Developing Scaffolds for full-thickness esophageal replacement

INTRODUCTION

Esophageal cancer is one of the most aggressive malignancies. Preoperative chemo(radio)therapy aims to reduce tumor size before surgical resection. Nevertheless, these treatments are burdened by severe toxicities and fail to elicit an effective tumor response in up to 60% of patients. To date, no predictive factors for tumor response to neoadjuvant treatments have been yet identified. Esophagectomy consists of the removal of the affected esophagus and lymph nodes. The alimentary tract is restored by replacing the esophageal resected segment with another hollow organ, typically, the stomach. The loss of the function of the transposed stomach adversely affects the nutritional capacity of patients, significantly impacting their quality of life.

The aim of our project was to develop and validate an in vitro tissue-engineered, patient-derived, full-thickness esophageal substitute for surgical replacement.

METHODS

The project was conducted in collaboration with the University of Torino. The development of the esophageal construct will was divided into two parts, reflecting the natural microscopic anatomy of the organ:

- A double-layer Gelatin-Methacrylol (GelMA) hydrogel seeded with patient-derived epithelial cells was designed. Air-Liquid Interface (ALI) 3D culture was planned in order to develop a stratified epithelium resembling the muscosal layer of the esophagus.
- A Polycaprolactone (PCL) 3D-printed scaffold with stent-like geometry was designed as a supportive layer, reflecting the structural function of the muscularis propria of the esophagus.

RESULTS

An Air-Liquid Interface (ALI) in vitro model was designed to promote the 3D culture of a stratified esophageal epithelium, resembling the human esophageal mucosa. Key steps include the

customization of the platform for the model and the selection of the appropriate substrate to support cell growth. Polycaprolactone (PCL) was used to fabricate customized inserts for 24- and 48-well plates using 3D printing via Fused Deposition Modeling (FDM).

Gelatin methacryloyl (GelMA) was selected for the hydrogel matrix due to its biocompatibility and adjustable mechanical properties. Various degrees of methacrylation (DOM) and different GelMA concentrations were tested to find the most suitable candidate. A photoinitiator was employed added to GelMA solutions to enable UV-induced photocrosslinking. The optimal formulation was identified by balancing mechanical properties and UV curing time. GelMA hydrogel cytotoxicity was assessed by culturing HEEC on eluates, showing no cytotoxic compound release. However, attempts to adhere and proliferate HEEC on GelMA hydrogels were unsuccessful, regardless of preconditioning. Further optimization of the GelMA hydrogel, its composition, cell seeding technique, and culturing conditions is needed.

A biomimetic stretchable biohybrid scaffold was designed for the muscular layer by combining natural and synthetic materials. 3D stretchable polymeric PCL meshes with stent-like geometry were be fabricated using FDM. A stent-like geometry was designed in two different configurations and printed as 2 or 4 layers to investigate the impact of geometry and thickness on the mechanical properties of the mesh. The mechanical characterization of PCL meshes, tested in both the longitudinal and transverse directions, was conducted using uniaxial traction tests, applying a displacement of 0.5 mm/min. Additionally, a finite element analysis model will be developed to analyze stress distribution on the structure.

4-layer meshes were filled with previously synthesized and characterized GelMA hydrogels to promote cell adhesion and tissue integration. The surface of PCL was functionalized with a DOPA coating to improve the polymer hydrophilicity and enhance coupling between PCL and GelMA. Traction tests were repeated on the resulting constructs to determine the optimal mesh configuration. Further optimization of the mesh configuration, suture techniques and fabrication methods using a rotating mandrel are needed.

Furthermore, traction test were performed on fresh esophageal tissue samples, harvested from surgical specimens of patients who underwent esophagectomy. The experimental data indicates that the mucosal layer exhibits the lowest stiffness values, making it the most elastic component of the tissue. In contrast, the muscular layer had the highest stiffness and was regarded as the most rigid component of the tissue. As a result, the full-thickness sample shoes intermediate values due to the combination of the aforementioned layers. Furthermore, both the mucosal layer and the full-thickness samples demonstrated greater elasticity when subjected to longitudinal tractions compared to transverse traction. Conversely, the muscular layer was found to be more rigid under longitudinal

traction than under transverse traction. The findings are further compared with the results of mechanical traction tests on mesh samples, revealing that the stiffness values of 4-layer meshes closely approximate those of full-thickness native fresh tissue. However, the values of transverse traction of the meshes were significantly lower than those of the native fresh full-thickness tissue. It is particular noteworthy that the reference material, derived from the literature, demonstrates significantly higher stiffness compared to fresh tissue.

CONCLUSIONS

This project represents an initial step towards the creation of a full-thickness, patient-specific, bioengineered esophageal substitute suitable for transplantation after esophagectomy. Both mucosal and muscular aspects were analyzed, highlighting the need to optimize scaffolds to support cell adhesion and viability, as well as the functional reproduction of smooth muscle.