Analysis of salivary factors related to the oral health status in children

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Abstract: Early detection of oral disease is important to reduce its severity and increase the likelihood of successful treatment. This study aimed to perform a quantitative assessment of the salivary components as a first stage of the research to screen oral homeostasis. Here, salivary secretions collected from children were evaluated, and their constituents were analyzed to investigate the potential correlations between the buffering capacity and a range of salivary factors. Subjects aged 3-16 years in the primary, mixed, or permanent dentition stage, were selected for this study. The following salivary factors were analyzed: flow rate, total protein, total sugar quantifications, and constituent analyses using RT-PCR and western blotting. The associations between each factor and the buffering capacity were then analyzed using multiple regression analysis. Flow rate, BPIFA2 RNA level, histatin 1 and BPIFB1 protein levels as well as female sex were positively associated with buffering capacity. In contrast, total sugar concentration and MUC7 RNA levels showed a negative relationship with the buffering capacity as well as histatin 1 and BPIFB1 protein levels. Decreased expression of cystatin S is observed in patients with Sjögren’s syndrome [12].

Introduction

Saliva plays an important role in the maintenance of oral health and physiology. It protects the functions of the oral cavity by promoting remineralization and retarding enamel demineralization, neutralizing acids, preventing microbial adherence, and inhibiting bacterial growth [1]. Saliva has been studied extensively as a diagnostic tool for systemic diseases because, similar to serum, it contains molecules that include hormones, antibodies, growth factors, enzymes, and other proteins. Some of these components access saliva from the blood; therefore, the physiological condition of the human body can be monitored with changes in its composition [2]. Saliva is easy to collect and can be accessed non-invasively without causing pain to the patient. Therefore, the analysis of saliva to monitor the health of infants, children, and other non-cooperative patients is a strategy that is attracting significant interest.

Saliva also contains water, various ions, sugars, lipids, and proteins that are candidate biomarkers for oral health status. The roles of some of these components are yet to be fully characterized, and some examples are described below.

Amylase produced in the various salivary glands, plays an important role in carbohydrate digestion. However, it can also promote streptococcal adhesion to the teeth and plaque formation [3]. In addition, amylase is a salivary marker for stress [4] and is a biomarker of diseases including type 2 diabetes [5].

Histatins produced in the parotid glands belong to the family of small, cationic, and histidine-rich proteins that inhibit proteases, exert bactericidal and fungicidal activities [6-8], and stimulate the healing of oral wounds [9]. Histatin-1 and histatin-3 are full-length precursor proteins encoded by HTN1 and HTN3, respectively, while further 10 smaller family members are derived from post-translational proteolysis of histatin-1 and histatin-3 [10]. The major histatins are histatin-1, histatin-3, and histatin-5 that are degradation products of histatin-3 [11].

Cystatin S is a defense protein that is primarily produced in the submandibular glands and is involved in innate immunity in the oral cavity. Decreased expression of cystatin S is observed in patients with Sjögren’s syndrome [12], indicating that this protein could be a candidate biomarker for other oral diseases.

BPIFB1 (BPI fold containing family B member 1, also known as LPLUNC1) expressed in the salivary glands is a member of the plate, lung and nasal epithelium clone (PLUNC) protein superfamily that has 102 members [13]. BPIFB1 has no direct bactericidal activity; however, it may be involved in the innate immune responses to oral bacteria [14,15]. BPIFB1 protein expression was recently detected in the saliva of non-obese diabetic mice but not in that from control mice [16]. Therefore, the detection and monitoring of salivary BPIFB1 levels can be a diagnostic marker for autoimmune diseases. BPIFA2 (BPI fold containing family A member 2, also known as SPLUNC2 or PSP) is expressed in human salivary glands and has been detected in the saliva. BPIFA2 has been observed to induce bacterial agglutination that indicates antibacterial function and can bind lipopolysaccharides, indicating an additional anti-inflammatory role [17].

Carbonic anhydrase VI (CA6) mainly produced in the parotid and submandibular glands is a secreted zinc metalloenzyme that catalyzes the hydration of carbon dioxide in the saliva and other body fluids. Some reports have suggested that polymorphisms in the CA6 gene are associated with the salivary buffering capacity [18,19].

WAP four-disulfide core domain 12 (WFDC12) expressed in the salivary glands is a whey-acidic-protein domain (WAP) family member that is highly expressed in the saliva. It has been predicted to exhibit antimicrobial activities through its WAP domains and is believed to play a critical role in mucosal defenses [20].

Mucins are glycoproteins with a variety of subtypes. Human salivary glands secrete two types of mucin, the high-molecular weight mucin 5B (MUC5B) and low-molecular weight mucin 7 (MUC7). Salivary mucins are major constituents of the biofilm that covers the oral mucosa and teeth surfaces [21]. Mucin 5B produced in various salivary glands plays important roles in strengthening the epithelial protective barrier, lubricating the oral cavity, and providing protection against bacterial and mechanical wear. In contrast, salivary mucin 7 encoding by MUC7 produced in the submandibular and sublingual glands protects the oral cavity from infections by binding to and clearing the microorganisms [22]. Some reports have suggested an association between the augmented levels of salivary mucins and the higher incidence of dental caries [23-25].

These salivary proteins are believed to indicate the oral homeostasis of the saliva; further, the buffering capacity of the saliva is the most important contributing factor in oral homeostasis. Here, further detailed analyses of these candidate oral health biomarkers were performed. First, their mRNA and protein expression levels in the saliva collected from a group of children were detected and quantified. The measured RNA levels in the saliva may include mRNAs that have originated from the salivary glands;
RT-PCR was performed to infer the protein expression in the saliva. Salivary secretion levels and other salivary constituents, such as total protein and sugar content were also measured. Therfore, the data were statistically analyzed to determine if there was any correlation between oral homeostasis and the levels of the salivary parameters that were studied. Furthermore, the salivary factors and/or the oral environment of children may differ from that in the past and may change again in the future. Therefore, the findings of this research can be enriched with future studies to investigate the changes in the saliva content of children over time in addition to any correlation with oral homeostasis and oral diseases.

Materials and Methods

Subject

Subjects aged 3-16 years (mean age: 9.27 years; 19 boys and 18 girls) in the primary, mixed, or permanent dentition stage were selected from among the patients of the Pediatric Dental Clinic at The Nippon Dental University Niigata Hospital in a random manner (the collecting period was Oct 2016 to Feb 2017). All the subjects were Japanese and resided in Niigata Prefecture; no patient had relevant medical history, and none was on antibiotic therapy. Before study initiation, informed consent was obtained from the parents or guardians of all the subjects. The study protocol was approved by the ethics committee of The Nippon Dental University School of Life Dentistry at Niigata (ECNG-R-274).

Measurements of the flow rate and the buffering capacity of the saliva

To measure the flow rate and buffering capacity of the stimulated saliva, the salivary buffer test CAT21 buf (Morita, Osaka, Japan) was used as per the manufacturer’s instructions. Each subject was asked to chew on an unflavored piece of paraffin wax for 5 min and expectorate saliva into a measuring cup over a period of 5 min. An aliquot of the collected saliva (1 mL) was added to a testing kit test tube, stopped and shaken, and the resultant color was compared with the color chart that was provided in the kit.

RNA extractions from the saliva

RNA was extracted from the saliva using the RNeasy Protect Saliva Mini Kit (QIAGEN, Hilden, Germany). Prior to RNA extraction, aliquots of saliva (0.2 mL) were pipetted into 1.5-mL tubes; RNA protect Saliva (0.2 mL) were pipetted into 1.5-mL tubes; RNA was extracted from the saliva using the RNeasy Protect Saliva Mini kit.

RT-PCR

Salivary mRNA expression levels were examined with semi-quantitative RT-PCR, as reported by Suda et al. [26]. The expression levels of the following five genes related to the oral environment were measured: CST4 (encoding cystatin S), HTN3 (encoding histatin-3), MUC5B (encoding mucin 5B), MUC7 (encoding mucin 7), and BPIFA2 (encoding BPI fold containing family A member 2). β-actin (ACTB) was used as an internal control. Histatin-3 was analyzed in RNA level, because histatin-3 is quickly digested by salivary proteases to make histatin-5 and other smaller histatins.

Total RNA isolated from the saliva (20 ng) was reverse transcribed using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics, Indianapolis, IN, USA) as per the manufacturer’s protocol. Resulting cDNA was amplified using PCR reaction mixtures (50 µL) containing 45 µL of Platinum PCR SuperMix High Fidelity (Invitrogen, Tokyo, Japan), 1 µL (25 pmol) of each primer (Invitrogen, summarized in Table 1), and 2 µL (1 ng) of cDNA. Cycling conditions were as follows: 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min using a Veriti 96 Well Thermal Cycler (Applied Biosystems, Waltham, MA, USA). The PCR products were analyzed on 1% agarose gels and detected using SYBR Green I (Lonza, Walkersville, MD, USA). The PCR product intensities were examined using NIH Image J, as reported previously [27].

Table 1: Primer sets used for RT-PCR

| Candidate gene | Primer pair (5'-3') | Product size (bp) |
|----------------|---------------------|-------------------|
| CST4          | ACTTGACACCTGTGCTTC  | 184               |
|               | GCATGACAGGCTGGGAGTT |                   |
| HTN3          | TCTTGGCTCTCATGCTTTCC| 182               |
|               | CGATTTGTCCATGCAAACCT|                   |
| MUC5B         | CACATCCACCTTCCAC    | 245               |
|               | GGCTAGTCTGCCCTG     |                   |
| MUC7          | CTGACACAGGAGAGCAGA  | 233               |
|               | CAGGGTTTGAGCAGACATT |                   |
| BPIFA2        | GATCAACAGCCTGAAGCA  | 179               |
|               | CCAAGTGAGCCTCCTATT  |                   |

Quantification of saliva total protein and total sugar

The saliva collected from each subject was divided into the following two samples: non-centrifuged samples (before centrifugation) and centrifuged samples (3,000 g, 10 min, 4°C) with the clear supernatants retained for analysis (after centrifugation). Total sugars were quantified in the non-centrifuged samples, and total sugar and protein quantifications were performed on the supernatants after centrifugation. Supernatants obtained after centrifugation were also analyzed using western blotting. Concentrations of total protein in the saliva were measured using the Bradford method [28] with Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Hercules, CA, USA). Total sugar concentrations in the saliva before or after centrifugation were measured with the phenol-sulfuric acid assay [29]. Saliva samples (50 µL) were mixed with 110 µL of sterile water and added to 160 µL of 5% (w/v) phenol. Thereafter, concentrated sulfuric acid (800 µL) was added. Sterile water (1 µL) was added to each sample immediately before reading their absorbance at 480 nm. The carbohydrate concentrations in each sample were calculated by performing a comparison with a maltose standard curve.

Western blotting analysis

The expression levels of seven proteins related to the oral environment, including amylase, histatin-1, cystatin S, BPIFA2, BPIFB1, CA6, and WDFC12, were examined using western blotting. Saliva samples (2 µg from each subject) were used for detecting each protein. The saliva samples were separately mixed with Laemmli sample buffer before SDS polyacrylamide gel electrophoresis (SDS-PAGE) using NuPAGE 10% Bis-Tris gels (Thermo Fisher Scientific, Waltham, MA, USA). Following SDS-PAGE, the separated proteins were electroblotted onto PVDF membranes using iBlot Gel Transfer Stacks (Thermo Fisher Scientific). Each membrane was then blocked and separately probed with specific antibodies against amylase α (a rabbit polyclonal antibody from Biovendor, Foster City, CA, USA), histatin-1 (a mouse polyclonal antibody from Abcam, Cambridge, UK), cystatin S (a rabbit polyclonal antiserum prepared by Isemura et al. [30]), BPIFA2 (a mouse monoclonal antibody from Gen Tex, Irvine, CA, USA), BPIFB1 (a mouse monoclonal antibody from Abcam), CA6 (a rabbit polyclonal antibody from Gene Tex), or WDFC12 (a rabbit polyclonal antibody from ABGENT, San Diego, CA, USA). The blots were subsequently probed with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized using ECL prime reagent (GE Healthcare, Little Chalfont, UK) and an Image Quant LAS500 imager (GE Healthcare). Additional saliva samples were treated with glycosidases (New England Bio Labs, Ipswich, MA, USA) before SDS-PAGE and immunoblotting of CA6: Saliva (3 µg protein) was treated with α1-2,4,6 fucosidase; thereafter, neuraminidase and O-glycosidase were added simultaneously to the reaction mixtures followed by N-glycosidase F as per the manufacturer’s instructions. The intensities of western blotting products were quantified using NIH Image J.

Statistical analyses

A multiple regression analysis was used to test for the associations between the variables analyzed in this study; the buffering capacity of saliva (Table 2) was analyzed using BelCurve for Excel (ver. 2.15, Social Survey Research Information Co. Ltd., Tokyo, Japan). A stepwise backward selection method was used for variable selection (Fin = Fout = 2.0) to yield the...
most appropriate regression equation.

**Results**

**Quantification of the RT-PCR products**

The results of the RT-PCR experiments for five subjects are shown in Fig. 1. The expression of CST4 (encoding cystatin S), HTN3 (encoding histatin-3), MUC5B (encoding mucin 5B), MUC7 (encoding mucin 7), and BPIFA2 (encoding BPI fold containing family A member 2) was detected using RT-PCR. (A) RT-PCR products for CST4 (encoding cystatin S), HTN3 (encoding histatin-3), MUC5B (encoding mucin 5B), MUC7 (encoding mucin 7), and BPIFA2 (encoding BPI fold containing family A member 2) analyzed with agarose gel electrophoresis. β-actin (ACTB) was used as an internal control. The expression of each mRNA under investigation was successfully detected using RT-PCR. (B) Bar graphs showing the relative intensity ratios of each RT-PCR product band for each of the five subjects who were tested. The profile of the intensities of the RT-PCR products was different for each subject who was tested. Sample 1: girl, mixed dentition, 10 years, buffering capacity = 5.6, dmft/DMFT = 3, flow rate = 0.43. Sample 2: girl, mixed dentition, 9 years, buffering capacity = 6.5, dmft/DMFT = 3, flow rate = 1.0. Sample 3: boy, mixed dentition, 6.83 years, buffering capacity = 6.2, dmft/DMFT = 0, flow rate = 0.2. Sample 4: girl, mixed dentition, 9 years, buffering capacity = 5.9, dmft/DMFT = 3, flow rate = 0.6. Sample 5: boy, mixed dentition, 7.08 years, buffering capacity = 6.5, dmft/DMFT = 0, flow rate = 2.0

**Quantification of the protein bands from the western blots**

The results of the quantification of the detected protein bands from western blots for the same five subjects are shown in Fig. 2A. The detected protein bands for the salivary proteins amylase, histatin-1, cystatin S, BPIFB1, BPIFB2, and WFDC12 were of the expected molecular weight. However, the CA6 antibody detected a protein with a higher molecular weight than expected. CA6 has carbohydrate chains; therefore, the saliva samples were subsequently treated with glycosidases before repeating the immunoblotting experiment. One clear band was subsequently detected at the expected molecular weight for CA6 (Fig. 2B). Analogous to the RT-PCR results, the profile intensities for each protein band of interest varied between the subjects (Fig. 2C).

**Multiple regression analysis of the variables that affected the buffering capacity of saliva**

The analysis of variance (ANOVA) table (Table 3A) indicated that the buffering capacity of saliva could be significantly predicted using the obtained regression model ($P < 0.001$). Table 3B indicated that the coefficient of determination (R square) was high (0.697). R square indicates the ratio of the explained variability with the obtained multiple regression equation to the total variability. Heteroscedasticity of the errors of the obtained model was tested with Breusch-Pagan and White methods, and both results showed that the null hypothesis of homoscedasticity could not be rejected ($P > 0.05$). Table 3C shows the resultant multiple regression equation obtained using the stepwise method. The variance inflation factor (VIF) values in Table 3C show that the multicollinearity in the obtained multiple regression equation can be ignored because the VIF values were much lower than 10. Partial regression coefficients indicate the degree to

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**Table 2. Variables analyzed in this study**

| Variables             | Sample size (n) | Average | Standard deviation | Minimum value | Maximum value |
|-----------------------|-----------------|---------|--------------------|---------------|---------------|
| sex*                  | 37              | 37      | 0.516              | 0.001         | 1             |
| age                   | 37              | 9.269   | 3.489              | 3.750         | 16.420        |
| dmft/DMFT             | 37              | 2.973   | 4.180              | 0.000         | 13.000        |
| dentition**           | 37              | 0.624   | 0.240              | 0.026         | 1.280         |
| total protein (ug/ul) | 37              | 0.368   | 0.110              | 0.110         | 0.773         |
| total sugar before cent. (ug/ul) | 37 | 1.848 | 0.778 | 0.061 | 3.695 |
| total sugar after cent. (ug/ul) | 37 | 0.173 | 0.451 | 0.001 | 2.042 |
| amylase α protein     | 37              | 0.979   | 0.317              | 0.380         | 2.020         |
| BPIFB1 protein        | 37              | 1.210   | 0.539              | 0.490         | 2.480         |
| CA6 protein           | 37              | 0.799   | 0.297              | 0.300         | 1.470         |
| histatin 1 protein    | 37              | 0.965   | 0.542              | 0.310         | 2.990         |
| cystatin S protein    | 37              | 1.896   | 0.870              | 0.220         | 3.830         |
| BPIFA2 protein        | 37              | 2.953   | 1.317              | 0.690         | 5.140         |
| WFDC12 protein        | 37              | 3.843   | 2.093              | 0.050         | 9.250         |
| CST4 RNA              | 37              | 0.123   | 0.144              | 0.001         | 0.529         |
| HTN3 RNA              | 37              | 0.624   | 0.240              | 0.026         | 1.280         |
| MUC5B RNA             | 37              | 0.096   | 0.121              | 0.002         | 0.462         |
| MUC7 RNA              | 37              | 0.126   | 0.098              | 0.005         | 0.521         |
| BPIFA2 RNA            | 37              | 0.083   | 0.064              | 0.014         | 0.280         |
| buffering capacity    | 37              | 6.095   | 0.471              | 4.800         | 6.500         |

*boys, 1 (51%); girls, 2 (49%). **primary dentition, 1 (11%); mixed dentition, 2 (65%); permanent dentition, 3 (24%)
which the dependent variable (in this case buffering capacity) varies with an independent variable (for example, the salivary proteins of interest) when all the other independent variables are kept constant. For example, the partial regression coefficient for the flow rate of stimulated salivama was 0.277. Thus, for every one-unit increase in the flow rate (mL/min), there was an increase in the buffering capacity (pH) of 0.277. The regression coefficients indicated that increasing RNA levels of BPIFA2, flow rate, histatin-1 protein, BPIFB1 protein, and female sex were positively associated with the buffering capacity. In contrast, increase in the total sugar content of the saliva after centrifugation and the RNA level of MUC7 showed a negative relationship with the buffering capacity. However, the presence of positive correlation does not always indicate causality. Thus, it is suggested that the same tendency as past research; a correlation between the buffering capacity and caries [31,32], is recognized in this study. The salivary flow rate significantly increased the buffering capacity (Table 3). Previous studies have reported that the salivary flow rate is slightly reduced in children with active caries [2,33]; the results of this investigation are also consistent with these findings. In this study, the average salivary flow rate was 1.0 mL/min. During the previous 20 years, a wide range of saliva flow rates in children has been reported. However, it remains to be established if there has been a mean overall change in the salivary flow rate of children during this time. It is necessary to measure and document the changes in the salivary flow over time.

Here, salivary RNA analysis was performed to analyze the levels of candidate biomarkers related to oral health status. It was demonstrated that these factors can be detected and potentially monitored using RT-PCR methods; however, it is sometimes challenging to analyze the protein level (e.g. mucins). Some previous studies have analyzed salivary RNA [34,35]. For example, salivary RNA has been used to detect oral microorganisms and disease markers that originate from the blood or carcinomas [34]. Thus, in this study, salivary RNA analysis was performed to know the expression level of salivary proteins that originate from the salivary glands. Total RNA from the saliva was extracted using the RNAeasy Protect Saliva Mini Kit, and it was routinely able to isolate >20 ng of total RNA from the saliva of each subject. These results suggest that the expression of BPIFA2 that encodes the antibacterial protein BPIFA2 produced in the salivary glands was positively associated with the buffering capacity. In contrast, the expression of MUC7, which encodes salivary mucin 7,
showed a negative relationship with the buffering capacity. Although some studies have shown that CA6 is associated with the saliva buffering capacity at the RNA level [18,19], there are reportedly no associations with the salivary buffering capacity and the protein level of CA6 [36]. In this study, CA6 protein was removed from the multiple regression equation during the stepwise variable selection process. However, a significant simple correlation ($P = 0.0324$) was observed between the CA6 protein and the salivary buffering capacity. Therefore, it is worthwhile to study the relationship between these two values.

The individual expression levels of the antimicrobial proteins analyzed in this study may be too low to have affected the pH of saliva, although salivary proteins could contribute to the saliva buffering capacity because of their ampholytic properties. However, the simultaneous increasing expression of the various proteins including the antibacterial proteins may result in salivary homeostasis. Therefore, these factors are suggested to reflect the conditions of salivary glands. Moreover, these factors are candidate biomarkers for detecting the tendency of dental caries. In contrast, the expressed protein BPIFB1 was not predicted to have a significant association with the buffering capacity. The RNA and protein did not show a significant correlation (Data not shown). It is possible that a proportion of this protein may have been degraded before the western blotting analysis. Thus, the analysis of salivary RNA may be a more effective strategy for the investigation of the oral health status.

In this study, total sugar, considered to be the endogenous carbohydrates that originate from salivary glycoproteins, was also measured. The increase in the total sugar in the saliva showed a negative relationship with the buffering capacity (Table 3), analogous to the result obtained for MUC7 expression. Thus, the decrease in the saliva total sugar, and the measurement of total sugar would be an effective test to check for the susceptibility to dental caries.

The western blotting analysis indicated that the increase in the proteins histatin-1 and BPIFB1 was positively associated with the buffering capacity (Table 3). Therefore, these proteins are also candidate biomarkers for oral health status. The mRNA expression of these factors was not examined in this study; therefore, additional experiments are required to investigate this issue in the future.

In conclusion, these results suggest that analyses of the correlation between the oral health status and various types of salivary factors are an effective strategy for monitoring the susceptibility of children to oral diseases.

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Conflict of interest

All authors have no conflict of interest.

References

1. Angwarowog O, Pitiphat W, Bolcher GMJ, Chaiyapit P (2015) Evaluation of salivary mucins in children with deciduous and mixed dentition: comparative analysis between high and low caries-risk groups. Clin Oral Investig 19, 1931-1937.
2. Preethi PB, Rethika D, Anand P (2010) Evaluation of flow rate, pH, buffering capacity, calcium, total proteins and total antioxidant capacity levels of saliva in caries free and caries active children: an in vivo study. Ind J Clin Biochem 25, 425-428.
3. Inoue H, Ono K, Masuda W, Inagaki T, Yokota M, Inoue K (2008) Rhelogical properties of human saliva and salivary mucins. J Oral Biosci 50, 134-141.
4. Kuroki H, Miyagawa Y, Shimomura-Kuroki J, Endo T, Shimomura H (2014) Identification of marker proteins by orthodontic treatment: relationship of RANKL in the gingival crevicular fluid and of amylase in whole saliva with orthodontic treatment. Odontology 102, 303-309.
5. Al-Sheikhaten ES, Elaeeed MA, Mansour HH (2017) Salivary changes in type 2 diabetic patients. Diabetes Metab Syndr 11, 637-641.
6. Oppenhen FG, Yang YC, Diamond RD, Hlyso D, Offner GD, Troxler RF (1986) The primary structure and functional characterization of the neutral histidine-rich polypeptide from human parotid secretion. J Biol Chem 261, 11171-11182.
7. Oppenhen FG, Xu T, Mcmillian FM, Levitz SM, Diamond RD, Offner GD et al. (1988) Histatin, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on Candida albicans. J Biol Chem 263, 7472-7477.
8. Guaman H, Travis J, Helmerhorst EJ, Potempa J, Troxler RE, Oppenhen FG (2001) Salivary histatin 5 is an inhibitor of both host and bacterial enzymes implicated in periodontal disease. Infect Immun 69, 1402-1408.
9. Sun X, Salih E, Oppenhen FG, Helmerhorst EJ (2009) Kinetics of histatin proteolysis in whole saliva and the effect on bioactive domains with metal-binding, antifungal, and wound healing properties. FASEB J 23, 2691-2701.
10. Sabatini LM, Azin EA (1989) Histatins, a family of salivary histidine-rich proteins, are encoded by at least two loci (HS11 and HS12). Biochem Biophys Res Commun 160, 495-502.
11. Ogata K (1990) Histatins, a family of histidine-rich polypeptides in human saliva; isolation and histamine-release activity. Jpn J Oral Biol 32, 671-685.
12. Martini D, Gallo A, Veilla S, Sernissi F, Cecchetti A, Luciano N et al. (2017) Cystatin S—a candidate biomarker for oral health and submandibular gland involvement in Sjögren’s syndrome. Rheumatology 56, 1031-1038.
13. Bingle CD, Craven C2 (2002) PLUNC: a novel family of candidate host defence proteins and a contributor to saliva buffering properties. FASEB J 12, 2691-2701.