UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

TATIANA MARTINI

Proteomics of acquired enamel pellicle in volunteers with gastroesophageal reflux with dental erosion or not

Proteoma da película adquirida em voluntários com refluxo gastresofágico com erosão dentária ou não

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DEDICATÓRIA

Aos meus pais ***Marcia e Richardi,***

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A minha família especialmente meus avós

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"Sonhe com aquilo que você quiser. Seja o que você quer ser, porque você possui apenas uma vida e nela só se tem uma chance de fazer aquilo que se quer. Tenha felicidade bastante para fazê-la forte. Tristeza para fazê-la humana. E esperança para fazê-la feliz. A felicidade aparece para aqueles que choram. Para aqueles que se machucam. Para aqueles que buscam e tentam sempre. E para aqueles que reconhecem a importância das pessoas que passam por suas vidas."

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"Tudo é considerado impossível, até acontecer."

Nelson Mandela

ABSTRACT

Proteomics of acquired enamel pellicle in volunteers with gastroesophageal reflux with dental erosion or not.

This study compared the protein profile of the acquired enamel pellicle (PAE) in 1) volunteers with gastroesophageal reflux disease (GERD) and dental erosion (BEWE ≥ 9 or grade 3 in the upper anterior sextant, all incisors affected; GE group); 2) volunteers with GERD without dental erosion (BEWE=0; GNE group) and 3) control volunteers (without GERD and dental erosion; BEWE = 0; C group). Twenty four subjects (8 in each group) participated. After dental prophylaxis, the AEP was allowed to form during 120 min and was then collected from the vestibular surface of the upper and lower teeth, with filter paper pre-soaked in 3% citric acid. After protein extraction, the samples were submitted to reverse phase liquid chromatography coupled to mass spectrometry (nLC-ESI-MS/MS). Label-free proteomic quantification was performed using Protein Lynx Global Service (PLGS) software. In total, 458 proteins were identified. Seventy-six proteins were common to all the groups. The proteomic profile of the AEP was quite different among the distinct groups. The numbers of proteins exclusively found in the C, GE and GNE groups were 113, 110 and 81, respectively. Most of the proteins exclusively identified in the C and GNE groups bind metals, while those in the GE group are mainly membrane proteins. Many proteins were found exclusively in the reflux groups. Heat-shock proteins were not found in GE. Histatins and Histones were not found in GNE, while Serine/threonine-protein kinases were only identified in GNE. In the quantitative analysis, when the GNE group was compared with the GE group, the proteins with the highest decreases were *Lysozyme C, Antileukoproteinase, Cathepsin G,* Neutrophil defensins and Basic salivary proline-rich proteins, while those with the highest increases were subunits of Hemoglobin, *Albumin* and isoforms of Cystatin. Profound alterations in the proteomic profile of the AEP were seen in GNE compared with GE volunteers, which might play a role in the resistance to dental erosion seen in the first.

Keywords: Acquired enamel pellicle; Dental erosion; Gastroesophageal reflux; Proteomics.

RESUMO

Este estudo comparou o perfil proteico da película adquirida do esmalte adquirida (PAE) em 1) voluntários com doença de refluxo gastroesofágico (DRGE) e erosão dentária (BEWE ≥ 9 ou grau 3 no sextante anterior superior, todos os incisivos afetados; 2) voluntários com DRGE sem erosão dentária (BEWE = 0; grupo GNE) e 3) voluntários de controle (sem DRGE e erosão dentária, BEWE = 0; grupo C). Participaram vinte e quatro indivíduos (8 em cada grupo). Após a profilaxia dentária, permitiu-se que a PAE se formasse durante 120 minutos e foi então coletada a partir da superfície vestibular dos dentes superiores e inferiores, com papel de filtro previamente embebido em ácido cítrico a 3%. Após a extração da proteína, as amostras foram submetidas a cromatografia líquida de fase reversa acoplada a espectrometria de massa (nLC-ESI-MS / MS). A quantificação proteômica livre de marcadores foi realizada utilizando o software de Protein Lynx Global Service (PLGS). No total, foram identificadas 458 proteínas. Setenta e seis proteínas foram comuns a todos os grupos. O perfil proteômico da AEP foi bastante diferente entre os grupos distintos. O número de proteínas encontradas exclusivamente nos grupos C, GERD com erosão e GERD sem erosão foi de 113, 110 e 81, respectivamente. A maioria das proteínas exclusivamente identificadas nos grupos C e GERD sem erosão se liga a metais, enquanto que as do grupo GERD com erosão são principalmente proteínas de membrana. Muitas proteínas foram encontradas exclusivamente nos grupos de refluxo. As proteínas Heat-shock não foram encontradas no GERD com erosão. Histatins e Histones não foram encontradas no GERD com erosão, enquanto Serine/threonine-protein kinases foram identificadas apenas no GERD sem erosão. Na análise quantitativa, quando o grupo GERD sem erosão foi comparado com o grupo GERD com erosão, as proteínas com as maiores diminuições foram *Lysozyme C, Antileukoproteinase, Cathepsin G, Neutrophil defensins* and *Basic salivary proline-rich proteins*, enquanto aquelas com os maiores aumentos foram subunidades de Hemoglobin, *Albumin* e isoformas de Cystatin. Maiores alterações no perfil proteômico da PAE foram observadas no GERD sem erosão em comparação com os voluntários GERD com erosão, o que pode ter um papel na resistência à erosão dentária observada anteriormente.

Palavras-chave: Película adquirida do esmalte; Erosão dentária; Refluxo gastroesofágico; Proteômica.

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1-INTRODUCTION

1 INTRODUCTION

Since the 1960´s, the decline in the prevalence of caries raise concern with tooth losses related to other causes, such as tooth wear (ATTIN; ZIRKEL; HELLWIG, 1998). Among the different types of tooth wear, erosion is one of the most documented injuries (MOSS, 1998; SMITH; KNIGHT, 1984). It is a chronic, localized lesion characterized by loss of hard tissue due to exposure to non-bacterial acids. Erosion is a type of tooth wear that must be differentiated from other non-carious lesions, such as abrasion, which occurs due to mechanical forces; attrition, which develops by tooth-to-tooth contact and abfraction, which occurs due to forces acting on the cervical region of the teeth (MISTRY; GRENBY, 1993; TEN CATE; IMFELD, 1996). It also differs from dental caries because there is no bacterial involvement in the loss of dental tissue (MOSS, 1998; NUNN, 1996; TEN CATE; IMFELD, 1996).

Dental erosion can be classified taking into account the type of acids involved that can be either extrinsic or intrinsic (IMFELD, 1996; LINNETT; SEOW, 2001; LUSSI, 1996; MAGALHAES et al., 2009; TEN CATE; IMFELD, 1996). Intrinsic erosion is the result of the action of endogenous acids from gastric reflux, chronic regurgitation, alcoholism, pregnancy, or disorders of the nervous system, such as anorexia and / or bulimia (IMFELD, 1996). It is due to the chronic action of gastric acid on the dental surface for a long period and on a regular basis (MEURMAN; TEN CATE, 1996; SCHEUTZEL, 1996). Extrinsic erosion is the result of the effect of exogenous acids, derived, for example, from the acids contained in the diet, as well as in drug formulations (LUSSI, 1996). The main extrinsic etiological factor of tooth erosion is derived from dietary acids (AINE; BAER; MAKI, 1993; IMFELD, 1996; ZERO, 1996). Most of the low pH food and beverages (pH below 4.5) would have the potential to cause tooth erosion, since at this pH the oral fluids are usually subsaturated in relation to hydroxyapatite and fluorapatite (MAGALHAES et al., 2009; ZERO, 1996).

All solid surfaces exposed in the oral cavity are covered by a proteinaceous layer called the acquired pellicle (HANNIG, C.; HANNIG; ATTIN, 2005; HANNIG, M.; BALZ, 1999; HANNING; JOINER, 2006; LENDENMANN; GROGAN; OPPENHEIM, 2000). It is an organic film, free of bacteria, covering soft and hard dental tissues. It is composed of glycoproteins and proteins, including several enzymes (HANNIG, C. et

al., 2005). More than 130 different proteins have been identified in the acquired pellicle formed *in vivo* on dental enamel (SIQUEIRA et al., 2007). Thus, the presence of protein covering the enamel or dentin, involved in lubrication and having buffering and remineralizing capabilities, makes the pellicle an important factor in the etiology of dental erosion (HANNIG, M.; BALZ, 1999). The acquired pellicles can protect against dental erosion by acting as a diffusion barrier or membrane, preventing the direct contact between the acids and the dental surface, thereby reducing the dissolution of hard dental tissues (AMAECHI et al., 1999; HANNIG, M.; BALZ, 2001; HANNIG, M. et al., 2004; HANNIG, M.; JOINER, 2006; HARA et al., 2006).

Recently, several studies have focused on the study of the protective impact of the acquired pellicle formed *in situ* on the enamel surface (HANNIG, M.; BALZ, 1999;2001; HANNIG, M. et al., 2004; HANNIG, M. et al., 2003), However, the acid resistance of the pellicle appears to be dependent on its formation time, since a 2-hour pellicle dissolves from the enamel surface more quickly than pellicle formed for 6, 12 or 24 hours. However, the acid resistance of the pellicle appears to be dependent on its formation time, since the 2 hour pellicle dissolves from the enamel surface more quickly than pellicle formed for 6, 12 and 24 hours (HANNIG, M. et al., 2003). It has been suggested that the pellicle should achieve optimum thickness to protect the dental tissues against acid challenges. Significant protection is achieved when the dental surface is exposed to saliva for at least 1 h, but this protection does not increase significantly if the pellicle undergoes a maturation process for 24 h. In addition, no significant differences were found in erosive changes when the pellicles were formed *in vivo* on blocks of bovine enamel for 24 h or 7 days. Smaller exposure periods (less than 30 min) led to pellicles that do not provide good protection against erosion [Hannig and Balz, 1999; Hannig et al., 2003; Wetton et al., 2006]. Moreover, after two hours the newly formed pellicle is still essentially free of bacterial colonization (SÖNJU; RÖLLA, 1973) and this provides an opportunity for collection of the bacterial plaque-free pellicle (YAO et al., 2001).

Gastroesophageal Reflux Disease (GERD) is the most common gastrointestinal disease (SANDLER et al., 2002), affecting about 1/3 of the population of industrialized countries (MEURMAN et al., 1994). It is a chronic and recurrent condition resulting from the retrograde flow of part of the gastroduodenal content into the esophagus or organs adjacent to it, causing a variable spectrum of esophageal and / or

extraesophageal symptoms and / or signs, associated or not to tissue lesions (TUTUIAN et al., 2008). About 24-48% of patients with gastroesophageal reflux have dental erosion (MEURMAN; TEN CATE, 1996; MUNOZ et al., 2003; SCHROEDER et al., 1995), caused by the low pH (1 to 3) of regurgitated gastric contents (MILOSEVIC; BRODIE; SLADE, 1997). Because of this low pH, an even greater prevalence of dental erosion among patients with DGRE would be expected, which indicates that patients with this disease that do not have dental erosion may have some protective factor. It has been reported that bulimic patients with dental erosion have a salivary buffer capacity after vomiting significantly lower than bulimic patients who do not have dental erosion. In addition, protease activities such as collagenase and pepsin in saliva are significantly higher in bulimic patients with dental erosion than in control patients and that peroxidase activity is significantly reduced by regular vomiting. It is believed that these proteolytic enzymes are relevant to the onset and progression of erosion directly, perhaps by direct hydrolysis of the demineralized structures or by modulation of the acquired pellicle (SCHLUETER et al., 2012). Another interesting finding is that the proteomic profiles of the esophageal mucosa of patients with non-erosive esophageal reflux disease (NERD) and erosive esophageal reflux disease (ERD) are different (CALABRESE et al., 2011), and some patients who develop ERD have a reduced ability to respond to insults caused by acid and pepsin. They are constituted by a weaker esophageal mucosal capacity, such as reduction in cell proliferation, cell migration, glucose metabolism, stress responses and probably esophageal keratinization (CALABRESE et al., 2009).

Therefore, it is possible that the protein composition of the acquired enamel pellicle (AEP) from patients with GERD and without tooth erosion is different from that of patients with the same disease, but that have erosion. The knowledge of this differential composition of the acquired pellicle may indicate possible proteins with protective potential against tooth erosion, which may be used in dental products to prevent these lesions in "acquired film engineering" procedures. It would be interesting to identify proteins within the AEP that are capable to increase the enamel resistance against dissolution by gastric acids. Thus, these proteins may in future be incorporated into dental products, such as dentifrices, mouthwash solutions or gels for topical application, in order to enrich the pellicle with them and thereby increase the protective potential of this pellicle against acids.

2-Article

2 ARTICLE

Proteomics of acquired pellicle in cases of reflux with dental erosion or not

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Short title: Acquired pellicle, GERD and dental erosion.

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Abstract

This study compared the protein profile of the acquired enamel pellicle (PAE) in 1) volunteers with gastroesophageal reflux disease (GERD) and dental erosion (BEWE ≥ 9 or grade 3 in the upper anterior sextant, all incisors affected; GE group); 2) volunteers with GERD without dental erosion (BEWE=0; GNE group) and 3) control volunteers (without GERD and dental erosion; BEWE = 0; C group). Twenty four subjects (8 in each group) participated. After dental prophylaxis, the AEP was allowed to form during 120 min and was then collected from the buccal surface of the upper and lower teeth, with filter paper pre-soaked in 3% citric acid. After protein extraction, the samples were submitted to reverse phase liquid chromatography coupled to mass spectrometry (nLC-ESI-MS/MS). Label-free proteomic quantification was performed using Protein Lynx Global Service (PLGS) software. In total, 458 proteins were identified. Seventy-six proteins were common to all the groups. The proteomic profile of the AEP was quite different among the distinct groups. The numbers of proteins exclusively found in the C, GE and GNE groups were 113, 110 and 81, respectively. Most of the proteins exclusively identified in the C and GNE groups bind metals, while those in the GE group are mainly membrane proteins. Many proteins were found exclusively in the reflux groups. Heat-shock proteins were not found in GE. Histatins and Histones were not found in GNE, while Serine/threonine-protein kinases were only identified in GNE. In the quantitative analysis, when the GNE group was compared with the GE group, the proteins with the highest decreases were *Lysozyme C, Antileukoproteinase, Cathepsin G,* Neutrophil defensins and Basic salivary proline-rich proteins, while those with the highest increases were subunits of Hemoglobin, *Albumin* and isoforms of Cystatin. Profound alterations in the proteomic profile of the AEP were seen in GNE compared with GE volunteers, which might play a role in the resistance to dental erosion seen in the first.

Keywords: Acquired enamel pellicle; Dental erosion; Gastroesophageal reflux; Proteomics.

1 Introduction

Erosive tooth wear, also known as dental erosion, is caused by non-bacterial acids that initially soften the dental surface. If the erosive challenge goes on, layer-by-layer dissolution of the enamel crystals occurs (LUSSI et al., 2011). These non-bacterial acids can have two origins: diet (extrinsic acids) or host (intrinsic acids). Intrinsic acids originate from the stomach, when gastric juice travels up through the esophagus and enters the mouth. This occurs in cases of vomiting or by regurgitation (involuntary movement of the gastric contents from the stomach into the mouth) (MOAZZEZ; BARTLETT, 2014), which typically occurs in gastroesophageal reflux disease (GERD) that affects around 10-20% of the population (DENT et al., 2005). The pH of the gastric acids is lower than that of dietary acids. In addition, the titratability of the former is greater than that of the latter, which leads to usually more severe destruction of the tooth structure (MOAZZEZ; BARTLETT, 2014). Thus, preventive measures against intrinsic erosion, comprising new prophylactic approaches and development of new dental products are highly desirable.

One of the most important preventive factors against dental erosion is the acquired enamel pellicle (AEP), an organic film, free of bacteria that covers all the hard and soft tissues in the oral cavity (BUZALAF, M. A. R.; HANNAS; KATO, 2012; VUKOSAVLJEVIC et al., 2014). The AEP is composed chiefly of proteins and glycoproteins arising mainly from saliva but also from the oral mucosa, bacteria and gingival crevicular fluid (SIQUEIRA; CUSTODIO; MCDONALD, 2012), besides containing some lipids in smaller extent (SLOMIANY et al., 1986). Due to its composition, the AEP is capable of protecting the underlying tooth structure, reducing the degree of acid dissolution (BUZALAF, M. A. R. et al., 2012; VUKOSAVLJEVIC et al., 2014). Recently, proteomic tools were employed to identify proteins within the AEP that are resistant to removal by citric acid (DELECRODE; SIQUEIRA; ZAIDAN; BELLINI; MOFFA; et al., 2015). However, intrinsic acids have a lower pH and are more difficult to be buffered than intrinsic acids (MOAZZEZ; BARTLETT, 2014), which means that the AEP proteins that are resistant to removal by dietary acids might not be resistant to removal by gastric acids and new players can be identified.

Around 24-48% of patients with GERD have dental erosion (MEURMAN et al., 1994; MOAZZEZ; BARTLETT; ANGGIANSAH, 2004; MUNOZ et al., 2003; SCHROEDER et al., 1995) due to the very low pH (1 to 3) of regurgitated gastric contents (MILOSEVIC et al., 1997). Because of this low pH, a greater prevalence of dental erosion among patients with GERD would be expected, which indicates that the patients with this disease who do not have dental erosion may have some protective factor. In addition, it has been reported that bulimic patients with dental erosion have a salivary buffer capacity after vomiting significantly lower than bulimic

patients who do not have dental erosion. In addition, protease activities, such as collagenase and pepsin in saliva, are significantly higher in bulimic patients with dental erosion than in control patients and peroxidase activity is significantly reduced by regular vomiting. It is believed that these proteolytic enzymes are relevant to the onset and progression of erosion directly, perhaps by direct hydrolysis of the demineralized structures or by modulation of the AEP (SCHLUETER et al., 2012). Another interesting finding is that the proteomic profiles of the esophageal mucosa of patients with non-erosive esophageal reflux disease (NERD) and erosive esophageal reflux disease (ERD) are different (CALABRESE et al., 2011), and patients who develop ERD have a reduced ability to respond to insults caused by acid and pepsin. These patients have a weaker capacity of the esophageal mucosa, such as reduction in cell proliferation, cell migration, glucose metabolism, stress responses and probably esophageal keratinization (CALABRESE et al., 2009). Therefore, it is plausible that the protein composition of the AEP from patients with GERD and without dental erosion is different from that of patients with the same disease, but with dental erosion. The knowledge of this differential composition of the AEP may indicate possible proteins with protective potential against dental erosion, which may be used in dental products to prevent these lesions, in the so-called "acquired pellicle engineering" procedures. Thus, the aim of the present study was to compare the protein composition of the AEP of GERD volunteers with dental erosion with that of GERD volunteers without dental erosion. Control patients (without GERD or dental erosion) were also evaluated for comparison. The null hypothesis was that there is no difference in the protein profile of the AEP of volunteers with GERD and dental erosion, as compared to those with GERD without dental erosion or controls (no GERD and no dental erosion).

2 Material and Methods

2.1 Ethical Aspects and Subjects

The protocol of this study was approved by the Ethics Committees of Bauru and Ribeirão Preto School of Dentistry, University of São Paulo, # CAAE 44007415.1.0000.5417 and 44007415.1.3001.5419, respectively). Prior to the beginning of the study the subjects signed an informed consent document. The sample size (n = 8 per group) was chosen based on previous studies that compared the proteomic profile of the AEP formed under different conditions or on distinct locations (DELECRODE; SIQUEIRA; ZAIDAN; BELLINI; LEITE; et al., 2015; LEE et al., 2013; VENTURA et al., 2017).

The volunteers from both genders (20-60 years of age) who participated of this *in vivo* study were non-smokers, had good general and oral health (without gingivitis, periodontitis or any other oral condition that could affect the composition of the oral fluids) and presented normal salivary flow (stimulated flow> 1 mL / minute). They were divided into 3 groups, as follows:

a) GERD-related symptoms and dental erosion (GE; n = 8): the inclusion criteria for GERD-related symptoms were heartburn and/or regurgitation for at least 1 year (frequency more than 2 times per week) and abnormal pH parameters of 24 h. Exclusion criteria were patients with malignant lesions in the esophagus or stomach, Barrett's esophagus, gastric or duodenal ulcer, previous gastric or esophageal surgery, and patients taking antisecretory or prokinetic drugs at least 15-30 days prior to the AEP collection (CALABRESE et al., 2011). These patients underwent esophageal pHmetry and endoscopy exams at Ribeirão Preto Medical School/University of São Paulo. The diagnosis of dental erosion was made using the BEWE (Basic Erosive Wear Examination) index. The inclusion criteria for dental erosion were BEWE ≥9 or grade 3 in the upper anterior sextant (with all incisors affected) (BARTLETT, D.; GANSS; LUSSI, 2008).

b) GERD-related symptoms without dental erosion (GNE; n = 8): the inclusion and exclusion criteria for GERD were the same as previously described. Patients without dental erosion were included in this group (BEWE = 0) (BARTLETT, D. et al., 2008).

c) Control group (C; n = 8): volunteers in this group did not have GERD, which was confirmed by esophageal pHmetry and previous endoscopy, since GERD can be silent with no signs and symptoms. They also did not have dental erosion (BEWE = 0) (BARTLETT, D. et al., 2008).

2.2 In vivo experiment

All the procedures were conducted during the morning in order to avoid circadian effects on the composition of the pellicle (ZIMMERMAN et al., 2013). The subjects were instructed not to eat or drink any type of food during the procedures AEP collection.

The volunteers were submitted to a dental prophylaxis with coarse pumice and a rubber cup. After 120 minutes, the dental surfaces were thoroughly rinsed with deionized water and dried with a jet of air. The AEP was collected from the buccal surface of the upper and lower teeth (second molar to second molar), from the middle and incisal/occlusal third of each tooth, using an electrode filter paper (Bio-Rad, Hercules, CA) of 5X10 mm pre-dipped in 3% citric

acid (SIQUEIRA et al., 2007). One filter paper was used for each quadrant and they were stored at -80°C until proteomic analysis.

2.3 Proteomic Analysis

For the extraction of proteins from the AEP, the papers collected from the 8 volunteers from the same group were cut into small pieces and grouped in a single microtube to constitute a *pool*. The procedures of preparation of AEP samples and shotgun proteomic analysis were performed exactly as described by Ventura et al. (VENTURA et al., 2017). The equipment used was a nanoACQUITY UPLC-Xevo QTof MS system (Waters, Manchester, UK), equipped with nanoACQUITY HSS T3, analytical reverse phase column (75 µm X 150 mm, 1.8 µm particle size, Waters). ProteinLynx Global Server (PLGS) version 3.0 (Waters Co., Manchester, UK) was used to process and search the continuum LC-MSE data. Proteins were identified with the embedded ion accounting algorithm in the software and a search of the *Homo sapiens* database (reviewed only, UniProtKB/Swiss-Prot) downloaded on February 2017 from UniProtKB (http://www.uniprot.org/). The identified proteins were classified and assigned by biological function (Rizon et al., 2000; Zimmermann et al., 2013), origin and molecular interaction (http://www.uniprot.org/).

For label-free quantitative proteome, three MS raw files from each pooled group were analysed using the PLGS version 3.0 software (Waters Co., Manchester, UK). All the proteins identified with a score with confidence greater than that 95% were included in the quantitative analysis. Identical peptides from each triplicate by sample were grouped based on mass accuracy (<10 ppm) and on time of retention tolerance <0.25 min, using the clustering software embedded in the PLGS software. Difference in expression among the groups was expressed as $p<0.05$ for down-regulated proteins and $1-p>0.95$ for up-regulated proteins. The relevant comparisons were GE X C, GNE X C and GNE X GE.

For bioinformatics analysis, Uniprot protein ID accession numbers were mapped back to their associated encoding Uniprot gene entries for each group (C, GE and GNE; Table S1). Gene Ontology annotation of Broad Molecular Function was performed using Cluego v2.3.2 + Clupedia v1.3.2 (BAUER-MEHREN, 2013; BINDEA; GALON; MLECNIK, 2013; BINDEA et al., 2009; MILLAN, 2013) plugin. Briefly, Uniprot IDs were uploaded from Table S1 analyzed with default parameters, which specify a Enrichment (Right-sided hypergeometric test) correction method using Bonferroni step down, analysis mode “Function” and load gene cluster list for Homon Sapiens (9606), Evidence Codes “All”, set networking specificity “medium” (GO levels 4 to 8), GO Term Fusion and KappaScoreThreshold 0.03.

3 Results

Table 1 shows the characterization of the volunteers according to age, gender and BEWE score. C and GE groups had both male and female volunteers, while GNE had only female volunteers. The mean age of the volunteers was quite similar among the group. Volunteers in GE group had a mean BEWE around 16.

The identified proteins when classified according to their function, molecular interaction and origin are displayed in Tables 2 and 3 and Supplementary table (S1). In total, 458 proteins were identified (Table S1). The highest and lowest numbers of proteins were identified in the C (260) and GNE group (193), while the GE group presented 235 proteins. Figure 1 shows the number of proteins common to the distinct groups, as well as the numbers of proteins found in only one of the groups. Seventy-six proteins were common to all the groups (Figure 1, Table S1). Among them are proteins typically found in the acquired enamel pellicle, such as isoforms of cystatin, isoforms of cytoskeletal keratin, isoforms of neutrophil defensin, isoforms of actin, isoforms of protein S100-A, isoforms of proline-rich protein, isoforms of albumin, isoforms of Ig A, isoforms of Ig G, *Lactotransferrin*, *Serotransferrin*, *Lysozyme C*, *Matrix Gla Protein* and *Annexin*. Isoforms of Hemoglobin, *Cathepsin G*, *Myeoblastin*, *Myeloperoxidase,* different members of the POTE ankyrin domain family, Isoforms of profilin and isoforms of Ig lambda that are not usually described in the acquired enamel pellicle were also identified in all the groups, as well as an Uncharacterized protein.

Figure 2 shows the functional classification of the proteins identified for each group, which was quite different among the groups. For the control group, the most frequent molecular functions found were cysteine-type endopeptidase inhibitor activity and serine-type endopeptidase activity. Regarding GE group, only 3 molecular functions were observed, with similar frequencies (serine-type endopeptidase activity, monocarboxylic acid binding and nucleosomal DNA binding). For GNE group, the most frequent molecular function observed was cysteine-type endopeptidase inhibitor activity, followed by monocarboxylic acid binding, nucleosomal DNA binding, phosphatidylcholine binding, phosphatidyl-4,5-biphosphate binding and serine-type endopeptidase activity that presented all similar frequencies.

The proteomic profile of the acquired pellicle was quite different among the distinct studied groups. The numbers of proteins exclusively found in the C, GE and GNE groups were 113, 110 and 81, respectively. Among the proteins exclusively found in the C group are zinc- (*ADAMTS-like protein* 1, *Utrophin*, *Cytidine deaminase* and *S phase cyclin A-associated protein in the endoplasmic reticulum*), calcium- (*CALM1 protein, Calmodulin* and *Reticulocalbin-3*), and copper-binding (*Ceruloplasmin*) proteins, *Cystatin-C*, *Histatin-3*, several isoforms of histones, DNA-, RNA- or chromatin-binding proteins (*High mobility group*

nucleosome-binding domain-containing protein 5, *Highly divergent homeobox*, *Serine/arginine repetitive matrix protein 2* and *La-related protein 1*), *Lipopolysaccharide-binding protein*, *Small proline-rich protein 3* and isoforms of Tubulin beta chain (Table 2).

Many of the proteins exclusively identified in GE group are membrane proteins (plasma, nuclear or organelles). Among them are *ATP synthase subunit beta_mitochondrial*, *Dystrotelin*, *Membrane-spanning 4-domains subfamily A member 12*, *Myeloid-associated differentiation marker, Multidrug resistance-associated protein 9, Nucleoporin p54, NRXN1 protein, Prolactin receptor, Protein tyrosine phosphatase_ receptor type_ C_ isoform CRA_d, Receptor-type tyrosine-protein phosphatase C, Reticulon, Reticulon-3*, *Semaphorin-6A*, *Sodium bicarbonate transporter-like protein 11*, *Tapasin*, *Tectonin beta-propeller repeat-containing protein 1* and *ATP-binding cassette sub-family A member 13.* Proteins associated with neutrophil degranulation were also identified uniquely in this group, such as *Armadillo repeat-containing protein 8, Serine protease 57*, *Transthyretin* and *Azurocidin.* Also C-terminus binding proteins such as *BAI1-associated protein 3, Peroxisomal targeting signal 1 receptor* and *Kinase suppressor of Ras 1*, as well as calmodulin-binding proteins (*CDK5 regulatory subunit-associated protein 2* and *MAP kinase-activated protein kinase 3*), actin-binding proteins (*Cingulin*), coiled coil domain proteins (*Coiled-coil domain-containing protein 159*, *Myomegalin (Fragment)* and *EF-hand and coiled-coil domain-containing protein 1*), calcium-binding proteins (*Integrin alpha-5* and *Membrane-associated phosphatidylinositol transfer protein 1*) and interleukin receptors (*Interleukin-11 receptor subunit alpha* and *Interleukin-6 receptor subunit beta*) were exclusively identified in this group. Isoforms of cuticular keratin, isoforms of Leucine-rich repeat proteins, isoforms of Liprin, isoforms of Zinc finger protein, isoforms of Rho GTPase-activating protein, as well as *Rho GDP-dissociation inhibitor 1*, *Mucin-4* and *Proline-rich protein 5, Protein FAM84B, Puromycin-sensitive aminopeptidase* were identified uniquely in the GE group (Table 2).

As for the proteins identified uniquely in the GNE group, similarly to what was found for the C group, many of them bind metals such as calcium (*Alpha-amylase 2B, 1-phosphatidylinositol 4_5-bisphosphate phosphodiesterase zeta-1, Calmodulin-like protein 6, Matrilin-4, Pancreatic alpha-amylase*), zinc (*A disintegrin and metalloproteinase with thrombospondin motifs 17, Matrin-3, Putative zinc finger and SCAN domain-containing protein 5D, V(D)J recombination-activating protein 2*) and magnesium (Various isoforms of Serine/threonine-protein kinase, *Geranylgeranyl pyrophosphate synthase, Probable phospholipid-transporting ATPase IIB, Pyruvate kinase, Pyruvate kinase PKM*). Some proteins bind actin (*Actin-related protein 2/3 complex subunit 5* and Unconventional myosin-Ig, *Cytoplasmic FMR1-interacting protein 1*). The number of membrane proteins was considerably smaller when compared with the GNE group (*Ankyrin repeat domain-containing protein 46,*

Dyslexia-associated protein KIAA0319-like protein, Protein GREB1, Roundabout homolog 3, Serine incorporator 1). Many serine-phosphorylated proteins were also exclusively identified in this group, such as *Activating transcription factor 7-interacting protein 1*, *Ankyrin repeat domain-containing protein 26, ATP-dependent RNA helicase DHX8, Breast cancer type 1 susceptibility protein, Centrosome-associated protein 350, Gametogenetin-binding protein 2, Myosin light chain 6B, Myosin phosphatase Rho-interacting protein, Neurofibromin, Pleckstrin homology domain-containing family G member 3, Polypyrimidine tract-binding protein 1, Proline synthase co-transcribed bacterial homolog protein, Protein Daple, Roundabout homolog 3, Serine incorporator 1, SWI/SNF complex subunit SMARCC1, Tyrosine-protein phosphatase non-receptor type 13, Zinc finger CCCH domain-containing protein 13*). Other unique proteins of this group include *Calpastatin, Legumain, Spermatogenesis-associated serine-rich protein 2,* and two uncharacterized proteins (Table 2).

Regarding the proteins identified exclusively in one or two of the groups, some findings must be highlighted: a) Heat-shock proteins were not found in GE; b) Histatins and Histones were not found in GNE; c) Leucine-rich repeat proteins, *Arginine-glutamic acid dipeptide repeats protein*, *Calpain-1 catalytic subunit (Fragment)*, *Sodium channel protein type 2 subunit alpha* and *Nuclear envelope phosphatase-regulatory subunit 1* were only found in reflux groups (GE and GNE); d) Most of the identified isoforms of 14-3-3 protein were only present in C and GE groups; e) Serine/threonine-protein kinase (various isoforms) were only identified in GNE (Table 2).

Regarding quantitative analysis, three comparisons were made among the groups (Table 3). For the comparison GE group *vs.* C group, 32 proteins were significantly increased and 14 proteins were significantly decreased in the first compared with the latter. Among the increased proteins are various isoforms of cytoskeletal keratin, isoforms of POTE ankyrin domain family, isoforms of Neutrophil defensin, isoforms of Actin, *Cathepsin G*, *Lysozyme C, Antileukoproteinase*, *Myeloperoxidase*, *Mucin-7* And *Lactotransferrin.* On the other hand, various proline-rich proteins, isoforms of Hemoglobin, isoforms of Cystatin, isoforms of Albumin and *Statherin* were reduced in the GE group compared with the C group. When the GNE group was compared with the C group, 13 and 20 proteins were increased and decreased, respectively, in the first in respect to the latter. The protein with the highest increase (more than 6-fold) was *Microtubule-associated protein*. Other proteins increased in a lesser extent include *Hemoglobin subunit beta*, isoforms of actin and immunoglobulins (G and lambda). Proteins with the greatest decreases were isoforms of Basic salivary proline-rich protein, *Zinc finger protein 532*, *Matrix Gla protein*, *Antileukoproteinase,* isoforms of proline-rich protein, isoforms of Neutrophil defensin, *Lysozyme C, Cathepsin G* and *isoforms of cystatin*. The most relevant comparison is GNE *vs.* GE. In this case, 8 proteins were increased

and 22 were decreased in the first compared with the latter. Remarkably, the proteins with the highest rates of increase (close to or higher than 3-fold) in GNE compared with GE were *Hemoglobin subunit alpha* and *Hemoglobin subunit beta*. Other increased proteins, despite in lower rates, were isoforms of albumin and isoforms of cystatin. The proteins with the highest decreases (close to or higher than 2-fold) were *Lysozyme C, Antileukoproteinase, Cathepsin G,* isoforms of Neutrophil defensins, *Matrix Gla protein,* Proline-rich protein 27, isoforms of Basic salivary-rich protein, *POTE ankyrin domain family member J* and various isoforms of cytoskeletal keratin. Proteins decreased in lower rates were *Lactotransferrin, Cystatin-B* and *Protein S100-A9*.

4 Discussion

To the best of our knowledge, this is the first study to compare the proteomic profile of the AEP of volunteers with GERD and dental erosion with that of volunteers with GERD but no erosion. A control group constituted of volunteers with no GERD and no erosion was also included, to allow the detection of changes in the proteomic profile of the AEP in function of GERD. The characteristics of the volunteers included in the study were quite similar in terms of age. Regarding gender, since this was a convenience sample, there was a higher number of female volunteers in all the groups and GNE group had only females (Table 1). However, there is no reason to suspect that this would influence in the results of the proteomic analysis of the AEP. The mean BEWE score of the volunteers was around 16, which denotes severe erosive tooth wear (BARTLETT, D. et al., 2008). The inclusion criteria for volunteers with GERD assured that all of them had symptoms for at least 1 year (frequency more than 2 times per week) (CALABRESE et al., 2011), which means that there was enough time for erosion to occur in all of them as a consequence of the intrinsic acids present in the oral cavity.

The protocol of protein extraction and proteomic analysis followed a recently developed methodology that increases the identification of proteins in the AEP samples (VENTURA et al., 2017). Accordingly, the number of identified proteins in the present study was 458, which is the highest number reported ever in studies involving in vivo analysis of AEP. The proteomic profiles of the AEPs collected in the 3 groups was quite different, as can be depicted from the high numbers of unique proteins in each of the groups (close to or higher than 100). Thus, the null hypothesis formulated was rejected. Most of the proteins identified in all the groups are proteins typically found in the AEP (Fig. 1, Table S1) and the majority of them presented differences in expression among the groups (Table 3). This means that GERD has a great impact on the proteomic profile of the AEP, which also changes remarkably in patients with GERD presenting dental erosion or not. This can also be seen when the molecular function of

the identified proteins is analyzed (Fig. 2). The GE group presented only 3 molecular functions (monocarboxylic acid binding, nucleosomal DNA binding and serine-type endopeptidase activity) and these functions were common to all the 3 groups. C and GNE groups had more diverse molecular functions when compared with GE. However, the types of molecular functions found for both of them was distinct, except for cysteine-type endopeptidase inhibitor activity that was the most frequent molecular function for both of them. It must be highlighted that the bioinformatics tools used refer mainly to intracellular functions, which in most of the cases does not apply to the AEP, since this integument contains both secreted proteins (derived from the salivary glands mainly but also from the gingival crevicular fluid) and intracellular proteins (originated from oral mucosa cells and from bacteria) (SIQUEIRA et al., 2012). It should be highlighted that bacterial proteins were not evaluated by the protocol used in the present study, since we used *Homo sapiens* database for protein identification. Information regarding the molecular functions of the identified proteins is interesting to show the diversities in the proteomes of the AEPs collected from the 3 distinct groups.

Many of the proteins identified exclusively in the groups with no erosion (C and GNE groups) are metal-binding proteins, while many of those exclusive to the GE group are membrane proteins. This suggests a higher degree of epithelial cell lysis in the GE group caused by the gastric acids, which is consistent with the higher incidence of lesions in the oral mucosa of patients with GERD (PREETHA et al., 2015; SUJATHA et al., 2016). Among the proteins exclusively found in the GE group are those related to neutrophil degranulation, which is in-line with findings of neutrophil infiltrates in eroded areas of the mucosa (LEONI et al., 2015). Some of the proteins that are stored in neutrophil cytoplasmic granules are secreted as active proteases in response to their stimulation, as it is the case for *Serine protease 57*. Another protein identified exclusively in the GE group was *Puromycin-sensitive aminopeptidase*. With broad substrate specificity for several peptides, this protein releases an N-terminal amino acid, preferably alanine from a wide range of peptides (UNIPROT). This proteolysis could change the structure of the AEP, reducing its protective ability against demineralization.

Among the proteins exclusively identified in the GNE group are those with sites of phosphorylation in serine, as well as various isoforms of serine/threonine-protein kinases (Table 2). Phosphorylation in serine confers negative charge to this amino acid. Hydroxyapatite binds proteins through both calcium (positive) and phosphate (negative) sites on the surface (KAWASAKI et al., 1986; KAWASAKI et al., 1987). Phosphorylated and negatively charged proteins, such as acidic proline-rich proteins, *Histatin 1* and *Statherin*, have strong affinity to hydroxyapatite and are included among the pellicle precursor proteins, constituting the basal layer of this integument (LENDENMANN et al., 2000). Most of the protection against

demineralization conferred by the AEP is attributed to its basal layer, since it is not removed after erosive challenge (HANNIG, C. et al., 2009). Thus, it is possible that the greater number of serine-phosphorylated proteins in the AEP of the GNE group might be responsible, at least in part, for the protection against erosion. On the other hand, histatins and histones were not found in GNE group. Histatins are primarily antimicrobial proteins (RAJ; EDGERTON; LEVINE, 1990), but more recently their role against acid injuries when they are adsorbed onto hydroxyapatite has been highlighted (SIQUEIRA et al., 2010). Despite histones have already been identified in the AEP (VENTURA et al., 2017), their function in this integument remains to be determined, as well as the reason why these two classes of proteins were not present in the GNE group. Other interesting findings related to the exclusive proteins were the fact that heat-shock proteins were not identified in the GE group and that most of the identified isoforms of 14-3-3 protein were present in C and GE groups. These findings are consistent with a study that evaluated the proteomic profile of the esophagus mucosa in patients with erosive and non-erosive GERD. The authors found higher expression of *Heat shock cognate 71 kDa protein* in patients with non-erosive GERD when compared to those with erosive GERD, as well as higher expression of 14-3-3 proteins in patients with reflux when compared to the healthy ones (CALABRESE et al., 2011). In addition, many proteins were exclusive of the GERD groups, regardless the presence of dental erosion. Thus, the occurrence of these proteins might be associated with this disease itself.

In the expression analyses performed in the present study, three comparisons were made. The first two of them involve comparison of the reflux groups with the control group. These comparisons likely reflect the proteins that have their rates of expression changed in function of the reflux, despite the occurrence of dental erosion or not also remarkably altered the pattern of protein expression. It is noteworthy that proteins that were lower in GE when compared with C were higher when GNE was compared with C, such as isoforms of hemoglobin. The opposite was also found, i.e., proteins higher in GE compared with C were lower in GNE compared with C, such as *Lysozyme C* and *Cathepsin G* and isoforms of neutrophil defensin. The GE group also had higher levels of various isoforms of cytoskeletal keratin. Higher levels of these proteins in the AEP of the GE group suggest higher degree of epithelial cell lysis (PREETHA et al., 2015; SUJATHA et al., 2016) and neutrophil degranulation (LEONI et al., 2015) in this group. It is also noteworthy that *Sthaterin*, a calcium-binding protein, was around 30% lower in the GE group when compared with control, which is consistent with a recent report of reduction of 35% in *Statherin* concentrations in patients with erosion (CARPENTER et al., 2014).

The most interesting comparison, considering the main aim of this study that is to find proteins in the GNE group that might be associated with protection against dental erosion, is

the comparison between GNE and GE groups. Among the proteins with the lowest rates of expression (more than 2-fold reduction) in GNE group when compared with GE group are *Lysozyme C, Antileukoproteinase* and *Cathepsin G*. Increases in lysozyme and cathepsins have been reported in patients with Barrett´s esophagus and esophageal adenocarcinoma induced by GERD (CHENG et al., 2005). The lower level of *Lysozyme C* in GNE compared with GE might be associated with a greater risk of caries development in the first, since it is an important antibacterial enzyme and reduced amounts of lysozyme in unstimulated saliva of children are related with early childhood caries (MOSLEMI et al., 2015). In addition, *Lysozyme C* was recently reported as an acid-resistant protein, since it was higher in the AEP after challenge with 1% citric acid (DELECRODE; SIQUEIRA; ZAIDAN; BELLINI; MOFFA; et al., 2015). In the present study this enzyme was increased in the GE group when compared with C, but it was decreased in the GNE group compared with control, which might suggest that volunteers in GE had a higher acid influx into the oral cavity when compared with GNE volunteers. The lower expression of *Cathepsin G* in GNE volunteers might be associated with lower rates of erosion progression in dentin, since the role of proteases, including matrix metalloproteinases and cysteine cathepsins in the degradation of the demineralized organic matrix and on the progression of erosion and caries into dentin has been emphasized (BUZALAF, M. A.; CHARONE; TJADERHANE, 2015; TJADERHANE et al., 2015). *Antileukoproteinase* is an acid-stable proteinase inhibitor with strong affinities for trypsin, chymotrypsin, elastase, and cathepsin G *(OHLSSON et al., 1983)*. Since it inhibits both *Trypsin* and *Cathepsin G*, it would be expected to be increased in the volunteers without erosion, since proteases activities were shown to be higher in bulimic patients with erosion (SCHLUETER et al., 2012) but in fact *Antileukoproteinase* was lower in the volunteers without erosion. In the present study, volunteers in GNE group presented slightly higher levels of different isoforms of cystatins when compared to their GE counterparts, but this was not the case for *Cystatin-B* that was lower in GNE when compared with GE. *Cystatin-B* was recently identified as an acid-resistant protein in the AEP that had its levels increased more than 20-fold when the AEP was challenged with 1% citric acid. This protein was then suggested as a potential candidate to be included in dental products to protect against extrinsic erosion. However, it seems that this might not be the case for intrinsic erosion. The gastric acids have a higher pH and titratability when compared with dietary acids, which usually leaves to more severe erosion (MOAZZEZ; BARTLETT, 2014). This means that protein candidates that seem promising to prevent extrinsic erosion might not work in the case of intrinsic erosion. Two isoforms of albumin were also increased in GNE volunteers when compared to GE volunteers. Albumin is able to bind calcium (SCHWEIGEL; WICHT; SCHWENDICKE, 2016) and has been suggested to reduce the dissolution of hydroxyapatite *in vitro* (HEMINGWAY et al., 2008; KOSORIC; HECTOR; ANDERSON, 2010).

An interesting finding of the present study was the higher level of distinct subunits of hemoglobin in the GNE group compared with the GE group. Hemoglobins are not classically included among the protein components of the AEP. The first study that reported the presence of hemoglobin in the AEP was recently published. This protein was found only in the AEP collected from the posterior region of the dental arches (VENTURA et al., 2017). This might be the reason why hemoglobin had not been described in the AEP before, because in the previous studies the AEP was collected from the anterior region only (DELECRODE; SIQUEIRA; ZAIDAN; BELLINI; MOFFA; et al., 2015; LEE et al., 2013; SIQUEIRA et al., 2007; ZIMMERMAN et al., 2013). At first glance, it could appear that the presence of hemoglobin in the AEP was due to contamination with the gingival crevicular fluid, but this is not the case, since in the collection we avoided the cervical third of the tooth surface. In addition, one of the exclusion criteria adopted in the present study was the absence of gingivitis and periodontitis. In fact, the affinity of hemoglobin by hydroxyapatite is known for a long time, since hydroxyapatite columns have been shown to have a good performance for purification of hemoglobin, among other proteins found in the present study (such as albumin, lysozyme and immunoglobulins) (KAWASAKI; TAKAHASHI; IKEDA, 1985). Due to its ability to adsorb hemoglobin, nanostructured hydroxyapatite microspheres (QI et al., 2013) or polyhedral (YU et al., 2017) have been developed to deliver this protein in a controlled manner. Interestingly, the adsorption rate of hemoglobin to hydroxyapatite increases as pH decreases, which may be explained by the electrostatic interactions between hemoglobin molecules and hydroxyapatite that occurs by van der Waals, electrostatic and hydrophobic forces. The isoelectric point (pI) of hydroxyapatite is around 6.8-70, which means that this protein becomes positively charged at pH below 6.8 (YU et al., 2017). GERD patients have an oral pH typically lower than that found in healthy people. A relationship has been reported between pH < 4 in the distal esophagus and pH < 5.5 in the mouth (BARTLETT, D. W. et al., 1996). Thus, the lower mouth pH in GERD patients might increase the chance of hemoglobin adsorption onto the dental surfaces, since this confers positive charge to hemoglobin thus increasing its electrostatic attraction by hydroxyapatite. This relationship does not seem to be so simplistic, however, because GE volunteers had lower levels of hemoglobin than controls. The reason why the hemoglobin levels in the AEP of GNE volunteers was around 3-fold higher than that of GE volunteers is not clear. Some possible explanations could be: 1) the GNE volunteers had higher concentrations of hemoglobin in saliva; 2) other proteins found only in the AEP of GNE patients might have stabilized hemoglobin adsorbed to hydroxyapatite. Both hypotheses deserve investigation in future studies. Anyway, the fact that hemoglobin was present in higher levels in GNE volunteers when compared with GE volunteers indicates that this protein might have protective role against dental erosion caused by intrinsic acids, which needs to be investigated in further researches. If confirmed, then this protein or peptides derived from it

that preserve the ability to bind to hydroxyapatite could be added to dental products to prevent dental erosion.

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Figure legends

Figure 1. Venn diagram showing the numbers of proteins identified in the control (no GERD no dental erosion), GE (GERD and dental erosion) and GNE (GERD and no dental erosion) groups.

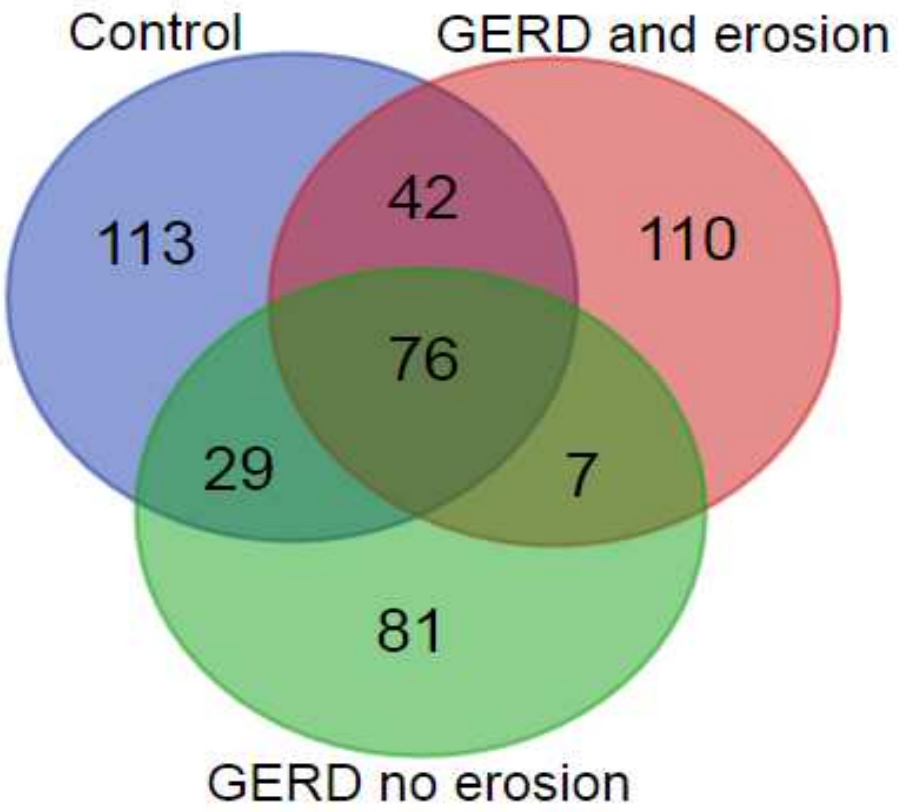

Figure 2. Functional distribution of proteins identified in the acquired enamel pellicle of volunteers with GERD and dental erosion (GE), GERD and no dental erosion (GNE) and in control volunteers (C; no GERD and no erosion). Uniprot protein ID accession numbers were mapped back to their associated encoding Uniprot gene entries for each group (Table S1). Gene Ontology annotation of Broad Molecular Function was performed using Cluego v2.3.2 + Clupedia v1.3.2 (Bauer-Mehren, 2013; Bindea et al., 2009; Bindea et al., 2013; Millan, 2013) plugin.

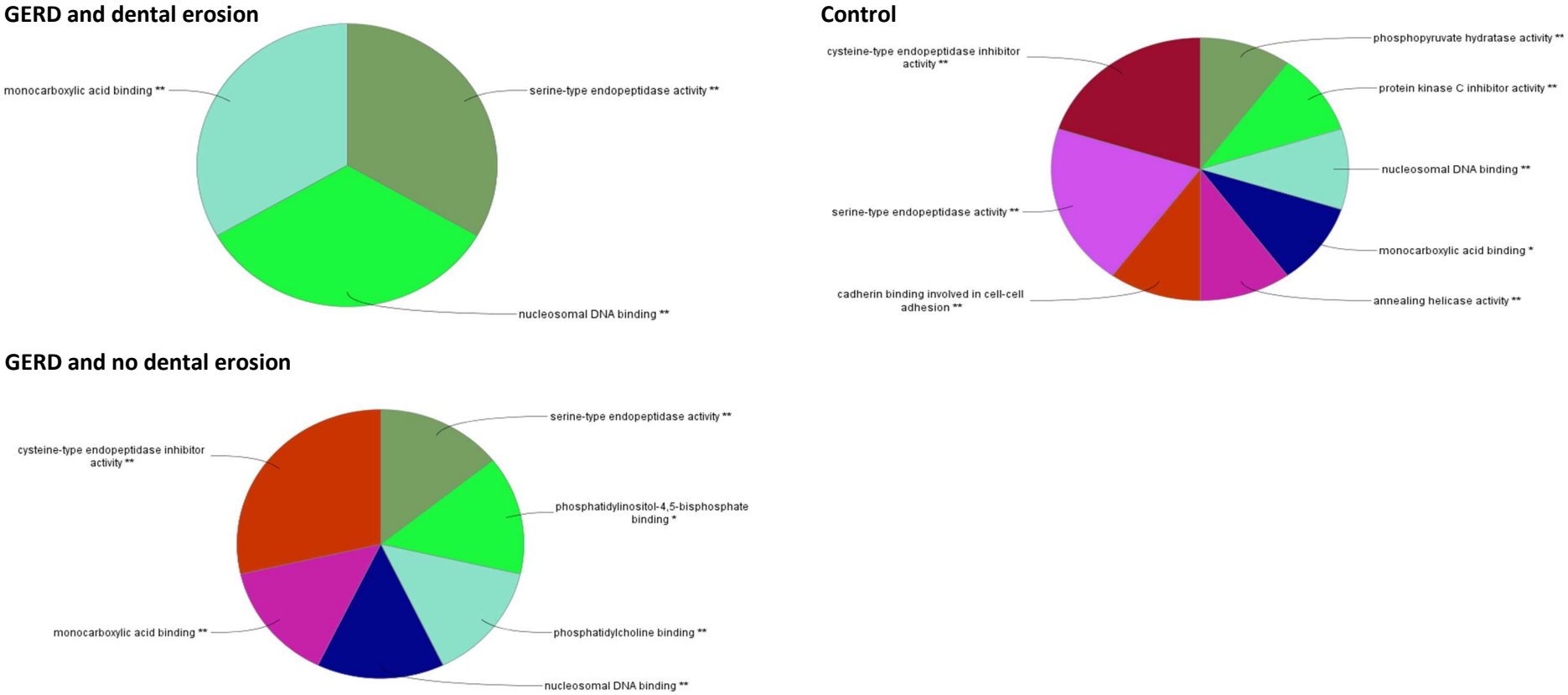

Table 1. Characterization of the volunteers according to age, gender and BEWE score.

	Gender	Meddle Ages ± DP	BEWE
C	5F 3M	31.37± 8.81	0
GE	7F 1M	32.37 ± 8.27	16.12± 2.08
GNE	8F	31.25 ± 12.72	0

Table 2. Classification of the proteins of the acquired enamel pellicle identified in only one of the groups evaluated.

Control	Accession number	Protein name and classification	Score
	R4GMU6	Adenomatous polyposis coli protein (Fragment)	168.55
	P02763	Alpha-1-acid glycoprotein 1	597.77
	H3BRI3	Alpha-mannosidase 2C1 (Fragment)	528.38
	Q9NUT2	ATP-binding cassette sub-family B member 8_ mitochondrial	290.21
	P46063	ATP-dependent DNA helicase Q1	165.87
	B5LMG6	Aurora borealis	581.8
	Q9NR09	Baculoviral IAP repeat-containing protein 6	217.01
	P13929	Beta-enolase	214.37
	Q9UQB8	Brain-specific angiogenesis inhibitor 1-associated protein 2	109.41
	Q02224	Centromere-associated protein E	231.39
	Q9P2H0	Centrosomal protein of 126 kDa	213.22
	P00450	Ceruloplasmin	529.78
	G3V1A4	Cofilin 1 (Non-muscle)_ isoform CRA_a	534.58
	P23528	Cofilin-1	534.58
	Q9Y281	Cofilin-2	534.58

Q5TFM2	Complement factor H	133.2
P01034	Cystatin-C	350.14
P17661	Desmin	222.4
A0A0B4J2C2	Discs_ large (Drosophila) homolog-associated protein 4_ isoform CRA_b	222.46
Q9Y2H0	Disks large-associated protein 4	243.46
Q9UDY4	DnaJ homolog subfamily B member 4	68.76
Q9UHY7	Enolase-phosphatase E1	133.85
P11678	Eosinophil peroxidase	272.78
O00591	Gamma-aminobutyric acid receptor subunit pi	112.24
Q8N335	Glycerol-3-phosphate dehydrogenase 1-like protein	117.65
A0A1B0GUA7	HCG40442_ isoform CRA_a	275.08
P82970	High mobility group nucleosome-binding domain-containing protein 5	76.27
Q7Z353	Highly divergent homeobox	272.34
P15516	Histatin-3	1281.93
A0M8Q6	Ig lambda-7 chain C region	573.11
P01602	Immunoglobulin kappa variable 1-5	91.29
P18428	Lipopolysaccharide-binding protein	102.98

Q659A1	Little elongation complex subunit 2	228.3
H7BZB9	Microtubule-associated protein 2 (Fragment)	114.17
O95297	Myelin protein zero-like protein 1	113.3
O95502	Neuronal pentraxin receptor	166.86
P08246	Neutrophil elastase	302.84
E9PLD1	Non-specific lipid-transfer protein	327.31
Q9H7P9	Pleckstrin homology domain-containing family G member 2	110.23
P40426	Pre-B-cell leukemia transcription factor 3	102.39
Q9NRD5	PRKCA-binding protein	113.81
P42694	Probable helicase with zinc finger domain	436.09
Q6PGQ7	Protein aurora borealis	581.8
Q13948	Protein CASP	96.01
A0A1B0GVX2	Protein LOC101929926	275.08
Q9NYP9	Protein Mis18-alpha	118.93
Q9Y2G9	Protein strawberry notch homolog 2	232.17
A0A075B6Z2	Protein TRAJ56 (Fragment)	3095.64
Q9UN71	Protocadherin gamma-B4	208.8

Q15276	Rab GTPase-binding effector protein 1	318.47
Q9P2R3	Rabankyrin-5	201.06
Q9BY12	S phase cyclin A-associated protein in the endoplasmic reticulum	340.73
H0YFR4	Scavenger receptor cysteine-rich type 1 protein M160 (Fragment)	243.57
Q14674	Separin	168.83
O75368	SH3 domain-binding glutamic acid-rich-like protein	416.53
Q92783	Signal transducing adapter molecule 1	568.43
Q9UBC9	Small proline-rich protein 3	408.15
Q9P1V8	Sterile alpha motif domain-containing protein 15	168.69
Q8NBJ7	Sulfatase-modifying factor 2	278.06
Q9NPQ8	Synembryn-A	102.43
I3NI44	TOM1-like protein 1	90.32
Q6ZXV5	Transmembrane and TPR repeat-containing protein 3	100.19
Q6ZMR5	Transmembrane protease serine 11A	106.65
Q6ZMB5	Transmembrane protein 184A	205.45
O15417	Trinucleotide repeat-containing gene 18 protein	135.99
Q6ZTA4	Tripartite motif-containing protein 67	153.58

	A0A075B736	Tubulin beta chain	209.19
	Q3ZCM7	Tubulin beta-8 chain	209.19
	P02774	Vitamin D-binding protein	373.64
	Q8WY21	VPS10 domain-containing receptor SorCS1	111.58
	Q96DA0	Zymogen granule protein 16 homolog B	739.5
GERD and erosion	**Accession number**	**Protein name and classification**	**Score**
	Q4L235	Acyl-CoA synthetase family member 4	219.63
	Q9UJX3	Anaphase-promoting complex subunit 7	82.32
	Q6ZW76	Ankyrin repeat and SAM domain-containing protein 3	222.85
	Q6P6B7	Ankyrin repeat domain-containing protein 16	89.25
	B7Z637	Armadillo repeat containing 8_ isoform CRA_g	242.03
	Q8IUR7	Armadillo repeat-containing protein 8	259.63
	P06576	ATP synthase subunit beta_ mitochondrial	77.33
	F5H7B7	ATP-binding cassette sub-family A member 13 (Fragment)	178.36
	O94812	BAI1-associated protein 3	193.63
	Q9BX70	BTB/POZ domain-containing protein 2	98.16

P51817	cAMP-dependent protein kinase catalytic subunit PRKX	159.81
Q8WXE0	Caskin-2	136.67
Q6ZRH7	Cation channel sperm-associated protein subunit gamma	194.98
Q96SN8	CDK5 regulatory subunit-associated protein 2	42.01
Q99638	Cell cycle checkpoint control protein RAD9A	133.04
Q9P2M7	Cingulin	175.79
P0C7I6	Coiled-coil domain-containing protein 159	100.12
Q9NZP8	Complement C1r subcomponent-like protein	200.8
Q96N67	Dedicator of cytokinesis protein 7	114.34
A2CJ06	Dystrotelin	97.11
Q9HA90	EF-hand and coiled-coil domain-containing protein 1	113.2
Q0PNE2	Elongator complex protein 6	395.38
Q9UPY3	Endoribonuclease Dicer	112.37
Q9BRP7	Ferredoxin-fold anticodon-binding domain-containing protein 1	225.79
H0Y5F3	Filamin-A (Fragment)	167.49
Q9H583	HEAT repeat-containing protein 1	218.76
Q5FC05	IL6ST nirs variant 3	257.3

Q9NSI5	Immunoglobulin superfamily member 5	134.72
Q8N201	Integrator complex subunit 1	141.41
P08648	Integrin alpha-5	411.96
G3V2J5	Interleukin-11 receptor subunit alpha	350.31
P40189	Interleukin-6 receptor subunit beta	265.96
Q15323	Keratin_ type I cuticular Ha1	208.14
Q14532	Keratin_ type I cuticular Ha2	208.14
Q92764	Keratin_ type I cuticular Ha5	208.14
O76013	Keratin_ type I cuticular Ha6	210.73
O76014	Keratin_ type I cuticular Ha7	208.14
O76015	Keratin_ type I cuticular Ha8	219.47
P05783	Keratin_ type I cytoskeletal 18	222.06
Q2M2I5	Keratin_ type I cytoskeletal 24	208.14
Q7Z3Y7	Keratin_ type I cytoskeletal 28	214.44
P35908	Keratin_ type II cytoskeletal 2 epidermal	123.81
Q8IVT5	Kinase suppressor of Ras 1	131.61
C9JDW2	Latent-transforming growth factor beta-binding protein 1 (Fragment)	132.56

Q96NI6	Leucine-rich repeat and fibronectin type-III domain-containing protein 5	127.84
Q05C16	Leucine-rich repeat-containing protein 63	109.8
H0YDW2	Liprin-alpha-1 (Fragment)	87.14
H0YHK3	Liprin-alpha-2	80.64
O75335	Liprin-alpha-4	59.75
Q16644	MAP kinase-activated protein kinase 3	122.01
Q9UHV7	Mediator of RNA polymerase II transcription subunit 13	23.9
O00562	Membrane-associated phosphatidylinositol transfer protein 1	152.77
Q9NXJ0	Membrane-spanning 4-domains subfamily A member 12	122.99
E7EW47	Mucin-4	309.91
Q96J65	Multidrug resistance-associated protein 9	141.86
Q96S97	Myeloid-associated differentiation marker	292.03
H0YCY0	Myomegalin (Fragment)	93.5
Q9HCE5	N6-adenosine-methyltransferase subunit METTL14	238.18
Q9GZT8	NIF3-like protein 1	180
Q08AH0	NRXN1 protein	166.54
Q7Z3B4	Nucleoporin p54	480.72

J3KPV0	Peroxisomal targeting signal 1 receptor (Fragment)	98.87
C9JLI1	Phosphatidylinositol 4-kinase alpha (Fragment)	254.2
P09619	Platelet-derived growth factor receptor beta	129.99
B7Z2X5	Post-GPI attachment to proteins factor 2	210.7
P16471	Prolactin receptor	164.27
P85299	Proline-rich protein 5	129.07
Q6SJ93	Protein FAM111B	128.13
Q96KN1	Protein FAM84B	132.37
Q9UN36	Protein NDRG2	286.94
B1AHC4	Protein PRR5-ARHGAP8	107.49
A0A0A0MT22	Protein tyrosine phosphatase_ receptor type_ C_ isoform CRA_d	113.07
P55786	Puromycin-sensitive aminopeptidase	203.76
O43930	Putative serine/threonine-protein kinase PRKY	139.53
P0C7V0	Putative uncharacterized protein encoded by LINC00271	120.07
P08575	Receptor-type tyrosine-protein phosphatase C	113.07
B7Z4M1	Reticulon	442.55
O95197	Reticulon-3	465.84

Q9Y2V3	Retinal homeobox protein Rx	119.01
P52565	Rho GDP-dissociation inhibitor 1	152.51
A1A4S6	Rho GTPase-activating protein 10	96.83
Q68EM7	Rho GTPase-activating protein 17	150.8
Q13017	Rho GTPase-activating protein 5	237.01
Q13950	Runt-related transcription factor 2	181.86
A0A0A0MQU6	Sema domain_ transmembrane domain (TM)_ and cytoplasmic domain_ (Semaphorin) 6A_ isoform CRA_d	163.47
Q9H2E6	Semaphorin-6A	175.74
Q6UWY2	Serine protease 57	218.9
Q8NBS3	Sodium bicarbonate transporter-like protein 11	206.08
Q15020	Squamous cell carcinoma antigen recognized by T-cells 3	187.41
O15533	Tapasin	133.05
A0A0D9SG95	T-complex protein 1 subunit eta	280.17
Q7Z6L1	Tectonin beta-propeller repeat-containing protein 1	154.47
Q86US8	Telomerase-binding protein EST1A	157.31
Q96RN1	Testis anion transporter 1	177.17
H0YF09	TRAF3-interacting JNK-activating modulator (Fragment)	251.6

	A9JX13	Transcription factor 20	91.19
	Q16514	Transcription initiation factor TFIID subunit 12	165.69
	P02766	Transthyretin	278.73
	Q7Z4G4	tRNA (guanine(10)-N2)-methyltransferase homolog	347.63
	Q8N841	Tubulin polyglutamylase TTLL6	126.92
	Q70CQ2	Ubiquitin carboxyl-terminal hydrolase 34	147.22
	Q8TAF7	Zinc finger protein 461	432.16
	E9PPS7	Zinc finger protein 707 (Fragment)	181
GERD no erosion	**Accession number**	**Protein name and classification**	**Score**
	Q86YW0	1-phosphatidylinositol 4_5-bisphosphate phosphodiesterase zeta-1	172.87
	X6RAY8	39S ribosomal protein L4_ mitochondrial	145.58
	Q8TE56	A disintegrin and metalloproteinase with thrombospondin motifs 17	353.38
	A0A087WWN5	Acetyl-CoA carboxylase 1 (Fragment)	358.38
	O15511	Actin-related protein 2/3 complex subunit 5	317.61
	Q6VMQ6	Activating transcription factor 7-interacting protein 1	194.44
	P19961	Alpha-amylase 2B	246.14
	Q9UPS8	Ankyrin repeat domain-containing protein 26	180.08

Q86W74	Ankyrin repeat domain-containing protein 46	151.2
P27540	Aryl hydrocarbon receptor nuclear translocator sapiens	112.55
Q14562	ATP-dependent RNA helicase DHX8	191.5
Q6ZP65	BICD family-like cargo adapter 1	75.69
Q8TD86	Calmodulin-like protein 6	166.1
E7EQA0	Calpastatin	186.6
F5GX99	Caseinolytic peptidase B protein	116.66
Q7L576	Cytoplasmic FMR1-interacting protein 1	141.77
Q9H1R2	Dual specificity protein phosphatase 15	241.52
Q9NYC9	Dynein heavy chain 9_ axonemal	142.28
Q8IZA0	Dyslexia-associated protein KIAA0319-like protein	140.11
F5H2B8	Epidermal growth factor receptor kinase substrate 8 (Fragment)	274.5
G3V1T0	Family with sequence similarity 55_ member A_ isoform CRA_a	150.7
Q8NFU4	Follicular dendritic cell secreted peptide	335.05
Q9H3C7	Gametogenetin-binding protein 2	147.19
O95749	Geranylgeranyl pyrophosphate synthase	325.44
A0A087WTW2	Golgin subfamily A member 4	227.74

E7ETV8	Importin-5 (Fragment)	95.29
A0A1B0GUE0	Janus kinase and microtubule-interacting protein 1	134.92
Q99538	Legumain	305.5
Q9P127	Leucine zipper protein 4	193.18
J3QLE7	Leucine-rich repeat-containing protein 37B (Fragment)	188.15
B1AKT2	Leucine-rich repeat-containing protein 7	192.24
O95460	Matrilin-4	74.31
P43243	Matrin-3	720.56
Q2M296	Methenyltetrahydrofolate synthase domain-containing protein	245.59
Q2PPL5	Mitochondrial 5-methylaminomethyl-2-thiouridylate-methyltransferase transcript variant 1	196.9
O75648	Mitochondrial tRNA-specific 2-thiouridylase 1	196.9
P14649	Myosin light chain 6B	280.34
Q6WCQ1	Myosin phosphatase Rho-interacting protein	166.39
Q8WTW4	Nitrogen permease regulator 2-like protein	161.13
Q8N323	NXPE family member 1	163.41
P50897	Palmitoyl-protein thioesterase 1	205.11
P04746	Pancreatic alpha-amylase	242.29

Q8N7S5	Phosphoinositide phospholipase C	172.87
A1L390	Pleckstrin homology domain-containing family G member 3	199.29
P26599	Polypyrimidine tract-binding protein 1	201.7
Q96KW2	POM121-like protein 2	273.49
O43861	Probable phospholipid-transporting ATPase IIB	154.56
O94903	Proline synthase co-transcribed bacterial homolog protein	79.56
Q9P219	Protein Daple	129.4
Q5JS89	Protein furry homolog	82.86
Q4ZG55	Protein GREB1	242.16
Q5T0J5	Protein TEX35 (Fragment)	214.82
Q5VTE0	Putative elongation factor 1-alpha-like 3	86.18
P0CG00	Putative zinc finger and SCAN domain-containing protein 5D	99.29
B4DNK4	Pyruvate kinase	960.56
P14618	Pyruvate kinase PKM	1276.73
Q96MS0	Roundabout homolog 3	82.66
Q9NRX5	Serine incorporator 1	121.44
Q13153	Serine/threonine-protein kinase PAK 1	163.39

I3L0W2	Serine/threonine-protein kinase SMG1	120.78
H3BPN6	Serine/threonine-protein kinase ULK3	197.89
Q6NVH2	SH3 domain and tetratricopeptide repeat-containing protein 1	146.91
H0YK24	Spatacsin (Fragment)	154.72
Q86XZ4	Spermatogenesis-associated serine-rich protein 2	289.71
B9ZVR7	Striated muscle preferentially-expressed protein kinase	309.75
Q92922	SWI/SNF complex subunit SMARCC1	332.95
S4R3C6	Transforming growth factor-beta-induced protein ig-h3 (Fragment)	124.95
Q12923	Tyrosine-protein phosphatase non-receptor type 13	108.17
H3BT81	Uncharacterized protein (Fragment)	293.48
Q9Y2V0	Uncharacterized protein C15orf41	213.71
B0I1T2	Unconventional myosin-Ig	368.34
P55895	V(D)J recombination-activating protein 2	268.67
H0YG53	Vacuolar protein sorting-associated protein 13A	203.62
Q9NYS7	WD repeat and SOCS box-containing protein 2	126.12

Proteins were classified according to: **General Function:** [a)] metabolism; [b)] biological process; [c)] transport; [d)] structure and structural organization; [e)] information pathways; [f)] miscellanea; **Function in AEP:** [g)] metabolism; [h)] tissue regeneration; [i)] antimicrobial; [j)] immune response; [k)] lubrication; [l)] biomineralization; [m)] unknown biological function; **Origin:** [n)] cytoplasm origin; [o)] extracellular origin; [p)] nucleus origin; [q)] cytoskeleton origin; [r)] intracellular origin; [s)] membrane origin; [t)] unknown protein origin; **Interaction:** [u)] protein/protein interaction; [v)] calcium/phosphate binding; [w)] other molecular interaction; [x)] unknown molecular interaction.

Table 3. Classification and relative quantification of proteins identified in the acquired enamel pellicle collected from volunteers with gastro-esophageal reflux and dental erosion (GE), GERD and no erosion (GNE) or control (no GERD, no erosion; C)

Accession number	Protein name	Ratio GE/C	*P*
Q01546	Keratin_ type II cytoskeletal 2 oral (d, m, p, o, u, w)	**3.13**	0.98
P08311	Cathepsin G (a, b, g, i, j, o, p, u)	**2.72**	1.00
P61626	Lysozyme C (a, b, g, i, j, o, u, w)	**2.66**	1.00
P0CG39	POTE ankyrin domain family member J (b, m, o, u)	**2.25**	1.00
P04259	Keratin, type II cytoskeletal 6B (b, i, o, u, w)	1.97	1.00
P02538	Keratin_ type II cytoskeletal 6A (b, d, m, o, u, w)	1.93	1.00
P48668	Keratin_ type II cytoskeletal 6C (d, m, o, u)	1.88	1.00
P02533	Keratin, type I cytoskeletal 14 (d, m, o, u, w)	1.84	1.00
P08779	Keratin, type I cytoskeletal 16 (b, m, o, u, w)	1.84	1.00
P13647	Keratin, type II cytoskeletal 5 (d, m, n, o, q, u)	1.84	0.99
P03973	Antileukoproteinase (a, b, g, i, j, o, u)	1.80	1.00
P13646	Keratin, type I cytoskeletal 13 (d, m, o, p, q, u)	1.72	1.00
P05164	Myeloperoxidase (a, b, g, j, r, u)	1.67	1.00
P0CG38	POTE ankyrin domain family member I(b, m, o, u)	1.67	0.99
P19012	Keratin, type I cytoskeletal 15 (b, m, o, u, w)	1.65	1.00

A5A3E0	POTE ankyrin domain family member F (f, m, n, u)	1.52	0.99
Q6S8J3	POTE ankyrin domain family member E (b, m, o, u)	1.52	0.98
P59665	Neutrophil defensin 1 (b, i, j, o, u)	1.51	1.00
P68133	Actin, alpha skeletal muscle (b, d, m, n, q, u, w)	1.49	1.00
Q8TAX7	Mucin-7 (b, i, k, o, u)	1.48	1.00
P62736	Actin, aortic smooth muscle (b, d, m, n, q, u)	1.46	1.00
P59666	Neutrophil defensin 3 (b, i, j, o, u)	1.46	1.00
P68032	Actin, alpha cardiac muscle 1 (d, m, n, q, u, w)	1.43	1.00
P63267	Actin, gamma-enteric smooth muscle (d, m, n, q, u)	1.40	1.00
P02788	Lactotransferrin (f, g, h, i, j, n, o, p, u, w)	1.40	1.00
Q5T3N1	Annexin (Fragment) (b, l, n, p, s, u)	1.31	0.99
P01859	Ig gamma-2 chain C region (b, j, o, u)	1.28	1.00
P60709	Actin_ cytoplasmic 1 (b, m, n, q, u, w)	1.22	1.00
P63261	Actin, cytoplasmic 2 (a, d, g, j, n, q, u, w)	1.22	1.00
P06702	Protein S100-A9 (a, b, g, i, j, n, o, q, s, u, w)	1.20	1.00
P01876	Ig alpha-1 chain C region (b, e, i, j, o, u)	1.14	0.98
P01877	Ig alpha-2 chain C region (b, e, i, j, o, u)	1.14	0.97
P02808	Statherin (b, e, i, l, o, u)	0.71	0.00

A0A0A0MT31	Proline-rich protein 4 (b, l, p, u)	0.70	0.00
P02810	Salivary acidic proline-rich phosphoprotein ½ (b, d, h, l, o, u, v)	0.70	0.00
A0A087WZY1	Uncharacterized protein (m,t,x)	0.70	0.00
P02812	Basic salivary proline-rich protein 2 (b, l, o, u)	0.70	0.00
C9JKR2	Albumin, isoform CRA_k (c, g, o, i, u)	0.68	0.00
P09228	Cystatin-SA (a, b, g, o, u)	0.66	0.00
P02768	Serum albumin (a, b, c, g, o, u, w)	0.66	0.00
P01037	Cystatin-SN (a, b, g, o, u)	0.65	0.00
P01036	Cystatin-S (a, b, g, o, u)	0.64	0.00
P68871	Hemoglobin subunit beta (b, c, m, n, o, u, w)	0.43	0.00
P69905	Hemoglobin subunit alpha (b, c, m, n, o, s, u)	0.41	0.00
G3V1N2	HCG1745306_ isoform CRA_a (b, c, m, r, u)	0.34	0.00
P04280	Basic salivary proline-rich protein 1 (b, l, o, u)	0.27	0.00

Accession number	**Protein name**	**Ratio GNE/GE**	***P***
P68871	Hemoglobin subunit beta (b, c, m, n, o, u, w)	**3.49**	1.00
P69905	Hemoglobin subunit alpha (b, c, m, n, o, s, u)	**2.94**	1.00
P02768	Serum albumin (a, b, c, g, o, u, w)	1.38	1.00
C9JKR2	Albumin, isoform CRA_k (c,g,o,i,u)	1.30	1.00

P01036	Cystatin-S (a, b, g, o, u)	1.26	1.00
P09228	Cystatin-SA (a, b, g, o, u)	1.26	0.98
P01037	Cystatin-SN (a, b, g, o, u)	1.21	1.00
P01857	Ig gamma-1 chain C region (b,j,o,u,w)	1.19	0.96
P06702	Protein S100-A9 (a, b, g, i, j, n, o, q, s, u, w)	0.89	0.00
P04080	Cystatin-B (a, g, n, p, u)	0.76	0.02
P13647	Keratin, type II cytoskeletal 5 (d, m, n, o, q, u)	0.64	0.03
P02788	Lactotransferrin (f, g, h, i, j, n, o, p, u, w)	0.62	0.00
P19012	Keratin, type I cytoskeletal 15 (b, m, o, u, w)	0.61	0.01
P02533	Keratin, type I cytoskeletal 14 (d, m, o, u, w)	0.55	0.00
P48668	Keratin, type II cytoskeletal 6C (d, m, o, u)	0.54	0.00
P13646	Keratin, type I cytoskeletal 13 (d, m, o, p, q, u)	0.53	0.00
P08779	Keratin, type I cytoskeletal 16 (b, m, o, u, w)	0.53	0.00
P19013	Keratin_ type II cytoskeletal 4 (d, m, q, u)	0.51	0.00
P02538	Keratin, type II cytoskeletal 6A (b, d, m, o, u, w)	0.51	0.00
P04280	Basic salivary proline-rich protein 1 (b, l, o, u)	0.47	0.00
P0CG39	POTE ankyrin domain family member J (b, m, o, u)	0.44	0.01
P04259	Keratin, type II cytoskeletal 6B (b, i, o, u, w)	0.43	0.00

P02812	Basic salivary proline-rich protein 2 (b, l, o, u)	0.38	0.00
Q6MZM9	Proline-rich protein 27 (b, l, o, x)	0.36	0.00
P08493	Matrix Gla protein (b, m, o, u)	0.25	0.00
P59666	Neutrophil defensin 3 (b, i, j, o, u)	0.24	0.00
P59665	Neutrophil defensin 1 (b, i, j, o, u)	0.23	0.00
P08311	Cathepsin G (a, b, g, i, j, o, p, u)	0.19	0.00
P03973	Antileukoproteinase (a, b, g, i, j, o, u)	0.18	0.00
P61626	Lysozyme C (a, b, g, i, j, o, u, w)	0.15	0.00

Accession number	**Protein name**	**Ratio GNE/C**	***P***
E7EVA0	Microtubule-associated protein (m,n,q,u,)	**6.05**	1.00
P68871	Hemoglobin subunit beta (b, c, m, n, o, u, w)	1.49	1.00
P01859	Ig gamma-2 chain C region (b, j, o, u)	1.46	1.00
P68133	Actin_ alpha skeletal muscle (d, m, n, q, u, w)	1.45	1.00
P68032	Actin_ alpha cardiac muscle 1 (b, m, n, q, u w)	1.43	1.00
P62736	Actin, aortic smooth muscle (b, d, m, n, q, u)	1.40	1.00
P63267	Actin, gamma-enteric smooth muscle (b, m, n, q, u, w)	1.39	1.00
A5A3E0	POTE ankyrin domain family member F (f, m, n, u)	1.35	0.98
P0CG04	Ig lambda-1 chain C regions (b, m, o, s, u, w)	1.34	0.97

P0CG05	Ig lambda-2 chain C regions (b, m, o, s, u, w)	1.31	0.97
P01857	Ig gamma-1 chain C region (b, m, o, u, w)	1.28	0.99
P63261	Actin, cytoplasmic 2 (a, d, g, j, n, q, u, w)	1.21	0.97
P60709	Actin_ cytoplasmic 1 (b, m, n, q, u, w)	1.19	0.99
P02768	Serum albumin (a, b, c, g, o, u, w)	0.90	0.02
C9JKR2	Albumin, isoform CRA_k (c,g,o,i,u)	0.90	0.04
P02788	Lactotransferrin (b,c,i,j,n,o,p,u,w)	0.87	0.03
P09228	Cystatin-SA (a, b, g, o, u)	0.83	0.01
P01036	Cystatin-S (a, b, g, o, u)	0.81	0.00
P04080	Cystatin-B (a, g, n, p, u)	0.80	0.04
P01037	Cystatin-SN (a, b, g, o, u)	0.79	0.00
P02810	Salivary acidic proline-rich phosphoprotein ½ (b, d, h, l, o, u, v)	0.77	0.00
A0A0A0MT31	Proline-rich protein 4 (b, l, p, u)	0.76	0.00
A0A087WZY1	Uncharacterized protein (m,t,x)	0.75	0.00
P08311	Cathepsin G (a, b, g, i, j, o, p, u)	0.51	0.00
P61626	Lysozyme C (a, b, g, i, j, o, u, w)	0.41	0.00
P59665	Neutrophil defensin 1 (b, i, j, o, u)	0.35	0.00
P59666	Neutrophil defensin 3 (b, i, j, o, u)	0.35	0.00

Q6MZM9	Proline-rich protein 27 [b, l, o, x]	0.32	0.00
P03973	Antileukoproteinase [a, b, g, i, j, o, u]	0.31	0.00
P08493	Matrix Gla protein [b, m, o, u]	0.30	0.00
P02812	Basic salivary proline-rich protein 2 [b, l, o, u]	0.27	0.00
Q9HCE3	Zinc finger protein 532 [b, m, p, u]	0.14	0.00
P04280	Basic salivary proline-rich protein 1[b, l, o, u]	0.13	0.00

Proteins were classified according to: **General Function:** [a] metabolism; [b] biological process; [c] transport; [d] structure and structural organization; [e] information pathways; [f] miscellanea; **Function in AEP:** [g] metabolism; [h] tissue regeneration; [i] antimicrobial; [j] immune response; [k] lubrication; [l] biomineralization; [m] unknown biological function; **Origin:** [n] cytoplasm origin; [o] extracellular origin; [p] nucleus origin; [q] cytoskeleton origin; [r] intracellular origin; [s] membrane origin; [t] unknown protein origin; **Interaction:** [u] protein/protein interaction; [v] calcium/phosphate binding; [w] other molecular interaction; [x] unknown molecular interaction.

Table S1. Classification of the identified proteins from the acquired enamel pellicle collected from the volunteers with gastro-esophageal reflux (GERD) and dental erosion (GE), GERD without dental erosion (GNE) and control (no GERD, no erosion; C)

Accession number	Protein name	C	GE	GNE
P63261	Actin_ cytoplasmic 2	Yes	Yes	Yes
P19961	Alpha-amylase 2B	-	-	Yes
P04083	Annexin A1	Yes	Yes	Yes
P0CG05	Ig lambda-2 chain C regions	Yes	Yes	Yes
A0A1B0GUE0	Janus kinase and microtubule-interacting protein 1	-	-	Yes
P31946	14-3-3 protein beta/alpha	Yes	Yes	-
P62258	14-3-3 protein epsilon	Yes	Yes	-
Q04917	14-3-3 protein eta	Yes	Yes	-
P61981	14-3-3 protein gamma	Yes	Yes	-
P31947	14-3-3 protein sigma	Yes	Yes	Yes
P27348	14-3-3 protein theta	Yes	Yes	-
P63104	14-3-3 protein zeta/delta	Yes	Yes	-
Q9C0C2	182 kDa tankyrase-1-binding protein	-	-	Yes
Q15147	1-phosphatidylinositol 4_5-bisphosphate phosphodiesterase beta-4	Yes	Yes	-
Q86YW0	1-phosphatidylinositol 4_5-bisphosphate phosphodiesterase zeta-1	-	-	Yes

X6RAY83	9S ribosomal protein L4_ mitochondrial	-	-	Yes
Q8TE56	A disintegrin and metalloproteinase with thrombospondin motifs 17	-	-	Yes
A0A087WWN5	Acetyl-CoA carboxylase 1 (Fragment)	-	-	Yes
P68133	Actin_ alpha skeletal muscle	Yes	Yes	Yes
P62736	Actin_ aortic smooth muscle	Yes	Yes	Yes
P60709	Actin_ cytoplasmic 1	Yes	Yes	Yes
P63267	Actin_ gamma-enteric smooth muscle	Yes	Yes	Yes
O15511	Actin-related protein 2/3 complex subunit 5	-	-	Yes
Q6VMQ6	Activating transcription factor 7-interacting protein 1	-	-	Yes
Q4L235	Acyl-CoA synthetase family member 4	-	Yes	-
Q8N6G6	ADAMTS-like protein 1	Yes	-	-
R4GMU6	Adenomatous polyposis coli protein (Fragment)	Yes	-	-
Q01518	Adenylyl cyclase-associated protein 1	Yes	Yes	Yes
Q12802	A-kinase anchor protein 13	-	Yes	Yes
C9JKR2	Albumin_ isoform CRA_k	Yes	Yes	Yes
P02763	Alpha-1-acid glycoprotein 1	Yes	-	-
P01009	Alpha-1-antitrypsin	Yes	-	Yes

P04745	Alpha-amylase 1	Yes	-	Yes
P06733	Alpha-enolase	Yes	Yes	Yes
Q16352	Alpha-internexin	Yes	-	Yes
H3BRI3	Alpha-mannosidase 2C1 (Fragment)	Yes	-	-
Q9UJX3	Anaphase-promoting complex subunit 7	-	Yes	-
Q6ZW76	Ankyrin repeat and SAM domain-containing protein 3	-	Yes	-
Q6UB98	Ankyrin repeat domain-containing protein 12	Yes	-	-
Q6P6B7	Ankyrin repeat domain-containing protein 16	-	Yes	-
Q9UPS8	Ankyrin repeat domain-containing protein 26	-	-	Yes
Q86W74	Ankyrin repeat domain-containing protein 46	-	-	Yes
Q12955	Ankyrin-3	-	Yes	-
Q5T3N1	Annexin (Fragment)	Yes	Yes	Yes
P03973	Antileukoproteinase	Yes	Yes	Yes
P02647	Apolipoprotein A-I	Yes	Yes	Yes
P02652	Apolipoprotein A-II	Yes	-	Yes
B1AKN3	Arginine-glutamic acid dipeptide (RE) repeats_ isoform CRA_b	Yes	-	-
Q9P2R6	Arginine-glutamic acid dipeptide repeats protein	-	Yes	Yes

B7Z637	Armadillo repeat containing 8_ isoform CRA_g	-	Yes	-
Q8IUR7	Armadillo repeat-containing protein 8	-	Yes	-
Q7L311	Armadillo repeat-containing X-linked protein 2	-	-	-
P27540	Aryl hydrocarbon receptor nuclear translocator sapiens	-	-	Yes
P06576	ATP synthase subunit beta_ mitochondrial	-	Yes	-
F5H7B7	ATP-binding cassette sub-family A member 13 (Fragment)	-	Yes	-
Q8IZY2	ATP-binding cassette sub-family A member 7	Yes	-	-
Q9NUT2	ATP-binding cassette sub-family B member 8_ mitochondrial	Yes	-	-
P46063	ATP-dependent DNA helicase Q1	Yes	-	-
Q14562	ATP-dependent RNA helicase DHX8	-	-	Yes
B5LMG6	Aurora borealis	Yes	-	-
P20160	Azurocidin	-	Yes	-
B9A064	B9A064 Immunoglobulin lambda-like polypeptide 5	Yes	Yes	Yes
Q9NR09	Baculoviral IAP repeat-containing protein 6	Yes	-	-
O94812	BAI1-associated protein 3	-	Yes	-
P04280	Basic salivary proline-rich protein 1	Yes	Yes	Yes
P02812	Basic salivary proline-rich protein 2	Yes	Yes	Yes

Q562R1	Beta-actin-like protein 2	Yes	Yes	Yes
P13929	Beta-enolase	Yes	-	-
Q6ZP65	BICD family-like cargo adapter 1	-	-	Yes
Q9UQB8	Brain-specific angiogenesis inhibitor 1-associated protein 2	Yes	-	-
P38398	Breast cancer type 1 susceptibility protein	-	-	Yes
Q9BX70	BTB/POZ domain-containing protein 2	-	Yes	-
Q96HY3	CALM1 protein	Yes	-	-
P62158	Calmodulin	Yes	-	-
P27482	Calmodulin-like protein 3	Yes	Yes	-
Q8TD86	Calmodulin-like protein 6	-	-	Yes
E9PLC9	Calpain-1 catalytic subunit (Fragment)	-	Yes	Yes
E7EQA0	Calpastatin	-	-	Yes
P51817	cAMP-dependent protein kinase catalytic subunit PRKX	-	Yes	-
F5GX99	Caseinolytic peptidase B protein	-	-	Yes
Q8WXE0	Caskin-2	-	Yes	-
P08311	Cathepsin G	Yes	Yes	Yes
Q6ZRH7	Cation channel sperm-associated protein subunit gamma	-	Yes	-

Q96SN8	CDK5 regulatory subunit-associated protein 2	-	Yes	-
Q99638	Cell cycle checkpoint control protein RAD9A	-	Yes	-
Q02224	Centromere-associated protein E	Yes	-	-
Q9P2H0	Centrosomal protein of 126 kDa	Yes	-	-
Q5SW79	Centrosomal protein of 170 kDa	Yes	Yes	-
Q5VT06	Centrosome-associated protein 350	-	-	Yes
P00450	Ceruloplasmin	Yes	-	-
Q9P2M7	Cingulin	-	Yes	-
G3V1A4	Cofilin 1 (Non-muscle)_ isoform CRA_a	Yes	-	-
P23528	Cofilin-1	Yes	-	-
Q9Y281	Cofilin-2	Yes	-	-
P0C7I6	Coiled-coil domain-containing protein 159	-	Yes	-
Q9NZP8	Complement C1r subcomponent-like protein	-	Yes	-
P01024	Complement C3	Yes	-	Yes
Q5TFM2	Complement factor H	Yes	-	-
Q9UBG3	Cornulin	Yes	-	-
P04080	Cystatin-B	Yes	Yes	Yes

P01034	Cystatin-C	Yes	-	-
P01036	Cystatin-S	Yes	Yes	Yes
P09228	Cystatin-SA	Yes	Yes	Yes
P01037	Cystatin-SN	Yes	Yes	Yes
P54108	Cysteine-rich secretory protein 3	Yes	Yes	-
P32320	Cytidine deaminase	Yes	-	-
Q7L576	Cytoplasmic FMR1-interacting protein 1	-	-	Yes
Q96N67	Dedicator of cytokinesis protein 7	-	Yes	-
P17661	Desmin	Yes	-	-
A0A0B4J2C2	Discs_ large (Drosophila) homolog-associated protein 4_ isoform CRA_b	Yes	-	-
Q9Y2H0	Disks large-associated protein 4	Yes	-	-
Q9UDY4	DnaJ homolog subfamily B member 4	Yes	-	-
Q9H1R2	Dual specificity protein phosphatase 15	-	-	Yes
Q9NYC9	Dynein heavy chain 9_ axonemal	-	-	Yes
Q8IZA0	Dyslexia-associated protein KIAA0319-like protein	-	-	Yes
A2CJ06	Dystrotelin	-	Yes	-
Q7Z6Z7	E3 ubiquitin-protein ligase HUWE1	Yes	-	-

Q05BV3	Echinoderm microtubule-associated protein-like 5	Yes	Yes	-
Q9HA90	EF-hand and coiled-coil domain-containing protein 1	-	Yes	-
Q0PNE2	Elongator complex protein 6	-	Yes	-
Q9UPY3	Endoribonuclease Dicer	-	Yes	-
Q9UHY7	Enolase-phosphatase E1	Yes	-	-
P11678	Eosinophil peroxidase	Yes	-	-
P54762	Ephrin type-B receptor 1	Yes	Yes	-
F5H2B8	Epidermal growth factor receptor kinase substrate 8 (Fragment)	-	-	Yes
G3V1T0	Family with sequence similarity 55_ member A_ isoform CRA_a	-	-	Yes
Q9BRP7	Ferredoxin-fold anticodon-binding domain-containing protein 1	-	Yes	-
H0Y5F3	Filamin-A (Fragment)	-	Yes	-
Q8NFU4	Follicular dendritic cell secreted peptide	-	-	Yes
Q9NZ56	Formin-2	Yes	Yes	-
J3KPS3	Fructose-bisphosphate aldolase	Yes	-	Yes
P04075	Fructose-bisphosphate aldolase A	Yes	-	Yes
P47929	Galectin-7	Yes	-	Yes
Q9H3C7	Gametogenetin-binding protein 2		-	Yes

O00591	Gamma-aminobutyric acid receptor subunit pi	Yes	-	-
P09104	Gamma-enolase	Yes	-	Yes
O95749	Geranylgeranyl pyrophosphate synthase	-	-	Yes
P04406	Glyceraldehyde-3-phosphate dehydrogenase	Yes	Yes	Yes
Q8N335	Glycerol-3-phosphate dehydrogenase 1-like protein	Yes	-	-
A0A087WTW2	Golgin subfamily A member 4	-	-	Yes
P00738	Haptoglobin	Yes	Yes	Yes
P00739	Haptoglobin-related protein	Yes	-	Yes
G3V1N2	HCG1745306_ isoform CRA_a	Yes	Yes	-
A0A0C4DGH4	HCG38864	Yes	-	-
A0A1B0GUA7	HCG40442_ isoform CRA_a	Yes	-	-
Q9H583	HEAT repeat-containing protein 1		Yes	-
P0DMV8	Heat shock 70 kDa protein 1A	Yes	-	Yes
P0DMV9	Heat shock 70 kDa protein 1B	Yes	-	Yes
P34931	Heat shock 70 kDa protein 1-like	Yes	-	Yes
P17066	Heat shock 70 kDa protein 6	Yes	-	Yes
P11142	Heat shock cognate 71 kDa protein	Yes	-	Yes

P04792	Heat shock protein beta-1	Yes	-	Yes
P54652	Heat shock-related 70 kDa protein 2	Yes	-	Yes
P69905	Hemoglobin subunit alpha	Yes	Yes	Yes
P68871	Hemoglobin subunit beta	Yes	Yes	Yes
P02042	Hemoglobin subunit delta	Yes	-	Yes
P82970	High mobility group nucleosome-binding domain-containing protein 5	Yes	-	-
Q7Z353	Highly divergent homeobox	Yes	-	-
P15515	Histatin-1	Yes	Yes	-
P15516	Histatin-3	Yes	-	-
C9J386	Histone H2A	Yes	Yes	-
P0C0S8	Histone H2A type 1	Yes	Yes	-
Q96QV6	Histone H2A type 1-A	Yes	Yes	-
P04908	Histone H2A type 1-B/E	Yes	Yes	-
Q93077	Histone H2A type 1-C	Yes	Yes	-
P20671	Histone H2A type 1-D	Yes	Yes	-
Q96KK5	Histone H2A type 1-H	Yes	Yes	-
Q99878	Histone H2A type 1-J	Yes	Yes	-

Q6FI13	Histone H2A type 2-A	Yes	Yes	-
Q8IUE6	Histone H2A type 2-B	Yes	Yes	-
Q16777	Histone H2A type 2-C	Yes	Yes	-
Q7L7L0	Histone H2A type 3	Yes	Yes	-
Q9BTM1	Histone H2A.J	Yes	Yes	-
Q71UI9	Histone H2A.V	Yes	Yes	-
P0C0S5	Histone H2A.Z	Yes	Yes	-
P16104	Histone H2AX	Yes	Yes	-
U3KQK0	Histone H2B	Yes	-	-
P33778	Histone H2B type 1-B	Yes	-	-
P62807	Histone H2B type 1-C/E/F/G/I	Yes	-	-
P58876	Histone H2B type 1-D	Yes	-	-
Q93079	Histone H2B type 1-H	Yes	-	-
P06899	Histone H2B type 1-J	Yes	-	-
O60814	Histone H2B type 1-K	Yes	-	-
Q99880	Histone H2B type 1-L	Yes	-	-
Q99879	Histone H2B type 1-M	Yes	-	-

Q99877	Histone H2B type 1-N	Yes	-	-
P23527	Histone H2B type 1-O	Yes	-	-
Q16778	Histone H2B type 2-E	Yes	-	-
Q5QNW6	Histone H2B type 2-F	Yes	-	-
Q8N257	Histone H2B type 3-B	Yes	-	-
P57053	Histone H2B type F-S	Yes	-	-
P62805	Histone H4	Yes	Yes	-
Q4G0P3	Hydrocephalus-inducing protein homolog	Yes	-	Yes
P01876	Ig alpha-1 chain C region	Yes	Yes	Yes
P01877	Ig alpha-2 chain C region	Yes	Yes	Yes
P01857	Ig gamma-1 chain C region	Yes	Yes	Yes
P01859	Ig gamma-2 chain C region	Yes	Yes	Yes
P01860	Ig gamma-3 chain C region	Yes	Yes	Yes
P01861	Ig gamma-4 chain C region	Yes	Yes	Yes
P01834	Ig kappa chain C region	Yes	Yes	Yes
P0CG04	Ig lambda-1 chain C regions	Yes	Yes	Yes
P0CG06	Ig lambda-3 chain C regions	Yes	Yes	Yes

P0CF74	Ig lambda-6 chain C region	Yes	Yes	Yes
A0M8Q6	Ig lambda-7 chain C region	Yes	-	-
Q5FC05	IL6ST nirs variant 3	-	Yes	-
P01602	Immunoglobulin kappa variable 1-5	Yes	-	-
Q9NSI5	Immunoglobulin superfamily member 5	-	Yes	-
E7ETV8	Importin-5 (Fragment)	-	-	Yes
Q8N201	Integrator complex subunit 1	-	Yes	-
Q75QN2	Integrator complex subunit 8	Yes	Yes	-
P08648	Integrin alpha-5	-	Yes	-
G3V2J5	Interleukin-11 receptor subunit alpha	-	Yes	-
P40189	Interleukin-6 receptor subunit beta	-	Yes	-
Q15323	Keratin_ type I cuticular Ha1	-	Yes	-
Q14532	Keratin_ type I cuticular Ha2	-	Yes	-
Q92764	Keratin_ type I cuticular Ha5	-	Yes	-
O76013	Keratin_ type I cuticular Ha6	-	Yes	-
O76014	Keratin_ type I cuticular Ha7	-	Yes	-
O76015	Keratin_ type I cuticular Ha8	-	Yes	-

P13645	Keratin_ type I cytoskeletal 10	Yes	Yes	Yes
P13646	Keratin_ type I cytoskeletal 13	Yes	Yes	Yes
P02533	Keratin_ type I cytoskeletal 14	Yes	Yes	Yes
P19012	Keratin_ type I cytoskeletal 15	Yes	Yes	Yes
P08779	Keratin_ type I cytoskeletal 16	Yes	Yes	Yes
Q04695	Keratin_ type I cytoskeletal 17	Yes	Yes	Yes
P05783	Keratin_ type I cytoskeletal 18	-	Yes	-
P08727	Keratin_ type I cytoskeletal 19	Yes	Yes	Yes
Q2M2I5	Keratin_ type I cytoskeletal 24	-	Yes	-
Q7Z3Y7	Keratin_ type I cytoskeletal 28	-	Yes	-
P35908	Keratin_ type II cytoskeletal 2 epidermal	-	Yes	-
Q01546	Keratin_ type II cytoskeletal 2 oral	Yes	Yes	-
P12035	Keratin_ type II cytoskeletal 3	-	Yes	Yes
P19013	Keratin_ type II cytoskeletal 4	Yes	Yes	Yes
P13647	Keratin_ type II cytoskeletal 5	Yes	Yes	Yes
P02538	Keratin_ type II cytoskeletal 6A	Yes	Yes	Yes
P04259	Keratin_ type II cytoskeletal 6B	Yes	Yes	Yes

P48668	Keratin_ type II cytoskeletal 6C	Yes	Yes	Yes
Q8IVT5	Kinase suppressor of Ras 1	-	Yes	-
P02788	Lactotransferrin	Yes	Yes	Yes
Q6PKG0	La-related protein 1	Yes	-	-
C9JDW2	Latent-transforming growth factor beta-binding protein 1 (Fragment)	-	Yes	-
Q99538	Legumain	-	-	Yes
Q9P127	Leucine zipper protein 4	-	-	Yes
Q96NI6	Leucine-rich repeat and fibronectin type-III domain-containing protein 5	-	Yes	-
Q38SD2	Leucine-rich repeat serine/threonine-protein kinase 1	-	Yes	-
J3QLE7	Leucine-rich repeat-containing protein 37B (Fragment)	-	-	Yes
Q05C16	Leucine-rich repeat-containing protein 63	-	Yes	-
B1AKT2	Leucine-rich repeat-containing protein 7	-	-	Yes
P18428	Lipopolysaccharide-binding protein	Yes	-	-
H0YDW2	Liprin-alpha-1 (Fragment)	-	Yes	-
H0YHK3	Liprin-alpha-2	-	Yes	-
O75335	Liprin-alpha-4	-	Yes	-
Q86W92	Liprin-beta-1	-	Yes	-

Q659A1	Little elongation complex subunit 2	Yes	-	-
P61626	Lysozyme C	Yes	Yes	Yes
Q6ZSS7	Major facilitator superfamily domain-containing protein 6	Yes	Yes	-
Q16644	MAP kinase-activated protein kinase 3	-	Yes	-
O95460	Matrilin-4	-	-	Yes
P43243	Matrin-3	-	-	Yes
P08493	Matrix Gla protein	Yes	Yes	Yes
Q9UHV7	Mediator of RNA polymerase II transcription subunit 13	-	Yes	-
O00562	Membrane-associated phosphatidylinositol transfer protein 1	-	Yes	-
Q9NXJ0	Membrane-spanning 4-domains subfamily A member 12	-	Yes	-
Q2M296	Methenyltetrahydrofolate synthase domain-containing protein	-	-	Yes
E7EVA0	Microtubule-associated protein	Yes	Yes	Yes
H7BZB9	Microtubule-associated protein 2 (Fragment)	Yes	-	-
P27816	Microtubule-associated protein 4	Yes	Yes	Yes
Q9Y2H9	Microtubule-associated serine/threonine-protein kinase 1	Yes	-	Yes
Q2PPL5	Mitochondrial 5-methylaminomethyl-2-thiouridylate-methyltransferase transcript variant 1	-	-	Yes
O75648	Mitochondrial tRNA-specific 2-thiouridylase 1	-	-	Yes

O60566	Mitotic checkpoint serine/threonine-protein kinase BUB1 beta	Yes	Yes	-
E7EW47	Mucin-4	-	Yes	-
Q8TAX7	Mucin-7	Yes	Yes	-
Q96J65	Multidrug resistance-associated protein 9	-	Yes	-
O95297	Myelin protein zero-like protein 1	Yes	-	-
P24158	Myeloblastin	Yes	Yes	Yes
Q96S97	Myeloid-associated differentiation marker	-	Yes	-
P05164	Myeloperoxidase	Yes	Yes	Yes
H0YCY0	Myomegalin (Fragment)	-	Yes	-
P14649	Myosin light chain 6B	-	-	Yes
P60660	Myosin light polypeptide 6	Yes	-	Yes
Q6WCQ1	Myosin phosphatase Rho-interacting protein	-	-	Yes
Q9HCE5	N6-adenosine-methyltransferase subunit METTL14	-	Yes	-
P18615	Negative elongation factor E	-	Yes	Yes
P21359	Neurofibromin	-	-	Yes
P07197	Neurofilament medium polypeptide	Yes	-	Yes
O95502	Neuronal pentraxin receptor	Yes	-	-

P59665	Neutrophil defensin 1	Yes	Yes	Yes
P59666	Neutrophil defensin 3	Yes	Yes	Yes
P08246	Neutrophil elastase	Yes	-	-
Q9GZT8	NIF3-like protein 1	-	Yes	-
Q8N4C6	Ninein	Yes	-	Yes
Q8WTW4	Nitrogen permease regulator 2-like protein	-	-	Yes
P05204	Non-histone chromosomal protein HMG-17	Yes	Yes	Yes
E9PLD1	Non-specific lipid-transfer protein	Yes	-	-
Q08AH0	NRXN1 protein	-	Yes	-
Q8N9A8	Nuclear envelope phosphatase-regulatory subunit 1	-	Yes	Yes
C9JE98	Nuclear receptor corepressor 2	-	Yes	-
Q7Z3B4	Nucleoporin p54	-	Yes	-
Q8N323	NXPE family member 1	-	-	Yes
P68032	P68032 Actin_ alpha cardiac muscle 1	Yes	Yes	Yes
P50897	Palmitoyl-protein thioesterase 1	-	-	Yes
P04746	Pancreatic alpha-amylase	-	-	Yes
P30044	Peroxiredoxin-5_ mitochondrial	Yes	-	-

J3KPV0	Peroxisomal targeting signal 1 receptor (Fragment)	-	Yes	-
C9JLI1	Phosphatidylinositol 4-kinase alpha (Fragment)	-	Yes	-
O00443	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha	-	Yes	-
Q8N7S5	Phosphoinositide phospholipase C	-	-	Yes
Q99569	Plakophilin-4	Yes	-	-
P09619	Platelet-derived growth factor receptor beta	-	Yes	-
Q9H7P9	Pleckstrin homology domain-containing family G member 2	Yes	-	-
A1L390	Pleckstrin homology domain-containing family G member 3	-	-	Yes
P01833	Polymeric immunoglobulin receptor	Yes	-	Yes
P26599	Polypyrimidine tract-binding protein 1	-	-	Yes
Q96KW2	POM121-like protein 2	-	-	Yes
B7Z2X5	Post-GPI attachment to proteins factor 2	-	Yes	-
Q6S8J3	POTE ankyrin domain family member E	Yes	Yes	Yes
A5A3E0	POTE ankyrin domain family member F	Yes	Yes	Yes
P0CG38	POTE ankyrin domain family member I	Yes	Yes	Yes
P0CG39	POTE ankyrin domain family member J	Yes	Yes	Yes
P40426	Pre-B-cell leukemia transcription factor 3	Yes	-	-

Q9NRD5	PRKCA-binding protein	Yes	-	-
P42694	Probable helicase with zinc finger domain	Yes	-	-
O43861	Probable phospholipid-transporting ATPase IIB	-	-	Yes
K7EJ44	Profilin	Yes	Yes	Yes
P07737	Profilin-1	Yes	Yes	Yes
P16471	Prolactin receptor	-	Yes	-
P12273	Prolactin-inducible protein	Yes	Yes	Yes
O94903	Proline synthase co-transcribed bacterial homolog protein	-	-	Yes
Q6MZM9	Proline-rich protein 27	Yes	Yes	Yes
A0A0A0MT31	Proline-rich protein 4	Yes	Yes	Yes
P85299	Proline-rich protein 5	-	Yes	-
Q6PGQ7	Protein aurora borealis	Yes	-	-
Q13948	Protein CASP	Yes	-	-
Q9P219	Protein Daple	-	-	Yes
Q6SJ93	Protein FAM111B	-	Yes	-
Q96KN1	Protein FAM84B	-	Yes	-
Q5JS89	Protein furry homolog	-	-	Yes

Q4ZG55	Protein GREB1	-	-	Yes
A0A1B0GVX2	Protein LOC101929926	Yes	-	-
Q9NYP9	Protein Mis18-alpha	Yes	-	-
Q9UN36	Protein NDRG2	-	Yes	-
Q5THK1	Protein PRR14L	Yes	-	-
B1AHC4	Protein PRR5-ARHGAP8	-	Yes	-
P31949	Protein S100-A11	Yes	-	Yes
P05109	Protein S100-A8	Yes	Yes	Yes
P06702	Protein S100-A9	Yes	Yes	Yes
Q9Y2G9	Protein strawberry notch homolog 2	Yes	-	-
Q5T0J5	Protein TEX35 (Fragment)	-	-	Yes
A0A075B6Z2	Protein TRAJ56 (Fragment)	Yes	-	-
A0A0A0MT22	Protein tyrosine phosphatase_ receptor type_ C_ isoform CRA_d	-	Yes	-
Q8NB66	Protein unc-13 homolog C	Yes	-	-
Q9UN71	Protocadherin gamma-B4	Yes	-	-
P55786	Puromycin-sensitive aminopeptidase	-	Yes	-
Q5VTE0	Putative elongation factor 1-alpha-like 3	-	-	Yes

P48741	Putative heat shock 70 kDa protein 7	Yes	-	Yes
O43930	Putative serine/threonine-protein kinase PRKY	-	Yes	-
P0C7V0	Putative uncharacterized protein encoded by LINC00271	-	Yes	-
P0CG00	Putative zinc finger and SCAN domain-containing protein 5D	-	-	Yes
B4DNK4	Pyruvate kinase	-	-	Yes
P14618	Pyruvate kinase PKM	-	-	Yes
Q15276	Rab GTPase-binding effector protein 1	Yes	-	-
Q4ADV7	RAB6A-GEF complex partner protein 1	Yes	Yes	-
Q9P2R3	Rabankyrin-5	Yes	-	-
P08575	Receptor-type tyrosine-protein phosphatase C	-	Yes	-
Q96D15	Reticulocalbin-3	Yes	-	-
B7Z4M1	Reticulon	-	Yes	-
O95197	Reticulon-3	-	Yes	-
Q9Y2V3	Retinal homeobox protein Rx	-	Yes	-
P52565	Rho GDP-dissociation inhibitor 1	-	Yes	-
A1A4S6	Rho GTPase-activating protein 10	-	Yes	-
Q68EM7	Rho GTPase-activating protein 17	-	Yes	-

Q13017	Rho GTPase-activating protein 5	-	Yes	-
P49756	RNA-binding protein 25	Yes	Yes	-
Q96MS0	Roundabout homolog 3	-	-	Yes
Q13950	Runt-related transcription factor 2	-	Yes	-
Q9BY12	S phase cyclin A-associated protein in the endoplasmic reticulum	Yes	-	-
P02810	Salivary acidic proline-rich phosphoprotein 1/2	Yes	Yes	Yes
H0YFR4	Scavenger receptor cysteine-rich type 1 protein M160 (Fragment)	Yes	-	-
A0A0A0MQU6	Sema domain_ transmembrane domain (TM)_ and cytoplasmic domain_ (Semaphorin) 6A_ isoform CRA_d	-	Yes	-
Q9H2E6	Semaphorin-6A	-	Yes	-
Q14674	Separin	Yes	-	-
Q9NRX5	Serine incorporator 1	-	-	Yes
Q6UWY2	Serine protease 57	-	Yes	-
Q9UQ35	Serine/arginine repetitive matrix protein 2	Yes	-	-
B4DTS2	Serine/threonine-protein kinase	-	-	Yes
Q9BZL6	Serine/threonine-protein kinase D2	-	-	Yes
Q13153	Serine/threonine-protein kinase PAK 1	-	-	Yes

I3L0W2	Serine/threonine-protein kinase SMG1	-	-	Yes
H3BPN6	Serine/threonine-protein kinase ULK3	-	-	Yes
P02787	Serotransferrin	Yes	Yes	Yes
P02768	Serum albumin	Yes	Yes	Yes
Q6NVH2	SH3 domain and tetratricopeptide repeat-containing protein 1	-	-	Yes
O75368	SH3 domain-binding glutamic acid-rich-like protein	Yes	-	-
Q92783	Signal transducing adapter molecule 1	Yes	-	-
Q9UBC9	Small proline-rich protein 3	Yes	-	-
Q8NBS3	Sodium bicarbonate transporter-like protein 11	-	Yes	-
A0A1B0GW40	Sodium channel protein type 2 subunit alpha	-	Yes	Yes
H0YK24	Spatacsin (Fragment)	-	-	Yes
Q86XZ4	Spermatogenesis-associated serine-rich protein 2	-	-	Yes
Q8WXA9	Splicing regulatory glutamine/lysine-rich protein 1	Yes	Yes	-
Q15020	Squamous cell carcinoma antigen recognized by T-cells 3	-	Yes	-
P02808	Statherin	Yes	Yes	-
Q9P1V8	Sterile alpha motif domain-containing protein 15	Yes	-	-
B9ZVR7	Striated muscle preferentially-expressed protein kinase	-	-	Yes

Q8NBJ7	Sulfatase-modifying factor 2	Yes	-	-
Q92922	SWI/SNF complex subunit SMARCC1	-	-	Yes
Q9NPQ8	Synembryn-A	Yes	-	-
O15533	Tapasin	-	Yes	-
A0A0D9SG95	T-complex protein 1 subunit eta	-	Yes	-
Q7Z6L1	Tectonin beta-propeller repeat-containing protein 1	-	Yes	-
Q86US8	Telomerase-binding protein EST1A	-	Yes	-
Q96RN1	Testis anion transporter 1	-	Yes	-
I3NI44	TOM1-like protein 1	Yes	-	-
H0YF09	TRAF3-interacting JNK-activating modulator (Fragment)	-	Yes	-
A9JX13	Transcription factor 20	-	Yes	-
Q16514	Transcription initiation factor TFIID subunit 12	-	Yes	-
P46100	Transcriptional regulator ATRX	Yes	-	-
S4R3C6	Transforming growth factor-beta-induced protein ig-h3 (Fragment)	-	-	Yes
P29401	Transketolase	Yes	-	-
Q6ZXV5	Transmembrane and TPR repeat-containing protein 3	Yes	-	-
Q6ZMR5	Transmembrane protease serine 11A	Yes	-	-

Q6ZMB5	Transmembrane protein 184A	Yes	-	-
Q12767	Transmembrane protein 94	Yes	-	Yes
P02766	Transthyretin	-	Yes	-
Q7Z2Z1	Treslin	Yes	-	-
O15417	Trinucleotide repeat-containing gene 18 protein	Yes	-	-
Q6ZTA4	Tripartite motif-containing protein 67	Yes	-	-
Q7Z4G4	tRNA (guanine(10)-N2)-methyltransferase homolog	-	Yes	-
A0A075B736	Tubulin beta chain	Yes	-	-
Q3ZCM7	Tubulin beta-8 chain	Yes	-	-
Q8N841	Tubulin polyglutamylase TTLL6	-	Yes	-
O15327	Type II inositol 3_4-bisphosphate 4-phosphatase	Yes	-	Yes
Q12923	Tyrosine-protein phosphatase non-receptor type 13	-	-	Yes
Q70CQ2	Ubiquitin carboxyl-terminal hydrolase 34	-	Yes	-
A0A087WZY1	Uncharacterized protein	Yes	Yes	Yes
H3BT81	Uncharacterized protein (Fragment)	-	-	Yes
Q9Y2V0	Uncharacterized protein C15orf41	-	-	Yes
B0I1T2	Unconventional myosin-Ig	-	-	Yes

B2RTY4	Unconventional myosin-IXa	Yes	-	-
P46939	Utrophin	Yes	-	-
P55895	V(D)J recombination-activating protein 2	-	-	Yes
H0YG53	Vacuolar protein sorting-associated protein 13A	-	-	Yes
P08670	Vimentin	Yes	Yes	Yes
P02774	Vitamin D-binding protein	Yes	-	-
Q8WY21	VPS10 domain-containing receptor SorCS1	Yes	-	-
Q9NYS7	WD repeat and SOCS box-containing protein 2	-	-	Yes
A2RRC6	ZFHX2 protein	Yes	-	-
Q9ULJ3	Zinc finger and BTB domain-containing protein 21	Yes	Yes	-
Q5T200	Zinc finger CCCH domain-containing protein 13	-	-	Yes
Q86VM9	Zinc finger CCCH domain-containing protein 18	Yes	-	-
Q9C0A1	Zinc finger homeobox protein 2	Yes	-	-
O14771	Zinc finger protein 213	-	Yes	-
Q8TAF7	Zinc finger protein 461	-	Yes	-
Q9HCE3	Zinc finger protein 532	Yes	-	Yes
E9PPS7	Zinc finger protein 707 (Fragment)	-	Yes	-

Q9Y6R6	Zinc finger protein 780B	Yes	-	-
P25311	Zinc-alpha-2-glycoprotein	Yes	-	-
Q96DA0	Zymogen granule protein 16 homolog B	Yes	-	-

Proteins were classified according to: **General Function:** [a)] metabolism; [b)] biological process; [c)] transport; [d)] structure and structural organization; [e)] information pathways; [f)] miscellanea; **Function in AEP:** [g)] metabolism; [h)] tissue regeneration; [i)] antimicrobial; [j)] immune response; [k)] lubrication; [l)] biomineralization; [m)] unknown biological function; **Origin:** [n)] cytoplasm origin; [o)] extracellular origin; [p)] nucleus origin; [q)] cytoskeleton origin; [r)] intracellular origin; [s)] membrane origin; [t)] unknown protein origin; **Interaction:** [u)] protein/protein interaction; [v)] calcium/phosphate binding; [w)] other molecular interaction; [x)] unknown molecular interaction.

3-Discussion

3 DISCUSSION

In the present study we used a different protocol to extract and prepare the AEP proteins for analyses, according to a new methodology recently developed. This protocol was selected because it increased the number of identified proteins (VENTURA et al., 2017).

Based on the literature, this is the first study that employed proteomic analysis to compare the protein profile of the AEP of volunteers who suffer with GERD and dental erosion with that of volunteers that have GERD but no dental erosion. One of the aims of the study was to detecte the changes in the proteomic profile of AEP in function of GERD, therefore a control group was also introduced, comprised of volunteers without GERD or dental erosion. Regarding the characteristics of the volunteers, there was a higher number of females in the all groups, and only females composed one of the groups (Table 1). Even with these differences about the gender, there is no reason to believe that this would influence in our results. In terms of age, this study had the participation of volunteers with a quite similar age.

The mean BEWE score of the volunteers was around 16, which denotes severe erosive tooth wear (BARTLETT, D. et al., 2008). According to the inclusion criteria for GERD, all volunteers should have heartburn and / or regurgitation for at least 1 year (frequency more than 2 times per week) (CALABRESE et al., 2011). This fact turns possible the presence of dental erosion, since there was enough time to occur the demineralization and softening of the dental hard tissues as a consequence of the intrinsic acids present in the oral cavity. We employed a different protocol to prepare and extract the AEP that increases the number of identified proteins (VENTURA et al., 2017). This protocol was effective and turned possible to identify nearly 460 proteins in the present study. This is the highest number of proteins ever identified in the AEP formed in vivo.

The proteins that there were found in the 3 groups had a high diversity, and most of these proteins are unique in each of the groups (approximately more than 100 in each group). Therefore, the null hypothesis to be tested was rejected. The majority of the proteins identified in all the groups are proteins that are found typically in the AEP (Fig. 1, Table S1) and most of them presented differences in expression among

the groups (Table 3). This demonstrates that GERD has a significant impact on the proteomic profile of the AEP that also changes considerably in patients with or without dental erosion. In the (fig. 2) can also be observed when the molecular function of the identified proteins is analyzed. Three molecular functions (monocarboxylic acid binding, nucleosomal DNA binding and serine-type endopeptidase activity) were present in the group GE and these functions were common to all the 3 groups. The groups C and GNE had more diverse molecular functions when compared with the GE. Even so, the molecular functions found for both of them (C and GNE) was distinct, despite cysteine-type endopeptidase inhibitor activity was the most frequent molecular function for both of them. It is important to note that that the bioinformatics tools used refer mainly to intracellular functions and in the majority of cases do not apply to the AEP, since this integument contains both secreted proteins (derived from the salivary glands mainly but also from the gingival crevicular fluid) and intracellular proteins (originated from oral mucosa cells and from bacteria) (SIQUEIRA et al., 2012). The protocol used in this study did not evaluate bacterial proteins, once we used the *Homo sapiens* database for the identification of proteins. The information on the molecular functions of the proteins identified is useful to show the proteome diversity of the AEP collected from the 3 distinct groups.

The majority of the proteins identified exclusively in the groups with no erosion (C and GNE groups) are metal-binding proteins, while most of those exclusive of the GE group are membrane proteins. This can suggest a higher degree of epithelial cell lysis in the GE group caused by the gastric acids, which is consistent with the higher incidence of lesions in the oral mucosa of patients with GERD (PREETHA et al., 2015). A few of the proteins that are stocked in neutrophil cytoplasmic granules are secreted as active proteases in response to their stimulation, as it is the case for *Serine protease 57*. In the GE group another unique protein identified was *Puromycin-sensitive aminopeptidase.* With broad substrate specificity for several peptides, this protein releases an N-terminal amino acid, preferably alanine, from a wide range of peptides (UNIPROT). Thus, this proteolysis can alter the structure of the AEP, reducing its protective ability against demineralization. Among the proteins exclusive of GNE group are those with sites of phosphorylation in serine, as well as various isoforms of serine/threonine-protein kinase (Table 2). So, phosphorylation in serine confers negative charge to this amino acid. Hydroxyapatite binds proteins through both calcium (positive) and phosphate (negative) sites on the surface (KAWASAKI et al., 1986;

KAWASAKI et al., 1987; PREETHA et al., 2015). Phosphorylated and negatively charged proteins, such as acidic proline-rich proteins, histatin 1 and statherin, have strong affinity to hydroxyapatite and are included among the pellicle precursor proteins, constituting the basal layer of this integument (LENDENMANN et al., 2000). The majority of protection versus demineralization conferred by the AEP is attributed to its basal layer, once it is not removed after erosive challenge (HANNIG, C. et al., 2009). In addition, proteins as histatins and histones were not found in GNE group. Histatins are primarily antimicrobial proteins (RAJ et al., 1990). Recently it was demonstrated their role against acid damage when adsorbed onto hydroxyapatite (SIQUEIRA et al., 2010). Although histones have already been identified in the AEP (VENTURA et al., 2017), the reason why these two classes are not present in the GNE group, as well as its function in this integument remains to be determined. Still regarding the unique proteins, the heat-shock proteins were not found in the GE group. Furthermore, the C and GE groups had a higher presence of isoforms of 14-3-3 protein. These results are consistent with a study that analyzed the profile proteomic of the esophageal mucosa in patients with erosive and non-erosive GERD, where higher expression of 14-3-3 proteins were seen in patients with reflux when compared to the healthy ones. As well as higher expression of *Heat shock cognate 71 kDa protein* in patients with non-erosive GERD when compared to those with erosive GERD (CALABRESE et al., 2011). The presence of these proteins may be related with presence with this disease itself, since many proteins were exclusive of the GERD groups, regardless the presence of dental erosion.

In the present study, three comparisons were made in the expression analyses. The first two comparisons involve comparison of the reflux groups with control group. Possibly these comparisons indicate the proteins that have their expression rates altered as a function of reflux, despite the occurrence of dental erosion or not also remarkably altered the pattern of protein expression. It is noteworthy that proteins higher in GE compared with C were lower in GNE compared with C, such as *Lysozyme C, Cathepsin G* and isoforms of neutrophil defensin. However, proteins that were lower in GE when compared with C were higher when GNE was compared with C, such as isoforms of hemoglobin. The GE group also had higher levels of various isoforms of cytoskeletal keratin. Higher levels in these proteins in the AEP of the GE group suggest higher degree of epithelial cell lysis (PREETHA et al., 2015; SUJATHA et al., 2016) and neutrophil degranulation (LEONI et al., 2015) in this group. It is important to

highlight that *Sthaterin*, a calcium-binding protein, as 30% lower when the GE group was compared to the C group, what is consistent with a recent report of reduction of 35% in *Statherin* concentrations in patients with erosion (CARPENTER et al., 2014).

Regarding the main objective of this study, which is to find proteins in the GNE group that could be associated with protection against dental erosion, the most important comparison is the one between the GNE and GE groups. Increases in lysozyme and cathepsins have been reported in patients with Barrett´s esophagus and esophageal adenocarcinoma induced by GERD (CHENG et al., 2005). Among the proteins with the lowest rates of expression (more than 2-fold reduction) in GNE group when compared with GE group are *Lysozyme C, Antileukoproteinase* and *Cathepsin G*. Besides, *Lysozyme C* was recently reported as an acid-resistant protein, since it was higher in the AEP after challenge with 1% citric acid (DELECRODE; SIQUEIRA; ZAIDAN; BELLINI; MOFFA; et al., 2015). The lower level of *Lysozyme C* in GNE compared with GE might be associated with a greater risk of caries development in the first, since it is an important antibacterial enzyme and reduced amounts of lysozyme in unstimulated saliva of children are related with early childhood caries (MOSLEMI et al., 2015). In addition, in the present study this enzyme was decreased in the GNE group compared with control. In relation to the GE group when compared to C, this enzyme was increased, which might suggest that volunteers in GE had a higher acid influx into the oral cavity when compared with GNE volunteers. *Antileukoproteinase* is an acid-stable proteinase inhibitor with strong affinities for trypsin, chymotrypsin, elastase, and cathepsin G *(OHLSSON et al., 1983)*. Once it inhibits both trypsin and cathepsin G, it would be expected to be increased in the volunteers without erosion, since proteases activities were shown to be higher in bulimic patients with erosion (SCHLUETER et al., 2012), but in the present study the GNE volunteers had lower levels of *Antileukoproteinase*.The smaller expression of *Cathepsin G* in GNE volunteers may be related with lower rates of erosion progression in dentin, once the role of proteases, including matrix metalloproteinases and cysteine cathepsins in the degradation of the demineralized organic matrix and on the progression of erosion and caries into dentin has been reported (BUZALAF, M. A. et al., 2015; TJADERHANE et al., 2015). Cystatin-B was recently identified as an acid-resistant protein in the AEP that had its levels increased more than 20-fold when the AEP was challenged with 1% citric acid. In the present study, slightly higher level of different isoforms of cystatins was seen in GNE volunteers when compared to their GE counterparts. But this did not

occur for Cystatin-B that was lower in GNE when compared with GE. This protein was suggested as a potential candidate to be included in dental products to protect against extrinsic erosion but it seems that this might not be the case for intrinsic erosion. The gastric acids have a lower pH and higher titratability when compared with dietary acids, which usually leads to more severe erosion (MOAZZEZ; BARTLETT, 2014). This means that protein candidates that seem promising to prevent extrinsic erosion might not work in the case of intrinsic erosion. Albumin is able to bind calcium (SCHWEIGEL et al., 2016) and has been suggested to reduce the dissolution of hydroxyapatite in vitro (HEMINGWAY et al., 2008; KOSORIC et al., 2010). When compared GNE volunteers and GE volunteers, two isoforms of albumin were also increased in the first.

An interesting finding of the present study when the GNE group compared to the GE group was the higher level of distinct subunits of hemoglobin in the first. Hemoglobins are not normally included among the protein components of the AEP. The first study that reported the presence of hemoglobin in the AEP was recently published. Moreover, this protein was found only in the AEP collected from the posterior region of the dental arches (VENTURA et al., 2017). In the previous studies, the AEP was collected from the anterior region only. This might be the reason why hemoglobin had not been described in the AEP before (DELECRODE; SIQUEIRA; ZAIDAN; BELLINI; MOFFA; et al., 2015; LEE et al., 2013; SIQUEIRA et al., 2007; ZIMMERMAN et al., 2013). It could appear that the presence of hemoglobin in the AEP was due to contamination with the gingival crevicular fluid, but this is not the case, since in the collection we avoided the cervical third of the tooth surface. Furthermore, one of the exclusion criteria adopted in the present study was the absence of gingivitis and periodontitis. Indeed, this affinity of hemoglobin for hydroxyapatite is acknowledged for a long time, since hydroxyapatite columns have been shown to have a good performance for purification of hemoglobin, among other proteins found in the present study (such as albumin, lysozyme and immunoglobulins) (KAWASAKI et al., 1985). Due to its ability to adsorb hemoglobin, nanostructured hydroxyapatite microspheres (QI et al., 2013) or polyhedral (YU et al., 2017) have been developed to deliver this protein. Interestingly, as pH decreases, the adsorption rate of hemoglobin to hydroxyapatite increases, which may be explained by the electrostatic interactions between hemoglobin molecules and hydroxyapatite that occurs by van der Waals, electrostatic and hydrophobic forces. The isoelectric point (pI) of hydroxyapatite is around 6.8-70, which means that this protein becomes positively charged at pH below

6.8 (YU et al., 2017). Besides, GERD patients have an oral pH typically lower than that found in healthy people. A relation has been reported between pH < 4 in the distal esophagus and pH < 5.5 in the mouth (BARTLETT, D. W. et al., 1996). Therefore, due to the lower intraoral pH in GERD patients, the chance to occur adsorption of hemoglobin onto the dental surfaces is higher, once this confers positive charge to hemoglobin thus increasing its electrostatic attraction by hydroxyapatite. GE volunteers had lower levels of hemoglobin than controls, showing that this relationship might not be as simplistic as it appears. The hemoglobin levels in the AEP of GNE volunteers was around 3-fold higher than that of GE volunteers but the reasons for this are still unclear. Some hypotheses could be: 1) the GNE volunteers had higher concentrations of hemoglobin in saliva; 2) other proteins found only in the AEP of GNE patients might have stabilized hemoglobin adsorbed to hydroxyapatite.

In conclusion, profound alterations in the proteomic profile of the AEP were seen in GNE compared with GE volunteers, which might play a role in the resistance to dental erosion seen in the first. Among the proteins differentially expressed between these two groups, hemoglobin drives attention, since it was increased around 3-fold in GNE volunteers when compared to their GE counterparts. Additional studies should evaluate the potential protective role of hemoglobin against intrinsic erosion, as well as the viability to add this protein into dental products to protect against intrinsic erosion.

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Annex

ANNEX 1

Universidade de São Paulo
Faculdade de Odontologia de Bauru

Departamento Ciências Biológicas
Disciplina de Bioquímica

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Vimos, por meio deste convidá-lo a participar de uma pesquisa onde serão analisadas as proteínas que restam sobre os dentes após contato com ácidos endógenos do refluxo gástrico que atingem a boca.

Essa pesquisa vai ser conduzida por Tatiana Martini, aluna do Curso de Pós-Graduação em Biologia Oral da Faculdade de Odontologia de Bauru- Universidade de São Paulo (FOB/USP), sob a orientação da Profa. Dra. Marília Afonso Rabelo Buzalaf (FOB/USP), com a colaboração das Dentistas Cintia Maria de Souza e Silva (FOB/USP) e Profa. Dr. Daniela Rios (FOB/USP), Profa. Dra. Regina Guenka Palma (Departamento de Odontologia Restauradora-FORP/USP) Faculdade de Odontologia de Ribeirão Preto e Prof. Dr. Ricardo Brandt de Oliveira (Departamento de Clínica Médica-FMRP/USP) Faculdade de Medicina de Ribeirão Preto. O experimento será realizado no período da manhã duas horas após a última refeição, tendo início às 8 horas da manhã. Inicialmente será feita a coleta da saliva, a higiene oral deverá ser feita 1 hora antes da coleta, após 15 minutos você fará um bochecho com água deionizada. Você deverá expectorar a saliva em um tubo de plástico por 5 minutos, após esse tempo a saliva será armazenada. Posteriormente para a coleta da película você receberá uma meticulosa profilaxia dentária com pedra pomes, para que a película adquirida (camada de proteínas originárias da saliva que se ligam à superfície do dente) se forme naturalmente sobre o esmalte dentário. Depois de 2 horas e após a formação da película adquirida, será aplicado 1 ml de ácido cítrico a 3%, (não causando nenhum dano ao esmalte do seu dente). A película será removida com um papel. Durante o período das duas coletas, você não poderá consumir alimentos ou bebidas. Após a coleta da película será feita a coleta de saliva estimulada onde você deverá mastigar um papel de parafilm e expectorar a saliva em um tubo plástico por 5 minutos. Depois do uso das amostras coletadas para as análises de dados, esta será devidamente descartada em local adequado e de forma segura. Quanto aos benefícios oferecidos a você, no início do estudo será feito um exame clínico em relação às suas condições bucais e o resultado deste exame será prontamente informado a você. Caso seja detectado algum problema, faremos o encaminhamento ao setor de Triagem da Clínica do Laboratório de Laser FORP USP (Voluntários Ribeirão Preto) ou Clínica da Farmacologia FOB USP (Voluntários Bauru), destacando que, uma vez encaminhado para triagem, deverá aguardar e respeitar as normas da Triagem de agendamento. Além disto, no final do estudo, serão dadas instruções sobre higiene bucal, por escrito e verbalmente. A participação será voluntária e entende-se que você poderá fazer qualquer pergunta sobre os procedimentos, sendo que será livre para desistir de participar a qualquer momento, sem nenhum prejuízo de sua parte. Em adição, você terá, também, por parte dos pesquisadores, a garantia do sigilo que assegura a sua privacidade. Destacamos ainda que não há risco nenhum à sua saúde com a participação nesta pesquisa. O desconforto que poderá ocorrer é sentir ânsia durante a mastigação do parafilm, coleta da saliva, ou o desconforto de permanecer com a boca aberta durante a profilaxia e coleta.

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Disciplina de Bioquímica

Concordando em participar, você entende que este estudo será realizado em benefício da ciência médica e odontológica, e desta forma concorda com a divulgação dos dados obtidos por meio de publicações científicas. Para maiores esclarecimentos de dúvidas sobre a pesquisa você pode, a qualquer momento, contatar a pesquisadora Tatiana Martini pelo telefone (14) 997402948. Caso tenha alguma despesa em relação ao transporte decorrente da participação na pesquisa, haverá ressarcimento na forma de passe de ônibus. Se ocorrer qualquer dano comprovadamente decorrente dos procedimentos aos quais o Sr.(a) será submetido, poderá solicitar o direito de indenização.

Pelo presente instrumento que atende às exigências legais, o Sr. (a) _____, portador da cédula de identidade _____, após leitura minuciosa das informações constantes neste **TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO**, devidamente explicada pelos profissionais em seus mínimos detalhes, ciente dos serviços e procedimentos aos quais será submetido, não restando quaisquer dúvidas a respeito do lido e explicado, **DECLARA** e **FIRMA** seu **CONSENTIMENTO LIVRE E ESCLARECIDO** concordando em participar da pesquisa proposta. Fica claro que o participante da pesquisa, pode a qualquer momento retirar seu **CONSENTIMENTO LIVRE E ESCLARECIDO** e deixar de participar desta pesquisa e ciente de que todas as informações prestadas tornar-se-ão confidenciais e guardadas por força de sigilo profissional (Art. 9º do Código de Ética Odontológica). Por fim, como pesquisadora responsável pela pesquisa, **DECLARO** o cumprimento do disposto na Resolução CNS nº 466 de 2012, contidos nos itens IV.3 e IV.4, este último se pertinente, item IV.5.a e na íntegra com a resolução CNS nº 466 de dezembro de 2012.

Por estarmos de acordo com o presente termo o firmamos em duas vias igualmente válidas que serão rubricadas em todas as suas páginas e assinadas ao seu término, conforme o disposto pela Resolução CNS nº 466 de 2012, itens IV.3.f e IV.5.d.

Bauru-SP, _____ de _____ de_____ .

_____ _____
Nome do Sujeito da Pesquisa Nome do Autor

_____ _____
Assinatura do Sujeito da Pesquisa Assinatura do Autor

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O **Comitê de Ética em Pesquisa – CEP**, organizado e criado pela **FOB-USP**, em 29/06/98 (**Portaria GD/0698/FOB**), previsto no item VII da Resolução nº 466/12 do Conselho Nacional de Saúde do Ministério da Saúde (publicada no DOU de 13/06/2013), é um Colegiado interdisciplinar e independente, de relevância pública, de caráter consultivo, deliberativo e educativo, criado para defender os interesses dos participantes da pesquisa em sua integridade e dignidade e para contribuir no desenvolvimento da pesquisa dentro de padrões éticos.
Qualquer denúncia e/ou reclamação sobre sua participação na pesquisa poderá ser reportada a este CEP:

Horário e local de funcionamento:
Comitê de Ética em Pesquisa
Faculdade de Odontologia de Bauru-USP - Prédio da Pós-Graduação (bloco E - pavimento superior), de segunda à sexta-feira, no horário das **13h30 às 17 horas**, em dias úteis.
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