Towards Cardiovascular Tissue Engineering:  
Macro to Micro Bioreactors

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Abstract — It has long been recognized that the combination of several factors, such as the electrical and mechanical stimulation, coupled with biochemical conditioning, is the most promising approach for in vitro development of fully functional engineered tissue-like structures having concrete chances of success when implanted in vivo for the repair of the impaired myocardium. In this scenario, the design of advanced technological platforms, i.e., bioreactors, represents a key element to define standardized, automated and repeatable bioprocessing steps replicating in vitro the physicochemical and mechanical cues triggering maturation of engineered cardiac tissues. We describe our technological advances in developing macro- and micro-scale bioreactors aimed at providing single or combined mechanical/electrical stimulation either to cultured cells or tissue constructs. Additional design requirements are: i) trading off the technical specifications with the needs of the final user’s environment, i.e. the biological lab; ii) the straightforward operability and the compliance with good laboratory practice; iii) a smart use of the concept of modularity, i.e. allowing simultaneous multiple cultures with independent stimulation patterns, which speeds up the experimental campaigns. The developed devices allow for a fine tuning of cellular electrical/mechanical stimulations, representing ideal tools for standardized and high-throughput analyses in cell biology and cardiac regenerative medicine.

I. THE ROLE OF BIOREACTORS IN MYOCARDIAL TISSUE ENGINEERING

In vitro (as in vivo) cells are extremely sensitive to the stimuli they receive from the external microenvironment: cell fate, differentiation and the accomplishment of cell specific functions can be tightly regulated in response to environmental factors. In particular, in myocardial tissue engineering, it has long been recognized that the combination of electrical and mechanical stimulation, conveniently coupled with biochemical conditioning, is the most promising approach for the in vitro development of functional cardiac tissue-like structures to be implanted in vivo for the repair of the impaired myocardium (Vunjak-Novakovic et al., 2009).

In the regenerative medicine perspective, the design of highly efficient technological platforms, i.e., bioreactors, able to provide cultured cells with proper stimulation patterns, represents a central element to define standardized, automated and repeatable bioprocessing steps replicating in vitro the physicochemical and mechanical cues triggering cardiomyogenic tissue maturation and functionalization. Although the encouraging results achieved in last few years by several groups worldwide, numerous drawbacks still exist in this research field, which require to be further elucidated.

We here present our technological advances in developing macro- and micro-bioreactors aimed at providing single or combined electro/mechanical stimulation to cultured cells/tissue constructs. This task was carried out along two parallel approaches involving the use of macro- and micro-scale platforms, therefore facing the problem at two different scales of investigation.

II. AN ELECTRICAL STIMULATION-BASED BIOREACTOR FOR STEM CELL CARDIOMYOGENIC DIFFERENTIATION

Cardiac cell-based therapy is to date emerging as a valid clinical and therapeutic approach to replace scarred, non-functional myocardium in the diseased heart. However, the elevated number of cells required to enable the structural and functional healing of the infarcted heart muscle dramatically hinders effective translation of cell-based approach to bedside. Adult cardiomyocytes, which are the ideal donor cells for the repair of the myocardium due to their intrinsic electrophysiological responsiveness, are characterized by poor proliferative capacity, besides limited availability. Several studies have therefore focused on the in vitro cardiogenic differentiation of stem cells (SCs) to provide a renewable and unlimited cell source to be employed in the manufacturing of bioartificial cardiac engineered tissue substitutes. In recent studies the goal of SC differentiation through the cardiac lineage has been
achieved by electrical stimulation of cells. Nevertheless, despite the application of electrical signals in cardiac tissue engineering enhances the functional coupling of the cells and the formation of synchronously contractile tissue constructs, the effect of different electrical stimulation patterns has not been fully investigated.

The main aim of current work was to further explore and compare the effects of different electrical field stimulation patterns in terms of SC cardiac differentiation enhancement. A multi-chamber culture systems equipped with electrical stimulation was developed (Pavesi et al., 2010a): the culture system (Fig. 1, left) features a chassis, including electrical wiring systems, equipped with housings for multiple culture chambers (15x30 mm), allowing for simultaneous multiple cell cultures with independent electrical stimulation patterns, also avoiding cross-contamination during experiments.

Stainless steel electrodes are embedded within the PDMS culture chambers (Fig. 1, right). Each chamber is completely removable from the chassis, to facilitate laboratory operations. The bioreactor is driven by dedicated graphical-interface software, wherein the operator can set and control the stimulation patterns delivered to the electrodes.

The electric field distribution within the 3D environment of the bioreactor was evaluated using a finite-element technique-based software (Comsol® multi-physics 3.5). The computational analysis was aimed at establishing stimulation conditions enabling a uniform electric field on the cell-seeded surface within the chamber (Fig. 2), which is mandatory to ensure the repeatability of the experimental conditions.

Preliminary in vitro tests were conducted by culturing adipose-tissue-derived SCs. Either square monophasic (2 ms, 1 Hz, 8 V amplitude) and biphasic (2 ms, 1 Hz, ±4 V amplitude) pulses were applied to the cultured cells (Tandon et al., 2009).

Results show that, at the end of a 72-h electrical stimulation period, cells exposed to a biphasic pulsed electric field expressed Connexin 43 (Cx-43, a specific protein of the gap junctions of cardiac muscle cells) in the peripheral cell region, thus suggesting the tendency to form the cell-cell connections involved in myocardial electric propagation. Expression of Cx-43 was poorly observed after monophasic stimulation. Cx-43 was completely absent in not-stimulated (control) samples (Fig. 3).

Experimental campaigns were conducted in collaboration with the Laboratory of Histology of the Faculty of Medical Science of Università del Piemonte Orientale.

III. ELECTRO-MECHANICAL STIMULATION OF 3D CARDIAC CONSTRUCTS

To date, one of the major difficulties encountered in developing functional cardiac equivalents in vitro resides in obtaining constructs featuring a contractile force comparable to that of the natural tissue. In vivo, myocardial tissue acts and resists against mechanical loading. Moreover, cardiac cells beat synchronously within the heart in response to electrical impulses. Together these stimuli provide cardiac cell functional contractility in the native heart. Therefore, it is reasonable to assume that electro-mechanical stimulation should be applied during in vitro tissue development, to provide a more physiologic-like environment for tissue maturation.

With the aim of providing cellular constructs with combined physical stimuli, a bioreactor was designed able to apply both electrical and mechanical stimulation to 3D engineered tissue constructs (Pavesi et al., 2009). The system (Fig. 4A), featuring both the mechanical actuator and electrodes for electrical stimulation, is meant to reside in an incubator and is equipped with housings for six independent culture chambers (Fig. 4B). Each culture chamber, consisting of a standard, sterile, disposable...
Falcon® tube (Fig. 5A), is completely removable from the chassis for facilitating under-hood manual operations. All parts inside the culture chamber are medical-grade silicone coated to enhance cytocompatibility specifications.

A 3D ring-shaped tissue construct can be housed across two grasps (Fig. 5B): one grasp is fixed to a static shaft featuring a fine pre-tension adjuster, while the other grasp is fixed to a moving shaft (connected to the actuator by a connecting rod), which applies the mechanical stimulus (uniaxial stretching, arrow in Fig. 5B) to the construct.

The mechanical simulation subsystem is piloted by a motorized linear slide actuator, driving six independent levers, each commanding a moving shaft belonging to each culture chamber complex (Fig. 5C): the fulcrum of each lever is displaced by operating on a linear guide (arrow in Fig. 5C) thus allowing the operator to scale the stroke of the mobile shaft.

IV. MICROFLUIDIC DEVICE FOR CELLS ELECTRO-MECHANICAL STIMULATION

Miniaturization of technological platforms able to provide controlled physical stimuli is a challenging issue. Indeed, it is becoming evident that conventional bioreactors, working on clinically relevant volumes of tissues and reagents, do not represent the proper tool for an economically-sustainable investigation of basic mechanisms of stem cell differentiation and tissue development. Microfluidic cell culture devices, on the contrary, enable both basic cell biology research and development of engineered tissues on an economically affordable scale. The possibility of automatically and individually addressing thousands of micro-bioreactor chambers renders microfluidics the ideal tool for high-throughput analyses in cell biology. PDMS microfluidic devices, in particular, represent promising and enabling technologies for cells based applications because of their versatility, biocompatibility, low processing costs and ease of technological transferability and experimental application.

A microfluidic cell culture device was realized equipped with 3D electrodes within a PDMS culture chamber (Fig. 6), and enabling for electro-mechanical stimulations (Pavesi et al., 2010b). For this purpose, a novel technology was developed (patent pending) aimed at embedding flexible electrodes (made of a nanocomposite material – PDMS and carbon nanotubes) without adding further steps in a traditional microfabrication process.

In vitro cytocompatibility tests were performed on H9c2 myoblasts, electrically stimulated with biphasic square electric pulses (2 ms, 1 Hz, ±1.5 V). After a 7-day culture period, no cytotoxic effects were detected (Fig. 7B).
In vitro tests were conducted in collaboration with the IRCCS Istituto Ortopedico Galeazzi Milano.

V. DISCUSSION AND CONCLUSIONS

Cardiac tissue engineering has improved immensely after the introduction of in vitro dynamic cell culture approach based on bioreactor technology. Bioreactors are to date used to perform studies in controlled conditions aimed at evaluating the effect of specific biological, chemical, mechanical and physical cues on cell behavior during in vitro cell cultures. Moreover, they constitute effective instruments by which piloting the activation of those intra-cellular pathways responsible for the development, maturation and functionalization of the engineered tissues.

Within this paper we described our technological advances in developing bioreactors for cardiac tissue engineering purpose, aimed at providing single or combined mechanical/electrical stimulation either to cultured cells or tissue constructs, therefore following a biomimetic approach, i.e., replicating in vitro the in vivo-like physicochemical and mechanical cues triggering maturation of engineered cardiac tissues.

The design of highly efficient technological platforms was carried out along two parallel pathways, involving the use of macro- and micro-scale bioreactors, facing the problem at two different scales of investigation. In detail, the developed devices allowed us to further investigate different aspects not still fully elucidated in the cardiac tissue engineering research field, and in particular, i) to compare the effect of different electrical stimulation patterns in terms of stem cell differentiation toward the cardiac lineage, ii) to combine electrical stimulation of 3D cardiac constructs with mechanical conditioning, and finally iii) to miniaturize technological platforms able to apply coupled electro-mechanical stimulation at the micro-scale, developing a cell culture device based on microfluidic technology.

Beyond the definition of specific technological requirements, additional design requirements involved: i) the trade off between technical specifications and the specifications related to the final user’s environment, i.e., the biological lab; ii) straightforward operability and the compliance with good laboratory practice; and iii) a smart use of the concept of modularity, i.e., allowing for simultaneous cell cultures within multiple units, with independent stimulation patterns.

The overall outcome was the development of laboratory-oriented tools, i.e., devoted to making systematic exploration feasible in the biological laboratory. Technically speaking, our bioreactors allow the user to design and apply single or combined mechanical and electrical stimulation in wide operating ranges, with the possibility to run a number of different tests in parallel, in separated chambers; the implementation of user-friendly graphical software interfaces facilitates the setting and the monitoring of the parameters during the experimental campaigns, with minimum technical skill requirements. Finally, the use of standard laboratory disposables was provided where possible.

In conclusion, we believe that the developed devices, allowing for a fine-tuning of cellular electrical/mechanical stimulations, represent ideal tools for standardized and high-throughput analyses in cell biology and cardiac regenerative medicine.

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