Novel thermosensitive poly$_{D,L}$ lactic acid 3D structure for cell growth and expansion

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Introduction: Stem cells are a promising approach in tissue engineering and regenerative medicine due to the self-renew potential of these cells, while retaining their differentiating potential. Nonetheless, a problem arises concerning the technologies currently available for cell expansion and proliferation. Cell culture is usually performed in 2D plates and enzymes or mechanical methods are used for cell detachment. Besides the use of aggressive conditions for cell recovery, which might inactivate the cells, these methods of cell culture do not respond to the large quantities of cells required for proper research and industrial use. Hereafter, we aim to develop of a novel technology for cell culture and expansion, based on thermo-responsive 3D substrates with large surface area allowing the growth of a higher number of cells than the traditional 2D culture plates and avoiding enzymatic or mechanical methods for cell recovery. The possibility of tunning the hydrophilicity/hydrophobicity of the matrixes can be successfully attained by the incorporation of poly(N-isopropylacrylamide) (PNIPAAm) in the substrate during its processing step. Due to the thermo-responsive properties of the substrates produced cells can be recovered, before confluency, simply but lowering the culturing medium temperature. In this work, we have developed a new thermosensitive 3D construct based on poly$_{D,L}$ lactic acid using supercritical fluid technology.

Methods: The 3D constructs were prepared by supercritical fluid foaming. Poly$_{D,L}$ lactic acid alone or loaded with poly-NIPAAm particles (5 or 10 wt%) were processed at 200 bar and 35 ºC. PNIPAAm was polymerized using carbon dioxide as reaction medium and AIBN (2,2-azobis isobutylnitrile) as initiator. N-isopropylacrylamide was polymerized with 1,2 wt% of cross-linking agent MBAM (N, N methylenebisacrylamide). The materials prepared were physico-chemically characterized by SEM, Micro-CT and FTIR. Further water uptake measurements at two different temperatures were carried out.

The cytotoxicity of the 3D constructs was evaluated through a test based on international standards with A mouse lung fibroblast cell line (L929 cell line, European Collection of Cell Cultures, UK). The cell detachment efficiency by cooling was evaluated by counting the cells detached after cooling with an hemocytometer. Five redings were taken and the results were averaged.

Results: In supercritical foaming process, the polymer is exposed to carbon dioxide, which plasticizes the polymer by reducing the glass transition temperature. Upon depressurization, thermodynamic instability causes supersaturation of the CO_2 dissolved in the polymer matrix and hence, nucleation of cells occurs. The foams prepared are characterized by 83% porosity and 49,5 μm mean pore size. Figure 1 represents a SEM image of the cross-section of the materials produced.

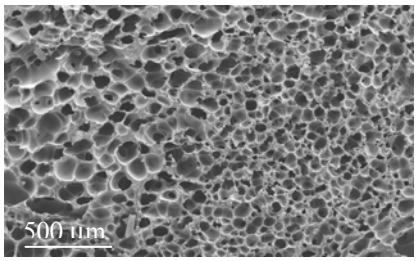

Figure 1: SEM image of P$_{D,L}$LA 3D structures prepared by supercritical fluid foaming at 200 bar and 35ºC.

The cell detachment efficiency was determined for scaffolds of P$_{D,L}$LA and P$_{D,L}$LA loaded with PNIPAAm (5 and 10 wt%), after 7 days of cell culture. The cells were detached by cooling, resuspended and counted. Figure 2 represents the cell detachment number from the different matrixes.

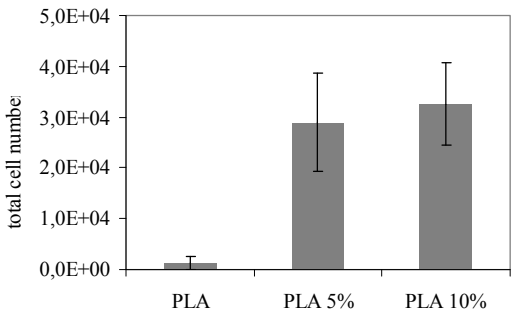

Figure 2: Cell detachment number on different 3D matrixes.

From figure 2 it is clear that the thermosensitive matrixes promoted the cell detachment. P$_{D,L}$LA scaffolds have therefore the potential to be used as substrates for cell growth and expansions avoiding enzymatic and mechanic methods of cell harvesting. The harvested cells were replated to evaluate their viability, which was not compromised.

Conclusions : In this work we developed a 3D structure with thermosensitive properties. An alternative technology, based on supercritical carbon dioxide was used to polymerize PNIPAAm and to foam P$_{D,L}$LA, creating therefore a thermosensitive 3D structure which has proven to have potential as substrate for cell growth and expansion.

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