Development of Novel 3D Thermosensitive Substrates for Cell Culture Using Supercritical Fluid Technology
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Introduction
Stem cells, due to their self-renew potential are a promising approach in tissue engineering and regenerative medicine. Even so, a problem arises concerning the currently available technologies for cell expansion and proliferation. Cell culture is usually performed in 2D surfaces and enzymes or mechanical methods are used for cell detachment. Besides the use of aggressive conditions for cell recovery, these culture methods do not respond to the large quantities of cells required for proper research and clinical use. After this, we aim to develop of a novel technology for cell culture and expansion, based on thermo-responsive 3D substrates. The thermo-responsive properties of the substrates produced allow cell recovery, before confluency, simply but lowering the culturing temperature. The possibility of tuning the hydrophilicity/hydrophobicity of the matrixes can be attained by the incorporation of poly(N-isopropylacrylamide) (PNIPAAm) in the substrate during its processing step, which has been successfully described specially for cell sheet engineering. In this work, we have developed a new thermosensitive 3D construct based on polyD,L lactic acid using supercritical fluid technology.

Materials and Methods
The 3D constructs were prepared by supercritical fluid foaming at 200 bar and 35 °C. PolyD,L lactic acid alone or loaded with poly-NIPAAm particles (5 or 10 wt%) were processed. PNIPAAm was polymerized using carbon dioxide as reaction medium and AIBN (2,2-azobisisobutylnitrile) 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 4°C, room temperature and 37°C 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 readings were taken and the results were averaged.

Results
P D,L LA constructs prepared present the same morphology independently of the presence of PNIPAAm. Micro-CT analysis was carried out in order to calculate structure porosity, interconnectivity and mean pore size, which were ~68%, ~55% and 138 μm, respectively.

Fig. 1 SEM image of the P D,L LA constructs prepared

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<th></th>
<th>PLA</th>
<th>PLA 5%</th>
<th>PLA 10%</th>
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Discussion and 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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