

Matricellular protein expression as a new parameter to test in vitro cytotoxicity of a 3D biomimetic material

Sandra Durante¹, Gabriella Teti¹, Alessandra Ruggeri¹, Viviana Salvatore¹, Stefano Focaroli¹, Monica Mattioli-Belmonte², Pietro Gobbi³ and Mirella Falconi¹

¹ Dipartimento di Scienze Biomediche e Neuromotorie (DIBINEM), Università di Bologna, 40126 Bologna, Italy

² Dipartimento di Scienze Cliniche e Molecolari, Università Politecnica delle Marche, 60121 Ancona, Italy

³ Dipartimento di Scienze della Terra, della Vita e dell'Ambiente, Università "Carlo Bo", 61010 Urbino, Italy

Up regulation of matricellular proteins are often involved in in-vitro cell suffering and in in-vivo encapsulation, foreign body reaction and inflammation response following scaffold implantation [1]. The cytotoxicity of biomaterials is generally tested according to the ISO standard 10993-5, based mainly on viability tests. Further assays, based on the improved biocompatibility knowledge, could be suggested to better analyze the cytotoxicity of implant materials.

The aim of the study was to propose matricellular protein expression as a new assay in the evaluation of cytotoxicity of implant materials. Tenascin C, osteocalcin and osteopontin belonged to the matricellular protein family and they were chosen as cytotoxicity markers. Viability test, Real Time PCR and western blot in mesenchymal stem cells seeded on collagen/hydroxyapatite scaffold were performed to evaluate gene/protein expression.

Transmission electron microscopy was carried out to evaluate morphological changes induced by cell/scaffold interactions.

Results showed a high viability of the cells during the time of culture and a good cell adhesion and morphology observed by electron microscopy. A lower expression of tenascin-c, osteonectin and osteopontin compared to cells cultured on tissue flasks was demonstrated both by Real Time PCR and western blot suggesting a better cell culture condition in 3D biomimetic scaffold compared to tissue flasks. Based on our results, we propose the analysis of matricellular protein expression as a new parameter for testing cytotoxicity of implant materials.

References

- [1] Kyriakides and Bornstein (2003) Matricellular proteins as modulators of wound healing and the foreign body response. *Thrombos Haemostasis* 90: 986-992.

Key words

Matricellular proteins, in vitro cytotoxicity, biomaterials, mesenchymal stem cells, R T- PCR.