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ENGINEERED RAT OESOPHAGI RETAIN SPECIALISED DIFFERENTIATION AFTER 2 WEEKS OF IN VIVO HETEROTOPIC IMPLANTATION IN AN IMMUNODEFICIENT MOUSE MODEL

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Aim of the Study: To test the effects of *in vivo* heterotopic implantation of an engineered rat oesophagus composed of extracellular matrix and seeded cells.

Methods: Rat oesophagi were decellularized using a detergent enzymatic protocol. Primary human mesangioblasts and rat oesophageal epithelial progenitors were used for repopulation. Cell engraftment, proliferation and differentiation were maximized using 3D culture in a bioreactor. Repopulated constructs (n=2) and unseeded scaffolds (n=3) were implanted in the omentum of immunodeficient mice. Scaffolds were harvested and analysed 2 weeks post-implantation (Figure A).

Main Results: Seeded mesangioblasts were able to generate smooth muscle cells in the external ring of the scaffold, as confirmed by histology and immunofluorescence. Oesophageal epithelium progenitors engrafted, proliferated and differentiated on the scaffold during the culture. The bioreactor allowed a better repopulation of the scaffold when compared with static culture, both in terms of distribution (bioreactor=0.593±0.3233, static=2.592±0.2083 cells/area, n=3, $p<0.05$) and differentiation of cells (bioreactor=56.6±1.56%, static=36.0±2.57% cells positive for smooth muscle marker SM22, n=3, $p<0.01$). 2 weeks after implantation in omentum, histological evidence of preservation of all scaffold layers was clear in all the implanted constructs. Cells were present within both seeded and unseeded groups highlighting mouse cell invasion. Nevertheless, seeded constructs showed higher number of cells and better orientation and distribution when compared with the unseeded scaffolds (Figure B: orientation and distribution of cells in the seeded scaffold). Most importantly, many cells were positive for human specific markers in the seeded constructs.

Conclusion: Tissue engineering may soon offer new therapeutic options for long gap oesophageal atresia. Here we report the results of heterotopic *in vivo* implantation of repopulated oesophageal extracellular matrix showing good integration and biocompatibility of the construct. Though further functional results are required, these results are promising for the development of artificial constructs for pre-clinical and clinical application in oesophageal replacement.