In vitro model of the human esophageal epithelium by tissue engineering tools

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Different pathologies, such as reflux and cancer, negatively affect esophagus functionalities, altering the integrity of the epithelium. Surgical resection of cancer tissue represents the main clinical approach, however it often causes early mortality and morbidity.

In vitro tissue-engineered models are useful tools to support the preclinical validation of new alternative therapies. *In vitro* models of epithelia have been frequently obtained by Air-Liquid Interface (ALI) cell culture in collagen hydrogels on commercially-available transwell inserts. However, commercial inserts have high cost and do not allow flexibility in the choice of composition and architecture, which are fundamental tools to counteract tissue contraction.

This work was aimed at designing a bilayered human *in vitro* model of esophageal epithelium by *in vitro* culture of a cellularized hydrogel on custom-designed inserts, fabricated by Melt Extrusion Additive Manufacturing (MEAM) from Poly(e-Caprolactone) (PCL). The hydrogel matrix was based on Gelatin Methacryloyl (GelMA), mimicking the stiffness and composition of the extracellular matrix of the esophageal submucosa. UV-mediated photocrosslinking time, using lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) initiator, was determined by photorheology. GelMA hydrogels showed permeability to a model dye molecule (Toluidine Blue O, TBO), and biocompatibility towards Human Esophageal Epithelial Cells (hEECs). *In vitro* models were obtained by culturing: a monolayer of hEEC on GelMA hydrogel i) not cellularised), or ii) cellularized with human epithelial fibroblasts (bilayered construct), under ALI condition.

The easily microfabricated inserts could avoid or minimize the contraction of the esophageal tissue model. Cell viability and tissue model formation were demonstrated by Live/Dead assay and immunofluorescence analysis of mucosa and submucosa markers, respectively. In the future the model will be used to screen new therapies.

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