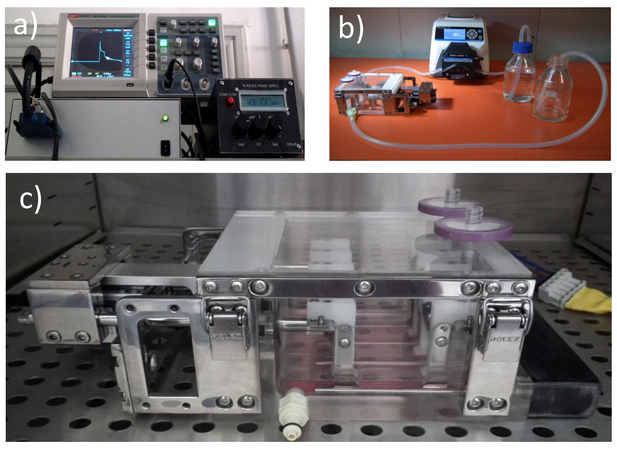

Figure 3. a) Oscilloscope and voltage source; **b)** CPDCD housing cell-seeded scaffolds within the incubator; **c)** Recirculation system.

Preliminary cellular tests

Enhanced Green Fluorescent Protein positive Rat Cardiac Progenitor Cells ($EGFP^{pos}$ CPCs) were seeded at a density of $4x10^4$ cells/cm^2 on poly-glycerol-sebacate (PGS) cardiac patches, some of which functionalized with GRGDSP (fibronectin). After 48h of static culture for allowing cell-patch adhesion, the patches underwent mechanical stimulation (strain 5%, frequency 1Hz) for 24 hours.

Results and Conclusions

In-house behaviour/operating tests were conducted and confirmed the suitability and the performances of the CPDCD: different set of polymeric cardiac patches were undergone to uniaxial tensile cyclic stretching in wet conditions (10%, 1 Hz, in PBS) for 5 days, the tests demonstrated the fittingness of chamber isolation, holding system, and motor running.

Preliminary cellular results demonstrated that the CPDCD guarantees the sterility and the viability of the cell culture. Moreover, $EGFP^{pos}$ CPCs seeded on functionalized PGS patches were observed distributed following stretching direction (Fig. 4) suggesting a role of mechanical stimulation in cell distribution.

Findings from preliminary cellular tests demonstrate the potentiality of this bioreactor to be used as a model system for 1) testing cytocompatibility and durability of seeded cardiac patches, and 2) investigating the influence of electro-mechanical stimulation on cells cultured on cardiac patches.

In conclusion, this device can be used as a multipurpose adaptable system for dynamic culture of cell-seeded patches for the production of functional engineered constructs.

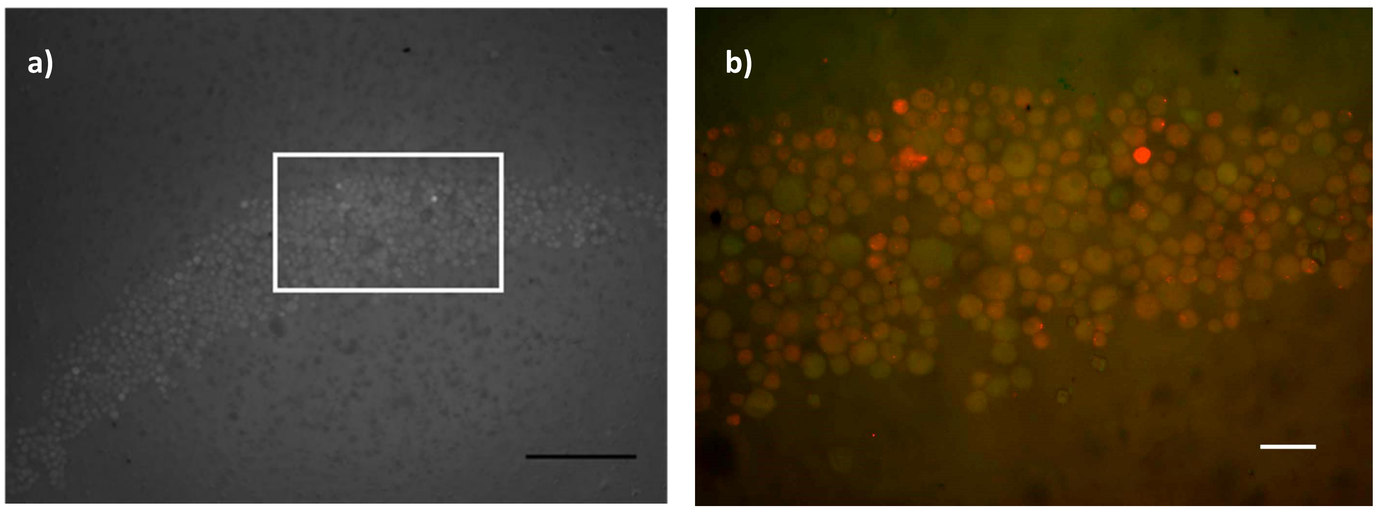

Figure 4. a) Phase contrast microphotograph shows $EGFP^{pos}$ CPCs adhered on functionalized PGS patches and distributed following stretching direction ; **b)** microphotograph magnification captured by fluorescence microscope. Scale bars: 500 µm (a), 100 µm (b).

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