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In vitro study on the generation of tympanic membrane substitutes via tissue engineering

Antonella Rocca[1], Delfo D'Alessandro[2], Federica Chiellini[3], Dinuccio Dinucci[3], Dario Puppi[3], Luisa Trombi[4], Stefano Berrettini[2], Amelio Dolfi[1] and Stefania Moscato[1]

[1] Department of Human Morphology and Applied Biology, University of Pisa, Pisa, Italy
[2] Department of Neurosciences, University of Pisa, Pisa, Italy
[3] Department of Chemistry and Industrial Chemistry, University of Pisa, BIOLab, San Piero a Grado, Pisa, Italy
[4] Department of Oncology and Transplants, University of Pisa, Pisa, Italy

The tympanic membrane (TM) is an anatomical structure with unique histological and physiological features playing a fundamental role in sound transmission. In particular, the middle layer of the pars tensa, which represents the widest and thickest surface portion of the TM, consists of connective tissue mainly composed of collagen types II and III fibers, while collagen type I is present at a lesser extent [1]. Several pathologies affect the TM, including otitis media, tympanosclerosis, cholesteatoma and perforation that require reconstructive surgery depending on the lesion extent [2]. To this purpose, the temporalis fascia is currently considered as the gold standard material. However, due to limited graft availability, fully synthetic substitutes are also applied, with poorly satisfactory outcomes. For these reasons new strategies for TM replacement are still needed.

In this study, we employed a tissue engineering (TE) approach for the regeneration of TM substitutes selecting some biocompatible and bioresorbable polymeric matrices to be cultured with human bone marrow-derived mesenchymal stem cells (MSCs). We set up a cell differentiation protocol using an appropriate mix of growing factors to obtain the in vitro differentiation of MSCs into TM fibroblasts. Furthermore, because of the role played by mechanical forces in TM motion, these engineered substitutes underwent mechanical stress during the culture.

The obtained biohybrid constructs were characterized about cellular viability assays, gene expression quantification as well as histochemical and immunohistochemical analyses. Moreover, native TMs from cadavers were investigated for assessment and optimization of the engineered constructs.

Our results showed that MSCs were able to grow and differentiate properly on the selected biomaterials and to synthesize appropriate extracellular matrix molecules. Moreover, the applied mechanical forces seem to promote TM-fibroblastic differentiation, increasing the production of collagen type II, that is a peculiarity of TM structure.

References

[1] D.J. Lim. Acta Otorhinolaryngol Belg 1995; 49(2):101-15.
[2] M. Tos "Manual of middle ear surgery". Thieme Medical Publishers, Inc. New York 1993 vol. 1, Part II cap. 8-13.

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