Materials Science and Engineering C 77 (2017) 521–533

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Polylactide- and polycaprolactone-based substrates enhance angiogenic potential of human umbilical cord-derived mesenchymal stem cells *in vitro* - implications for cardiovascular repair

Małgorzata Sekuła [a,b,1], Patrycja Domalik-Pyzik [c,1], Anna Morawska-Chochół [c], Sylwia Bobis-Wozowicz [a], Elżbieta Karnas [a,b], Sylwia Noga [a,b], Dariusz Boruczkowski [d], Marta Adamiak [a], Zbigniew Madeja [a], Jan Chłopek [c,*], Ewa K. Zuba-Surma [a,*]

[a] Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 7 Gronostajowa St., 30-387 Krakow, Poland
[b] Malopolska Centre of Biotechnology, Jagiellonian University, 7A Gronostajowa St., 30-387 Krakow, Poland
[c] Department of Biomaterials, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, 30 Mickiewicza Ave., 30-059 Krakow, Poland
[d] Polish Stem Cell Bank, 29 Jana Pawła II Ave., 00-867 Warsaw, Poland

ARTICLE INFO

Article history:
Received 22 November 2016
Received in revised form 4 February 2017
Accepted 29 March 2017
Available online 30 March 2017

Keywords:
Human umbilical cord mesenchymal stem cells
Polylactide
Polycaprolactone
Angiogenesis
Cardiovascular repair
Blood vessel regeneration

ABSTRACT

Recent approaches in tissue regeneration focus on combining innovative achievements of stem cell biology and biomaterial sciences to develop novel therapeutic strategies for patients. Growing recent evidence indicates that mesenchymal stem cells harvested from human umbilical cord Wharton's jelly (hUC-MSCs) are a new valuable source of cells for autologous as well as allogeneic therapies in humans. hUC-MSCs are multipotent, highly proliferating cells with prominent immunoregulatory activity. In this study, we evaluated the impact of widely used FDA approved poly(α-esters) including polylactide (PLA) and polycaprolactone (PCL) on selected biological properties of hUC-MSCs *in vitro*. We found that both polymers can be used as non-toxic substrates for *ex vivo* propagation of hUC-MSCs as shown by no major impact on cell proliferation or viability. Moreover, PCL significantly enhanced the migratory capacity of hUC-MSCs. Importantly, genetic analysis indicated that both polymers promoted the angiogenic differentiation potential of hUC-MSCs with no additional chemical stimulation.
These results indicate that PLA and PCL enhance selected biological properties of hUC-MSCs essential for their regenerative capacity including migratory and proangiogenic potential, which are required for effective vascular repair *in vivo*. Thus, PLA and PCL-based scaffolds combined with hUC-MSCs may be potentially employed as future novel grafts in tissue regeneration such as blood vessel reconstruction.
© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Stem cells (SCs) are a unique cell type that possesses the ability of self-renewal and differentiation into various mature tissues. SCs have been widely recognized as promising cells for biomedical applications including tissue and organ regeneration. Due to several legal restrictions and biological obstacles related to the use of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPS) in human treatment, particular attention has been given to adult SCs including mesenchymal stem cells (MSCs) of various origins. MSCs may be easily isolated from several tissue sources including bone marrow (BM), adipose tissue (AT), peripheral blood (PB), cord blood (CB) as well as recently from umbilical cord Wharton's jelly (UC) [1,2].

Human UC-MSCs are multipotent, highly proliferating cells with low immunogenicity, which makes them potentially valuable for tissue repair and regeneration not only in autologous as well as allogeneic recipients [3,4]. Importantly, hUC-MSCs do not raise any ethical considerations or questions about teratoma formation, which are still important concerns in the case of clinical applications of ESCs and iPS [5,6]. Therefore, similarly to MSCs from other tissues, hUC-MSCs possibly exhibit wide clinical applicability in the treatment of numerous diseases including degenerative diseases, tissue injury, diabetes, limb ischemia, osteonecrosis, bone damage as well as burn-induced skin defects and myocardial infarction [2,7,8]. However, the regenerative efficacy of hUC-MSCs would strongly rely on their proliferative and differentiation capacities at the side of transplantation, which may be enhanced *via* several *in vitro* approaches such as utilization of supportive biocompatible scaffolds. Importantly, it has been shown that the physicochemical properties of culture surfaces may play a significant role in regulation of cell behavior, proliferation,

Abbreviations: MSCs, mesenchymal stem cells; hUC-MSCs, human umbilical cord Wharton's jelly mesenchymal stem cells; PLA, poly(L-lactide); PCL, polycaprolactone; CV, cardiovascular.

* Corresponding authors.
E-mail addresses: chlopek@agh.edu.pl (J. Chłopek), ewa.zuba-surma@uj.edu.pl (E.K. Zuba-Surma).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.msec.2017.03.281
0928-4931/© 2017 Elsevier B.V. All rights reserved.

differentiation potential and cell-to-cell communication, which opens new perspectives in tissue regeneration [9].

One of potential application of hUC-MSCs and novel supporting biomaterials is the development of blood vessel substitutes useful for vascular repair required for multiple disorders including cardiovascular (CV) diseases. Current therapies are often based on surgical replacement with autologous vascular graft or the use of synthetic materials that are still insufficient for a long lasting treatment solution [10]. Bioengineering of functional vascular networks is still a challenge in regenerative medicine field. Therefore, the interdisciplinary approach of combining biodegradable scaffolds as artificial niches modulating SCs fate, including their proangiogenic capacity, may be a promising method for the treatment of CV injury.

Several biodegradable materials have been developed as one of the key components for tissue engineering and novel biomedical technologies such as regenerative medicine and modern drug delivery [11]. The most commonly used biomaterials in medical applications are polymers, especially poly(α-esters) including polylactides, polyglycolide, polydioxanone and polycaprolactone (PCL) [12–15].

Poly(L-lactide) (PLA) represents a slow-degrading, semi-crystalline polymer with high tensile strength, low extension and high modulus [14]. Depending on the processing of the PLA affecting its mechanical properties, this material may be utilized in many applications ranging from load-bearing orthopedic devices to blood vessel conduits. PCL represents a semi-crystalline, highly processable polymer with low tensile strength, but very high elongation at break. PCL was tested and approved for use in *e.g.* drug delivery vehicles or tissue engineering scaffolds [14]. Both of these FDA-approved polymers are biocompatible, which makes them interesting for medical applications [11,16]. Moreover, PLA and PCL have been successfully used as a matrix component of many different composite biomaterials including those supporting cell growth and fate [17].

Thus, the aim of this study was to investigate the impact of the selected PLA- and PCL-based polymers on several biological properties of hUC-MSCs that are currently envisioned as a new important source of SCs capable for tissue regeneration under both autologous and allogeneic conditions. Interactions of these type of SCs with biomaterials used for tissue repair, have not been well studied. Therefore, we utilized 2D film-formed biodegradable variants of the polymers, which are FDA approved for clinical use to support *in vitro* propagation and proangiogenic capacity of hUC-MSCs. This experimental model allowed the removal of external factors that may affect cell microenvironment and induce changes in hUC-MSCs behavior including viability and capacity to differentiate into endothelial cells. A combined approach may open new perspectives for vascular regeneration by employing novel hUC-MSCs and well-established biocompatible scaffolds.

2. Materials and methods

2.1. Fabrication of PLA and PCL substrates

Poly(L-lactide) (Ingeo™ 3051D, Nature Works LCC) - PLA, poly(ε-caprolactone) (Sigma-Aldrich, USA, Mn = 80,000) - PCL, 1.4-dioxan (Pure P.A., Avantor Performance Materials Poland S.A.) and acetic acid (99.5%–99.9% Pure P.A., Avantor Performance Materials Poland S.A.) were used as delivered, without further purification.

Samples were prepared by the solvent casting method. To form 2.5% (w/v), 5% (w/v), 7.5% (w/v) and 10% (w/v) solutions, PLA and PCL were dissolved in 1,4-dioxan and acetic acid, respectively by mixing on a magnetic stirrer for 24 h (300 rpm, room temperature). Polymer solutions were casted onto glass Petri dishes (30 ml/dish), 12-well plates (for cell experiments) or glass coverslips with subsequent drying at room temperature (22 °C ± 2 °C) for 96 h. 2.5PLA, 5PLA, 7.5PLA, 10PLA and 2.5PCL, 5PCL, 7.5PCL, 10PCL are used to indicate the samples. All the polymer films were rinsed with cell culture medium DMEM/F12 (Sigma Aldrich, Missouri, USA) supplemented with antibiotics (300 U/ml penicillin, 300 μg/ml streptomycin; Thermo Fisher Scientific, Massachusetts, USA) prior to cell culture.

2.2. Physicochemical analysis of polymers

2.2.1. Differential scanning calorimetry (DSC)

For thermal analysis 3.6–4.0 mg of each sample was sealed in an aluminum pan, and placed in the equipment sample chamber (Mettler Toledo DSC 1, Switzerland). PLA and PCL samples were analyzed from 0 °C to 220 °C or from −100 °C to 220 °C (10 °C/min), respectively (atmosphere: nitrogen; intercooler for PCL).

2.2.2. X-ray diffractometry (XRD)

X-ray diffractometry analyses were performed for both semi-crystalline polymers – PLA and PCL. XRD patterns were taken with an X'Pert Pro diffractometer (Philips, PANalytical, Netherlands), equipped with a Cu anode ($K\alpha1 = 1.5406$ Å).

2.2.3. Atomic force microscopy (AFM)

Surface topography of the PLA and PCL samples dedicated for hUC-MSCs growth was investigated by an atomic force microscope (AFM, Bruker, Germany). Images were collected with the MultiMode® 8 Bruker AFM system, using Peak Force Tapping mode. Surface parameters were determined by NanoScope software (Bruker, Germany).

2.2.4. X-ray photoelectron spectroscopy (XPS)

The surface composition and chemistry of the polymer substrates were characterized using X-ray photoelectron spectroscopy (XPS). XPS spectra were recorded on a spectrometer (Vacuum Systems Workshop Ltd., England) with a Mg anode, using K-alpha radiation (1253.6 eV) under a vacuum of about 3×10^{-8} mbar, at an electron takeoff angle of 15°. The analysis depth was 3–10 nm. X-ray power of about 200 W was used during analysis. For calibration of the energy scale binding energy of 284.6 eV was used for the C—C and C—H. The high-resolution spectra were taken in the constant analyzer energy mode with a 22 eV pass energy. The spectra were fitted with Gaussian–Lorentzian peaks using XPSPEAK 4.1 (Prof. Raymund W.M. Kwok, Chinese University of Hong Kong).

2.2.5. Wettability

Water contact angle (WCA) was measured by the sessile drop method (DSA 10 Mk2, Krüss, Germany). Measurements were done at room temperature. Typically, ten 0.2 μl water droplets were used on the carefully cleaned PLA and PCL surfaces to determine the contact angle.

2.2.6. Mechanical testing

Tensile properties were tested according to the ASTM D 882 standard. The measurements were done using a universal testing machine with a 5 kN load cell (1435 Zwick/Roell, Germany). Six specimens per each sample type were used.

2.3. Isolation and cell culture of hUC-MSCs

hUC-MSCs were isolated from human umbilical cord Whartons' jelly using an explant method. Clinical material was provided by The Polish Stem Cell Bank S.A. with Ethical Committee permissions required for human tissue harvest. Umbilical cords (UC) were collected into tubes containing sterile phosphate buffered saline (PBS; GE Healthcare Life Sciences, Utah, USA) supplemented with antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin) and antimycotic (10 μl/ml; Sigma-Aldrich, Missouri, USA). After dissection and removal of the UC arteries and vein, the tissue was mechanically cut into small fragments of approximately 2 mm^2. Several explants were placed in tissue-culture polystyrene dishes (TCPS) in DMEM/F12 medium (Sigma Aldrich, Missouri, USA) supplemented with 10% heat-inactivated FBS (Sigma Aldrich, Missouri, USA), 100 IU/ml penicillin, 10 μg/ml

Download English Version:

https://daneshyari.com/en/article/5434552

Download Persian Version:

https://daneshyari.com/article/5434552

Daneshyari.com