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Preliminary Study of Inkjet Printed Sensors for Monitoring Cell Cultures

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Abstract

An extremely promising methodology able to obtain feedbacks from cell cultures is represented by the direct integration within culture substrates of specific sensitive elements capable to provide information related to cell adhesion, migration, differentiation and growth. At present, the most common materials used in the implementation of sensors monitoring 2D cell culture are noble metals. However, printed electronics allow instead an innovative approach, from both sensor realization technique and utilization of sensitive materials. This project aims to develop and test 2D ink-jet printed sensors, focusing on biocompatible substrates and conductive inks. Both biocompatibility and printability of two different sensor designs were evaluated, followed by electronic measurements that estimate fibroblast adhesion. Preliminary findings show a good biocompatibility of the Kapton® substrate coupled with PEDOT:PSS ink. This solution allowed us to correlate cell adhesion with an increase of impedance module, in agreement with the optical observation. On-going works rely on the evaluation of different materials used for both substrates and inks, addressing the possibility to monitor cardiomyocyte activity.

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

1. Introduction

Recently, a growing interest in pharmacology, regenerative medicine, and tissue engineering has been related to the identification of measuring systems and non-invasive methods that can provide real-time quantitative information

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regarding cell growth and differentiation, in order to obtain a feedback of cell functions and tissue development. An extremely promising methodology is represented by the direct integration into culture plates and scaffolds of sensitive elements capable of providing information relating to cell growth, migration, differentiation or to record cellular electrical activity. In this perspective, cell-substrate impedance measurements (ECIS) are usually adopted [1, 2]. In general, the materials used so far in the realization of this type of sensors are noble metals (i.e. gold or platinum), for both two dimensional (2D) electrodes and sensitive elements (mainly nanoparticles or wires) implemented in three dimensional (3D) environment [3].

Printed electronics represent instead an innovative approach, in terms of both implementation (inkjet printing) and use of sensitive materials (i.e. conductive inks), aiming to reduce production costs and improving the biocompatibility and the adaptability of these devices to deformable structures [4].

Concerning 2D sensing applications, of particular interest has been the introduction of organic electronics (e.g. PEDOT:PSS, carbon) and of stretchable surfaces to investigate cardiomyocyte and neural cell behavior, innovating traditional technology such as Micro Electrode Arrays (MEAs) [5]. Similarly, in 3D sensing applications, nanostructured materials (e.g. carbon nanotubes), PEDOT:PSS, and conductive materials are emerging as promising techniques to obtain feedbacks from cells mimicking a physiological environment.

The main goal of this study was to realize and characterize 2D printed sensors for monitoring cell culture adhesion and growth, by means of ink-jet printing technology, with a particular attention to the evaluation of substrate and ink biocompatibility.

2. Materials and Methods

Different substrates: polyimide (Kapton®), polyethylene terephthalate (PET), PET treated to improve printability (Novele™ and Coveme®), thermoplastic polyurethane (TPU from Nagase®) and inks for inkjet printing (PEDOT: PSS, carbon, silver) were considered. After evaluating biocompatibility and printability, ECIS measurements were performed to evaluate the ability to monitor cell adhesion.

2.1. Qualitative and quantitative evaluation of cytocompatibility

After preparing samples of proper dimensions and printing strips of inks, substrates were attached to the bottom of a cell culture well using biocompatible high vacuum grease (Dow Corning), sterilized by exposure to UV rays, and finally seeded with fibroblasts. After 24 hours, the samples were observed with an optical microscope to evaluate cell adhesion.

Cell adhesion quantification was then performed using a specific dye, Neutral Red, able to stain cellular lysosomes. In details, culture medium of the cultured samples was replaced with a Neutral Red solution and the samples were incubated at 37 °C with controlled humidity and 5% CO_2. Once washed, samples were immersed in a de-staining solution, under constant agitation, and then the absorbance of the solution was quantified using a spectrophotometer.

Quantification of cells adherent on each substrate was calculated referring to a calibration curve obtained by incubating a specific number of cells in the same assay. As blank samples, a set of substrates without any seeded cell was used to assess the amount of absorption due to the material itself.

2.2. Design and production of sensors and measuring system

Two different designs of the sensors (monopolar and interdigitated) were printed using a home ink-jet printer (Epson XP 225) and the following combinations: PEDOT: PSS on Kapton® and on PET substrates, silver on treated PET (Novele™). Before proceeding with the printing process, Kapton® and untreated PET underwent specific O_2 plasma treatment to increase surface hydrophilicity and facilitate ink absorption. In order to perform ECIS measurements, two thin copper wires were soldered on working and counter electrodes. After that, sensors were glued to the bottom of Petri dishes using the adhesive biocompatible high vacuum grease, sterilized under UV radiation and finally seeded with fibroblasts. After 24 hours, cell adhesion and viability were qualitatively and quantitatively assessed as described in the previous paragraph. Results were then compared with the data obtained from ECIS.

2.3. Impedance Measurements

Preliminary impedance measurements were carried out using a standardized protocol before and after seeding fibroblasts on sensors, in order to correlate variations in the electrical quantities with cell adhesion and migration. Control tests were performed before cell seeding for all the samples, to evaluate impedance due to cell culture medium. Duplicate measurements for each sensor were performed by using the impedance analyzer HP4194A recording impedance magnitude and phase, in a frequency range between 200 Hz and 2 MHz.

3. Results

3.1. Qualitative and quantitative evaluation of cytocompatibility

Results from the qualitative and quantitative evaluation of cytocompatibility on samples of substrates and inks showed a good accordance (Fig. 1A). Polyimide appeared to be the most compatible material, PET and Coveme® showed an acceptable cytocompatibility, while Novele™ and TPU substrates the poorest one. Among the inks, PEDOT:PSS and carbon showed the best biocompatibility, while a poorer adhesion was observed on silver. The same evaluation performed on the complete ink-jet printed sensors highlighted polymide and PEDOT:PSS combination as the most compatible one: cells appeared to be well adherent and uniformly distributed on all the surface of the sensor. On sensors printed with PEDOT:PSS on untreated PET, a less uniform cell adhesion could be observed: interestingly, cells tended to migrate on the PEDOT:PSS printed pattern, showing instead a poorer adhesion on the other part of the sensor. Sensors printed using Silver ink on Novele™ substrates instead showed the poorest cell adhesion (Fig. 1B).

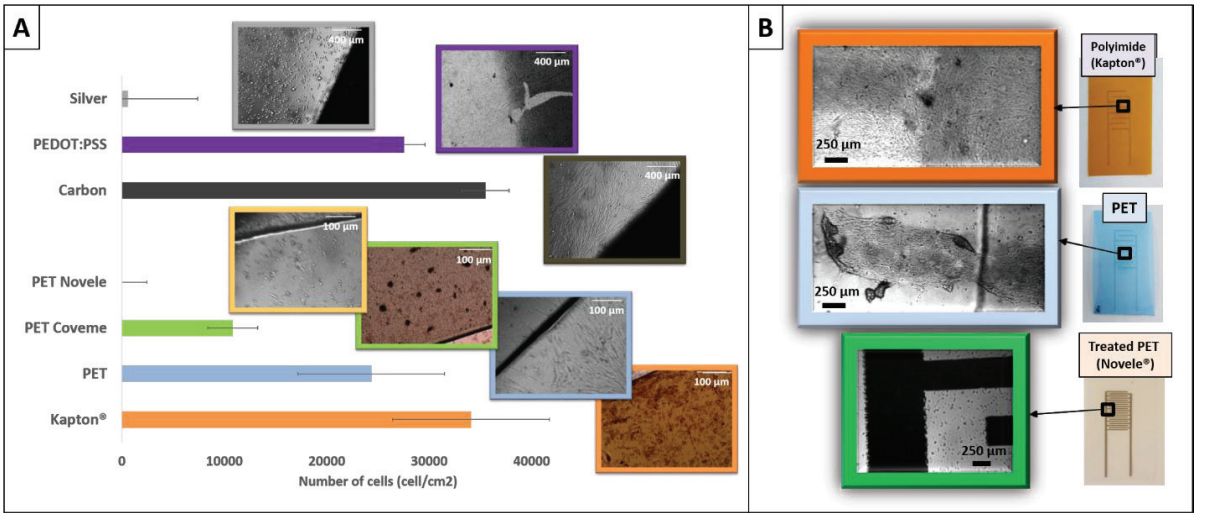

Figure 1: Cytocompatibility evaluation of cell adhesion on substrate and ink samples (A) and on the final ink-jet printed sensors, with different ink and substrate combinations (B).

3.2. Impedance Measurements

ECIS measurements showed results in accordance to what could be observed from the optical analysis, suggesting the possibility to use this technique to assess cell adhesion and growth (Fig. 3). In particular, measurements performed on sensors realized using Kapton® and PET after cell seeding recorded an increase of impedance module in the order of a few hundred ohms compared to measurements taken with only culture medium. On the contrary, ECIS measurements performed on Novele® substrates, where the optical analysis identified a poorer cell adhesion, showed no significant changes comparing impedance before and after cell seeding.

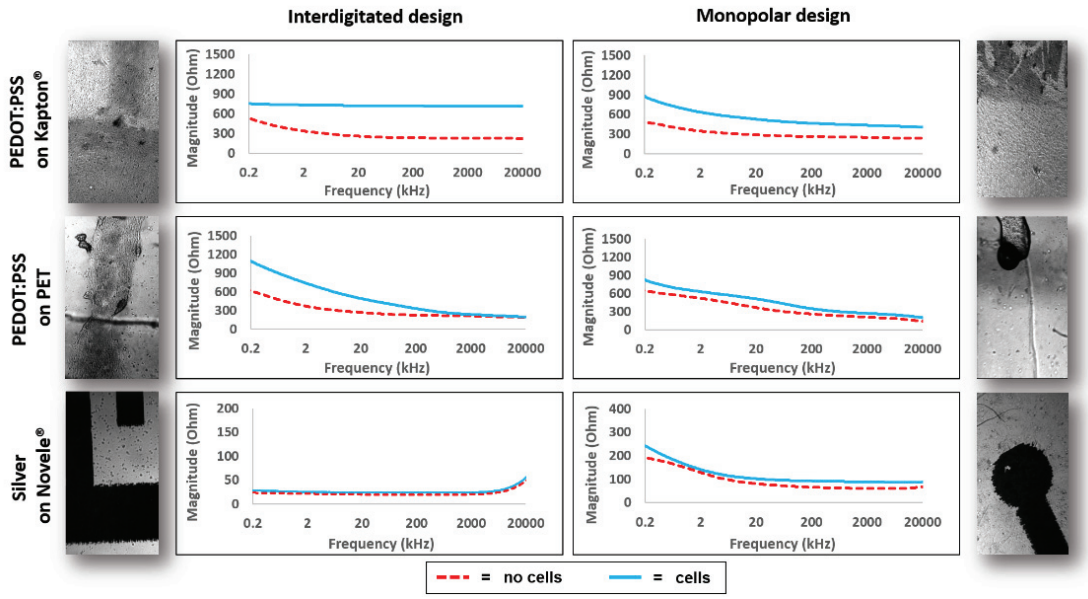

Figure 2 : Module acquired during ECIS measurements performed using both sensors designs for different material combinations (PEDOT:PSS on Kapton®, PEDOT:PSS on PET, Silver on Novele™

4. Conclusion and future outlooks

Results obtained from qualitative and quantitative evaluation of cell adhesion suggested the possibility to pursue in the investigation of the described materials and technology. On-going work refers to the quantification of cell adhesion using the protocol described for different set of combination of substrates and inks previously described. Once confirmed the ability of impedance measurements to assess cell adhesion through the ECIS measurements described, subsequent activities will concern the evaluation of the differences between impedance in the course of cell growth, with the objective of correlating changes in impedance to specific events of the cell cycle.

Stretchable materials in particular will be evaluated as possible substrates, aiming to a final application for monitoring cardiomyocyte activity under mechanical conditioning. Further research interests for future development may focus on the integration of sensing elements in 3D scaffolds, in order to monitor cell functions in an environment more similar to physiological conditions.

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