Carbon on poly(ε-caprolactone) (PCL) Ink-jet Printed Sensor for Monitoring Cell Cultures of Myoblasts

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Abstract- **Nowadays techniques for sensitive non-invasive, real-time monitoring of cell differentiation and maturation are highly demanded. In light of this, the development of electrochemical printed sensors impedance-based could represent a promising tool. In the present work, we developed 2D ink-jet printed sensors for myoblasts adhesion monitoring, using carbon-based ink on a substrate consisting in non-woven electrospun mats made in crosslinked poly(ε-caprolactone) (PCL). First of all, sensors printability was optimized and the biocompatibility tested. In order to determine the possibility to employ the prepared systems as scaffolds for dynamic cellular cultures, the mechanical response of the PCL scaffold was evaluated through the application of cyclic deformation tests. After that, electrical characterization of ink and substrate was performed, followed by electrochemical impedance-based measurements to evaluate myoblasts adhesion. Biocompatibility assessment showed good results for both carbon and PCL. Mechanical tests findings suggested that a training of 50 cycles and a proper value of strain should be applied before the cell seeding, in order to ensure a subsequent controlled strain amplitude. The sensorized scaffold allowed us to correlate cell adhesion with an increase of impedance module, in agreement with biocompatibility testing. Thus, this first preliminary testing suggested that this non-invasive impedance spectroscopy-based measurement system can be used for sensitive monitoring of cells adhesion, in static and moreover, as suggested from mechanical characterization, in dynamic conditions.**

Keywords- **Electrochemical Cell-substrate Impedance Spectroscopy (ECIS), cell monitoring, ink-jet printed sensors.**

I. Introduction

In recent years, techniques for sensitive non-invasive, real-time monitoring of cell growth and differentiation are highly demanded. A promising field is represented by electrochemical sensors that are capable of providing real-time quantitative information relating to cell growth and differentiation [1,2]. About this, it has long been recognized that Electrochemical Cell-substrate Impedance Spectroscopy (ECIS) is a valid detecting technique for cell adhesion [3]. More recently, ECIS has been employed to monitor many types of cells including cardiomyocytes [4].

Ink-jet printing technology, a type of printing that recreates 2D patterns by propelling droplets of ink onto substrates, including several types of materials, has found application for the synthesis of electrochemical sensors [5]. The use of biocompatible and conductive inks (carbon, PEDOT:PSS) on biocompatible and stretchable surfaces (PCL, poly(lactide-co-caprolactone) (PLCL)), elastic and resistant to cyclic strain, allows the development of sensors addressing the possibility to monitor cell cultures of myoblasts and cardiomyocytes under dynamic conditions [6].

In this work, in order to monitor myoblast adhesion, a sensor was produced by printing carbon ink pattern on electrospun mats composed by crosslinked PCL. Electrical, thermo-mechanical characteristics, dynamic features and biocompatibility of the sensor were evaluated, in order to validate the possibility to use this system for cell culture monitoring under mechanical conditioning.

II. Materials and Methods

A. Preparation of PCL scaffolds

The scaffolds were prepared as electrospun mats of crosslinked poly(ε-caprolactone) (PCL), obtained starting from α,ω-triethoxysilane-terminated PCL and crosslinked through sol-gel reaction of the triethoxysilane end-caps. The scaffold preparation route was carried out by an early partial crosslinking of the PCL precursors, followed by electrospinning at room temperature and by a later crosslinking

step to achieve an optimized network structure [7]. The scaffold was obtained as a 50 µm thick mat, constituted by continuous randomly oriented fibers, with diameters of about 2 µm.

B. Design and production of sensors and measuring system

After the optimization of sensor geometry, the PCL mat was cut into regular rectangles, fixed on a supporting sheet and sensors were then realized using carbon ink (Novacentrix) with a commercial ink-jet printer (Epson C88+). After curing sensors for 15 minutes at 110 °C, two copper wires were soldered to the electrodes, in order to perform electrical measurements.

C. Electrical and thermo-mechanical tests on sensors

To evaluate electrical features, impedance measurements were carried out in dry and wet (with culture medium) conditions. In addition, thermo-mechanical tests were carried out through differential scanning calorimetry and dynamic mechanical analysis.

D. Preliminary mechanical characterization of the scaffolds

To evaluate the future employment of the sensors for dynamic cell cultures, a preliminary mechanical response of the PCL scaffolds was carried out through the application of cyclic deformation test. The tests were performed at 37 °C under tensile conditions on specimens cut as strips (overall length = 30 mm; average gauge length = 15 mm; average width = 5 mm) from the mats. A dynamic mechanical analyzer DMA Q800 (TA Instruments) was employed under load-controlled conditions, and the specimens were subjected to 100 subsequent loading-unloading cycles at 7 N/mm, between a maximum load (ranging from 1 N to 3.1 N) and a minimum load equal to 0.05 N. The minimum load was chosen in order to avoid lack of load control at the end of unloading due to specimen buckling. Stress and strain values were calculated as engineering values from the values of force and displacement recorded during the tests.

E. Qualitative evaluation of cytocompatibility

To prepare sensors for cell seeding, they were washed with phosphate buffered saline (PBS) and fixed to the bottom of culture dishes using a biocompatible high vacuum grease that prevents their floating in the culture medium. After that, they were sterilized under UV radiation and finally coated with gelatin from porcine skin (Sigma Aldrich) to improve cells adhesion. After that for preliminary studies, sensors were seeded with L6 rat myoblast (30000 cells/cm^2) and incubated for 24 hours at 37 °C with 5% CO_2.

Cell adhesion and vitality evaluation were then performed using DAPI (4',6-diamidino-2-phenylindole) that is a DNA-intercalating fluorescent dye. Precisely, culture medium was removed and, after washing with PBS, myoblasts were fixed for 10 minutes with Immunofix (Bio-Optica). Cells were then incubated for ten minutes with DAPI (Life Technologies) and adherent myoblasts were observed under fluorescence microscopy (Axiovert 200M epifluorescent microscope, Zeiss). Furthermore, we performed Neutral Red and MTT assay.

F. Impedance Measurements

Impedance measurements were carried out using a standardized protocol before and after cell seeding in order to correlate variations in the electrical quantities with cell adhesion. The pre-seeding tests were carried out with medium after gelatin coating to obtain a value of reference, comparable with the measurements after-seeding. The measurements were performed by using the impedance analyzer HP4194A, keeping the sensor in a horizontal position. For each sensor two measures have been performed, recording as the impedance magnitude and phase, in a range of frequencies between 100 Hz and 10 MHz. The tests were performed in

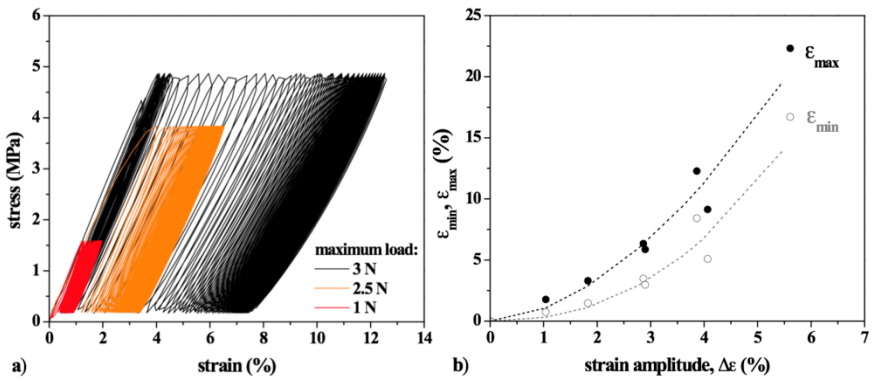

Fig. 1: a) Cyclic stress vs. strain curves for the crosslinked PCL scaffold subjected to various values of maximum load; b) Maximum and minimum strain values required to assure a given strain amplitude

triplicate.

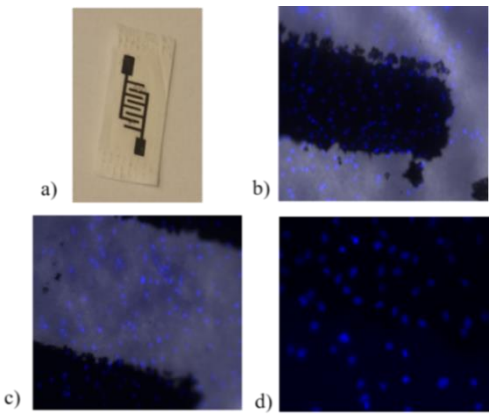

Fig. 2: a) Representative image of carbon on PCL sensor; b) Representative image of sensor with myoblasts stained with DAPI. The myoblasts appear adherent and evenly distributed both on PCL (c) and Carbon (d).

III. Results

A. Electrical and thermo-mechanical tests on sensors

Results from electrical characterization showed a capacitive behavior of sensors in the test performed with dry sensor and a resistive behavior when immersed in cell culture medium.

Thermo-mechanical tests revealed for the mat a melting temperature, T_m = 55°C, a crystallization temperature, T_c = 31°C, and the presence of a rubbery behavior above T_m. This latter result confirms the achievement of a stable cross-linked structure, which ensure the possibility to treat the material above T_m during the ink-printing step without the loss of the microfibrous structure, as confirmed by micro-structural characterization at the scanning electron microscope [7].

B. Preliminary mechanical characterization of the scaffolds

The material response is represented in terms of loading-unloading cycles in Fig. 1(a) for three maximum levels of force applied. The curves show that under each maximum stress conditions the specimens undergo mechanical hysteresis, and the effect becomes more important with the maximum stress applied. This determines a progressive shift of the cycle towards higher levels of strain, in particular for the first 20-30 cycles, reaching a more stable behavior in the following ones. Further, it is shown that the presence of a certain irreversible strain (i.e. not recovered at unloading) at unloading (ε_{min}) is found; as a consequence, the proper strain amplitude ($\Delta\varepsilon = \varepsilon_{min} - \varepsilon_{max}$) experienced by the film within each cycle is lower than the maximum strain applied (ε_{max}). In Fig. 1(b) the average values of ε_{min} and ε_{max}, measured after the first 50 cycles, are represented as a function of the strain amplitude, and this representation may serve to guide the choice of the parameters for the dynamic culture.

C. Qualitative evaluation of cytocompatibility

Results from DAPI cytocompatibility evaluation showed a good cell viability on all the sensors. Myoblasts appeared to be adherent and evenly distributed on the surface of the sensor, both on carbon and PCL, suggesting a good biocompatibility of both ink and substrate (Fig. 2). Due to the interference of the substrate, Neutral Red and MTT assay testing could not be reliably used for cytocompatibility assessment.

D. Impedance Measurement

Results obtained from impedance evaluation before and after cell seeding suggested the possibility to correlate cell adhesion to impedance changes. In particular, the carbon on PCL sensor showed a good trend with an increase of impedance after the cell seeding compared to the pre-seeding and in agreement with DAPI assay results (Fig. 3). It can be appreciated how the presence of the cell changes the measured impedance in the range of 10^2-10^6 Hz, with the biggest change recorded at frequencies in a range near to 10^3 Hz in all sensors tested (Fig. 3). An equivalent circuit-based fitting of the magnitude and phase angle spectra was performed resulting in the following parameters: *R electrode* and *C dl* (double layer capacitance) for the electrode, *Z Warburg* and *R solution* for the effect of electrolytic solution and *R Cell*, *C Cell* and *R gap* due to cell coverage.

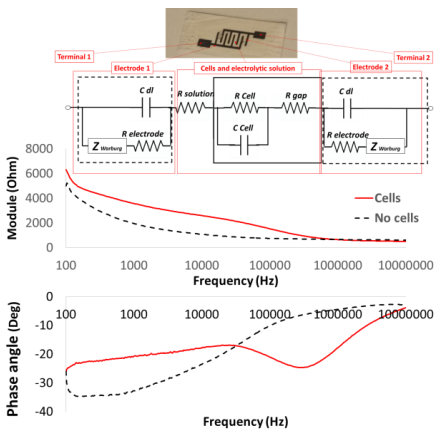

Fig. 3: Comparison between impedance module and phase angle with and without seeded cells and a schematic representation of the equivalent circuit model.

IV. Discussion

Despite cell cultures represent a well-accepted model for drug testing and pathology development, a critical point is still represented by the possibility to monitor in real-time cell adhesion, growth and differentiation processes. In fact, often, testing methods are invasive, time- and cost-consuming and, in general, destructive [8]. In this field, the use of electrochemical sensor coupled to ECIS represent an excellent tool for the continuous screening of cell properties and behavior, without affecting standard culture conditions. More recently, ECIS research turned to excitable cells, such as cardiomyocytes [4].

In this work, an ink-jet printed sensor was used to analyze the adhesion of myoblasts. The choice of carbon-ink and PCL-substrate was performed after previous studies where different materials and inks were compared [9]. This new study confirms the cytocompatibility of both the identified ink and substrate. In addition, in order to address the specific application, thermo-mechanical tests confirm the achievement of a stable crosslinked structure of PCL, which ensure the possibility to treat the material above T_m during the ink-printing step without the loss of the microfibrous structure. Moreover, the preliminary results obtained from ECIS show an increase of impedance after cell seeding consistent with the optical analysis and the DAPI assay, making the sensor suitable for cell monitoring. For all sensors, the highest impedance increase could be measured, in accordance with previous works [10, 11], in a window of frequency around 10^3 Hz. The comparison between data with and without cells performed using equivalent circuit, highlighted that the effective cell coverage, can be electronically schematized with the introduction of a RC parallel circuit, in agreement with several literature findings [1].

As it is well known, cardiomyocytes in the body undergo cyclic mechanical strain induced by the rhythmic heart beating. Further, numerous studies show that mechanical conditioning promotes structural and functional maturation of mouse and human cardiomyocytes [4, 12]. In light of this, results of mechanical stress confirm the possibility to apply strain in the physiological ranges. More specifically, the characterization showed that in order to ensure a certain strain amplitude requested from the dynamic cellular culture: i) it would be preferable to train the scaffold for at least 50 cycles before the culture; ii) a proper value of strain maximum has to be applied.

V. Conclusions and Future Outlooks

In conclusion, we developed a sensor capable of providing information about myoblasts adhesion, representing a promising strategy to control cardiomyocytes biological activities under mechanical conditioning. Thus, future studies will focus on the use of the described stretchable system to study contractile cells mimicking the physiological conditions. In the close future, the proposed system will allow complete monitoring, correlating impedance variations not only to cell adhesion, but also to cell growth and differentiation.

Conflict of Interest

The authors declare that they have no conflict of interest

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