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A COMBINATION OF HUMAN MESANGIOBLASTS AND FIBROBLASTS MAXIMIZES CELL ENGRAFTMENT FOR THE DEVELOPMENT OF ENGINEERED OESOPHAGI

Luca Urbani[1], Carlotta Camilli[1], Claire Crowley[1], Rui Rachel Wong[1], Federico Scottoni[1], Edward Hannon[1], Ji Luo[1], Anna Urciuolo[1], Salvatore Aruta[1], Koichi Deguchi[1], Simon Eaton[1], Giulio Cossu[2], Paolo De Coppi[1]
[1]UCL Institute of Child Health and Great Ormond Street Hospital, London, UK, [2]Institute of Inflammation and Repair, University of Manchester, Manchester, UK

Aim of the Study: Tissue engineering is an emerging clinical reality for the repair of long-gap oesophageal atresia, with the aim of developing substitutes combining biomaterials and stem cells. The aim of this study is to mature a functional oesophageal replacement starting from decellularized matrices, natural templates that preserve the tissue-specific extracellular matrix. A combination of mesoangioblasts (hMABs) and fibroblasts (mFBs) was chosen to repopulate the scaffold to engineer the smooth muscle layer of the oesophagus using static and dynamic 3D-culture.

Methods: Rat oesophagi were decellularized with an established detergent-enzymatic protocol. Following ethical approval, hMABs were isolated from human skeletal muscle biopsies; mFBs were obtained from mouse hindlimb muscles by enzymatic digestion. Injection of different hMABs:mFBs ratios in the oesophageal muscle layer was tested after 11 days of static or dynamic culture using customised bioreactors.

Main Results: Fibroblasts increased engraftment and invasion of hMABs throughout the culture, favouring an overall homogeneous distribution within the scaffold layers. In particular, a combination of 85% hMABs and 15% mFBs enhanced cell engraftment without over-proliferation of stromal cells. hMAB proliferation and differentiation were not affected by the presence of fibroblasts, as detected with Ki67 and SM22 staining. The majority of smooth muscle differentiated hMABs (SM22-positive) was detected within the muscle layer of the scaffold, suggesting a strong cell-matrix influence. Fibroblasts' positive effect on tissue re-colonization was observed also in the dynamic system, where the bioreactor provided the proper 3D-culture setup, significantly enhancing growth and differentiation of hMABs (figure).

Conclusion: In this study, hMABs were identified as a successful cell source for oesophageal engineering. A finely regulated co-culture with tissue-specific fibroblasts maximized hMABs migration capacity, leading to optimal matrix maturation without fibrotic effects. The crosstalk between mesoangioblasts, fibroblasts and oesophageal matrix could be the key for the development of functional engineered constructs for oesophageal replacement.