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Studies of endothelial monolayer formation on irradiated poly-L-lactide acid with ions of different stopping power and velocity

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ABSTRACT

In this work we study cell viability, proliferation and morphology of bovine aortic endothelial cells (BAEC) cultured on poly-L-lactide acid (PLLA) modified by heavy ion irradiation. In a previous study comparing ions beams with the same stopping power we observed an increase in cell density and a better cell morphology at higher ion velocities. In the present work we continued this study using heavy ions beam with different stopping power and ion velocities. To this end thin films of 50 μm thickness were irradiated with 2 MeV/u and 0.10 MeV/u ion beams provided the Tandar (Buenos Aires, Argentina) and Tandetron (Porto Alegre, Brazil) accelerators, respectively. The results suggest that a more dense and elongated cell shapes, similar to the BAEC cells on the internal surface of bovine aorta, was obtained for stopping power of 18.2–22.1 MeV cm^2 mg^{-1} and ion velocity of 2 MeV/u. On the other hand, for low ion velocity 0.10 MeV/u the cells present a more globular shapes.

1. Introduction

Cell sheet engineering has arisen as an attractive approach to tissue engineering. In this approach, the formation of an endothelial cell monolayer and the confluent cell cultures are harvested from a variety of substrates as intact, tissue-like sheets consisting of the cells and their associated extracellular matrix [1]. The control of the substrate surface properties is a substantial step in the development and improvement of biomaterials for clinical applications. Interaction of the surface with the biochemical or biological environments is crucial for firm attachment of anchorage-dependent cells under in vitro culture conditions; further cellular processes such as proliferation, survival, differentiation and migration are driven by the polymer surface characteristics [2]. Cells ability to anchor on a particular medium depends on the characteristics of the substrate as well as the culture conditions, cell type used and culture medium composition [3,2].

Among others, the most common substrate which is non-cytotoxic and biodegradable is polystyrene, commonly used to culture plates and three dimensional matrices. For preclinical studies in situ in experimental animals or in vitro in cell cultures, non resorbable synthetic polymers (polystyrene, polypropylene, polyethylene) are used [4,5]. Furthermore, many aliphatic types of polyesters have been extensively used for biomedical applications [6–9].

In particular, poly-L-lactide acid (PLLA) is a biodegradable polymer of great importance used in bioabsorbable sutures, drug delivery systems, biodegradable scaffolds and tissue-engineered blood vessels [10,11]. As the slow degradation of PLLA occurs by non enzymatic hydrolysis, it produces as end products, lactic acid and glycolic acid which are subsequently metabolized in the human body [12]. Nevertheless, there are drawbacks that result from their low bioaffinity. The difference in physico-chemical properties between hydrophobic polyesters and hydrophilic bio-active signals has a deep consequence on biomedical applications [13].

In order to achieve a higher attached cell density and to promote cell culture on biomedical materials, many studies have been reported to modify these biodegradable polymers using various approaches [14–18]. In particular, ion irradiation at different energies is an effective method used to provide specific surface properties in different polymer materials [19] and, in recent years, in PLLA [20,21]. The ion beam irradiation method modifies the surface characteristic and induces a more hydrophilic surface, without changing the degradation properties of PLLA [22]. In our previous

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work [23] we studied the cell adhesion and proliferation as a function of ion beam fluence and the result was that a better monolayer formation was obtained with fluence larger than 4×10^{10} ions/cm^2.

The purpose of the present work is to explore the effects on cell adhesion and proliferation with other physical parameters such as: stopping power and ion velocity. To this end we irradiated foils of PLLA with a constant ion fluence of 7×10^{10} ions/cm^2 and used different ion beams with stopping power from 5 up to 22.1 MeV cm^2 mg^{-1} and with ion velocities of 2 and about of 0.10 MeV/u. The ion velocity determined the size of the ion spot and then induced different energy densities on the irradiated surface. In this work we study the endothelial cell proliferation, morphology and monolayer formation on irradiated PLLA with heavy ion beams during 1, 3 and 7 days of cell culture.

2. Experimental procedure

2.1. Polymer irradiation

Commercial grade films of PLLA polymer with a thickness of 50 μm manufactured by Goodfellow (England) were used "as received". The samples were irradiated with ion beams perpendicular to the surfaces in a vacuum of 10^6–10^7 Pa. The irradiation times for each sample varied as a function of the current intensity and the ion beam fluence was 7×10^{10} ions/cm^2. The irradiations were performed in the Tandar accelerator, Buenos Aires, Argentina (2 MeV/u) and the Tandetron accelerator, Porto Alegre, Brazil (0.10 MeV/u). Table 1 lists the ions, stopping power, velocity and energies used in the different experiments. To minimize sample heating during irradiation the current density was kept between 0.25 and 1.50 nA/cm^2. After irradiation, the samples were stored in a nitrogen atmosphere.

2.2. Cell culture and conditions

The adhesion and proliferation of BAEC endothelial cell line derived from the tunica intima of bovine aorta were studied in vitro, following the same procedure described in our previous work [23]. Cells were routinely cultured using the Polystyrene (TCPS P100) with a solution of Dulbecco Modified Eagles Medium (DMEM GIBCO) high glucose supplemented with Fetal Bovine Serum and antibiotic.

Then the cells were incubated at 37 °C with 5% of CO_2/95% of air and relative humidity of 95%. After a treatment with trypsine, the cells were harvested. Both, irradiated and control pristine PLLA samples, were sterilized prior to inoculation of cells. The samples were placed on the bottom of 24 wells TCPS with a density of 10^4 cells/cm^2 and covered with a sterilized Viton® ring to prevent floating. The culture wells were incubated under the same experimental conditions.

2.3. EC adhesion and biochemical activity

The cells were seeded onto irradiated and non irradiated PLLA films and then incubated for 1, 3 and 7 days. After these periods of time, we monitored the viability and biochemical activity of cell populations following the MTT assay. This test allows to determine the mitochondrial functionality and therefore the viability of the adhered cells. These cell viability measurements are proportional to the number of alive cells attached to the surface. This method only senses cells attached and alive and it is preferred over others which only counts cells.

Each of the relative cell viability measurements was obtained from the ratio between the average intensity of three wells which a near non irradiated PLLA (pristine sample) for normalization purpose. These procedures tried to avoid any possible difference of non homogeneity on the batch recipient during the culture experiments. The final reported cell viability values resulted from the average of six independent measurements, involving a total of 18 cells and 6 pristine samples, and the errors were calculated as standard deviations.

Table 1
Stopping power, ion beams and ion beam velocity used to irradiate the PLLA samples.

dE/dx (MeV cm^2 mg^{-1})	Ion	Energy (MeV)	Velocity (MeV/u)	Ion	Energy (MeV)	Velocity (MeV/u)
5.0	^{12}C	24	2	^{16}O	0.8	0.05
7.8	^{16}O	32	2	^{16}O	1.7	0.10
18.2	^{28}Si	56	2	^{63}Cu	7.5	0.12
22.1	^{32}S	64	2	^{63}Cu	9.5	0.15

2.4. Morphology and spreading

Morphology of endothelial cells cultured on the tested surface was then evaluated on micro photographs taken with an Olympus BX51 microscope. More experimental details are described in our previous work [23].

3. Results

The purpose of this work was to measure the number of cells attached to the irradiated surface as a function of different physical parameters such as: stopping power and ion velocity keeping the ion fluence constant at 7×10^{10} ions/cm^2.

Fig. 1 shows the cell viability values normalized to the pristine sample (100%) for three culture times (1, 3 and 7 days) as a function of the stopping power and for two ion beam velocities. Fig. 1 (a) shows the viability values for the ion velocity of 2 MeV/u; as it can be seen, in the first day of culturing time, the cells adhesion is less than 200% independent of the stopping power. In particular for the 5 and 7.8 MeV cm^2 mg^{-1} stopping powers the cell viability values fluctuated close to the pristine ones (100%). On the other hand, for the 18.2 and 22.1 MeV cm^2 mg^{-1} stopping powers, a sudden increase is shown in the number of cells which almost doubled their number at each culture time. Fig. 1(b) shows the relative cell viability for the average ion velocity of 0.10 MeV/u. For the first day of culture time, the result of an adhesion intensity of 250% up to 310% is nearly independent of the stopping power. For the 3 and 7 days of culture times the cells proliferated up to a value of 500%, except for 5.0 MeV cm^2 mg^{-1} stopping power, when they double the initial number of cells reaching 600%.

In addition to the cell viability intensity a relevant parameter to evaluate is the morphology of cells on a surface. To this end Fig. 2 shows the pictures of the BAEC cells over different substrates that reach, after 7 days of culture time the largest cell viabilities values of 600%. Fig. 2(b and e) compare the 1 and 7 days of culture time for 22.1 MeV cm^2 mg^{-1} stopping power and 2 MeV/u velocity and Fig. 2(c and f) for 5 MeV cm^2 mg^{-1} stopping power and 0.10 MeV/u velocity.

In Fig. 2(b and c) it can be appreciated the difference in cell densities, between the 2 and 0.10 MeV/u velocity, after 1 day of seeding. This difference is a factor of about three (see Fig. 1). In Fig. 2(e and f) the cells after 7 days of proliferation can be seen. The compactness of the cells indicate that there are so many cells that they are about to cover the total available surface. When the cells cover the total surface they develop a monolayer and the closed contact between this particular type of cells inhibit the proliferation process.