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Preliminary observations on scleral ossicles in performing functionalized 3D vascularized scaffolds for "critical-size" bone defect healing

Marta Checchi [1] - Alberto Smargiassi [1] - Marzia Ferretti [1] - Paola Sena [1] - Marta Benincasa [1] - Francesco Cavani [1] - Marco Sola [2] - Antonio Ranieri [2] - Stefania Mitola [3] - Carla Palumbo [1]

[1] Università di Modena e Reggio Emilia, Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Modena, Italia - [2] Università di Modena e Reggio Emilia, Dipartimento di Scienze della Vita, Modena, Italia - [3] Università degli Studi di Brescia, Dipartimento di Medicina Molecolare e Traslazionale, Brescia, Italia

The problem of "critical-size" bone defects occurs when a severe lesion is difficult to be self-recovered. Many strategies of regenerative medicine were used in the last decade, with translational approaches, to mimic both structure and function of the native bone tissue, making use of synthetic materials, nanotechnologies, bio/synthetic constructs or some of their combination. The main obstacle to engineering strategies is mostly due to the lack of a proper vascularization of the construct used. In this feasibility study, our attention is directed towards the main tissue engineering items: scaffolds, cells and conditioning factors. We propose the use of scleral ossicles of lower vertebrates (1), as natural scaffolds which will be functionalized to allow the best adhesion of endothelial cells along a geometrically controlled pattern on the bony surface of the construct; successively, on the functionalized scaffold, osteogenic cell lines will be cultured. In the preliminary phases of the study, the ossicles were scratched to remove soft tissue residues, variously flattened with different methods to reach a regular morphology on both sides, and finally autoclaved to eliminate cellular remnants and to annul antigenic properties. Ossicles were observed under SEM and subjected to micro-assay, to establish the best scaffold preparation and to characterize morphological properties more suitable for engineering phases. Functionalization will be made by immobilizing on the engineered ossicles specific growth factors for endothelial cells; later, mouse primary lung endothelial cells (ECs) and immortalized osteogenic cells (IDG-SW3) will be used. As expected results ECs should adhere to the ossicle surface and organize to form lumenized microvascular-like structures; later, supported by the vascular-like network, the osteogenic lineage should produce bone matrix on the construct. The production of newly-formed bone around vascular-like buds will be verify. 3D tissue constructs generated in vitro will be used in successive in vivo study for the healing of "critical-size" bone defects experimentally induced in mice.

References

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