Materials Science and Engineering C 34 (2014) 130–139

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Design of 3D scaffolds for tissue engineering testing a tough polylactide-based graft copolymer

R. Dorati [a,c,*], C. Colonna [a,c], C. Tomasi [b], I. Genta [a,c], G. Bruni [b], B. Conti [a,c]

[a] Department of Drug Sciences, University of Pavia, V.le Taramelli 12, 27100 Pavia, Italy
[b] C.S.G.I., Department of Chemistry, Division of Physical Chemistry, University of Pavia, V.le Taramelli 16 I, 27100 Pavia, Italy
[c] Center for Tissue Engineering (CIT), University of Pavia, Via Ferrata 1, 27100 Pavia, Italy

ARTICLE INFO

Article history:
Received 10 January 2013
Received in revised form 29 July 2013
Accepted 29 August 2013
Available online 6 September 2013

Keywords:
Scaffold
Tissue regeneration
Cell adhesion
Biodegradation
Tensile properties

ABSTRACT

The aim of this research was to investigate a tough polymer to develop 3D scaffolds and 2D films for tissue engineering applications, in particular to repair urethral strictures or defects. The polymer tested was a graft copolymer of polylactic acid (PLA) synthesized with the rationale to improve the toughness of the related PLA homopolymer. The LMP-3055 graft copolymer (in bulk) demonstrated to have negligible cytotoxicity (bioavailability >85%, MTT test). Moreover, the LMP-3055 sterilized through gamma rays resulted to be cytocompatible and non-toxic, and it has a positive effect on cell biofunctionality, promoting the cell growth. 3D scaffolds and 2D film were prepared using different LMP-3055 polymer concentrations (7.5, 10, 12.5 and 15%, w/v), and the effect of polymer concentration on pore size, porosity and interconnectivity of the 3D scaffolds and 2D film was investigated. 3D scaffolds got better results for fulfilling structural and biofunctional requirements: porosity, pore size and interconnectivity, cell attachment and proliferation. 3D scaffolds obtained with 10 and 12.5% polymer solutions (3D-2 and 3D-3, respectively) were identified as the most suitable construct for the cell attachment and proliferation presenting pore size ranged between 100 and 400 μm, high porosity (77–78%) and well interconnected pores. *In vitro* cell studies demonstrated that all the selected scaffolds were able to support the cell proliferation, the cell attachment and growth resulting to their dependency on the polymer concentration and structural features. The degradation test revealed that the degradation of polymer matrix (ΔMw) and water uptake of 3D scaffolds exceed those of 2D film and raw polymer (used as control reference), while the mass loss of samples (3D scaffold and 2D film) resulted to be controlled, they showed good stability and capacity to maintain the physical integrity during the incubation time.

1. Introduction

Extracellular matrix (ECM) represents a support for cells providing a natural environment for the proliferation, the differentiation of cells and for the morphogenesis contributing to the organogenesis and cell-based tissue regeneration. In case of large tissue defects, it is unlikely that the damaged tissue initiates the regeneration process exclusively supplying cells to the diseased area because both cells and ECM as well as the surrounding environment are lost [1–3].

For this reason, the design and the development of local cell environment, namely artificial scaffolds, gain importance because they can initially assist the cell attachment and subsequently promote proliferation and differentiation, inducing cell-based tissue regeneration [1]. Several biomaterials have been studied and used to develop 3D scaffold and 2D film [4–6]. It is generally established that a biomaterial to be used in TE needs to have specific features such as biocompatibility, suitable surface chemistry, defined mechanical properties and biodegradability.

The choice of biomaterials for TE applications is dictated by the final application and consequently requires severe considerations regarding the physical, chemical and mechanical properties of selected material [7–9]. The prolonged permanence of the polymer in contact with biological fluids and the response of the immediate surroundings (tissues) determine the choice between biostable (non-biodegradable) and biodegradable polymers. Moreover, the biological interactions seem to influence the selection of naturally derived polymers vs synthetic ones, encouraging the combination of several materials and eventually their functionalization with specific bio-molecules to promote cell adhesion and proliferation [10].

A massive number of different natural materials have been studied and proposed for the development of 3D scaffold for TE, they present good biodegradability, low toxicity, low manufacture and disposal cost, and renewability. Moreover, these natural materials offer several advantages in TE area such as biological signaling, cell adhesion, and cell response degradation and re-modeling. However, their use as a unique scaffold constituent is generally minimized by i) the high solubility of

* Corresponding author at: Department of Drug Sciences, University of Pavia, V.le Taramelli 12, 27100 Pavia, Italy. Tel.: +39 0382 987786; fax: +39 0382 422975.
E-mail address: rossella.dorati@unipv.it (R. Dorati).

0928-4931/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2013.08.037

these materials and their ability to dissolve into the physiological fluids, ii) the rapid degradation and iii) the potential loss of intrinsic biological features during the formulation process. Furthermore, there are several concerns about the immune-rejection and disease transmission when natural materials are used [11]. Potential risks such as toxicity, immunogenicity and infections are reduced for pure synthetic materials which consist into well-known simple structures [12].

Synthetic materials exhibit predictable and reproducible physical, chemical and degradation properties that can be modified and tuned to satisfy the structural and functional requirements for the intended application. Moreover, they are easily processable into desired and specific shapes and sizes as well.

Poly(lactic acid) (PLA) is a biodegradable aliphatic polyester, and it has extensively been used as a biomaterial for use in the human body because of its adsorbability and non-toxicity after degradation [13,14]. PLA basically exhibits brittleness and its fracture behavior strongly depends on the crystal structure. Therefore, improving the toughness of PLA appears to be an important topic in the field of biomaterial and in particular for TE application [15,16].

The aim of this research was to explore the feasibility of using the LMP-3055 in the biological environment and to investigate the capability of using LMP-3055 as substrates for TE. In particular, the interest was focused on the design and development of 3D scaffolds and 2D film for tissue engineering. The polymer studied and tested was a graft copolymer of polylactic acid (PLA) supplied by the Marc Hillmyer research group (University of Minnesota). The copolymer was synthesized with the rationale to improve the toughness and tensile properties of the related PLA homopolymer; indeed, the TE applications of the PLA homopolymer are limited by its brittle nature [15].

The approach used to improve the toughness tensile properties of PLA homopolymer consists in the addition of rubbery domains into the backbone of PLA homopolymer. The rubbery phase makes available supplementary dissipation energy during the deformation process, resulting into an increase of block copolymer toughness [13].

The Mark Hillmyer group synthesized a graft copolymer (LMP-3055) containing 5% weight of the rubbery phase and 95% of PLA homopolymer with excellent toughness tensile properties. LMP-3055 is the poly(cyclooctadiene-co-norbornenemethanol-graft-lactide) with C200N3L95 composition, where the numbers respectively define the degree of polymerization of the cyclooctadiene (COD), the norborneneMeOH (N) copolymerized with COD and the D,L-lactide in the synthesized graft copolymer. The mechanical behavior of LMP-3055 graft copolymer was assessed by tensile testing of compression-molded bars using PLA samples as control. The PLA sample failed after a restricted deformation (13 ± 4%), with no evidence of neck formation and stress whitening: this behavior is intrinsic of a brittle material. On the opposite, LMP-3055 graft copolymer exhibited stress whitening, neck formation, and cold drawing with high elongation percentage (238 ± 43%) [15].

The toughness and tensile properties exhibited by LMP-3055 addressed the present study aimed to investigate on the use of LMP-3055 as substrate for tissue engineering application, as urethral reconstruction. LMP-3055 polymers could be useful as raw material to formulate biodegradable scaffolds for urethral reconstruction. Even though several advancements have been made in the surgical techniques for the urethral reconstruction, the nature of material used as substitute substrate in the repair of urethral strictures or defects remains the most challenging problem in this field. Various autologous grafts or flaps from skin or mucosa have been proposed for urethra structure repair, and today the buccal mucosa (BM) is considered as the best tissue for urethral substitution [17]. It is defined as the gold-standard for its good tensile properties, its stability to urine and excellent resistance to infections. Limits related to the use of autologous tissue are donor-site morbidity and time-consuming harvesting. To mimic the complex structure of urethral tissue the substrate will be prepared combining LMP-3055 with other biocompatible and biodegradable polymers.

In the first part of this work, attention was focused on the evaluation of the cytotoxicity of the LMP-3055 graft copolymer (in bulk) on adult fibroblasts as a healthy cell model by MTT assay. Moreover, the cytotoxicity of the graft copolymer was assessed after sterilization by gamma rays at 25 kGy dose, to verify the effects of the sterilization technique on biological performances, such as cell adhesion and growth. Gamma irradiation was selected because it is defined as the most appropriate technique for the sterilization of polymeric device, micro and nanoparticulate systems, implant and scaffolds [18].

The second part of the work was aimed to design and prepare 3D scaffold and 2D film. Scaffolds were produced using specific preparation procedures which were set up and optimized to attend the compulsory requirements of systems intended for the TE [12]. Scaffolds were prepared using different LMP-3055 concentrations and characterized in terms of shape, size, apparent density, pore size and porosity. The *in vitro* degradation properties of 3D scaffolds and 2D film were assessed through gel permeation chromatography (GPC) and monitoring the mass loss, water uptake and buffer pH shifts. The mechanical properties of LMP-3055 3D scaffolds and 2D film were measured by an electromagnetic testing machine. Biological tests were performed on 3D scaffolds and 2D film using human fibroblasts and evaluating both cell adhesion and growth.

2. Experimental section

2.1. Materials

The polymer LMP 3055 (95% mol of D,L lactide and, 5% mol of poly(1,5 cyclooctadiene-co-5-norbornene-2-methanol)), Mw 500 kDa and polydispersity 1.5, inherent viscosity 0.7 dl/g (~0.5% w/v in $CHCl_3$ at 30 °C) was synthesized by the Marc Hillmyer research group (University of Minnesota). Tetrahydrofuran (THF) and 1,4 dioxane, hexane, cyclohexane and ethanol were purchased from Sigma-Aldrich Corporation (Milan, Italy). Krebs–Ringers Hepes (KRS): sodium chloride (NaCl, 0.1 M), potassium chloride (KCl, 3.6 mM), sodium bicarbonate ($NaHCO_3$, 5 mM), monosodium phosphate (NaH_2PO_4, 0.49 mM), magnesium chloride anhydrous ($MgCl_2$, 0.01 M), glucose ($C_6H_{12}O_6$, 2.5 mM), bovine serum albumin (BSA, 0.0007 mM), N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES, 9.98 mM), sodium azide (NaN_3, 0.15 M) and monosodium phosphate (NaH_2PO4, Mw 119.98 g/mol, 0.49 mM) were obtained from Carlo Erba, Milan (Italy). Dulbecco modified Eagle's medium (DMEM) with 4.5 g/l glucose and glutamine was purchased from Lonza, Milan (Italy). Fetal bovine serum (FBS, Eu approved) was purchased from EuroClone, Milan (Italy). Human adult dermal fibroblasts as primary cells were purchased from International PBI, Milan (Italy). All the reagents were of analytical grade.

2.2. Methods

2.2.1. Cytotoxicity studies

The cytotoxicity of the LMP-3055 polymer was evaluated on two sets of samples: i) polymer suspension in DMEM at different concentrations (ranging from 0.01875 to 0.6 mg/100 μl of the working cell medium) and ii) LMP-3055 films (corresponding to LMP-3055 polymer amounts from 0.84 to 3.18 mg/100 μl of the working cell medium and prepared as reported below). Two sets of samples (polymer suspension and films) were considered because LMP-3055 polymer (as raw material) was like heterogeneous agglomerates, thus making difficult sample preparation.

The effects of LMP-3055 polymer on cell viability were assessed with the 3-(4,5-dimethyl-2-thiazolyl)-2,5 diphenyl-2H-tetrazolium (MTT) assay, using 96 Well Cell Culture Cluster with 10,000 fibroblasts plated in contact to 100 μl of polymer suspensions and polymer film samples [19].

Briefly, fibroblasts were cultured in wells in DMEM supplemented with FBS for 24 h at 37 °C, then media were removed and fresh