Note

A novel technique capable of taking 'protected' biopsies for reliable assessment of the distribution of microbiota along the colonic mucosa

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ABSTRACT

We evaluated a novel 'protected' biopsy method to reliably ascertain the spatial distribution of the mucosa-adherent colonic microbiota. Apart from minor differences at genus level, overall similarities along the colon were high between the various areas, irrespective of protected or unprotected sampling.

An aberrant gut microbiota composition has been found to be implicated in various chronic and metabolic diseases, including inflammatory bowel disease (IBD) (Sokol et al., 2006; Ottman et al., 2012). The gut microbiota plays a key role in inflammation and hence possibly also in the development of the mucosal lesions that are characteristic of IBD. Characterization of the gut microbial ecological patterns may be essential to the understanding of the pathophysiology of inflammation in IBD. In current IBD research, gut microbiota profiling is performed on fecal samples (Kolho et al., 2015; Norman et al., 2015), samples of the mucus layer (Johansson et al., 2014), and colonoscopic biopsy samples (Rossen et al., 2015). Several sampling methods have been described, but currently there is no validated method for accurate and systematic collection and processing of samples (Zhang et al., 2017).

Various studies have reported differences in luminal and mucosal microbiota profiles along the intestines of healthy subjects and IBD patients (Lavelle et al., 2013; Lavelle et al., 2015; Donaldson et al., 2016). Controversy exists concerning the spatial variation of the microbial communities along the length of the colonic mucosa, however. Whereas some studies report no spatial differences along the colonic mucosa (Lavelle et al., 2013; Lavelle et al., 2015; Zoetendal et al., 2002; Eckburg et al., 2006; Hong et al., 2011), others have observed a biogeographical gradient of colonic mucosa-associated microbiota (De Cárcer et al., 2011; Zhang et al., 2014; Flynn et al., 2018). We hypothesize that contamination of proximally collected intestinal samples with microbiota residing in distal colonic sites may partly explain these different observations. In this study, we evaluated a new sampling technique for characterization of the colonic microbiota profiles which allows for collection of 'protected biopsies', thereby preventing contamination of the working channel of the endoscope and subsequently the biopsy forceps with microbiota residing in distal colonic sites while being advanced through the working channel of the endoscope for sampling of more proximal colonic sites (Fig. 1). Aiming to move towards a better understanding of the microbial spatial distribution implicated in the pathophysiology of IBD, the primary objective in this study was to assess whether there are true differences in mucosa-adherent microbiota along different locations in the bowel in a patient group without active inflammatory disease. To this end, we hypothesized that if there are true differences in microbial composition along the colon, we would find differences between the protected biopsies of the most proximal and the most distal sites that we would not find between the unprotected biopsies of these sites, due to the expected high risk of contamination of the working channel upon insertion of the endoscope.
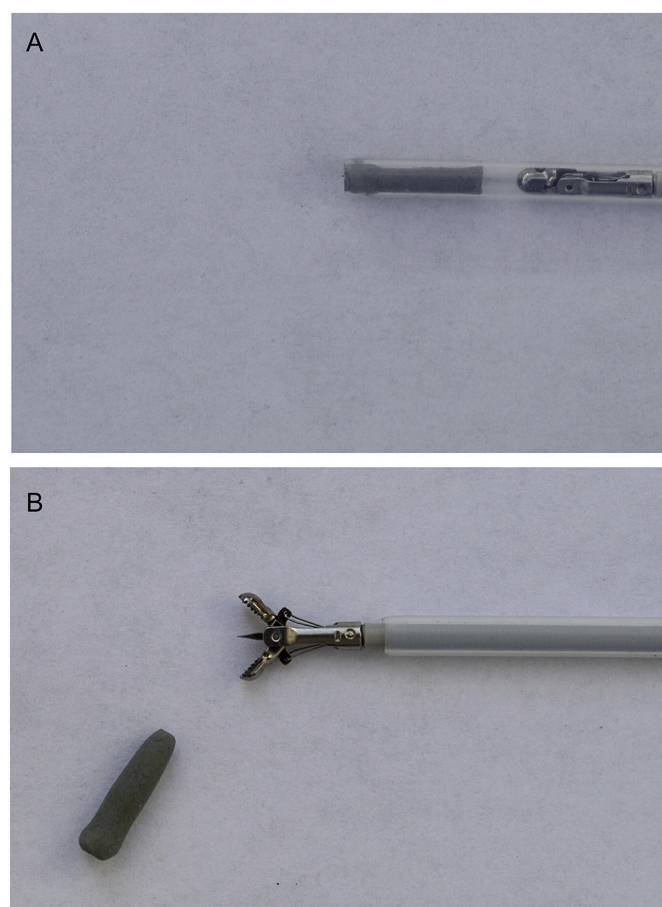
Fig. 1. The 'protected' biopsy device. **A** – Protected biopsy configuration. **B** – The cap is pushed out of the catheter by the biopsy forceps just before taking the biopsy.

Eleven patients scheduled for colonoscopy for diagnostic purposes were included in the study (five men and six women [age range 34–76 years old, median 54]). The indications for colonoscopy included rectal bleeding, iron deficiency anemia, neoplasia surveillance and altered bowel pattern. Patients received bowel lavage with either Picoprep or Moviprep. Their median total Boston Bowel Preparation Scale (BBPS) score was 8 (range 3–9). Additional demographics and details of performed colonoscopies are listed in Supplementary Table 1. Patients were sampled in the terminal ileum (TI), the ascending colon (CA), and the rectosigmoid (RS). At each location two samples were collected; one 'unprotected' (U) mucosal biopsy following the routine biopsy procedure, and one 'protected' (P) mucosal biopsy which was collected using a P biopsy device that was assembled as follows. Under sterile conditions, a standard 2.4 mm Captura biopsy forceps (Wilson-Cook Medical, Winston-Salem, NC, US) was premounted in a 3.7 mm HCP-C hemostasis delivery catheter (Wilson-Cook Medical, Winston-Salem, NC, US), and sealed off with a silicone cap. The premounted system was sterilized with ethylene oxide and packed in a sealed bag, which was subsequently exposed to UV-light in order to destroy any bacterial DNA. The extent to which the P biopsy method protects from contamination was examined by 16S rRNA gene qPCR analysis as described by Fuentes et al. (2017) on the presence of bacterial DNA that was collected on the biopsy forceps during advancement of a P and subsequently U biopsy device up until 5 cm before the tip of a used endoscope channel right after colonoscopy with suboptimal bowel preparation ($n = 4$). The average bacterial DNA abundance of the U samples was $1.47*10^5$ times higher than of the P samples, whereas of the P samples bacterial DNA abundance was $3.22*10^3$ times higher than of the negative air control samples. No

bacterial DNA was detected on devices that were not advanced. These results confirmed that the P biopsy method protects from contamination.

Upon collection of the P mucosal biopsy, the catheter was advanced through the working channel of the endoscope, and the cap was pushed off by the forceps at the mucosal site of interest. Care was taken to select an area devoid of residual luminal feces. After collection of one biopsy, the biopsy forceps was retrieved through the catheter. The biopsy sample was immediately stored in liquid nitrogen. A new biopsy device was used for each mucosal biopsy sample.

After thawing, specimens microbiota composition and diversity were determined using the Human Intestinal Tract Chip (HITChip) following the method previously described in other studies (Rajilic-Stojanovic et al., 2009; Lahti et al., 2011; Lahti et al., 2013), as well as through partial 16S rRNA (V1-V2) gene sequencing by Illumina MiSeq and analysis using SILVA v.132 and mothur MiSeq SOP v.1.42.3 (Kozich et al., 2013), as previously described by James et al. (2020). The HITChip platform is a phylogenetic microarray designed by Rajilic-Stojanovic et al. (2009) following a systematic probe design approach. Genus-like level data is generated following data organization according to specificity of probes for higher phylogenetic groups, which allows for analysis of differences between samples in terms of genus abundances. Statistical significance was calculated using a student's *t*-test for the Pearson correlations on the 16S rRNA gene read data and the HITChip probe level data following the example set by Rajilic-Stojanovic et al. (2009), and a Wilcoxon rank sum test with Bonferroni-Holm correction for the genus-like level data, following the method previously carried out by Zoetendal et al. (2002).

The results showed no significant differences in microbiota composition between P and U biopsies at HITChip probe level, nor did they show spatial differences between different (ileo)colonic sites (Fig. 2A). Comparisons of inter-site Pearson correlations of the samples confirmed that similarity between sites was not significantly different in P biopsies compared to U biopsies (Fig. 2B).

Both analyses on HITChip probe signals and 16S rRNA sequence read showed that similarity between TI and CA and TI and RS was not significantly different between U and P biopsies. PCoA-analysis revealed no clear separation between P and U samples of TI, CA and RS (Fig. 3A). However, a significant difference was found between the P and U biopsies of the CA and RS (Fig. 3B, $p = 0.04$). Correspondingly, neither the 16S rRNA sequencing data nor the HITChip data showed significant taxonomic differences at genus-like level (data not shown) between P and U biopsies along the different colonic locations (Supplementary Fig. S1).

Although likely to be evenly spread throughout the intestines, a potentially microbiota-composition altering effect of bowel lavage must be taken into account with interpretation of the results (Harrell et al., 2012; O'Brien et al., 2013) Unprepared mucosa may exhibit overall higher microbiota phylotype richness and diversity (O'Brien et al., 2013).

In summary, our data show high similarity in microbiota composition between the TI, the CA, as well as the RS, and thereby confirm findings from earlier studies (Lavelle et al., 2013; Lavelle et al., 2015; Zoetendal et al., 2002; Eckburg et al., 2006; Hong et al., 2011). We conclude that in individuals without colonic inflammation, no significant spatial variation of the mucosa-associated microbiota profile along the colon exists. Hence, taking extra precautions by using a protected biopsies SOP such as ours may be warranted only when location-specific differences, such as inflammatory lesions versus normal adjacent mucosa, are sought after.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2021.106204.
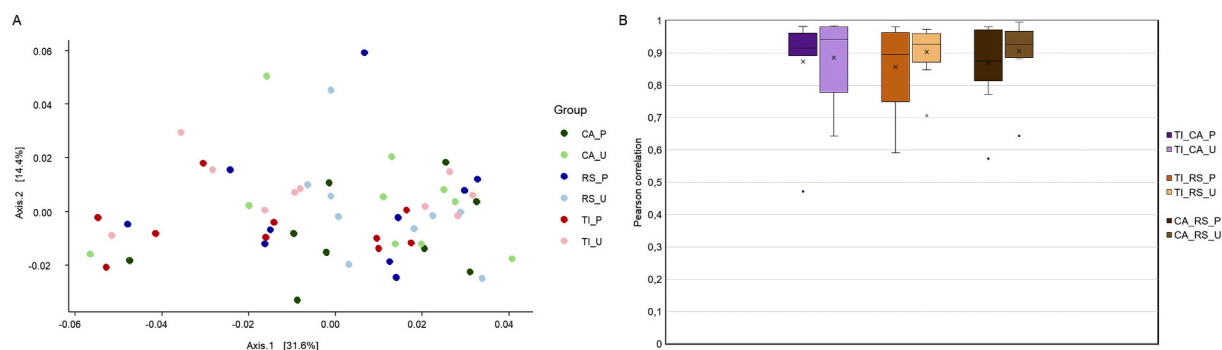
Fig. 2. HITChip probe level data. **A** – PCoA plot of Bray Curtis dissimilarities of HITChip probe level data. **B** – Comparisons of inter-site HITChip probe level data Pearson correlations between P and U samples of TI, CA and RS. TI_CA_P is an inter-site comparison of the P biopsies of TI and CA, TI_CA_U of the U biopsies of TI and CA, etc. Statistical significance was accepted at $p < 0.05$. Data points represented by dots are outliers.
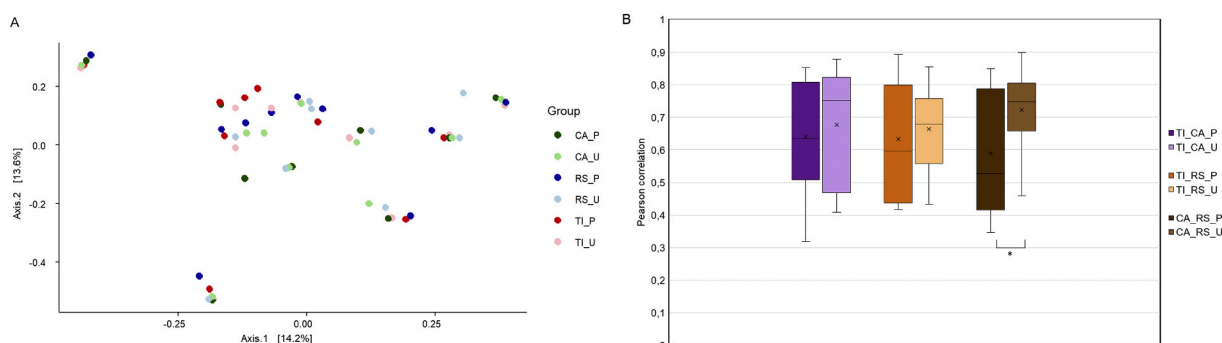

Fig. 3. 16S rRNA gene sequencing data. **A** – PCoA plot of Bray Curtis dissimilarities the 16S rRNA data. **B** – Comparisons of inter-site 16S rRNA data Pearson correlations between P and U samples of TI, CA and RS. TI_CA_P is an inter-site comparison of the P biopsies of TI and CA, TI_CA_U of the U biopsies of TI and CA, etc. Statistical significance was accepted at $p < 0.05$.

Ethical approval

This study was approved by the Medical Research Ethics Committee of the Academic Medical Center.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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