

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

Federica Riva¹, Claudia Omes², Lorenzo Fassina³, Patrizia Vaghi⁴, Marcella Reguzzoni⁵, Marco Casasco¹, Antonia Icaro Cornaglia¹ and Andrea Casasco¹

¹Dip. Sanità Pubblica, Medicina Sperimentale e Forense, Unità di Istologia ed Embriologia generale, Università di Pavia

²IRCCS Fondazione Policlinico S. Matteo di Pavia, Centro di Procreazione Medicalmente Assistita, Ostetricia e Ginecologia

³Dip. di Ingegneria Industriale e dell'Informazione, Università di Pavia, C.I.T.

⁴Centro Grandi Strumenti, Università di Pavia

⁵Dip. Scienze Chirurgiche e Morfologiche, Università dell'Insubria

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 sustain 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.

References

- [1] Hutmacher D.W. (2001) Scaffold design and fabrication technologies for engineering tissues—state of art and future perspectives. *J Biomater Sci Polym Ed*, 12: 107-124.
- [2] Dubruel P. et al. (2007) Porous gelatine hydrogel: 2. *In vitro* cell interaction study. *Biomacromolecules* 8: 338-344.
- [3] Pittenger MF et al. (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143-147.
- [4] Zuk PA et al. (2002) Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell* 13: 4279-4295.
- [5] Riva F et al. (2008) Characterization of putative mesenchymal stem cells from human follicular fluid. *Int J Anat Embryol* 113: 241.

Keywords:

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