In vitro development of cell-derived extracellular matrix scaffolds using PLA microparticles for bone regeneration

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Introduction: Microstructure complexity and heterogeneity of macromolecules found in cellular environment entangle a great challenge when conceiving biomaterials for regenerative therapies. Native extracellular matrices (ECM), obtained from decellularized heart, nerves, liver, among others (1), are proposed as the best scaffolds for the generation of functional tissues. However, their limited availability forces the investigation for alternatives.

Cells cultured *in vitro* are able to secrete their own and specific ECM. Bone marrow mesenchymal stem cells (MSC) have been shown to enhance osteoblast-specific genes when seeded on cell-derived ECM scaffolds even without differentiating medium. Moreover, higher calcium deposition was found in these scaffolds (2).

The purpose of our research is to obtain biological scaffolds based on the ECM from cells cultured on biodegradable polylactic acid (PLA) microparticles (MPs), and its further use for the production of functional living tissue.

Materials and Methods: PLA MPs were prepared using a green-solvent-based method (3), and coated covalently with collagen-I. MSC culture was maintained for 21 days. Cell proliferation was evaluated with a resazurin assay. Deposited ECM was analyzed by Scanning Electron Microscope (SEM) and immunofluorescence, to detect representative matrix proteins such as collagen-I, fibronectin and laminin.

Results: MPs with sizes ranging between 60 and 120 um were produced. Proliferation was positive and constant during the first week, and then remained steady, demonstrating MPs are suitable for cell culture.

Immunofluorescence after 21 days revealed the presence of collagen, laminin and fibronectin. ECM distribution within the scaffold was also evaluated by SEM micrographs. A dense matrix entrapping the MPs was formed. The presence of cells and matrix inside the construct could also be seen by cross-sections. Cells penetrated 0.75 mm from the scaffold surface.

Discussion and conclusion: It is of utmost importance that MP dimensions allow cell-cell interaction for the formation of the ECM. Different approaches have used diverse MP sizes such as 20-60 um (4), 130-380 um (5), 400-700 um (6). All of them reported the deposition of a dense matrix on the outer region of the construct corresponding to what we have obtained.

Cell penetration in our scaffold stays within the limits reviewed by Yeatts et al (7). Deeper invasion faces mass transfer issues. In order to create bigger scaffolds, flow perfusion strategies have shown to enhance interior distribution (5).

These results state the bases for the production of cell-derived ECMs by the use of PLA MPs as they allow cell proliferation and spreading. Moreover, ECM formation is clearly observed by immunofluorescence and SEM. Further evaluation of the ECM components will be done, as well as decellularization studies. The impact on the osteoblastic differentiation of MSCs will also be evaluated.

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