Showa Univ J Med Sci **31**(4), 365～372, December 2019

Original

Comparison of Protein Profiles of Gingival Crevicular Fluids Collected from Incisors, Canines, and Molars

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Abstract: Many studies have shown that gingival crevicular fluid (GCF) reflects the inflammatory state of local periodontal tissues. GCF has been collected from several types of teeth in previous studies. However, there is no report that characterizes GCF by the type of tooth. In the present study, the protein profiles of GCF from different sites were comprehensively compared with each other. GCF was sampled from six healthy adult men (21–31 years old) with healthy periodontal tissues. Three separate GCF samples were collected at the maxillary central incisor, canine, and first molar of each individual. The protein profiles of GCF were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis and liquid chromatogram-tandem mass spectrometry (LC-MS/MS). The band patterns on the sodium dodecyl sulfate polyacrylamide gel electrophoresis from the set of three GCF samples from each individual were similar, regardless of the type of tooth. The proteins contained in each band were identified by LC-MS/MS analysis, and they were found to be the same among the three GCF samples. A comprehensive and quantitative analysis of proteins in the GCF samples was performed by LC-MS/MS using isobaric tag labeling. In total, 86 proteins were identified in GCF. A small number of proteins were increased or decreased in GCF from the first molars compared with the other types of teeth in one or two individuals. However, overall, no proteins were found to exhibit a reproducibly different composition in any of the individuals. These analyses show that the protein profiles of GCF in healthy periodontal tissues are similar, regardless of the type of tooth.

Key words: gingival crevicular fluids, protein profiles, quantitative proteomics, isobaric tag labeling

Introduction

Gingival crevicular fluid (GCF) is a biological fluid derived from plasma, which passes through the gingival plexus and the junctional epithelium to exude into the gingival sulcus. GCF can be collected from the oral cavity non-invasively. It contains plasma components, various antibacterial substances, and cell components. Although GCF is an exudate, leukocytes, including neutrophils

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and macrophages, which have migrated through the junctional epithelium, and epithelial cells, detached from the periodontal tissue, are observed in GCF. GCF functions as a biological defense mechanism for periodontal tissue. Research on GCF has a long history. GCF was reported in the mid-20th century, when the composition and flow of GCF were described[1]. Several inflammatory mediators are present in GCF, suggesting that GCF reflects the inflammatory status of local periodontal tissues, including gingivitis and periodontitis[2].

In our previous study, the presence of low-density lipoprotein (LDL) and oxidized LDL (oxLDL) was reported in GCF for the first time[3]. LDL and oxLDL in GCF can be determined by ELISA using antibodies against apolipoprotein B (apoB) and oxidized phosphatidylcholine. The concentration of apoB in GCF from patients with diabetes mellitus was higher than in normal controls[4]. Further, levels of apoB and oxLDL in GCF were higher at sites with periodontitis than at healthy sites, and these levels decreased following initial preparation of the sites of periodontitis[5]. These observations suggest that GCF is a sensitive marker for hyperglycemic conditions, and it is useful for the examination of not only the oral status of patients but also certain systemic conditions.

A recent proteome technique enables the analysis of protein profiles of several biological fluids, including plasma, saliva, and urine. Using liquid chromatogram-tandem mass spectrometry (LC-MS/MS), the composition of protein components can be analyzed from samples, even in small quantities, with GCF proteins having been used for proteome analysis for the last two decades. Several studies have identified hundreds of human proteins and more than 20 bacterial proteins either from healthy individuals or patients with periodontitis[6–10]. The GCF contents, including albumin, serotransferrin, and α-2-macroglobulin, reflect the serum origin of GCF.

In our previous study, GCF samples were collected from the permanent and deciduous teeth in healthy gingivae of the same child with mixed dentition, and the GCF samples were compared using proteomic analysis[11]. The proteins contained in GCF were markedly different between the permanent and deciduous teeth. GCF from deciduous teeth contains an abundance of neutrophil-derived proteins, whereas GCF from permanent teeth contains larger quantities of immunoglobulins. From these results, it is possible that GCF of different types of teeth may have different compositions, reflecting the condition of the tissues.

The pathology of localized aggressive periodontitis, a type of aggressive periodontal disease whose classification in periodontal diseases still remains controversial, is characterized by site-selective progression. It is widely recognized that aggressive periodontitis becomes apparent mainly at the first molars or incisors[12], although periodontitis has no site-specificity in general. Thus, there is merit in investigating whether there is a difference in periodontal tissues depending on the type of tooth.

In previous reports describing the proteomic analysis of GCF, the sampling sites of GCF varied. Baliban et al analyzed GCF collected from the mesiobuccal sites of first molars[6, 13]. Moriya et al collected GCF from the central incisors and deciduous cuspids in the maxilla separately[11]. Ishizuka et al collected GCF from the incisors in the maxilla[5]. However, there are several reports that do not specify the type of tooth and lack a precise description of GCF

collection sites. For example, certain studies specified collection sites only by classification with periodontal pocket depth, whereas others designated only regions, including the maxillary buccal side and anterior teeth. Some reports have specified the type of tooth ; however, each report was collected from only one type of tooth without a rationale for selecting that type of tooth. As there has been no report that compared the components of GCF according to the type of tooth, it is unclear whether these previous data can be compared.

Therefore, in the present study, the following three permanent teeth were compared: central incisors, canines (successional teeth of the deciduous cuspids), and first molars (the additional teeth). The purpose of the present study was to clarify whether GCF has different characteristics depending on the type of tooth by comprehensively comparing the protein components of GCF.

Material and methods

This study was approved by the School of Dentistry, Showa University Ethics Committee (No. 2017-003). GCF samples were collected from six healthy adult men (21–31 years old) with healthy periodontal tissues at the maxillary central incisors, canines, and first molars. The participants were neither smokers nor seeking orthodontic treatment. The sampling teeth were free of prostheses, tooth mobility, endodontic lesions, severe caries, or trauma history.

Collection of GCF and sample preparation

GCF samples were collected from all around both maxillary central incisors, canines, and first molars using paper points (Dentsply International Inc., York, PA, USA). The sampling sites were isolated from the saliva with cotton rolls and air-dried using an air syringe. The paper points were gently inserted into the sulcus and left in place for 1 min. A total of 10 paper points were used for collecting GCF from each tooth, following which the paper points were removed and immersed in 200 µl phosphate buffered saline containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA ; final 5% v/v) in 1.5 ml sample tubes. The pairs of GCF samples from both sides of the maxillary central incisors, canines, or first molars were combined and kept frozen at −80°C for future analysis. The protein concentrations of GCF samples were measured using BCA protein assay reagents (Thermo Scientific, Rockford, IL, USA).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of the GCF samples (5 µg/lane) was performed using e-PAGEL (12.5% polyacrylamide ; Atto, Tokyo, Japan) with buffer solution containing 100 mM Tris, 50 mM Tricine, and 0.1% SDS for electrophoresis, according to the manufacturer's protocol. The gels were stained with Flamingo™ fluorescent gel stain (Bio-Rad Lab., Hercules, CA, USA) for 1 hr and washed with purified water. After staining, the protein bands on the gels were recorded using Fluoro-PhoreStar 3000 (Anatech, Tokyo, Japan).

In-gel protein mass spectrometry

Using the gel picker (1.8-mm diameter) associated with FluoroPhoreStar 3000, three gel pieces were punched out from each band and transferred into a new 1.5 ml sample tube. In-gel protein digestion was performed according to the method previously reported[14]. The gel pieces were treated with 50 µl of 10 mM dithiothreitol/100 mM NH_4HCO_3 and incubated at 56°C for 1 hr. The reagent solution was replaced with 50 µl of freshly prepared 55 mM iodoacetamide/100 mM NH_4HCO_3 and incubated at room temperature for 45 min under light protection. The gel pieces were then washed with 100 mM NH_4HCO_3 followed by 50 mM NH_4HCO_3 in 50% acetonitrile, and completely dried using a centrifugal concentrator VC-360 (Taitec, Saitama, Japan). The dried gel pieces were immersed in 8 µl of 10 mM NH_4HCO_3 containing 2 µg of trypsin and incubated overnight at 37°C. The resulting tryptic peptides were extracted twice with 30 µl of 50% acetonitrile/0.1% formic acid, and the extract was dried using a centrifugal concentrator. The sample was dissolved in 30 µl of 2% acetonitrile/0.1% formic acid and analyzed by LC-MS/MS as described below.

Isobaric tag analysis for relative quantitation (iTRAQ) labeling

iTRAQ labeling was performed according to the manufacturer's protocol (Sciex, Foster City, CA, USA) as previously described[11]. Briefly, the GCF samples (5 µg each) were reduced, alkylated, and digested with 1 µg of trypsin overnight at 37°C. Tryptic peptides of the GCF samples from the central incisors, canines, and first molars were labeled with iTRAQ 114, 115, and 116 reagents, respectively. The iTRAQ reagents were dissolved in 70 µl ethanol and mixed with the tryptic peptide sample at room temperature for 1 hr, and the set of three GCF samples from a single individual was mixed into a new 1.5 ml sample tube. Unlabeled iTRAQ reagents were removed by cation-exchange chromatography. The mixed iTRAQ-labeled peptides were eluted from the column and dried using a centrifugal concentrator. Labeled peptides were resuspended in 50 µl of 0.1% formic acid containing 2% acetonitrile and analyzed in duplicate with a DiNa nano-LC system (KYA Tech Co., Tokyo, Japan) coupled online with Triple TOF 5600 (Sciex). Calibration was performed using tryptic digests of bovine serum albumin.

Results

GCF samples were collected from six healthy adult men with healthy periodontal tissues. Figure 1 shows the results of SDS-PAGE for the six sets of three GCF samples collected from the maxillary central incisors, canines, and first molars of each individual. Although there was a slight difference between individuals, the band patterns of the three GCF samples were similar, regardless of the type of tooth. The bands were excised, and the proteins contained in each band were identified by LC-MS/MS analysis. The most abundant protein was serum albumin, and several other serum proteins were identified.

A comprehensive and quantitative analysis of the proteins in all the GCF samples was performed by the iTRAQ method. In total, 86 proteins were identified in GCF, and the relative ratio for the type of tooth for each of the 86 proteins was calculated (Table 1). The solid

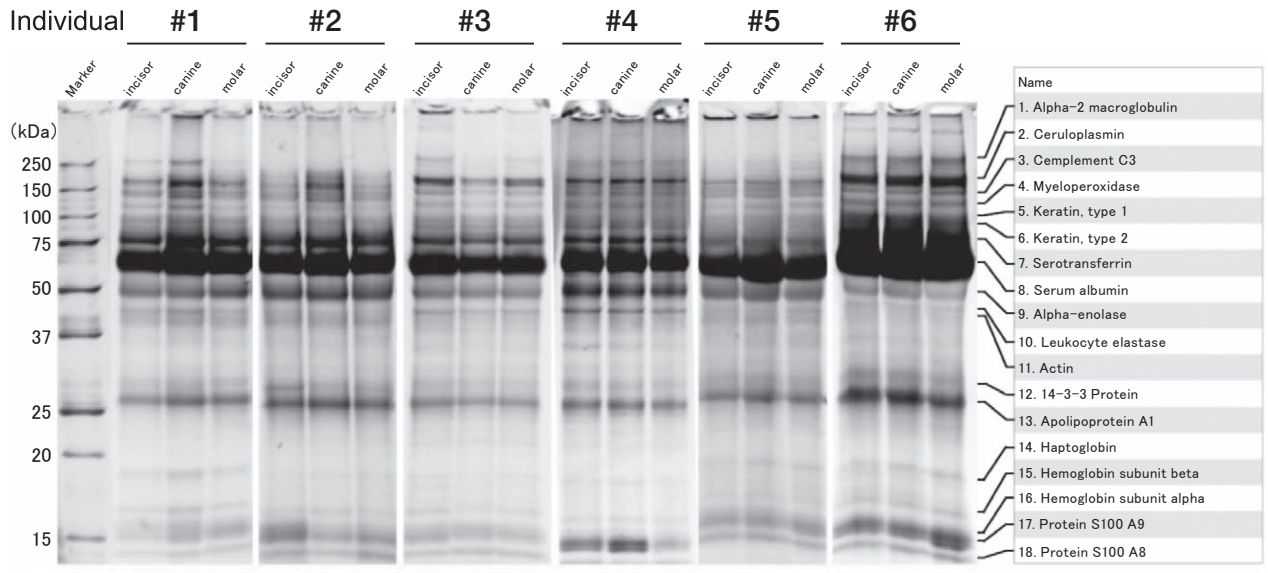

Fig. 1. Protein profiles of gingival crevicular fluid (GCF) samples collected from three types of teeth and the identification of proteins in each band. Sets of three GCF samples collected from the maxillary central incisors (incisor), canines (canine), and first molars (molar) of six individuals were analyzed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (5 μg GCF sample/lane), then the band patterns were visualized by Flamingo staining. The proteins in the major bands were identified with liquid chromatogram-tandem mass spectrometry analysis of gel pieces punched out from each band. The list of identified proteins in each band is attached.

numbers in the table indicate that the calculated values are highly reliable, based on the MS/MS spectra of identified peptide fragments, and the gray numbers are those with lower reliability. When the ratio (central incisor vs. first molar, or canine vs. first molar) was lower than 2/3, the numbers were colored in blue, indicating the protein is more enriched in GCF from the first molars compared to the other teeth. A ratio higher than 3/2, colored in magenta, indicates the protein in GCF from the first molars is less than that from the other teeth. There was a change in protein ratios between the different types of teeth in one or two individuals. There was a tendency for only a small difference between GCF from the canines and first molars, whereas a larger difference was apparent between GCF from the central incisors and first molars. For example, the ratios of central incisor/first molar for α-1-antitrypsin 1 and apoA1 were higher than 1.5 in 2 individuals but lower than 0.67 in another two. However, no protein showed a distinct reproducible distribution in any of the three types of teeth for all the participants.

Discussion

In the present study, it was found that the proteins in GCF and its composition are similar regardless of the type of tooth, based on the band patterns on SDS-PAGE of the three GCF samples from each individual and the result of LC-MS/MS analysis. No GCF protein was found to have a distinct distribution in any of the three types of teeth. These findings suggest that there are no certain differences in the GCF protein profiles between the three types of teeth

Table 1. Relative quantification of gingival crevicular fluid (GCF) proteins by liquid chromatogram-tandem mass spectrometry (LC-MS/MS) analysis with isobaric tag analysis for relative quantitation (iTRAQ) labeling

	central incisor/first molar						canine/first molar					
	#1	#2	#3	#4	#5	#6	#1	#2	#3	#4	#5	#6
14-3-3 protein sigma	1.07	0.82	0.88	1.06	1.34	1.73	1.38	0.87	0.83	1.00	1.49	1.26
Actin, cytoplasmic 2	1.43		0.79	0.94	1.70	1.41	1.72		0.87	0.96	1.12	0.97
Alpha-1-acid glycoprotein 1	9.91	0.89	1.20	1.32	0.61	0.71	7.38	1.17	1.08	1.16	0.68	0.81
Alpha-1-antichymotrypsin		0.64		0.99	0.40	0.46		1.31		0.97	0.81	0.53
Alpha-1-antitrypsin	5.01	0.80	1.99	1.38	0.50	0.46	1.12	1.22	1.27	1.60	0.85	0.57
Alpha-1B-glycoprotein	1.03	1.06	1.21	1.26	0.69	0.62	0.66	1.15	1.23	1.27	0.85	0.80
Alpha-2-HS-glycoprotein	1.00	0.75	1.21	1.09	0.70	0.55	0.49	1.20	1.29	1.25	0.95	0.77
Alpha-2-macroglobulin	7.52	0.83	1.46	1.36	0.81	0.52	3.10	1.10	1.30	1.43	0.81	0.73
Alpha-enolase	1.05	1.01	0.81	1.11	1.50	1.39	2.23	0.82	0.82	0.99	1.37	1.05
Angiotensinogen	1.27		1.84	1.22	0.84	0.74	1.09		1.08	1.27	0.76	0.77
Annexin A1	0.90	1.59	1.10	0.41	1.88	1.22	0.82	1.41	1.35	0.41	1.41	1.21
Antithrombin-III	2.49	0.92	1.18	1.55	0.68	0.71	1.92	1.06	1.45	1.50	0.86	0.79
Apolipoprotein A-I	10.86	0.57	1.69	0.79	0.33	0.57	2.91	1.19	1.27	1.14	0.54	0.60
Apolipoprotein A-II	4.66	0.73	1.19	1.11	0.33	0.53	2.29	1.28	1.05	1.40	0.56	0.66
Apolipoprotein A-IV	1.54				0.49	0.66	1.12				0.79	0.73
Beta-2-glycoprotein 1	1.49	0.74	1.14	1.41	0.84	0.44	0.82	1.04	1.41	1.29	0.84	0.70
Calmodulin-like protein 3	1.10		0.93	1.19		1.70	1.50		0.84	1.14		1.34
Carbonic anhydrase 1	4.66		0.64	1.25	0.34	0.50	4.92		2.22	1.82	0.30	0.56
Cathelicidin antimicrobial peptide	0.52		1.21	0.44	1.03	1.41	0.21		1.23	0.52	0.97	1.16
Cathepsin G	0.96	1.06	1.26	0.56	1.87	1.26	0.93	0.81	0.98	0.56	0.97	1.74
Ceruloplasmin	2.58	0.99	1.34	1.22	0.60	0.65	1.51	1.14	1.19	1.19	0.79	0.76
Complement C3	4.17	0.89	1.01	1.05	0.78	0.54	0.86	1.10	1.17	1.13	0.88	0.69
Complement C4-A (B/)						0.56						0.72
Complement factor B	1.49	0.82	1.20	1.00	0.61	0.53	0.79	0.88	1.18	1.14	1.02	0.70
Complement factor H	1.51	0.92	1.16	1.22	0.98	0.46	0.91	1.04	1.24	1.22	0.91	0.74
Coronin-1A	0.95	0.92	0.63	1.03	1.08	1.10	0.90	0.75	0.85	1.04	1.41	0.92
Cystatin-B	0.65	0.91	0.92	0.99	1.44	1.47	0.90	0.67	0.92	1.18	1.25	1.75
Elongation factor 2	1.36		0.65	0.69		1.00	1.32		0.79	0.74		0.89
Ezrin	0.39		0.62	0.75	0.83	1.11	0.92		0.77	0.66	0.70	1.05
Fatty acid-binding protein, epidermal	1.19	1.18	0.65	1.30	1.69	1.38	3.13	1.42	0.83	1.26	1.31	1.36
Fibrinogen alpha chain	0.32		0.59	0.90	1.09	0.48	0.36		0.85	1.13	1.11	0.62
Fibrinogen beta chain	2.49	1.30	0.74	0.81	0.91	0.38	1.74	1.05	0.80	1.32	1.00	0.54
Fibronectin	1.29	1.19	1.09	1.08	1.16	0.54	0.44	1.09	1.30	1.03	0.84	0.81
Fructose-bisphosphate aldolase A	1.22	0.96	0.73	1.05	1.02	1.04	0.84	0.87	0.88	1.03	1.27	1.02
Glucose-6-phosphate isomerase	1.22	0.84	0.94	0.95	1.10	0.66	0.84	0.87	0.94	1.04	0.92	0.79
Glutathione S-transferase P	1.77	1.04	0.84	1.25	1.82	1.24	1.31	0.98	0.77	1.01	1.45	0.99
Glyceraldehyde-3-phosphate dehydrogenase	0.55	0.60	0.79	1.29	1.77	1.95	1.38	0.97	0.63	1.19	1.19	1.30
Haptoglobin	4.88	0.81	1.22	1.51	0.86	0.52	1.67	1.02	1.38	1.56	0.99	0.74
Heat shock 70 kDa protein 1A/1B	0.66		0.64	0.87	0.69	0.89	1.06		0.68	0.87	0.89	0.88
Heat shock protein beta-1	0.49		0.39	0.53	0.78	1.01	0.86		0.55	0.53	1.12	1.33
Hemoglobin subunit alpha	0.94	2.97	0.56	1.22	0.76	0.30	0.96	3.99	2.76	1.61	0.69	0.39
Hemoglobin subunit beta	1.84	1.48	0.47	1.11	0.68	0.39	2.47	2.19	2.70	1.44	0.67	0.48
Hemoglobin subunit delta	2.03		0.77			0.42	2.19		2.54			0.47
Hemopexin	4.70	0.93	1.39	1.33	0.68	0.73	2.83	1.11	1.21	1.29	0.83	0.83
Ig alpha-1 chain C region	1.11	0.89	1.15	1.09	0.56	0.57	0.74	0.93	1.13	1.23	0.95	0.90
Ig gamma-1 chain C region	6.19	1.06	1.15	1.51	1.01	0.56	5.55	1.02	1.22	1.44	1.00	0.92
Ig gamma-2 chain C region	0.95	1.43	1.24	2.20	0.62	0.48	0.94	1.19	1.34	2.12	1.10	0.87
Ig kappa chain C region	1.92	1.22	1.00	1.57	0.74	0.53	1.75	1.11	1.12	1.50	1.05	0.86
Ig lambda chain V-III region LOI	0.80		1.11	1.09			0.82		1.10	1.22		
Ig lambda-2 chain C regions	1.80		1.17	1.38	0.60	0.65	1.33		1.12	1.43	1.07	0.96
Ig mu chain C region	1.51	0.78	1.18	1.21	0.67	0.64	0.74	1.13	1.28	1.23	0.85	0.77
Inter-alpha-trypsin inhibitor heavy chain H2	0.90	0.63	1.12	1.25	0.70	0.56	0.88	1.00	1.42	1.47	0.84	0.70
Inter-alpha-trypsin inhibitor heavy chain H4	0.99			1.09	0.53	0.62	0.86			1.26	0.72	0.76
Isoform 3 of Glucose-6-phosphate 1-dehydrogenas				0.69						0.83		
Isoform Gamma-A of Fibrinogen gamma chain						0.42						0.55
Isoform LMW of Kininogen-1		0.79	1.14	1.21	0.54			1.05	1.39	1.33	0.76	
Isoform M1 of Pyruvate kinase isozymes M1/M2			0.56						0.54			
Keratin, type I cytoskeletal 16	0.37	0.52		0.44			0.30	0.91		0.36		
Keratin, type II cytoskeletal 4		0.37	0.88	0.27		1.35		1.12	0.95	0.20		2.76
L-lactate dehydrogenase A chain	0.44		0.94	0.79	1.40	1.56	0.77		0.73	0.72	1.62	1.25
Lysozyme C	1.07	1.42	1.09	0.89	1.89	1.24	1.03	1.07	1.14	0.90	1.07	1.49
Macrophage-capping protein	0.69	0.96	0.56	0.67			0.86	0.87	0.87	0.67		
Moesin	0.61	1.04	0.68	0.80	1.51	0.99	0.64	0.93	0.98	0.79	1.18	0.89
Myeloperoxidase	1.41	1.10	1.31	0.88	1.01	1.04	2.17	0.96	0.93	0.86	0.82	1.53
Myosin-9	0.90	1.29	0.84	0.63		1.45	0.93	1.25	0.68	0.68		1.20
Neutrophil defensin 3		1.22	1.09	0.34	2.25	1.76		1.02	0.66	0.38	1.39	1.51
Neutrophil gelatinase-associated lipocalin	1.87	1.13	1.47	0.99	1.03	1.39	1.96	1.10	0.96	0.94	0.68	0.97
Peptidyl-prolyl cis-trans isomerase A	0.44	1.26	0.84	0.98	1.40	1.24	0.57	0.99	0.82	0.99	1.88	1.22
Peroxiredoxin-2	0.90	0.91	0.67	0.93	0.78	0.56	0.99	1.02	2.34	0.97	0.69	0.64
Plasma protease C1 inhibitor	1.41	1.23	1.19	1.15	0.73	0.54	1.16	1.35	1.00	1.25	0.73	0.67
Plastin-2	1.04	0.68	0.82	0.67	1.11	0.83	1.03	0.95	0.97	0.85	0.90	0.81
Prolactin-inducible protein		0.41	0.46	0.61		2.67		0.55	0.35	0.74		1.39
Protein S100-A11	3.91		1.03	0.62	0.75	1.26	4.41		0.88	0.60	0.66	0.89
Protein S100-A8	5.30		1.61	0.78	0.97	0.80	8.79		1.11	0.72	0.56	1.10
Protein S100-A9	1.29	3.24	1.33	0.86	1.54	0.69	2.75	1.55	1.03	0.80	0.67	1.05
Putative annexin A2-like protein				0.59						0.61		
Pyruvate kinase isozymes M1/M2	0.32	0.87		1.03	1.50	1.52	0.58	0.80		0.96	1.30	1.16
Rab GDP dissociation inhibitor beta	1.01	1.21	0.88	0.74	1.21	1.01	1.02	0.89	1.07	0.84	1.63	0.98
Serum albumin	1.06	0.91	1.56	1.52	0.76	0.49	1.04	1.22	1.40	1.50	0.91	0.83
SH3 domain-binding glutamic acid-rich-like protein 3	0.38		0.71	1.19		1.94	0.43		0.74	1.01		1.14
Transaldolase	0.54	1.32	0.64	1.16	1.13	1.52	0.68	0.86	0.92	1.05	1.16	1.07
Transthyretin			1.21	0.40	0.71	0.67			0.67	0.38	0.90	0.64
Vimentin	0.66		1.08	0.63	1.52	1.50	0.59		0.93	0.58	1.34	1.04
Vitamin D-binding protein	1.34	0.72	1.11	1.29	0.78	0.59	0.88	0.96	1.37	1.31	0.97	0.87
Vitronectin	1.09	0.95	1.55	0.89	0.61	0.44	0.86	1.32	1.22	0.92	1.05	0.69
Zymogen granule protein 16 homolog B	1.46	0.48		0.65		1.09	1.41	0.68		0.91		1.10

>4
>2
>1.5
<0.67
<0.5
<0.25

A comprehensive and quantitative analysis of proteins in all the GCF samples was performed by LC-MS/MS analysis using iTRAQ labeling. For each of the 86 proteins identified in GCF, relative ratios of the amounts present in GCF for incisors/first molars, and for canines/first molars, were calculated. The reliability of the MS data is evaluated by the abundance of the peptide fragment signals. The solid numbers indicate that the calculated ratios are highly reliable, whereas the gray numbers have lower reliability. Ratios lower than 2/3 are colored in blue, indicating the protein is more enriched in GCF from the first molars compared to the other teeth. Ratios higher than 3/2 are colored in magenta.

examined in healthy periodontal tissue.

Our previous observation that the protein patterns differ markedly between GCF collected from permanent and deciduous teeth[11], raises the possibility that GCF protein compositions could differ depending on the type of tooth. However, the present study did not show differences between GCF samples from three different types of teeth. One possible explanation of this result is that GCF protein profiles are constant when the periodontal tissues are healthy. The replacement of deciduous teeth with permanent teeth is likely to impose a marked stress on periodontal tissue and may provide a special condition in the surrounding gingival tissue. It is interesting to note that GCF protein profiles may change under certain disease conditions. Future investigation of GCF from several diseases, including periodontitis or diabetes mellitus, would answer this question.

Aggressive periodontitis is a rapid and severe progressing form of periodontitis, without contributory medical history. The defining characteristic of localized aggressive periodontitis is clinical attachment loss at mainly first molars or incisors. It is possible that gingival tissue at the canines is different from that of the other two teeth, so that GCF from canines may reflect this difference. However, this appears not to be the case. At least in healthy people, there is no difference in GCF protein components due to the type of tooth. In patients with aggressive periodontitis, GCF may change as the disease progresses. Future studies are necessary to directly investigate the GCF of patients with aggressive periodontitis.

From our current results, there are no distinct differences in the protein patterns in GCF from the three different teeth ; however, there are some individual deviations in some proteins. The concentration of some proteins in GCF from the central incisors, such as α-1-antitrypsin 1 and apoA1, seem to be highly variable among individuals. GCF is thought to originate from plasma exudate through capillaries in gingival epithelium. The transfer of plasma proteins through capillaries and epithelium may be controlled differently in different individuals.

The present study shows that there is no difference in GCF proteins among the three types of teeth in the healthy periodontal tissue of healthy people. Thus, two important points are raised. First, the protein profiles of GCF samples collected from various teeth can be compared with each other so that we can make use of the GCF data reported in a number of previous studies. Second, GCF proteins may reflect changes in gingival tissue conditions, but not the type of tooth.

Conflict of interest disclosure

The authors declare that they have no conflicts of interest.

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[Received January 14, 2019 : Accepted September 11, 2019]