Vol. 118, n. 2 (Supplement): 162, 2013

## 3D culture of multipotent cells derived from waste human ovarian follicular liquid and seeded onto gelatin cryogel

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Current tissue engineering uses 3D biomaterials in combination with stem cells, since mature cells are often not available in sufficient amounts or quality. Biomaterial scaffolds have been widely used in reconstructive bone surgery not only as cell carriers providing mechanical support, but also as promoters of cell attachment and proliferation (1). In particular, gelatine cryogel scaffolds are promising new biomaterials owing to their biocompatibility and to substain the differentiation of mesenchymal stromal stem cells (MSCs) (2). Human MSC proliferate onto the surfaces with fibroblastic morphology and can differentiate into osteoblasts, chondrocytes and adipocytes (3). These cells can be isolated from several sources, including bone marrow and adipose tissue (4). Our previously studies showed the possibility to obtain MSCs also from the human ovarian follicular liquid (FL) that is usually wasted during in vitro fertilization (5). In this study, we tested the ability of these FL cells to grow and differentiate on gelatine cryogel in comparison with MSCs derived from human bone marrow. Samples and controls were analyzed with confocal and scanning electron microscopes. Results demonstrated that FL cells could grow on the biomaterial not only on the top but also in the layers below till 60mm of deepness. Data suggested that the observed cells are mesenchymal since positive for vimentin and CD44 (a typical MSC marker). Preliminary results showed also the capability of induced FL cells to osteogenic differentiation to produce bone extracellular matrix, expressing some specific proteins (i.e.osteopontin). In conclusion, MSCs derived from waste human ovarian follicular liquid showed promising affinity with 3D gelatine cryogel, opening new potential developments in biotech and medical applications.

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## Keywords:

Mesenchymal Stem Cells, Waste Human Ovarian Follicular Liquid, Gelatin Cryogel Scaffold, Proliferation and Differentiation, Immunostaining Analysis, Confocal Microscopy and Scanning Electron Microscopy Analysis.