UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

TALITA MENDES DA SILVA VENTURA

Changes in the composition of the acquired enamel pellicle according to its location in the oral cavity: proteomic study

Detecção de alterações na composição da película adquirida em função da sua localização na cavidade bucal: estudo proteômico

BAURU
2016

TALITA MENDES DA SILVA VENTURA

Changes in the composition of the acquired enamel pellicle according to its location in the oral cavity: proteomic study

Detecção de alterações na composição da película adquirida em função da sua localização na cavidade bucal: estudo proteômico

Dissertation presented to the Bauru School of Dentistry of the University of São Paulo to obtain the degree of Master in Science in the Applied Dental Science Program, Stomatology and Oral Biology concentration area.

Supervisor: Prof. Drª Marília Afonso Rabelo Buzalaf

Dissertação apresentada à Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Mestre em Ciências no Programa de Ciências Odontológicas Aplicadas, área de concentração Estomatologia e Biologia Oral.

Orientadora: Prof. Drª Marília Afonso Rabelo Buzalaf

BAURU
2016

Ventura, Talita Mendes da Silva

V567c Changes in the composition of the acquired enamel pellicle according to its location in the oral cavity: proteomic study / Talita Mendes da Silva Ventura – Bauru, 2016.

109 p. : il. ; 31cm.

Dissertação (Mestrado) – Faculdade de Odontologia de Bauru. Universidade de São Paulo

Orientadora: Prof. Drª Marília Afonso Rabelo Buzalaf

Comitê de Ética da FOB-USP

Protocolo nº: 48094715.0.0000.5417

Data: 21/10/2015

FOLHA DE APROVAÇÃO

DEDICATÓRIA

À Deus,

Pelo amor incondicional, por estar presente constantemente em minha vida, por ser minha luz, meu refúgio, por não me abandonar nos momentos difíceis, por me mostrar os caminhos a seguir e me conduzir quando muitas vezes o fardo tornou-se árduo, por ser o Senhor em todos os sentidos da minha vida.

"Tudo posso naquele que me fortalece".
Filipenses 4,13

Aos meus queridos pais **Orí** *e* **Germana**,

Por sempre lutarem para me proporcionar uma boa educação, por me ensinarem a sonhar e, além disso, ser exemplos de como conquistar nossos sonhos da melhor maneira possível, colocando Deus acima de tudo. Vocês são o meu alicerce e porto seguro. A vocês, minha eterna gratidão. Sinto-me abençoada por ter vocês como meus pais.

Ao meu único irmão **João Vitor**,

Que sempre me apoiou em cada dificuldade, por sempre acreditar em mim. Passamos por muitas coisas juntos, sorrimos, choramos, ajudamos, lutamos, vencemos, perdemos e sempre juntos. Obrigada por ser o melhor irmão e por nossa amizade, meu amor por você é imensurável.

Ao meu noivo **Caío**,

Pelo amor sincero, por me apoiar em todos os momentos, por não medir esforços para me ver feliz. Por estar sempre perto, mesmo quando a distância nos deixa longe, pelo amor, carinho, respeito, compreensão nos meus momentos de ausência e de estresse, quando me amou ainda mais, mesmo quando tudo era tempestade, pela preocupação e dedicação de sempre.

AGRADECIMENTOS

À Deus,

Por me proporcionar nascer em uma família maravilhosa, com muito amor, princípios e fé. Por colocar pessoas maravilhosas em meu caminho, onde na ausência dos meus pais foram também a minha família em Bauru. Obrigada por ser o Senhor da minha vida. Ele é o maior responsável por essa conquista, sendo minha força, me concedendo sabedoria, esperança, serenidade e, além disso, saúde, para que eu conseguisse vencer mais uma etapa da mina vida. Eu te amo Senhor, Tu és o meu tudo.

Aos meus pais **Ori** e **Germana,**

Como é difícil encontrar palavras que descrevam tamanha gratidão e felicidade. A vocês, que a cada derrota, me encheram de força e coragem para continuar. E em cada prova da vida aguentaram a minha tensão e meus dias difíceis. Além de pais, foram amigos e fiéis companheiros, mesmo quando meus ideais pareciam distantes e inatingíveis, quando ninguém mais acreditou em mim, vocês acreditaram e vibraram de alegria comigo quando tudo foi alcançado. A vocês que muitas vezes choraram pela minha ausência e por toda a preocupação, mas ainda assim me encheram de tranquilidade e segurança. Iluminaram o meu futuro com o que ninguém jamais poderá tirar: o estudo. A vocês que muitas vezes sacrificaram os seus sonhos a favor dos meus. Acredito que de todas as palavras, as mais sinceras e simples são essas: eu não sou nada sem vocês! Obrigada por me ensinarem a desejar, a buscar e a ter esperança sempre, de que posso realizar todos os meus sonhos. Obrigada por cada oração, por me dar um lar cheio de amor, uma verdadeira sagrada família. Obrigada por me darem a alegria de ter os melhores pais do mundo. Vocês sempre serão os meus maiores exemplos em tudo. Amo vocês incondicionalmente!

Á meu querido irmão **João Vitor**,

Obrigada por todos os conselhos, por ser mais que um irmão, um verdadeiro amigo que sei que estará comigo em todos os percursos da minha vida. Às vezes a distância tenta nos afastar, mas ao mesmo tempo estamos unidos pelo coração.

Levo-te comigo sempre, no meu coração e nas minhas orações. Tenho orgulho de ser sua irmã e de ter me espelhado em você, por sua garra, perseverança, fé, por ser essa pessoa maravilhosa e de um coração imenso, acho que um dos maiores que já conheci. Eu te admiro e amo muito, você é um pedacinho de mim.

Ao meu noivo **Caio**,

Obrigada por ser mais que um noivo. Obrigada por ser meu amigo, companheiro, confidente, que vibra por mim, que não mede esforços para me ver feliz. Você que me ouviu em cada dificuldade deste trabalho, que rezou por mim e não me fez desanimar quando tudo parecia não ter mais respostas. Obrigada por não me fazer desistir, por acreditar em mim, por ser tão carinhoso. Eu te admiro muito por quem você é, você é uma pessoa maravilhosa, o meu presente de Deus! Obrigada por me ensinar que não existe amor sem sacrifícios. Eu te amo.

A minha queria e amada avó **Altamira** (*in memorian*),

Que sempre foi um referencial de força e garra para mim. Obrigada por me ensinar a lutar, acreditar e me incentivar sempre. Hoje meu coração se enche de saudades, mas também só de lembranças boas. A alegria que tinha em viver, me faz querer ser diferente todos os dias. Te amo muito avozinha, eu sei que o céu é o teu lugar!

À minha cunhada **Natália**,

Ná, obrigada pela sua amizade. Obrigada pelas alegrias que trouxe para nossa família, por ser tão atenciosa, carinhosa e amiga. Agradeço a Deus por ter você em nossas vidas.

À minha segunda família, minha sogra **Izilda**, meu sogro **Marcos** e minha cunhada **Gabriela**,

Obrigada por me darem força quando eu achei que tudo estava tão inatingível, obrigada pelas orações e por tanto amor. Obrigada por me ajudarem a crescer na minha fé, pelos momentos de descontração, que muitas vezes me fizeram esquecer as dificuldades pelo caminho. Eu só tenho que agradecer a Deus por ter vocês em minha vida! Amo vocês de todo coração.

À toda minha **Família**,

Que simplesmente por se orgulharem de mim, me fizeram sentir capaz de ir além. Obrigada pelo carinho e pelo incentivo. Espero poder ainda dividir com vocês muitas conquistas. Amo cada um!

As minhas queridas amigas, **Mariane Dias**, **Bianca Muniz**, **Kamila Cardoso** e **Luana Antonelli**,

Obrigada pelos momentos de descontração, pelos conselhos, pelos puxões de orelha e por vibrarem comigo em cada conquista, mas também por me apoiarem em cada derrota, por todo amor. Dez anos de amizade não é para qualquer um (risos). Obrigada por estarem presentes em todos os momentos da minha vida. Amo vocês, meu quinteto!

À minha amiga e companheira de casa **Cláudia**,

Claudinha, obrigada por ser minha família aqui em Bauru. Por compartilhar momentos tão alegres, mas também os momentos difíceis. Nunca vou me esquecer dos momentos de dificuldades que passamos juntas, por estar longe de casa, mas sermos irmãs e demonstrar em todo momento que temos uma a outra. Por entender as vezes que me trancava no quarto para estudar e muitas vezes não te dar nem boa noite, preocupada com os seminários e experimentos. Obrigada não só por dividir o apartamento comigo, mas também por dividir sua vida e seu coração.

A minha querida amiga **Mariane Dias**,

Mari, minha amiga, ou melhor, minha irmã. Obrigada por tudo o que é para mim, você que ouviu cada choro e muitas vezes acabou guardando suas lágrimas para enxugar as minhas, você que me encorajou em cada etapa desse mestrado, mas também em cada caminho da minha vida. Mesmo longe, se fez e se faz tão presente. Obrigada por tanto amor, por tanto carinho. Por ser aquela amiga que diz verdades, mesmo quando essas podem doer por alguns momentos, mas que são necessárias. Você é um carinho de Deus na minha vida, não tenho palavras para explicar tamanha admiração e carinho que tenho por você. Eu te amo amiga e estarei aqui do seu lado, para todo o sempre.

Aos meus **amigos do Ministério Universidades Renovadas** e do **Grupo de Oração Arca de Noé**, em especial, **Letícia, Augusto, Diana, Larissa, Isabela, Ana Enilda, Eduarda, Heliton, Elton, André, Cidinha e Fernando**. Obrigada por ser essa família linda que Deus me deu, pelos momentos de oração, alegria e missão. Sei que posso contar com vocês em qualquer momento da minha vida, vou leva-los sempre no meu coração e nas minhas orações por onde eu for. Amo cada um de vocês, obrigada por me ajudarem a conhecer um amor tão lindo, um amor que vem de Deus.

Aos meus **queridos colegas** e **amigos do Laboratório de Bioquímica**,
Cíntia Tokuhara, Mariana Santesso, Aline Dionízio, Isabela Tomazini, Aline Braga, Beatriz Souza, Tatiana Martini, Éven Taira, Luiza Cassiano, Vinícius Taioqui, Daiana Moreli, João Paulo Domezi, Adriana Matos, Flávia Amadeu, David Dezidério, Maria Aparecida, Juliana Pires, Juliana Trevizol, Daniela Komatsu, Mileni Fernandez, Cíntia Souza, Carlos, Zohaib, Priscila Salomão, Polliana Scaffa, Senda Charone, Flávia Levy, Flávia Iano, Heloisa Pereira, Amanda Amaral, Cristiane Baldini e Gabriela Neubern. Obrigada pelos momentos que passados juntos no laboratório, por compartilharem suas experiências acadêmicas e de vida. Por cada disciplina juntos, pela torcida dos experimentos e pelos momentos de descontração. Ter vocês nessa caminhada tornou-se tudo mais leve! Família Bioquímica, vocês estarão sempre em meu coração.

Aos **amigos da cultura celular** e **funcionários do Centro Integrado de Pesquisa - CIP**,

Pepe Burgos, Karem Pinke, Cintía Tokuhara, Heliton Lima, Amanda Amaral, Gabriela Neubern, Mariana Santesso, Flávida Amadeu e Adriana Arruda, foi junto com vocês que dei os primeiros passos na pesquisa, companheiros de laboratório e fluxo laminar, obrigada por me ajudar a crescer como profissional desde a iniciação científica e por todos os momentos de descontração que passamos juntos.

À querida amiga e orientadora de Iniciação Científica **Camila Peres Buzalaf**,

Camila, lembro como se fosse hoje a primeira vez que fui ao Laboratório de Bioquímica. Lembro que quando cheguei, sabia que era ali onde queria estar, e lá se foram quase cinco anos no laboratório, em meio a estágios, iniciação científica, mestrado e muito aprendizado. Obrigada por ter sido e por ser tão maravilhosa, por todo apoio que sempre me deu desde daquele primeiro dia, por toda paciência em me ensinar cada detalhe, cada técnica com a maior paciência e brilhantismo do mundo. Vou te levar comigo sempre, pois foi com você que aprendi o que é ter amor pela profissão. Obrigada pelos conselhos, pelos puxões de orelha, que foram necessários para meu crescimento. Obrigada por não ter me deixado desistir quando achei que tudo estava perdido, por ter sido essa orientadora maravilhosa e amiga, que me conduziu nos melhores caminhos. Serei eternamente grata a você!

À queria amiga de pesquisa **Cíntia**,

Obrigada por todos os ensinamentos, pelo companheirismo, por lutar comigo em cada protocolo dessa pesquisa, por chorar comigo, mas também por vibrar em cada conquista. Obrigada por cada conselho e paciência por me explicar assuntos tão específicos em relação à área da odontologia, onde muitas vezes me sentia um “peixe fora d’agua” (risos). Obrigada por tudo sempre, mas principalmente pela amizade que construímos.

À querida amiga de pesquisa **Luiza Cassiano**,

Lú, quem diria que hoje nos tornaríamos tão próximas assim. Só tenho que agradecer a Deus por ter te colocado no meu caminho, obrigada por me ajudar nas coletas, tabelas, picotar papeis e tantos outros obstáculos que encontrados durante este trabalho. Obrigada pelos momentos de risada, conversas, que tornou tudo mais fácil. Sei que nossa amizade vai além dos consultórios e bancadas do laboratório. Obrigada pela sua amizade e pelo apoio de sempre. Te levo com muito carinho no meu coração.

À querida amiga de pesquisa **Éven Taira**,

Éven, obrigada por toda ajuda na realização deste trabalho. Pelos dias incansáveis de coletas, por toda ajuda em picotar aqueles papéis infinitos e por todo zelo que sempre teve. Estarei sempre aqui para quando precisar.

À queria especialista do Laboratório de Bioquímica e amiga **Aline Leite**,

Line, minha gratidão por você sempre será imensurável. Posso dizer que tudo o que aprendi em relação às técnicas do proteoma, você tem um papel fundamental, obrigada por cada explicação, cada hora e hora passada na bancada, por cada conversa, conselhos, aprendizados e trocas de experiências. Por segurar as barras, por ter assumido o fardo desse protocolo junto conosco, por não me deixar desistir, por ser tempestade quando necessário, mas calmaria nas horas de desespero, por me deixar chorar na bancada ao seu lado quando tudo parecia perdido, por me dar chocolates e dizer que tudo daria certo. Li, vou te levar comigo para qualquer lugar que for, pois seus ensinamentos, sempre estarão gravados em mim. Obrigada por tanto carinho, por atender as ligações desesperadas em meio aos experimentos e por retirar cada dúvida que surgia. Você é um anjo, com seu jeitinho Aline de ser. Peço a Deus que abençoe seus caminhos sempre, te desejo todo sucesso do mundo!

Á minha querida amiga **Isabela Tomazini**,

Isinha, obrigada por ser essa pessoa maravilhosa. Obrigada por ser uma verdadeira amiga, por me ouvir nos momentos difíceis, pelos conselhos, pelas mensagens, ligações que me acalmavam diante de tudo e me dava coragem para enfrentar os obstáculos. Você é um presente de Deus para mim! Eu te amo e estarei sempre aqui.

A minha querida amiga **Aline Dionizio**,

Line, obrigada pela sua amizade. Por ser tão atenciosa e cuidadosa comigo. Pelas ligações, mensagens, conselhos, pelas risadas e por enfrentar comigo todos os obstáculos do mestrado, por cada disciplina que fizemos juntas, cada dúvida que surgia ao longo do caminho e por me ajudar em cada detalhe dessa trajetória. Você é muito especial para mim. Sua amizade me dá forças!

Aos amigos de graduação **Geisa Dias**, **Rodrigo Almeida**, **Mariana Santesso e Juliana Mauad,**

Obrigada por serem companheiros e amigos desde a graduação. Foi com vocês os meus primeiros passos na profissão. Sonhamos e lutamos juntos por nosso lugar ao sol. Eu torço por vocês e peço para Deus abençoar a vida de cada um. Vocês são muito importantes e especiais para mim! Amo muito vocês.

Ás técnicas e especialistas do Laboratório de Bioquímica, **Larissa, Thelma e Aline**,

Por todo conhecimento, atenção e presteza. Sempre dispostas a ajudar. Obrigada por compartilharem seus conhecimentos durante todos esses anos no laboratório, levarei cada ensinamento comigo, pois me fizeram crescer como profissional. Muito obrigada!

Aos professores da Disciplina de Bioquímica, **Professor Dr. Rodrigo Cardoso de Oliveira** e **Professora Dra. Ana Carolina Magalhães**,

Professores, muito obrigada por cada ensinamento compartilhado, pelos conselhos repassados a nós, por se mostrarem tão competentes e atenciosos. Por cada conselho também apresentados nas disciplinas, vocês me fizeram crescer como profissional e levarei todos eles por toda a vida.

A **Universidade Paulista – UNIP**, todos os funcionários e professores, em especial,

Á **Professora Dra. Patrícia Carvalho Garcia**, à **Profa. Michele Janegitz Acorci Valerio**, ao **Professor Dr. Renato Massaharu Hassunuma**, à **Bióloga Tatiane Andrea Lionete,** à **Biomédica Lívia Ferreira dos Santos** e a funcionária **Helen Thiago**. Serei eternamente grata por todos os ensinamentos compartilhados desde a graduação, por ter a honra de viver uma experiência única de lecionar nessa grande instituição, que me fez crescer na minha vida profissional e pessoal. Eu tenho o maior orgulho em dizer que me formei no melhor curso de Biomedicina do Brasil.

Aos minhas **colegas de Pós-graduação**, por todo apoio em cada disciplina e por compartilharem suas experiências acadêmicas.

As secretárias do Departamento **Dalva Ribeiro de Oliveira** e ex-secretária **Vera Lucia Rufino**,

Obrigada por toda atenção dedicada a nós alunos, a presença de vocês são imprescindíveis nas nossas vidas. Obrigada por toda paciência e presteza de sempre.

Às secretárias da Pós-graduação **Fátima, Leila, Letícia, Meg e Maristela**,

Pela atenção em responder todas as dúvidas, pela disposição de responder a e-mails, telefonemas e ajudar com assuntos relacionados à documentação, prazos e relatórios. Muito obrigada pela presteza de sempre!

Aos **voluntários** desta pesquisa,

Vocês foram fundamentais para que esse trabalho fosse realizado. Sem vocês nada seria possível, serei eternamente grata a cada um de vocês. Obrigada por terem aceitado fazer parte da pesquisa, por toda dedicação e carinho que demonstraram. Muito obrigada!

Ao **Professor Dr. Carlos Ferreira dos Santos**,

Por abrir as portar da clínica do laboratório de farmacologia para que as coletas fossem realizadas. Agradeço todos os alunos, funcionários e professores, por toda atenção e esforço em nos ajudar com agendamentos de horários e as entradas e saídas da clínica.

À **Faculdade de Odontologia de Bauru- FOB/USP**, na pessoa da diretora **Prof.ª Drª Maria Aparecida Moreira Machado,**

É um honra poder realizar a pós-graduação em nível de Mestrado nesta grande instituição, que nos dá total suporte e estrutura indiscutível. Obrigada FOB-USP por tudo que me proporcionou até hoje.

Á todos os **funcionários da FOB-USP** que colaboraram de forma direta ou indireta no desenvolvimento deste trabalho, que contribuíram para que a minha trajetória pelo mestrado se tornasse mais alegre e leve.

À **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES),** pela concessão da minha bolsa de Mestrado, importante para meu aprimoramento profissional e pessoal.

"O que somos é presente de Deus, no que nos transformamos é o nosso presente a ele".

São João Bosco

AGRADECIMENTOS ESPECIAIS

Agradeço à minha orientadora **Profª Drª Marília Afonso Rabelo Buzalaf,**

Ainda me lembro com muita clareza quando entrei na sua sala com tamanha timidez para conversamos sobre o projeto de mestrado, naquele momento a senhora disse sobre um trabalho relacionado com película adquirida, minha reação por não ser dentista e não ter o menor conhecimento sobre o assunto, uma vez que minha experiência na iniciação científica era em uma área totalmente diferente, lembro que pensei: “Película Adquirida, o que é isso?” (risos), mas naquele momento a senhora me encorajou e disse que eu seria capaz, a partir de então entendi o porquê da senhora ser essa luz na vida das pessoas ao seu redor.

Professora, tudo isso mostra o quanto eu cresci desde então. Obrigada por confiar no meu trabalho e acreditar na minha competência. Obrigada por ser essa pessoa fantástica, na qual não mede esforços para ver seus alunos crescerem. Obrigada por toda paciência, carinho e dedicação ao ensinar. Obrigada por me receber em sua sala tão prontamente todas às vezes, por responder e-mails e mensagens, mesmo com tantos alunos e compromissos. Apesar dos grandes títulos, premiações e grande conhecimento que possui, está sempre de portas abertas, literalmente, para nos receber. Obrigada por todos os ensinamentos que servirão para a vida toda e que não se resumem somente na vida profissional, mas também ao pessoal. Conviver com a senhora é presenciar seu brilhantismo na área cientifica, sua excelente capacidade de raciocínio, senso critico em discutir resultados e acima de tudo, transbordando amor em tudo que faz. Obrigada por me confiar um trabalho tão complexo e tão valioso, por me incentivar em cada obstáculo, cada piloto, cada decepção, mas também por vibrar comigo em cada conquista e obstáculo que vencemos. Obrigada por este trabalho, pois tive um crescimento profissional e pessoal incomparável! Eu te admiro e a senhora para mim é uma inspiração, esse amor no qual coloca em cada projeto seu só poderia ter como resultado grandes sucessos. Eu agradeço a Deus todos os dias por ter colocado a senhora em meu caminho. Que Jesus e Nossa Senhora te abençoem imensamente! Tê-la como orientadora, só pode ter sido presente de Deus. É uma grande honra dizer que sou sua aluna e poder trabalhar ao seu lado, sua humildade e simplicidade a torna totalmente merecedora por estar onde esta. Obrigada por tudo, sempre!

"O que eu faço é uma gota no meio de um oceano.
Mas sem ela, o oceano será menor".

Madre Teresa de Calcutá

ABSTRACT

Changes in the composition of the acquired enamel pellicle according to its location in the oral cavity: proteomic study

The acquired enamel pellicle (AEP) is a bacteria-free organic film formed in vivo as a result of selective adsorption of salivary proteins on the surface of the tooth. It also contains glycoproteins and lipids. The presence of proteins in the AEP forms a protective interface on the tooth surface that participates in the interfacial events that occur in the oral cavity. The objective of this study was to detect changes in the protein profile of the AEP formed in vivo according to its location in the dental arches. Nine volunteers, aged 18 to 35 years, non-smokers, with good general and oral health participated in the study. The acquired pellicle was formed in the morning, for 120 minutes, after prophylaxis with pumice. Pellicle formed at upper and lower anterior labial (ULALa; teeth 13-23 and 33-43), upper anterior palatal (UAPa; teeth 13-23), lower anterior lingual (LALi; teeth 33-43), upper and lower posterior labial (ULPLa; teeth 14-17 24 to 27, 34 to 37 and 44 to 47), upper posterior palatal (UPPa; teeth 14 to 17 and 24 to 27) and lower posterior lingual (LPLi; teeth 34 to 37 and 44 to 47) regions was collected separately for analysis. After its formation, the pellicle was collected with filter paper soaked in 3% citric acid and processed for analysis by LC-ESI-MS/MS. The MS/MS spectra obtained were compared with human protein databases (SWISS PROT). Label-free quantification was done using the PLGs software. A total of 363 proteins were found, of which 252 are unique proteins for one of the regions, while 25 proteins care to all of them, including Protein S100-A8, Lysozyme C, Lactoferrin, Sthatherin, Ig alpha-2 chain C, ALB protein, Myeloperoxidase and Submaxillary gland androgen-regulated protein 3B. In the quantitative analysis, 9 comparisons were made and many proteins were differently expressed among the groups, thus demonstrating that the location in the dental arches can change the composition of the AEP. Some proteins not previously found in the AEP were identified and their function in the AEP was inferred from the literature. In conclusion, the composition of the AEP changes as a function of its location in the dental arches. These data should be taken into account when we think

about the protective potential of the acquired pellicle against tooth demineralization and provide important insights for understanding the differential protective roles of the AEP as a function of its location in the dental arches.

.

Keywords: Proteomics. Acquired Pellicle. Enamel. Saliva. Protein. Dental Arches.

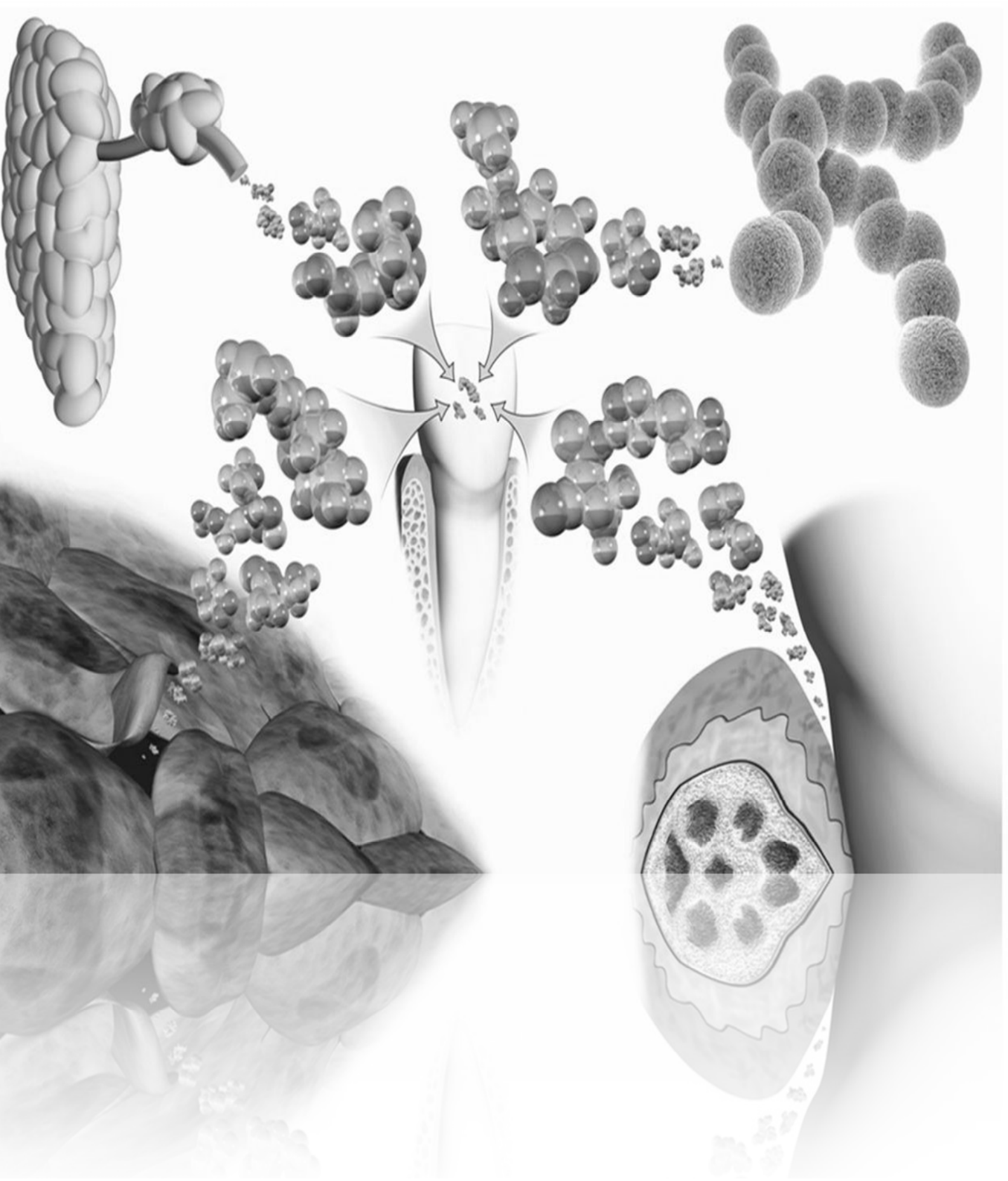
RESUMO

A película adquirida do esmalte (PAE) é um filme orgânico, livre de bactérias, formado *in vivo* como resultado da adsorção seletiva de proteínas salivares sobre a superfície do dente, contendo também glicoproteínas e lipídeos. A presença de proteínas na PAE forma uma interface protetora sobre a superfície do dente, participando em todos os eventos interfaciais que ocorrem na cavidade bucal. O objetivo deste trabalho foi detectar alterações no perfil proteico da PAE formada *in vivo* de acordo com a sua localização nos arcos dentários. Fizeram parte da pesquisa 9 voluntários, com idade entre 18 e 35 anos, não fumantes, com bom estado de saúde geral e bucal. A película adquirida foi formada no período da manhã, por 120 minutos, após profilaxia com pedra pomes. Películas formadas nas regiões anterior vestibular superior e inferior (ULALa; dentes 13-23 e 33-43), anterior palatina superior (UAPa; dentes 13-23), anterior lingual inferior (LALi; dentes 33-43), posterior vestibular superior e inferior (ULPLa; dentes 14-17, 24-27, 34-37 e 44-47), posterior palatina superior (UPPa; dentes 14-17 e 24-27) e posterior lingual inferior (LPLi; dentes 34-37 e 44-47) foram coletadas separadamente para análise. Após a sua formação, a película foi coletada em papel filtro embebido em ácido cítrico a 3% e processada para análise por LC-ESI-MS/MS. Os espectros MS/MS obtidos foram confrontados com bases de dados de proteínas humanas (SWISS PROT). A quantificação livre de marcadores foi feita utilizando o software PLGS. Um total de 363 proteínas foi encontrado, sendo 252 proteínas únicas de cada grupo e 25 proteínas comuns entre eles (como Protein S100-A8, Lysozyme C, Lactoferrin, Sthatherin, Ig alpha-2 chain C, ALB protein, Myeloperoxidase and Submaxillary gland androgen-regulated protein 3B). Na análise quantitativa, nove comparações foram realizadas e muitas proteínas foram diferentemente expressas entre os grupos, demonstrando assim que a localização na cavidade bucal pode alterar a composição da película adquirida do esmalte. Foram encontradas tanto proteínas típicas da película quanto proteínas não anteriormente descritas na película, cuja função na película foi inferida com base na literatura. Em conclusão houve diferença na composição proteica da película adquirida de acordo com a localização dos arcos dentários. Esses dados devem ser levados em conta quando se pensa no potencial protetor da película adquirida contra a desmineralização dentária, uma vez que

esses resultados fornecem informações importantes para a compreensão dos diferentes papeis protetores da AEP dependendo da sua localização nos arcos dentários.

Palavras-chave: Proteômica. Película Adquirida. Esmalte Dentário. Saliva. Proteínas. Arcos Dentários.

TABLE OF CONTENTS



1-INTRODUCTION

1 INTRODUCTION

The acquired enamel pellicle (AEP) is an organic, acellular, protein-rich film with unique composition and properties. Its formation occurs by selective adsorption of proteins originated from glandular secretions, microorganisms, oral mucosa and gingival crevicular fluid on the surface of the enamel (JENSEN; LAMKIN; OPPENHEIM, 1992; SIQUEIRA; CUSTODIO; MCDONALD, 2012; SIQUEIRA; OPPENHEIM, 2009; YAO et al., 2003; YAO et al., 2001). The pellicle was first described in 1839 by Alexander Nasmyth, who claimed that the it was like an organic film, which was presented as a delicate membrane on the enamel surface (NASMYTH, 1839).

This membrane has been described as a "persistent dental capsule" and a long time ago it was believed that it was of embryonic origin. After much investigation, in 1968 Amostrong showed that AEP is a complex of a salivary mucoproteinaceous matrix of submandibular gland origin, which is formed at the interface between the teeth and the salivary environment, with important biological and clinical interest (ARMSTRONG, 1968). Thus, AEP was shown as an organic film that covers all surfaces of the teeth, and it became evident that this membrane is formed after tooth eruption, reason why it is called “acquired pellicle”. The AEP structure can be influenced by factors such as oral microbiota composition, chemical and physical properties of the tooth surface, different locations of formation, circadian cycles and proteolytic capacity of the oral environment (LENDENMANN; GROGAN; OPPENHEIM, 2000).

The AEP forms a protective interface between the tooth surface and the oral cavity, reducing the friction and abrasion by acting as a semipermeable barrier. AEP has a role of contribute in the mineralization/demineralization processes, modulating mineral precipitation and adherence of microorganisms into the tooth surface (HANNIG; JOINER, 2006; LENDENMANN et al., 2000). It also lubricates the tooth surface, reducing friction between the teeth and the mucosa, participates in bacterial adhesion, being very important in the maintenance of tooth integrity by contributing the dynamic dissolution of the enamel surface and giving a certain resistance and stability to chemical dissolutions and to attacks by acid agents (HANNIG; JOINER, 2006; HARA; ZERO, 2010; LENDENMANN et al., 2000).

The precursor proteins of the pellicle are derived from glandular secretions, such as the smaller glands, but mainly parotid, submandibular gland and sublingual glands, as well as gingival crevicular fluid, secretions from the nasal cavity, blood, mucosa and epithelium which comprise the whole saliva (VAN NIEUW AMERONGEN; BOLSCHER; VEERMAN, 2004). Among the main components identified are the proteins and glycoproteins, but carbohydrates, neutral lipids, phospholipids and glycolipids are also found (HANNIG; JOINER, 2006; HARA; ZERO, 2010).

The formation of the AEP is characterized by stages. In the first stage, the spontaneous adsorption of salivary proteins on the teeth surface occurs. The calcium ions of the enamel crystals, in contact with whole saliva, tend to dissolve. As a result, the remaining phosphate ions give a negative charge to this surface, which is then coated with a layer of positively charged calcium ions dissolved. Thus, this electropositive surface interact with the electronegative proteins (HANNIG; JOINER, 2006). At this stage, phosphoproteins with high affinity such PRP-3, PRP-4 as well as Statherin, instantly adsorb. These proteins are capable of interacting with the calcium and phosphate ions from enamel via ionic interactions, van der Waals and hydrophobic interactions. In the second step, slower interaction with hydroxyapatite occurs. Proteins participating in this step are α-amylase, PRPg and cystatin (HANNIG; JOINER, 2006; LAMKIN; ARANCILLO; OPPENHEIM, 1996). In the third stage there is a continuous adsorption of saliva biopolymers. It also occurs interaction between the salivary proteins and precursor proteins of the primary pellicle, which is a more complex phase of rapid adsorption, followed by a slower phase. The proteins that participate in that last step are PRP-1, PRP-2 and Histatin (HANNIG; JOINER, 2006; LAMKIN et al., 1996). Studies suggest that this phase does not reach its peak at 2 hours, showing that the formation of AEP is not complete at this time (HANNIG; JOINER, 2006; LAMKIN et al., 1996). The equilibrium then occurs close to 2 hours of pellicle formation and bacteria can be found only after 4 hours (HANNIG; JOINER, 2006; LENDENMANN et al., 2000) (FIGURE 1).

In vitro studies showed that the thickness of the AEP varies within the dental arch and tooth surface, being thinner in the palatal face anterior maxillary teeth and thicker on the lingual surface of the lower posterior teeth (AMAECHI et al., 1999). In the lower arch, when the thickness of the AEP formed on the the labial and lingual surfaces is compared, the pellicle is significantly thicker on the lingual surface of

anterior and posterior teeth. However, in the upper arch, the pellicle formed on the palatal surface of the anterior teeth is significantly thinner than that formed on the labial regions. The same applies to the posterior teeth (HANNIG, 1999). The difference in thickness of the AEP according to the dental arch and tooth surface is justified by several factors. First, the dorsum of tongue is keratinized and therefore more abrasive than its venter. Thus, the dorsum of the tongue may limit the thickness of AEP in the palatal surface of the upper teeth and be partly responsible for a thinner pellicle in this region. This fact does not occur in the lower arch. Moreover, the presence of thicker AEP on the lingual aspect of lower teeth may be because this region is constantly irrigated by saliva from the submandibular and sublingual glands, which secrete mucin that is one of the salivary proteins that is present in the AEP (AMAECHI et al., 1999; HANNIG, 1999). Therefore, the pellicle thickness varies significantly between the dental arches, and can be interindividual variation, which could explain the site-specificity of dental erosion, as a significant inverse relationship is observed between the pellicle thickness and the degree of erosion (AMAECHI et al., 1999). Dental erosion shows a typical distribution pattern between dental arches, and the palatal and occlusal surfaces most commonly affected (BARTLETT et al., 1998; HOUSE et al., 1981; JAEGGI; LUSSI, 2014; JARVINEN; RYTOMAA; MEURMAN, 1992; ROBB; SMITH; GEIDRYS-LEEPER, 1995; SCHEUTZEL, 1996). This pattern occurs both adults and children (JAEGGI; LUSSI, 2014; MILLWARD et al., 1994; MILOSEVIC; YOUNG; LENNON, 1994). The study by Amaechi et al. (1999) indicates that the pellicle thickness is as a determining factor in dental erosion, since a thicker pellicle (1.06 μm) is observed in the lower lingual surfaces, while thinner pellicles (0.3 μm) were found in palatal anterior superior region, which are the area most commonly affected by dental erosion, as described above.

Due to their intimate contact with the tooth surface, AEP is of great value to maintain tooth integrity (HANNIG; JOINER, 2006; SIQUEIRA et al., 2007). Due to this, it is considered of great importance and biological and clinical interest (ARMSTRONG, 1968). However, the precise mechanism that regulates the diffusion of ions from the enamel surface and the oral environment in the presence of AEP is not yet completely understood (HANNIG; JOINER, 2006). Therefore, knowledge about the formation, composition and function of the AEP can clarify the process of adsorption of salivary proteins on the tooth surface, as well as the demineralization/remineralization processes and of bacterial adhesion and

antimicrobial activity. Limitations due to the small amount of material that can be collected from the tooth surface slow down the progress of research on this topic. However, with the development of sensitive proteomic techniques in recent years, it was possible to characterize the organic material such as proteins and peptides in the AEP (SIQUEIRA et al., 2007). Thus, considering that one of the main functions of the AEP is the protection against demineralization of enamel, it is of great importance to study the role of its components in this process.

The proteomic studies have a major advance in understanding the protein composition of AEP, as well as the impact of different proteins found in the pellicle in the prevention of caries and dental erosion (DELECRODE et al., 2015; LEE et al., 2013; SIQUEIRA; OPPENHEIM, 2009; SIQUEIRA et al., 2007; VITORINO et al., 2007; VUKOSAVLJEVIC et al., 2014; ZIMMERMAN et al., 2013). The proteomic analysis is a set of tools used to characterize quantitatively and qualitatively a set of proteins expressed by a given genome, and this set of proteins was called proteome (WILKINS et al., 1996).

However, all the results related to this analytical tool until now focus on the pellicle formed on the vestibular surface of teeth (DELECRODE et al., 2015; SIQUEIRA; OPPENHEIM, 2009; ZIMMERMAN et al., 2013). Knowing that the AEP is formed following direct contact with saliva and the distribution and fluid composition of salivary glands is different (HANNIG et al., 2004), it is expected that the composition of the AEP formed on other regions of the dental arches might distinct composition. However, the protein composition of the pellicle formed at different sites in the dental arches has never been studied using proteomic approaches. Proteomic studies in pellicles formed in different regions of the dental arches can provide important information about the proteins that are related to greater protection against erosion and caries depending on the forming site of the AEP. Thus, this knowledge becomes important so that we can better understand the site-specificity of dental caries and erosion and possibly identify potentially acid-resistant proteins that can be added to dental products in the future, aiming to enrich the AEP with them for a better protection against dental caries and erosion.

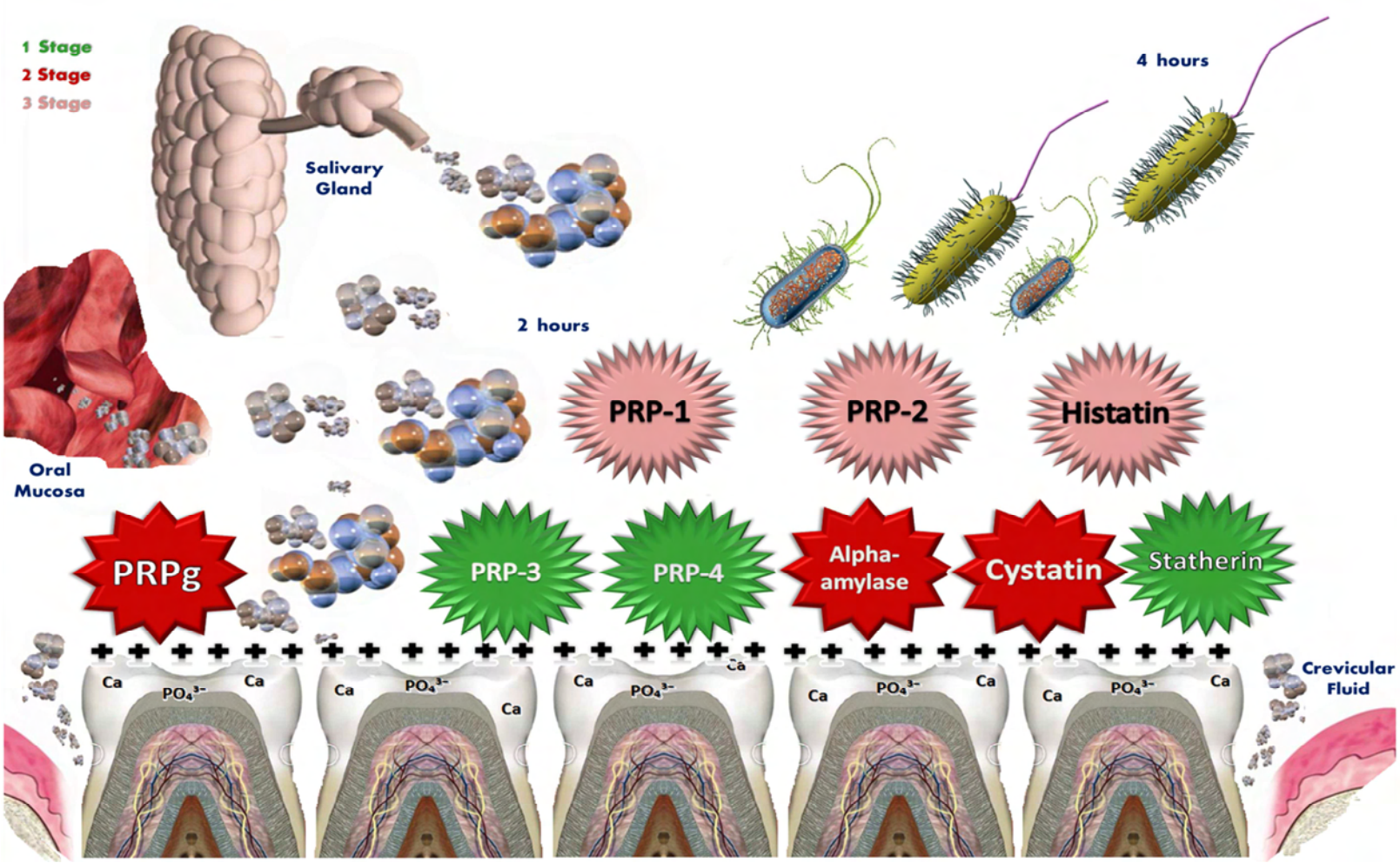
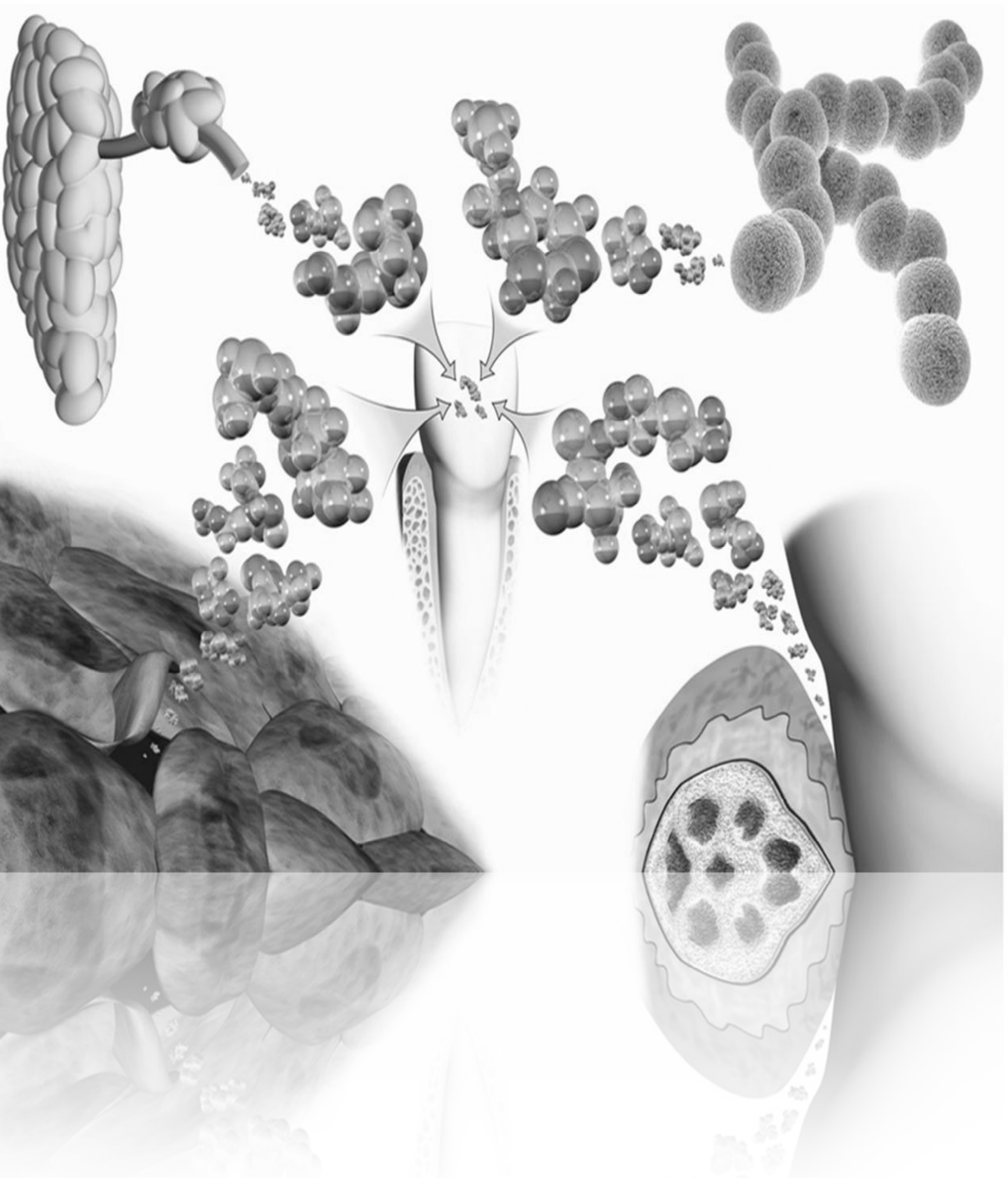


Figure 1. Stages of formation of the acquired enamel pellicle. In the first stage (green), the spontaneous adsorption of salivary proteins on the teeth surface occurs. The calcium ions of the enamel crystals, in contact with whole saliva, tend to dissolve. As a result, the remaining phosphate ions give a negative charge to this surface, which is then coated with a layer of positively charged calcium ions dissolved. Thus, this electropositive surface interact with the

electronegative proteins (HANNIG; JOINER, 2006). At this stage, phosphoproteins with high affinity such PRP-3, PRP-4 as well as Statherin, instantly adsorb. These proteins are capable of interacting with the calcium and phosphate ions from enamel via ionic interactions, van der Waals and hydrophobic interactions. In the second step (red), slower interaction with hydroxyapatite occurs. Proteins participating in this step are α-amylase, PRPg and cystatin (HANNIG; JOINER, 2006; LAMKIN; ARANCILLO; OPPENHEIM, 1996). In the third stage (pink) there is a continuous adsorption of saliva biopolymers. It also occurs interaction between the salivary proteins and precursor proteins of the primary pellicle, which is a more complex phase of rapid adsorption, followed by a slower phase. The proteins that participate in that last step are PRP-1, PRP-2 and Histatin (HANNIG; JOINER, 2006; LAMKIN et al., 1996). Studies suggest that this phase does not reach its peak at 2 hours, showing that the formation of AEP is not complete at this time (HANNIG; JOINER, 2006; LAMKIN et al., 1996). The equilibrium then occurs close to 2 hours of pellicle formation and bacteria can be found only after 4 hours (HANNIG; JOINER, 2006; LENDENMANN et al., 2000).
Adapted Figure: SIQUEIRA et al., 2012.



2-Article

2 ARTICLE

Article formatted according to Journal of Dentistry

The proteomic profile of the acquired enamel pellicle changes according to its location in the dental arches.

Ventura TMS, Cassiano LPS, Silva CMS, Taira EA, Leite AL, Rios D, Buzalaf MAR*

Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, SP, Brazil.

Running title: Proteomics of acquired enamel pellicle changes in the dental arches
Keywords: Proteomics; Acquired Pellicle; Enamel; Saliva.

***Corresponding author:** Present address: Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo, Bauru, SP. Al. Octávio Pinheiro Brisolla, 9-75. Bauru-SP, 17012-901, Brazil. Tel. + 55 14 32358346 Fax + 55 14 32271486 E-mail: mbuzalaf@fob.usp.br

Abstract

Objective: This study evaluated changes in the protein profile of the acquired enamel pellicle (AEP) formed *in vivo* according to its location in the dental arches. Methods: Nine subjects with good oral conditions took part in the study. The AEP was formed in the morning, for 120 minutes, after prophylaxis with pumice. Pellicle formed at upper and lower anterior labial (ULALa; teeth 13-23 and 33-43), upper anterior palatal (UAPa; teeth 13-23), lower anterior lingual (LALi; teeth 33-43), upper and lower posterior labial (ULPLa; teeth 14-17 24 to 27, 34 to 37 and 44 to 47), upper posterior palatal (UPPa; teeth 14 to 17 and 24 to 27) and lower posterior lingual (LPLi; teeth 34 to 37 and 44 to 47) regions was collected separately with filter paper soaked in 3% citric acid and processed for analysis by LC-ESI-MS/MS. Results: A total of 363 proteins were identified in the AEP collected from all the regions. Twenty-five proteins were identified in all the locations, such as Protein S100-A8, Lysozyme C, Lactoferrin, Sthatherin, Ig alpha-2 chain C, ALB protein, Myeloperoxidase and Submaxillary gland androgen-regulated protein 3B. Many proteins were found exclusively in the AEP collected from one of the regions (46-UAPa, 33-LALi, 59-ULALa, 31-ULPLa, 44-LPLi and 39-UPPa). Conclusion: There were differences in the protein composition of the AEP due to its location in the dental arches. These results provide important insights for understanding the differential protective roles of the AEP as a function of its location in the dental arches.

Introduction

The acquired enamel pellicle (AEP) is a bacteria-free organic layer formed in vivo as a result of selective adsorption of salivary proteins on the surface of the enamel [1]. Its formation is a dynamic process, influenced by several factors such as circadian rhythm, composition of the oral microbiota, proteolytic capacity of the oral environment, physicochemical properties of the enamel surfaces and tooth location in the oral cavity [2].

The main components identified in the AEP are proteins and glycoproteins, but carbohydrates, neutral lipids, phospholipids and glycolipids are also found [3, 4]. Each protein plays a particular function in the pellicle and is of great importance to understanding the role of this organic film [3-6]. Due to its composition, the AEP forms a protective interface between the tooth surface and the oral cavity, reducing friction and abrasion. AEP also acts as a semi-permeable barrier, which modulates the mineralization/demineralization processes, modulating mineral precipitation and adherence of microorganisms to the dental surface [3, 5, 7, 8].

The AEP is thickest on the lingual surfaces of the lower teeth, since this region is constantly bathed in saliva excreted from submandibular and sublingual glands [9]. On the other hand, it is thinnest on the palatal face of maxillary anterior teeth because these surface are exposed to shear forces from the rubbing action of the tongue and are poorly bathed in saliva [10]. Therefore, the pellicle thickness varies significantly between the dental arches, and can undergo variation among individuals, which could explain the site-specificity of dental erosion, since a significant inverse relationship is observed between the pellicle thickness and the degree of erosion [10]. Dental erosion shows a typical distribution pattern between the dental arches, and the palatal and occlusal surfaces are most commonly affected [11-16]. In addition, this pattern is repeated both in adults and children [13, 17, 18].

The proteins of the AEP are derived mainly from glandular secretions (major glands as well as minor glands) but also originate from the crevicular fluid, oral mucosa and microorganisms [4]. Saliva is the most contributor for the protein composition of the AEP [19]. Whole saliva is produced and secreted by the various salivary glands, such as the submandibular, sublingual and parotid glands [20]. Each type of salivary gland secretes a characteristic spectrum of proteins. The complete arsenal of antimicrobial proteins present in whole saliva is thus the sum of contributions from different glands [21]. The secretion of a specific gland type of saliva has a characteristic protein composition. Parotid saliva is characterized by intensely stained amylase and proline-rich protein bands, but contains minor

amounts of cystatins, lysozyme and the extra-parotid glycoprotein. Sublingual saliva is characterized by high concentrations of both types of salivary mucins, MG1 and MG2, and contain relatively high levels of lysozyme. Submandibular saliva contains the highest concentration of cystatin S. Palatine secretions contain high molecular weight mucins and a relatively high amylase concentration [22]. The submandibular and sublingual glands are located in the floor of the mouth, where the ducts empty into. Their secretion is mixed (serous and mucous), and the percentage production of each type of saliva can vary depending on the amount and type of cells that form the glands [23]. Since the ducts of different glands open into different locations in the oral cavity, this can also be a source of variation in the composition of the AEP, with impact on its protective potential.

However, the protein composition of the pellicle formed at different sites in the oral cavity has never been studied using proteomic approach. This knowledge is essential in order that we can better understand the site-specificity of dental erosion and possibly identify potentially acid-resistant proteins, which may be added to dental products in the future, in order to increase the protective ability of the AEP. Thus, this study used state-of-the-art proteomic tools to compare the protein composition of the AEP formed in different locations of the dental arches.

Materials and Methods

Ethical Aspects and Subjects

Nine young adult subjects (1 male, 8 female) took part in the study, after approval by the local Institutional Ethics Committee (No. 48094715.0.0000.54-17). Sample size was based on in vivo studies conducted with similar research protocol [24, 25]. The inclusion criteria were: non-smokers, good oral (without caries, gingivitis, periodontitis and other oral conditions that could affect the composition of oral fluids, stimulated salivary flow > 1 mL/min, unstimulated salivary flow > 0.25 mL/min; salivary pH > 6) and overall health (pregnant women, patients with systemic diseases and using chronic medication were not eligible to participate). Participants who had risk factors for dental erosion, such as excessive consumption of carbonated drinks, fruit juices or acidic fruits, swimmers, or that had gastric disorders such as bulimia and gastroesophageal reflux were excluded. Subjects signed an informed consent document prior to the beginning of the study.

AEP formation and collection

The experiment began in the morning to avoid circadian effects on the composition of the pellicle [26]. The subjects underwent a dental prophylaxis employing coarse pumice containing no additives. The volunteers waited 120 minutes deprived of food and beverage consumption, to allow the formation of acquired pellicle on enamel and to avoid possible bacterial aggregation [27]. After a period of 120 minutes, each quadrant of the mouth was rinsed and dried with compressed air twice and isolated with cotton rolls. The obtained pellicle was then collected with the aid of 5X10 mm electrodes filter paper (filter paper wick electrode, Bio-Rad, Hercules, CA) pre-dipped in 3% citric acid (pH 2.5; Sigma-Aldrich, USA). The filter paper was rubbed (without pressure) on the two-thirds coronal (to avoid contamination of the gingival margin) of the surfaces of the teeth with tweezers [27]. The dental arches were divided into 6 regions, according to the location of the ducts of the salivary glands that could contribute to the formation of the AEP. One filter paper for each of these regions was used, as follows: upper (U) and lower (L) anterior (A) labial (La) (teeth 13 to 23 and 33 to 43; ULALa), U A palatal (Pa) (teeth 13 to 23; UAPa), L A lingual (Li) (teeth 33 to 43; LALi), U and L posterior (P) La (teeth 14 to 17, 24 to 27, 34 to 37 and 44 to 47; ULPLa), UPPa (teeth 14 to 17 and 24 to 27) and LPLi (teeth 34 to 37 and 44 to 47). The wick filters were placed in 2 mL cryotubes and stored at -80 until used for proteomic analysis. The filters collected from the same region from all the participants were pooled, totalling 6 pools, corresponding to the 6 groups described above. The experiment was performed on 3 different. Samples collected each day, from each region, from each volunteer, were assembled in the same pool, in order to ensure sufficient sample to be analysed.

Preparation of the AEP samples

The Eppendorf tubes containing the filter papers were removed from the -80° C freezer. After defrost, the papers were cut into small pieces with the aid of sterile scissors and tweezers. These small pieces were put together in an Eppendorf tube, constituting a pool for each group. To the tubes containing the chopped papers approximately 400 uL (up to cover all the chopped papers) of a solution containing 6 M urea, 2 M thiourea in NH4HCO3 50 mM pH 7.8 was added, vortexed for 10 minutes at 4°C, sonicated for 5 minutes and centrifuged for 10 minutes at 14,000 g at 4°C. The supernatant was then collected and transferred to a new tube. This procedure was repeated once more for a perfect recovery of the samples. Thus,

the papers were placed in tube filters (Corning Costar®Spin-X® Plastic Centrifuge Tube Filters, Sigma-Aldrich, New York, USA) and centrifuged at 14,000 g for 10 minutes at 4°C. The supernatant was recovered and added to that previously collected. The collected supernatants were centrifuged once more at 14,000 g at 4°C, the supernatant was collected and transferred to a 15-ml falcon tube. This procedure was performed to eliminate all the fibers coming out of filter papers. Then, 50 mM NH4HCO3 (volume corresponding to 1.5 X the sample volume) was added to dilute the urea and thiourea. The samples were transferred to Falcon Amicon tubes (Amicon Ultra - 15 Centrifugal Filter Units - Merck Millipore, Tallagreen, Ireland) centrifuged at 5000 g at 4°C and concentrated to approximately 150 µL. Samples were then reduced by adding 5 mM dithiothreitol (DTT)) followed by incubation for 40 minutes at 37ºC and then alkylated by adding 10 mM iodoacetamide (IAA) and incubated in the dark for 30 minutes. After this procedure, 100 µL of 50 mM NH4HCO3 was added and samples were digested for 14 h at 37ºC by adding 2% (p/p) trypsin (Promega, Madison, USA). After this time lapsed, 10 µL of 5% formic acid was added to stop the action of trypsin. Samples were then desalted and purified using C18 Spin columns (Thermo Scientific, United States). Then an aliquot of 1 µL from each samples was removed and protein quantification was performed using the Bradford method (Bio-Rad Bradford Assays, United States). The total amount of protein obtained ranged between 29 (LALi region) to 88 (ULPLa region) µg, depending on the region of AEP collection. The samples were resuspended in a solution containing 3% acetonitrile and 0.1% formic acid to be submitted to nano LC-ESI-MS / MS.

Shotgun Label-free Quantitative Proteomic Analysis

Peptides identification was performed on a nanoACQUITY UPLC-Xevo QTof MS system (Waters, Manchester, UK). The nanoACQUITY UPLC, was equipped with nanoACQUITY HSS T3, analytical reverse phase column (75 µm X 150 mm, 1.8 µm particle size, Waters). The column was equilibrated with mobile phase A (0.1 % formic acid in water). Then, the peptides were separated with a linear gradient of 7-85% mobile phase B (0.1 % formic acid in ACN) for 70 min at a flow rate of 0.35 µL/min. The column temperature was maintained at 55°C. The Xevo G2 Q-TOF mass spectrometer was operated in positive nanoelectrospray ion mode and data were collected using the MSE method in elevated energy (19-45 V), which allows data acquisition of both precursor and fragment ions, in one injection. Source conditions used included capillary voltage, 2.5 kV; sample cone, 30 V;

extraction cone, 5.0 V and source temperature, 80°C. Data acquisition occurred over 70 min and the scan range was 50–2000 Da. The lockspray, used to ensure accuracy and reproducibility, was run with a [Glu1] fibrinopeptide solution (1 pmol/μL) at a flow rate of 1 μL/min, as a reference ion in positive mode at m/z 785.8427. ProteinLynx Global Server (PLGS) version 3.0 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 June 2015 from UniProtKB (http://www.uniprot.org/). The identified proteins were classified and assigned by biological function 26, 28, 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 Protein Lynx Global Service (PLGS, v 2.2.5, Waters Co., Manchester, UK) software. 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. Difference in expression among the groups was expressed as $p<0.05$ for down-regulated proteins and $1\text{-}p>0.95$ for up-regulated proteins. The following relevant comparisons were made: ULALa X LALi, LPLi X LALi, UAPa X LALi, ULALa X UAPa, UPPa X UAPa, ULPLa X ULALa, UPPa X LPLi, ULPLa X LPLi, ULPLa X UPPa.

Results

A total of 363 proteins were identified in the AEP collected from all the regions (Table S1). Figure 1 shows the numbers of proteins common to the different groups, as well as the numbers of proteins found in only one of the groups. Twenty-five proteins were identified in all the locations, such as Protein S100-A8, Lysozyme C, Lactoferrin, Sthatherin, Ig alpha-2 chain C, ALB protein, Myeloperoxidase and Submaxillary gland androgen-regulated protein 3B (Figure 1, Table 1). Ig alpha-1 chain C, Cystatin-B and Histatin-1 were identified in all the regions, except in LPLa. Cystatin-S was identified only in the AEP collected from the anterior teeth (ULALa, UAPa and LALi), while different isoforms of Hemoglobin subunits were found only in the AEP collected from the posterior teeth (LPLi, UPPa, ULPLa). Many proteins (252), however, were found exclusively in the AEP collected from one of the regions

(46 for UAPa, 33 for LALi, 59 for ULALa, 31 for ULPLa, 44 for LPLi and 39 for UPPa) (Figure 1, Table 2).

Regarding quantitative analysis, nine comparisons were made among the six groups, as displayed above. Two of them involved the anterior labial region (both upper and lower; ULALa) with its lingual (LALi) or palatal (UAPa) counterpart. For the comparison ULALa X LALi, 5 and 7 proteins were significantly increased and decreased in the first compared with the latter. Among the proteins differentially expressed, Cystatin-B was increased, while PRP 27 and Lysozyme C were decreased in ULALa (Table 3). When the ULALa region was compared with the UAPa region, 9 proteins were significantly increased and 4 significantly decreased in the first compared to the latter. Among the proteins differentlially expressed are 2 isoforms of Ig alpha, Keratin and Neutrophil defensin, as well as Histatin-1 and Statherin that were increased in the ULALa region, while Myeloperoxidase and Protein S100-A8 were decreased. The anterior labial region (ULALa) was also compared with the posterior labial region (ULPLa). In this case, 4 and 8 proteins were significantly increased or decreased, respectively, in the posterior region in comparison to the anterior one. Albumin isoform CRA and Myeloperoxidase were increased in the posterior region, while 2 isoform of Ig alpha, Statherin, Lysozyme C, Submaxillary gland androgen-regulated protein 3B, Protein S100-A8 and Histatin-1 were decreased (Table 3).

For the comparison of the anterior palatal region (UAPa) with its lingual counterpart (LALi), 8 proteins were significantly increased and another 8 significantly decreased in UAPa region compared with LALi region. The AEP formed on the palatal teeth (UAPa) had significantly higher concentration of Myeloperoxidase, Protein S100-A8, Submaxillary gland androgen-regulated protein 3B and Ig gamma-2 chain C region, while the concentrations of Statherin, Myeloblastin, Lactotransferrin, Lysozyme C and 2 isoform of Neutrophil defensing were significantly lower. When the AEP formed on the palatal surfaces of the anterior teeth (UAPa) was compared with that formed on the posterior counterpart (UPPa), 9 proteins were significantly increased, while 12 were significantly decreased in the posterior region. Ig alpha-1 chain C region, Myeloblastin, Lactotransferrin, 3 isoforms of Albumin and 2 of neutrophil defensing were increased, while Lysozyme C, Histatin-1, Submaxillary gland androgen-regulated protein, Protein S100-A8, Cystatin-B, Statherin and Myeloperoxidase were decreased in the posterior region compared with the anterior one. As for the comparison between the AEP formed on the lingual surface of the anterior teeth (LALi) with that formed on the posterior counterpart (LPLi), 5 proteins were increased and 11 decreased in the latter compared with the first. Myeloperoxidase was increased, while Ig alpha-2 chain C region,

Azurocidin, Protein S100-A8, Sthaterin, Lactotransferrin, Lysozyme C and Neutrophil defensins were reduced in the posterior region compared to the anterior one (Table 3).

Two of the comparisons made involved the posterior labial region (upper and lower analyzed together; ULPLa) with its palatal (UPPa) or lingual (LPLi) counterparts. When ULPLa was compared with UPPa, 9 proteins were increased and another 9 were decreased in labial region, compared with the palatal one. Histatin-1, Cystatin-B and Lysozyme C were increased, while Submaxillary gland androgen-regulated protein 3B, Myeloperoxidase, Lactotransferrin, Protein S100-A8 and isoforms of IgA were decreased in the labial region, when compared with the palatal one. As for the comparison between the posterior labial region (ULPLa) and its lingual counterpart (LPLi), two proteins, including Lysozyme C, were increased, while 13 proteins, including Submaxillary gland androgen-regulated protein 3B and Myeloperoxidase were decreased in the labial region compared with the lingual one (Table 3).

Finally, when the posterior palatal region (UPPa) was compared with its lingual counterpart (LPLi), 10 proteins were increased, including Protein S100-A8. Lactotransferrin and Neutrophil defensins were increased, while 26 proteins, including Myeloperoxidase, was descreased in the palatal region when compared with the lingual one (Table 3).

Discussion

The analysis of the protein composition of the AEP using proteomic approaches is recent [27]. Moreover, all the studies available in the literature so far collected AEP from the labial surface of the teeth only [24-26, 29]. It is likely that the composition of the AEP might vary depending on the location of the teeth in the dental arches, as function of the contribution of the different salivary glands. This, together with the different thickeness of the AEP from different regions [10] might have an impact on the distinct prevalence of dental erosion in the different dental arches, since the palatal and occlusal surfaces are the most commonly affected, both in adults and children [13-15, 17, 18]. To our knowledge, this is the first study to compare the protein composition of the AEP collected from different regions of the dental arches using proteomic tools. We divided the areas of AEP collection based on the location of the opening of the ducts of the salivary glands that could contribute to the formation of the AEP. For the labial region, collections were made together both for upper and lower teeth (anterior and posterior separately). The upper posterior region receives contribution mainly from the parotid gland, while the upper anterior region is mainly influenced by the

composition of the minor salivary glands. Pellicles formed at the palatal and lingual regions were collected separately for the upper and lower teeth, because the lingual area is abundantly bathed by saliva coming from submandibular and sublingual glands.

One of the shortcomes when conducting proteomic analysis of the AEP is the amount of protein obtained that is very tiny and not enough to allow individual analysis of the volunteers. In order to overcome this, an acceptable approach is to pool samples collected from all the volunteers and then analyze this pool in triplicate in order to allow quantitative comparisons [24]. In the present study, since we wanted to collect samples from 6 different regions, we had to collect samples on 3 different days from each region from each volunteer. We used a special protocol to extract the AEP proteins that allowed us to obtain enough amount of protein (29 – 88 μg) to be identified and quantified from all the 6 regions. In previous studies, AEP samples were extracted with NH4HCO3 [24, 25] or water [29]. In the present protocol, urea and thiourea were added to the extraction solution in order to keep the proteins in their primary structure. Another important step in our extraction protocol was the use of Falcon Amicon tubes to concentrate the samples. This increased the rate of protein recovery (data not shown). In addition, samples were alkylated with IAA prior to digestion, which might have improved the action of trypsin. Moreover, to desalt and purify the samples prior to mass spectrometry, we used C18 Spin columns instead of ZipTip™, commonly used in previous protocols [24-26]. This also increased the degree of protein recovery. In summary, the protein extraction protocol was considered excellent, since it allowed the identification of 363 proteins in total. This is the highest number of proteins that have been identified in the AEP using proteomic approaches so far. The other studies available in the literature typically identify ~ 100-150 different proteins [24-27] in the in vivo formed AEP. The highest idenfication rate of the present study may be in part due to the different extraction protocol employed, as mentioned above, but also to the collection of AEP from different regions of the dental arches, since all the studies published so far collected AEP from the labial surface of the teeth only [24-26, 29].

In the present study, 25 proteins were identified in all the locations. Among them are proteins typically described in the AEP, such as Protein S100-A8, Lysozyme C, Lactoferrin, Sthatherin, Ig alpha-2 chain C, ALB protein, Myeloperoxidase and Proline-rich protein 3, which corroborates the adequacy of the extraction protocol employed.

Of special interest are the proteins that were only found in specific regions, since they can help to explain why the protective properties of the AEP against acid dissolution vary in distinct areas of the dental arches. For example, regarding cystatins, that have been reported to

be acid-resistant [24] and can also inhibit cysteine-type endopeptidases, Cystatin-B was not identified in the LPLi region, while Cystatin SN was not detected in the LPLi and ULPLa regions and Cystatin-S was not found in the LPLi, UPPa and ULPLa regions. This means that this Cystatin was not found in the AEP collected from the posterior teeth. Recently, the role of cysteine cathepsins on the progression of dentin caries and erosion has been enphasyzed [30, 31]. The presence of cystatins might be important to reduce the rate of degradation of the demineralized organic matrix, reducing the progression of caries and erosion in dentin. Besides this study evaluated enamel, the same pattern might be expected for dentin. This means that the anterior teeth might be more protected when we consider that cystatins are more abundantly found in the acquired pellicle formed at the anterior region. This, in addition to the high salivary flow, is consistent with the lower prevalence of erosion in the lower lingual teeth [13]. On the other hand, hemoglobins were only identified in the AEP collected from the posterior teeth, both in the labial, palatal and lingual areas. Despite the volunteers had good conditions of oral health, we cannot rule out the possibility that this protein was derived from contamination [32]. So far the function of hemoglobin in the AEP is not known.

Some interesting proteins not previously identified in the AEP were found in the present study. Among them are Azurocidin, a protein with antibacterial function (UNIPROT) that was found in the LALi, PLi, UPPa and ULPLa regions. This protein can be a potential new antimicrobial player in the oral cavity, showing the importance the pellicle to protect also against caries, and should be better evaluated in further studies. Another one is Cathepsin G, a cysteine cathepsin that could play a role in the degradation of the demineralized organic matrix in dentin [30].

Of interest are the proteins that were identified exclusively in one of the regions evaluated. Protein S100-A9 was only found in the UAPa. This family of proteins is associated with calcium and zinc binding, playing a role in the regulation of inflammatory processes and immune response, with antimicrobial function (UNIPROT). In relation to erosion, Protein S100-A9 was also identified in the AEP after exposure to citric and lactic acids [24], which, combined with its ability to bind calcium, might indicate a potential to protect against acid challenges. Despite Protein S100-A9 was identified in the UAPa region only, its homolog Protein S100-A8 was identified in all the regions. Protein S100-A8 is commonly identified in the AEP collected under different conditions [26, 33]. Interestingly, Ras GTPase-activating-like protein that bind calcium and also S100 proteins (UNIPROT) was only found in the AEP collected from the LALi region, which has lower risk to develop erosive lesions. Many proteins identified solely in the ULALa region are involved in immune response, such as Ig

kappa chain V-III region CLL, Ig kappa chain V-I region Lay, Ig kappa chain V-III region POM, Ig heavy chain V-III region TEI and Ig kappa chain V-III region VH. Another exclusive protein of this region was Prolactin-inducible protein, involved in the detection of chemical stimulus involved in sensory perception of bitter taste (UNIPROT).

As for the proteins found exclusively in the posterior region, Protein SAAL1 (Serum amyloid A-like 1) was only identifided in the ULPLa. There is not much information available in the literature regarding this protein, since it was cloned in 2012 [34] and its function in the AEP cannot be accurately predicted. However, this is an extracellular protein whose predicted functional partners are keratin and carbonic anhydrase (STRING). Thus, it might have a function in the homeostasis of the oral cavity, which should be investigated in future studies. In the LPLi region, proteins involved in immune response were also detected exclusively, such as Ig heavy chain V-III region TIL, Ig heavy chain V-III region WAS and Ig heavy chain V-III region TUR. In the UPPa region, it was identified exclusively the protein ADAMT59, a disintegrin and metalloproteinase with thrombospondin motifs 9, which cleaves the large aggregating proteoglycans, aggrecan and versican (UNIPROT) and could be involved in the degradation of dentin and alveolar bone. Another interesting protein exclusive of this region was Calpain-8 that is a calcium-regulated non-lysosomal thiol-protease with digestive function. Calpains-8 and -9 are members of Ca+2-dependent intracellular proteases that form a functional complex "G-calpain", expressed specifically in the mucus-producing cells [35]. They might come to the oral cavity originating from the stomach after gastroesophageal-reflux episodes. Interestingly, the UPPa region is highly affected by intrinsic erosion [36].

In conclusion, the present study showed important differences in the protein profile of the AEP collected from different regions of the dental arches. These results provide important insights for understanding the differential protective roles of the AEP as a function of its location in the dental arches. Moreover, future studies involving collection of the AEP for proteomic analysis should harvest this integument from all the regions of the dental arches.

Acknowledgements

CAPES provided a Master fellowship to the first author of this study. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

References

1. Dawes C, Jenkins GN, Tongue CH. The nomenclature of the integuments of the enamel surface of the teeth. British Dental Journal 1963;115:65-8.
2. Lendenmann U, Grogan J, Oppenheim FG. Saliva and dental pellicle--a review. Advances in Dental Research 2000;14:22-8.
3. Hannig M, Joiner A. The structure, function and properties of the acquired pellicle. Monographis in Oral Science 2006;19:29-64.
4. Siqueira WL, Custodio W, McDonald EE. New Insights into the Composition and Functions of the Acquired Enamel Pellicle. Journal of Dental Research 2012;91(12):1110-18.
5. Buzalaf MAR, Hannas AR, Kato MT. Saliva and dental erosion. Journal of Applied Oral Science 2012;20(5):493-502.
6. Vitorino R, Calheiros-Lobo MJ, Williams J, Ferrer-Correia AJ, Tomer KB, Duarte JA, et al. Peptidomic analysis of human acquired enamel pellicle. Biomedical Chromatography 2007;21(11):1107-17.
7. Hara AT, Zero DT. The caries environment: saliva, pellicle, diet, and hard tissue ultrastructure. Dental Clinics of North America 2010;54(3):455-67.
8. Vukosavljevic D, Custodio W, Buzalaf MAR, Hara AT, Siqueira WL. Acquired pellicle as a modulator for dental erosion. Archives of Oral Biology 2014;59(6):631-8.
9. Carlen A, Borjesson AC, Nikdel K, Olsson J. Composition of pellicles formed in vivo on tooth surfaces in different parts of the dentition, and in vitro on hydroxyapatite. Caries Research 1998;32(6):447-55.
10. Amaechi BT, Higham SM, Edgar WM, Milosevic A. Thickness of acquired salivary pellicle as a determinant of the sites of dental erosion. Journal of Dental Research 1999;78(12):1821-8.
11. Bartlett DW, Coward PY, Nikkah C, Wilson RF. The prevalence of tooth wear in a cluster sample of adolescent schoolchildren and its relationship with potential explanatory factors. British Dental Journal 1998;184(3):125-9.
12. House RC, Grisius R, Bliziotes MM, Licht JH. Perimolysis: unveiling the surreptitious vomiter. Oral Surgery Oral Medicine Oral Pathology 1981;51(2):152-5.
13. Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. Monographs in Oral Science 2014;25:55-73.
14. Jarvinen V, Rytomaa I, Meurman JH. Location of dental erosion in a referred population. Caries Research 1992;26(5):391-6.
15. Robb ND, Smith BG, Geidrys-Leeper E. The distribution of erosion in the dentitions of patients with eating disorders. British Dental Journal 1995;178(5):171-5.
16. Scheutzel P. Etiology of dental erosion--intrinsic factors. European Journal of Oral Science 1996;104(2 (Pt 2)):178-90.
17. Millward A, Shaw L, Smith AJ, Rippin JW, Harrington E. The distribution and severity of tooth wear and the relationship between erosion and dietary constituents in a group of children. International Journal of Paediatric Dentistry 1994;4(3):151-7.
18. Milosevic A, Young PJ, Lennon MA. The prevalence of tooth wear in 14-year-old school children in Liverpool. Community Dental Health 1994;11(2):83-6.
19. Hannig M, Fiebiger M, Guntzer M, Dobert A, Zimehl R, Nekrashevych Y. Protective effect of the in situ formed short-term salivary pellicle. Archives of Oral Biology 2004;49(11):903-10.
20. van Nieuw Amerongen A, Veerman ECI, Vissink A. Salivary properties and functions. In: Wong DT, editor. Salivary diagnostics. 1st ed. Iowa: Wiley-Blackwell; 2008. p.27-36.

21. Van Nieuw Amerongen A, Bolscher JG, Veerman EC. Salivary proteins: protective and diagnostic value in cariology? Caries Research 2004;38(3):247-53.
22. Veerman EC, van den Keybus PA, Vissink A, Nieuw Amerongen AV. Human glandular salivas: their separate collection and analysis. European Journal of Oral Sciences 1996;104(4 (Pt 1)):346-52.
23. Know SM, Hoffman MP. Salivary gland development and regeneration. In: Wong DT, editor. Salivary disgnostics. 1st ed. Iowa: Wiley-Blackwell; 2008. p. 3-13.
24. Delecrode TR, Siqueira WL, Zaidan FC, Bellini MR, Moffa EB, Mussi MC, et al. Identification of acid-resistant proteins in acquired enamel pellicle. Journal of Dentistry 2015;43(12):1470-5.
25. Lee YH, Zimmerman JN, Custodio W, Xiao Y, Basiri T, Hatibovic-Kofman S, et al. Proteomic evaluation of acquired enamel pellicle during in vivo formation. PLoS One 2013;8(7):e67919.
26. Zimmerman JN, Custodio W, Hatibovic-Kofman S, Lee YH, Xiao Y, Siqueira WL. Proteome and peptidome of human acquired enamel pellicle on deciduous teeth. International Journal of Molecular Science 2013;14(1):920-34.
27. Siqueira WL, Zhang W, Helmerhorst EJ, Gygi SP, Oppenheim FG. Identification of protein components in in vivo human acquired enamel pellicle using LC-ESI-MS/MS. Journal of Proteome Research 2007;6(6):2152-60.
28. Rison SC, Hodgman TC, Thornton JM. Comparison of functional annotation schemes for genomes. Functional Integrated Genomics 2000;1(1):56-69.
29. Siqueira WL, Oppenheim FG. Small molecular weight proteins/peptides present in the in vivo formed human acquired enamel pellicle. Archives of Oral Biology 2009;54(5):437-44.
30. Buzalaf MA, Charone S, Tjaderhäne L. Role of host-derived proteinases in dentine caries and erosion. Caries Research 2015;49 Suppl 1:30-7.
31. Tjaderhäne L, Buzalaf MA, Carrilho M, Chaussain C. Matrix metalloproteinases and other matrix proteinases in relation to cariology: the era of 'dentin degradomics'. Caries Research 2015;49(3):193-208.
32. Maeng YJ, Kim BR, Jung HI, Jung UW, Kim HE, Kim BI. Diagnostic accuracy of a combination of salivary hemoglobin levels, self-report questionnaires, and age in periodontitis screening. Journal of Periodontal Implant Science 2016;46(1):10-21.
33. Masson N, Domingues RR, Cury JA, Leme AFP. Acidulated Phosphate Fluoride Application Changes the Protein Composition of Human Acquired Enamel Pellicle. Caries Research 2013;47(3):251-58.
34. Revathy KS, Umasuthan N, Whang I, Lee Y, Lee S, Oh MJ, et al. A novel acute phase reactant, serum amyloid A-like 1, from Oplegnathus fasciatus: Genomic and molecular characterization and transcriptional expression analysis. Developmental and Comparative Immunology 2012;37(2):294-305.
35. Hata S, Sorimachi H. [Physiological importance of calpains in gastric mucosal defense]. Nihon Rinsho 2011;69(6):1116-22.
36. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R, Global Consensus G. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. American Journal of Gastroenterology 2006;101(8):1900-20.

Figure legend

Figure 1. Organogram showing the number os proteins identified in the acquired enamel pellicle collected in the different regions of the dental arches. U- upper, L- lower; A- anterior; P-posterior; Pa- palatal; La- labial; Li- lingual.

Table 1. Proteins identified in the acquired enamel pellicle collected in all the regions evaluated.

Acession number	Protein name and classification
P05109	Protein S100-A8 [b, e, i, j, l, n, o, q, s, u, w]
P24158	Myeloblastin [a, g, n, o, s, u, w]
P61626	Lysozyme C [a, b, g, i, j, o, u, w]
C9JKR2	Albumin_ isoform CRA_k [c, m, o, u]
P63261	Actin_ cytoplasmic 2 [a, d, g, j, n, q, u, w]
P02788	Lactotransferrin [f, g, h, i, j, n, o, p, u, w]
Q9BYX7	Putative beta-actin-like protein 3 [a, m, n, q, u, w]
P02768	Serum albumin [a, b, c, g , o ,u, w]
P68133	Actin_ alpha skeletal muscle [b, m, n, q, u, w]
P63267	Actin_ gamma-enteric smooth muscle [b, m, n, q, u, w]
A5A3E0	POTE ankyrin domain family member F [b, m, n, u]
P02808	Statherin [b, e, l, o, u]
P62736	Actin_ aortic smooth muscle [b, m, n, q, u, w]
P60709	Actin_ cytoplasmic 1 [b, m, n, q, u, w]
P68032	Actin_ alpha cardiac muscle 1 [b, m, n, q, u, w]
P08670	Vimentin [b, j, n, u, w]
Q6S8J3	POTE ankyrin domain family member E [b, m, o, u]
J3KT65	Actin_ cytoplasmic 2_ N-terminally processed [b, m, t, u]
P01877	Ig alpha-2 chain C region [b, j, o, u]
P25311	Zinc-alpha-2-glycoprotein [a, b, g, o, u, w]
Q8IUK7	ALB protein [c, m, o, u, w]
P05164	Myeloperoxidase [a, b, g, j, r, u]
Q562R1	Beta-actin-like protein 2 [b, m, n, u, w]
P0CG38	POTE ankyrin domain family member I [b, m, o, u]
P02814	Submaxillary gland androgen-regulated protein 3B [a, g, o, u, w]

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 2. Classification of the proteins of the acquired enamel pellicle identified in only one of the regions evaluated. U- upper, L- lower; A- anterior; P-posterior; Pa- palatal; La- labial; Li- lingual.

UAPa	Access number	Protein name and classification
	Q16777	Histone H2A type 2-C [b, m, p, u, w]
	P11137	Microtubule-associated protein 2 [d, m, n, q, u, w]
	Q9BV73	Centrosome-associated protein CEP250 [b, m, n, q, u, w]
	Q49AC9	PTPRM protein [a, g, t, u]
	Q8WXA9	Splicing regulatory glutamine/lysine-rich protein 1 [b, m, p, u, w]
	Q9HCG7	Non-lysosomal glucosylceramidase [a, m, s, u]
	Q96G74	OTU domain-containing protein 5 [a, g, n, u, w]
	Q9NTJ3	Structural maintenance of chromosomes protein 4 [b, m, n, p, u, w]
	Q96L93	Kinesin-like protein KIF16B [c, m, n, q, s]
	P16104	Histone H2AX [b, e, m, p, u, w]
	P25705	ATP synthase subunit alpha_ mitochondrial [a, c, m, s, u, w]
	Q14687	Genetic suppressor element 1 [m, t, u, w]
	Q99878	Histone H2A type 1-J [b, m, p, u, w]
	Q08495	Dematin [d, m, n, q, u, w]
	P02763	Alpha-1-acid glycoprotein 1 [b, c, j, o, u]
	J3KNI2	UPF0598 protein C8orf82 [m, t, x]
	F1T0F2	T-cell leukemia homeobox protein 2 [b, m, t, x]
	H7C242	Doublecortin domain-containing protein 2C (Fragment) [e, m, r, u]
	P14678	Small nuclear ribonucleoprotein-associated proteins B and B' [b, m, n, p, u, w]
	Q96QV6	Histone H2A type 1-A [b, m, p, u, w]

P61576	Endogenous retrovirus group K member 104 Rec protein [b, m, n, p, w]
Q6FI13	Histone H2A type 2-A [b, m, p, u, w]
Q96DA0	Zymogen granule protein 16 homolog B [b, m, o, u]
P0C0S8	Histone H2A type 1 [b, m, p, u, w]
Q96BD5	PHD finger protein 21A [p, m, p, u, w]
Q9BX84	Transient receptor potential cation channel subfamily M member 6 [c, m, s, u, w]
Q12923	Tyrosine-protein phosphatase non-receptor type 13 [e, m, n, p, u, w]
P04908	Histone H2A type 1-B/E [b, m, p, u, w]
P21333	Filamin-A [d, m, n, q, u, w]
P21817	Ryanodine receptor 1 [c, e, m, s, u, w]
P20671	Histone H2A type 1-D [b, m, p, u, w]
Q9BTM1	Histone H2A.J [b, m, p, u, w]
Q96KK5	Histone H2A type 1-H [b, m, p, u, w]
Q9BXP5	Serrate RNA effector molecule homolog [b, e, m, n, p, u, w]
Q4G0P3	Hydrocephalus-inducing protein homolog [b, m, q, u]
Q7L7L0	Histone H2A type 3 [b, m, p, u, w]
E7EMT6	TRAF family member-associated NF-kappa-B activator (Fragment) [m, n, u]
Q93077	Histone H2A type 1-C [b, m, p, u, w]
P06702	Protein S100-A9 [a, b, g, i, j, n, o, q, s, u, w]
E9PD53	Structural maintenance of chromosomes protein [b, m, p, u]
Q9Y5H0	Protocadherin gamma-A3 [b, m, s, u]
O75531	Barrier-to-autointegration fator [b, m, n, p, u, w]
E7EPS8	Receptor-type tyrosine-protein phosphatase mu [a, g, s, u]
Q8N1F7	Nuclear pore complex protein Nup93 [c, d, m, p, s, u, w]

	Q13315	Serine-protein kinase ATM [a, b, c, g, n, p, u, w]
	O75376	Nuclear receptor corepressor 1 [b, m, p, u, w]
LALi	**Access number**	**Protein name and classification**
	Q6UW56	All-trans retinoic acid-induced differentiation factor [b, l,p, s, u]
	I3L3I0	Actin_ cytoplasmic 2_ N-terminally processed (Fragment) [m, t, u]
	Q92600	Cell differentiation protein RCD1 homolog [b, m, n, p, u, w]
	Q8N8A2	Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit B [b, e, m, t, u]
	Q9BQ39	ATP-dependent RNA helicase DDX50 [b, m, p, u]
	Q9H7C4	Syncoilin [d, m, n, u]
	Q9NPI0	Transmembrane protein 138 [b, m, s, u]
	Q9BRR9	Rho GTPase-activating protein 9 [g, h, k, m, o]
	J3KPQ4	Rho GTPase activating protein 9_ isoform CRA_a [a, m, n, u]
	P09871	Complement C1s subcomponente [a, e, g, j, o, u, w]
	Q07092	Collagen alpha-1(XVI) chain [b, g, o, u, w]
	E9PMD0	Uncharacterized protein (Fragment) [b, m, t, u]
	P46940	Ras GTPase-activating-like protein IQGAP1 [a, g, s, u]
	Q12955	Ankyrin-3 [b, d, m, n, q, s, u, w]
	Q9NY43	BarH-like 2 homeobox protein [b, m, n, p, u]
	E7EWM2	Centrosomal protein of 170 kDa [m, n, x]
	Q96NU1	Sterile alpha motif domain-containing protein 11 [e, m, p, u]
	X6R5P2	Astrotactin-2 [m, t, x]
	Q9BUP0	EF-hand domain-containing protein D1 [a, m, o, u]
	O75448	Mediator of RNA polymerase II transcription subunit 24 [b, m, p, u, w]
	Q5BKV1	MYH9 protein [b, m, q, u]

	Q9Y6D0	Selenoprotein K [c, g, s, u, w]
	P35579	Myosin-9 [d, m, n, q, u, w]
	P08493	Matrix Gla protein [b, m, o, u]
	Q13017	Rho GTPase-activating protein 5 [b, m, n, s, u, w]
	Q9H2K8	Serine/threonine-protein kinase TAO3 [b, m, n, s, u, w]
	Q96L14	Cep170-like protein [b, m, t, u, w]
	Q8ND90	Paraneoplastic antigen Ma1 [b, m, p, u, w]
	Q9UI17	Dimethylglycine dehydrogenase_ mitochondrial [a, g, r, u]
	Q86WA9	Sodium-independent sulfate anion transporter [c, m, s, u]
	A0A575	Protein TRBV2 (Fragment) [m, t, x]
	P23763	Vesicle-associated membrane protein 1 [c, m, s, u, w]
	Q96P50	Arf-GAP with coiled-coil_ ANK repeat and PH domain-containing protein 3 [a, m, t, u]
ULALa	**Access number**	**Protein name and classification**
	Q8N4C8	Misshapen-like kinase 1 [b, c, m, n, s, u, w]
	E7EX83	Mitogen-activated protein kinase kinase kinase kinase 4 [a, b, g, t, u]
	E7EPM6	Long-chain-fatty-acid--CoA ligase 1 [a, g, s, u]
	Q9BXR0	Queuine tRNA-ribosyltransferase [a, g, n, p, u]
	P12830	Cadherin-1 [b, m, s, u, w]
	P01766	Ig heavy chain V-III region BRO [b, j, o, x]
	P09565	Putative insulin-like growth factor 2-associated protein [b, m, t, u]
	A5D8W1	Cilia- and flagella-associated protein 69 [m, t, x]
	S4R460	Protein IGHV3OR16-9 [b, j, o, w]
	A8MTA8	Protein FAM166B [m, t, u]
	P04207	Ig kappa chain V-III region CLL [b, j, o, x]

P09104	Gamma-enolase [a, g, n, s, u]
A0A075B6H7	Protein IGKV3-7 (Fragment) [m, t, u]
Q3V6T2	Girdin [b, m, n, s, u, w]
A0A087WYC5	Ig gamma-1 chain C region [m, t, x]
F8WBX7	Protein CFAP69 [m, t, x]
P01605	Ig kappa chain V-I region Lay [b, j, o, x]
P19013	Keratin_ type II cytoskeletal 4 [d, m, q, u]
Q9BVG4	Protein PBDC1 [m, t, u]
M0QZK5	Neuropathy target esterase (Fragment) [d, m, s, u]
A0A087WT11	Protein IGKV3OR2-268 (Fragment) [m, t, u]
P40200	T-cell surface protein tactile [b, j, s, u]
P13929	Beta-enolase [a, g, n, u]
Q9BUA5	GNPTAB protein [m, s, u]
H0Y6Q4	Centrosome-associated protein 350 (Fragment) [d, m, r, x]
A0A0C4DH90	Protein IGKV2-28 [m, t, x]
P30101	Protein disulfide-isomerase A3 [a, b, g, o, p, r, u]
F8VQW2	N-acetylglucosamine-1-phosphotransferase subunit alpha [m, s, u]
F5H2I9	Tyrosine-protein kinase STYK1 (Fragment) [m, s, u]
B2RTY4	Unconventional myosin-IXa [d, m, s, u]
P08236	Beta-glucuronidase [a, g, o, u, w]
Q3T906	N-acetylglucosamine-1-phosphotransferase subunits alpha/beta [a, c, g, s, u]
Q9Y2V7	Conserved oligomeric Golgi complex subunit 6 [c, m, s, u]
Q9Y236	Oxidative stress-induced growth inhibitor 2 [b, m, t, u]
P17036	Zinc finger protein 3 [b, m, p, u]

Q9UQB3	Catenin delta-2 [e, m, p, u, w]
M0QYN9	IgG receptor FcRn large subunit p51 [b, j, t, u]
Q92823	Neuronal cell adhesion molecule [b, m, s, u, w]
Q00839	Heterogeneous nuclear ribonucleoprotein U [b, m, n, p, u]
Q8WUE2	CD96 antigen_ isoform CRA_b [m, t, x]
Q96N21	AP-4 complex accessory subunit tepsin [m, n, u, w]
P60660	Myosin light polypeptide 6 [d, m, n, o, s, u]
F5GY88	Mediator of RNA polymerase II transcription subunit 24 [b, m, t, u]
Q8NEU8	DCC-interacting protein 13-beta [b, m, p, s, u, w]
F5H0C8	Enolase [a, m, t, u]
F8W0C2	Neuronal PAS domain-containing protein 3 1 [b, m, t, u]
Q13615	Myotubularin-related protein 3 [a, m, n, s, u]
P01624	Ig kappa chain V-III region POM [b, j, o, x]
Q8IV96	DDX6 protein [b, m, n, u]
H0Y9L8	Bifunctional lysine-specific demethylase and histdyl-hydroxylase MINA (Fragment) [b, m, n, p, u]
P29401	Transketolase [c, g, n, o, u]
P12273	Prolactin-inducible protein [b, d, m, o, u]
Q13409	Cytoplasmic dynein 1 intermediate chain 2 [c, n, q, u]
Q5T3J3	Ligand-dependent nuclear receptor-interacting factor 1 [b, d, p, u]
P01777	Ig heavy chain V-III region TEI [b, e, j, o, x]
Q8IYM2	Schlafen family member 12 [a, g, t, u]
H3BSE9	Polycystin-1 (Fragment) [m, t, x]
A0A087WW89	Protein IGHV3-72 [m, t, x]
P04434	Ig kappa chain V-III region VH (Fragment) [b, e, j, o, x]

ULPLa	Access number	Protein name and classification
	O15453	Next to BRCA1 gene 2 protein [m, t, u]
	I3L4J3	KAT8 regulatory NSL complex subunit 1 [m, x, u]
	A0A087X2J2	ADP-ribosylation factor-like protein 8A [b, c, e, r, u]
	O60486	Plexin-C1 [b, m, s, u, w]
	A0A0A0MR55	Kallikrein-11 [a, b, m, t, x]
	O77932	Decapping and exoribonuclease protein [a, b, f, p, u]
	G3V584	Cation channel sperm-associated protein subunit beta (Fragment) [b, m, t, u]
	C9J3U1	3-oxo-5-beta-steroid 4-dehydrogenase (Fragment) [m, t, x]
	H0YFC3	Autophagy-related protein 16-2 (Fragment) [m, t, u]
	A0A0A0MTS5	HCG1811249_ isoform CRA_f 1 [b, m, t, u]
	A6NMS7	Leucine-rich repeat-containing protein 37A [m, s, u]
	O95996	Adenomatous polyposis coli protein 2 [b, e, m, n, q, u, w]
	Q9Y2J4	Angiomotin-like protein 2 [b, m, n, u]
	Q9Y520	Protein PRRC2C [m, s, u]
	Q4G0U5	Cilia- and flagella-associated protein 221 [d, m, n, q, u, w]
	Q15034	Probable E3 ubiquitin-protein ligase HERC3 [a, d, g, n , u]
	C9JFP8	SH3 and multiple ankyrin repeat domains protein 2 [m, t, u]
	Q9H841	NIPA-like protein 2 [c, m, s, u]
	Q96ER3	Protein SAAL1 [m, o, t, u]
	V9GY79	Spectrin alpha chain_ erythrocytic 1 (Fragment) [m, t, x]
	Q13634	Cadherin-18 [a, m, s, u]
	P35609	Alpha-actinin-2 [d, m, n, u, w]
	C9JEA7	Phosphoinositide phospholipase C [a, e, g, n, s, u]

	Q9BRC7	1-phosphatidylinositol 4_5-bisphosphate phosphodiesterase delta-4 [a, g, n, p, s, u]
	P12814	Alpha-actinin-1 [b, d, m, n, q, s, u, w]
	Q01484	Ankyrin-2 [c, d, m, n, q, s, u, w]
	P42336	Phosphatidylinositol 4_5-bisphosphate 3-kinase catalytic subunit alpha isoform [f, j, n, u, w]
	Q86UE3	Zinc finger protein 546 [b, m, p, u]
	Q6P050	F-box and leucine-rich protein 22 [a, m, n, u]
	Q16787	Laminin subunit alpha-3 [b, d, m, o, u, w]
	Q13884	Beta-1-syntrophin [d, m, n, q, s, u, w]

LPLi	**Access number**	**Protein name and classification**
	Q3KRA9	Alpha-ketoglutarate-dependent dioxygenase alkB homolog 6 [a, g, n, p, u]
	K9N163	Interleukin 15 receptor alpha isoform IC2 [m, t, u]
	Q6NVZ8	CBWD5 protein [m, t, u]
	P14867	Gamma-aminobutyric acid receptor subunit alpha-1 [a, c, g, s]
	Q6ZU80	Centrosomal protein of 128 kDa [m, n, q, u]
	Q8NBZ9	Putative uncharacterized protein NEXN-AS1 [m, t, x]
	Q13261	Interleukin-15 receptor subunit alpha [b, e, m, n, p, s, u, w]
	Q03692	Collagen alpha-1(X) chain [a, b, d, h, o, u]
	Q9NVM1	Protein eva-1 homolog B [b, m, s, u]
	P01765	Ig heavy chain V-III region TIL [b, j, o, x]
	P01776	Ig heavy chain V-III region WAS [b, j, o, x]
	H0Y5V3	Putative COBW domain-containing protein 7 [m, t, u]
	J3QQL2	Integrin beta (Fragment) [b, m, s, u]
	F8WCZ4	DnaJ homolog subfamily B member 6 [m, t, u]

K7EQZ3	Uncharacterized protein (Fragment) [a, g, p, x]
K9N2S2	Interleukin 15 receptor alpha isoform EM2 [b, e, m, t, u]
P19484	Transcription factor EB [b, j, n, p, u]
F8WBN0	Sn1-specific diacylglycerol lipase beta [m, s, u]
Q5JTY5	COBW domain-containing protein 3 [b, m, t, u]
O95714	E3 ubiquitin-protein ligase HERC2 [b, m, n, p, q, u, w]
O75953	DnaJ homolog subfamily B member 5 [d, m, n, p, u, w]
Q52LA3	Protein lin-52 homolog [b, m, p, u, w]
O95352	Ubiquitin-like modifier-activating enzyme ATG7 [b, c, g, n, u, w]
P06326	Ig heavy chain V-I region Mot [b, j, o, x]
A0A096LP88	Protein LOC102725121 [m, t, x]
Q16825	Tyrosine-protein phosphatase non-receptor type 21 [a, g, n, q, u]
Q4V339	COBW domain-containing protein 6 [b, m, t, u]
Q9Y2F9	BTB/POZ domain-containing protein 3 [b, m, n, p, u]
Q96IU2	Zinc finger BED domain-containing protein 3 [e, m, n, s, u]
P01779	Ig heavy chain V-III region TUR [b, j, o, x]
H0YJE3	Centrosomal protein of 128 kDa (Fragment) [m, t, u]
K9N0C7	Interleukin 15 receptor alpha isoform IC4 [m, t, u]
K9N1J3	Interleukin 15 receptor alpha isoform IC5 [m, t, u]
P51587	Breast cancer type 2 susceptibility protein [b, m, n, p, q, u, w]
Q5RIA9	COBW domain-containing protein 5 [b, m, t, u]
P27482	Calmodulin-like protein 3 [a, g, o ,u]
Q8TAF3	WD repeat-containing protein 48 [b, e, m, n, p, u, w]
O75604	Ubiquitin carboxyl-terminal hydrolase 2 [a, b, g, n, p, u, w]

	F5H6X5	Zinc finger protein 84 [b, m r, u]
	K9N2Q6	Interleukin 15 receptor alpha isoform IC3 [m, t, u]
	Q9H5L6	DNA transposase THAP9 [b, c, m, t, u]
	Q9UBC5	Unconventional myosin-Ia [b, d, m, n, o, u]
	Q3SXR2	Uncharacterized protein C3orf36 [m, t, u]
	Q7Z6Z7	E3 ubiquitin-protein ligase HUWE1 [b, e, m, n, p, u, w]
UPPa	**Access number**	**Protein name and classification**
	P11678	Eosinophil peroxidase [a, m, n, u]
	Q9P2N4	A disintegrin and metalloproteinase with thrombospondin motifs 9 [a, g, o, u]
	Q9Y2Z0	Suppressor of G2 allele of SKP1 homolog [b, j, n, p, u, w]
	Q06455	Protein CBFA2T1 [a, b, g, p, u, w]
	R9WNI0	Fragile X mental retardation 1 [b, m, t, u]
	Q6UWW8	Carboxylesterase 3 1 [a, g, o, r, u]
	K7EQY5	Megakaryocyte-associated tyrosine kinase_ isoform CRA_a [a, c, m, t, u]
	Q06787	Fragile X mental retardation protein 1 [b, c, m, n, p, u]
	I3L4Q0	HCG1818442_ isoform CRA_a [m, t, x]
	E7ETU7	39S ribosomal protein L3_ mitochondrial [d, m, r, x]
	Q5TBA9	Protein furry homolog [b, m, n, q, u, w]
	F6W7H3	Heat shock 70 kDa protein 1A/1B [b, m , r, u]
	Q53EL9	Seizure protein 6 homolog [b, m, s, u]
	Q16695	Histone H3.1t [b, m, p, u, w]
	Q7L1V2	Vacuolar fusion protein MON1 homolog B [b, m, n, u, w]
	I3L3P4	Tumor necrosis factor receptor superfamily member 12 [m, t, x]
	P84243	Histone H3.3 [b, m, p, u, w]
	A6NHC0	Calpain-8 [a, g, n, u]

M0QZQ7	Cell growth regulator with RING finger domain protein 1 (Fragment) [m, t, x]
Q8NDV7	Trinucleotide repeat-containing gene 6A protein [b, m, n, u, w]
C9JC32	Trafficking kinesin-binding protein 1 [m, t, u]
P68431	Histone H3.1 [m, t, u]
H3BQ30	Glycine cleavage system H protein_ mitochondrial (Fragment) [b, m, t, u]
B4DEB1	Histone H3 [b, m, p, u, w]
Q9Y263	Phospholipase A-2-activating protein [b, m, o, u, w]
K7EM90	Enolase (Fragment) [a, b, g, t, u]
Q14590	Zinc finger protein 235 [b, m, p, u]
P07437	Tubulin beta chain [b, d, m, n, q, u, w]
A0A0A0MT02	Negative elongation factor E (Fragment) [b, m, t, u]
Q6NXT2	Histone H3.3C [b, m, p, u, w]
Q6ZRH7	Cation channel sperm-associated protein subunit gamma [b, m, s, u]
Q9NX62	Inositol monophosphatase 3 [a, b, m, s, u]
G3V0J0	Fragile X mental retardation 1_ isoform CRA_e [b, m, n, x]
Q8IXW7	FMR1 protein [b, m, t, u]
Q12952	Forkhead box protein L1 [b, m, p, u]
Q9ULL4	Plexin-B3 [b, m, s, u, w]
B8ZZI7	Uncharacterized protein [b, m, s, u]
Q9H1J1	Regulator of nonsense transcripts 3A [b, m, n, p, w]
P42679	Megakaryocyte-associated tyrosine-protein kinase [b, c, e, g, w]

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 different regions of the dental arches. U- upper, L- lower; A- anterior; P-posterior; Pa- palatal; La- labial; Li- lingual.

Accession number	**Protein name**	**Ratio ULALa/LALi**	***P***
P04080	Cystatin-B [a, g, n, p, u]	**2.71**	1.00
P04792	Heat shock protein beta-1 [b, d, m, n, p, q, u, w]	1.87	1.00
P01834	Ig kappa chain C region [b, j, o, u]	1.59	1.00
A0A087WWV8	Protein IGKV1-8 [m, t, x]	1.58	1.00
A0A087WZW8	Protein IGKV3-11 [m, t, x]	1.56	1.00
P08670	Vimentin [b, j, n, u, w]	0.67	0.03
Q6MZM9	Proline-rich protein 27 [b, l, o, x]	0.67	0.00
P05164	Myeloperoxidase [a, b, g, j, r, u]	0.66	0.00
P61626	Lysozyme C [a, b, g, i, j, o, w]	0.52	0.00
P03973	Antileukoproteinase [a, b, g, i, j, o, u]	0.45	0.00
P59665	Neutrophil defensin 1 [b, i, j, o, u]	0.34	0.00
P59666	Neutrophil defensin 3 [b, i, j, o, u]	0.34	0.00
Accession number	**Protein name**	**Ratio ULALa/UAPa**	***P***

P01876	Ig alpha-1 chain C region [b, i, j, o, u]	**3.22**	1.00
P15515	Histatin-1 [b, i, l, o, u]	**3.18**	1.00
P19012	Keratin, type I cytoskeletal 15 [b, m, o, u, w]	**2.18**	0.97
P01877	Ig alpha-2 chain C region [b, j, o, u]	**2.15**	1.00
P13647	Keratin, type II cytoskeletal 5 [d, m, n, o, q, u]	**2.07**	0.96
P59665	Neutrophil defensin 1 [b, i, j, o, u]	1.84	1.00
P61626	Lysozyme C [a, b, g, i, j, o, u, w]	1.75	1.00
P59666	Neutrophil defensin 3 [b, i, j, o, u]	1.75	1.00
P02808	Statherin [b, e, l, o, u]	1.29	0.96
P60709	Actin, cytoplasmic 1 [b, m, n, q, u, w]	0.79	0.00
P63261	Actin, cytoplasmic 2 [a, d, g, j, n, q, u, w]	0.79	0.00
P05109	Protein S100-A8 [b, e, i, j, l, n, o, q, s, u, w]	0.71	0,01
P05164	Myeloperoxidase [a, b, g, j, r, u]	0.53	0.00

Accession number	**Protein name**	**Ratio ULPLa/ULALa**	***P***
C9JKR2	Albumin, isoform CRA_k [c, m, o, u]	**2.05**	0.97

P05164	Myeloperoxidase [a, b, g, j, r, u]	1.80	1.00
P0CG38	POTE ankyrin domain family member I [b, m, o, u]	1.43	0.97
P63261	Actin, cytoplasmic 2 [a, d, g, j, n, q, u, w]	1.16	0.96
P02768	Serum albumin [a, b, c, g, o, u, w]	0.76	0.03
P01877	Ig alpha-2 chain C region [b, j, o, u]	0.59	0.00
P02808	Statherin [b, e, l, o, u]	0.59	0.00
P61626	Lysozyme C [a, b, g, i, j, o, u, w]	0.57	0.00
P01876	Ig alpha-1 chain C region [b, i, j, o, u]	0.55	0.00
P02814	Submaxillary gland androgen-regulated protein 3B [a, g, o, u, w]	0.54	0.00
P05109	Protein S100-A8 [b, e, i, j, l, n, o, q, s, u, w]	0.44	0.00
P15515	Histatin-1 [b, i, l, o, u]	0.32	0.00

Accession number	**Protein name**	**Ratio UAPa/LALi**	***P***
P05164	Myeloperoxidase [a, b, g, j, r, u]	1.39	1.00
Q9BYX7	Putative beta-actin-like protein 3 [a, m, n, q, u, w]	1.39	1.00
P01859	Ig gamma-2 chain C region [b, j, o, u]	1.37	0.96

Q562R1	Beta-actin-like protein 2 [b, m, n, u, w]	1.36	0.99
P05109	Protein S100-A8 [b, e, i, j, l, n, o, q, s, u, w]	1.32	1.00
P60709	Actin, cytoplasmic 1 [b, m, n, q, u, w]	1.16	1.00
P63261	Actin, cytoplasmic 2 [a, d, g, j, n, q, u, w]	1.15	1.00
P02814	Submaxillary gland androgen-regulated protein 3B [a, g, o, u, w]	1.15	1.00
P02808	Statherin [b, e, l, o, u]	0.78	0.00
P08670	Vimentin [b, j, n, u, w]	0.67	0.01
P24158	Myeloblastin [a, g, n, o, s, u, w]	0.62	0.00
P02788	Lactotransferrin [f, g, h, i, j, n, o, p, u, w]	0.58	0.00
P61626	Lysozyme C [a, b, g, i, j, o, u, w]	0.29	0.00
P59665	Neutrophil defensin 1 [b, i, j, o, u]	0.15	0.00
P59666	Neutrophil defensin 3 [b, i, j, o, u]	0.13	0.00

Accession number	**Protein name**	**Ratio UPPa/UAPa**	***P***
P01876	Ig alpha-1 chain C region [b, i, j, o, u]	**2.80**	1.00
P24158	Myeloblastin [a, g, n, o, s, u, w]	1.68	1.00

P59666	Neutrophil defensin 3 [b, i, j, o, u]	1.61	1.00
P59665	Neutrophil defensin 1 [b, i, j, o, u]	1.59	1.00
P08670	Vimentin [b, j, n, u, w]	1.50	1.00
P02768	Serum albumin [a, b, c, g, o, u, w]	1.39	1.00
P02788	Lactotransferrin [f, g, h, i, j, n, o, p, u, w]	1.27	1.00
C9JKR2	Albumin, isoform CRA_k [c, m, o, u]	1.19	0.98
Q8IUK7	ALB protein [c, m, o, u, w]	1.18	0.98
P60709	Actin, cytoplasmic 1 [b, m, n, q, u, w]	0.86	0.00
P63261	Actin, cytoplasmic 2 [a, d, g, j, n, q, u, w]	0.86	0.00
P05164	Myeloperoxidase [a, b, g, j, r, u]	0.84	0.01
P02808	Statherin [b, e, l, o, u]	0.61	0.00
P04080	Cystatin-B [a, g, n, p, u]	0.60	0.01
P05109	Protein S100-A8 [b, e, i, j, l, n, o, q, s, u, w]	0.60	0.00
P02814	Submaxillary gland androgen-regulated protein [a, g, o, u, w]	0.59	0.00
P25311	Zinc-alpha-2-glycoprotein [a, b, g, o, u, w]	0.53	0.00

P62805	Histone H4 [b, m, p, u]	0.45	0.00
P15515	Histatin-1 [b, i, l, o, u]	0.39	0.03
P61626	Lysozyme C [a, b, g, i, j, o, u, w]	0.37	0.00
Q9UKM9	RNA-binding protein Raly [b, m, p, u]	0.07	0.00

Accession number	**Protein name**	**Ratio LPLi/LALi**	***P***
P63261	Actin, cytoplasmic 2 [a, d, g, j, n, q, u, w]	**2.63**	1.00
P05164	Myeloperoxidase [a, b, g, j, r, u]	**2.61**	1.00
Q8IUK7	ALB protein [c, m, o, u, w]	1.87	1.00
Q562R1	Beta-actin-like protein 2 [b, m, n, u, w]	1.12	0.96
Q9BYX7	Putative beta-actin-like protein 3 [a, m, n, q, u, w]	1.12	0.96
P01877	Ig alpha-2 chain C region [b, j, o, u]	0.66	0.00
P05109	Protein S100-A8 [b, e, i, j, l, n, o, q, s, u, w]	0.53	0.00
P20160	Azurocidin [a, b, g, i, s, u, w]	0.51	0.00
P08670	Vimentin [b, j, n, u, w]	0.46	0.00
C9JKR2	Albumin, isoform CRA_k [c, m, o, u]	0.44	0.00

P02808	Statherin [b, e, l, o, u]	0.43	0.01
P02788	Lactotransferrin [g, h, i, j, n, o, p, u, w]	0.39	0.00
P02768	Serum albumin [a, b, c, g, o, u, w]	0.37	0.00
P61626	Lysozyme C [a, b, g, i, j, o, u, w]	0.15	0.00
P59665	Neutrophil defensin 1 [b, i, j, o, u]	0.10	0,03
P59666	Neutrophil defensin 3 [b, i, j, o, u]	0.09	0.00

Accession number	**Protein name**	**Ratio ULPLa/UPPa**	***P***
P15515	Histatin-1 [b, i, l, o, u]	**2.07**	1.00
P04080	Cystatin-B [a, g, n, p, u]	1.89	0.98
P61626	Lysozyme C [a, b, g, i, j, o, u, w]	1.64	0.97
P02042	Hemoglobin subunit delta [b, c, h, n, o, u, w]	1.43	1.00
P69892	Hemoglobin subunit gamma-2 [b, c, m, n, u]	1.41	0.98
P69891	Hemoglobin subunit gamma-1 [b, c, h, n, o, u, w]	1.39	0.97
P02100	Hemoglobin subunit epsilon [b, c, m, n, u]	1.37	0.99
P0CG38	POTE ankyrin domain family member I [b, m, o, u]	1.29	1.00

J3KT65	Actin, cytoplasmic 2, N-terminally processed [b, m, t, u]	1.28	0.98
P02814	Submaxillary gland androgen-regulated protein 3B [a, g, o, u, w]	0.83	0.01
P05164	Myeloperoxidase [a, b, g, j, r, u]	0.82	0.02
P02788	Lactotransferrin [f, g, h, i, j, n, o, p, u, w]	0.77	0.00
P01877	Ig alpha-2 chain C region [b, j, o, u]	0.69	0.00
P01876	Ig alpha-1 chain C region [b, i, j, o, u]	0.61	0.00
Q562R1	Beta-actin-like protein 2 [b, m, n, u, w,]	0.59	0.00
Q9BYX7	Putative beta-actin-like protein 3 [a, m, n, q, u, w]	0.59	0.00
P02768	Serum albumin [a, b, c, g, o, u, w]	0.54	0.00
P05109	Protein S100-A8 [b, e, i, j, l, n, o, q, s, u, w]	0.49	0.00

Accession number	**Protein name**	**Ratio ULPLa/LPLi**	***P***
P61626	Lysozyme C [a, b, g, i, j, o, u, w]	1.63	0.98
P02768	Serum albumin [a, b, c, g, o, u, w]	1.34	1.00
F8W6P5	LVV-hemorphin-7 (Fragment) [b, c, m, o, u]	0.74	0.00
P68871	Hemoglobin subunit beta [b, c, m, n, o, u, w]	0.72	0.00

P02814	Submaxillary gland androgen-regulated protein 3B [a, g, o, u, w]	0.71	0.00
P69891	Hemoglobin subunit gamma-1 [b, c, h, n, o, u, w]	0.67	0.01
P02042	Hemoglobin subunit delta [b, c, h, n, o, u, w]	0.65	0.00
P02100	Hemoglobin subunit epsilon [b, c, m, n, u]	0.65	0.00
P69892	Hemoglobin subunit gamma-2 [b, c, m, n, u]	0.65	0.00
Q562R1	Beta-actin-like protein 2 [b, m, n, u, w]	0.65	0.01
Q9BYX7	Putative beta-actin-like protein 3 [a, m, n, q, u, w]	0.63	0.01
P08670	Vimentin [b, j, n, u, w]	0.60	0.04
P63261	Actin, cytoplasmic 2 [a, d, g, j, n, q, u, w]	0.36	0.00
P60709	Actin, cytoplasmic 1 [b, m, n, q, u, w]	0.36	0.00
P05164	Myeloperoxidase [a, b, g, j, r, u]	0.33	0.00

Accession number	**Protein name**	**Ratio UPPa/LPLi**	***P***
P02768	Serum albumin [a, b, c, g, o, u, w]	**4.05**	1.00
P59666	Neutrophil defensin 3 [b, i, j, o, u]	**3.00**	1.00
P59665	Neutrophil defensin 1 [b, i, j, o, u]	**2.91**	1.00

P05109	Protein S100-A8 [b, e, i, j, l, n, o, q, s, u, w]	1.73	1.00
P69905	Hemoglobin subunit alpha [b, c, m, n, o, s, u]	1.71	0.97
P02788	Lactotransferrin [f, g, h, i, j, n, o, p, u, w]	1.55	1.00
A5A3E0	POTE ankyrin domain family member F [b, m, n, u]	1.20	0.98
P62736	Actin, aortic smooth muscle [b, m, n, q, u, w]	1.20	0.97
P63267	Actin, gamma-enteric smooth muscle [b, m, n, q, u, w]	1.20	0.97
P68133	Actin, alpha skeletal muscle [b, m, n, q, u, w]	1.20	0.96
F8W6P5	LVV-hemorphin-7 (Fragment) [b, c, m, o, u]	0.65	0.00
P68871	Hemoglobin subunit beta [b, c, m, n, o, u, w]	0.65	0.00
P69891	Hemoglobin subunit gamma-1 [b, c, h, n, o, u, w]	0.52	0.00
P02100	Hemoglobin subunit épsilon [b, c, m, n, u]	0.50	0.00
P69892	Hemoglobin subunit gamma-2 [b, c, m, n, u]	0.50	0.00
P02042	Hemoglobin subunit delta [b, c, h, n, o, u, w]	0.48	0.00
P05164	Myeloperoxidase [a, b, g, j, r, u]	0.44	0.00
K7EQR6	Cation channel sperm-associated protein subunit gamma [m, t, x]	0.41	0.01

P60709	Actin, cytoplasmic 1 [b, m, n, q, u, w]	0.41	0.00
P63261	Actin, cytoplasmic 2 [a, d, g, j, n, q, u, w]	0.40	0.00
P80188	Neutrophil gelatinase-associated lipocalin [c, j, o, u]	0.32	0.00
O60814	Histone H2B type 1-K [b, i, p, u]	0.25	0.02
Q99880	Histone H2B type 1-L [b, m, p, u]	0.25	0.03
U3KQK0	Histone H2B [b, m, p, u]	0.24	0.01
Q8N257	Histone H2B type 3-B [b, m, p, u]	0.24	0.01
P33778	Histone H2B type 1-B [b, m, p, u]	0.24	0.02
Q99877	Histone H2B type 1-N [b, m, p, u]	0.24	0.02
P06899	Histone H2B type 1-J [b, i, p, u]	0.24	0.01
P57053	Histone H2B type F-S [b, i, p, u]	0.24	0.01
Q99879	Histone H2B type 1-M [b, m, p, u]	0.23	0.00
P58876	Histone H2B type 1-D [b, m, p, u]	0.23	0.00
Q93079	Histone H2B type 1-H [b, m, p, u]	0.23	0.01
Q5QNW6	Histone H2B type 2-F [b, m, p, u]	0.23	0.01

P23527	Histone H2B type 1-O [b, m, p, u]	0.23	0.00
P62807	Histone H2B type 1-C/E/F/G/I [b, i, p, u]	0.22	0.01
Q16778	Histone H2B type 2-E [b, i, p, u]	0.22	0.01

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.

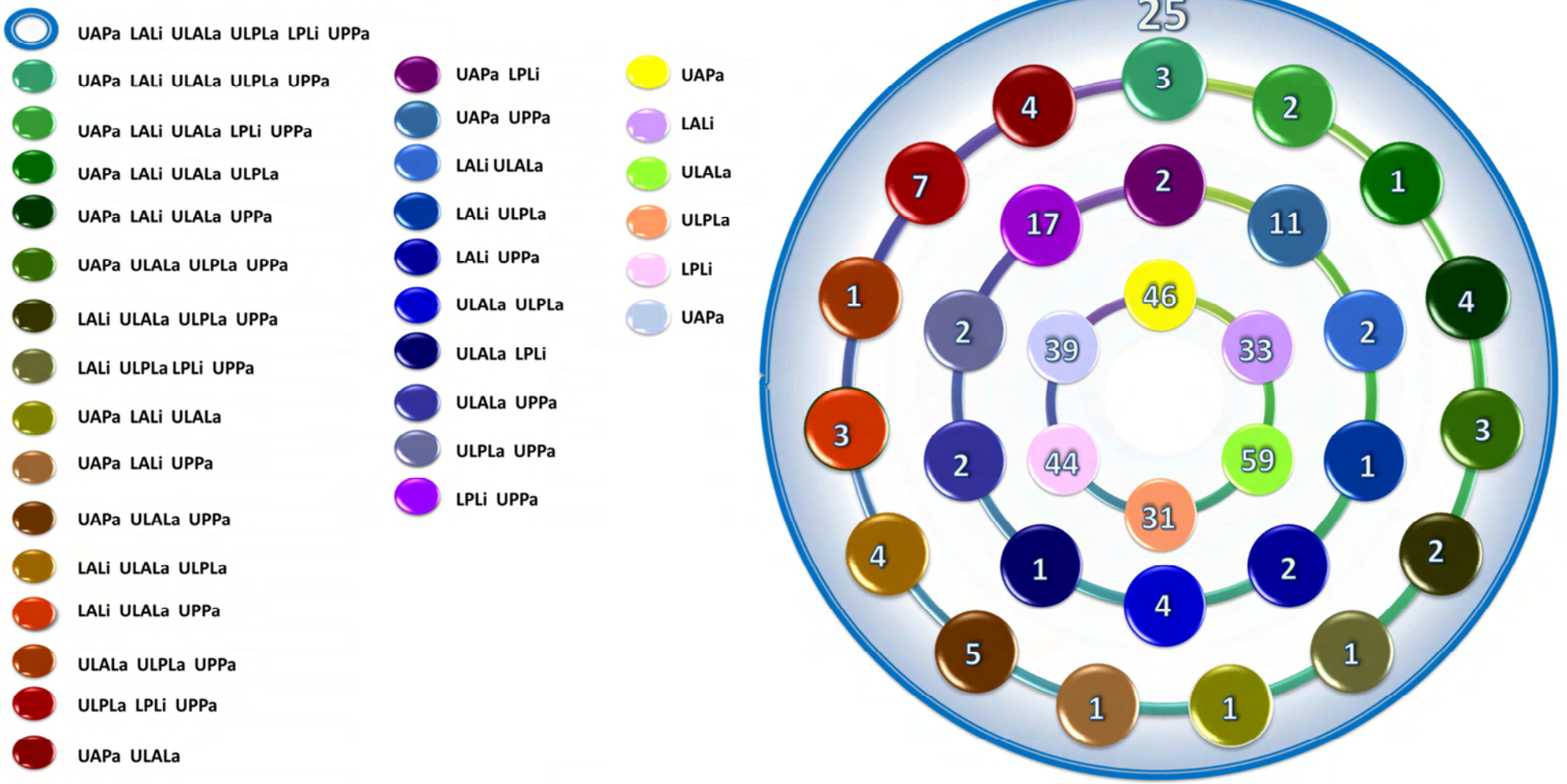

UAPa LALi ULALa ULPLa LPLi UPPa
UAPa LALi ULALa ULPLa UPPa
UAPa LALi ULALa LPLi UPPa
UAPa LALi ULALa ULPLa
UAPa LALi ULALa UPPa
UAPa ULALa ULPLa UPPa
LALi ULALa ULPLa UPPa
LALi ULPLa LPLi UPPa
UAPa LALi ULALa
UAPa LALi UPPa
UAPa ULALa UPPa
LALi ULALa ULPLa
LALi ULALa UPPa
ULALa ULPLa UPPa
ULPLa LPLi UPPa
UAPa ULALa
UAPa LPLi
UAPa UPPa
LALi ULALa
LALi ULPLa
LALi UPPa
ULALa ULPLa
ULALa LPLi
ULALa UPPa
ULPLa UPPa
LPLi UPPa
UAPa
LALi
ULALa
ULPLa
LPLi
UAPa
25
3
2
1
4
2
11
17
7
4
1
2
46
39
33
2
4
3
2
44
59
1
3
31
4
1
2
2
4
5
1
1
1

Table S1. Classification of identified proteins from the acquired enamel pellicle collected from all the regions of dental arches. U- upper, L- lower; A- anterior; P-posterior; Pa- palatal; La- labial; Li- lingual.

Accession number	Protein Name	UAPa	LALi	ULALa	ULPLa	LPLa	UPPa
Q9BRC7	1-phosphatidylinositol 4_5-bisphosphate phosphodiesterase delta-4 [a, g, n, p, s, u]	-	-	-	Yes	-	-
E7ETU7	39S ribosomal protein L3_ mitochondrial [d, m, r, x]	-	-	-	-	-	Yes
C9J3U1	3-oxo-5-beta-steroid 4-dehydrogenase (Fragment) [m, t, x]	-	-	-	Yes	-	-
Q9P2N4	A disintegrin and metalloproteinase with thrombospondin motifs 9 [a, g, o, u]	-	-	-	-	-	Yes
P68032	Actin_ alpha cardiac muscle 1 [b, m, n, q , u, w]	Yes	Yes	Yes	Yes	Yes	Yes
P68133	Actin_ alpha skeletal muscle [b, m, n, q, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
P62736	Actin_ aortic smooth muscle [b, m, n, q, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
P60709	Actin_ cytoplasmic 1 [b, m, n, q, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
P63261	Actin_ cytoplasmic 2 [a, d, g, j, n, q, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
J3KT65	Actin_ cytoplasmic 2_ N-terminally processed [b, m, t, u]	Yes	Yes	Yes	Yes	Yes	Yes
I3L3I0	Actin_ cytoplasmic 2_ N-terminally processed (Fragment) [m, t, u]	-	Yes	-	-	-	-
P63267	Actin_ gamma-enteric smooth muscle [b, m, n, q, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
O95996	Adenomatous polyposis coli protein 2 [b, e, m, n, q, u, w]	-	-	-	Yes	-	-
A0A087X2J2	ADP-ribosylation factor-like protein 8A [b, c, e, r, u]	-	-	-	Yes	-	-
Q8IUK7	ALB protein [c, m, o, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
C9JKR2	Albumin_ isoform CRA_k [c, m, o, u]	Yes	Yes	Yes	Yes	Yes	Yes

Q6UW56	All-trans retinoic acid-induced differentiation factor [b, l, p, s, u]	-	Yes	-	-	-	-
P02763	Alpha-1-acid glycoprotein 1 [b, c, j, o, u]	Yes	-	-	-	-	-
P12814	Alpha-actinin-1 [b, d, m, n, q, s, u, w]	-	-	-	Yes	-	-
P35609	Alpha-actinin-2 [d, m, n, u, w]	-	-	-	Yes	-	-
Q5T085	Alpha-amylase (Fragment) [a, g, t, u]	Yes	-	Yes	Yes	-	Yes
P04745	Alpha-amylase 1 [a, g, o, u]	Yes	-	Yes	Yes	-	Yes
P19961	Alpha-amylase 2B [a, g, o, u]	Yes	-	Yes	Yes	-	Yes
P06733	Alpha-enolase [a, b, g, n, p, s, u, w]	Yes	-	Yes	-	-	Yes
Q3KRA9	Alpha-ketoglutarate-dependent dioxygenase alkB homolog 6 [a, g, n, p, u]	-	-	-	-	Yes	-
Q9Y2J4	Angiomotin-like protein 2 [b, m, n, u]	-	-	-	Yes	-	-
Q01484	Ankyrin-2 [c, d, m, n, q, s, u, w]	-	-	-	Yes	-	-
Q12955	Ankyrin-3 [b, d, m, n, q, s, u, w]	-	Yes	-	-	-	-
Q5T3N1	Annexin (Fragment) [b, l, n, p, s, u]	Yes	-	Yes	-	-	-
P04083	Annexin A1 [a, b, g, j, n, o, p, s, u, w]	Yes	-	Yes	-	-	-
P03973	Antileukoproteinase [a, b, g, i, j, o, u]	-	Yes	Yes	-	-	-
Q96N21	AP-4 complex accessory subunit tepsin [m, n, u, w]	-	-	Yes	-	-	-
Q96P50	Arf-GAP with coiled-coil_ ANK repeat and PH domain-containing protein 3 [a, m, t, u]	-	Yes	-	-	-	-
X6R5P2	Astrotactin-2 [m, t, x]	-	Yes	-	-	-	-
P25705	ATP synthase subunit alpha_ mitochondrial [a, c, m, s, u, w]	Yes	-	-	-	-	-

Q9BQ39	ATP-dependent RNA helicase DDX50 [b, m, p, u]	-	Yes	-	-	-	-
H0YFC3	Autophagy-related protein 16-2 (Fragment) [m, t, u]	-	-	-	Yes	-	-
P20160	Azurocidin [a, b, g, i, s, u, w]	-	Yes	-	Yes	Yes	Yes
O75531	Barrier-to-autointegration fator [b, m, n, p, u, w]	Yes	-	-	-	-	-
Q13884	Beta-1-syntrophin [d, m, n, q, s, u, w]	-	-	-	Yes	-	-
Q562R1	Beta-actin-like protein 2 [b, m, n, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
P13929	Beta-enolase [a, g, n, u]	-	-	Yes	-	-	-
P08236	Beta-glucuronidase [a, g, o, u, w]	-	-	Yes	-	-	-
H0Y9L8	Bifunctional lysine-specific demethylase and histdyl-hydroxylase MINA (Fragment) [b, m, n, p, u]	-	-	Yes	-	-	-
P51587	Breast cancer type 2 susceptibility protein [b, m, n, p, q, u, w]	-	-	-	-	Yes	-
Q9Y2F9	BTB/POZ domain-containing protein 3 [b, m, n, p, u]	-	-	-	-	Yes	-
P12830	Cadherin-1 [b, m, s, u, w]	-	-	Yes	-	-	-
Q13634	Cadherin-18 [a, m, s, u]	-	-	-	Yes	-	-
P27482	Calmodulin-like protein 3 [a, g, o, u]	-	-	-	-	Yes	-
A6NHC0	Calpain-8 [a, g, n, u]	-	-	-	-	-	Yes
Q6UWW8	Carboxylesterase 3 1 [a, g, o, r, u]	-	-	-	-	-	Yes
Q9UQB3	Catenin delta-2 [e, m, p, u, w]	-	-	Yes	-	-	-
P08311	Cathepsin G [a, b, g, i, j, o, p, u]	-	-	Yes	Yes	-	Yes
G3V584	Cation channel sperm-associated protein subunit beta (Fragment) [b, m, t]	-	-	-	Yes	-	-

K7EQR6	Cation channel sperm-associated protein subunit gamma [m, t, x]	-	-	-	-	Yes	Yes
Q6ZRH7	Cation channel sperm-associated protein subunit gamma [b, m, s, u]	-	-	-	-	-	Yes
Q6NVZ8	CBWD5 protein [m, t, u]	-	-	-	-	Yes	-
Q8WUE2	CD96 antigen_ isoform CRA_b [m, t, u]	-	-	Yes	-	-	-
Q92600	Cell differentiation protein RCD1 homolog [b, m, ,n, p, u, w]	-	Yes	-	-	-	-
M0QZQ7	Cell growth regulator with RING finger domain protein 1 (Fragment) [m, t, x]	-	-	-	-	-	Yes
Q6ZU80	Centrosomal protein of 128 kDa [m, n, q, u]	-	-	-	-	Yes	-
H0YJE3	Centrosomal protein of 128 kDa (Fragment) [m, t, u]	-	-	-	-	Yes	-
E7EWM2	Centrosomal protein of 170 kDa [m, n, x]	-	Yes	-	-	-	-
H0Y6Q4	Centrosome-associated protein 350 (Fragment) [d, m, r, i, x]	-	-	Yes	-	-	-
Q9BV73	Centrosome-associated protein CEP250 [b, m, n, q, u, w]	Yes	-	-	-	-	-
Q96L14	Cep170-like protein [b, m, t, u, w]	-	Yes	-	-	-	-
Q4G0U5	Cilia- and flagella-associated protein 221 [d, m, n, q, u, w]	-	-	-	Yes	-	-
A5D8W1	Cilia- and flagella-associated protein 69 [m, t, x]	-	-	Yes	-	-	-
Q5JTY5	COBW domain-containing protein 3 [b, m, t, u]	-	-	-	-	Yes	-
Q5RIA9	COBW domain-containing protein 5 [b, m, t, u]	-	-	-	-	Yes	-
Q4V339	COBW domain-containing protein 6 [b, m, t, u]	-	-	-	-	Yes	-
Q03692	Collagen alpha-1(X) chain [a, b, d, h, o, u]	-	-	-	-	Yes	-
Q07092	Collagen alpha-1(XVI) chain [b, g, o, u, w]	-	Yes	-	-	-	-

P09871	Complement C1s subcomponente [a, e, g, j, o, u, w]	-	Yes	-	-	-	-
Q9Y2V7	Conserved oligomeric Golgi complex subunit 6 [c, m, s, u]	-	-	Yes	-	-	-
P04080	Cystatin-B [a, g, n, p, u]	Yes	Yes	Yes	Yes	-	Yes
P01036	Cystatin-S [a, b, g, o, u]	Yes	Yes	Yes	-	-	-
P01037	Cystatin-SN [a, b, g, o, u]	Yes	Yes	Yes	-	-	Yes
Q13409	Cytoplasmic dynein 1 intermediate chain 2 [c, n, q, u]	-	-	Yes	-	-	-
Q8NEU8	DCC-interacting protein 13-beta [b, m, p, s, u, w]	-	-	Yes	-	-	-
Q8IV96	DDX6 protein [b, m, n, u]	-	-	Yes	-	-	-
O77932	Decapping and exoribonuclease protein [a, b, f, p, u]	-	-	-	Yes	-	-
Q08495	Dematin [d, m, n, q, u, w]	Yes	-	-	-	-	-
Q9UI17	Dimethylglycine dehydrogenase_ mitochondrial [a, g, r, u]	-	Yes	-	-	-	-
Q9H5L6	DNA transposase THAP9 [b, c, m, t, u]	-	-	-	-	Yes	-
O75953	DnaJ homolog subfamily B member 5 [d, m, n, p, u, w]	-	-	-	-	Yes	-
F8WCZ4	DnaJ homolog subfamily B member 6 [m, t, u]	-	-	-	-	Yes	-
H7C242	Doublecortin domain-containing protein 2C (Fragment) [e, m, r, u]	Yes	-	-	-	-	-
O95714	E3 ubiquitin-protein ligase HERC2 [b, m, n, p, q, u, w]	-	-	-	-	Yes	-
Q7Z6Z7	E3 ubiquitin-protein ligase HUWE1 [b, e, m, n, p, u, w]	-	-	-	-	Yes	-
Q9BUP0	EF-hand domain-containing protein D1 [a, m, o, u]	-	Yes	-	-	-	-
P61576	Endogenous retrovirus group K member 104 Rec protein [b, m, n, p, w]	Yes	-	-	-	-	-

F5H0C8	Enolase [a, m, t, u]	-	-	Yes	-	-	-
K7EM90	Enolase (Fragment) [a, b, g, t, u]	-	-	-	-	-	Yes
P11678	Eosinophil peroxidase [a, m, n, u]	-	-	-	-	-	Yes
Q6P050	F-box and leucine-rich protein 22 [a, m, n, u]	-	-	-	Yes	-	-
P21333	Filamin-A [d, m, n, q, u, w]	Yes	-	-	-	-	-
Q8IXW7	FMR1 protein [b, m, t, u]	-	-	-	-	-	Yes
Q12952	Forkhead box protein L1 [b, m, p, u]	-	-	-	-	-	Yes
R9WNI0	Fragile X mental retardation 1 [b, m, t, u]	-	-	-	-	-	Yes
G3V0J0	Fragile X mental retardation 1_ isoform CRA_e [b, m, n, x]	-	-	-	-	-	Yes
Q06787	Fragile X mental retardation protein 1 [b, c, m, n, p, u]	-	-	-	-	-	Yes
P47929	Galectin-7 [b, m, n, o, p, u]	-	Yes	Yes	-	-	-
P14867	Gamma-aminobutyric acid receptor subunit alpha-1 [a, c, g, s, u]	-	-	-	-	Yes	-
P09104	Gamma-enolase [a, g, n, s, u]	-	-	Yes	-	-	-
Q14687	Genetic suppressor element 1 [m, t, u, w]	Yes	-	-	-	-	-
Q3V6T2	Girdin [b, m, n, s, u, w]	-	-	Yes	-	-	-
H3BQ30	Glycine cleavage system H protein_ mitochondrial (Fragment) [b, m, t, u]	-	-	-	-	-	Yes
Q9BUA5	GNPTAB protein [m, s, u]	-	-	Yes	-	-	-
Q14789	Golgin subfamily B member 1 [d, m, s, u]	-	-	Yes	-	Yes	-
P00738	Haptoglobin [a, b, g, i, j, o, u, w]	Yes	-	Yes	-	-	-

A0A0A0MTS5	HCG1811249_ isoform CRA_f 1 [b, m, t, u]	-	-	-	Yes	-	-
I3L4Q0	HCG1818442_ isoform CRA_a [m, t, x]	-	-	-	-	-	Yes
F6W7H3	Heat shock 70 kDa protein 1A/1B [b, m, r, u]	-	-	-	-	-	Yes
P34931	Heat shock 70 kDa protein 1-like [b, m, o, u, w]	Yes	-	-	-	-	Yes
P17066	Heat shock 70 kDa protein 6 [b, e, m, n, o, u]	Yes	-	-	-	-	Yes
P11142	Heat shock cognate 71 kDa protein [b, d, m, n, t, s, u, w]	Yes	-	-	-	-	Yes
P04792	Heat shock protein beta-1 [b, d, m, n, p, q, u, w]	Yes	Yes	Yes	Yes	-	-
P54652	Heat shock-related 70 kDa protein 2 [b, m, n, q, u, w]	Yes	-	-	-	-	Yes
P69905	Hemoglobin subunit alpha [b, c, m, n, o, s, u]	-	-	-	Yes	Yes	Yes
P68871	Hemoglobin subunit beta [b, c, m, n, o, u, w]	-	-	-	Yes	Yes	Yes
P02042	Hemoglobin subunit delta [b, c, h, n, o, u, w]	-	-	-	Yes	Yes	Yes
P02100	Hemoglobin subunit épsilon [b, c, m, n, u]	-	-	-	Yes	Yes	Yes
P69891	Hemoglobin subunit gamma-1 [b, c, h, n, o, u, w]	-	-	-	Yes	Yes	Yes
P69892	Hemoglobin subunit gamma-2 [b, c, m, n, u]	-	-	-	Yes	Yes	Yes
Q00839	Heterogeneous nuclear ribonucleoprotein U [b, m, p, u]	-	-	Yes	-	-	-
P15515	Histatin-1 [b, i, l, o, u]	Yes	Yes	Yes	Yes	-	Yes
C9J0D1	Histone H2A [b, m, p, u]	Yes	-	-	-	-	Yes
P0C0S8	Histone H2A type 1 [b, m, p, u, w]	Yes	-	-	-	-	-
Q96QV6	Histone H2A type 1-A [b, m, p, u, w]	Yes	-	-	-	-	-

P04908	Histone H2A type 1-B/E [b, m, p, u, w]	Yes	-	-	-	-	-
Q93077	Histone H2A type 1-C [b, m, p, u, w]	Yes	-	-	-	-	-
P20671	Histone H2A type 1-D [b, m, p, u, w]	Yes	-	-	-	-	-
Q96KK5	Histone H2A type 1-H [b, m, p, u, w]	Yes	-	-	-	-	-
Q99878	Histone H2A type 1-J [b, m, p, u, w]	Yes	-	-	-	-	-
Q6FI13	Histone H2A type 2-A [b, m, p, u, w]	Yes	-	-	-	-	-
Q16777	Histone H2A type 2-C [b, m, p, u, w]	Yes	-	-	-	-	-
Q7L7L0	Histone H2A type 3 [b, m, p, u, w]	Yes	-	-	-	-	-
Q9BTM1	Histone H2A.J [b, m, p, u, w]	Yes	-	-	-	-	-
Q71UI9	Histone H2A.V [b, m, p, u]	Yes	-	-	-	-	Yes
P0C0S5	Histone H2A.Z [b, m, p, u, w]	Yes	-	-	-	-	Yes
P16104	Histone H2AX [b, e, m, p, u, w]	Yes	-	-	-	-	-
U3KQK0	Histone H2B [b, m, p, u]	-	-	-	-	Yes	Yes
P33778	Histone H2B type 1-B [b, m, p, u]	-	-	-	-	Yes	Yes
P62807	Histone H2B type 1-C/E/F/G/I [b, i, p, u]	-	-	-	-	Yes	Yes
P58876	Histone H2B type 1-D [b, m, p, u]	-	-	-	-	Yes	Yes
Q93079	Histone H2B type 1-H [b, m, p, u]	-	-	-	-	Yes	Yes
P06899	Histone H2B type 1-J [b, i, p, u]	-	-	-	-	Yes	Yes
O60814	Histone H2B type 1-K [b, i, p, u]	-	-	-	-	Yes	Yes

Q99880	Histone H2B type 1-L [b, m, p, u]	-	-	-	-	Yes	Yes
Q99879	Histone H2B type 1-M [b, m, p, u]	-	-	-	-	Yes	Yes
Q99877	Histone H2B type 1-N [b, m, p, u]	-	-	-	-	Yes	Yes
P23527	Histone H2B type 1-O [b, m, p, u]	-	-	-	-	Yes	Yes
Q16778	Histone H2B type 2-E [b, i, p, u]	-	-	-	-	Yes	Yes
Q5QNW6	Histone H2B type 2-F [b, m, p, u]	-	-	-	-	Yes	Yes
Q8N257	Histone H2B type 3-B [b, m, p, u]	-	-	-	-	Yes	Yes
P57053	Histone H2B type F-S [b, i, p, u]	-	-	-	-	Yes	Yes
B4DEB1	Histone H3 [b, m, p, u, w]	-	-	-	-	-	Yes
P68431	Histone H3.1 [m, t, u]	-	-	-	-	-	Yes
Q16695	Histone H3.1t [b, m, p, u, w]	-	-	-	-	-	Yes
Q71DI3	Histone H3.2 [b, m, p, u]	Yes	-	-	-	-	Yes
P84243	Histone H3.3 [b, m, p, u, w]	-	-	-	-	-	Yes
Q6NXT2	Histone H3.3C [g, h, k, m, o]	-	-	-	-	-	Yes
P62805	Histone H4 [b, m, p, u]	Yes	Yes	-	-	-	Yes
Q4G0P3	Hydrocephalus-inducing protein homolog [b, m, q, u]	Yes	-	-	-	-	-
P01876	Ig alpha-1 chain C region [b, i, j, o, u]	Yes	Yes	Yes	Yes	-	Yes
P01877	Ig alpha-2 chain C region [b, j, o, u]	Yes	Yes	Yes	Yes	Yes	Yes
A0A087WYC5	Ig gamma-1 chain C region [m, t, x]	-	-	Yes	-	-	-

P01859	Ig gamma-2 chain C region [b, j, o, u]	Yes	Yes	Yes	-	-	Yes
P01860	Ig gamma-3 chain C region [b, j, o, u]	Yes	Yes	Yes	-	-	Yes
P01861	Ig gamma-4 chain C region [b, j, o, u]	Yes	Yes	Yes	-	-	Yes
P06326	Ig heavy chain V-I region Mot [b, j, o, x]	-	-	-	-	Yes	-
P01766	Ig heavy chain V-III region BRO [b, j, o, x]	-	-	Yes	-	-	-
P01774	Ig heavy chain V-III region POM [b, j, o, x]	Yes	-	-	-	Yes	-
P01777	Ig heavy chain V-III region TEI [b, e, j, o, x]	-	-	Yes	-	-	-
P01765	Ig heavy chain V-III region TIL [b, j, o, x]	-	-	-	-	Yes	-
P01779	Ig heavy chain V-III region TUR [b, j, o, x]	-	-	-	-	Yes	-
P01776	Ig heavy chain V-III region WAS [b, j, o, x]	-	-	-	-	Yes	-
P01834	Ig kappa chain C region [b, j, o, u]	-	Yes	Yes	-	-	Yes
P01605	Ig kappa chain V-I region Lay [b, j, o, x]	-	-	Yes	-	-	-
P04207	Ig kappa chain V-III region CLL [b, j, o, x]	-	-	Yes	-	-	-
P04206	Ig kappa chain V-III region GOL [b, j, o, u]	-	Yes	Yes	Yes	-	-
P01624	Ig kappa chain V-III region POM [b, j, o, x]	-	-	Yes	-	-	-
P01620	Ig kappa chain V-III region SIE [b, j, o, u]	-	Yes	Yes	Yes	-	-
P01622	Ig kappa chain V-III region Ti [b, j, o, u]	-	Yes	Yes	Yes	-	-
P04434	Ig kappa chain V-III region VH (Fragment) [e, j, o, x]	-	-	Yes	-	-	-
P01623	Ig kappa chain V-III region WOL [b, j, o, u]	-	Yes	Yes	Yes	-	-

P01871	Ig mu chain C region [b, i, j, o, s, u, w]	-	Yes	-	Yes	-	-
M0QYN9	IgG receptor FcRn large subunit p51 [b, j, t, u]	-	-	Yes	-	-	-
Q9NX62	Inositol monophosphatase 3 [a, b, m, s, u]	-	-	-	-	-	Yes
J3QQL2	Integrin beta (Fragment) [b, m, s, u]	-	-	-	-	Yes	-
K9N2S2	Interleukin 15 receptor alpha isoform EM2 [b, e, m, t, u]	-	-	-	-	Yes	-
K9N163	Interleukin 15 receptor alpha isoform IC2 [m, t, u]	-	-	-	-	Yes	-
K9N2Q6	Interleukin 15 receptor alpha isoform IC3 [m, t, u]	-	-	-	-	Yes	-
K9N0C7	Interleukin 15 receptor alpha isoform IC4 [m, t, u]	-	-	-	-	Yes	-
K9N1J3	Interleukin 15 receptor alpha isoform IC5 [m, t, u]	-	-	-	-	Yes	-
Q13261	Interleukin-15 receptor subunit alpha [b, e, m, n, p, s, u, w]	-	-	-	-	Yes	-
A0A0A0MR55	Kallikrein-11 [b, m, t, x]	-	-	-	Yes	-	-
I3L4J3	KAT8 regulatory NSL complex subunit 1 [m, x, u]	-	-	-	Yes	-	-
P13645	Keratin_ type I cytoskeletal 10 [d, m, n, o, p, s, u]	-	-	Yes	Yes	-	-
P13646	Keratin_ type I cytoskeletal 13 [d, m, o, u, w]	-	-	Yes	Yes	-	-
P19012	Keratin_ type I cytoskeletal 15 [b, m, o, u, w]	Yes	-	Yes	-	-	-
P19013	Keratin_ type II cytoskeletal 4 [d, m, q, u]	-	-	Yes	-	-	-
P13647	Keratin_ type II cytoskeletal 5 [d, m, n, o, q, u]	Yes	-	Yes	-	-	Yes
P02538	Keratin_ type II cytoskeletal 6A [b, d, m, o, u, w]	Yes	-	Yes	-	-	Yes
P04259	Keratin_ type II cytoskeletal 6B [b, i, o, u, w]	Yes	-	Yes	-	-	Yes

P48668	Keratin_ type II cytoskeletal 6C [d, m, o, u]	Yes	-	Yes	-	-	Yes
Q96L93	Kinesin-like protein KIF16B [c, m, n, q, s]	Yes	-	-	-	-	-
P02788	Lactotransferrin [f, g, h, i, j, n, o, p, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
Q16787	Laminin subunit alpha-3 [b, d, m, o, u, w]	-	-	-	Yes	-	-
A6NMS7	Leucine-rich repeat-containing protein 37A [m, s, u]	-	-	-	Yes	-	-
Q5T3J3	Ligand-dependent nuclear receptor-interacting factor 1 [b, d, p, u]	-	-	Yes	-	-	-
E7EPM6	Long-chain-fatty-acid--CoA ligase 1 [a, g, s, u]	-	-	Yes	-	-	-
F8W6P5	LVV-hemorphin-7 (Fragment) [b, c, m, o, u]	-	-	-	Yes	Yes	Yes
P61626	Lysozyme C [a, b, g, i, j, o, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
P08493	Matrix Gla protein [b, m, o, u]	-	Yes	-	-	-	-
P14780	Matrix metalloproteinase-9 [a, g, o, u, w]	-	-	-	Yes	-	Yes
O75448	Mediator of RNA polymerase II transcription subunit 24 [b, m, p, u, w]	-	Yes	-	-	-	-
F5GY88	Mediator of RNA polymerase II transcription subunit 24 [b, m, t, u]	-	-	Yes	-	-	-
K7EQY5	Megakaryocyte-associated tyrosine kinase_ isoform CRA_a [a, c, m, t, u]	-	-	-	-	-	Yes
P42679	Megakaryocyte-associated tyrosine-protein kinase [b, c, e, g, w]	-	-	-	-	-	Yes
P11137	Microtubule-associated protein 2 [d, m, n, q, u, w]	Yes	-	-	-	-	-
Q8N4C8	Misshapen-like kinase 1 [b, c, m, n, s, u, w]	-	-	Yes	-	-	-
E7EX83	Mitogen-activated protein kinase kinase kinase kinase 4 [a, b, g, t, u]	-	-	Yes	-	-	-
P24158	Myeloblastin [a, g, n, o, s, u, w]	Yes	Yes	Yes	Yes	Yes	Yes

P05164	Myeloperoxidase [a, b, g, j, r, u]	Yes	Yes	Yes	Yes	Yes	Yes
Q5BKV1	MYH9 protein [b, m, q, u]	-	Yes	-	-	-	-
P60660	Myosin light polypeptide 6 [d, m, n, o, s, u]	-	-	Yes	-	-	-
P35579	Myosin-9 [d, m, n, q, u, w]	-	Yes	-	-	-	-
Q13615	Myotubularin-related protein 3 [a, m, n, s, u]	-	-	Yes	-	-	-
F8VQW2	N-acetylglucosamine-1-phosphotransferase subunit alpha [m, s, u]	-	-	Yes	-	-	-
Q3T906	N-acetylglucosamine-1-phosphotransferase subunits alpha/beta [a, c, g, s, u]	-	-	Yes	-	-	-
A0A0A0MT02	Negative elongation factor E (Fragment) [b, m, t, u]	-	-	-	-	-	Yes
Q92823	Neuronal cell adhesion molecule [b, m, s, u, w]	-	-	Yes	-	-	-
F8W0C2	Neuronal PAS domain-containing protein 3 1 [b, m, t, u]	-	-	Yes	-	-	-
M0QZK5	Neuropathy target esterase (Fragment) [d, m, s, u]	-	-	Yes	-	-	-
P59665	Neutrophil defensin 1 [b, i, j, o, u]	Yes	Yes	Yes	-	Yes	Yes
P59666	Neutrophil defensin 3 [b, i, j, o, u]	Yes	Yes	Yes	-	Yes	Yes
P08246	Neutrophil elastase [a, b, j, n, o, u, w]	-	Yes	-	Yes	-	-
P80188	Neutrophil gelatinase-associated lipocalin [c, j, o, u]	-	-	-	-	Yes	Yes
O15453	Next to BRCA1 gene 2 protein [m, t, u]	-	-	-	Yes	-	-
Q9H841	NIPA-like protein 2 [c, m, s, w]	-	-	-	Yes	-	-
Q9HCG7	Non-lysosomal glucosylceramidase [a, m, s, u]	Yes	-	-	-	-	-
Q8N1F7	Nuclear pore complex protein Nup93 [c, d, m, p, s, u, w]	Yes	-	-	-	-	-

O75376	Nuclear receptor corepressor 1 [b, m, p, u, w]	Yes	-	-	-	-	-
Q9NY43	BarH-like 2 homeobox protein [b, m, n, p, u]	-	Yes	-	-	-	-
Q96G74	OTU domain-containing protein 5 [a, g,, n, u, w]	Yes	-	-	-	-	-
Q9Y236	Oxidative stress-induced growth inhibitor 2 [b, m, t, u]	-	-	Yes	-	-	-
P04746	Pancreatic alpha-amylase [a, g, o, u]	Yes	-	-	-	-	Yes
Q8ND90	Paraneoplastic antigen Ma1 [b, m, p, u, w]	-	Yes	-	-	-	-
O75594	Peptidoglycan recognition protein 1 [b, i, j, n, o, u]	-	-	-	Yes	-	Yes
F8WE65	Peptidyl-prolyl cis-trans isomerase [d, m, t, u]	-	-	Yes	Yes	-	-
P62937	Peptidyl-prolyl cis-trans isomerase A [b, d, m, n, o, u, w]	-	-	Yes	Yes	-	-
Q96BD5	PHD finger protein 21A [b, m, p, u, w]	Yes	-	-	-	-	-
P42336	Phosphatidylinositol 4_5-bisphosphate 3-kinase catalytic subunit alpha isoform [f, j, n, u, w]	-	-	-	Yes	-	-
C9JEA7	Phosphoinositide phospholipase C [a, e, g, n, s, u]	-	-	-	Yes	-	-
Q9Y263	Phospholipase A-2-activating protein [b, m, o, u, w]	-	-	-	-	-	Yes
Q9ULL4	Plexin-B3 [b, m, s, u, w]	-	-	-	-	-	Yes
O60486	Plexin-C1 [b, m, s, u, w]	-	-	-	Yes	-	-
H3BSE9	Polycystin-1 (Fragment) [m, t, x]	-	-	Yes	-	-	-
Q6S8J3	POTE ankyrin domain family member E [b, m, o, u]	Yes	Yes	Yes	Yes	Yes	Yes
A5A3E0	POTE ankyrin domain family member F [b, m, n, u]	Yes	Yes	Yes	Yes	Yes	Yes
P0CG38	POTE ankyrin domain family member I [b, m, o, u]	Yes	Yes	Yes	Yes	Yes	Yes

P0CG39	POTE ankyrin domain family member J [b, m, o, u]	Yes	-	-	-	Yes	-
Q15034	Probable E3 ubiquitin-protein ligase HERC3 [a, d, g, n, u]	-	-	-	Yes	-	-
P12273	Prolactin-inducible protein [b, d, m, o, u]	-	-	Yes	-	-	-
Q6MZM9	Proline-rich protein 27 [b, l, o, x]	-	Yes	Yes	Yes	-	Yes
Q06455	Protein CBFA2T1 [a, b, g, p, u, w]	-	-	-	-	-	Yes
F8WBX7	Protein CFAP69 [m, t, x]	-	-	Yes	-	-	-
P30101	Protein disulfide-isomerase A3 [a, b, g, o, p, r, u]	-	-	Yes	-	-	-
Q9NVM1	Protein eva-1 homolog B [b, m, s, u]	-	-	-	-	Yes	-
A8MTA8	Protein FAM166B [m, t, u]	-	-	Yes	-	-	-
Q5TBA9	Protein furry homolog [b, m, n, q, u, w]	-	-	-	-	-	Yes
A0A087WW89	Protein IGHV3-72 [m, t, x]	-	-	Yes	-	-	-
S4R460	Protein IGHV3OR16-9 [b, j, o, w]	-	-	Yes	-	-	-
A0A087WWV8	Protein IGKV1-8 [m, t, x]	-	Yes	Yes	-	-	Yes
A0A087X1V9	Protein IGKV2-28 [m, t, x]	-	-	Yes	-	-	-
A0A087WZW8	Protein IGKV3-11 [m, t, x]	-	Yes	Yes	-	-	Yes
A0A075B6H7	Protein IGKV3-7 (Fragment) [m, t, u]	-	-	Yes	-	-	-
A0A0C4DH90	Protein IGKV3OR2-268 (Fragment) [m, t, u]	-	-	Yes	-	-	-
Q52LA3	Protein lin-52 homolog [b, m, p, u]	-	-	-	-	Yes	-
A0A096LP88	Protein LOC102725121 [m, t, x]	-	-	-	-	Yes	-

Q9BVG4	Protein PBDC1 [m, t, u]	-	-	Yes	-	-	-
Q9Y520	Protein PRRC2C [m, s, u]	-	-	-	Yes	-	-
P05109	Protein S100-A8 [b, e, i, j, l, n, o, q, s, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
P06702	Protein S100-A9 [a, b, g, i, j, n, o, q, s, u, w]	Yes	-	-	-	-	-
Q96ER3	Protein SAAL1 [m, o, t, u]	-	-	-	Yes	-	-
A0A575	Protein TRBV2 (Fragment) [m, t, x]	-	Yes	-	-	-	-
Q9Y5H0	Protocadherin gamma-A3 [b, m, s, u]	Yes	-	-	-	-	-
Q49AC9	PTPRM protein [a, g, t, u]	Yes	-	-	-	-	-
Q9BYX7	Putative beta-actin-like protein 3 [a, m, n, q, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
H0Y5V3	Putative COBW domain-containing protein 7 [m, t, u]	-	-	-	-	Yes	-
P48741	Putative heat shock 70 kDa protein 7 [b, m, o, u]	Yes	-	-	-	-	Yes
P09565	Putative insulin-like growth factor 2-associated protein [b, m, t, u]	-	-	Yes	-	-	-
Q8NBZ9	Putative uncharacterized protein NEXN-AS1 [m, t, x]	-	-	-	-	Yes	-
Q9BXR0	Queuine tRNA-ribosyltransferase [a, g, n, p, u]	-	-	Yes	-	-	-
P46940	Ras GTPase-activating-like protein IQGAP1 [a, g, s, u]	-	Yes	-	-	-	-
E7EPS8	Receptor-type tyrosine-protein phosphatase mu [a, g, s, u]	Yes	-	-	-	-	-
Q9H1J1	Regulator of nonsense transcripts 3A [b, m, n, p, w]	-	-	-	-	-	Yes
J3KPQ4	Rho GTPase activating protein 9_ isoform CRA_a [e, m, t, u]	-	Yes	-	-	-	-
Q13017	Rho GTPase-activating protein 5 [b, m, n, s, u, w]	-	Yes	-	-	-	-

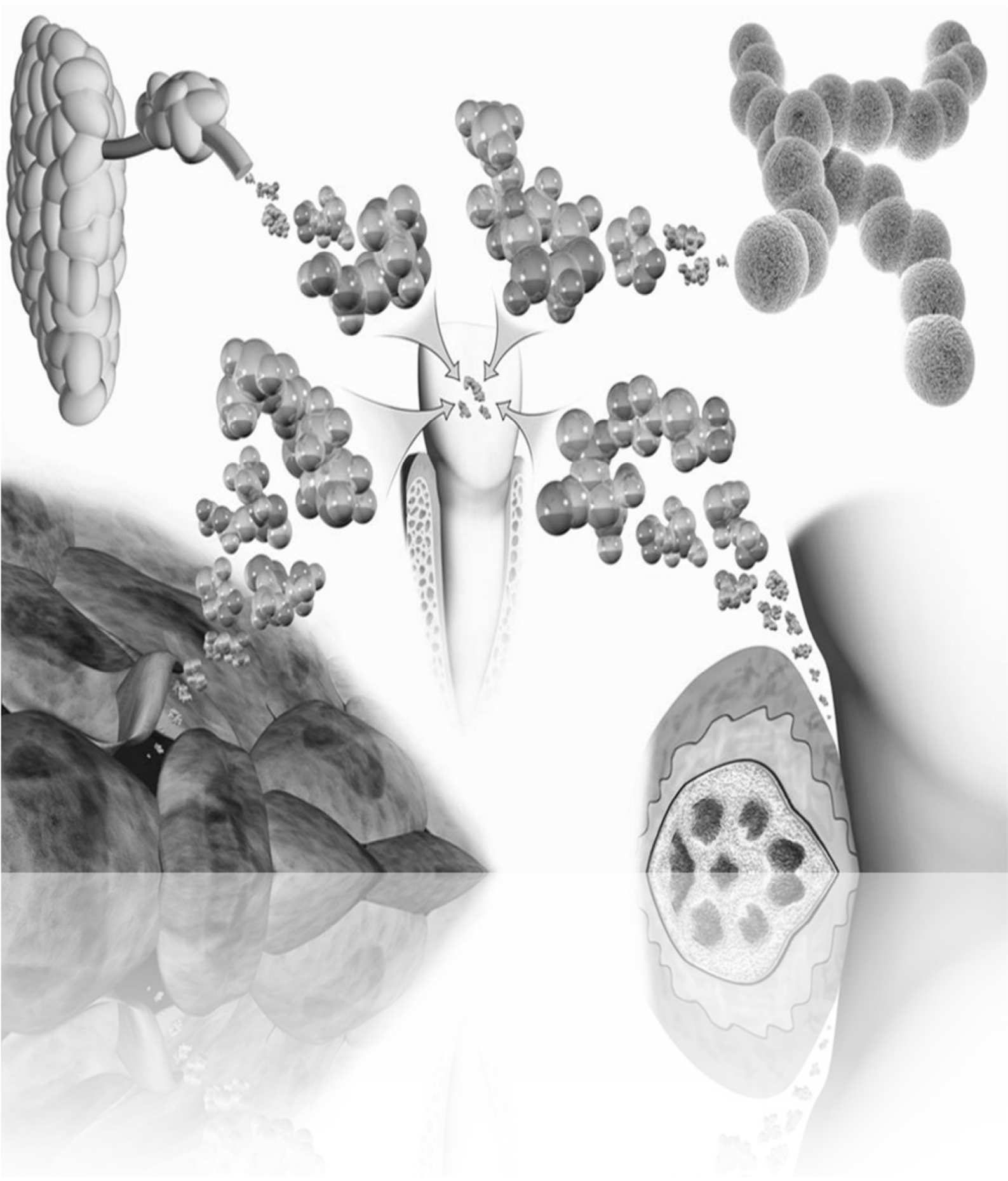
Q9BRR9	Rho GTPase-activating protein 9 [a, m, n, u]	-	Yes	-	-	-	-
Q9UKM9	RNA-binding protein Raly [b, m, p, u]	Yes	-	-	-	-	Yes
P21817	Ryanodine receptor 1 [c, e, m, s, u, w]	Yes	-	-	-	-	-
Q8IYM2	Schlafen family member 12 [a, g, t, u]	-	-	Yes	-	-	-
Q53EL9	Seizure protein 6 homolog [b, m, s, u]	-	-	-	-	-	Yes
Q9Y6D0	Selenoprotein K [c, g, s, u, w]	-	Yes	-	-	-	-
Q9H2K8	Serine/threonine-protein kinase TAO3 [b, m, n, s, u, w]	-	Yes	-	-	-	-
Q8N8A2	Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit B [b, e, m, t, u]	-	Yes	-	-	-	-
Q13315	Serine-protein kinase ATM [a, b, c, g, n, p, u, w]	Yes	-	-	-	-	-
P02787	Serotransferrin [c, d, g, o, u, w]	-	Yes	Yes	Yes	-	Yes
Q9BXP5	Serrate RNA effector molecule homolog [b, e, m, n, p, u, w]	Yes	-	-	-	-	-
P02768	Serum albumin [a, b, c, g, o, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
C9JFP8	SH3 and multiple ankyrin repeat domains protein 2 [m, t, u]	-	-	-	Yes	-	-
P14678	Small nuclear ribonucleoprotein-associated proteins B and B' [b, m, n, p, u, w]	Yes	-	-	-	-	-
F8WBN0	Sn1-specific diacylglycerol lipase beta [m, s, u]	-	-	-	-	Yes	-
Q86WA9	Sodium-independent sulfate anion transporter [c, m, s, u]	-	Yes	-	-	-	-
V9GY79	Spectrin alpha chain_ erythrocytic 1 (Fragment) [m, t, x]	-	-	-	Yes	-	-
Q96R06	Sperm-associated antigen 5 [b, m, n, p, q, u, w]	-	Yes	-	Yes	-	-
Q8WXA9	Splicing regulatory glutamine/lysine-rich protein 1 [b, m, p, u, w]	Yes	-	-	-	-	-

P02808	Statherin [b, e, l, o, u]	Yes	Yes	Yes	Yes	Yes	Yes
Q96NU1	Sterile alpha motif domain-containing protein 11 [e, m, p, u]	-	Yes	-	-	-	-
Q15772	Striated muscle preferentially expressed protein kinase [b, c, m, p, u]	-	-	Yes	-	-	Yes
E9PD53	Structural maintenance of chromosomes protein [b, m, p, u]	Yes	-	-	-	-	-
Q9NTJ3	Structural maintenance of chromosomes protein 4 [b, m, n, p, u, w]	Yes	-	-	-	-	-
P02814	Submaxillary gland androgen-regulated protein 3B [a, g, o, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
Q9Y2Z0	Suppressor of G2 allele of SKP1 homolog [b, j, n, p, u, w]	-	-	-	-	-	Yes
Q9H7C4	Syncoilin [d, m, n, u]	-	Yes	-	-	-	-
F1T0F2	T-cell leukemia homeobox protein 2 [b, m, t, x]	Yes	-	-	-	-	-
P40200	T-cell surface protein tactile [b, j, s, u]	-	-	Yes	-	-	-
Q9BUZ4	TNF receptor-associated factor 4 [b, e, m, n, p, q, s, u, w]	-	-	Yes	-	-	Yes
E7EMT6	TRAF family member-associated NF-kappa-B activator (Fragment) [m, n, u]	Yes	-	-	-	-	-
C9JC32	Trafficking kinesin-binding protein 1 [m, t, u]	-	-	-	-	-	Yes
P19484	Transcription factor EB [b, j, n, p, u]	-	-	-	-	Yes	-
Q9BX84	Transient receptor potential cation channel subfamily M member 6 [c, m, s, u, w]	Yes	-	-	-	-	-
P29401	Transketolase [c, g, n, o, u]	-	-	Yes	-	-	-
Q9NPI0	Transmembrane protein 138 [b, m, s, u]	-	Yes	-	-	-	-
Q8NDV7	Trinucleotide repeat-containing gene 6A protein [b, m, n, u, w]	-	-	-	-	-	Yes
P07437	Tubulin beta chain [b, d, m, n, q, u, w]	-	-	-	-	-	Yes

I3L3P4	Tumor necrosis factor receptor superfamily member 12a [m, t, x]	-	-	-	-	-	Yes
F5H2I9	Tyrosine-protein kinase STYK1 (Fragment) [m, s, u]	-	-	Yes	-	-	-
Q12923	Tyrosine-protein phosphatase non-receptor type 13 [e, m, n, p, u, w]	Yes	-	-	-	-	-
Q16825	Tyrosine-protein phosphatase non-receptor type 21 [a, g, n, q, u]	-	-	-	-	Yes	-
O75604	Ubiquitin carboxyl-terminal hydrolase 2 [a, b, g, n, p, u, w]	-	-	-	-	Yes	-
O95352	Ubiquitin-like modifier-activating enzyme ATG7 [b, c, g, n, u, w]	-	-	-	-	Yes	-
B8ZZI7	Uncharacterized protein [b, m, s, u]	-	-	-	-	-	Yes
E9PMD0	Uncharacterized protein (Fragment) [b, m, t, u]	-	Yes	-	-	-	-
K7EQZ3	Uncharacterized protein (Fragment) [a, g, p, x]	-	-	-	-	Yes	-
Q3SXR2	Uncharacterized protein C3orf36 [g, h, k, m, o]	-	-	-	-	Yes	-
Q9UBC5	Unconventional myosin-Ia [m, t, u]	-	-	-	-	Yes	-
B2RTY4	Unconventional myosin-IXa [d, m, s, u]	-	-	Yes	-	-	-
J3KNI2	UPF0598 protein C8orf82 [m, t, x]	Yes	-	-	-	-	-
Q7L1V2	Vacuolar fusion protein MON1 homolog B [b, m, n, u, w]	-	-	-	-	-	Yes
P23763	Vesicle-associated membrane protein 1 [c, m, s, u, w]	-	Yes	-	-	-	-
P08670	Vimentin [b, j, n, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
Q8TAF3	WD repeat-containing protein 48 [b, e, m, n, p, u, w]	-	-	-	-	Yes	-
Q96IU2	Zinc finger BED domain-containing protein 3 [e, m, n, s, u]	-	-	-	-	Yes	-
Q14590	Zinc finger protein 235 [b, m, p, u]	-	-	-	-	-	Yes

P17036	Zinc finger protein 3 [b, m, p, u]	-	-	Yes	-	-	-
Q86UE3	Zinc finger protein 546 [b, m, p, u]	-	-	-	Yes	-	-
F5H6X5	Zinc finger protein 84 [b, m, r, u]	-	-	-	-	Yes	-
P25311	Zinc-alpha-2-glycoprotein [a, b, g, o, u, w]	Yes	Yes	Yes	Yes	Yes	Yes
Q96DA0	Zymogen granule protein 16 homolog B [b, m, o, u]	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

One of the biggest obstacles and challenges in studying the composition of the AEP are the very small amounts of pellicle that can be collected. For this reason, it is not possible to individually analyze the material collected from each volunteer. As a consequence, it is necessary to pool the material from the different volunteers to get enough material to be analyzed. State-of-the-art proteomic tools now allow identification and quantification of material providing from these pools with great success (DELECRODE et al., 2015).

In the present study, the main objective was to compare the proteomic profile of the AEP formed on different locations of the dental arches, since the main origin of the pellicle proteins are the salivary glands and the saliva produced by each of them has a distinct composition. This, together with the difference in thickness of the AEP from different regions (AMAECHI et al., 1999) may have a different impact on the prevalence of dental erosion in different regions of the dental arches, since the occlusal and palatal surfaces are the most often affected, both in adults and children (JAEGGI; LUSSI, 2014; JARVINEN et al., 1992; MILLWARD et al., 1994; MILOSEVIC et al., 1994; ROBB et al., 1995). The process of dental erosion progresses with sustained release, layer by layer, of the enamel crystals leading to an irreversible loss of tooth. In addition, a softened layer remains on the surface of tissue. In the advanced stages, dentin can be exposed progressively (LUSSI et al., 2011). So, any structure that involves the tooth surface, avoiding the direct contact of the acid with it, might have a protective effect against erosion. This is the case of the AEP, which acts as a diffusion barrier or semi-permeable membrane, reducing the degree of diffusion of calcium and phosphate ions in the surrounding liquid after exposure to acids, protecting against demineralization (HANNIG; BALZ, 1999; HANNIG et al., 2004; HARA et al., 2006)

Regarding the AEP formation area, for this study we divided the area of the pellicle collection, based on the opening of channels of the salivary glands, which could contribute to the formation of the AEP in different places. For the labial region, the samples were taken together, both from the upper and lower teeth (anterior and posterior) separately. This was done because the upper posterior region receives

contribution mainly from the parotid gland, while the upper anterior region is mainly influenced by the composition of the minor salivary glands. Thus, pellicle formed on the palatal and lingual regions were collected separately for the upper and lower teeth, because the lingual area is abundantly bathed by saliva from submandibular and sublingual glands (CARLEN et al., 1998).

As mentioned above, in the proteomic analysis of the AEP the quantity of proteins obtained is often scarce, which requires that samples collected from different volunteers are pooled (DELECRODE et al., 2015). Therefore, in this study pellicle samples were taken from each volunteer from 6 different regions and pooled. We used tongue cleaners to avoid any interference, especially for the pellicles collected in the lingual regions.

In relation to proteomic analysis, a special protocol was used, which allowed us considerable protein extraction (28-88 µg) to be identified and quantified. Thus, in this protocol we used urea and thiourea for the extraction of the proteins from the papers, in order to maintain the protein in its primary structure throughout the extraction process. We also used Falcon Amicon tubes for protein concentration. Moreover, the samples were alkylated with iodoacetamide (IAA) before digestion, which might have improved the action of trypsin, since IAA avoid the reformation of dissulphide bridges. Another important step regarding the protocol used for desalination and purification of the proteins was the use of C18 Spin Columns, increasing the degree of recovery of the peptides, since when the ZipTip™ method was used (DELECRODE et al., 2015; ZIMMERMAN et al., 2013), a great loss of peptides may occur, especially when using samples with small amounts of proteins, as in the case of pellicle. For all these reasons, we believe that the protein extraction protocol used in proteomic analysis was of great excellence, because it allowed us to identify 363 proteins in total, making this the largest number of proteins identified in AEP using proteomics approach. Available studies have identified approximately 100-150 different proteins (DELECRODE et al., 2015; SIQUEIRA et al., 2007; ZIMMERMAN et al., 2013) in AEP formed in vivo, so the large amount of protein identification in this study may be due to the differential protocol we used, but it is noteworthy that the satisfactory result may also have resulted from the procedure of AEP collection from different regions of the dental arches. Previous studies published so far collected AEP only from the vestibular region of the teeth (DELECRODE et al.,

2015; LEE et al., 2013; SIQUEIRA; OPPENHEIM, 2009; ZIMMERMAN et al., 2013).

Still in relation to the proteins identified in this study, 25 proteins were identified in all regions. Among them are proteins which are normally already described in the AEP, such as Protein S100-A8, Lysozyme C, Lactotransferrin, Sthatherin, Ig alpha-2 chain C, ALB protein, Myeloperoxidase and Proline-rich protein 3. This once again demonstrates the success of the extraction protocol used. It is worth mentioning the proteins that have been identified in specific regions, and this may explain the protective properties of AEP that vary in different areas of dental arches. Among them, the cystatins, that have been reported to be acid-resistant (DELECRODE et al., 2015) and can also inhibit cysteine-type endopeptidases, Cystatin-B was not identified in the LPLi region, while Cystatin SN was not detected in the LPLi and ULPLa regions and Cystatin-S was not found in the LPLi, UPPa and ULPLa regions, which means that this protein was not found in the AEP collected from the posterior teeth. It was shown recently the role of cysteine cathepsins in the progression of dental caries and erosion (BUZALAF; CHARONE; TJADERHANE, 2015; TJADERHANE et al., 2015). Besides this study evaluated enamel, the same pattern might be expected for dentin and the presence of cystatins might be important to reduce the rate of degradation of the demineralized organic matrix, reducing the progression of caries and erosion. Thus, it becomes important because the anterior teeth may be more protected, when we consider that cystatins are abundantly found in the AEP formed in the anterior region. Moreover, hemoglobins have been found only in the pellicle formed on the posterior teeth, both the labial, lingual and palatal areas. Despite the volunteers had good conditions of oral health, we cannot rule out the possibility that this protein was derived from contamination (MAENG et al., 2016), but so far the function of hemoglobin in the AEP is not known.

Many proteins (252), however, were found exclusively in the AEP collected from one of the regions (46 for UAPa, 33 for LALi, 59 for ULALa, 31 for ULPLa, 44 for LPLi and 39 for UPPa). These proteins are of great importance and some of them deserve special mention. The S100-A9 protein was found only in the AEP formed in the UAPa region. This protein is associated with the calcium and zinc binding, and plays an important role in regulating inflammatory processes and immune response, also presenting antimicrobial function (UNIPROT). In relation to erosion, this Protein S100-A9 was also identified in the AEP formed after exposure to citric and lactic

acids (DELECRODE et al., 2015), which is related to its calcium binding capacity and can thus indicate its great potential to protect against acid challenges. While the S100-A9 protein has been identified only in the UAPa region, S100-A8 protein, another isoform was found in all the regions. The S100-A8 protein is also usually identified in the AEP under different conditions (MASSON et al., 2013; SIQUEIRA; OPPENHEIM, 2009; ZIMMERMAN et al., 2013). Another important protein, Ras GTPase-activating-like, which binds to calcium and S100 protein (UNIPROT) was identified only in LALi region, which has low risk to develop erosion lesions.

Many proteins that are involved with immune responses were found exclusively in ULALa region, such as Ig kappa chain V-III region CLL, Ig kappa chain V-I region Lay, Ig kappa chain V-III region POM, Ig heavy chain V-III region TEI and Ig kappa chain V-III region VH. Another exclusive protein of this region was Prolactin-inducible, which is involved in chemical stimulus detection and in the perception of the bitter taste (UNIPROT).

With regard to the proteins found only in the posterior region, Protein SAAL1 (Serum amyloid A-like 1) was identified only in ULPLa, but there is not much information available in the literature for this protein, since it was cloned in 2012 (REVATHY et al., 2012) and its role in AEP cannot be precisely described. However, this protein is extracellular and interacts with carbonic anhydrase and keratin (UNIPROT). Thus, it can be involved in the homeostasis in the oral cavity, which should be investigated in further studies.

In the LPLi region, proteins that are involved in the immune response were also uniquely identified, such as Ig heavy chain V-III region TIL, Ig heavy chain V-III region WAS and Ig heavy chain V-III region TUR.

In the UPPa region, among the unique proteins identified is ADAMT59, a disintegrin and metalloproteinase with thrombospondin motifs 9, which cleaves the large aggregating proteoglycans, aggrecan and versican (UNIPROT) and could be involved in the degradation of dentin and alveolar bone. Another interesting protein found in this region was Calpain-8 that is a calcium-regulated non-lysosomal thiol protease with digestive function. Calpains-8 and -9 are members of Ca^{+2}-dependent intracellular prostheses that form a functional complex "G-calpain," expressed specifically in the mucus-producing cells (HATA; SORIMACHI, 2011). They might come to the oral cavity originating from the stomach after gastroesophageal-reflux

episodes. Interestingly, the UPPa region is highly affected by intrinsic erosion (VAKIL et al., 2006).

In this study, quantitative analysis was also carried out regarding the proteins found so nine comparison was made between the six groups. Two of them involved the anterior labial region (both upper and lower; ULALa) with its lingual (LALi) or palatal (UAPa) counterpart. For the comparison ULALa X LALi, 5 and 7 proteins were significantly increased and decreased in the first compared with the latter. Among the proteins differentially expressed, Cystatin-B was increased, while PRP 27 and Lysozyme C were decreased in ULALa (Table 3). When the ULALa region was compared with the UAPa region, 9 proteins were significantly increased and 4 significantly decreased in the first compared to the latter. Among the proteins differentlially expressed are 2 isoforms of Ig alpha, Keratin and Neutrophil defensin, as well as Histatin-1 and Statherin that were increased in the ULALa region, while Myeloperoxidase and Protein S100-A8 were decreased. The anterior labial region (ULALa) was also compared with the posterior labial region (ULPLa). In this case, 4 and 8 proteins were significantly increased or decreased, respectively, in the posterior region in comparison to the anterior one. Albumin isoform CRA and Myeloperoxidase were increased in the posterior region, while 2 isoform of Ig alpha, Statherin, Lysozyme C, Submaxillary gland androgen-regulated protein 3B, Protein S100-A8 and Histatin-1 were decreased (Table 3).

For the comparison of the anterior palatal region (UAPa) with its lingual counterpart (LALi), 8 proteins were significantly increased and another 8 significantly decreased in UAPa region compared with LALi region. The AEP formed on the palatal teeth (UAPa) had significantly higher concentration of Myeloperoxidase, Protein S100-A8, Submaxillary gland androgen-regulated protein 3B and Ig gamma-2 chain C region, while the concentrations of Statherin, Myeloblastin, Lactotransferrin, Lysozyme C and 2 isoform of Neutrophil defensing were significantly lower. When the AEP formed on the palatal surfaces of the anterior teeth (UAPa) was compared with that formed on the posterior counterpart (UPPa), 9 proteins were significantly increased, while 12 were significantly decreased in the posterior region. Ig alpha-1 chain C region, Myeloblastin, Lactotransferrin, 3 isoforms of Albumin and 2 of neutrophil defensing were increased, while Lysozyme C, Histatin-1, Submaxillary gland androgen-regulated protein, Protein S100-A8, Cystatin-B, Statherin and Myeloperoxidase were decreased in the posterior region compared with the anterior

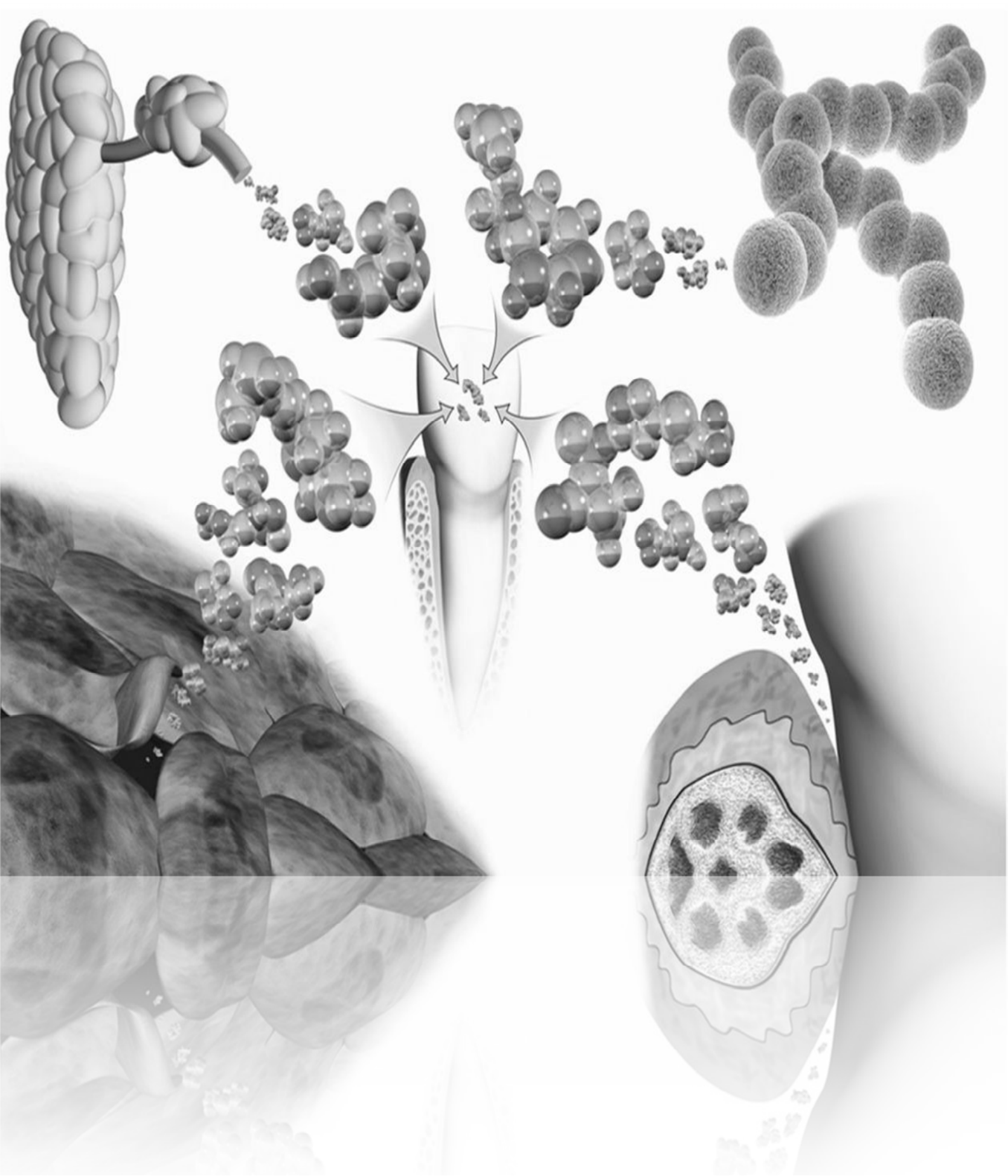
one. As for the comparison between the AEP formed on the lingual surface of the anterior teeth (LALi) with that formed on the posterior counterpart (LPLi), 5 proteins were increased and 11 decreased in the latter compared with the first. Myeloperoxidase was increased, while Ig alpha-2 chain C region, Azurocidin, Protein S100-A8, Sthaterin, Lactotransferrin, Lysozyme C and Neutrophil defensins were reduced in the posterior region compared to the anterior one (Table 3).

Two of the comparisons made involved the posterior labial region (upper and lower analyzed together; ULPLa) with its palatal (UPPa) or lingual (LPLi) counterparts. When ULPLa was compared with UPPa, 9 proteins were increased and another 9 were decreased in labial region, compared with the palatal one. Histatin-1, Cystatin-B and Lysozyme C were increased, while Submaxillary gland androgen-regulated protein 3B, Myeloperoxidase, Lactotransferrin, Protein S100-A8 and isoforms of IgA were decreased in the labial region, when compared with the palatal one. As for the comparison between the posterior labial region (ULPLa) and its lingual counterpart (LPLi), two proteins, including Lysozyme C, were increased, while 13 proteins, including Submaxillary gland androgen-regulated protein 3B and Myeloperoxidase were decreased in the labial region compared with the lingual one (Table 3). And when the posterior palatal region (UPPa) was compared with its lingual counterpart (LPLi), 10 proteins were increased, including Protein S100-A8. Lactotransferrin and Neutrophil defensins were increased, while 26 proteins, including Myeloperoxidase, was descreased in the palatal region when compared with the lingual one (Table 3).

Finally, the present study found some interesting proteins that were not previously described in the AEP. Among them are Azurocidin, a protein with antibacterial function (UNIPROT) which was found in LALi, PLi, UPPa and ULPLa regions. This protein may be a major new antimicrobial potential in the oral cavity, showing the importance of the AEP also for dental caries and should be further evaluated in further studies. Another protein is Cathepsin G, a cysteine cathepsin could play a role in the degradation of the organic matrix in demineralized dentin (BUZALAF et al., 2015).

In conclusion, this study demonstrated the great and important differences in the protein profile of the AEP formed in different regions of the dental arches. These results help to explain the different protective roles of AEP depending on its location and can be involved with the site-specific nature of the occurrence of dental caries

erosion. In addition, it shows the importance of, in future studies that involve proteomic analysis of the AEP, collecting the samples from all the regions of the dental arches.



References

REFERENCES

AMAECHI, B. T. et al. Thickness of acquired salivary pellicle as a determinant of the sites of dental erosion. **Journal of Dental Research,** v. 78, n. 12, p. 1821-1828, Dec 1999.

ARMSTRONG, W. G. Origin and nature of the acquired pellicle. **Proc R Soc Med,** v. 61, n. 9, p. 923-30, Sep 1968.

BARTLETT, D. W. et al. The prevalence of tooth wear in a cluster sample of adolescent schoolchildren and its relationship with potential explanatory factors. **Br Dent J,** v. 184, n. 3, p. 125-9, Feb 14 1998.

BUZALAF, M. A.; CHARONE, S.; TJADERHANE, L. Role of host-derived proteinases in dentine caries and erosion. **Caries Res,** v. 49 Suppl 1, p. 30-7, 2015.

CARLEN, A. et al. Composition of pellicles formed in vivo on tooth surfaces in different parts of the dentition, and in vitro on hydroxyapatite. **Caries Res,** v. 32, n. 6, p. 447-55, 1998.

DELECRODE, T. R. et al. Identification of acid-resistant proteins in acquired enamel pellicle. **J Dent,** v. 43, n. 12, p. 1470-5, Dec 2015.

HANNIG, M. Ultrastructural investigation of pellicle morphogenesis at two different intraoral sites during a 24-h period. **Clin Oral Investig,** v. 3, n. 2, p. 88-95, Jun 1999.

HANNIG, M.; BALZ, M. Influence of in vivo formed salivary pellicle on enamel erosion. **Caries Res,** v. 33, n. 5, p. 372-9, Sep-Oct 1999.

HANNIG, M. et al. Protective effect of the in situ formed short-term salivary pellicle. **Arch Oral Biol,** v. 49, n. 11, p. 903-10, Nov 2004.

HANNIG, M.; JOINER, A. The structure, function and properties of the acquired pellicle. **Monogr Oral Sci,** v. 19, p. 29-64, 2006.

HARA, A. T. et al. Protective effect of the dental pellicle against erosive challenges in situ. **J Dent Res,** v. 85, n. 7, p. 612-6, Jul 2006.

HARA, A. T.; ZERO, D. T. The caries environment: saliva, pellicle, diet, and hard tissue ultrastructure. **Dent Clin North Am,** v. 54, n. 3, p. 455-67, Jul 2010.

HATA, S.; SORIMACHI, H. [Physiological importance of calpains in gastric mucosal defense]. **Nihon Rinsho,** v. 69, n. 6, p. 1116-22, Jun 2011.

HOUSE, R. C. et al. Perimolysis: unveiling the surreptitious vomiter. **Oral Surg Oral Med Oral Pathol,** v. 51, n. 2, p. 152-5, Feb 1981.

JAEGGI, T.; LUSSI, A. Prevalence, incidence and distribution of erosion. **Monogr Oral Sci,** v. 25, p. 55-73, 2014.

JARVINEN, V.; RYTOMAA, I.; MEURMAN, J. H. Location of dental erosion in a referred population. **Caries Res,** v. 26, n. 5, p. 391-6, 1992.

JENSEN, J. L.; LAMKIN, M. S.; OPPENHEIM, F. G. Adsorption of human salivary proteins to hydroxyapatite: a comparison between whole saliva and glandular salivary secretions. **J Dent Res,** v. 71, n. 9, p. 1569-76, Sep 1992.

LAMKIN, M. S.; ARANCILLO, A. A.; OPPENHEIM, F. G. Temporal and compositional characteristics of salivary protein adsorption to hydroxyapatite. **J Dent Res,** v. 75, n. 2, p. 803-8, Feb 1996.

LEE, Y. H. et al. Proteomic evaluation of acquired enamel pellicle during in vivo formation. **PLoS One,** v. 8, n. 7, p. e67919, 2013.

LENDENMANN, U.; GROGAN, J.; OPPENHEIM, F. G. Saliva and dental pellicle--a review. **Adv Dent Res,** v. 14, p. 22-8, Dec 2000.

LUSSI, A. et al. Dental erosion--an overview with emphasis on chemical and histopathological aspects. **Caries Res,** v. 45 Suppl 1, p. 2-12, 2011.

MAENG, Y. J. et al. Diagnostic accuracy of a combination of salivary hemoglobin levels, self-report questionnaires, and age in periodontitis screening. **J Periodontal Implant Sci,** v. 46, n. 1, p. 10-21, Feb 2016.

MASSON, N. et al. Acidulated phosphate fluoride application changes the protein composition of human acquired enamel pellicle. **Caries Res,** v. 47, n. 3, p. 251-8, 2013.

MILLWARD, A. et al. The distribution and severity of tooth wear and the relationship between erosion and dietary constituents in a group of children. **Int J Paediatr Dent,** v. 4, n. 3, p. 151-7, Sep 1994.

MILOSEVIC, A.; YOUNG, P. J.; LENNON, M. A. The prevalence of tooth wear in 14-year-old school children in Liverpool. **Community Dent Health,** v. 11, n. 2, p. 83-6, Jun 1994.

NASMYTH, A. On the structure, physiology, and pathology of the persistent capsular investments and pulp of the tooth. **Med Chir Trans,** v. 22, p. 310-28, 1839.

REVATHY, K. S. et al. A novel acute phase reactant, serum amyloid A-like 1, from Oplegnathus fasciatus: Genomic and molecular characterization and transcriptional expression analysis. **Developmental and Comparative Immunology,** v. 37, n. 2, p. 294-305, Jun 2012.

ROBB, N. D.; SMITH, B. G.; GEIDRYS-LEEPER, E. The distribution of erosion in the dentitions of patients with eating disorders. **Br Dent J,** v. 178, n. 5, p. 171-5, Mar 11 1995.

SCHEUTZEL, P. Etiology of dental erosion--intrinsic factors. **Eur J Oral Sci,** v. 104, n. 2 (Pt 2), p. 178-90, Apr 1996.

SIQUEIRA, W. L.; CUSTODIO, W.; MCDONALD, E. E. New insights into the composition and functions of the acquired enamel pellicle. **J Dent Res,** v. 91, n. 12, p. 1110-8, Dec 2012.

SIQUEIRA, W. L.; OPPENHEIM, F. G. Small molecular weight proteins/peptides present in the in vivo formed human acquired enamel pellicle. **Arch Oral Biol,** v. 54, n. 5, p. 437-44, May 2009.

SIQUEIRA, W. L. et al. Identification of protein components in in vivo human acquired enamel pellicle using LC-ESI-MS/MS. **J Proteome Res,** v. 6, n. 6, p. 2152-60, Jun 2007.

TJADERHANE, L. et al. Matrix metalloproteinases and other matrix proteinases in relation to cariology: the era of 'dentin degradomics'. **Caries Res,** v. 49, n. 3, p. 193-208, 2015.

VAKIL, N. et al. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. **Am J Gastroenterol,** v. 101, n. 8, p. 1900-20; quiz 1943, Aug 2006.

VAN NIEUW AMERONGEN, A.; BOLSCHER, J. G.; VEERMAN, E. C. Salivary proteins: protective and diagnostic value in cariology? **Caries Res,** v. 38, n. 3, p. 247-53, May-Jun 2004.

VITORINO, R. et al. Peptidomic analysis of human acquired enamel pellicle. **Biomed Chromatogr,** v. 21, n. 11, p. 1107-17, Nov 2007.

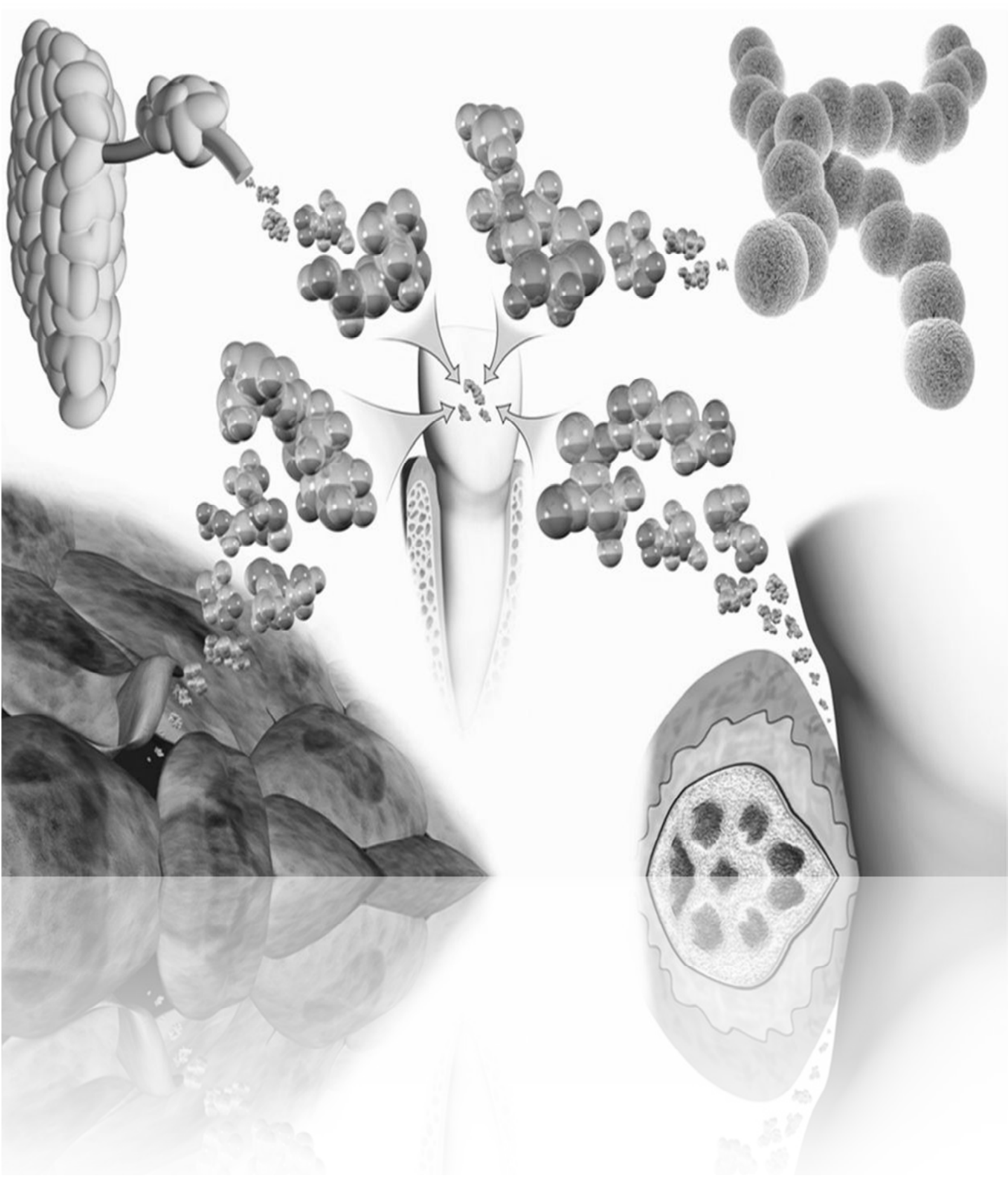
VUKOSAVLJEVIC, D. et al. Acquired pellicle as a modulator for dental erosion. **Arch Oral Biol,** v. 59, n. 6, p. 631-8, Jun 2014.

WILKINS, M. R. et al. Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. **Biotechnol Genet Eng Rev,** v. 13, p. 19-50, 1996.

YAO, Y. et al. Identification of protein components in human acquired enamel pellicle and whole saliva using novel proteomics approaches. **J Biol Chem,** v. 278, n. 7, p. 5300-8, Feb 14 2003.

YAO, Y. et al. Compositional analysis of human acquired enamel pellicle by mass spectrometry. **Arch Oral Biol,** v. 46, n. 4, p. 293-303, Apr 2001.

ZIMMERMAN, J. N. et al. Proteome and peptidome of human acquired enamel pellicle on deciduous teeth. **Int J Mol Sci,** v. 14, n. 1, p. 920-34, 2013.



Annex

ANNEX 1

Universidade de São Paulo
Faculdade de Odontologia de Bauru

Comitê de Ética em Pesquisa

DECLARAÇÃO

DECLARAMOS, que o projeto de pesquisa intitulado ***Detecção de alterações na composição da película adquirida em função da sua localização na cavidade bucal: estudo proteômico***, tendo como Pesquisadora Responsável, a pós-graduanda *Talita Mendes da Silva Ventura*, CAAE nº 48094715.0.0000.5417 foi analisado e aprovado por este Comitê de Ética em Pesquisa, conforme normativas da Resolução CNS 466/12, em reunião ordinária realizada no dia 21.10.2015,.

Bauru, 28 de junho de 2016.

Izabel Regina F. Rubira Bullen

Prof.a Dr.a Izabel Regina Fischer Rubira de Bullen
Coordenadora do Comitê de Ética em Pesquisa FOB-USP

Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-901 – C.P. 73
e-mail: cep@fob.usp.br – Fone/Fax (0xx14) 3235-8356
http://www.fob.usp.br

ANNEX 2

Universidade de São Paulo
Faculdade de Odontologia de Bauru

Departamento de Ciências Biológicas

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Caro aluno de pós-graduação da Faculdade de Odontologia de Bauru gostaria de convidá-lo a participar de uma pesquisa, onde serão analisadas as proteínas sobre os dentes em diferentes localidades da cavidade bucal, o trabalho experimental é intitulado "Detecção de alterações na composição da película adquirida em função da sua localização na cavidade bucal: estudo proteômico" e tem como objetivo detectar alterações no perfil proteico (mudanças nas proteínas) em películas adquiridas formadas *in vivo* em função da localização na cavidade bucal (em dentes diferentes). Você deverá comparecer ao laboratório de Bioquímica da Faculdade de Odontologia de Bauru FOB/USP, no dia e horário que será estabelecido pelo pesquisador, que entrará em contato com você no dia anterior da coleta da película adquirida, totalizando três dias que serão necessários a sua presença.

A película adquirida é uma fina camada transparente formada sobre os dentes e começam aparecer assim quando entram em contato com a saliva. É sobre essa camada que se forma a placa dentária.

O experimento consiste na coleta da película adquirida formada naturalmente sobre os seus dentes e para isso, realizaremos a coleta da saliva não estimulada durante 10 minutos em recipientes plásticos pré-pesados de 50 mL para primeiramente fazer o cálculo do seu fluxo salivar em mL/minuto e pH. Logo após a saliva será descartada em local apropriado segundo as normas de descarte de material biológico. Você receberá uma meticulosa profilaxia dentária com pedra pomes (não contendo aditivos), e em seguida aguardará por 120 minutos para que a película adquirida se forme naturalmente sobre os dentes, durante este período, você não poderá realizar o consumo de alimentos e bebidas. Assim, logo após 120 minutos a coleta da película formada em seus dentes será feita para se evitar possível agregação bacteriana. O período de coleta será realizado em três dias consecutivos (para obter amostras em triplicata, ou seja, 3 amostras iguais), tendo início às 8 horas da manhã. Todos os procedimentos para cada coleta serão idênticos para ambos os dias. Toda amostra de película adquirida coletada será utilizada para a realização da pesquisa, portanto não haverá descarte das mesmas.

Este projeto traz como benefícios á importância do conhecimento sobre quais proteínas da película adquirida do esmalte são formadas em diferentes locais da cavidade bucal, que irá nos permitir elaborar novas estratégias preventivas para proteção contra a erosão dentária.

Em relação aos benefícios oferecidos a você, no início do estudo será realizado um exame clínico em relação às condições bucais e o resultado deste exame será prontamente avisado a você. Caso algum problema seja detectado, faremos o encaminhamento para a triagem, segundo agendamento na clínica responsável da FOB. Além disto, você receberá a profilaxia dentária e serão fornecidas instruções sobre higiene bucal, por escrito e verbalmente.

Em relação aos riscos inerentes a pesquisa, destacamos que pode ocorrer um possível desconforto pela espera de 120 minutos sem o consumo de alimentos e bebidas, esse período é necessário para que a película adquirida se forme naturalmente sobre os seus dentes, vale ressaltar que não há risco a sua saúde com a participação nesta pesquisa, já que a coleta da película adquirida não é um método invasivo e você receberá a profilaxia dentária com pedra pomes sem o uso de aditivos, similar ao procedimento utilizado na rotina de uma clínica odontológica.

Concordando em participar, você entende que este estudo será realizado em benefício das ciências médicas e odontológicas, e desta forma concorda com a divulgação dos dados obtidos por meio de publicações científicas. A participação será voluntária, e entenda-se que você poderá fazer qualquer pergunta sobre cada etapa dos procedimentos, sendo que será livre para desistir de participar a qualquer momento mesmo após assinar este termo, caso você mude de idéia e queira sair da pesquisa, poderá fazê-lo, sem nenhum prejuízo de sua parte. Contudo, você terá também a garantia do sigilo que assegura a sua privacidade, adicionalmente, este termo de consentimento livre e esclarecido constará de duas vias, uma permanecerá com o pesquisador e outra será entregue a você. Importante ressaltar que não está sendo considerado nenhum pagamento ou recompensa material pela sua participação neste estudo. O participante não terá despesas pela participação da pesquisa, os gastos que forem gerados por este trabalho ficarão a cargo da responsável pelo projeto, caso você necessite de ajuda financeira de transporte para participar desta pesquisa ela poderá ser ressarcida pelo pesquisador. Você terá garantido

Rubrica do Participante da Pesquisa | Rubrica do Pesquisador Responsável

Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-901 – C.P. 73
e-mail: drolivei@fob.usp.br – Fone/FAX (0xx14) 3235-8271
http://www.fob.usp.br

Universidade de São Paulo
Faculdade de Odontologia de Bauru

Departamento de Ciências Biológicas

o direito de indenização compensatória caso fique comprovado que a sua participação na pesquisa acarretou algum problema a você.
Para esclarecimentos de dúvidas sobre sua participação na pesquisa poderá entrar em contato com pesquisador por meio do endereço institucional da Alameda Dr. Octávio Pinheiro Brisolla, 9-75, Departamento de Ciências Biológicos no Laboratório de Bioquímica, telefone (14) 3235-8246/ (14) 99769-5390 ou por e-mail: talitaventura@usp.br e, para denúncias e/ou reclamações entrar em contato com Comitê de Ética em Pesquisa-FOB/USP, à Alameda Dr. Octávio Pinheiro Brisolla, 9-75, Vila Universitária, ou pelo telefone (14)3235-8356, e-mail: cep@fob.usp.br, e a forma de contato com CONEP, pelo endereço SEPN 510 NORTE, BLOCO A, 3°Andar, Edifício Ex-INAN – Unidade II – Ministério da Saúde – Brasília-DF, telefone (61) 3315-5878 ou por e-mail: cns@saude.gov.br, quando pertinente.
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 (uma via para o participante da pesquisa e outra para o pesquisador) 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 _____.

_____
Assinatura do Participante da Pesquisa

_____
Talita Mendes da Silva Ventura
Assinatura da Pesquisadora Responsável

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 CNS 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 **14hs às 17 horas**, em dias úteis.
Alameda Dr. Octávio Pinheiro Brisolla, 9-75
Vila Universitária – Bauru – SP – CEP 17012-901
Telefone/FAX(14)3235-8356
e-mail: cep@fob.usp.br