A Novel Sampling Device For Collecting Mucocellular Material From The Rectum

J Booth[1], J Lacy-Colson[2], M Norwood[3], C Murray[1]

1 Origin Sciences Limited, Unit 1 Riverside, Granta Park, Cambridge, CB21 6AD, UK
2 Department of Surgery, Royal Shrewsbury Hospital, Mytton Oak Road, Shrewsbury, SY3 8XQ, UK
3 Department of Surgery, The Leicester Royal Infirmary, University Hospitals of Leicester NHS Trust, Leicester, LE1 5WW, UK

Introduction

Earlier detection of colorectal and other gastrointestinal malignancies is an urgent objective. Currently much effort is directed at the development of *in vitro* diagnostic tests that evaluate informative protein or DNA biomarkers in stool or blood samples. Stool samples are inconvenient to collect, require special handling facilities, and suffer from contamination that may interfere with molecular assays.

Blood samples, while more convenient, may not be as informative early in the disease process. Several studies have shown that significant numbers of exfoliated cells and their products are retained in a mucocellular layer overlying the colonic mucosa but distinct from the stool itself, and that this material flows toward the rectum, where it can be captured for analysis.[1,2]

We have developed a novel sampling device to capture this material and present data demonstrating its potential utility in gastrointestinal research.

Materials & Methods

Sample Collection: The sampling device incorporates a nitrile membrane that, upon insertion into the unprepared rectum via a standard proctoscope, is inflated with air using a syringe. The inflated membrane remains in contact with the rectal mucosa for 10 seconds and is then deflated and retracted into the device prior to removal from the patient (Figure 1). Patient samples were collected and either smeared directly onto slides and fixed, or preserved by the immediate addition of RNA*Retain*® (Assuragen) to the inverted membrane. Samples were stored at 4°C prior to transport to the laboratory. Material was removed from the membrane and lysed by incubation with lysis buffer (10 mM Tris, 150 mM NaCl, 0.1% Triton X-100, pH7.5) at ambient temperature for 30 min with rotation. The recovered material was stored at -20°C prior to analysis. Laboratory testing *in vitro* was performed using cultured HT29 colorectal adenocarcinoma cells grown as monolayers. The device was inserted into the culture flask and the membrane inflated for 10 seconds to make contact with the cell monolayer.

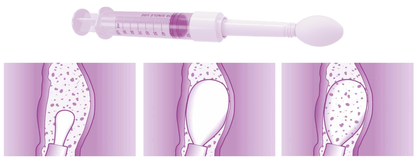

Figure 1. Device, with syringe attached (top) is inserted into the rectum via a standard proctocope. The membrane is inflated and makes contact with the rectal mucosa to collect material from the surface.

Patient Acceptability: Immediately after sampling, patients were asked if they experienced any pain during insertion of the proctoscope or during inflation of the membrane. Patients were also asked to rate the acceptability of the whole procedure on a visual analogue scale (VAS), by making a vertical mark on a pre-printed 100 mm horizontal line, running from 0 (the most uncomfortable procedure imaginable) to 100 (completely comfortable). The distance from the left end of the scale to the mark was measured and recorded.

Sample Analysis: Total protein content was measured using the Bradford Assay (Sigma) and in-house ELISA assays used to detect autoantibodies. Total DNA was extracted and purified using the QIAamp MinElute Media kit (Qiagen) and quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies). The percentage of human DNA was estimated using Real-Time ß-Globin PCR.[3] The absolute equivalent amount of DNA in each sample was determined by use of a calibration curve with serial dilutions of genomic DNA.

Results

Patient Acceptability: The sampler has been tested in over 2500 patients, including healthy volunteers, and has shown excellent patient acceptability. Over 85% of patients did not experience any pain either during the insertion of the proctoscope or inflation of the membrane (Figure 2) and the average acceptability score was 81.9 (min 2, max 100).

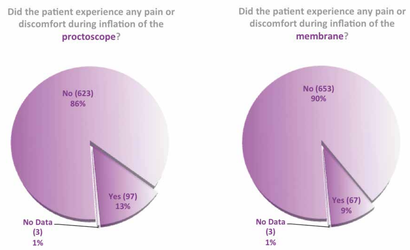

Figure 2. Summary of the acceptability data for the sampling process, assessed in a cohort of 723 patients.

***In Vitro* Modelling of Sampling:** Experiments with monolayers of cultured human colon adenocarcinoma cells (HT29) demonstrate the capture of intact cells on the membrane surface (Figure 3), which can be easily removed for further investigation.

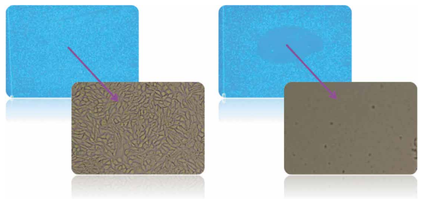

Figure 3. HT29 cell monolayer before and after contact with the OriCol™ membrane, visualised under UV light with NucBlue® dye and light microscopy.

Histological Evaluation of Captured Material: Intact cells can also be identified in samples collected from the rectum of healthy and diseased individuals and smeared directly from the membrane onto slides prior to staining with haematoxylin and eosin[4] (Figure 4).

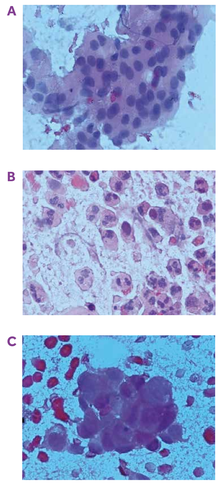

Figure 4. Examples of cells collected from the surface of human rectal mucosa. (A) Exfoliated fragment of normal-looking colonic epithelium. (B) Abundance of neutrophils in material taken from a patient with ulcerative colitis. Several apoptotic bodies are also present. (C) Cluster of malignant cells from a patient with rectal cancer. Erythrocytes present around the cell cluster.

Molecular Characterisation of Captured Material: Mucous-associated soluble material captured by the device is rich in both protein and nucleic acids. In a study of 40 patients, levels of total soluble protein present in the buffer varied between 90 and 3000 µg/ml, with a mean of 710 µg/ml. Various protein biomarkers have been detected in the rectal mucosal samples collected using the device[5] and, as part of a program to identify novel cancer biomarkers, we have recently demonstrated the presence of informative autoantibodies. Data from healthy volunteers suggests that the autoantibody profile is stable over time and thus any changes detected in disease states may have useful diagnostic applications (Figure 5).

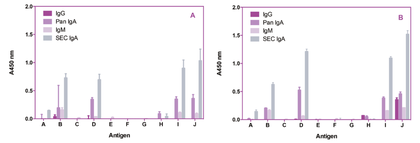

Figure 5. Autoantibody profiles of samples taken from the same healthy volunteer at time = 0 (A) and time = 0+14 weeks (B).

The same preparation is also rich in nucleic acids. The total amount of DNA from a cohort of 40 patients ranged from 0.5 to 30 µg/ml, with a mean value of 6 µg/ml (total volume 5 ml). The proportion of human DNA in these samples ranged from 1-60% with mean of 22% (Figure 6). The DNA has also been shown to be suitable for Next Generation Sequencing (data not shown).

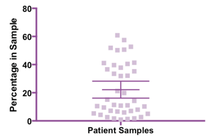

Figure 6. Scatter plot depicting the percentage human DNA in a cohort of 40 patient samples. Bars represent mean ±95% CI.

Conclusion

The sampling device represents a novel and minimally invasive means of capturing biomarker-rich material from the unprepared rectum. Since there is minimal contamination by stool, the material collected is readily analysable, in principle lending itself to point-of-care tests for a wide range of indications, including infectious and inflammatory diseases of the GI tract in addition to malignancy. The device can be used as a robust means of collecting material for later analysis by a wide range of technologies.

References

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The sample device is being marketed as OriCol™ by Origin Sciences. OriCol™ is CE-marked in accordance with the requirements of the Medical Device Directive 93/42/EEC.

For further information please contact Dr Booth at **jodie.booth@originsciences.com**