



Brief communication

Thermosensitive polymeric matrices for three-dimensional cell culture strategies

Ana Rita C. Duarte*, João F. Mano, Rui L. Reis

Biomaterials, Biodegradables and Biomimetics Research Group, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal
 Institute for Biotechnology and Bioengineering, PT Government Associated Laboratory, Guimarães, Portugal

ARTICLE INFO

Article history:

Received 7 July 2010

Received in revised form 13 September 2010

Accepted 24 September 2010

Available online 29 September 2010

Keywords:

Supercritical fluids

Cell expansion

Tissue engineering

Thermoresponsive matrices

Poly-lactic acid

ABSTRACT

A completely new strategy for cell culture focusing on the design of three-dimensional (3D) smart surfaces by supercritical fluid technology has been developed. This approach might overcome the limitations on cell expansion and proliferation of currently existing techniques. An alternative technology, based on supercritical carbon dioxide, was used to polymerize poly(N-isopropylacrylamide) (PNIPAAm) and to foam poly(D,L-lactic acid) ($P_{D,L}LA$), creating a thermosensitive 3D structure which has proven to have potential as a substrate for cell growth and expansion. We demonstrated that the thermosensitive matrices promoted cell detachment, thus $P_{D,L}LA$ scaffolds have the potential to be used as substrates for cell growth and expansion avoiding enzymatic and mechanical methods of cell harvesting. The harvested cells were replated to evaluate their viability, which was not compromised. A major advantage of this technology is the fact that the prepared materials can be recovered and reused. Therefore, the same substrate can be recycled and reused for different batches. An indirect impact of the technology developed is related to the field of biotechnology, as this novel technology for cell expansion can be applied to any adherent cell cultures.

© 2010 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The use of adequate cell sources in tissue engineering and regenerative medicine strategies is crucial for their success [1,2]. In most cases the number of cells harvested from a patient to be used in subsequent transplantation is insufficient, necessitating their expansion, while retaining their phenotype. A problem arises concerning the technologies currently available for cell expansion and proliferation [3]. Cell culture is usually performed in two-dimensional (2D) plates and enzymatic or mechanical methods are used to induce cell detachment. Besides the use of aggressive conditions for cell recovery, which might inactivate the cells, these methods of cell culture are inadequate for the production of the large numbers of cells required for research and industrial use.

This work aims to develop thermoresponsive 3D substrates, prepared using supercritical fluid techniques, as a new technology for cell expansion and proliferation. 3D thermoresponsive substrates with a large surface area allow the growth of a greater number of cells than the traditional 2D culture plates and avoid enzymatic or mechanical methods of cell recovery. Particularly interesting are thermoresponsive systems based on poly(N-isopropylacrylamide) (PNIPAAm), which are among the most widely investigated [4]. This

polymer presents, in aqueous solution, a lower critical solution temperature (LCST) of around 32 °C, which makes it extremely interesting for biomedical applications. Below the LCST it presents a flexible extended coil conformation, which makes it hydrophilic. In contrast, close to the LCST it becomes hydrophobic. The preparation of smart surfaces which can be tuned between hydrophobic and hydrophilic can either promote cell adhesion or detachment depending solely on the temperature (Scheme 1).

The preparation of the poly(D,L-lactic acid) ($P_{D,L}LA$) constructs was based on a supercritical fluid (SCF) technology which is well documented in the literature [5,6]. Supercritical fluids, especially supercritical carbon dioxide ($scCO_2$), have been identified as prime candidates to develop alternative clean processes, as they offer many advantages. CO_2 is readily available, environmentally acceptable, non-flammable, has low critical constants and leaves no toxic residue. The unique solvent tuneability of supercritical fluids, from gas-like to liquid-like properties, also offers the intriguing possibility of precise control over the processing conditions, leading to new interesting applications [7].

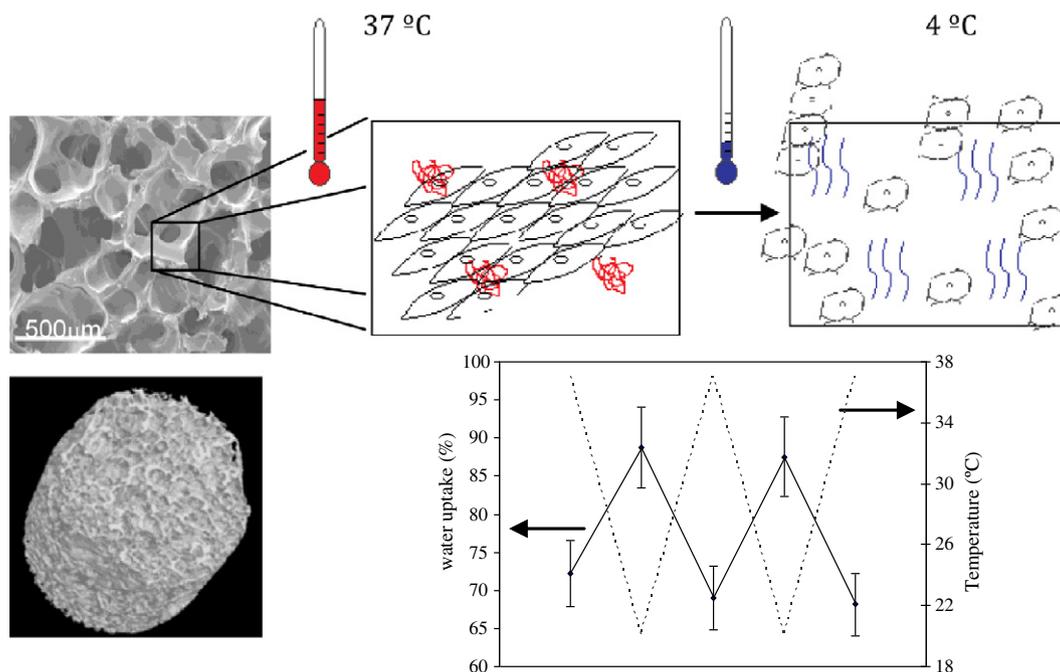
2. Experimental procedure

2.1. Supercritical fluid polymerization

PNIPAAm hydrogels were polymerized in a high pressure apparatus. Monomer, cross-linking agent (1.2 wt.%) and initiator

* Corresponding author at: Biomaterials, Biodegradables and Biomimetics Research Group, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal. Tel.: +351 253 510 900; fax: +351 253 510 909.

E-mail address: aduarte@dep.uminho.pt (A.R.C. Duarte).



Scheme 1. Illustration of the principle underlying the proposal in this work, thermoresponsive 3D substrates. At 37 °C the surface is hydrophobic, allowing cell attachment and growth. At lower temperatures the surface becomes hydrophilic, promoting cell detachment.

(2 wt.%) were loaded into the high pressure vessel, which was purged before the reaction was allowed to take place.

Based on the results presented by Cao et al. [8] and Temtem et al. [9], the percentage of cross-linking agent was chosen to be 1.2 wt.%, as this percentage yields 90% reaction conversion. Further, the PNIPAAm hydrogel prepared under these conditions has a LCST of 32 °C.

Polymerization was carried out, under stirring, at 65 °C and 20.0 MPa for 24 h. The operating conditions were chosen based on results described in the literature [8,9]. These experimental conditions ensure a homogeneous phase and that all the reagents are soluble in the supercritical phase.

2.2. Supercritical fluid foaming

The scaffolds were prepared by SCF foaming at 200 bar and 35 °C. In each experiment approximately 100 mg of PDLLA or a mixture PDLLA + 10 wt.% PNIPAAm was loaded into a mould, which was placed inside a high pressure vessel. The vessel was heated by means of a thin band electric heater (Ogden) connected to a temperature controller, which maintained the temperature within ± 1 °C. Carbon dioxide was pumped into the vessel using a high pressure piston pump (P-200A, Thar Technologies) until the operational pressure was attained. The pressure inside the vessel was measured with a pressure transducer. The system was closed for 30 min to allow plasticization of the polymer. Afterwards the system was slowly depressurized (~ 5 bar min^{-1}).

3. Results and discussion

The technique used to produce the 3D matrices is based on gas foaming technology, which takes advantage of the plasticizing properties of carbon dioxide. $P_{D,L}LA$ matrices were produced at 200 bar and 35 °C. A smart material can be successfully produced by incorporation of PNIPAAm into the substrate during processing. This single step procedure is of the utmost importance for the industrial production of these systems. Additionally, PNIPAAm can also be processed

using SCF technology [8,9]. In this work PNIPAAm cross-linked with *N,N*-methylenebisacrylamide was polymerized at 200 bar and 65 °C for 24 h. Penetration of the thermoresponsive hydrogel into the $P_{D,L}LA$ construct was confirmed by Fourier transform infrared spectroscopy (Fig. S1).

The morphological analysis was carried out by scanning electron microscopy (SEM) and micro-computed tomography, which allows determination of the porosity, interconnectivity and mean pore size of the materials, which were found to be 68%, 55% and 138 μm, respectively. The matrices present a rough inner structure with micro and macropores, which can encourage cell attachment and proliferation. Furthermore, the matrices are highly interconnected and thus nutrients and oxygen are accessible to the cells and cell waste can be eliminated. The pore size of the material is another important key issue, so that cells are able to penetrate the pores and then be released from the interior upon lowering the temperature.

Fig. 1 shows SEM images of the $P_{D,L}LA$ and $P_{D,L}LA$ + 10% PNIPAAm matrices colonized by L929 cells after 7 days culture. The insets represent magnified images of the materials, which show proliferation of the cells into the bulk of the matrix in more detail. Samples fixed with 4% formalin and dehydrated in a series of ethanol solutions were fractured using liquid nitrogen before being sputter coated with gold.

Fig. 1 also presents a confocal microscopic image of fibroblast-like cells after 7 days culture. Cells were stained with calcein and observed under a confocal microscope in order to evaluate the cell distribution within the construct. Green fluorescence indicates viability of the cells cultured on the matrices, and from the images we can conclude that there was good integration between the cells and the polymer.

Due to the thermoresponsive properties of the substrates, i.e. the possibility of tuning the hydrophilicity/hydrophobicity, recovery of the cells is possible simply by lowering the culturing medium temperature. The potential use of these materials in biomedical applications greatly depends on their cytotoxicity. Therefore, a cytotoxicity assessment of the matrices leachables was carried out as a preliminary approach to assessing their

ID	Title	Pages
959	Thermosensitive polymeric matrices for three-dimensional cell culture strategies	4

Download Full-Text Now



<http://fulltext.study/article/959>



-  **Categorized Journals**
Thousands of scientific journals broken down into different categories to simplify your search
-  **Full-Text Access**
The full-text version of all the articles are available for you to purchase at the lowest price
-  **Free Downloadable Articles**
In each journal some of the articles are available to download for free
-  **Free PDF Preview**
A preview of the first 2 pages of each article is available for you to download for free

<http://FullText.Study>