UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

Caroline Loureiro

Análise comparativa do perfil proteômico da polpa dentária em condição normal, inflamada e necrótica

Araçatuba
2019

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Análise comparativa do perfil proteômico da polpa dentária em condição normal, inflamada e necrótica

Dissertação apresentada à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Mestre em Endodontia.

Orientador: Prof. Ass. Dr. Rogério de Castilho Jacinto

Araçatuba
2019

Catalogação na Publicação (CIP)

Diretoria Técnica de Biblioteca e Documentação – FOA / UNESP

Loureiro, Caroline.
L892a Análise comparativa do perfil proteômico da polpa dentária em condição normal, inflamada e necrótica / Caroline Loureiro. - Araçatuba, 2019
83 f. : il. ; tab.

Dissertação (Mestrado) – Universidade Estadual Paulista, Faculdade de Odontologia de Araçatuba
Orientador: Prof. Rogério de Castilho Jacinto

1. Proteômica 2. Endodontia 3. Polpa dentária 3. Pulpite 4. Necrose da polpa dentária I. T.

Black D24
CDD 617.67

Claudio Hideo Matsumoto CRB-8/5550

Dados Curriculares

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Mestrado 2017-2019

Mestrado em Ciências Odontológicas

Área de Endodontia

Faculdade de Odontologia de Araçatuba

UNESP

Agradecimentos

Aos familiares

Aos meus pais, **Jaime Aparecido Loureiro e Maria Iracema Chagas de Brito Loureiro**, por estarem ao meu lado em todas as etapas da minha vida, mesmo morando longe, vocês estão sempre presentes no meu dia-a-dia. Agradeço por cuidarem de mim, por me ensinarem a dar valor às coisas que realmente tem valor e pela dedicação em me ensinar o caminho correto a seguir. Vocês são tudo na minha vida, obrigada por tudo que fizeram e fazem por mim. Tudo que tenho devo a vocês, agradeço por terem me dado a oportunidade de ser quem eu sou hoje. Sempre presentes, mesmo com a distância, me apoiam em todas as minhas decisões. Com vocês aprendi o real sentido de amor, obrigada por terem me ensinado com maestria a ser uma pessoa íntegra, honesta e humilde. Pelo empenho na minha criação e educação, pelo cuidado, carinho e amor. Se hoje sou uma mulher forte, isso reflete toda força de vocês depositada em mim. Obrigada por confiarem nos meus sonhos e sonharem junto comigo. Amo muito vocês.

Ao meu amor, **Lucas Marcondes de Mello**, você mudou minha vida para melhor e te agradeço por ser para mim mais do que eu podia esperar em um homem e um companheiro. Quando penso que foi graças à pós-graduação que nos encontramos, vejo o real sentido disso tudo, nos conhecemos no tempo certo, momento perfeito e em uma fase propícia. Acho que nada é por acaso, e se hoje sou mais madura, mais mulher, mais forte, eu devo grande parte disso a você. Agradeço por ter me ajudado com tanta paciência ao longo desses anos de pós-graduação. Por ter me incentivado todas as vezes que pensei em desistir, por ter me ouvido em todas as vésperas de seminários ou aulas, por ter me abraçado nos momentos difíceis e por acreditar em mim e nos meus sonhos.

Aos meus irmãos, **Gustavo Loureiro** e **Rodrigo Loureiro**, pelo incentivo mesmo de longe sempre torcendo por mim. Meu coração fica apertado longe de vocês, mas o importante é que sempre nos reunimos para matar a saudade e lembrar o quanto nossa família é importante. Amo vocês. Às minhas cunhadas,

Karina Marco Loureiro e **Jessica de Oliveira Loureiro**, por sempre me apoiaram e ficarem na torcida pelos meus objetivos, obrigada.

Aos mestres

Ao meu orientador, **Prof. Rogério de Castilho Jacinto**, minha eterna gratidão por contribuir imensamente para a minha formação acadêmica, sendo sempre solícito, paciente e cuidadoso. Agradeço a confiança que o senhor depositou em mim e o estímulo que me fez crescer tanto pessoal como profissionalmente. Agradeço por me ajudar e incentivar dentro da pós-graduação. Quando entramos na pós-graduação, vira consenso ter medo do orientador, e eu (como mera mortal) tive no início, simplesmente por não o conhecer e por ter um bloqueio gerado inconscientemente pelo medo infundado, mas hoje, graças a nossa convivência, vejo o senhor como um amigo e agradeço pela sua dedicação como orientador, o senhor é o exemplo de profissional que eu almejo ser. Muito obrigada por tudo e por aceitar continuar me orientando no doutorado, sou muito grata por isso.

Ao **Prof. João Eduardo Gomes Filho**, pelo apreço e vontade de passar os conhecimentos da melhor forma, o senhor teve grande participação no meu crescimento como endodontista e hoje, como mestre. Obrigada por me acolher nos momentos de desespero, sua sensibilidade e humanidade conseguiram me fazer ver além da carreira acadêmica. Não tenho palavras para agradecer o que o senhor fez por mim, obrigada por ter me feito confrontar meus próprios medos, sem dúvida as nossas conversas me guiaram ao meu real objetivo, permitindo que eu enxergasse meus próprios resolvendo algumas pendências e er essa pessoa incrível e um profissional excelente, sou muito grata por todas as conversas e incentivos.

Ao **Prof. Elói Dezan Junior**, presente nos meus agradecimentos desde a graduação, meu tutor desde o primeiro ano da faculdade e meu amigo pessoal. O senhor consegue transmitir com maestria seus conhecimentos. Obrigada pelo

incentivo, é um prazer enorme fazer parte do seu time desde a graduação, a convivência com o senhor torna o ambiente da pós-graduação mais leve e agradável. Obrigada por tudo professor.

Ao **Prof. Luciano Ângelo Tavares Cintra** por ser um dos profissionais mais completos que eu já tive o prazer de conhecer e conviver. O senhor é um professor excepcional e um clínico inigualável, tenho grande admiração e agradeço pelos momentos de descontração e pelo incentivo.

Ao **Prof. Gustavo Sivieri de Araújo** pela amizade e conselhos cedidos com tanto carinho. Muito obrigada por todas as dicas, considerações e preocupação. Tenho o senhor como um amigo e um professor disponível para qualquer tipo de assunto, seja pessoal ou profissional.

Ao **Prof. Juliano Pelim Pessan**, um professor excepcional, que sempre está disposto a ajudar em qualquer situação. Muito obrigada pelas conversas descontraídas, por ajudar tanto no desenvolvimento desse trabalho e por ser uma pessoa tão divertida e agradável, o senhor é um exemplo de professor para mim.

À **Prof. Marília Afonso Rabelo Buzalaf** pelo acolhimento, desde o nosso primeiro contato me recepcionou muito bem, abriu a porta da sua casa e com a leveza de quem domina e ama o que faz, me ajudou em tudo que precisei. Muito obrigada pela parceria e ajuda.

Ao **Prof. Paulo Carvalho Tobias Duarte** por ser uma pessoa tão alegre, pelos momentos de descontração, pelas conversas e conselhos. É um prazer poder contar com você na minha banca de mestrado, nada melhor do que perpetuar esse momento ao lado de pessoas cercada por pessoas queridas e competentes.

Aos professores **Antônio Hernandes Chaves Neto** e **Cristiane Duque**, agradeço por terem feito parte do meu exame de qualificação. Fiquei extremamente agradecida pelas considerações e dicas cedidas por vocês. Minha admiração por vocês só aumentou frente a vossa dedicação em melhorar o nosso trabalho. Muito obrigada, acatamos todas as considerações.

Aos amigos

Um agradecimento especial à **Flávia Plazza,** primeiro por ter me convencido entrar na pós-graduação dizendo que não queria passar por essa sozinha, aqui estamos. Obrigada amiga por ser tão parceira, você não imagina o quanto eu te admiro, como amiga, como endodontista e como professora. Acho que você, quase sem querer, fez o trabalho de semear em mim a vontade de ser professora e faz o meu dia-a-dia da pós-graduação ser mais leve. Não é à toa que dizem que temos sintonia, quero perpetuar essa parceria em tudo que eu fizer. A nossa união me faz forte, amo você.

Às amizades feitas em 2018, obrigada **Pedro Henrique Chaves, Daniela Marques Tavares, Cristiane Cantiga, Lariana Camacho, Arianny Kessia, Juliana Trizzi e Felipe Moraes**. A turma de vocês trouxe leveza para o nosso departamento e cada um de vocês contribuiu muito para isso. Espero aprender muito com vocês ainda nesses próximos anos e projetos.

Aos amigos de pós-graduação, **Amanda Andolfatto, Camila Ambrósio, Francine Benetti, Ana Maria Veiga, Marina Cury, Ana Paula Fernandes, Nathália Machado, Flávio Duarte, Henrique Banci** pela convivência, ajuda mútua e por todos momentos de descontração.

Ao **Carlos Bueno, Leopoldo Cosme e Renan Dal Fabro** por estarem sempre dispostos a me ajudar, vocês são exemplos de profissionais em que almejo me tornar, competentes e humildes. Obrigada por vocês me salvarem sempre que precisei.

À **Talita Ventura** e **Vinicius Pelá** da USP-Bauru por todo cuidado com nosso trabalho. Agradeço a excelente recepção, vocês me ensinaram tudo sobre análise proteômica, repetidas vezes quando necessário. De coração, agradeço por tornarem mais fácil essa fase tão complicada de processamento, tabelas e tabelas de proteínas, vocês foram essenciais.

Aos funcionários

Agradeço a toda equipe do Departamento de Odontologia Restaurada, em especial ao **Peterson, Carlos e Jorge** por tornarem nossos dias mais agradáveis e pela boa convivência. Obrigada serem sempre muito solícitos, pacientes e por me ajudarem com tudo no departamento, vocês são incríveis. Às funcionárias da Seção de pós-graduação, **Cristiane e Valéria**, pela paciência e ajuda com os prazos e documentações, obrigada por tudo.

Ao apoio das agências de fomento

Obrigada às agências de fomento CNPq (169451/2017-8) pela concessão da bolsa de estudos durante o mestrado e FAPESP (18/18741-0) pelo auxílio para realização desse projeto.

Resumo Geral

Loureiro, C. **Análise comparativa do perfil proteômico da polpa dentária em condição normal, inflamada e necrótica.** 2019**.** 83f. Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2019.

RESUMO

Este estudo teve como objetivo comparar quantitativamente a diferença de expressão proteica na progressão da patogênese pulpar, bem como correlacionar as funções biológicas das proteínas identificadas no tecido pulpar normal, inflamado ou necrótico. As amostras foram obtidas de pacientes atendidos na Clínica Endodôntica da Faculdade de Odontologia de Araçatuba para tratamento endodôntico, sendo divididos em três grupos: grupo de polpa normal, com amostras do tecido pulpar obtidas a partir de dentes extraídos por indicação ortodôntica (n = 2); grupo de polpa inflamada, com amostras obtidas de pacientes com diagnóstico de pulpite irreversível (n = 2) e grupo de polpa necrótica, cujas amostras foram obtidas de pacientes com diagnóstico de periodontite apical crônica (n = 2). Após o preparo proteômico prévio, as amostras de polpa dentária foram processadas para análise proteômica quantitativa livre de marcadores em um sistema nanoACQUITY UPLC-Xevo QTof MS. A diferença na expressão entre os grupos de polpa normal e inflamada e grupos de polpa inflamada e necrótica foi calculada com o software Protein Lynx Global Service, usando o algoritmo Monte-Carlo, e expressa como p <0,05 para proteínas presentes em menor abundância e 1-p> 0,95 para proteínas presentes em maior abundância. Um total de 465 proteínas humanas foram identificadas em todos os grupos. Nos grupos normal, inflamado e necrótico, foram encontradas 241, 240 e 124 proteínas, respectivamente. Na análise quantitativa, as proteínas mais expressas foram hemoglobinas, peroxirredoxinas e imunoglobulinas, enquanto as menos expressas foram as tubulinas no grupo de polpa inflamada em relação ao grupo de polpa normal. Já, no grupo de polpa necrótica em relação ao de polpa normal, foram encontradas em expressão aumentada as albuminas, imunoglobulinas e alpha-2-macroglobulina, enquanto as menos expressas foram hemoglobinas e actinas. Quanto a análise qualitativa, as proteínas identificadas no grupo pulpar normal estavam envolvidas nas vias metabólicas e energéticas. No grupo de polpa inflamado, as funções proteicas mais prevalentes foram: comunicação celular e

transdução de sinal; e regulação e reparo de DNA/RNA, enquanto no grupo da polpa necrótica prevaleceram proteínas associadas à resposta imune. Sendo assim, a análise proteômica mostrou diferenças quantitativas na expressão proteica em diferentes tipos de condições pulpares e revelou que a inflamação pulpar induz à maior expressão de proteínas relacionadas a comunicação e transdução de sinal. No entanto, com o avanço para a necrose pulpar as proteínas estavam associadas à resposta imunológica.

Palavras-chave: análise proteômica, condições pulpares, endodontia.

Abstract

LOUREIRO, C. **Comparative analysis of the proteomic profile of dental pulp under normal, inflamed and necrotic conditions.** 2019**.** 83f. Dissertação (Mestrado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2019.

ABSTRACT

This study aimed to quantitatively compare the difference in protein expression in the progression of pulp pathogenesis, as well as to correlate the biological functions of proteins identified in normal, inflamed or necrotic pulp tissue. The samples were obtained from patients treated at the Endodontic Clinic of the Araçatuba Dental School for endodontic treatment, and were divided into three groups: normal pulp group with pulp tissue samples obtained from orthodontic teeth (n = 2) ; inflamed pulp group, whose samples were obtained from patients diagnosed with irreversible pulpitis (n = 2) and necrotic pulp group, whose samples were obtained from patients diagnosed with chronic apical periodontitis (n = 2). After previous proteomic preparation, dental pulp samples were processed for label-free quantitative proteomic analysis in a nanoACQUITY UPLC-Xevo QTof MS system. The difference in expression between the normal and inflamed pulp groups and groups of inflamed and necrotic pulp was calculated using the Protein Lynx Global Service software using the Monte Carlo algorithm and expressed as p <0.05 for proteins present in lower abundance and 1-p> 0.95 for proteins present in greater abundance. A total of 465 human proteins were identified in all groups. In the normal, inflamed and necrotic groups, 241, 240 and 124 proteins were found, respectively. In the quantitative analysis, the most expressed proteins were hemoglobin, peroxiredoxins and immunoglobulins, whereas the less expressed were the tubulins in the inflamed pulp group in relation to the normal pulp group. Expression of albumins, immunoglobulins and alpha-2-macroglobulin were increased in the necrotic pulp group when compared to normal pulp, whereas hemoglobin and actin were less expressed. As for the qualitative analysis, the proteins identified in the normal pulp group were involved in the metabolic and energetic pathways. In the inflamed group the most prevalent protein functions were: cellular communication and signal transduction; and regulation and repair of DNA / RNA, while

in the necrotic pulp group proteins associated with the immune response prevailed. Thus, proteomic analysis showed quantitative differences in protein expression in different types of pulp conditions, and revealed that pulp inflammation induced increased expression of proteins related to cellular communication and signal transduction. Nevertheless, with the progression to pulp necrosis, the proteins were associated with immune response.

Keywords: proteomic analysis, pulpal conditions, endodontics.

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1. Introduction

Dental pulp is a complex specialized tissue with nourishing, restorative, sensorial and defensive functions. The responses of pulp tissue to aggressive agents may be inflammatory or degenerative, depending on the characteristics of the irritant. Several etiological factors can cause pulp damage. Among them, the biological factor is the most relevant one and represented by all the polymicrobial communities, usually organized as biofilm adhered to the walls of the root canal (Ricucci & Siqueira 2010, Signoretti et al. 2013). The characteristic events of the inflammatory process, such as the recruitment of defense cells from the innate and adaptive immune response, and the presence of chemical mediators alter the physiology of the dental pulp in the attempt to remove the aggressive agent. Damage to the pulp tissue can occur in the persistence of the irritant agent, de-structuring the tissue and leading to a process of necrosis. In this context, bacteria colonize the root canal system and start releasing potential antigens into the periapical tissues, thus triggering the development of apical diseases (Siqueira & Rôças 2009, Jacinto et al. 2003).

Proteomic analysis is the study of the whole set of proteins expressed by an organism in a particular environment at a specific stage of the cell cycle (Wilkins et al. 1996). It also covers their relative abundance, distribution, functions and interaction with other macromolecules (Hakkinen et al. 2009). This approach has been widely applied in medical microbiology. The information collected in relation to bacterial resistance and virulence has been used in the development of new diagnostic and therapeutic applications for the treatment of infectious diseases (Macarthur & Jacques 2003). Proteomics techniques are important tools for investigating the progression of pulp alterations (Eckhardt et al. 2014), since they allow molecular analyzes and biochemical studies, which can be applied for the identification of virulence factors (Shi et al. 2006); study of the host / pathogen response to infections (Mirrashidi et al. 2015); and analysis of parameters such as formation of pulp tissue related to pulpal regeneration and tissue engineering (Li et al. 2017).

Characterization and expression of proteins using liquid chromatography (LC) / mass spectrometry (MS / MS) has gained prominence in proteomic analysis of pulp tissue, since this method provides the necessary technology for the study of small amounts of samples from complex biological systems (Motoyama & Yates 2008). This

method involves the proteolytic digestion of all proteins in the samples and their subsequent identification using a database of individual peptides, reducing sample handling time and eliminating the need for individual protein processing (Nikolov et al. 2012). The direct identification of proteins expressed in pulp and periapical diseases, involving descriptive analysis of pulp pathogenesis, focusing on: primary and persistent infections (Nandakumar et al. 2009); endodontic abscesses (Alfenas et al. 2017); and cases of failure of endodontic treatment (Francisco et al. 2018) has allowed the identification of several human proteins, which were mainly related to cellular processes, metabolism and immune defense.

Although the above-mentioned studies provided significant contribution to the comprehension of endodontic infections, they employed qualitative identification approaches, which do not allow a direct quantitative comparison of the expression of proteins of human origin. Furthermore, to date no study has assessed the proteomic profile of the human pulp tissue with different degrees of microbial injury, especially determining quantitatively the sub- and supra-regulated proteins. Therefore, considering the scarcity of proteomic studies of pulp diseases, this study aimed to compare the protein profile at different clinical stages during the progression of pulp diseases, as well as to correlate the biological functions of the proteins detected with the pulp status (normal, inflamed or necrotic pulp tissue).

2. Material and methods

2.1. Patient Selection

This study was approved by the Research Ethics Committee of the Araçatuba School of Dentistry – UNESP (Nº 91331518.7.0000.5420). All patients signed an informed consent form. Samples were taken from patients with no history of systemic diseases, who attended the Endodontic Clinic of the Araçatuba Dental School for root canal treatment, and were divided into three groups: normal pulp group with pulp tissue samples obtained from teeth extracted for orthodontic reasons (n = 2); inflamed pulp group - samples obtained from patients diagnosed with irreversible pulpitis (n = 2), and necrotic pulp group - samples obtained from patients diagnosed with chronic apical periodontitis (n = 2). Clinical and radiographic characteristics and a detailed anamnesis

of the patient's health conditions were recorded. The samples were divided into three groups according to the clinical characteristics and the state of the pulp tissues (Table 1).

Table 1. Clinical and radiographic characteristics of patients included in each group.

Groups	Age	Gender	Tooth	Thermal stimuli	Radiographic examination	Clinical examination
Normal pulp	27	Female	15	Positive (normal)	Normal periapical structures and complete root formation	No cavities and normal probing depth
	19	Male	34	Positive (normal)		
Inflamed pulp	15	Male	36	Positive (acute)	There was no PDL* widening and no periapical radiolucency	Carious lesion without pulp exposure
	13	Female	16	Positive (acute)		Extensive carious lesion and spontaneous pain
Necrotic pulp	28	Female	46	Negative	Periapical radiolucency on the mesial and distal roots	Sensitive to vertical percussion
	25	Female	26	Negative	Extensive periapical radiolucency PDL widening	Extensive restoration with caries recurrence

*PDL: periodontal ligament

2.2. Sample Collection

The collection was done aseptically. Firstly, the crown of the tooth to be sampled was cleaned with pumice paste and water, followed by removal of the restoration and/or carious tissue without exposing the root canals. Then, the tooth was individually isolated from the oral cavity with a rubber dam, except for the normal pulp sample. The tooth and the surrounding field were cleaned with 30% hydrogen peroxide and decontaminated with 2.5% sodium hypochlorite solution for 30 seconds each, followed by neutralization of the solution with 5% sodium thiosulfate (Jacinto et al. 2003). The

access to the pulp cavity was performed with sterile carbide bur without water spray. Irrigation during the access phase was done with sterile saline solution.

Normal pulp - To obtain the normal pulp sample, teeth with healthy pulp and without periodontal disease, indicated for orthodontic extraction, were selected. Immediately after the extraction, the pulp tissue was carefully removed with the aid of sterile manual files (Hedstroem file size #15, Dentsply Sirona, Ballaigues, Switzerland), avoiding contamination and complete disruption of the pulp tissue.

Inflamed pulp - In cases of irreversible pulpitis, consistent pulp tissue was collected from the palatal or distal canal with Hedstroem file, complemented by three sterile paper points introduced into the apparent length of the palatal canal determined on diagnostic radiographs, and held in place for 60 seconds each, without any irrigation.

Necrotic pulp tissue - Samples of the teeth with pulp necrosis, with radiographic lesion (chronic apical periodontitis), were obtained immediately after exposure of the pulp chamber. A sterile K-type file was introduced with minimal instrumentation, without the use of any irrigant to disrupt biofilms of the canal wall; then three sterile paper points were introduced into the apparent length of the canal determined on diagnostic radiographs and held in place for 60 seconds each (Jacinto et al. 2008). If the canal was completely dry, a drop of sterile saline was placed before removing the paper point. In cases of teeth with more than one canal, the sample was collected only from the wider canal, since it was associated with the apical lesion (otherwise the tooth would not be included in the study), to confine the analysis to a single environment.

After the collection, the paper points and tissue samples were placed in sterile, DNA-free and RNA-free cryotubes, which were frozen at -80 ° C until use for proteomic analysis.

2.3. Proteomic Analysis - Preparation of pulp samples

The paper points were cut and samples corresponding to the same groups were pooled. In the tubes containing the paper points an extraction solution containing 6M urea, 2M thiourea in 50mM NH_4HCO_3 pH 7.8 was added until the papers were covered. The samples were then vortexed for 10 min at 4°C, followed by sonication for 5 min and centrifugation at 14000 g for 10 min at 4°C. The supernatant was collected,

and this procedure was repeated once more. The papers were placed in filter tubes (Corning® Costar® Spin-X® Plastic Centrifuge Tube Filters Sigma-Aldrich, New York, USA) and centrifuged at 14,000 g for 10 min at 4°C. The supernatant was collected and added to the previously collected supernatant. Soon after, 1.5 volume of 50 mM NH4HCO3 was added to the samples. The samples were then placed in Falcon Amicon Ultra-4 10k tubes (Merck Millipore, Ireland) and centrifuged at 14,000 g at 4°C to approximately 150 µL.

After this time, 5mM dithiothreitol (DTT) was added to the samples and they were incubated at 37°C for 40 min. After this time, 10 mM iodoacetamide (IAA) was added and the samples were incubated for 30 min in the dark. After the incubations, 100 µL of 50 mM NH4HCO3 were added and shortly thereafter the tryptic digestion was performed for 14 h at 37°C by the addition of 2% (w/w) trypsin (Promega, Madison, USA). After the digestion, 5% formic acid was added to stop the action of trypsin and the procedures were performed with the C18 spin column (Thermo Scientific, United States) for desalting and purifying the samples. Thus, an aliquot of each sample (1 µL) was removed and protein quantification was performed by the Bradford method (Bio-Rad Bradford Assays). The remnants were dried to approximately 1 µL in SpeedVac (Thermo Scientific, United States). After drying the samples were resuspended in 3% acetonitrile and 0.1% formic acid for the application to the nano Liquid Chromatography Electron Spray Ionization Tandem Mass Spectrometer (nLC-ESI-MS / MS) (Ventura et al. 2017).

2.4. Shotgun label-free quantitative proteomic analysis

Peptides identification was performed on a nanoACQUITY UPLC-Xevo QTof MS system (Waters, Manchester, New Hampshire, 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, Manchester, New Hampshire, UK). 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, 80øC. Data acquisition occurred over 70 min and the scan range was 50–2000 Da. The lock spray, 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 LC-MSE continuum data. Proteins were identified with the embedded ion accounting algorithm in the software and a search of the Homo sapiens database (UniProtKB/Swiss-Prot) downloaded on April 2017 from UniProtKB (http://www.uniprot.org/).

For label-free quantitative proteome, three MS raw files from normal, inflamed and necrotic pulp groups were analyzed 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 statistical analysis embedded in the PLGS software. 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 normal and inflamed pulp groups and inflamed and necrotic groups was calculated using Monte-Carlo algorithm and expressed as $p < 0.05$ for proteins present in lower abundance and $1\text{-}p>0.95$ for proteins present in higher abundance.

In the quantitative analysis, two comparisons were made among the groups: The first comparison was between the normal and inflamed pulp groups, and the second was between the inflamed and necrotic pulp groups. Proteins expressed at a ratio > 2.0 in relation to the group of comparison were regarded as supra-regulated, while proteins expressed at a ratio < 0.5 were regarded as sub-regulated proteins. Proteins expressed with ratio between 0.5 and 2 were disregarded. The identified proteins were classified according to their biological functions using Homo sapiens database (UniProtKB/Swiss-Prot).

3. Results

Overall, 465 proteins were identified from the samples in all groups. Among these, 30 were common to all groups, including six isoforms of Actin, Albumin, Alpha-1_4 glucan phosphorylase, three isoforms of Glycogen and six isoforms of Hemoglobin, Serum albumin, among other proteins such as Apolipoprotein A-II, Haptoglobin and three isoforms of Immunoglobulin (Figure 1).

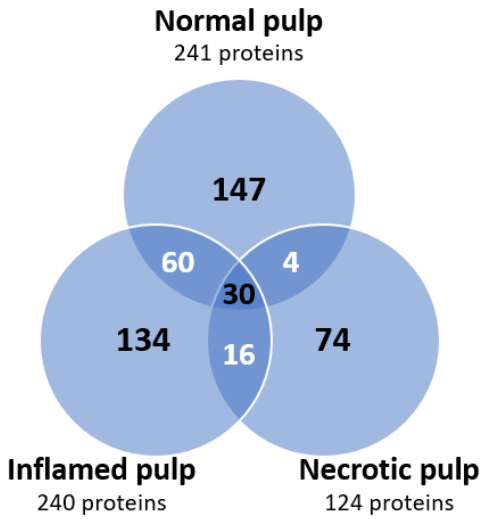

Figure 1. Venn diagram of proteins identified in all groups and the relation between them.

The proteins expression were divided into 12 categories: metabolism and energy pathways, immune response, transport, structure, DNA/RNA regulation and repair, cell communication and signal transduction, cell growth and/or maintenance, differentiation of neural cells, apoptosis, stress response, ions regulation and binding and proteins of unknown function (Eckhardt et al. 2014) (Tables 2 and 3).

When comparing the inflamed pulp group with the normal pulp group, there was an increase in 39 proteins in the first, among which 18 were increased more than 2-fold. Among these are 4 subunits of Hemoglobin (increased more than 100-fold), 2 isoforms of Peroxiredoxin, (increased more than 20-fold), 3 isoforms of Immunoglobulin (increased more than 10-fold), Nuclear mitotic apparatus protein, Apolipoprotein A-II, Haptoglobin, Serum albumin, Triosephosphate isomerase and Glyceraldehyde-3-phosphate dehydrogenase. On the other hand, 41 proteins were decreased in inflamed pulp group in comparison to normal pulp group, among which 17 were isoforms of Tubulin, besides other cytoskeletal proteins, such as Desmin.

Serum albumin and neurofilament proteins, such as Alpha-internexin, Neurofilament medium polypeptide were also reduced (Table 2).

When necrotic pulp group was compared with inflamed pulp group, 8 proteins were increased and 26 were decreased in the first. Among the increased proteins, 8 of them were increased more than 2-fold (2 isoforms of Serum albumin, Immunoglobulin heavy constant gamma 1 and Alpha-2-macroglobulin). As for the decreased proteins, 13 were decreased more than 2-fold (various isoforms of Hemoglobin, various isoforms of POTE ankyrin domain, various isoforms of Actin and Bromodomain-containing protein 3) (Table 2).

Table 3 shows the proteins exclusively identified in each one of the groups. Most of the proteins identified in the normal pulp group were involved in metabolic and energy pathways (20.4%) and in cell communication and signal transduction (20.4%). While in the inflamed pulp group, the cellular communication and signal transduction function (19.4%) also presented a higher percentage, followed by regulation and repair of DNA / RNA (17.9%). Finally, in the necrotic pulp group, most proteins were involved in the immune response (24.3%). Some proteins had unknown functions (7.5%, 3% and 10.8%, respectively, in normal, inflamed and necrotic groups) (Figure 2).

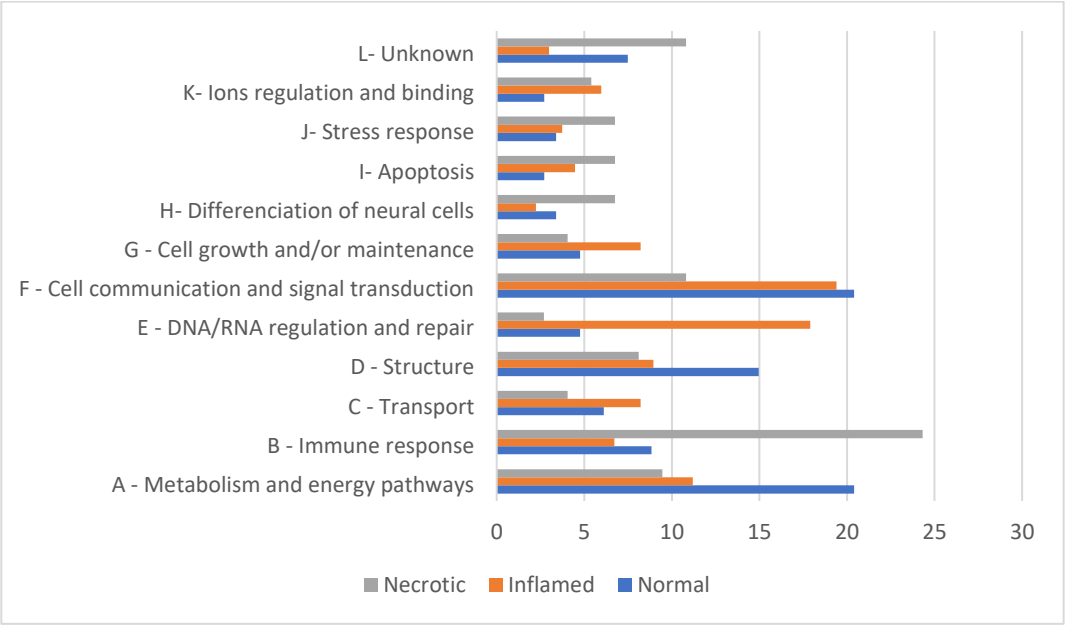

Figure 2. Biological function (Uniprot database) of proteins exclusively identified in normal, necrotic of inflamed pulp groups (%).

Alpha-2-macroglobulin, Transthyretin and Apolipoprotein A-I were not identified in the normal pulp group, while Beta-actin-like protein 2 was not found in necrotic pulp group. Isoforms of Neutrophil defensin were not found in the groups of normal and inflamed pulp, while Serotransferrin was identified in the groups of normal and necrotic pulp (Table 4).

4. Discussion

Proteomic techniques have helped to improve understanding of the biology, function and pathology of the pulpal tissue. Tissue formation, diagnosis, identification of risk factors, tissue engineering and pathogenesis of endodontic infections represent some of the most important themes investigated in proteomic studies of the pulp tissue (Murakami et al. 2012). Knowledge of proteins and their functions provide insights into the complex host-pathogen relationship and host antimicrobial strategies to combat infections (Eckhardt et al. 2014). To our knowledge, this is the first study that described and quantified the proteome of the pulp tissue in relation to the progression of pulp diseases, comparing the protein profile of different pulp diagnoses and their relationship with the characteristic events of each diagnosis. The nano Liquid Chromatography Electron Spray Ionization Tandem Mass Spectrometry (nLC-ESI-MS/MS) method allowed the identification of 465 different proteins. The high sensitivity of the method makes it possible to identify and comparatively quantify proteins in tiny amounts of samples, such as samples for root canals, allowing the study of its pathological processes (Murad & Rech 2012). For a more effective and satisfactory detection of proteins, a pool of two samples from each diagnosis (normal, inflamed or necrotic pulp) was carried out, as already done in other studies (Provenzano et al. 2013).

To understand the mechanisms related to the host according to the progression of the pulp disease, two comparisons were made between the groups. The first comparison (normal vs inflamed tissue) showed a significant supra-regulation of 4 subunits of Hemoglobin. Approximately one third of the mass of a human red blood cell (RBCs) is hemoglobin (Malka et al. 2014). RBCs participate in the vascular system and its increase is related to the diagnosis of the pulp. The inflammation of the dental pulp causes an immediate increase in blood flow, along with vasodilation, increased blood supply and microcirculation (Heyeraas & Kvinnsland 1992).

The immunoinflammatory response is intended to restore the structural and functional integrity of the injured tissue by eliminating irritants as quickly as possible (Speer et al. 1977). Indicating the activity of the immune response in inflamed pulp, three immunoglobulin isoforms were present in greater amounts in the inflamed tissue than in the healthy tissue samples. The increase of Nuclear mitotic apparatus protein indicates cell division, probably related to the proliferation of immunoinflammatory cells.

Tissue damage might occur by the release of reactive oxygen species (ROS) by disintegrated neutrophils and macrophages within the pulp tissue. ROS are mandatory byproducts of the metabolic activities of living aerobic organisms (Rhee 2016). Removal of ROS is done by peroxiredoxins - a family of antioxidant proteins that catalyze these substances. Two isoforms of Peroxiredoxins were supra-regulated in the inflamed pulp group when compared to the normal tissue, which are responsible for the protection of cellular components against oxidative damage. They are involved in processes such as cell proliferation and differentiation, protection of free radical-sensitive proteins, hemoglobin metabolism and intracellular signaling (Fisher 2011).

Throughout the inflammatory process maintained by the aggressive agent, damage to the pulp tissue occurs, with consequent cell death and destruction of the extracellular matrix (Hannas et al. 2007). The sub-regulation of 17 isoforms of Tubulin in the inflamed pulp group shows the disorganization and destruction of the structural portion of the cell in front of this process (Monteiro 2011). Neurofilament proteins, such as Alpha-internexin and Neurofilament medium polypeptide were also sub-regulated, which could be predicted due to the intense inflammation present in irreversible pulpitis. Some other proteins were also found with increased expression in inflamed pulpal tissue, most of them participating in biological processes related to transport, and metabolism and energy pathways.

As a result of the evolution of the inflammatory process of the pulp, the vital functions of the pulp are compromised, followed by hypoxia and tissue necrosis. In this context, there are also changes in blood microcirculation that led to reduced pulp blood flow, explaining the sub-regulation of hemoglobin in the necrotic pulp group, when compared with the inflamed one (Abbott & Yu 2007). The sub-regulation of 4 isoforms of actins show that the mortification process of the pulp tissue leads to destruction of

the cytoskeleton and rupture of actin microfilaments. Actin is a protein involved in structuring the cytoskeleton. The actin microfilaments participate in the generation of forces and cell adhesion, stabilizing the cell and determining the shape of the plasma membrane (Taniguchi et al. 2010).

Among the proteins supra-regulated in the necrotic group, serum albumin, albumin, immunoglobulin, and alpha-2-macroglobulin were found. Proteins derived from albumin and serum albumin are constituents of fluids and exudates that infiltrate the apical and lateral foramen of the root canal. Albumin and immunoglobulins may be related to the immune response as they participate in reducing the diffusion of antigens when they adhere to the dentinal tubules (Hahn & Best 2006).

Immunity-related proteins, such as immunoglobulins and protease inhibitors, involved in antigen presentation, defense cell activation and stress response can be identified in necrotic pulps, suggesting that host cells react to root canal system infections (Provenzano et al. 2013). One of the increased proteins in this group, Alpha-2-macroglobulin (α2M) protects the body against bacterial endotoxins, regulating apoptosis and inhibiting the generation of hydrogen peroxide. In addition, α2M can be used as a biomarker for the diagnosis and prognosis of various diseases (Rehman et al. 2013).

Bromodomain protein 3, classified as protein scaffold, mitotic markers, cell cycle regulator and transcription control was sub-regulated in necrotic pulp tissue. Transcription control mechanisms involve the regulatory processes of modification (phosphorylation, acetylation, ubiquitylation), turnover, nuclear compartmentalization, feedback regulation and signaling pathway specificity (Ren et al. 2017).

The most recurrent biological processes found in normal pulp tissue were metabolism and energy pathways, followed by cellular communication and signal transduction. These processes provide tissue balance, including maintenance, renewal and energy supply for cellular interaction. To maintain normal pulp tissue conditions, metabolic proteins and energy pathways must be in balance (Langellotti et al. 2014). In the inflamed pulp group, there was an increase in the percentage of proteins involved in the regulation and repair of DNA / RNA in order to allow cell viability. This may have occurred due to the damage suffered by the cells of the pulp tissue during the inflammatory process. Meanwhile, the increase of proteins associated

with metabolism and energetic pathways is directly related to the greater cellular activity for the elimination of the aggressive agent (D'Alessandro et al. 2016). Moreover, samples representing the infected pulp had a higher percentage of proteins with biological function related to the immune response, similarly to which was described by Provenzano et al. (2013), revealing the presence of viable host cells at the site of infection. These results contribute to the understanding of the complex pathogen-host relationship, the host's antimicrobial strategies to fight the infections and shed light into the pathogenesis of the disease.

5. Conclusion

The present study provided, for the first time, robust qualitative and quantitative analysis of proteins differentially expressed in normal, inflamed and necrotic pulp, thus contributing to the understanding of the complex pathogen-host relationship underlying the progression of the pulp diseases. Thus, proteomic analysis showed quantitative differences in protein expression in different types of pulp conditions, and revealed that pulp inflammation induced increased expression of proteins related to cellular communication and signal transduction. Nevertheless, with the progression to pulp necrosis, the proteins were associated with immune response.

6. Acknowledgements

We are thankful to the Biochemistry Department from the Bauru School of Dentistry, University of São Paulo. This study was supported by the Brazilian agencies FAPESP (18/18741-0) and CNPq (169451/2017-8).

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TABLES

Table 2 – Human proteins increased or decreased more than 2-fold in inflamed (I) compared with normal (NO) pulp and necrotic (NE) compared with inflamed pulp (I).

Accession	*Description*	*Biologic process*	*Score*	*Ratio I/NO*
P68871	Hemoglobin subunit beta [C]	Oxygen transport	25560	262.43
P02042	Hemoglobin subunit delta [C]	Oxygen transport	6892	164.02
G3V1N2	HCG1745306_ isoform CRA_a [C]	Oxygen transport	6068	139.77
P69905	Hemoglobin subunit alpha [C]	Oxygen transport	16796	139.77
Q14980	Nuclear mitotic apparatus protein 1 [F]	Cell division	282	29.96
P32119	Peroxiredoxin-2 [F]	Cell redox homeostasis	558	24.05
Q06830	Peroxiredoxin-1 [F]	Cell redox homeostasis	558	22.20
P01876	Immunoglobulin heavy constant alpha 1 [B]	Adaptive immunity	338	14.44
P02652	Apolipoprotein A-II [C]	Transport	1124	12.94
P01877	Immunoglobulin heavy constant alpha 2 [B]	Adaptive immunity	338	12.81
P00738	Haptoglobin [B]	Acute phase. Immunity	842	11.94
P01857	Immunoglobulin heavy constant gamma 1 [B]	Adaptative immunity	822	11.70
Q9Y2L5	Trafficking protein particle complex subunit 8 [C]	Transport	426	9.39
Q7Z2K6	Endoplasmic reticulum metallopeptidase 1 [A]	Catalytic activity	573	6.05
P60174	Triosephosphate isomerase [A]	Glycolysis	216	3.19
Q9Y4G6	Talin-2 [D]	Structural constituent of cytoskeleton	333	2.48
P04406	Glyceraldehyde-3-phosphate dehydrogenase [J]	Oxidoreductase	312	2.23
Q562R1	Beta-actin-like protein 2 [D]	Structural constituent of cytoskeleton	2959	0.44
P17661	Desmin [D]	Cytoskeleton organization	4264	0.39
Q8TAI7	GTPase RhebL1 [F]	Transcription factor activity	277	0.39
Q16352	Alpha-internexin [H]	Differentiation. Neurogenesis	3973	0.38
P07197	Neurofilament medium polypeptide [D]	Structural constituent of cytoskeleton	3973	0.38
Q9BY44	Eukaryotic translation initiation factor 2A [D]	Translation regulation	444	0.36
Q9BUF5	Tubulin beta-6 chain [D]	Microtubule-based process	5168	0.35
A6NNZ2	Tubulin beta-8 chain-like protein LOC260334 [D]	Microtubule-based process	5281	0.34
P02768	Serum albumin [C]	Transport	14783	0.32
P68371	Tubulin beta-4B chain [D]	Microtubule-based process	23514	0.32
P04350	Tubulin beta-4A chain [D]	Microtubule-based process	21996	0.32
Q3ZCM7	Tubulin beta-8 chain [D]	Structural constituent of cytoskeleton	8805	0.32
Q9BVA1	Tubulin beta-2B chain [D]	Microtubule-based process	26225	0.28

P07437	Tubulin beta chain [D]	Microtubule-based process	26437	0.28
Q13885	Tubulin beta-2A chain [D]	Microtubule-based process	26225	0.28
Q13509	Tubulin beta-3 chain [D]	Microtubule-based process	19550	0.24
A0A0B4J269	Uncharacterized protein [D]	Microtubule-based process	8751	0.24
P08729	Keratin_ type II cytoskeletal 7 [D]	Structural constituent of cytoskeleton	478	0.23
Q9NY65	Tubulin alpha-8 chain [D]	Microtubule-based process	11965	0.23
Q71U36	Tubulin alpha-1A chain [D]	Microtubule-based process	38018	0.22
G3V3R4	HCG1983504_ isoform CRA_c [D]	Microtubule-based process	16551	0.21
Q13748	Tubulin alpha-3C/D chain [D]	Microtubule-based process	12692	0.21
Q6PEY2	Tubulin alpha-3E chain [D]	Microtubule-based process	8817	0.21
P68363	Tubulin alpha-1B chain [D]	Microtubule-based process	37783	0.21
G3V2N6	HCG1983504_ isoform CRA_d [D]	Microtubule-based process	16551	0.21
P68366	Tubulin alpha-4A chain [D]	Microtubule-based process	12442	0.20
Q9BQE3	Tubulin alpha-1C chain [D]	Microtubule-based process	34846	0.20
F5H5D3	Tubulin alpha chain [D]	Microtubule-based process	34846	0.20
Q9UMX9	Membrane-associated transporter protein [F]	Sensory transduction	800	0.08

Accession	*Description*	*Biologic process*	*Score*	*Ratio NE:I*
P02768	Serum albumin [C]	Transport	72906	25.53
C9JKR2	Albumin_ isoform CRA_k [C]	Transport	28569	18.17
P01857	Immunoglobulin heavy constant gamma 1 [B]	Adaptive immunity	12257	3.00
P01023	Alpha-2-macroglobulin [G]	Regulation of complement activation	1004	2.23
P68032	Actin_ alpha cardiac muscle 1 [D]	Structural constituent of cytoskeleton	1208	0.49
P68133	Actin_ alpha skeletal muscle [D]	Structural constituent of cytoskeleton	1190	0.49
P63267	Actin_ gamma-enteric smooth muscle [D]	Structural constituent of cytoskeleton	1208	0.49
P62736	Actin_ aortic smooth muscle [D]	Structural constituent of cytoskeleton	1208	0.48

Q6S8J3	POTE ankyrin domain family member E [L]	Unknown	1142	0.44
A5A3E0	POTE ankyrin domain family member F [L]	Unknown	1132	0.44
P0CG38	POTE ankyrin domain family member I [L]	Unknown	1085	0.41
P0CG39	POTE ankyrin domain family member J [L]	Unknown	568	0.35
P02042	Hemoglobin subunit delta [C]	Oxygen transport	3864	0.11
Q15059	Bromodomain-containing protein 3 [F]	Transcription regulation	2487	0.10
G3V1N2	HCG1745306_ isoform CRA_a [C]	Oxygen transport	3312	0.01
P69905	Hemoglobin subunit alpha [C]	Oxygen transport	8134	0.01
P68871	Hemoglobin subunit beta [C]	Oxygen transport	8969	0.00

Proteins were classified according to Uniprot Homo sapiens database: A – Metabolism and energy pathways; B - Immune response; C – Transport; D – Structure; E - DNA/RNA regulation and repair; F - Cell communication and signal transduction; G - Cell growth and/or maintenance; H- Differentiation of neural cells; I- Apoptosis; J- Stress response; K- Ions regulation and binding; L- Unknown. Proteins with difference in expression higher than 2-fold are colored in blue while those smaller than 2-fold in pink.

Table 3 – Human proteins identified exclusively in normal, inflamed or necrotic pulp.

Normal pulp

Accession	***Description***	***Biologic process***	***Score***
E2QRG7	4-hydroxybenzoate polyprenyltransferase_ mitochondrial [A]	Ubiquinone biosynthesis	424.48
Q8IUX7	Adipocyte enhancer-binding protein 1 [F]	Transcription regulation	281.92
F2Z324	Aldehyde dehydrogenase 1 family member L1 isoform 2 [L]	Unknown	354.48
P02765	Alpha-2-HS-glycoprotein [B]	Acute-phase response	334
H0YMF8	Ammonium transporter Rh type C [D]	Component of membrane	751.41
P00966	Argininosuccinate synthase [A]	Arginine biosynthesis	178.45
H0YH81	ATP synthase subunit beta (Fragment) [A]	ATP synthesis	289.75
P06576	ATP synthase subunit beta_ mitochondrial [C]	Transport	548.43
Q9NUQ8	ATP-binding cassette sub-family F member 3 [B]	Antiviral defense	217.99
P08237	ATP-dependent 6-phosphofructokinase_ muscle type [A]	Glycolysis	439.21
P49407	Beta-arrestin-1 [F]	Transcription regulation	243.29
P21810	Biglycan [D]	Extracellular matrix structural constituent	277.04
P22223	Cadherin-3 [F]	Sensory transduction	414.89
Q9H9S4	Calcium-binding protein 39-like [G]	Cell cycle arrest	785.13
P29762	Cellular retinoic acid-binding protein 1 [C]	Transport	1319.56
X6RIU2	Cilia- and flagella-associated protein 221 (Fragment) [D]	Component of cytoskeleton	158.14
Q96N23	Cilia- and flagella-associated protein 54 [D]	Component of cytoskeleton	864.57

P26441	Ciliary neurotrophic factor [H]	Differentiation. Neurogenesis. Growth factor	150.4
A0A0A0MT56	Cleavage stimulation factor subunit 2 (Fragment) [E]	mRNA-processing	428.34
Q9GZT6	Coiled-coil domain-containing protein 90B_ mitochondrial [D]	Mitochondrial membrane	271
J3QT66	COP9 signalosome complex subunit 7b [B]	Host-virus interaction	847.96
Q2NKJ3	CST complex subunit CTC1 [G]	Regulation of fibroblast proliferation	586.38
K7EJ26	CUGBP Elav-like family member 4 (Fragment) [E]	mRNA-processing	247.83
O75891	Cytosolic 10-formyltetrahydrofolate dehydrogenase [J]	Oxidoreductase	362.58
Q07507	Dermatopontin [D]	Extracellular matrix structural constituent	1412.86
O75907	Diacylglycerol O-acyltransferase 1 [A]	Acyltransferase. Transferase	290.99
Q16555	Dihydropyrimidinase-related protein 2 [H]	Differentiation. Neurogenesis	169.43
P53602	Diphosphomevalonate decarboxylase [G]	Regulation of cell proliferation	552.3
H7C0V2	DNA repair protein RAD50 (Fragment) [B]	Host-virus interaction	234.66
P38935	DNA-binding protein SMUBP-2 [F]	Transcription regulation	197.26
A0PK19	EPGN protein [G]	Regulation of cell proliferation	1566.9
Q6UW88	Epigen [G]	Regulation of cell proliferation	1566.9
P60842	Eukaryotic initiation factor 4A-I [B]	Host-virus interaction	175.34
Q04637	Eukaryotic translation initiation factor 4 gamma 1 [B]	Host-virus interaction	280.67
Q9NPD3	Exosome complex component RRP41 [E]	rRNA processing	340.1
Q9UK22	F-box only protein 2 [A]	Regulation of protein ubiquitination	280.14
Q969U6	F-box/WD repeat-containing protein 5 [A]	Regulation of protein ubiquitination	335.47
A0A0A0MS75	FGF receptor activating protein 1_ isoform CRA_e [D]	Integral component of membrane	229.6
Q06828	Fibromodulin [D]	Extracellular matrix structural constituent	484.9
G5E9X3	Fibronectin type III domain containing 3A_ isoform CRA_f [F]	Cell-cell adhesion	263.94
Q9Y2H6	Fibronectin type-III domain-containing protein 3A [F]	Cell-cell adhesion	283.11
H3BQN4	Fructose-bisphosphate aldolase [A]	Glycolytic process	577.74
P04075	Fructose-bisphosphate aldolase A [A]	Glycolytic process	591.64
P09382	Galectin-1 [I]	Apoptosis	4761.72
Q9UBS5	Gamma-aminobutyric acid type B receptor subunit 1 [A]	G-protein coupled receptor	433.53
Q99501	GAS2-like protein 1 [D]	Component of cytoskeleton	299.88
H7C4Q8	General transcription factor II-I repeat domain-containing protein 1 (Fragment) [F]	Transcription regulation	168.04
Q14687	Genetic suppressor element 1 [L]	Unknown	118.64
Q86VD9	GPI mannosyltransferase 4 [A]	GPI-anchor biosynthesis	218.44

G3V3J6	HCG1983504_ isoform CRA_b [D]	Microtubule-based process	10798.74
Q9H583	HEAT repeat-containing protein 1 [F]	Transcription regulation	391.17
Q86XA9	HEAT repeat-containing protein 5A [F]	Transcription regulation	238.55
P04792	Heat shock protein beta-1 [J]	Stress response	1700.43
Q9Y5N1	Histamine H3 receptor [A]	G-protein coupled receptor	188.39
Q96A08	Histone H2B type 1-A [B]	Inflammatory response	1914.55
Q14571	Inositol 1_4_5-trisphosphate receptor type 2 [C]	Transport	271.01
P19823	Inter-alpha-trypsin inhibitor heavy chain H2 [A]	Cellular protein metabolic process	371.22
V9GXZ7	Kin of IRRE-like protein 2 (Fragment) [L]	Unknown	462.22
Q96Q89	Kinesin-like protein KIF20B [G]	Cell division. mitosis	844.1
H0YEH0	Large neutral amino acids transporter small subunit 3 (Fragment) [D]	Integral component of membrane	736.6
O75845	Lathosterol oxidase [A]	Lipid metabolism	169.98
Q8N653	Leucine-zipper-like transcriptional regulator 1 [F]	Transcription factor	161.39
Q8NHJ6	Leukocyte immunoglobulin-like receptor subfamily B member 4 [B]	Adaptive immunity	315.03
P51884	Lumican [D]	Extracellular matrix structural constituent	5091.27
Q6UWQ5	Lysozyme-like protein 1 [B]	Defense response to bacteria	847.95
Q7Z4W2	Lysozyme-like protein 2 [B]	Defense response to bacteria	847.95
Q14168	MAGUK p55 subfamily member 2 [F]	Excitatory postsynaptic potential	700.69
Q9H3U5	Major facilitator superfamily domain-containing protein 1 [C]	Transport	167.24
C9JQX2	Mannosyltransferase [A]	Glycosyltransferase	210.23
E5RJR3	Methionine adenosyltransferase 2 subunit beta [A]	One-carbon metabolism	683.62
P25189	Myelin protein P0 [I]	Regulation of apoptotic process	366.82
P60660	Myosin light polypeptide 6 [K]	Calcium ion binding	430.43
Q9UK23	N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase [A]	Protein glycosylation	246.65
A0A087WYD0	NADH dehydrogenase (Ubiquinone) 1 beta subcomplex_ 5_ 16kDa_ isoform CRA_g [C]	Transport	379.52
O43674	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5_ mitochondrial [C]	Transport	379.52
O00308	NEDD4-like E3 ubiquitin-protein ligase WWP2 [B]	Host-virus interaction	261.84
O00533	Neural cell adhesion molecule L1-like protein [H]	Differentiation. Neurogenesis	238.35
P12036	Neurofilament heavy polypeptide [D]	Structural constituent of cytoskeleton	471.82
P07196	Neurofilament light polypeptide [D]	Structural constituent of cytoskeleton	645.68
Q9NZ94	Neuroligin-3 [F]	Cell adhesion	470.51
Q7RTR2	NLR family CARD domain-containing protein 3 [B]	Regulation of inflammatory response	180.39

H3BLT9	NOD3 protein_ isoform CRA_d [D]	Microtubule organizing center	180.39
Q14980	Nuclear mitotic apparatus protein 1 [F]	Cell division	281.88
Q14686	Nuclear receptor coactivator 6 [F]	Transcription regulation	253.67
A0A126GWK9	Olfactory receptor [F]	Sensory transduction	305.24
O43869	Olfactory receptor 2T1 [F]	Sensory transduction	305.24
Q6IFN5	Olfactory receptor 7E24 [F]	Sensory transduction	220.6
H0Y2Y4	Palmitoyltransferase (Fragment) [A]	Kinase activity	212.83
O95497	Pantetheinase [J]	Response to oxidative stress	239.94
P26022	Pentraxin-related protein PTX3 [B]	Inflammatory response	159.12
Q9Y536	Peptidyl-prolyl cis-trans isomerase A-like 4A [A]	Catalytic activity	511.68
Q8NEB9	Phosphatidylinositol 3-kinase catalytic subunit type 3 [I]	Autophagy	518.24
Q9UHJ9	Post-GPI attachment to proteins factor 2 [A]	GPI-anchor biosynthesis	229.6
Q01860	POU domain_ class 5_ transcription factor 1 [F]	Transcription regulation	190.11
P02545	Prelamin-A/C [A]	Regulation of apoptotic signaling pathway	405.48
Q9UMS4	Pre-mRNA-processing factor 19 [E]	mRNA processing	353.75
Q96I59	Probable asparagine--tRNA ligase_ mitochondrial [A]	Protein biosynthesis	191.32
Q15034	Probable E3 ubiquitin-protein ligase HERC3 [A]	Ubl conjugation pathway	82.2
Q16651	Prostasin [K]	Regulation of sodium ion transport	275.33
H0YHR3	Protein phosphatase Slingshot homolog 1 (Fragment) [G]	Actin cytoskeleton organization	255.57
Q99497	Protein/nucleic acid deglycase DJ-1 [J]	Stress response	784.37
H3BRZ0	Putative sodium-coupled neutral amino acid transporter 7 (Fragment) [D]	Integral component of membrane	672.03
B4DNK4	Pyruvate kinase [A]	Kinase activity	458.75
P14618	Pyruvate kinase PKM [A]	Kinase activity	458.75
P26374	Rab proteins geranylgeranyltransferase component A 2 [C]	Intracellular protein transport	618.53
P21860	Receptor tyrosine-protein kinase erbB-3 [D]	Receptor of cell membrane	271.71
Q12913	Receptor-type tyrosine-protein phosphatase eta [D]	Receptor of cell membrane	436.47
E9PGT3	Ribosomal protein S6 kinase [F]	Intracellular signal transduction	415.96
Q15418	Ribosomal protein S6 kinase alpha-1 [F]	Intracellular signal transduction	430.79
Q6P3W7	SCY1-like protein 2 [C]	Endosome to lisossome transport	144.69
P59797	Selenoprotein V [L]	Unknown	3591.64
Q9H3S1	Semaphorin-4A [H]	Differentiation. Neurogenesis	898.66
O95754	Semaphorin-4F [H]	Differentiation. Neurogenesis	257.33
Q05519	Serine/arginine-rich splicing factor 11 [E]	mRNA processing	1467.45
Q8WU08	Serine/threonine-protein kinase 32A [F]	Intracellular signal transduction	292.95

Q9H2K8	Serine/threonine-protein kinase TAO3 [E]	DNA repair	607.16
O43147	Small G protein signaling modulator 2 [C]	Intracellular protein transport	738.5
Q8N4F4	Solute carrier family 22 member 24 [K]	Ion transport	317.43
P00441	Superoxide dismutase [Cu-Zn] [J]	Antioxidant. Oxidoreductase	1233.87
Q9UMS6	Synaptopodin-2 [F]	Regulation of cell migration	289.93
Q9Y6H5	Synphilin-1 [A]	Cellular protein metabolic process	224.06
Q9NYW2	Taste receptor type 2 member 8 [A]	G-protein coupled receptor	173.03
Q8IWY7	Tau-tubulin kinase [A]	Kinase activity	231.03
P24821	Tenascin [F]	Cell adhesion	788.89
Q5R3I4	Tetratricopeptide repeat protein 38 [L]	Unknown	164.5
Q96FV9	THO complex subunit 1 [F]	Transcription regulation	165.16
Q14135	Transcription cofactor vestigial-like protein 4 [F]	Transcription regulation	320.85
Q15582	Transforming growth factor-beta-induced protein ig-h3 [F]	Cell adhesion	699.53
Q7Z6W1	Transmembrane and coiled-coil domain-containing protein 2 [D]	Integral component of membrane	383.03
Q9H4B7	Tubulin beta-1 chain [D]	Structural constituent of cytoskeleton	1944.92
Q9UJT1	Tubulin delta chain [D]	Structural constituent of cytoskeleton	493.65
A8MXF1	Tyrosine-protein phosphatase non-receptor type 5 [A]	Protein phosphatase	355.82
A0A0B4J269	Uncharacterized protein [D]	Microtubule-based process	8750.79
Q8NBR9	Uncharacterized protein C11orf72 [L]	Unknown	310.42
V9GY35	Uncharacterized protein C1orf109 (Fragment) [L]	Unknown	240.6
Q6ZUG5	Uncharacterized protein FLJ43738 [L]	Unknown	992.96
D6RBZ9	Uncharacterized protein FLJ43738 (Fragment) [L]	Unknown	990.13
Q92628	Uncharacterized protein KIAA0232 [L]	Unknown	583.39
D6REK0	Uncharacterized protein KIAA0232 [L]	Unknown	568.86
P10746	Uroporphyrinogen-III synthase [A]	Heme biosynthesis	212.17
P04004	Vitronectin [F]	Cell adhesion	728.59
Q06432	Voltage-dependent calcium channel gamma-1 subunit [K]	Calcium transport	219.37
Q5MNZ6	WD repeat domain phosphoinositide-interacting protein 3 [I]	Autophagy of nucleus	228.89
Q13105	Zinc finger and BTB domain-containing protein 17 [F]	Transcription regulation	302.68
Q6PJT7	Zinc finger CCCH domain-containing protein 14 [E]	Regulation of mRNA stability	331.85
Q09FC8	Zinc finger protein 415 [F]	Transcription regulation	192.42
Q96MU6	Zinc finger protein 778 [F]	Transcription regulation	562.65

Inflamed pulp

Accession	*Description*	*Biologic process*	*Score*
P62280	40S ribosomal protein S11 [F]	Translational initiation	351.39
Q13085	Acetyl-CoA carboxylase 1 [A]	Lipid metabolism	394.31

P78348	Acid-sensing ion channel 1 [K]	Ion transport	1387.23
A0A1X7SBU6	Adhesion G protein-coupled receptor V1 (Fragment) [D]	Integral component of membrane	208.16
Q9NVD7	Alpha-parvin [G]	Angiogenesis	174.54
Q86UQ4	ATP-binding cassette sub-family A member 13 (Fragment) [C]	Transport	281.18
O94911	ATP-binding cassette sub-family A member 8 (Fragment) [C]	Transmembrane transport	190.11
Q8WXE1	ATR-interacting protein [E]	Response to DNA damage	636.8
P17213	Bactericidal permeability-increasing protein [B]	Antibacterial humoral response	284.17
P02730	Band 3 anion transport protein [k]	Ion transport	243.95
Q96T60	Bifunctional polynucleotide phosphatase/kinase [E]	Response to DNA damage	229.66
P00915	Carbonic anhydrase 1 [K]	Bicarbonate transport	364.93
P00918	Carbonic anhydrase 2 [K]	Bicarbonate transport	641.72
Q9NS85	Carbonic anhydrase-related protein 10 [K]	Zinc ion binding	179.75
P51948	CDK-activating kinase assembly factor MAT1 [F]	Transcription regulation	228.15
P00451	Coagulation factor VIII [B]	Acute-phase response	548.5
H0YK65	Coiled-coil domain-containing 9B (Fragment) [L]	Unknown	372.31
Q9Y2V7	Conserved oligomeric Golgi complex subunit 6 [C]	Protein transport	382.37
Q8TEY5	Cyclic AMP-responsive element-binding protein 3-like protein 4 [F]	Transcription regulation	314.59
P55273	Cyclin-dependent kinase 4 inhibitor D [I]	Autophagic cell death	360.12
Q68DD2	Cytosolic phospholipase A2 zeta [A]	Lipid metabolism	279.68
P30038	Delta-1-pyrroline-5-carboxylate dehydrogenase_ mitochondrial [J]	Oxidoreductase. proline metabolism	191.36
P78352	Disks large homolog 4 [F]	Cell adhesion	233.8
P36507	Dual specificity mitogen-activated protein kinase 2 [A]	Activation of protein kinase activity	240.42
H0YD30	Dynein assembly factor 3_ axonemal (Fragment) [F]	Axonemal dynein complex assembly	309.68
Q14118	Dystroglycan [G]	Angiogenesis	383.01
Q8N2H9	E3 ubiquitin-protein ligase pellino homolog 3 [A]	Ubl conjugation pathway	1102.45
K7EII6	Echinoderm microtubule-associated protein-like 2 (Fragment) [F]	Regulation of microtubule nucleation	230.16
Q05BV3	Echinoderm microtubule-associated protein-like 5 [D]	Microtubule binding	308.9
P43897	Elongation factor Ts_ mitochondrial [G]	Protein biosynthesis	215.14
Q5T6L9	Endoplasmic reticulum membrane-associated RNA degradation protein [G]	Developmental protein	440.87
O15360	Fanconi anemia group A protein [E]	Response to DNA damage	156.29
Q14CZ7	FAST kinase domain-containing protein 3_ mitochondrial [A]	Protein kinase activity	216.49

O94887	FERM_ ARHGEF and pleckstrin domain-containing protein 2 [G]	Osteoclast differentiation	394.64
P02679	Fibrinogen gamma chain [F]	Hemostasis	117.71
Q4L180	Filamin A-interacting protein 1-like [I]	Regulation of apoptosis process	259.28
Q9NSN8	Gamma-1-syntrophin [F]	Cell communication	273.12
P36383	Gap junction gamma-1 protein [G]	Cell development. Vasculogenesis	179.45
Q5T442	Gap junction gamma-2 protein [G]	Cell development. Vasculogenesis	307.56
Q13630	GDP-L-fucose synthase [J]	Oxidoreductase	120.68
Q6UWF4	GLGQ5807 [D]	Membrane component	325.59
Q92805	Golgin subfamily A member 1 [D]	Structural component	197.06
A0A1W2PNZ5	GPI transamidase component PIG-T [I]	Neuron apoptotic process	369.7
P62826	GTP-binding nuclear protein Ran [G]	Cell division	306.27
A0A0A6YYF2	HCG1811249_ isoform CRA_e [F]	Regulation of cell adhesion	277.46
A0A0A0MTS5	HCG1811249_ isoform CRA_f [F]	Regulation of cell adhesion	274.02
A0A0U1RR32	Histone H2A [E]	DNA-binding	176.75
P0C0S8	Histone H2A type 1 [E]	DNA-binding	176.75
Q96QV6	Histone H2A type 1-A [E]	DNA-binding	176.75
P04908	Histone H2A type 1-B/E [E]	DNA-binding	176.75
Q93077	Histone H2A type 1-C [E]	DNA-binding	176.75
P20671	Histone H2A type 1-D [E]	DNA-binding	176.75
Q96KK5	Histone H2A type 1-H [E]	DNA-binding	176.75
Q99878	Histone H2A type 1-J [E]	DNA-binding	176.75
Q6FI13	Histone H2A type 2-A [E]	DNA-binding	176.75
Q8IUE6	Histone H2A type 2-B [E]	DNA-binding	176.75
Q16777	Histone H2A type 2-C [E]	DNA-binding	176.75
Q7L7L0	Histone H2A type 3 [E]	DNA-binding	176.75
Q9BTM1	Histone H2A.J [E]	DNA-binding	176.75
Q71UI9	Histone H2A.V [E]	DNA-binding	176.75
P0C0S5	Histone H2A.Z [E]	DNA-binding	176.75
P16104	Histone H2AX [E]	DNA-binding	176.75
P62805	Histone H4 [E]	DNA-binding	1795.98
I3L3R1	Homeobox B8_ isoform CRA_a [F]	Transcription regulation	236.2
P17481	Homeobox protein Hox-B8 [F]	Transcription regulation	236.2
P31273	Homeobox protein Hox-C8 [F]	Transcription regulation	236.2
J3QL30	Hydrocephalus-inducing protein homolog (Fragment) [L]	Unknown	536.84
Q7Z5J1	Hydroxysteroid 11-beta-dehydrogenase 1-like protein [J]	Oxidoreductase	388.82
A0A0B4J2B6	Immunoglobulin heavy variable 2/OR16-5 (non-functional) (Fragment) [B]	Innate immune response	410.2
H0YBQ1	Integrator complex subunit 8 (Fragment) [E]	snRNA processing	244.63
Q8IU57	Interferon lambda receptor 1 [B]	Antiviral defense	262.8

A0A1B0GTI5	Interleukin-10 receptor subunit beta (Fragment) [B]	Inflammatory response	326.35
Q15811	Intersectin-1 [I]	Neuron apoptotic process	241.91
A0A1W2PQS2	IQ motif and SEC7 domain-containing protein 2 (Fragment) [D]	Structural component of cytoskeleton	385.21
Q16787	Laminin subunit alpha-3 [F]	Cell adhesion	282.82
Q16363	Laminin subunit alpha-4 [F]	Cell adhesion	215.34
Q2I0M4	Leucine-rich repeat-containing protein 26 [C]	Ion transport	231.68
Q9Y2P4	Long-chain fatty acid transport protein 6 [A]	Lipid metabolism	216.78
Q9Y561	Low-density lipoprotein receptor-related protein 12 [A]	Endocytosis	243.95
O95711	Lymphocyte antigen 86 [B]	Innate immune response	473.09
Q9BVV7	Mitochondrial import inner membrane translocase subunit Tim21 [C]	Protein transport	251.37
F8VYZ2	Monocarboxylate transporter 2 [C]	Transport	306.67
O75970	Multiple PDZ domain protein [F]	Cell adhesion	276.49
Q8IY17	Neuropathy target esterase [A]	Lipid metabolism	222.84
Q8NH81	Olfactory receptor 10G6 [F]	Sensory transduction	252.13
P09131	P3 protein [C]	Transport	201.68
O95428	Papilin [G]	Protease inhibitor	187.48
A6NIW5	Peroxiredoxin 2_ isoform CRA_a [J]	Cell redox homeostasis	776.51
P30041	Peroxiredoxin-6 [J]	Antioxidant. Lipid metabolism	1218.39
Q6IQ23	Pleckstrin homology domain-containing family A member 7 [D]	Cellular component	270.31
Q3KNV8	Polycomb group RING finger protein 3 [F]	Transcription regulation	192.63
Q5H9U9	Probable ATP-dependent RNA helicase DDX60-like [E]	RNA-binding	270.08
Q8IZL8	Proline-_ glutamic acid- and leucine-rich protein 1 [F]	Transcription	511.17
Q9H8V3	Protein ECT2 [H]	Differentiation. Neurogenesis	324.14
U3KQD2	Protein GPR107 [C]	Protein transport	186.68
Q9Y6F6	Protein MRVI1 [D]	Membrane component	270.56
Q8WXB1	Protein N-lysine methyltransferase METTL21A [F]	Transferase	236.44
A0A0A6YY99	Protein TNFSF12-TNFSF13 [B]	Immune response	490.49
O94855	Protein transport protein Sec24D [C]	Protein transport	271.08
Q69YN4	Protein virilizer homolog [E]	mRNA processing	321.84
A8MUN3	Putative uncharacterized protein ENSP00000381830 [L]	Unknown	672.41
Q96D71	RalBP1-associated Eps domain-containing protein 1 [K]	Calcium binding	277.02
Q92619	Rho GTPase-activating protein 45 [F]	Intracellular signal transduction	290.3
E5RI70	Rho GTPase-activating protein 7 (Fragment) [I]	Apoptosis process	1145.15

Q9NRP7	Serine/threonine-protein kinase 36 [A]	Kinase activity	197.99
Q96BR1	Serine/threonine-protein kinase Sgk3 [A]	Kinase activity	163.05
P30154	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform [I]	Apoptotic process	273.47
Q8TE82	SH3 domain and tetratricopeptide repeat-containing protein 1 [L]	Unknown	248.71
Q8TCT6	Signal peptide peptidase-like 3 [A]	T cell receptor signaling pathway	203.96
O94813	Slit homolog 2 protein [H]	Differentiation. Neurogenesis	187.71
P05023	Sodium/potassium-transporting ATPase subunit alpha-1 [K]	Ion transport	328.76
Q8WUM9	Sodium-dependent phosphate transporter 1 [K]	Phosphate ion transport	571.51
P02549	Spectrin alpha chain_ erythrocytic 1 [D]	Structural component of cytoskeleton	335.76
A0A087WXB8	ST3 beta-galactoside alpha-2_3-sialyltransferase 6_ isoform CRA_b [A]	Protein glycosylation	277.21
Q8IVG5	Sterile alpha motif domain-containing protein 9-like [G]	Regulation of growth factor	456.51
P57105	Synaptojanin-2-binding protein [G]	Regulation of growth factor	169.06
Q86Y82	Syntaxin-12 [C]	Protein transport	371.79
Q6PGP7	Tetratricopeptide repeat protein 37 [A]	Catabolic process	262.47
A0A0U1RQW3	Three prime repair exonuclease 1_ isoform CRA_a [E]	DNA damage checkpoint	636.8
I3L3T4	TOM1-like protein 1 [C]	Intracellular protein transport	194.25
O75204	Transmembrane protein 127 [D]	Endosome organization	791.15
Q14CX5	Transmembrane protein 180 [D]	Membrane component	341.01
Q9NX78	Transmembrane protein 260 [D]	Membrane component	121.58
Q9BTW9	Tubulin-specific chaperone D [D]	Microtubule cytoskeleton organization	328.09
Q2QBA2	Tumor necrosis factor (Ligand) superfamily member 13 transcript variant delta [B]	Immune response	490.49
O75888	Tumor necrosis factor ligand superfamily member 13 [B]	Immune response	490.49
Q9Y274	Type 2 lactosamine alpha-2_3-sialyltransferase [A]	Metabolic process	277.21
A0A0B4J269	Uncharacterized protein [H]	Neuron differentiation	526.49
S4R451	WD repeat-containing protein 11 [A]	Signaling pathway to ciliogenesis	385.34
Q9UII5	Zinc finger protein 107 [F]	Transcription regulation	162.73
Q8NHY6	Zinc finger protein 28 homolog [F]	Transcription regulation	415.19
F8WAL3	Zinc finger protein 528 [F]	Transcription regulation	269.74
Q86YE8	Zinc finger protein 573 [F]	Transcription regulation	246.76
Q03923	Zinc finger protein 85 [F]	Transcription regulation	289.34

Necrotic pulp

Accession	*Description*	*Biologic process*	*Score*
P31946	14-3-3 protein beta/alpha [B]	Host-virus interaction	731.1
Q6N063	2-oxoglutarate and iron-dependent oxygenase domain-containing protein 2 [J]	Oxidoreductase activity	350.12
Q86U10	60 kDa lysophospholipase [A]	Lipid metabolism	653.91
E9PNY0	Adenine DNA glycosylase [E]	DNA repair	1337.74
P55196	Afadin [F]	Cell adhesion	546.31
P02763	Alpha-1-acid glycoprotein 1 [B]	Regulation of immune system	588.98
P04217	Alpha-1B-glycoprotein [B]	Neutrophil degranulation	418.04
P01019	Angiotensinogen [G]	Growth factor activity	772.78
Q6ZTN6	Ankyrin repeat domain-containing protein 13D [F]	Ubiquitin-binding protein	491.4
K7EL63	Ankyrin repeat domain-containing protein 29 (Fragment) [L]	Unknown	358.5
C9JP59	Ankyrin repeat_ SAM and basic leucine zipper domain-containing protein 1 (Fragment) [L]	Unknown	859.6
Q01484	Ankyrin-2 [C]	Protein transport	58.02
Q9Y2F9	BTB/POZ domain-containing protein 3 [H]	Neurogenesis	645.18
Q9BXU9	Calcium-binding protein 8 [K]	Calcium ion binding	451.01
B4E1Z4	cDNA FLJ55673_ highly similar to Complement factor B (EC 3.4.21.47) [B]	Complement activation	503.52
A0A087X2B6	Cell cycle and apoptosis regulator protein 2 [I]	Regulation of apoptotic process	704.06
A6PVI9	Centrosomal protein 250kDa [G]	Cell cycle	461.65
Q9BV73	Centrosome-associated protein CEP250 [G]	Cell cycle	490.84
P00450	Ceruloplasmin [K]	Ion transport	161
H3BN91	C-Jun-amino-terminal kinase-interacting protein 3 (Fragment) [H]	Axon regeneration	327.76
P01024	Complement C3 [A]	Complement pathway	788.64
P00751	Complement factor B [B]	Innate immunity	503.52
O15315	DNA repair protein RAD51 homolog 2 [E]	DNA repair	292.13
Q7L591	Docking protein 3 [F]	Signal transduction	282.57
Q9NRD9	Dual oxidase 1 [J]	Oxidoreductase	1227.04
Q0PNE2	Elongator complex protein 6 [F]	Transcription regulation	406.73
E7EU71	Ephrin type-A receptor 6 [D]	Membrane component	466.19
Q9NRG7	Epimerase family protein SDR39U1 [J]	Oxidoreductase	346.17
I6L9I8	EPN3 protein [L]	Unknown	628.04
Q9H201	Epsin-3 [F]	Lipid-binding	650.19
C9JLC0	F-box/SPRY domain-containing protein 1 [H]	Neurogenesis	326.5
P02675	Fibrinogen beta chain [B]	Innate immunity	518.5
P00739	Haptoglobin-related protein [I]	Regulation of cell death	1026.1

A0A0B4J1V2	Immunoglobulin heavy variable 2-26 [B]	Adaptive immunity	663.4
P01834	Immunoglobulin kappa constant [B]	Adaptive immunity	3257.26
P01619	Immunoglobulin kappa variable 3-20 [B]	Adaptive immunity	613.37
P0CG04	Immunoglobulin lambda constant 1 [B]	Adaptive immunity	4803.8
P0DOY2	Immunoglobulin lambda constant 2 [B]	Adaptive immunity	4803.8
P0DOY3	Immunoglobulin lambda constant 3 [B]	Adaptive immunity	2697.85
P0CF74	Immunoglobulin lambda constant 6 [B]	Adaptive immunity	1652.51
A0M8Q6	Immunoglobulin lambda constant 7 [B]	Adaptive immunity	402.02
B9A064	Immunoglobulin lambda-like polypeptide 5 [B]	Innate immune response	4803.8
Q53G59	Kelch-like protein 12 [A]	Ubl conjugation pathway	237.11
P02788	Lactotransferrin [K]	Ion transport	256.65
Q6ZSS7	Major facilitator superfamily domain-containing protein 6 [D]	Membrane component	148.16
B5MC10	MpV17 mitochondrial inner membrane protein isoform 2 [J]	Regulation of reactive oxygen	563.58
E9PK80	NAD-dependent protein deacetylase [A]	Catalytic activity	1042.07
Q9NTG7	NAD-dependent protein deacetylase sirtuin-3_ mitochondrial [A]	Catalytic activity	1060.08
Q9UBB6	Neurochondrin [F]	Signal transduction	490.42
P59665	Neutrophil defensin 1 [B]	Antiviral defense	7785.6
P59666	Neutrophil defensin 3 [B]	Antiviral defense	1433.54
P08246	Neutrophil elastase [A]	Catalytic activity	614.34
Q15155	Nodal modulator 1 [D]	Membrane component	523.61
Q5JPE7	Nodal modulator 2 [D]	Membrane component	45.57
P69849	Nodal modulator 3 [D]	Membrane component	533.01
A0A0J9YWI0	Peroxisomal 2_4-dienoyl-CoA reductase (Fragment) [J]	Oxidoreductase	424.99
P27986	Phosphatidylinositol 3-kinase regulatory subunit alpha [I]	Regulation of apoptotic process	462.89
O00443	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha [C]	Endocytosis	586
A0A0U1RQS1	Probable global transcription activator SNF2L2 (Fragment) [H]	Neurogenesis	986.63
Q9BVM2	Protein DPCD [D]	Formation of ciliated cells	225.08
C9K0C0	Protein FAM71F2 [L]	Unknown	1745.9
P06702	Protein S100-A9 [I]	Apoptosis	4809.99
Q96I85	Putative uncharacterized protein C14orf144 [L]	Unknown	1071.1
Q86TS7	Putative UPF0730 protein encoded by LINC00643 [L]	Unknown	506.3

Q9UHV5	Rap guanine nucleotide exchange factor-like 1 [H]	Nervous system development	657.95
P52565	Rho GDP-dissociation inhibitor 1 [I]	Regulation of apoptotic process	241.48
J3KPQ4	Rho GTPase activating protein 9_ isoform CRA_a [F]	Signal transduction	421.35
Q9BRR9	Rho GTPase-activating protein 9 [F]	Signal transduction	421.35
O15393	Transmembrane protease serine 2 [A]	Catalytic activity	300.54
A0A1W2PQJ5	Uncharacterized protein [L]	Unknown	331.97
H0Y8H3	Uncharacterized protein C3orf67 (Fragment) [L]	Unknown	864.21
P02774	Vitamin D-binding protein [C]	Transport	470.82
E9PNL3	V-type proton ATPase 21 kDa proteolipid subunit [K]	Ion transport	222.07

Proteins were classified according to Uniprot Homo sapiens database: A – Metabolism and energy pathways; B - Immune response; C – Transport; D – Structure; E - DNA/RNA regulation and repair; F - Cell communication and signal transduction; G - Cell growth and/or maintenance; H- Differentiation of neural cells; I- Apoptosis; J- Stress response; K- Ions regulation and binding; L- Unknown.

Table 4 – Human proteins identified in normal (NO), inflamed (I) and/or necrotic (NE) pulp.

Accession	*Description*	*Score*	*NO*	*I*	*NE*
P31946	14-3-3 protein beta/alpha	731.1			x
Q6N063	2-oxoglutarate and iron-dependent oxygenase domain-containing protein 2	350.12			x
P62280	40S ribosomal protein S11	351.39		x	
E2QRG7	4-hydroxybenzoate polyprenyltransferase_ mitochondrial	424.48	x		
P10809	60 kDa heat shock protein_ mitochondrial	315.54	x	x	
Q86U10	60 kDa lysophospholipase	653.91			x
A0A087WYK6	Acetyl-CoA carboxylase 1	394.31		x	
P78348	Acid-sensing ion channel 1	1387.23		x	
P68032	Actin_ alpha cardiac muscle 1	23566.06	x	x	x
P68133	Actin_ alpha skeletal muscle	23540.28	x	x	x
P62736	Actin_ aortic smooth muscle	23566.06	x	x	x
P60709	Actin_ cytoplasmic 1	30939.39	x	x	x
P63261	Actin_ cytoplasmic 2	30939.39	x	x	x
P63267	Actin_ gamma-enteric smooth muscle	23566.06	x	x	x
E9PNY0	Adenine DNA glycosylase	1337.74			x
A0A1X7SBU6	Adhesion G protein-coupled receptor V1 (Fragment)	208.16		x	
Q8IUX7	Adipocyte enhancer-binding protein 1	281.92	x		
P55196	Afadin	546.31			x
C9JKR2	Albumin_ isoform CRA_k	8751.58	x	x	x
F2Z324	Aldehyde dehydrogenase 1 family member L1 isoform 2	354.48	x		
P02763	Alpha-1-acid glycoprotein 1	588.98			x
P01009	Alpha-1-antitrypsin	977.72	x	x	x
E9PK47	Alpha-1_4 glucan phosphorylase	4192.58	x	x	x
P04217	Alpha-1B-glycoprotein	418.04			x

P02765	Alpha-2-HS-glycoprotein	334	x		
P01023	Alpha-2-macroglobulin	86.39		x	x
Q16352	Alpha-internexin	3973.03	x	x	
Q9NVD7	Alpha-parvin	174.54		x	
H0YMF8	Ammonium transporter Rh type C	751.41	x		
P01019	Angiotensinogen	772.78			x
Q6ZTN6	Ankyrin repeat domain-containing protein 13D	491.4			x
K7EL63	Ankyrin repeat domain-containing protein 29 (Fragment)	358.5			x
C9JP59	Ankyrin repeat_ SAM and basic leucine zipper domain-containing protein 1 (Fragment)	859.6			x
Q01484	Ankyrin-2	58.02			x
P02647	Apolipoprotein A-I	716.81		x	x
P02652	Apolipoprotein A-II	1123.97	x	x	x
Q96P47	Arf-GAP with GTPase_ ANK repeat and PH domain-containing protein 3	647.64		x	x
P00966	Argininosuccinate synthase	178.45	x		
H0YH81	ATP synthase subunit beta (Fragment)	289.75	x		
P06576	ATP synthase subunit beta_ mitochondrial	548.43	x		
H7BZI9	ATP-binding cassette sub-family A member 13 (Fragment)	281.18		x	
K7ELK9	ATP-binding cassette sub-family A member 8 (Fragment)	190.11		x	
Q9NUQ8	ATP-binding cassette sub-family F member 3	217.99	x		
P08237	ATP-dependent 6-phosphofructokinase_ muscle type	439.21	x		
Q8WXE1	ATR-interacting protein	636.8		x	
P17213	Bactericidal permeability-increasing protein	284.17		x	
P02730	Band 3 anion transport protein	243.95		x	
Q562R1	Beta-actin-like protein 2	2959.17	x	x	
P49407	Beta-arrestin-1	243.29	x		
Q96T60	Bifunctional polynucleotide phosphatase/kinase	229.66		x	
P21810	Biglycan	277.04	x		
Q15059	Bromodomain-containing protein 3	693.33		x	x
Q9Y2F9	BTB/POZ domain-containing protein 3	645.18			x
P22223	Cadherin-3	414.89	x		
Q9H9S4	Calcium-binding protein 39-like	785.13	x		
Q9BXU9	Calcium-binding protein 8	451.01			x
P00915	Carbonic anhydrase 1	364.93		x	
P00918	Carbonic anhydrase 2	641.72		x	
Q9NS85	Carbonic anhydrase-related protein 10	179.75		x	
P51948	CDK-activating kinase assembly factor MAT1	228.15		x	
B4E1Z4	cDNA FLJ55673_ highly similar to Complement factor B (EC 3.4.21.47)	503.52			x
A0A087X2B6	Cell cycle and apoptosis regulator protein 2	704.06			x
P29762	Cellular retinoic acid-binding protein 1	1319.56	x		
A6PVI9	Centrosomal protein 250kDa	461.65			x
Q9BV73	Centrosome-associated protein CEP250	490.84			x
P00450	Ceruloplasmin	161			x
X6RIU2	Cilia- and flagella-associated protein 221 (Fragment)	158.14	x		
Q96N23	Cilia- and flagella-associated protein 54	864.57	x		

P26441	Ciliary neurotrophic factor	150.4	x		
H3BN91	C-Jun-amino-terminal kinase-interacting protein 3 (Fragment)	327.76			x
A0A0A0MT56	Cleavage stimulation factor subunit 2 (Fragment)	428.34	x		
P00451	Coagulation factor VIII	548.5		x	
H0YK65	Coiled-coil domain-containing 9B (Fragment)	372.31		x	
Q9GZT6	Coiled-coil domain-containing protein 90B_ mitochondrial	271	x		
P01024	Complement C3	788.64			x
P00751	Complement factor B	503.52			x
Q9Y2V7	Conserved oligomeric Golgi complex subunit 6	382.37		x	
J3QT66	COP9 signalosome complex subunit 7b	847.96	x		
Q2NKJ3	CST complex subunit CTC1	586.38	x		
K7EJ26	CUGBP Elav-like family member 4 (Fragment)	247.83	x		
Q8TEY5	Cyclic AMP-responsive element-binding protein 3-like protein 4	314.59		x	
P55273	Cyclin-dependent kinase 4 inhibitor D	360.12		x	
O75891	Cytosolic 10-formyltetrahydrofolate dehydrogenase	362.58	x		
Q68DD2	Cytosolic phospholipase A2 zeta	279.68		x	
P30038	Delta-1-pyrroline-5-carboxylate dehydrogenase_ mitochondrial	191.36		x	
Q07507	Dermatopontin	1412.86	x		
P17661	Desmin	4264.47	x	x	
O75907	Diacylglycerol O-acyltransferase 1	290.99	x		
Q16555	Dihydropyrimidinase-related protein 2	169.43	x		
P53602	Diphosphomevalonate decarboxylase	552.3	x		
P78352	Disks large homolog 4	233.8		x	
H7C0V2	DNA repair protein RAD50 (Fragment)	234.66	x		
O15315	DNA repair protein RAD51 homolog 2	292.13			x
P38935	DNA-binding protein SMUBP-2	197.26	x		
Q7L591	Docking protein 3	282.57			x
Q9NRD9	Dual oxidase 1	1227.04			x
P36507	Dual specificity mitogen-activated protein kinase 2	240.42		x	
H0YD30	Dynein assembly factor 3_ axonemal (Fragment)	309.68		x	
Q14118	Dystroglycan	383.01		x	
Q8N2H9	E3 ubiquitin-protein ligase pellino homolog 3	1102.45		x	
K7EII6	Echinoderm microtubule-associated protein-like 2 (Fragment)	230.16		x	
Q05BV3	Echinoderm microtubule-associated protein-like 5	308.9		x	
P43897	Elongation factor Ts_ mitochondrial	215.14		x	
Q0PNE2	Elongator complex protein 6	406.73			x
Q5T6L9	Endoplasmic reticulum membrane-associated RNA degradation protein	440.87		x	
Q7Z2K6	Endoplasmic reticulum metallopeptidase 1	187.1	x	x	x
A0PK19	EPGN protein	1566.9	x		
E7EU71	Ephrin type-A receptor 6	466.19			x
Q6UW88	Epigen	1566.9	x		
Q9NRG7	Epimerase family protein SDR39U1	346.17			x
I6L9I8	EPN3 protein	628.04			x
Q9H201	Epsin-3	650.19			x

P60842	Eukaryotic initiation factor 4A-I	175.34	x		
Q9BY44	Eukaryotic translation initiation factor 2A	443.73	x	x	
Q04637	Eukaryotic translation initiation factor 4 gamma 1	280.67	x		
Q9BQ95	Evolutionarily conserved signaling intermediate in Toll pathway_ mitochondrial	464.91	x	x	
Q9NPD3	Exosome complex component RRP41	340.1	x		
O15360	Fanconi anemia group A protein	156.29		x	
Q14CZ7	FAST kinase domain-containing protein 3_ mitochondrial	216.49		x	
Q9UK22	F-box only protein 2	280.14	x		
C9JLC0	F-box/SPRY domain-containing protein 1	326.5			x
Q969U6	F-box/WD repeat-containing protein 5	335.47	x		
O94887	FERM_ ARHGEF and pleckstrin domain-containing protein 2	394.64		x	
A0A0A0MS75	FGF receptor activating protein 1_ isoform CRA_e	229.6	x		
P02675	Fibrinogen beta chain	518.5			x
P02679	Fibrinogen gamma chain	117.71		x	
Q06828	Fibromodulin	484.9	x		
G5E9X3	Fibronectin type III domain containing 3A_ isoform CRA_f	263.94	x		
Q9Y2H6	Fibronectin type-III domain-containing protein 3A	283.11	x		
Q4L180	Filamin A-interacting protein 1-like	259.28		x	
H3BQN4	Fructose-bisphosphate aldolase	577.74	x		
P04075	Fructose-bisphosphate aldolase A	591.64	x		
P09382	Galectin-1	4761.72	x		
Q9NSN8	Gamma-1-syntrophin	273.12		x	
Q9UBS5	Gamma-aminobutyric acid type B receptor subunit 1	433.53	x		
P36383	Gap junction gamma-1 protein	179.45		x	
Q5T442	Gap junction gamma-2 protein	307.56		x	
Q99501	GAS2-like protein 1	299.88	x		
Q13630	GDP-L-fucose synthase	120.68		x	
H7C4Q8	General transcription factor II-I repeat domain-containing protein 1 (Fragment)	168.04	x		
Q14687	Genetic suppressor element 1	118.64	x		
Q6UWF4	GLGQ5807	325.59		x	
P04406	Glyceraldehyde-3-phosphate dehydrogenase	311.62	x	x	
P11216	Glycogen phosphorylase_ brain form	6210.93	x	x	x
P06737	Glycogen phosphorylase_ liver form	4192.58	x	x	x
P11217	Glycogen phosphorylase_ muscle form	15413.33	x	x	x
Q92805	Golgin subfamily A member 1	197.06		x	
Q86VD9	GPI mannosyltransferase 4	218.44	x		
A0A1W2PNZ5	GPI transamidase component PIG-T	369.7		x	
Q8TAI7	GTPase RhebL1	277.05	x	x	
P62826	GTP-binding nuclear protein Ran	306.27		x	
P00738	Haptoglobin	8038	x	x	x
P00739	Haptoglobin-related protein	1026.1			x
G3V1N2	HCG1745306_ isoform CRA_a	6067.66	x	x	x
A0A0A6YYF2	HCG1811249_ isoform CRA_e	277.46		x	
A0A0A0MTS5	HCG1811249_ isoform CRA_f	274.02		x	

G3V3J6	HCG1983504_ isoform CRA_b	10798.74	x		
G3V3R4	HCG1983504_ isoform CRA_c	16550.79	x	x	
G3V2N6	HCG1983504_ isoform CRA_d	16550.79	x	x	
G3V2R8	HCG1983504_ isoform CRA_e	16550.79	x	x	
Q9H583	HEAT repeat-containing protein 1	391.17	x		
Q86XA9	HEAT repeat-containing protein 5A	238.55	x		
P04792	Heat shock protein beta-1	1700.43	x		
P69905	Hemoglobin subunit alpha	16795.5	x	x	x
P68871	Hemoglobin subunit beta	25559.88	x	x	x
P02042	Hemoglobin subunit delta	6891.86	x	x	x
P02100	Hemoglobin subunit epsilon	5396.91	x	x	x
P69891	Hemoglobin subunit gamma-1	5396.91	x	x	x
P69892	Hemoglobin subunit gamma-2	5396.91	x	x	x
Q9Y5N1	Histamine H3 receptor	188.39	x		
A0A0U1RR32	Histone H2A	176.75		x	
P0C0S8	Histone H2A type 1	176.75		x	
Q96QV6	Histone H2A type 1-A	176.75		x	
P04908	Histone H2A type 1-B/E	176.75		x	
Q93077	Histone H2A type 1-C	176.75		x	
P20671	Histone H2A type 1-D	176.75		x	
Q96KK5	Histone H2A type 1-H	176.75		x	
Q99878	Histone H2A type 1-J	176.75		x	
Q6FI13	Histone H2A type 2-A	176.75		x	
Q8IUE6	Histone H2A type 2-B	176.75		x	
Q16777	Histone H2A type 2-C	176.75		x	
Q7L7L0	Histone H2A type 3	176.75		x	
Q9BTM1	Histone H2A.J	176.75		x	
Q71UI9	Histone H2A.V	176.75		x	
P0C0S5	Histone H2A.Z	176.75		x	
P16104	Histone H2AX	176.75		x	
U3KQK0	Histone H2B	2872.74	x	x	
Q96A08	Histone H2B type 1-A	1914.55	x		
P33778	Histone H2B type 1-B	2872.74	x	x	
P62807	Histone H2B type 1-C/E/F/G/I	508.67	x	x	
P58876	Histone H2B type 1-D	2872.74	x	x	
Q93079	Histone H2B type 1-H	2872.74	x	x	
P06899	Histone H2B type 1-J	2872.74	x	x	
O60814	Histone H2B type 1-K	2872.74	x	x	
Q99880	Histone H2B type 1-L	2872.74	x	x	
Q99879	Histone H2B type 1-M	2872.74	x	x	
Q99877	Histone H2B type 1-N	2872.74	x	x	
P23527	Histone H2B type 1-O	508.67	x	x	
Q16778	Histone H2B type 2-E	2872.74	x	x	
Q5QNW6	Histone H2B type 2-F	2872.74	x	x	

Q8N257	Histone H2B type 3-B	267.14	x	x	
P57053	Histone H2B type F-S	2872.74	x	x	
Q5TEC6	Histone H3	260.08		x	x
P68431	Histone H3.1	910.49		x	x
Q16695	Histone H3.1t	737.84		x	x
Q71DI3	Histone H3.2	269.25		x	x
P84243	Histone H3.3	269.25		x	x
Q6NXT2	Histone H3.3C	269.25		x	x
P62805	Histone H4	1795.98		x	
I3L3R1	Homeobox B8_ isoform CRA_a	236.2		x	
P17481	Homeobox protein Hox-B8	236.2		x	
P31273	Homeobox protein Hox-C8	236.2		x	
J3QL30	Hydrocephalus-inducing protein homolog (Fragment)	536.84		x	
Q7Z5J1	Hydroxysteroid 11-beta-dehydrogenase 1-like protein	388.82		x	
P01876	Immunoglobulin heavy constant alpha 1	338.1	x	x	x
P01877	Immunoglobulin heavy constant alpha 2	612.25	x	x	x
P01857	Immunoglobulin heavy constant gamma 1	821.58	x	x	x
P01859	Immunoglobulin heavy constant gamma 2	580.61	x		x
P01860	Immunoglobulin heavy constant gamma 3	3537.37	x		x
P01861	Immunoglobulin heavy constant gamma 4	2908.43			x
A0A0B4J2B6	Immunoglobulin heavy variable 2/OR16-5 (non-functional) (Fragment)	410.2		x	
A0A0B4J1V2	Immunoglobulin heavy variable 2-26	663.4			x
P01834	Immunoglobulin kappa constant	3257.26			x
A0A0C4DH90	Immunoglobulin kappa variable 3/OR2-268 (non-functional) (Fragment)	306.71		x	x
P01624	Immunoglobulin kappa variable 3-15	1001.37		x	x
P01619	Immunoglobulin kappa variable 3-20	613.37			x
A0A075B6H7	Immunoglobulin kappa variable 3-7 (non-functional) (Fragment)	306.71		x	x
A0A0C4DH55	Immunoglobulin kappa variable 3D-7	306.71		x	x
P0CG04	Immunoglobulin lambda constant 1	4803.8			x
P0DOY2	Immunoglobulin lambda constant 2	4803.8			x
P0DOY3	Immunoglobulin lambda constant 3	2697.85			x
P0CF74	Immunoglobulin lambda constant 6	1652.51			x
A0M8Q6	Immunoglobulin lambda constant 7	402.02			x
B9A064	Immunoglobulin lambda-like polypeptide 5	4803.8			x
Q14571	Inositol 1_4_5-trisphosphate receptor type 2	271.01	x		
H0YBQ1	Integrator complex subunit 8 (Fragment)	244.63		x	
P19823	Inter-alpha-trypsin inhibitor heavy chain H2	371.22	x		
Q8IU57	Interferon lambda receptor 1	262.8		x	
A0A1B0GTI5	Interleukin-10 receptor subunit beta (Fragment)	326.35		x	
Q15811	Intersectin-1	241.91		x	
A0A1W2PQS2	IQ motif and SEC7 domain-containing protein 2 (Fragment)	385.21		x	
Q53G59	Kelch-like protein 12	237.11			x
V9GXZ7	Kin of IRRE-like protein 2 (Fragment)	462.22	x		
Q86UP2	Kinectin	318.27	x	x	

Q96Q89	Kinesin-like protein KIF20B	844.1	x		
P02788	Lactotransferrin	256.65			x
Q16787	Laminin subunit alpha-3	282.82		x	
Q16363	Laminin subunit alpha-4	215.34		x	
H0YEH0	Large neutral amino acids transporter small subunit 3 (Fragment)	736.6	x		
O75845	Lathosterol oxidase	169.98	x		
Q2I0M4	Leucine-rich repeat-containing protein 26	231.68		x	
Q8N653	Leucine-zipper-like transcriptional regulator 1	161.39	x		
Q8NHJ6	Leukocyte immunoglobulin-like receptor subfamily B member 4	315.03	x		
Q9Y2P4	Long-chain fatty acid transport protein 6	216.78		x	
Q9Y561	Low-density lipoprotein receptor-related protein 12	243.95		x	
P51884	Lumican	5091.27	x		
O95711	Lymphocyte antigen 86	473.09		x	
Q6UWQ5	Lysozyme-like protein 1	847.95	x		
Q7Z4W2	Lysozyme-like protein 2	847.95	x		
P14174	Macrophage migration inhibitory factor	644.94	x	x	
A0A0D9SFF8	MAGUK p55 subfamily member 2	700.69	x		
Q9H3U5	Major facilitator superfamily domain-containing protein 1	167.24	x		
Q6ZSS7	Major facilitator superfamily domain-containing protein 6	148.16			x
C9JQX2	Mannosyltransferase	210.23	x		
Q9UMX9	Membrane-associated transporter protein	671.2	x	x	
E5RJR3	Methionine adenosyltransferase 2 subunit beta	683.62	x		
Q9UBK8	Methionine synthase reductase	233.79	x	x	
E7EVA0	Microtubule-associated protein	750.64	x	x	
P27816	Microtubule-associated protein 4	122.57	x	x	
Q9BVV7	Mitochondrial import inner membrane translocase subunit Tim21	251.37		x	
F8VYZ2	Monocarboxylate transporter 2	306.67		x	
B5MC10	MpV17 mitochondrial inner membrane protein isoform 2	563.58			x
O75970	Multiple PDZ domain protein	276.49		x	
P25189	Myelin protein P0	366.82	x		
P60660	Myosin light polypeptide 6	430.43	x		
Q9UK23	N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase	246.65	x		
E9PK80	NAD-dependent protein deacetylase	1042.07			x
Q9NTG7	NAD-dependent protein deacetylase sirtuin-3_ mitochondrial	1060.08			x
A0A087WYD0	NADH dehydrogenase (Ubiquinone) 1 beta subcomplex_ 5_ 16kDa_ isoform CRA_g	379.52	x		
O43674	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5_ mitochondrial	379.52	x		
O00308	NEDD4-like E3 ubiquitin-protein ligase WWP2	261.84	x		
P18615	Negative elongation factor E	340.06	x		x
O00533	Neural cell adhesion molecule L1-like protein	238.35	x		
Q9UBB6	Neurochondrin	490.42			x
P12036	Neurofilament heavy polypeptide	471.82	x		
P07196	Neurofilament light polypeptide	645.68	x		
P07197	Neurofilament medium polypeptide	609	x	x	

A0A087WW27	Neuroligin-3	470.51	x		
Q8IY17	Neuropathy target esterase	222.84		x	
P59665	Neutrophil defensin 1	7785.6			x
P59666	Neutrophil defensin 3	1433.54			x
P08246	Neutrophil elastase	614.34			x
Q7RTR2	NLR family CARD domain-containing protein 3	180.39	x		
H3BLT9	NOD3 protein_ isoform CRA_d	180.39	x		
Q15155	Nodal modulator 1	523.61			x
Q5JPE7	Nodal modulator 2	45.57			x
P69849	Nodal modulator 3	533.01			x
Q14980	Nuclear mitotic apparatus protein 1	281.88	x		
F6M2K2	Nuclear receptor coactivator 6	253.67	x		
A0A126GWK9	Olfactory receptor	305.24	x		
Q8NH81	Olfactory receptor 10G6	252.13		x	
O43869	Olfactory receptor 2T1	305.24	x		
Q6IFN5	Olfactory receptor 7E24	220.6	x		
P09131	P3 protein	201.68		x	
H0Y2Y4	Palmitoyltransferase (Fragment)	212.83	x		
O95497	Pantetheinase	239.94	x		
O95428	Papilin	187.48		x	
P26022	Pentraxin-related protein PTX3	159.12	x		
C9J5S7	Peptidyl-prolyl cis-trans isomerase	1087.52	x	x	
P62937	Peptidyl-prolyl cis-trans isomerase A	924.38	x	x	
Q9Y536	Peptidyl-prolyl cis-trans isomerase A-like 4A	511.68	x		
P41219	Peripherin	3802	x	x	
A6NIW5	Peroxiredoxin 2_ isoform CRA_a	776.51		x	
Q06830	Peroxiredoxin-1	558.33	x	x	
P32119	Peroxiredoxin-2	558.33	x	x	
P30041	Peroxiredoxin-6	1218.39		x	
A0A0J9YWI0	Peroxisomal 2_4-dienoyl-CoA reductase (Fragment)	424.99			x
Q8NEB9	Phosphatidylinositol 3-kinase catalytic subunit type 3	518.24	x		
P27986	Phosphatidylinositol 3-kinase regulatory subunit alpha	462.89			x
O00443	Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha	586			x
Q6IQ23	Pleckstrin homology domain-containing family A member 7	270.31		x	
Q3KNV8	Polycomb group RING finger protein 3	192.63		x	
Q9UHJ9	Post-GPI attachment to proteins factor 2	229.6	x		
Q6S8J3	POTE ankyrin domain family member E	11353.69	x	x	x
A5A3E0	POTE ankyrin domain family member F	11345.5	x	x	x
P0CG38	POTE ankyrin domain family member I	11284.24	x	x	x
P0CG39	POTE ankyrin domain family member J	7922.04	x	x	x
Q01860	POU domain_ class 5_ transcription factor 1	190.11	x		
P02545	Prelamin-A/C	405.48	x		
Q9UMS4	Pre-mRNA-processing factor 19	353.75	x		
Q96I59	Probable asparagine--tRNA ligase_ mitochondrial	191.32	x		

Q5H9U9	Probable ATP-dependent RNA helicase DDX60-like	270.08		x	
Q15034	Probable E3 ubiquitin-protein ligase HERC3	82.2	x		
A0A0U1RQS1	Probable global transcription activator SNF2L2 (Fragment)	986.63			x
Q8IZL8	Proline-_ glutamic acid- and leucine-rich protein 1	511.17		x	
Q16651	Prostasin	275.33	x		
Q9BVM2	Protein DPCD	225.08			x
Q9H8V3	Protein ECT2	324.14		x	
C9K0C0	Protein FAM71F2	1745.9			x
U3KQD2	Protein GPR107	186.68		x	
Q9Y6F6	Protein MRVI1	270.56		x	
Q8WXB1	Protein N-lysine methyltransferase METTL21A	236.44		x	
H0YHR3	Protein phosphatase Slingshot homolog 1 (Fragment)	255.57	x		
P06702	Protein S100-A9	4809.99			x
A0A0A6YY99	Protein TNFSF12-TNFSF13	490.49		x	
O94855	Protein transport protein Sec24D	271.08		x	
Q69YN4	Protein virilizer homolog	321.84		x	
Q99497	Protein/nucleic acid deglycase DJ-1	784.37	x		
H3BRZ0	Putative sodium-coupled neutral amino acid transporter 7 (Fragment)	672.03	x		
Q96I85	Putative uncharacterized protein C14orf144	1071.1			x
A8MUN3	Putative uncharacterized protein ENSP00000381830	672.41		x	
Q86TS7	Putative UPF0730 protein encoded by LINC00643	506.3			x
B4DNK4	Pyruvate kinase	458.75	x		
P14618	Pyruvate kinase PKM	458.75	x		
P26374	Rab proteins geranylgeranyltransferase component A 2	618.53	x		
Q96D71	RalBP1-associated Eps domain-containing protein 1	277.02		x	
Q9UHV5	Rap guanine nucleotide exchange factor-like 1	657.95			x
P21860	Receptor tyrosine-protein kinase erbB-3	271.71	x		
Q12913	Receptor-type tyrosine-protein phosphatase eta	436.47	x		
P52565	Rho GDP-dissociation inhibitor 1	241.48			x
J3KPQ4	Rho GTPase activating protein 9_ isoform CRA_a	421.35			x
Q92619	Rho GTPase-activating protein 45	290.3		x	
E5RI70	Rho GTPase-activating protein 7 (Fragment)	1145.15		x	
Q9BRR9	Rho GTPase-activating protein 9	421.35			x
E9PGT3	Ribosomal protein S6 kinase	415.96	x		
Q15418	Ribosomal protein S6 kinase alpha-1	430.79	x		
Q6P3W7	SCY1-like protein 2	144.69	x		
P59797	Selenoprotein V	3591.64	x		
Q9H3S1	Semaphorin-4A	898.66	x		
O95754	Semaphorin-4F	257.33	x		
Q05519	Serine/arginine-rich splicing factor 11	1467.45	x		
Q8WU08	Serine/threonine-protein kinase 32A	292.95	x		
Q9NRP7	Serine/threonine-protein kinase 36	197.99		x	
Q96BR1	Serine/threonine-protein kinase Sgk3	163.05		x	
Q9H2K8	Serine/threonine-protein kinase TAO3	607.16	x		

P30154	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	273.47		x	
P02787	Serotransferrin	16118.53	x		x
P02768	Serum albumin	14782.73	x	x	x
Q8TE82	SH3 domain and tetratricopeptide repeat-containing protein 1	248.71		x	
Q8TCT6	Signal peptide peptidase-like 3	203.96		x	
O94813	Slit homolog 2 protein	187.71		x	
O43147	Small G protein signaling modulator 2	738.5	x		
P05023	Sodium/potassium-transporting ATPase subunit alpha-1	328.76		x	
Q8WUM9	Sodium-dependent phosphate transporter 1	571.51		x	
A0A087WWM3	Solute carrier family 22 member 24	317.43	x		
P02549	Spectrin alpha chain_ erythrocytic 1	335.76		x	
Q8WXA9	Splicing regulatory glutamine/lysine-rich protein 1	202.85	x	x	
A0A087WXB8	ST3 beta-galactoside alpha-2_3-sialyltransferase 6_ isoform CRA_b	277.21		x	
Q8IVG5	Sterile alpha motif domain-containing protein 9-like	456.51		x	
P00441	Superoxide dismutase [Cu-Zn]	1233.87	x		
P57105	Synaptojanin-2-binding protein	169.06		x	
Q9UMS6	Synaptopodin-2	289.93	x		
Q9Y6H5	Synphilin-1	224.06	x		
Q86Y82	Syntaxin-12	371.79		x	
Q9Y4G6	Talin-2	332.52	x	x	
Q9NYW2	Taste receptor type 2 member 8	173.03	x		
Q8IWY7	Tau-tubulin kinase	231.03	x		
P24821	Tenascin	788.89	x		
Q6PGP7	Tetratricopeptide repeat protein 37	262.47		x	
Q5R3I4	Tetratricopeptide repeat protein 38	164.5	x		
J3QQZ3	THO complex subunit 1	165.16	x		
A0A0U1RQW3	Three prime repair exonuclease 1_ isoform CRA_a	636.8		x	
I3L3T4	TOM1-like protein 1	194.25		x	
Q9Y2L5	Trafficking protein particle complex subunit 8	426.03	x	x	
Q14135	Transcription cofactor vestigial-like protein 4	320.85	x		
Q15582	Transforming growth factor-beta-induced protein ig-h3	699.53	x		
Q7Z6W1	Transmembrane and coiled-coil domain-containing protein 2	383.03	x		
O15393	Transmembrane protease serine 2	300.54			x
O75204	Transmembrane protein 127	791.15		x	
Q14CX5	Transmembrane protein 180	341.01		x	
Q5JRV8	Transmembrane protein 255A	268.12		x	x
Q9NX78	Transmembrane protein 260	121.58		x	
P02766	Transthyretin	1092.4		x	x
P60174	Triosephosphate isomerase	216.16	x	x	
F5H5D3	Tubulin alpha chain	34846.15	x	x	
Q71U36	Tubulin alpha-1A chain	38017.8	x	x	
P68363	Tubulin alpha-1B chain	37782.63	x	x	
Q9BQE3	Tubulin alpha-1C chain	34846.15	x	x	
Q13748	Tubulin alpha-3C/D chain	12691.5	x	x	

Q6PEY2	Tubulin alpha-3E chain	8816.79	x	x	
P68366	Tubulin alpha-4A chain	12442.36	x	x	
Q9NY65	Tubulin alpha-8 chain	11965.06	x	x	
P07437	Tubulin beta chain	26436.61	x	x	
Q9H4B7	Tubulin beta-1 chain	1944.92	x		
Q13885	Tubulin beta-2A chain	26225.01	x	x	
Q9BVA1	Tubulin beta-2B chain	923.82	x	x	
Q13509	Tubulin beta-3 chain	19549.53	x	x	
P04350	Tubulin beta-4A chain	913.4	x	x	
P68371	Tubulin beta-4B chain	923.9	x	x	
Q9BUF5	Tubulin beta-6 chain	439.06	x	x	
Q3ZCM7	Tubulin beta-8 chain	8805.07	x	x	
A6NNZ2	Tubulin beta-8 chain-like protein LOC260334	5280.73	x	x	
Q9UJT1	Tubulin delta chain	493.65	x		
Q9BTW9	Tubulin-specific chaperone D	328.09		x	
Q2QBA2	Tumor necrosis factor (Ligand) superfamily member 13 transcript variant delta	490.49		x	
O75888	Tumor necrosis factor ligand superfamily member 13	490.49		x	
Q9Y274	Type 2 lactosamine alpha-2_3-sialyltransferase	277.21		x	
A8MXF1	Tyrosine-protein phosphatase non-receptor type 5	355.82	x		
A0A0B4J269	Uncharacterized protein	8750.79	x		
A0A0B4J269	Uncharacterized protein	526.49		x	
A0A1W2PQJ5	Uncharacterized protein	331.97			x
Q8NBR9	Uncharacterized protein C11orf72	310.42	x		
V9GY35	Uncharacterized protein C1orf109 (Fragment)	240.6	x		
H0Y8H3	Uncharacterized protein C3orf67 (Fragment)	864.21			x
Q6ZUG5	Uncharacterized protein FLJ43738	992.96	x		
D6RBZ9	Uncharacterized protein FLJ43738 (Fragment)	990.13	x		
Q92628	Uncharacterized protein KIAA0232	583.39	x		
D6REK0	Uncharacterized protein KIAA0232	568.86	x		
P10746	Uroporphyrinogen-III synthase	212.17	x		
P08670	Vimentin	26074.13	x	x	
P02774	Vitamin D-binding protein	470.82			x
P04004	Vitronectin	728.59	x		
Q06432	Voltage-dependent calcium channel gamma-1 subunit	219.37	x		
E9PNL3	V-type proton ATPase 21 kDa proteolipid subunit	222.07			x
Q5MNZ6	WD repeat domain phosphoinositide-interacting protein 3	228.89	x		
S4R451	WD repeat-containing protein 11	385.34		x	
H0Y6X2	Zinc finger and BTB domain-containing protein 17	302.68	x		
Q6PJT7	Zinc finger CCCH domain-containing protein 14	331.85	x		
Q9UII5	Zinc finger protein 107	162.73		x	
Q8NHY6	Zinc finger protein 28 homolog	415.19		x	
Q09FC8	Zinc finger protein 415	192.42	x		
F8WAL3	Zinc finger protein 528	269.74		x	
Q86YE8	Zinc finger protein 573	246.76		x	

Q96MU6	Zinc finger protein 778	562.65	x	
Q03923	Zinc finger protein 85	289.34		x

Anexo 1 – Guidelines for authors – International Endodontic Journal

1. GENERAL

International Endodontic Journal publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology; the branch of dental sciences dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in International Endodontic Journal. Authors are encouraged to visit Wiley Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

International Endodontic Journal adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.

International Endodontic Journal adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisiation of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Please find more information on the conflict of interest form in section 2.6.

2.2. Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study. The authors MUST upload a copy of the ethical approval letter when submitting their manuscript and a separate English translation. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

2.3 Clinical Trials

The International Endodontic Journal asks that authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following public clinical trials registries: www.clinicaltrials.gov, https://www.clinicaltrialsregister.eu/, http://isrctn.org/. Other primary registries if named in the WHO network will also be considered acceptable. The clinical trial registration number and name of the trial register should be included in the Acknowledgements at the submission stage.

2.3.1 Randomised control clinical trials

Randomised control clinical trials should be reported using the guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

2.3.2 Epidemiological observational trials

Submitting authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this should be detailed in the materials and methods section. (www.strobe-statement.org)

2.4 Systematic Reviews

Authors submitting a systematic review should register the protocol in a readily-accessible source at the time of project inception (e.g. PROSPERO database, previously published review protocol in journal). The protocol registration number, name of the database or journal reference should be provided in the 'Acknowledgements' at the submission stage. Systematic review should be reported using the PRISMA guidelines (http://www.prisma-statement.org/). A PRISMA checklist and flow diagram (as a Figure) should also be included in the submission material.

2.5 DNA Sequences and Crystallographic Structure Determinations

Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

2.6 Conflict of Interest and Source of Funding

International Endodontic Journal requires that all authors (both the corresponding author and co-authors) disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If authors are unsure whether a past or present affiliation or relationship should be disclosed in the manuscript, please contact the editorial office at iejeditor@cardiff.ac.uk. The existence of a conflict of interest does not preclude publication in this journal.

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Conflict of Interest Disclosure Form

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3. MANUSCRIPT SUBMISSION PROCEDURE

Manuscripts should be submitted electronically via the online submission site http://mc.manuscriptcentral.com/iej. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequentially speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete instructions for submitting a paper is available online and below. Further assistance can be obtained from iejeditor@cardiff.ac.uk.

3.1. Getting Started

• Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's online Submission Site: http://mc.manuscriptcentral.com/iej

• Log-in, or if you are a new user, click on 'register here'.

• If you are registering as a new user.

- After clicking on 'register here', enter your name and e-mail information and click 'Next'. Your e-mail information is very important.

- Enter your institution and address information as appropriate, and then click 'Next.'

- Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your areas of expertise. Click 'Finish'.

• If you are registered, but have forgotten your log in details, please enter your e-mail address under 'Password Help'. The system will send you an automatic user ID and a new temporary password.

• Log-in and select 'Author Centre '

3.2. Submitting Your Manuscript

• After you have logged into your 'Author Centre', submit your manuscript by clicking on the submission link under 'Author Resources'.

• Enter data and answer questions as appropriate. You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter.

• Click the 'Next' button on each screen to save your work and advance to the next screen.

• You are required to upload your files.

- Click on the 'Browse' button and locate the file on your computer.

- Select the designation of each file in the drop down next to the Browse button.

- When you have selected all files you wish to upload, click the 'Upload Files' button.

• Review your submission (in HTML and PDF format) before completing your submission by sending it to the Journal. Click the 'Submit' button when you are finished reviewing.

3.3. Manuscript Files Accepted

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing. The files will be automatically converted to HTML and PDF on upload and will be used for the review process. The text file must contain the abstract, main text, references, tables, and figure legends, but no embedded figures or Title page. The Title page should be uploaded as a separate file. In the main text, please reference figures as for instance 'Figure 1', 'Figure 2' etc to match the tag name you choose for the individual figure files uploaded. Manuscripts should be formatted as described in the Author Guidelines below.

3.4. Blinded Review

Manuscript that do not conform to the general aims and scope of the journal will be returned immediately without review. All other manuscripts will be reviewed by experts in the field (generally two referees). International Endodontic Journal aims to forward referees´ comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for fast-track publication under special circumstances after consultation with the Editor.

International Endodontic Journal uses double blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper and the name(s) of the author(s) will not be disclosed to the reviewers.

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Please upload:

• Your manuscript without title page under the file designation 'main document'

• Figure files under the file designation 'figures'

• The title page and Acknowledgements where applicable, should be uploaded under the file designation 'title page'

All documents uploaded under the file designation 'title page' will not be viewable in the html and pdf format you are asked to review in the end of the submission process. The files viewable in the html and pdf format are the files available to the reviewer in the review process.

3.5. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.6. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

3.7. Manuscript Status

You can access ScholarOne Manuscripts any time to check your 'Author Centre' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.8. Submission of Revised Manuscripts

To submit a revised manuscript, locate your manuscript under 'Manuscripts with Decisions' and click on 'Submit a Revision'. Please remember to delete any old files uploaded when you upload your revised manuscript.

4. MANUSCRIPT TYPES ACCEPTED

Original Scientific Articles: must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original Scientific Articles must conform to the highest international standards in the field.

Review Articles: are accepted for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should generally include a clearly defined search strategy and take a broad view of the field rather than merely summarizing the authors´ own previous work. Extensive or unbalanced citation of the authors´ own publications is discouraged.

Clinical Articles: are suited to describe significant improvements in clinical practice such as the report of a novel technique, a breakthrough in technology or practical approaches to recognised clinical challenges. They should conform to the highest scientific and clinical practice standards.

Case Reports: illustrating unusual and clinically relevant observations are acceptable but they must be of sufficiently high quality to be considered worthy of publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered. Illustrative material must be of the highest quality and healing outcomes, if appropriate, should be demonstrated.

Supporting Information: International Endodontic Journal encourages submission of adjuncts to printed papers via the supporting information website (see submission of supporting information below). It is encouraged that authors wishing to describe novel procedures or illustrate cases more fully with figures and/or video may wish to utilise this facility.

Letters to the Editor: are also acceptable.

Meeting Reports: are also acceptable.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Format

Language: The language of publication is English. It is preferred that manuscript is professionally edited. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

Presentation: Authors should pay special attention to the presentation of their research findings or clinical reports so that they may be communicated clearly. Technical jargon should be avoided as much as possible and clearly explained where its use is unavoidable. Abbreviations should also be kept to a minimum, particularly those that are not standard. The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Titles and abstracts especially should be written in language that will be readily intelligible to any scientist.

Abbreviations: International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

5.2. Structure

All manuscripts submitted to International Endodontic Journal should include Title Page, Abstract, Main Text, References and Acknowledgements, Tables, Figures and Figure Legends as appropriate

Title Page: The title page should bear: (i) Title, which should be concise as well as descriptive; (ii) Initial(s) and last (family) name of each author; (iii) Name and address of department, hospital or institution to which work should be attributed; (iv) Running title (no more than 30 letters and spaces); (v) No more than six keywords (in alphabetical order); (vi) Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence.

Abstract for Original Scientific Articles should be no more than 350 words giving details of what was done using the following structure:

• Aim: Give a clear statement of the main aim of the study and the main hypothesis tested, if any.

• Methodology: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.

• Results: Give the main results of the study, including the outcome of any statistical analysis.

• Conclusions: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Systematic Review Articles should be no more than 350 words giving details of what was done using the following structure where applicable:

• Background: Provide a brief introduction of the subject and why it is important.

• Aim: Give a clear statement of the main aim of the study and the main hypothesis tested, if any.

• Data sources: Describe the databases searched.

• Study eligibility criteria, participants, and interventions: Briefly describe the methods adopted including exclusion/inclusion criteria.

• Study appraisal and synthesis methods: Describe bias, study type and quality

• Results: Give the main results of the review, including the outcome of any statistical meta-analysis.

• Limitations: Highlight problems with the current review end research area

• Conclusions and implications of key findings: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Review Articles (narrative)

The Abstract should be unstructured and no more than 350 words.

Abstract for Case Reports should be no more than 350 words using the following structure:

• Aim: Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• Summary: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.

• Key learning points: Provide up to 5 short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

Abstract for Clinical Articles should be no more than 350 words using the following structure:

• Aim: Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• Methodology: Describe the methods adopted.

• Results: Give the main results of the study.

• Conclusions: State the primary conclusions of the study.

Main Text of Original Scientific Article should include Introduction, Materials and Methods, Results, Discussion and Conclusion

Introduction: should be focused, outlining the historical or logical origins of the study and gaps in knowledge. Exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation, or hypothesis to be tested.

Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.

(i) Clinical Trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

(ii) Experimental Subjects: experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each

subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

(iii) Suppliers: Suppliers of materials should be named and their location (Company, town/city, state, country) included.

Results: should present the observations with minimal reference to earlier literature or to possible interpretations. Data should not be duplicated in Tables and Figures.

Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.

Conclusion: should contain a summary of the findings.

Main Text of Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

Main Text of Clinical Reports and Clinical Articles should be divided into Introduction, Report, Discussion and Conclusion,. They should be well illustrated with clinical images, radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality

Acknowledgements: International Endodontic Journal requires that all sources of institutional, private and corporate financial support for the work within the manuscript

must be fully acknowledged, and any potential conflicts of interest noted. Grant or contribution numbers may be acknowledged, and principal grant holders should be listed. Acknowledgments should be brief and should not include thanks to anonymous referees and editors. See also above under Ethical Guidelines.

5.3. References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. The EndNote reference style can be obtained upon request to the editorial office (iejeditor@cardiff.ac.uk). Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

In the text: single or double authors should be acknowledged together with the year of publication, e.g. (Pitt Ford & Roberts 1990). If more than two authors the first author followed by et al. is sufficient, e.g. (Tobias et al. 1991). If more than 1 paper is cited the references should be in year order and separated by "," e.g. (Pitt Ford & Roberts 1990, Tobias et al. 1991).

Reference list: All references should be brought together at the end of the paper in alphabetical order and should be in the following form.

(i) Names and initials of up to six authors. When there are seven or more, list the first three and add et al.

(ii)Year of publication in parentheses

(iii) Full title of paper followed by a full stop (.)

(iv) Title of journal in full (in italics)

(v) Volume number (bold) followed by a comma (,)

(vi) First and last pages

Examples of correct forms of reference follow:

Standard journal article

Bergenholtz G, Nagaoka S, Jontell M (1991) Class II antigen-expressing cells in experimentally induced pulpitis. International Endodontic Journal 24, 8-14.

Corporate author

British Endodontic Society (1983) Guidelines for root canal treatment. International Endodontic Journal 16, 192-5.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). Blood 54 (Suppl. 1), 26a.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) Surgical Endodontics, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. Endodontics in Clinical Practice, 3rd edn; pp. 186-223. London, UK: Butterworth.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.

Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html

[accessed on 7 November 2003]

5.4. Tables, Figures and Figure Legends

Tables: Tables should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.

Figures: All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm), and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction, symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final reproduction size. In general, multi-part figures should be arranged as they would appear in the final version. Reduction to the scale that will be used on the page is not necessary, but any special requirements (such as the separation distance of stereo pairs) should be clearly specified.

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size.

Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by a thin space (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

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the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible). For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

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7. Guidelines for reporting of DNA microarray data

The International Endodontic Journal gives authors notice that, with effect from 1st January 2011, submission to the International Endodontic Journal requires the reporting of microarray data to conform to the MIAME guidelines. After this date, submissions will be assessed according to MIAME standards. The complete current guidelines are available at http://www.mged.org/Workgroups/MIAME/miame_2.0.html. Also, manuscripts will be published only after the complete data has been submitted into the public repositories, such as GEO (http://www.ncbi.nlm.nih.gov/geo/) or ArrayExpress (http://www.ebi.ac.uk/microarray/submissions_overview.html), in MIAME compliant format, with the data accession number (the identification number of the data set in the database) quoted in the manuscript. Both databases are committed to keeping the data private until the associated manuscript is published, if requested.

Anexo 2 – Comitê de Ética em Pesquisa em Humanos

UNESP - FACULDADE DE ODONTOLOGIA-CAMPUS DE ARAÇATUBA/ UNIVERSIDADE

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Perfil proteômico das infecções endodônticas
Pesquisador: Rogério de Castilho Jacinto
Área Temática:
Versão: 1
CAAE: 91331518.7.0000.5420
Instituição Proponente: Faculdade de Odontologia do Campus de Araçatuba - UNESP
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.745.028

Apresentação do Projeto:
Desenho:
Serão coletadas amostras de 18 pacientes encaminhados para tratamento de canal radicular ou tratamento de emergência na Faculdade de
Odontologia de Araçatuba (FOA – UNESP). A seleção dos pacientes será determinada pelo histórico odontológico, bem como pelo exame clínico e
radiográfico. Todos pacientes que concordarem em participar da pesquisa assinarão o Termo de Consentimento Livre e Esclarecido. As seguintes características serão observadas para cada paciente: idade, gênero, estado dentário e pulpar, natureza da dor, história de
dor prévia, dor espontânea sensibilidade à percussão, dor à palpação, mobilidade, presença de fístula e sua origem, presença de inchaço dos
tecidos periodontais, e profundidade da bolsa periodontal. Após o diagnóstico baseado nos critérios clínicos e radiográficos acima os pacientes
serão divididos em dois grupos: dentes com periodontite apical sintomática (n= 8) e dentes com periodontite apical assintomática (n= 8). Serão
incluídos pacientes de 18 a 60 anos de idade, de ambos os sexos. Serão incluídos apenas dentes com infecção endodôntica primária e presença de
lesão periapical visível na radiografia. Serão excluídos do estudo pacientes que receberam antibioticoterapia nos últimos 3 meses, com patologia

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associada à doença periodontal, pacientes com dentes cuja coroa esteja destruída impedindo o isolamento absoluto, dentes cujo canal radicular
esteja exposto na cavidade oral, dentes com canais atrésicos que não permitam a coleta de material. Também serão excluídos pacientes com sinais
de disseminação da infecção endodôntica como febre, linfadenopatia, trismo e mal-estar geral.

Objetivo da Pesquisa:
Objetivo Primário:
Analisar o perfil proteômico das infecções endodônticas de pacientes normais e portadores de doenças sistêmicas.
Objetivo Secundário:
Ampliar o entendimento do papel do perfil proteômico na patogênese das doenças periapicais utilizando cromatografia líquida associada à
espectrometria de massas e investigar a correlação da composição das proteínas bacterianas detectadas nos canais radiculares em diferentes tipos
de infecções endodônticas.

Avaliação dos Riscos e Benefícios:
Riscos:
A participação nesta pesquisa não infringe as normas legais e éticas. O risco é mínimo, a participação nesta pesquisa não infringe as normas legais
e ética (A coleta da amostra é feita sob efeito de anestesia local). Os procedimentos adotados nesta pesquisa obedecem aos Critérios da Ética em
Pesquisa com Seres Humanos e nenhum dos procedimentos usados oferece riscos à sua dignidade.
Benefícios:
Ao participar desta pesquisa o(a) sr.(a) não terá nenhum benefício direto. Entretanto, esperamos que o conhecimento construído a partir desta
pesquisa possa tornar o tratamento endodôntico mais efetivo, onde pesquisador se compromete a divulgar os resultados obtidos, respeitando-se o
sigilo das informações coletadas

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Comentários e Considerações sobre a Pesquisa:

Pesquisa apresenta-se apta para a sua realização.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos foram apresentados de acorco com a resolução 466/12 do CNS.

Recomendações:

não há.

Conclusões ou Pendências e Lista de Inadequações:

Pesquisa apresenta-se apta para a sua realização.

Considerações Finais a critério do CEP:

Salientamos que, de acordo com a Resolução 466 CNS, de 12/12/2012 (título X, seção X.1., art. 3, item b, e, título XI, seção XI.2., item d), há necessidade de apresentação de relatórios semestrais, devendo o primeiro relatório ser enviado até 01/01/2019.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1152596.pdf	07/06/2018 17:42:09		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	07/06/2018 17:41:02	CAROLINE LOUREIRO	Aceito
Cronograma	Cronograma.docx	07/06/2018 17:40:34	CAROLINE LOUREIRO	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Proteoma_Comite.docx	07/06/2018 15:43:28	CAROLINE LOUREIRO	Aceito
Folha de Rosto	folhaderosto.pdf	07/06/2018 15:40:39	CAROLINE LOUREIRO	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

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