Development of an anti-TFF3 functionalized surface to capture of Barrett's oesophagus cells

Team : Gianmarco Contino (Rebecca Fitzgerald Lab, MRC-CU, Cambridge University), designed the project and provide the biological expertize and reagents to design and optimize the functionalized surface. **Miranda Robbins,** (Department of Chemical Engineering and Biotechnology, University of Cambridge) manufactures the functionalized surface and collaborate to the testing.

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Summary

We have successfully developed a non-endoscopic diagnostic test involving a cell-collection device that, coupled with the biomarker trefoil factor 3 (TFF-3), diagnoses Barrett's oesophagus, the only known precursor of oesophageal adenocarcinoma. We propose to fabricate an anti-TFF3 functionalized surface to capture of Barrett's oesophagus cells. This prototype will provide the backbone for the development of microfluidic device for capture of Barrett's oesophagus cells and further characterization rom Cytopsponge samples.

Proposal

Patients with oesophageal adenocarcinoma have a median survival of 1 year, despite advances in treatment. The burden of oesophageal adenocarcinoma could be reduced by diagnosing more cases of the precursor lesion Barrett's oesophagus while identifying individuals with dysplasia and increased risk for cancer development before treating them endoscopically. However, this is a formidable task given that the incidence of gastro-oesophageal reflux is approximately five per 1000 person-years (15% incidence) in the UK and Europe (Vaughan and Fitzgerald 2015; Ross-Innes et al. 2016).

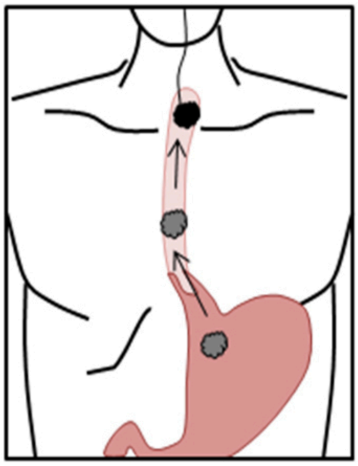

Figure 1. Cytosponge pulled from the stomach and collecting cells from the oesophageal lining.

We have successfully developed a non-endoscopic diagnostic test involving a cell-collection device that, coupled with the biomarker trefoil factor 3 (TFF3), diagnoses Barrett's oesophagus (BMJ 2010; Nature Genetics 2014; PLOS Medicine 2015; Nature Genetics 2015). The device, called Cytosponge, comprises a medical-grade foam sphere on a string compressed within a gelatine capsule that is swallowed while holding onto the string. After 5 min, the capsule dissolves in the stomach, allowing the foam sphere to expand before being pulled from the stomach through the oesophagus to the mouth. Cells are collected along the entire oesophageal lining, minimising the sampling bias that is inevitable with endoscopic biopsies. The sample is transported to the laboratory in preservative at room temperature and processed to paraffin for TFF3 biomarker assessment. A large scale randomised trial to diagnose Barrett's is underway in the UK as the final step towards presenting the evidence to NICE and introducing the technology into routine clinical practice (Registered Trial BEST3).

To ensure consistency and standardization of Cytosponge analysis there is a need to automate the analysis of the specimens. We have devised a project to **develop a companion diagnostic for the Cytosponge to identify Barrett's (TFF3 positive) patients.**

Main Aim of the project

We aim to design a microfluidic device to process Cytosponge samples and separation into a single cell suspension and enrich for TFF-3 positive cells. For the purpose of the BioMaker challenge, we will focus on the development a functionalized surface with TFF-3 antibodies to capture Barrett's oesophagus cells.

Specific aims

Aim 1. Fabricate a functionalized surface with anti-TFF-3 antibodies

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We will fabricate a PDMS substrate and we will covalently attach a commercially available anti-TFF3 antibody.

Aim 2. Test and optimize the capture of TFF-3 positive cells

We will test the device using a TFF-3 positive Barrett's Oesophagus cell line (CP-A) a TFF-3 negative immortalized Oesophageal squamous cell line (Het-1). Both cell lines are available in our lab. We will label with a fluorescent membrane marker the TFF-3 positive cells and dilute them at different concentrations with the unmarked TTF-3 negative cells. We will measure the sensitivity and specificity of Barrett's Oesophagus cell capture of the device with fluorescent microscopy. This experiment will also provide preliminary information on the optimal yield of cells that should be provided by the Cytosponge sample to obtain a diagnosis. If needed, we will revise the design of the device with the aim to achieve a capture cells at a dilution of 1:1000.

Expected results

This project will inform on the ability of a TFF-3 coated surface to capture Barrett's oesophagus cells. If successful, we aim to develop a microfluidic device to capture Barrett's oesophagus cells and perform further diagnostic tests. There is huge potential to add assays for risk stratification which can be implemented as a second tier test on the same sample used for diagnosis. We have promising data for such an approach however further work is required to optimise the assays for stratification and ensure clinical applicability.

Future developments of this platform will include:

1) **perform cytology on chip with staining for other molecular markers such as P53 and Aurora Kinase A**
2) **Development of an algorithm for recognition and analysis of captured cells based on existing platforms of automated image capture using the data generated in Aim 1.**
3) **On-chip isolation of nucleic acids for sequencing and methylation/expression assays**

Components and budget to complete the project

1) APTES 55.50 GBP
2) Membrane fluorescent markers 350 GBP
3) PDMS substrate fabrication 250 GBP