Preliminary Comparison of Endoscopic Brush and Net Catheters as the Sampling Tool to Analyze the Intestinal Mucus in the Rectum with Ulcerative Colitis Patients

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Keywords
Microbiome · Sampling · Brush · Ulcerative colitis · Mass spectrometry

Abstract
Background: The pathophysiology of ulcerative colitis (UC) remains unclear, but early lesions on the colorectal mucosal surface may play an important role in its etiology. Intestinal mucus samples, including inner and outer layers, are collected by net or brush catheters, but the quality of the samples obtained by each method has not been fully investigated.

Objective: The purpose of this study was to compare the microbiome and protein content of intestinal mucus collected by net and brush catheters during colonoscopy.

Methods: Intestinal mucus samples from the lower rectum of 4 patients with UC were collected using a net catheter, a brush catheter, and intestinal fluid suction. Microbiome and protein content were analyzed using 16S rRNA gene sequencing and mass spectrometry.

Results: The patients demonstrated significant differences in microbiome alpha diversity ($p < 0.05$), but this difference was not observed between the sampling methods. Net catheter samples demonstrated higher total protein concentrations than brush catheter samples. The brush catheter group had more \textit{Lachnospira}, a butyrate-producing bacterium, when compared to the net group. The brush catheter group also had more oral bacteria of \textit{Staphylococcus} and \textit{Dialister} in those with active phase when compared to the net group. Conclusions: Brush catheters are more likely to collect the intestinal mucus inner layer, whereas net catheters are more likely to collect larger samples that include the outer mucus layer, as well as the intestinal fluid. Two sampling methods with different types of collection of the mucosa may lead to different results among patients with mucosal vulnerabilities.

Introduction
The incidence of ulcerative colitis (UC) has continuously increased, but its pathophysiology has yet to be elucidated. Studies have proposed that the intestinal immune abnormalities in inflammatory bowel disease may...
be related to genetic and environmental factors [1, 2]. In particular, mental stress and overconsumption of fatty foods induce sympathetic hyperactivity, which reduces surface intestinal mucus. Reduced intestinal mucus results in increased antigen presentation from diet and bacteria and a lower anti-inflammatory response [3]. Decreased parasympathetic activity has also been shown to contribute to a collapse of the intestinal mucosal immune system [3]. The intestinal mucosal system, as well as the gut microbiome, is currently considered as the main mediator behind UC [4, 5].

Clinical studies have examined the gut microbiome through fecal samples [6, 7]. However, it is difficult to identify which part of the large intestine influences the microbiome data from fecal samples. Moreover, large interindividual differences in gut microbiome data make it difficult to identify specific UC-causing organisms [8]. The gut microbiome can be examined by intestinal mucus sampling during gastrointestinal endoscopy, wherein a sample of intestinal mucus is extracted with a brush or net catheter [9]. The brush catheter is used more frequently [10], but its collecting tip is small, which sometimes makes acquiring an adequate amount of samples more difficult. In contrast, the net catheter utilizes a loop that is approximately 2.5 cm in size. In clinical practice, it is used to retrieve resected colorectal polyps, but the intestinal mucus attached to the loop may be analyzed separately. Such attached mucus may be applied for experimental materials.

The mucus layer of the large intestine has inner and outer layers [11]. The inner layer is thicker and more resistant to bacteria, whereas the outer layer is less dense and more susceptible to bacterial colonization [12]. Brush sampling is more likely to provide information about the inner layer because it samples the mucosa perpendicular to the intestinal wall, whereas net sampling takes samples parallel to the intestinal wall. It may be more useful for collecting larger amounts of intestinal mucus. In previous reports of endoscopic sampling, biopsy forceps and brushes were mainly used. As far as we could find, there was only one report of net-based sampling by Nagayama et al. [9]. In this report, they took samples by either brush or biopsy forceps from a patient. A comparison of methods was not reported. It has been reported that the presence of dysbiosis on the mucosa in patients with UC also causes changes in intestinal metabolites and serum antibody levels, which are also associated with active UC [13]. Earley et al. [14] reported that abundance of Akkermansia muciniphila, a key symbiont member of the microbiota, was reduced in active UC. The Clinical Activity Index (CAI) is an indicator of active UC, and it will be important to be able to evaluate sufficient microbiome and metabolome even in cases with high CAI.

The aim of this study was to collect samples of intestinal mucus obtained during colonoscopy and analyze these based on whether they were collected by a brush or net catheter in UC. Microbiome and protein data were analyzed through 16S rRNA gene sequencing and mass spectrometry, respectively.

**Materials and Methods**

This was a single-center study that examined 4 patients with UC. We collected intestinal mucus samples in the lower rectum through colonoscopy. Three samples were taken from each patient (Fig. 1). Intestinal mucus from the anterior and right rectal walls
Sample Collection and 16S rRNA Gene Sequencing

Gene sequencing was performed based on previously published methodology [17]. Samples were immediately stored at −80°C. Isolated DNA were amplified using universal primers (forward: 5′-TGCTCGGCAAGTCAGATGTGTATAAGACAGGACTACHVGGGTATCTATACT-3′ and reverse: 5′-GCTCGGAGATGTGTATAAGACAGGACTACHVGGGTATCTATACTC-3′) to target the V3–4 regions of the bacterial 16S rRNA. Sequencing data were obtained using the MiSeq Reagent Kit v3 (Illunina, San Diego, CA, USA), with 2 × 300 reads and 600 cycles for microbial analysis. We followed the instruction tutorials and used QIME2 [18] and DADA2; Greengenes was used as a reference. The details are shown in the online supplementary information 1.

Mass Spectrometry

The samples were lysed using the Minute Total Protein Extraction Kit (Funakoshi, Tokyo, Japan). The supernatants were collected after centrifugation, and the protein concentrations of the supernatants were determined by bicinchoninic protein assay to determine the total amount of proteins in the samples. After reduction and alkylation, the proteins were digested with trypsin for 16 h at 37°C. The peptides were analyzed by liquid chromatography mass spectrometry using an Orbitrap Fusion Mass Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled to an UltiMate 3000 RSLCnano LC System (Dionex Co., Amsterdam, the Netherlands) using a nano high-performance LC capillary column, 150 mm × 75 μm i.d. (Nikkoyo Technos Co., Tokyo, Japan) and a nano-electrospray ion source. Reversed-phase chromatography was performed with a linear gradient (0 min, 5% B; 100 min, 40% B) with solvents A (2% acetonitrile with 0.1% formic acid) and B (95% acetonitrile with 0.1% formic acid) at an estimated flow rate of 300 nL/min. A precursor scan was performed using a 400–1,600 mass-to-charge ratio (m/z) prior to MS/MS analysis. Tandem MS was performed by isolation at 0.8 Th with the quadrupole, high-energy collisional dissociation fragmentation at a normalized collision energy of 30%, and rapid-scan MS analysis was performed in the ion trap. Only precursors with charge states of 2–6 were sampled for MS2. The dynamic exclusion duration was set to 15 s with a 10-ppm tolerance. The instrument was operated in the top speed mode with 3 s cycles.

Statistical Analysis

Categorical variables were compared using the χ² or Fisher’s exact test, whereas continuous variables were compared using the Mann-Whitney U test. Data were analyzed using IBM SPSS Statistics version 24 (SPSS Inc., Chicago, IL, USA).

Microbiome data were visualized and statistically analyzed using MicrobiomeAnalyst [19]. The alpha diversities of the observed species were calculated using the Shannon-Wiener and Simpson indices. The analysis was performed with the default settings, but the filtering and taxonomic levels were set as the feature level. Low and variance filters were not used because they take miner bacteria. Beta diversity was calculated using an analysis of similarities, a nonparametric statistical test, and the taxonomic level was selected as the feature and genus level. The alpha diversities of the bacteria at the phylum level were compared using the Kruskal-Wallis H test, whereas the alpha diversities of the bacteria at the genus level were compared using LEfSe in the default setting.

Protein data were processed using Proteome Discoverer 1.4 (Thermo Fisher Scientific) and identified with the MASCOT search engine version 2.6.0 (Matrix Science Inc., Boston, MA, USA). The peptides and proteins in the samples were identified against the human protein database in UniProt (release 2020_03), with precursor mass and fragment ion mass tolerances fixed at 10 ppm and 0.8 Da, respectively. Fixed modification was set with cysteine carbamidomethylation, and variable modifications were set for the oxidation of methionine. Two missed cleavages with trypsin were used.

Table 1. Demographic and basic characteristics of patients

|                          | (N = 4)        |
|--------------------------|---------------|
| Gender, female/male, N   | 3/1           |
| Age, median, [range]     | 46 [22–70]    |
| Duration of diseasemonth [range] | 90 [24–144] |
| Disease location, N      | 3/1/0         |
| Extensive/left-sided/proctitis |              |
| Treatment, N             |               |
| Oral 5-aminosalicycic acid, N (%) | 4 (100) |
| Corticosteroids, N (%)   | 1 (25)        |
| Biologic agents, N (%)   | 1 (25)        |
| Immunomodulators, N (%)  | 1 (25)        |
| Calcineurin inhibitors, N (%) | 0 (0) |
| Topical agents, N (%)    | 0 (0)         |
| CRP, median [range], mg/dL | 0.12 [0.01–0.39] |
| Alb, median [range], g/dL | 4.3 [4.2–4.4] |
| Rachmilewitz CAI,* median [range] | 5 [0–9] |
| UCEIS, ** median [range] | 3 [2–4]      |

* CAI: Clinical Activity Index. ** UCEIS, Ulcerative Colitis Endoscopy Index of Severity.
Table 2. Demultiplexed sequence counts (paired-end reads) with 948 features identified by 16S rRNA gene sequencing

| Patient | Sequence count | Feature count | Simpson | Chao1 | Shannon | Observed | Match rate (genus level, %) |
|---------|----------------|---------------|---------|-------|---------|----------|-----------------------------|
|         | Brush          | Net           | Fluid   |       |         |          | Brush | Net | Fluid |
| Patient 1 | 137,043       | 98,629        | 0.8690262 | 158   | 2.812758| 158      | –    | 85.9 | 91.2 |
|          | 147,142       | 101,024       | 0.8807727 | 121   | 2.863048| 121      | –    | –   | 85.3 |
|          | 169,781       | 118,025       | 0.8765724 | 118   | 2.852898| 118      | –    | –   | –    |
| Patient 2 | 76,634        | 56,846        | 0.8840762 | 94    | 2.629995| 94       | –    | 80.6 | 71.9 |
|          | 116,490       | 85,041        | 0.9031833 | 108   | 2.83076 | 108      | –    | –   | 82.3 |
|          | 79,657        | 57,825        | 0.8997583 | 103   | 2.857096| 103      | –    | –   | –    |
| Patient 3 | 146,611       | 105,408       | 0.9195404 | 172   | 3.386226| 172      | –    | 93.2 | 75.1 |
|          | 193,501       | 124,311       | 0.9407875 | 182   | 3.56386 | 182      | –    | –   | 78.5 |
|          | 139,854       | 86,681        | 0.9549685 | 167   | 3.653543| 167      | –    | –   | –    |
| Patient 4 | 97,864        | 55,733        | 0.9432242 | 231   | 3.740973| 231      | –    | 83.9 | 84.8 |
|          | 136,630       | 85,734        | 0.9646357 | 271   | 4.085237| 271      | –    | –   | 93.6 |
|          | 88,905        | 55,730        | 0.9635789 | 262   | 4.074341| 262      | –    | –   | –    |
| Average  | 114,538       | 79,154        | 1        | 164   | 3       | 164      | –    | 85.9 | 80.75|
|          | 148,441       | 99,028        | 1        | 171   | 3       | 171      | –    | –   | 84.925|
|          | 119,549       | 79,565        | 1        | 163   | 3       | 163      | –    | –   | –    |

Results

Patient Data

The clinical backgrounds of the 4 patients with UC are shown in Table 1. For 16S rRNA gene sequencing in microbiome analysis, we obtained 1,530,112 demultiplexed sequence counts (paired-end reads) with 948 features identified. The median sequencing depth was 86,207.5, and the minimum frequency was 55,730 (Table 2). Both sequencing and feature data showed more variability when analyzed among patients than among the methods (online suppl. information 2). However, all samples had sufficient sequence counts and quality for the analysis.

Alpha Diversity and Relative Bacterial Abundance

The alpha diversity of each patient is shown in Table 2. The alpha diversity was significantly different among patients (p < 0.05), but there was no significant difference in the alpha diversities between the sampling methods (online suppl. information 3, 4). This difference can be clearly observed in the principal coordinates analysis plot because the samples formed clusters for each patient. This finding suggested that the microbiome data were more influenced by the patient than the method (online suppl. information 5).

The percentages of phylum-level bacteria in each sample are shown in Figure 2a. The microbiome was classified into nine phyla, and Bacteroidetes, Firmicutes, and Proteobacteria were the most dominant. The major dominance differed among patients but not among methods. There was no significant difference in the phylum-level bacteria detected among the sampling methods (online suppl. information 6).

The percentages of genus-level bacteria in each sample are different among patients and sampling methods. However, the heatmap suggested that the differences were more significant among patients (Fig. 2c). The results from the LEfSe demonstrated significant bacterial differences among patients but not among methods (online suppl. information 7).

Bacterial Data by the Sampling Method

Each sampling method demonstrated a similar microbiome for each patient, but the microbiome data showed variability among the patients. At the genus level, data from the brush and net catheter, brush catheter and intestinal fluid, and net catheter and intestinal fluid groups matched 85.9%, 80.75%, and 84.93% of the time, respectively.

While the types of bacteria varied greatly among the patients, some bacteria consistently demonstrated a higher relative abundance when sampled with the net catheter compared to the brush catheter. Statistical comparison of each bacterium at the genus level is difficult because we only had 4 patients. Therefore, instead of comparing the mean of each bacterium, we searched for bacteria that...
In all patients, no bacteria were detected closer to the mucosal surface than brush catheters. In net catheters and brush catheters, respectively. This also cant and representative amount of bacteria, followed by such, intestinal fluid samples collected the most signifi-
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tinal fluid samples than in the brush catheter samples. As such, intestinal fluid samples collected the most signifi-
cant and representative amount of bacteria, followed by net catheters and brush catheters, respectively. This also suggested that net catheters may be collecting samples closer to the mucosal surface than brush catheters. In contrast, in all patients, no bacteria were detected with higher relative abundance in the brush than in the net.

**Mass Spectrometry**
Specimens were collected from the anterior and right walls of the rectal mucosa using brush and net catheters, respectively. Patient characteristics are shown in Table 1. We identified the proteins in the net and brush catheter samples using mass spectrometry. Significantly higher protein concentrations were detected in the net catheter samples than the brush catheter samples (Fig. 3a). We used these sample concentrations to identify the total number of proteins in each patient. The median number of identified proteins was 1,183 (range 815–1,537) for brush samples and 1,104 (range 689–1,423) for net samples. Analysis of mucus samples collected from ex vivo colon biopsies by tandem mass spectrometry identified 29 core proteins. Consistent with this report, MUC2 mucin, the main constituent of intestinal mucus, and other mucins, such as MUC5 and MUC13A, and IgGfC-binding protein (FCGBP) were also detected and demonstrated similar concentrations regardless of the sampling method.

Inflammatory protein markers (protein S100-A8 and protein S100-A9) and a neurotroph protein (myeloperoxidase) were also detected, but their protein levels were similar between the net and brush catheter samples (Fig. 3b). Some proteins were only identified in the net catheter samples; these are shown in Table 3.
**Fig. 3.** 

**a** Protein concentrations in the net and brush catheter samples. ● Brush sample, ■ net sample. 

**b** Proteins collected by the net and brush catheter samples. ● Brush sample, ■ net sample.
Table 3. Proteins detected in net catheter samples only

| Accession | Protein name                                           | MW   | Score     |
|-----------|--------------------------------------------------------|------|-----------|
| P8217     | Chymotrypsin-like elastase family member 2A            | 85.4 | 8,965.87  |
| P09923    | Intestinal-type alkaline phosphatase                   | 72.5 | 2,977.77  |
| P04118    | Colipase                                               | 35.4 | 2,679.83  |
| P08861    | Chymotrypsin-like elastase family member 3B            | 141.5| 2,611.26  |
| P13688    | Carcinoembryonic antigen-related cell adhesion molecule 1 | 28.9 | 1,804.4   |
| P22748    | Carbonic anhydrase 4                                   | 57.5 | 1,700.48  |
| P04054    | Phospholipase A2                                       | 15.3 | 1,693.51  |
| Q9BYE9    | Cadherin-related family member 2                       | 25.6 | 1,667.72  |
| P05107    | Integrin beta 2                                        | 28.9 | 1,371.29  |
| P35580    | Myosin-10                                              | 29.2 | 1,252.92  |
| P13797    | Plastin-3                                              | 191.5| 1,023.11  |
| A0A075B6K5| Immunoglobulin lambda variable 3–9                     | 11.9 | 869.48    |
| P49913    | Cathelicidin antimicrobial peptide                     | 60.1 | 851.99    |
| P14136    | Gial fibrillary acidic protein                          | 63.1 | 840.2     |
| O43451    | Malate glucoamylase, intestinal                         | 36.2 | 804.48    |
| Q00610    | Clathrin heavy chains 1                                | 50.1 | 784.71    |
| P08575    | Receptor-type tyrosine-protein phosphatase C           | 39.1 | 644.21    |
| P14923    | Junction plakoglobin                                   | 16.1 | 536.23    |
| A0A075B6S6| Immunoglobulin kappa variable 2D-30                    | 49.8 | 535.85    |
| Q09666    | Neuroblast differentiation-associated protein AHNK      | 78.8 | 474.64    |
| P51970    | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex subunit 8 | 12.7 | 372.85    |
| O75830    | Serpin 12                                              | 13.5 | 358.93    |
| P62834    | Ras-related protein Rap-1A                             | 13.2 | 357.57    |
| P08962    | CD63 antigen                                           | 12.6 | 320.95    |
| P29350    | Tyrosine-protein phosphatase nonreceptor type 6         | 12.5 | 305.96    |
| Q06210    | Glutamine-fructose-6-phosphate aminotransferase (isomerizing) 1 | 12.6 | 301.47    |
| Q6UXB2    | Ly6/PLAUR domain-containing protein 8                  | 12.3 | 293.9     |
| P19075    | Tetraspanin-8                                          | 12.4 | 290.21    |
| P26641    | Elongation factor 1-gamma                              | 84.7 | 290.03    |
| Q06323    | Proteasome activator complex subunit 1                 | 56.8 | 278.75    |
| Q9UFN0    | Protein NipSnap homolog 3A                             | 81.7 | 262.03    |
| P19338    | Nucleolin                                              | 38.9 | 259.06    |
| O75131    | Copine-3                                               | 25.2 | 252.41    |
| O00584    | Ribonuclease T2                                        | 209.7| 219.07    |
| Q8WZ42    | Titin                                                  | 46.6 | 206.99    |
| Q99798    | Aconitate hydratase, mitochondrial                     | 83.6 | 197.84    |
| P27216    | Annexin A13                                            | 228.9| 193.28    |
| Q6UXH1    | Cysteine-rich with EGF-like domain protein 2            | 20.1 | 160.39    |
| P30048    | Thioredoxin-dependent peroxide reductase, mitochondrial | 628.7| 153.36    |
| O75477    | Erlin-1                                                | 76.6 | 147.2     |
| P11310    | Medium-chain-specific acyl-CoA dehydrogenase, mitochondrial | 48.5 | 146.29    |
| A0A087WSY6| Immunoglobulin kappa variable 3D-15                    | 16.3 | 138.52    |
| P61457    | Pterin-4-alpha-carboxylamine dehydratase               | 70.8 | 125.63    |
| Q9H4A4    | Aminopeptidase B                                       | 28.7 | 124.13    |
| Q5TZA2    | Rootletin                                              | 28.4 | 123.3     |
| Q5TOJ7    | Tests-expressed sequence 35 protein                    | 12   | 118.9     |
| P01709    | Immunoglobulin lambda variable 2–8                     | 21   | 116.05    |
| P01706    | Immunoglobulin lambda variable 2–11                    | 147.4| 115.43    |
| Q16881    | Thioredoxin reductase 1                                | 29.5 | 106.31    |
| A0A0A0MRZ8| Immunoglobulin kappa variable 3D-11                    | 228.4| 105.43    |
| A0A084J1U7| Immunoglobulin heavy variable 61                      | 46.1 | 91.95     |
| P01742    | Immunoglobulin heavy variable 1–69                     | 26.5 | 87.15     |
| Q860Q8    | Copine-8                                               | 26   | 84.42     |
| Q15645    | Pachytenec checkpoint protein 2 homolog                | 70.9 | 81.42     |
| Q16891    | MICOS complex subunit MIC60                           | 56.5 | 56.71     |
| Q05315    | Galectin-10                                            | 3,813.7| 56.69    |
| Q860E4    | Leucine zipper protein 2                               | 67.5 | 47.66     |

MW, molecular weight.
Relationship between Microbiome and Mass Spectrometry for Each Patient

Of the 4 enrolled patients, only patient No. 2 had a CAI of 0, which means no active UC. The CAI was higher in patient No. 1, 4, 3, and 2, in that order. Mucin-2, Mucin-5B, Mucin-13, and FCGBP associated with the intestinal mucosa of each patient are shown in Figure 4a. Patient No. 1, No. 3, and No. 4 with active UC had higher abundance of these proteins collected in the net than in the brush. On the other hand, in the inactive UC patient No. 2, more of these proteins were collected with the brush.

Nine bacteria that showed a similar trend are shown in online supplementary information 9. Only patient No. 2 showed a different trend when comparing brushes and nets. The bacteria with the highest relative abundance are shown in Figure 4b. An adequate amount of *Bifidobacterium* was detected in patient No. 1, No. 3, and No. 4, but the percentage detected in patient No. 2 was very low. The genus *Lachnospira*, a butyrate-producing bacterium, was higher in the brushes of patient No. 2 than in the nets, while the other patients had the opposite occur. On the other hand, oral bacteria *Staphylococcus* and *Dialister* were found at a higher concentration on the brush than on the net in 3 patients. If brushes are collected from a deeper mucus layer than nets, this may indicate that there is less *Bifidobacterium* and more oral bacteria in the deeper mucus layer of active patients. B: proteins collected by the net and brush catheter samples. CAI: Clinical Activity Index; UCEIS: Ulcerative Colitis Endoscopic Index of Severity.

(Figure continued on next page.)
Discussion

Recent studies have demonstrated that the intestinal microbiome of patients with UC differ from healthy controls [20] because the mucosal inflammation in UC changes the mucosa-associated microbiota. Ulceration of the colonic epithelium further aggravates this condition, which perpetuates the cycle. Nishino et al. [21] reported that the relative abundance of Firmicutes and Proteobacteria in intestinal surface mucus was different among patients with UC and Crohn’s disease and normal controls. Fecal microbiota transplantation, probiotics, prebiotics, or synbiotics have been shown to be effective for UC [22, 23]; however, the pathogenesis of UC must be clarified before new therapeutic microbiome-based strategies are introduced.

The gut microbiome differs depending on the location in the large intestine. As such, we only collected samples from a single site, the lower rectum. Our endoscopic samples demonstrated a large amount of human DNA but little bacterial DNA [24]. Among the sampling techniques, brush catheter sampling has been reported to provide more bacterial DNA than biopsies, even if the amount of DNA obtained through brush catheter sampling is lower. We also expected that the brush catheter would acquire a smaller sample of proteins, which would make protein analysis difficult.

The colonic mucus layer has inner and outer layers. Both layers have the same protein profile; however, the inner mucus layer is denser and does not contain any bacteria or bacterial metabolites [11]. Therefore, the microbiome data are not expected to change, even if a larger
proportion of the inner layer is sampled. In contrast, there is a difference in the microbiome of the mucus layer and intestinal fluid [25]. Each of our sampling techniques may have collected different proportions of mucus and intestinal fluid content, which would explain the difference in the microbiome data identified in our study. While both brushes scrape the mucus layer to collect a sample, the net catheter scrapes over a larger area, which may catch more intestinal fluid and mucus than the brush catheter.

Lavelle et al. also analyzed whether biopsy, brush, and laser capture microdissection affected the mucosa-associated microbiota data in 5 patients with UC and 4 controls [26]. Similar to our results, they detected large interpatient variabilities. While they were not able to identify definite UC-causing bacteria, their data did show higher relative abundance of Coriobacteriaceae, Bacteroidaceae, Ruminococcaceae [27], and Family XIII Incertae Sedis in the mucus of patients with UC than in controls.

Our sample also showed a great variation among patients. Therefore, it was difficult to compare between patients in our study as well. However, comparison of brush and net proteins may suggest an association with activity. Abnormalities in mucus production have been reported in active UC. Abnormalities of the mucus system have been described in active UC. Reduction of MUC2 and FCGBP in the colonic mucus occurs prior to the onset of inflammation of UC and has been suggested to be related to the pathogenesis of UC. Mucins, a component of mucus, are classified into two different types: transmembrane mucins and gel-forming mucins. MUC2 and MUC5B are gel-forming secreted mucins, and MUC13 is transmembrane mucin. Both types of mucins are secreted by the goblet cells. In samples from patients with active UC, more than twice as much protein associated with mucus was detected in the net. On the other hand, brush samples collected more protein associated with mucus in patient 2, who had less active disease. This may indicate that the horizontal force of the net may cause the mucus to peel off easier, especially for the outer mucin layer. In other words, these differences indicate mucosal friability, which may be associated with higher clinical activity of UC.

Nishino et al. [21] showed that Bifidobacterium levels were higher in UC patients than healthy controls using brush samples. Lavelle A [26] also showed that UC patients had higher Bifidobacterium than controls and also showed higher abundance in the mucus than in the lumen. This is in agreement with our finding that only highly active patients had higher relative abundance of Bifidobacterium. The lower abundance of Lachnospira, a butyrate-producing bacterium collected by net in less active UC patients than in brushes may indicate that the bacterium has settled in the inner layer, in which only the brush catheter can collect that mucus. On the other hand, in active UC patients, Staphylococcus, a resident oral bacterium, was collected more abundantly by brushes than nets, which may indicate that the brush catheter can catch the bacteria in the inner mucus layer in active UC.

Studies have proposed that patients with UC have different microbiomes compared to healthy controls, but whether this is the cause or result of the disease process remains unknown. Various bacteria have been also been identified as the possible triggers for UC, but these have yet to be proven. Fecal microbiota transplantation has been suggested as an effective therapy for UC because it increases the production of short-chain fatty acids, particularly butyric acid [28]. Butyric acid decreases intestinal permeability and maintains the integrity of the intestinal epithelium, which reduces overall disease severity [29]. Our analysis was able to identify genus Butyrivibrio bacteria, a butyric acid bacterium, in our samples. Rothia and Streptococcus are commonly found in the oral cavity and are expected to be present near the mucosal lumen surface. The net catheter has a high potential for future research because sampling with the net catheter obtained more feature counts than sampling with the brush in all patients.

Our study also demonstrated that net and brush catheter sampling collected the same types and amounts of mucosal proteins and inflammatory markers, except for leucine-rich alpha-2-glycoprotein [30], which was detected in the brush catheter samples alone. As mentioned previously, the brush catheter collects samples perpendicular to the intestinal mucosa and is more likely to obtain portions of the inner mucus layer. This presumes that leucine-rich alpha-2-glycoprotein may be more predominant in the inner layer rather than the mucosal surface.

This study has several limitations. First, our sample size was small. Second, we tried to perform a multi-omics analysis; however, there were significant differences in the microbiomes among the patients, which made the analysis impossible. Lastly, we did not analyze the proteins that were only detected in small amounts.

In conclusion, our study demonstrated the bacterial and protein content of intestinal mucosal samples taken with net and brush catheters among patients with UC. Brush catheters were more likely to acquire samples from the inner mucus layer, whereas net catheters were more likely to collect larger samples that include the outer mucus layer and intestinal fluid.
Acknowledgments

We wish to thank Ms. Akina Ooishi of the Department of Gastroenterology and Hepatology of the Nagoya University Graduate School of Medicine for technical assistance in DNA isolation and 16S rRNA gene sequencing.

Statement of Ethics

The study was reviewed and approved by the Ethics Committee of Nagoya University Hospital (protocol number 2015-0420, August 30, 2016). The study was registered in the University Hospital Medical Information Network and in a clinical trial registry (UMIN000020269). Written informed consent was obtained from all patients prior to their enrollment in accordance with the Declaration of Helsinki.

Conflict of Interest Statement

All the authors had no conflict of interest relevant to this submission.

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Funding Sources

A part of this study was covered by Grant-in-Aid for Scientific Research (KAKENHI), ID 20K07801.

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Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
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