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Temas Libres 1

Activation of pSTAT3 on and CD45^{+} cells in Human Gingival Tissues

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Objectives Periodontitis is a chronic inflammatory disease in which the locally deployed immune response against a dysbiotic subgingival microbiome results in alveolar bone resorption and tooth loss. The transcription factor signal transducer and activator of transcription 3 (STAT3) integrates and transduces the signaling of multiple pro-inflammatory cytokines associated with periodontitis. STAT3 is also crucial for Th17 cell differentiation, a critical CD4^{+} T cell subset in periodontitis immunopathogenesis. Despite the importance of this protein, there is a gap in our knowledge regarding which cell type activates STAT3 (pSAT3) in human periodontal tissues. Hence, this study aimed to evaluate the STAT3 activation on hematopoietic (CD45^{+}) cells in gingival tissues from healthy and periodontitis subjects.
Methods After clinical evaluation, diagnosis, selection, and consent, a standardized gingival tissue sample was obtained from each volunteer. Gingival tissues were fixed, included in paraffin, and activation of STAT3 and immune cells was evaluated via immunofluorescence using an anti-pSTAT3 and anti-CD45 antibody. Immuno-positive cells were counted in 20 tissues (10 healthy and 10 periodontitis) using the Image J software. Data are shown in mean ± SEM. P-values of <0.05 were considered statistically significant.
Results Total percentages of pSTAT3 and CD45 immune-positive cells were significantly higher in the periodontitis tissues than compared with the healthy gingival tissues (54,1± 28,4% pSTAT3 and 59,3 ± 36,9%CD45). pSTAT3 was highly detected in the epithelium of periodontitis tissues and distributed within the basal layer cells.
Conclusions Immune cells and STAT3 transcription factor were more activated in gingival tissues obtained from subjects with periodontitis than healthy. Furthermore, STAT3 activation was higher and more widely distributed in basal layer cells of epithelium.

Characterization of Cx43 and Epithelial-Mesenchymal Transition Proteins in Oral Cancer

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Objectives The epithelial-mesenchymal transition (EMT) is a process of transcriptional reprogramming of epithelial cells with a crucial role in early events, such as oncogenesis and invasion, and late events, such as migration and metastasis. The hallmark is the alteration of the expression of adhesion molecules (downregulation of E-cadherin and upregulation of N-cadherin), which is regulated by complex signaling pathways, not entirely known, and understood. Cx43 is a gap junction protein, responsible for the communication of neighboring cells, but its role in EMT of oral cancer is poorly understood.
For this reason, we studied the immunohistochemical expression of the Cx43 in paraffin-embedded primary and metastatic tumors. Posteriorly, the Cx43, E-cadherin, and N-cadherin expression was measured in normal or keratinocytes (NOK) and cancer cell lines (SCC9 and SCC25).
Methods Five non-metastatic primary tumors, five metastatic primary tumors, and their lymph nodes were stained for fluorescent immunohistochemistry. DAPI was used for nuclei and CD45 for lymphocytes.
After this, cell lines from primary tongue cancer (SCC9 and SCC25) and one primary cell line from normal oral keratinocytes (NOK) were studied. The expression of Cx43, E-cadherin, and N-cadherin was measured by western blot and immunocytochemistry.
Results The expression of Cx43 was higher in non-metastatic primary tumors when compared with metastatic primary tumors. Surprisingly, a well-defined net of Cx43 was evidenced in lymph nodes.
In cell lines, a correlation in the expression of Cx43 and E-cadherin in NOK was observed, but disorganization of connexin expression in cancer cell lines is associated with the upregulation of N-cadherin. In these cells, a previously not reported expression of Cx43 in the nuclei was observed.
Conclusions These findings demonstrate that the E-Cadherin and N-Cadherin switch observed in the EMT of oral cancer cells also affects the expression of Cx43. Based on the results, we suggest that a probable translocation of Cx43 to the nucleus can play a role in the regulation of the adhesion molecules switch in the EMT of oral squamous cell carcinoma.

ACE2 Expression And Immunity Development In Saliva Of COVID-19 Subjects

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Objectives To provide a more comprehensive understanding of the COVID-19 infection on the oral cavity and their possible involvement in the transmission of SARS-CoV-2 by the study of the salivary expression of angiotensin-converting enzyme 2 (ACE2) and the antibodies IgG and IgA specific for SARS-CoV-2 in positive COVID-19 subjects and their inhouse close contacts.
Methods There was performed a prospective cohort study with 114 primary cases diagnosed by nasopharyngeal swabs and their inhouse close contacts (n=239). All subjects were visited at their

homes, underwent an anamnesis, symptoms questionnaire and periodontal condition self-report. Unstimulated saliva samples from primary cases and their inhouse close contacts (symptomatic and asymptomatic) at 7, 14, and 45-60-days post-infection were collected and the expression of (ACE2) and antibodies (IgG and IgA) expression in COVID-19 (+) patients was determined by ELISA assays. Fisher exact and T Student tests were used to evaluating the association among variables.
Results 353 subjects were recruited, 228 were positives, and 122 were negatives for SARS-CoV-2 infection. In those positive for COVID-19, 54.6% express ACE2, a 98.2% IgA and a 47.3% IgG in saliva samples. A positive association between saliva concentration of ACE2 (p=0,0176) and symptoms quantity (p=0.05) was observed. The positive contact presented a higher salivary concentration of ACE2 (p-value=0.0345). Also, ACE2 was related to self-reported periodontal disease (p=0.00486), bleeding gums (p=0.0397), and tooth mobility (p=0.0185). IgA was related with the viral load in saliva (p=0.0006) and symptomatic cases have higher expression of IgA compared to asymptomatic subjects (p=0.0185). Also, positives overweight subjects have a lower expression of IgG (p=0.03).
Conclusions The oral cavity and saliva is involved in the transmission of COVID-19, and the present results confirm the expression of SARS-CoV-2 receptor ACE2 and the development of specific antibodies against SARS-CoV-2 in saliva samples.

The evaluation of buccal drug delivery route for analgesics using microneedle array geometries compared by finite element method.

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Objectives The transdermal drug delivery systems, microneedles (MN), have played an important role in recent medical and pharmaceutical applications, presenting themselves as a painless, efficient, and easy-to-apply alternative in the administration of drugs. This MN consists of matrix systems of needles on a micro-scale, which are lodged in the dermis or epidermis. The present work evaluated four different geometries presentations of MNs for buccal local anesthetics delivery.
Methods The MNs were compared to hollow MNs, keeping all the required mechanical parameters for height (600 µm), basal diameter (300 µm), and dose release for each channel (90 µm). The analysis was validated through numerical simulations through finite elements methods (FEM), both the total deformations, Von Mises stresses, and dynamics of fluids (CFD) using ANSYS software (ANSYS 2021 R1, Maule, Chile).
Results The strengths simulated range force application of 2mN to 30mN. The behavior of local drug delivery of anesthetic agents was established for MNs systems, and efforts were considered for the multilayer system of the buccal mucosa. Within the simulations, a maximum deformation of 43 µm and the stresses of 70 MPa were found.
Conclusions

This new MN consists of a matrix system of needles with acceptable mechanical valuables as a new and improved drug delivery method in dentistry.

TLR9 gene-promoter's hypomethylation regulates its active transcription in periapical inflammation

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Objectives To assess the methylation pattern of TLR9 at the body gene and promoter region, and its association with its transcriptional regulation in periapical inflammation
Methods Cross-sectional study. Apical tissues were obtained from apical lesions of endodontic origin (ALEOs) (n=61) and healthy periodontal ligament (HPL) (n=15) from volunteers having indication of tooth extraction. All samples were homogenized, and both total RNA and DNA were extracted. Analysis of TLR9 gene revealed CpG sites within the gene promoter; and three CpG islands on its exon 2 within the gene body. DNA was bisulfited, amplified by PCR using a validated primer sets and their products were sequenced. The results were analyzed by BiQanalyzer. TLR9 mRNA levels were quantified by qPCR
Results TLR9 expression levels were higher in ALEOs compared to healthy controls ($p<0.05$). Global methylation % of the TLR9 gene promoter and body-island 1 were lower in ALEOs compared to HPL ($p<0.05$), while body-islands 2 and 3 tended to be totally methylated independently of the periapical status ($p>0.05$). Only the promoter's methylation % was confirmed to influence gene expression ($p<0.05$). Analysis of CpG single-sites showed that positions -805, -736, -683, -546, -488, -481, -389, -105, -78, -35 and +2086 were hypomethylated in ALEOs compared to HPL ($p<0.05$), whereas a higher expression of TLR9 was associated with unmethylated -736 and -683 CpG single-sites within the TLR9 gene promoter in apical tissues ($p<0.05$). The TLR9 gene expression influence the methylation status of -736 and -683 CpG sites from TLR9 gene promoter in apical tissues, after adjusting by smoking and gender ($p<0.05$)
Conclusions CpG island 1 from exon 2 and CpG sites within the promoter of the TLR9 gene were hypomethylated in ALEOs compared to HPL. TLR9 gene promoter hypomethylation, specifically at CpG single-sites near CRE-binding, were involved in higher active transcription of TLR9 in ALEOs

CD73-bearing Treg-derived extracellular vesicles modulate *in vitro* and *in vivo* immune response and prevent alveolar bone loss during periodontitis

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Objectives The CD73 ectoenzyme promotes immune regulation through the conversion of adenosine-mono phosphate (AMP) into adenosine, which after interaction with its receptors (A2aR and A2bR expressed on immune cells) upholds anti-inflammatory responses. T regulatory cells (Treg)-derived extracellular vesicles (TEVs) have been shown to be loaded with CD73, contributing to their immunomodulatory capacity in a cell-free manner. Periodontitis, a chronic inflammatory disease, develops a deregulated host immune response, where the formation of a inflammatory infiltrate and accumulation of bone pro-resorptive factors result in tooth-supporting tissue breakdown and alveolar bone loss. This study aimed to evaluate the effect of CD73-bearing TEVs on T-cell proliferation, activation, and phenotype in vitro and on periodontitis-induced immune response and alveolar bone resorption.
Methods Magnetically isolated murine $CD4^+$ T cells were differentiated into Tregs and characterized by flow cytometry (FC). From their recovered supernatant, TEVs were isolated by differential centrifugation then, quantified and characterized by Nanoparticle-Tracking-Analysis and Western Blot. The TEV immunosuppressive and modulatory capacities were evaluated *in vitro* on $CD4^+$ and $CD8^+$ T cells studying their effect on proliferation, activation, and phenotype. Besides, using a ligature-induced periodontitis mice model, the TEVs effect on periodontal immune response was assessed quantifying leukocyte infiltration by FC and the extent of alveolar bone loss by morphometric analysis.
Results Tregs showed high expression of lineage markers Foxp3, CD25, and CD73. Isolated TEVs expressed CD73, displayed canonical EVs characteristics, downregulated the $CD4^+$ and upregulated the $CD8^+$ T cell proliferation and activation. During periodontitis, TEVs increased the frequency of $CD73^+$ leukocytes, promoted anti-inflammatory response, and prevented alveolar bone loss.
Conclusions CD73-bearing TEVs modulate $CD4^+$ and $CD8^+$ T cell response, impacts on local immune response and prevent peridontitis-induced alveolar bone loss.

Temas Libres 2

Bioethical Reports of Animal Studies published in Dental Journals.

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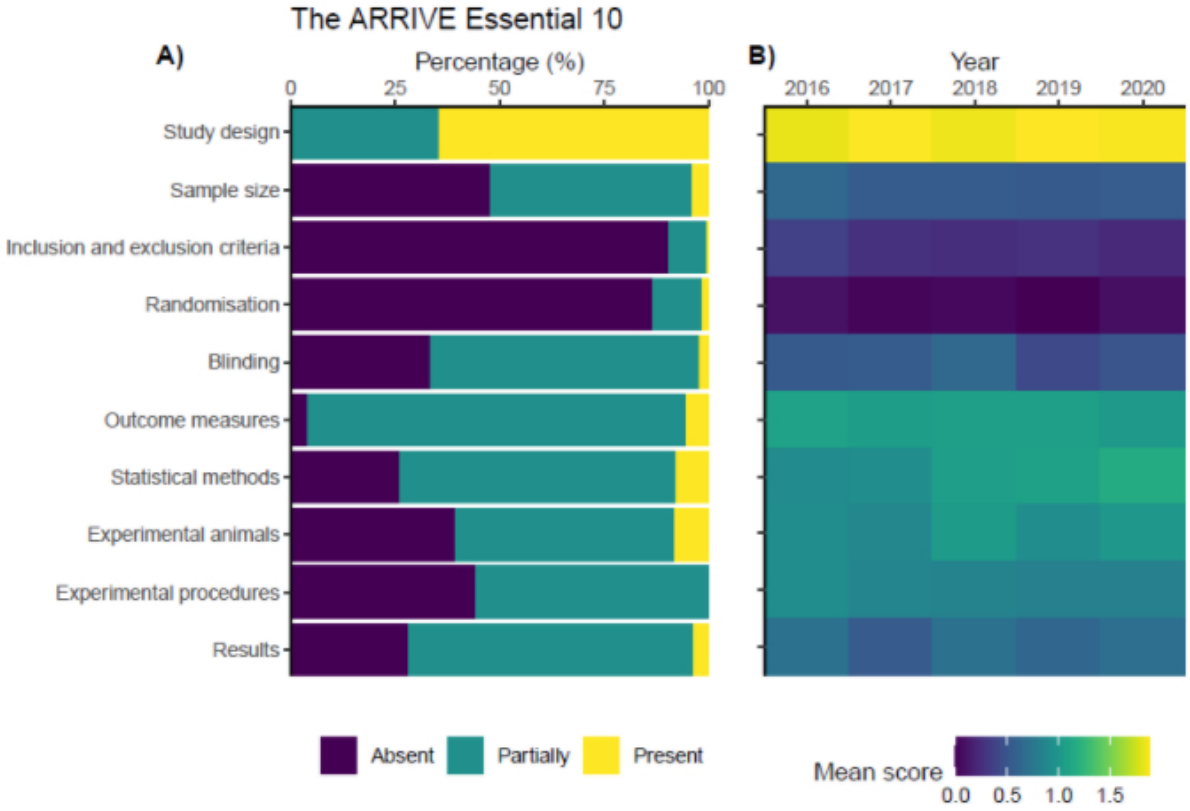
Objectives Experimental animal models are widely used in dentistry. The ARRIVE guidelines are relevant to any study involving live animals. In Dentistry, there is no evidence available on the reporting of criteria and bioethical aspects with animals. This study aimed to determine the quality of bioethical reports in animal studies published in the dental journals with the highest impact index in 2016-2020 using the ARRIVE 2020 guideline.
Methods We searched the five dental journals with the highest impact index in the Web of Science database (table 1). Inclusion and exclusion criteria were used for the selection of articles. The screening was carried out independently by four authors (P.R., C.V., N.M., and D.H.). Disagreements were resolved by discussion, and the level of agreement was by saturation calibration. To categorize each guideline of ARRIVE 2020: three categories were assigned: 0: does not comply, 1: partially complies, and 2: fully complies. For data management and analysis, we use RStudio 4.0.5. Additionally, averages, dispersion measures, and frequencies were calculated.
Results 490 articles included, 75% and 60% partially report the description of results and experimental procedures, respectively. The items with the lowest compliance describe the size and selection of the sample of the animal model, inclusion-exclusion criteria, randomization, care, housing upbringing, and access to data (graphs 1 and 2).
Conclusions In the analyzed journals, partial compliance was observed; this indicates that there is a margin to improve the report to the extent that they adhere to the ARRIVE guidelines in a complete way, improving the quality and reproducibility of the studies, as well as the translation of the basic science to applied science improving future treatments.



A. Categorization "Essential 10" items ARRIVE guideline 2020.
B. Categorization "Essential 10" items ARRIVE guideline 2020 between the years 2016 - 2020.

C. Categorization "Recommended Set" items ARRIVE guideline 2020.
D. Categorization "Recommended Set" items ARRIVE guideline 2020 between the years 2016 and 2020.

Table 1. Journals with the highest impact index in the Web of Science database year 2020.

Rank	Full title of the journal	Impact of the journal year 2020
1	Periodontology 2000	7.718
2	Journal Of Clinical Periodontology	5.241
3	Journal Of Dental Research	4.914
4	Dental Materials	4.495
5	Oral Oncology	3.979

6	International Endodontic Journal	3.801

Corticosteroids for third-molar extractions: An evidence map using LOVE platform

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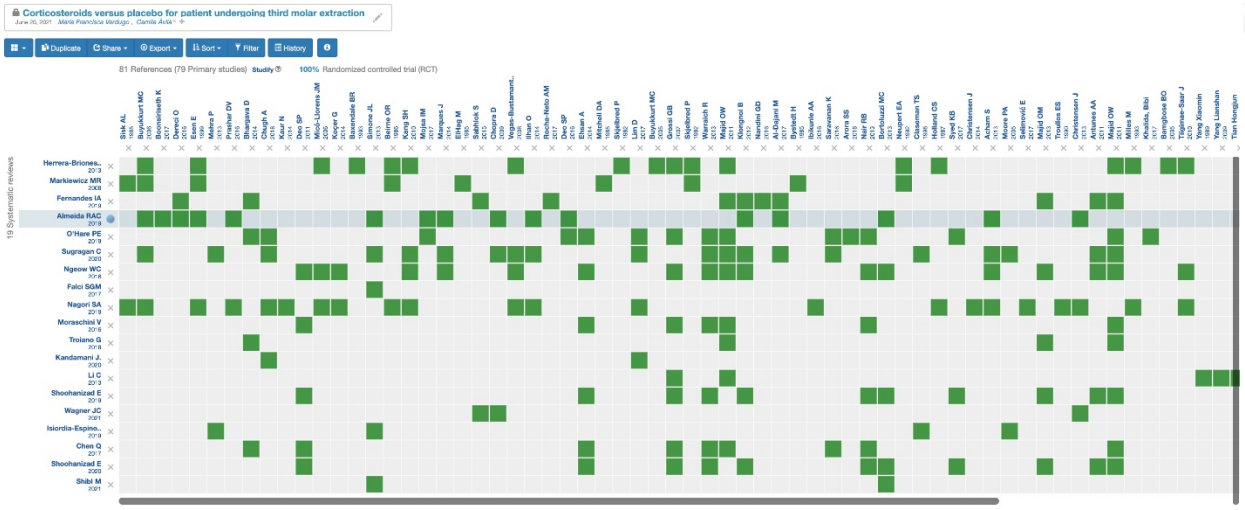
Objectives Formulating evidence-based recommendations in oral health is challenging. Identifying and synthesizing the evidence to inform these recommendations has become tantalizingly difficult. Answering simple questions such as; should we use corticosteroids for managing postoperative pain when removing third molars, requires an enormous effort.
We aimed to map the evidence available of this question, to illustrate the overlap and shortcomings of the evidence syntheses in this area.
Methods
We conducted systematic searches in the LOVE (Living OVerview of Evidence) platform for Third Molar, a system that maps PICO questions to a repository maintained through regular searches. Our main search source for systematic reviews (SR) was Epistemonikos database. An additional search was performed on PubMed in order to identify RCTs not included in reviews. We included SR of corticosteroids (injected, v.o.,or any other measure of administration) versus no treatment or placebo for patients undergoing third molar extractions. Two reviewers independently evaluated potentially eligible studies. Searches had no language restrictions and covered the period until July, 2021.
We built a matrix of evidence using Epistemonikos to compare the studies included in the reviews. A matrix of evidence is a table displaying all the RSs answering a question, and all the studies answering the question of interest included in these reviews.
Results We identified 20 systematic reviews including 79 RCTs overall answering the question of interest. The number of primary studies identified by each particular review ranged from 1 to 25. Our search in LOVE found 28 RCTs eligible that were not identified by any of the reviews.
Conclusions None of the SRs included the totality of RCTs, which may be partly explained by serious limitations on the quality of the reviews and the search date. This also highlights how fast published reviews become obsolete if they are not continuously updated.
SRs are considered the gold standard to make sense of multiple trials addressing a similar scientific question, but the traditional model for conducting reviews has several limitations so alternative models are needed.



Oral Health Analysis Among Patients With And Without Movement Disorders

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Objectives - Compare the presence of temporomandibular disorders in patients.
with and without movement disorders.
- Compare the state of oral health among patients with disorders of the
movement and patients without movement disorders on examination
intraoral hard and soft tissue
Methods A case and control study was carried out, with a quantitative and comparative approach, oral health exams were performed in patients with and without movement disorders, applying surveys, clinical guidelines, to determine the state of oral health and the significant differences between groups.
Results The results obtained showed a minimal significance between the two groups, however it could be observed that there is a greater deterioration of oral health in patients with movement disorders, showing a greater difference in COPD.
Conclusions In conclusion, there is a greater deterioration of oral health in patients with movement disorders, but the significance is minimal, so it can be concluded that this condition is not decisive.
There is a greater difference in the measurement points of COPD and xerostomia

Inflammatory and TLR2-Methylation Profiles of PBMCs in Apical Periodontitis

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Objectives To determine the inflammatory profile, TLR2 gene methylation patterns, and expression in peripheral mononuclear blood cells (PBMCs) from individuals with apical periodontitis (AP) and controls.
Methods Cross-sectional study. Otherwise healthy individuals with AP (n=27) and controls (n=30) who consulted at the Faculty of Dentistry, Universidad de Chile were included. PMBCs were isolated by Ficoll gradient, cultured for 24 hours, and both RNA and DNA were extracted. DNA was bisulfite-treated, and specific sites at the promoter region of the TLR2 gene were amplified by PCR and sequenced. mRNA expression of TLR2 was determined by qPCR. The levels of TNF-α, IL-6, IL-10, IL-6Rα, IL-1β and IL-12p70 were measured in the supernatants by Multiplex assay. The results were analyzed with the software STATA V.12 ($p<0.05$).
Results PBMCs demonstrated a proinflammatory profile showing higher soluble levels of TNF-α, IL-6 and IL-1β in AP compared to controls ($p<0.05$). Higher TLR2 expression was also found in AP compared to controls ($p<0.05$), although the global methylation pattern of the promoter region of the gene showed no differences ($p>0.05$). The CpG individual sites at positions -69, -16 and -12 were 100% demethylated in controls, being significantly different compared to AP ($p<0.05$). However, only the unmethylated state of CpG -16 and -12 sites was associated with increased TLR2 mRNA expression in both groups ($p<0.05$).
Conclusions PBMCs from AP individuals demonstrate higher cytokine and TLR2 expression in association with the methylation state of the gene promoter's single sites. Consequently, PBMCs contribute to the sustained systemic inflammatory load in individuals with periapical endodontic diseases.

Molecular And Nanomechanical Characterization Of Honey-derived Exosomes Against *Streptococcus mutans*

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Objectives Recently, it has been shown that honey contains exosome-like extracellular vesicles (EVs) with strong antibacterial and antibiofilm effects against gram positive bacteria. The aim of our study was to characterize molecular and nanomechanical properties of EVs derived from *A. mellifera* honey (HEc-EVs) to get an idea of the possible mechanism of action of these EVs on

gram positive bacterial strains.
Methods *A. mellifera* honey-derived EVs from *Eucryphia cordifolia* (HEc-EVs) were isolated by ultracentrifugation and molecularly characterized with Western Blot and ELISA. To explore the nanomechanical and ultrastructural properties of HEc-EVs, atomic force microscopy (AFM) was employed.
Results The characterization at the molecular level of the HEc-EVs allowed identification of the exosomal markers CD63 and syntenin. In addition, the antibacterial molecules MRJP1, defensin-1 and jellein-3 were found as intra-vesicular cargo. Nanomechanical results revealed that EVs were mostly <150nm, and low elastic modulus values comparable to EVs from other biological sources. AFM nanocharacterization showed alterations consistent with membrane damage on *S. mutans* when treated with EVs.
Conclusions This is the first study reporting characterization of HEc-EVs exosomal markers and antibacterial peptides as cargo molecules. AFM nanocharacterization shows structural bacterial membrane alterations that explain potential mechanisms for bacterial viability inhibition when treated with HEc-EVs.

CPP-ACP in Milk, Chewing-Gum, and Candies in Dental Caries: Systematic-Review

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Objectives Casein is one of the most studied caries-related proteins, with promising remineralizing effects when used in the form of casein phosphopeptide--amorphous calcium phosphate-nanocomplexes (CPP-ACP). However, there is little evidence on the anticaries potential of CPP-ACP when added to foods, such as milk or candies. Hence, this study aimed to review the available evidence on CPP-ACP added to milk, chewing-gums or candies, and determine its effect on caries.
Methods A systematic review of the literature was conducted by searching three databases (Medline via PubMed, SCOPUS and Web of Science). A predefined searching strategy was developed according to a PICO-question framework. No limits in publication year or language were applied. Study screening, selection and data extraction were carried out independently by two researchers. Eligible in vivo or in situ studies were included.
Results Out of 210 titles found, 23 were selected for full-text review. From those, 16 studies were included (14 in situ, 2 in vivo). Outcomes included enamel remineralization and biofilm activity. Ninety two percent of the studies show enamel remineralizing potential from CPP-ACP. At the biofilm level, CPP-ACP showed an ability to retain within the biofilm and release calcium and phosphate, increasing the proportion of alkali-producing bacteria with an antibacterial activity. The overall quality of the evidence was judged as moderate.
Conclusions The available evidence suggests that CPP-ACP added to milk, chewing-gums or candies has a remineralizing potential in enamel, with biofilm controlling activity. Additional clinical studies are needed to confirm a clinical effect (PROSPERO CRD42020215024).

Inflammatory Phenotype of Syncytiotrophoblasts Stimulated with Periodontal Bacteria and Hyperglycemia

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Objectives To assess a protocol for syncyalization of a BeWo cells.
To assess BeWo-SYN mRNA expression of TNF-α, IL-1β, TLR-2, TLR-4 and TLR-9 upon inactivated *P. gingivalis* and *F. nucleatum* in vitro stimulation.
To assess BeWo-SYN secretion of Adiponectin, IL-8, PLGF, CRP, IL-6, Leptin and TNF-α upon inactivated *P. gingivalis* and *F. nucleatum* and hyperglycemia in vitro stimulation.
Methods BeWo cell line was syncytialized by the use of forskolin at different concentrations and time, evaluation of syncytialization was assessed by syncytialization ratio by immunofluorescence and expression of LGALS13 and PLGF mRNA by RT-qPCR. BeWo-SYN were stimulated with hyperglycemia, or heat-inactivated *P.gingivalis* and *F.nucleatum* (MOI 200 each and 100, both) for 15 min, 2, 24 and 48 h. mRNA expression of TNF-α, IL-1β, TLR-2, TLR-4 and TLR-9 quantified through RT-qPCR. Secreted adiponectin, IL-8, PLGF, CRP, IL-6, Leptin and TNF-α in conditioned medium at 24 and 48 hours was quantified by multiplex immunoassay. Comparisons of multiple variables at different time points was performed by ANOVA two-way.
Results Syncytialization ratio of BeWo cells increased with time and concentration of forskolin with statistical differences ($p<0.0001$). LGALS13 and PLGF mRNA expression was higher at 48 h of 50 uM FSK. BeWo-SYN did not express TLR-2 mRNA. *P.gingivalis* MOI200 had a peak of TLR-4 and TLR-9 mRNA expression at 15 min, *P.ginvialis/F.nucleatum* MOI100 of TLR-9 at 2h, *F.nucleatum* MOI200 of TLR-4 and TLR-9 at 48 h. TNF-a mRNA expression was higher in control until 2 h, then it increased in *F.nucleatum* MOI200 at 24 h. Only IL-6 and Leptin were quantifiable inside the curve and leptin concentration had differences between bacterial stimulation groups (p-value=0.0324).
Conclusions BeWo-SYN model of syncytiotrophoblast did not increase pro-inflammatory protein in CM upon bacterial and hyperglycemia stimuli, which is congruent with mRNA expression of pro-inflammatory markers. Bacterial translocation into placenta may provoke a proinflammatory stimuli, but BeWo SYN may have other hormonal responses, like reduced leptin secretion upon P.gingivalis stimulus.

Exploring the Genetic Etiology of Molar Root-Incisor Malformation.

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Objectives Molar root-incisor malformation (MRIM) is a recently described anomaly that presents alterations in the root structure of deciduous second molars, permanent first molars and crown of permanent upper central incisors. Its etiology is undetermined, but it has recently been associated with alteration of the *TCTEX1D2* gene. The objective of this study was to detect a 2.2 Kb deletion of the *TCTEX1D2* gene, which includes the entire exon 3 plus flanking intron sequences, in patients diagnosed with MRIM and healthy control subjects.
Methods In this cross-sectional study, a family composed of a healthy father and mother and a child affected with MRIM were recruited, who signed an informed consent. As controls, anonymized samples of 100 subjects without MRIM were included. Genomic DNA was purified from blood using the QIAamp DNA Blood Midi Kit, according to the manufacturer's instructions. The deletion was identified by Polymerase Chain Reaction (PCR) with specific primers, designed with the Primer3 v4.0 program, which originate a 310 bp amplicon corresponding to exon 3. If there is a deletion, there will be no amplification and if the sequence is normal, the 310 bp fragment is obtained. After determining the optimal alignment conditions of the primers and identity of the amplified fragment, the gDNA samples were subjected to PCR. Visualization of the amplicon was performed by agarose gel electrophoresis, safeview staining, and UV transillumination.
Results Amplification of exon 3 of the TCTEX1D2 gene in 103 samples revealed that both the proband and his parents, and all control samples, presented the 310 bp fragment, corroborating the absence of this mutation. However, the existence of mutations in other regions of the gene cannot be ruled out.
Conclusions The analyzed family does not present the 2.2 Kb deletion mutation that includes exon 3 of the *TCTEX1D2* gene.

Expression of Cdk5/p35 and co-localization with its substrates in murine dental pulp cells

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Objectives Molecular mechanisms involved in dental pain are poorly understood, although there recent evidences that odontoblast cells participate in this process. We earlier demonstrated that Cdk5, a kinase implicate in orofacial pain, is expressed in mouse odontoblast-like cells. Here we evaluate the expression of Cdk5 and its activator p35, and its co-localization with known protein targets of Cdk5 involved in pain in murine dental pulp cells.
Methods By western blot we evaluated protein expression of Cdk5 and p35 together with its phosphorylation targets (P2X2, TRPV1 and TRPA1) in adult rat dental pulps. In addition, by immunofluorescence we evaluated immunolocalization of Cdk5, p35, P2X2, TRPV1 and TRPA1 in primary culture of mouse differentiated dental pulp cells.
Results Cdk5 and p35 are expressed in less amount in rat dental pulp cells compare to rat

trigeminal ganglia. In addition, we found that P2X2, TRPV1 and TRPA1 were expressed in dental pulp tissues and in primary culture of differentiated dental pulp cells.
Conclusions the expression of Cdk5/p35 and co-localization with their phosphorylation targets in murine dental pulp cells suggest a possible role for Cdk5 in odontoblast cells physiology and consequently in dental pain.

Temas Libres 3

Periodontal health and COVID-19 infection: A cohort study

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Objectives There is increasing evidence that many patients with COVID-19 symptoms are related to the oral cavity, but the connection with periodontal health is poorly understood. We aim to provide a more comprehensive understanding of the COVID-19 infection effects on oral health, we performed a questionnaire survey of the periodontal condition and analyzed expression in saliva and SARS-CoV-2 levels in COVID-19 patients and their close contacts.
Methods Unstimulated saliva samples from cases and contacts subjects and oropharyngeal swabs were collected. SARS-CoV-2 nucleic acids in saliva and oropharyngeal swabs were detected by real-time polymerase chain reaction (RT-PCR). Additionally, a questionnaire survey on COVID symptoms, periodontitis auto report was carried on COVID-19 patients and their respective close contacts.
Results 353 patients were recruited into this study; 176 males and 183 females. 228 positive and 122 negative cases, 114 of them correspond to the primary case of the study (32.08%), and 239 are close contacts who live in the same home (67.28%). Cases are significantly younger than close contacts (p-value=0.0400). The age of positive cases was 36.39 years, versus 40.21 years in contacts. When comparing the viral CT among patients <30-years-old and ≥30-years-old (N=39 and 75, respectively), mean CT was 30.97 (CI:29.72-32.23) in younger patients and 30.17 (CI:29.19-31.15) in older patients, although not statistically significant. Regarding periodontal status, CT mean values were 29.84 (CI:28.26-31.42) in patients with periodontal disease and 30.73 (CI:29.84-31.61) in individuals without the disease. In negative patients, 196 had periodontal disease (83.76%), 84 (73.04%) in positive cases. There was a positive association between periodontal disease and positivity ($p<0.05$). 75.7% of cases (87/115) self-reported periodontitis-related problems, 81.9% in contacts (199/243). 18.93% of symptomatic patients report having gum disease versus 6.67% in asymptomatic; 8.44% and 10.08% report having a diagnosis of periodontitis, respectively.
Conclusions To our knowledge, this is the first report on COVID-19 and periodontal health

among contacts and confirmed cases in Chile. Notably, there was an association between periodontal disease and COVID positivity. Moreover, confirmed cases are younger than contacts. T

Periodontitis and Alzheimer's Experimental Models: Neuroinflammation and Cognitive Status Comparison

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Objectives Periodontitis is a chronic non-transmissible disease characterized by a low-grade inflammatory state, caused by the dysbiosis of subgingival microbiota. The low-grade inflammatory state caused by bacteria associates periodontitis with others diseases, such as Alzheimer's disease (AD). Recently, it was demonstrated that the experimental periodontitis induced by *Porphyromonas gingivalis* (*P. gingivalis*) triggers an AD-like pathology in Sprague-Dawley rats. This study aimed to determine if experimental periodontitis and an experimental model of AD display similar levels of neuroinflammation, astrogliosis and cognitive defects.
Methods 30 *Sprague-Dawley* male rats 6 weeks age were divided into the following groups: 1) experimental periodontitis, induced by palatine mucosa inoculation of *P. gingivalis*, 2) experimental AD, induced by bilateral intra-hipocampal inoculation of amyloid β (Aβ) oligomers, 3) periodontitis control, by palatal inoculation of medium without bacteria, 4) AD control, by intra-hippocampal injection of saline solution, and 5) naïve rats. All animals were trained in the OASIS Maze task to test memory and spatial learning. The cytokines and Aβ42 levels and lipoperoxidation in serum and hippocampus were quantified. The Tau and phosphorylated Tau protein, and the morphology and number of astrocytes were observed by immunofluorescence.
Results In both periodontitis and AD models, an increase in pro-inflammatory cytokines, Aβ42 and lipoperoxidation levels were detected compared with controls. Also, a learning and spatial memory decline was detected in both experimental models, compared to their respective controls. Finally, an increase in the Tau/pTau ratio was detected in both experimental conditions compared with controls. No significant differences were found between the experimental models investigated.
Conclusions Periodontitis and AD models have a similar neuroinflammation, astrogliosis and cognitive decline pattern. Periodontitis trigger an AD-like pathology in a similar way than experimental AD.

Identification of Cell Death-Associated and TLR-Regulator Proteins During Clinical Progression of Periodontitis

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Objectives Periodontitis is triggered by dysbiotic biofilms that elicit a local response with immuno-inflammatory dysregulation leading to a progressive tooth-support tissue destruction. There is no clear understanding of the cell death-associated and TLR-regulating molecular events occurring in the periodontal microenvironment during disease progression. **Objective:** To identify the presence of cell death-associated- and TLR-regulator proteins in the proteome of human GCF from progressive periodontal sites.
Methods 16 periodontal patients were weekly monitored in their progression of periodontal destruction by clinical attachment loss and GCF samples were obtained. Three groups were established: Progression(PG); Pre-Progression(PP) and Non-Progression(NP). Proteins were processed using high-throughput proteomic approaches and label-free analysis determined their relative abundances. Proteins were identified at SwissProt database. Génie was searched for progression biomarkers. String and Gene Ontology Databases were searched for functional enrichment analysis and an integrated bioinformatics study of *in silico* and *in vivo* data was performed to determine proteins related to TLR-regulation, cell death and disease progression.
Results 1572 proteins were identified: 1554, 1549 and 1550 in PP, PG and NP groups respectively. Proteomic characterization showed a higher abundance of IL1RN, IL36A and Phosphatases (PPP2CA, PTPN6 and MAP2K1) in NP group. A higher abundance of IL23R was found in PP group, while CA1, CA2 and several DAMPs (HSP90AA1, PRDX2, LGALS1, HEME, APOA1, NMI and SNCA) were more abundant in PG. The apoptotic-related proteins ANXA5 and H6PD were more abundant in NP vs. PG. The anti-ferroptosis-related protein FTH1 was exclusively identified in NP vs PG, and 4-fold increase in NP vs. PP; and the Ferroptosis-related protein GPX4 was more abundant in PG vs. NP.
Conclusions There are quantitative and qualitative differences in the proteome of human GCF from periodontal sites. Different profiles of apoptosis-, ferroptosis-associated-, TLRs-regulator- and DAMPs proteins were identified according to the state of clinical progression of periodontitis. The understanding of their etiopathogenic role in progressive periodontitis may help to develop new diagnostic and therapeutic approaches.

Rapid maxillary expansion: Dimensional changes of the upper airway

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Objectives The purpose of this review was to evaluate dimensional changes of the upper airway and transverse width in orthodontic patients after rapid maxillary expansion therapy (RME).
Methods 10 patients (10 to 18 years old) underwent pre and post RME treatment evaluations with a bimaxillary type tomography, obtained with a 9500 Cone Beam equipment. The maxillary width before and after the disjunction, in the coronal image, it was measured in the palatal alveolar table at the level of the first permanent upper molars. The data was exported to the STATA software version 14. To determine if there are statistical differences, the student's T-test was used.
Results The area corresponding to the P-plane shows statistically significant changes in all the subjects studied. The lower linear planes of the upper airway, the EP plane and the SP plane, did not show statistically significant changes.
Conclusions The results of this study confirm the findings of previous research in which RME produces a significant expansion of the maxilla and there is an increase in total volume of the upper airway in all subjects studied, which would be explained by a direct relationship with the changes obtained in relation to the upper limit of the retropalatine segment.

Use of Teledentistry by Elderly Population and their Caregivers: Systematic-Review

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Objectives People's oral health has worsened with the sanitary crisis due to COVID-19, revealing inequalities in dental access, especially in underserved populations like the elderly. Teledentistry emerges as a tool to improve the oral health of this population. However, its utilization and access have been scarcely studied within this age group and their caregivers. This systematic review revised the evidence about their experiences of teledentistry use compared to traditional interventions or their previous experiences.
Methods A systematic review was conducted by searching in three databases (Medline via PubMed, SCOPUS, and Web of Science). A predefined searching strategy was developed according to a PICO-question framework. All types of clinical studies were included, without limits in publication year or language. Both the selection and the data extraction were carried out independently and in duplicate.
Results From 1,516 potentially eligible titles, 18 articles were selected to read as full-text. After confirming their eligibility, five studies were included (quasi-experimental (n=4) and observational (n=1)). The interventions used were video calls (n=3), online platform (n=1) or application (n=1). Studies reported formal (n=2) or informal (n=1) caregivers. Most studies were a qualitative evaluation of the experiences, perceptions, knowledge, and practices of oral hygiene (n=4), satisfaction degree (n=2), acceptance (n=1), usability (n=1), and quality of life associated with oral health (n=1). Overall, studies classified the experiences of using teledentistry as positive. Just one study reported that caregivers presented difficulties on the implementation. The bias of risk was judged as moderate using ROBINS-I. The descriptive nature of data, plus the

heterogenicity among studies, prevented us from performing a meta-analysis.
Conclusions Our findings show that teledentistry is perceived as a positive and acceptable approach; however, the available evidence on this age group is still limited. Further research should consider the socio-economic and cultural context of the population on which it is applied (PROSPERO CRD42021243722).

Posters 1

Activated Charcoal-Containing Toothpastes: Chemically-Available Fluoride and pH in commercial formulations

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Objectives Activated charcoal-containing toothpastes have emerged as a novel and attractive formulation due to their potential putative beneficial effects for oral health. Due to its unspecific adsorbent properties charcoal might reduce the concentrations chemically-available fluoride found in toothpastes as ion fluoride (F^-) or ion monofluorophosphate (FPO_3^{2-}), from sodium fluoride (NaF) or sodium monofluorophosphate (Na_2FPO_3) salts, respectively. Furthermore, a low pH has been described to increase the adsorption potential of charcoal. To test this hypothesis, we assessed the concentration of available F and pH in charcoal-containing toothpastes marketed in the USA and in Chile.
Methods Toothpastes (n=16) were acquired in duplicate with different lot number. According to the label, toothpastes contained NaF (68.8%), or Na_2FPO_3 (31.2%) as the source of F. Silica was used as abrasive in all tested products. Chemically-available F (total soluble F; TSF), total F (TF), and ionic F (IF), were determined using a calibrated specific F electrode coupled to a potentiometer. pH was determined on slurries using a calibrated pH-meter. Mean values of F and pH (n=4) were calculated for each toothpaste.
Results In agreement with the manufacturers, ion FPO_3^2 was exclusively found in samples labeled as containing Na_2FPO_3.TSF ranged from 970.5 to 1170.2 ppm F (µg F/g toothpaste), with only one sample slightly below 1,000 ppm F, considered the lower threshold for an anticaries effect by toothpastes. TSF concentrations were similar to the measured and declared TF concentrations. Overall, the pH of the fluoridated charcoal-containing toothpastes was basic (6.45 to 8.14).
Conclusions Although we found that activated charcoal contained in fluoridated toothpastes does not decrease the concentration of available F, their stability overtime needs to be checked. In the absence of scientific evidence supporting their effectiveness on caries or potential erosive effects, activated charcoal-containing toothpastes should not be recommended to replace currently used F toothpastes.

Autologous Platelet Concentrates Efficacy in Regenerative Endodontics: Systematic Review

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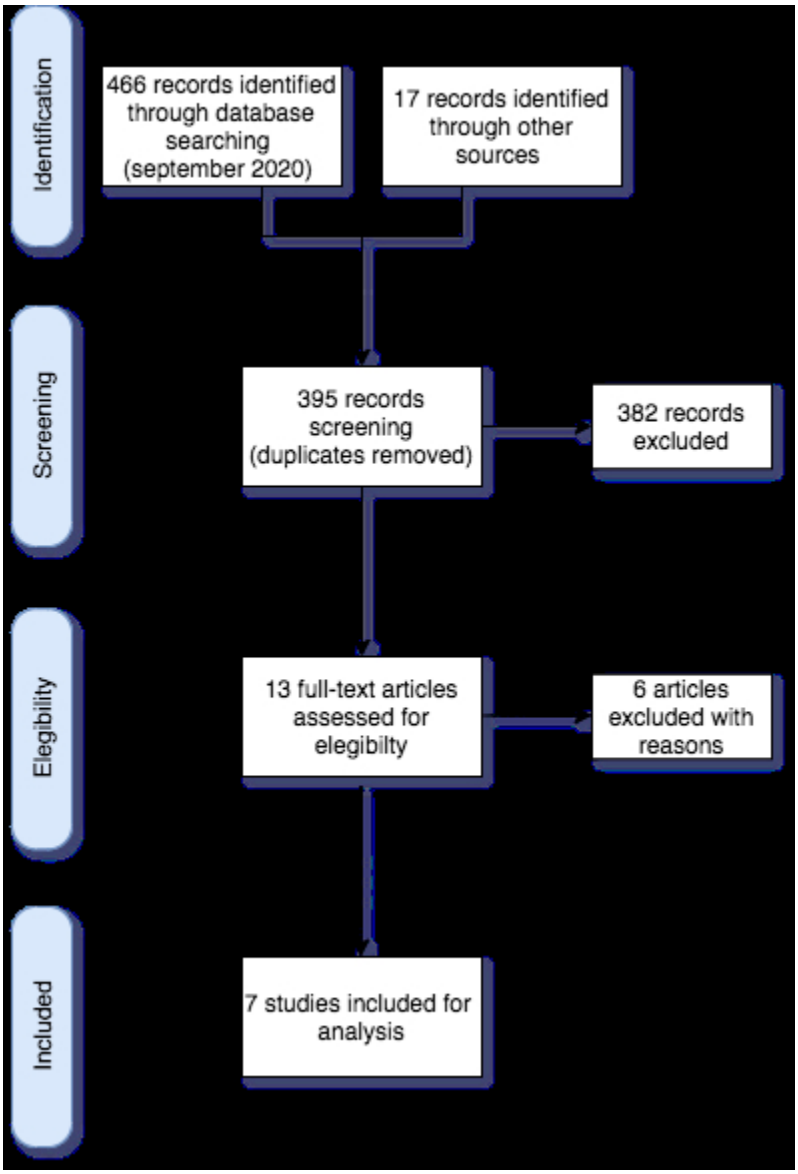
Objectives To analyze the quality of the evidence from studies that compare the clinical efficacy of blood clot (BC) versus Autologous Platelet Concentrates (APCs) in non-vital immature teeth treated with regenerative endodontics (RE).
Methods Randomized Clinical Trials (RCTs) comparing CPAs with BC in patients with non-vital immature teeth treated with ER were included. A systematic electronic search was performed in MEDLINE, THE COCRHANE LIBRARY, EMBASE, ScienceDirect, Scielo, LILACS, OpenGrey, ClinicalTrials.gov, WHO International Clinical Trials Registry Platform. A hand search was also performed in journals and conferences minutes or abstracts related to endodontics. The search included all publications without a year or language limit. Quality of the evidence was assessment through the "Risk of Bias 2" Cochrane tool.
Results Seven articles corresponding to RCTs were included for analysis (Figure 1). Four out of seven studies reported better CPA performance over CS. One low risk of bias study reports statistically better results of PRP in apical healing, apical closure, lengthening and root thickening. Two "some concerns" risk of bias studies, reports a better performance of PRP, one in apical healing and the other in lengthening root. One high risk of bias study inform significantly greater apical healing, lengthening and root thickening of PRF over CS.
Conclusions Included studies have a moderate to low quality of evidence, therefore RCTs with protocols and standardized outcome measures are needed, along with longer follow-up times.



Four-year Effects of Copper-nanoparticles on Durability of Resin/Dentin Interfaces

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Objectives To evaluate the effect of addition of copper nanoparticles at different concentrations into an etchand- rinse adhesive (ER) on the immediate (IM) and 4-year (4y) resin-dentine bond strength (µTBS), nanoleakage (NL) and presence of copper within hybrid layer.
Methods Seven experimental ER adhesives were formulated according to the amount of copper nanoparticles incorporated into the adhesives (0 [control], 0.0075 to 1 wt.%). The adhesives were

applied to flat occlusal dentine surfaces after acid etching. After resin build-ups, specimens were longitudinally sectioned to obtain beam-like resin-dentine specimens (0.8 mm2), which were used for evaluation of μTBS, nanoleakage and presence of copper within hybrid layer at the IM and 4y periods. Data were submitted to appropriate statistical analyses (α=0.05).
Results After 4y, significantly higher μTBS values significantly lower nanoleakage values were observed for copper containing adhesives compared to the control group. For presence of copper within hybrid layer, concentrations from 0.06 up to 1 wt.% present copper after 4y.
Conclusions Copper nanoparticles addition up to 1 wt.% prevent the degradation of the ER adhesives interface.

EXPERIENCE IN TELEATENTION OF TMD-OFP PATIENTS DURING THE COVID-19 PANDEMIC

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Objectives To characterize the experience of care through telemedicine to patients who consulted for Temporomandibular Disorders and Orofacial Pain (TMD-OFP) in COVID-19 pandemic period, in relation to the particularities of patients who consulted as well as the level of satisfaction reported with the care modality.
Methods May to September 2020, students of TMD-OFP program of the Andrés Bello University, provided free teleconsultations and telecare to patients requesting support. They conducted a full remote interview, mimicking the DC/TMD examination and collected Axis II Symptom questionnaires using electronic surveys. A 13-question Likert-scale survey assessed the system's experience, information sharing, consumer focus and overall satisfaction. All data was digitally recorded and descriptively analyzed.
Results 63 Men (15.8%) and 335 Women (84.2%) were attended, average age of 30.96 ± 10.96 years old (min 18-max 78). 75.6% presented pain in their face, temple or joint, with an average duration of 67.63 ± 57.20 days. 30.9% of the sample presented chronic pain (3 months cut-off). 62.2% had joint noises, 39.4% had a jaw lock history, 21.6% were currently jaw limited, and 12.4% presented open lock in the previous month. The average level of depression was 10.06 ± 6.29 (moderate depression) and anxiety 9.46 ± 4.81 (mild-moderate). Regarding user satisfaction, the domain focused on the consumer shows that 74.7% of patients could obtain positive results from their consultation. 95.14% of those who responded to the satisfaction survey would recommend this type of oral health-care to another person with their symptoms.
Conclusions Patients who during the COVID-19 pandemic demanded TMD-OFP care through University e-Health care were mostly middle-aged women with acute or chronic pain of moderate-intensity and moderate anxiety and depression. Also, telemedicine can be a useful and well-received alternative between the patient, user satisfaction aspect, and the oral health professional. More research is needed on examination, diagnosis and remote treatment of TMD-OFP.

Masseteric Atrophy Evoked by Botulinum Toxin Early Impairs Mandibular Microstructure

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Objectives Masseter muscle injection with Botulinum Toxin Type A (BoNTA) generates muscle paralysis 1d after the intervention. In the mandibular condyle, a significant impairment in bone microstructure at the BoNTA-side has been demonstrated after 14d, with a significant increase in the expression of a molecular marker of bone resorption in just 2d. Microstructure studies in early stages of BoNTA-induced muscle paralysis (2d and 7d) are not reported. Our aim is to compare the bone microstructure parameters of the mouse mandibular condyle 2d and 7d after a BoNTA injection in masseter muscle.
Methods The unilateral masseter hypofunction was induced by a single injection of BoNTA (0.2U/10 µl) in the right masseter and saline solution in the left masseter of adult male BALB/c mice. After 2d (T1) or 7d (T2), masseter muscles were processed for qPCR detection of atrophy molecular markers (Atrogin1, Murf1) or fibers diameter. Mandibles were scanned by micro-tomography (microCT, voxel size 8.43 µm). The microstructure parameters trabecular thickness (Tb.Th) and bone volume fraction (BV/TV), comparing the BoNTA-side with the control-side, were addressed using BoneJ.
Results A reduction in muscle mass and fibers diameter was observed in the BoNTA-injected side at T1 and T2. A significant increase ($p<0.05$) in the expression level of both Atrogin1 and Murf1 was also detected in BoNTA-injected muscles at T1 and T2. There was a tendency to decrease in Tb.Th. and BV/TV on the BoNTA side at T2; changes were not statistically significant, but the reduction in the BoNTA vs control-side was observed in all the animals addressed. No tendency of changes in bone parameters was observed at T1.
Conclusions We here demonstrated that BoNTA injection in masseter muscle early evokes muscle atrophy. Our data suggest that masseteric atrophy leads to an impairment in mandibular condyle microstructure of the ipsilateral side as early as 7d after the intervention.

Characterization of Accessory Mental Foramen in an Adults Chilean Population

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Universidad Austral de Chile

Objectives The recognition of key anatomical structures is decisive to avoid complications in the dental clinical practice. Cone Beam Computed Tomography (CBCT) is a complementary exam recommended for the planning of different procedures in dentistry. With this exam, anatomical variants can be identified, such as the Accessory Mental Foramen (AMF). The objective was to determine the frequency and characteristics of AMF in an adult Chilean population from Valdivia city, Chile.
Methods This is an observational study that included 247 CBCT exams from different radiological centers from Valdivia. According to the selection criteria, 143 CBCT exams were included. This study was approved by the Scientific Ethics Committee of the Valdivia Health Service.
Results AMF was identified in 25 patients representing 17.48%. From this result, 80% were found in females. AMF was most frequently identified in patients from 18 to 39 years old. The average distance between AMF and Mental Foramen (MF) was 5.76 mm, which corresponds to 2 mm the minimum distance and 11.5 mm the maximum distance. The average distance between AMF and the nearest dental apex was 5.36 mm, and the minimum and maximum distance were 0.8 mm and 10.2 mm, respectively. The root apex of the second premolar was most frequently associated with the AMF, representing 60% (n=15).
Conclusions This study confirms the importance of the correct evaluation of the mental region before initiating procedures in the area, which is vital to prevent injuries associated with this anatomical variant.

Effect Of Submandibulectomy On Interradicular Bone Of Rats Undergoing Tooth Movement

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Objectives To assess the effect of SMx on interradicular bone and periodontal ligament (LP) in two stages of orthodontic tooth movement (O).
Methods 24 adult male Wistar rats were divided into 4 groups: Control (C) and Orthodontic (O), both subbmited to Sham and SMx, which were performed 7 days before T0. At T0, O was induced by a NiTi spring attached from the right upper 1st molar (1M) to the upper incisors, and euthanasia took place 48hs and 7 days after. Microcomputed tomography scans were taken to determine the microarchitecture of the cervical half of the interradicular bone of the 1M by analizing: bone volume (BVp), trabecular thickness (Tb.Th), intertrabecular distance (Tb.Sp), number of trabeculae (Tb.N) and LP space. Statistical analysis was performed by Student t test and statistical difference was considered when $p<0.05$.
Results At 48hs BVp was lower in C+SMx (1,22±0,02) and in O (1,13±0,03) vs C (1,3±0,04). Also O+SMx (0,9±0,02) was lower vs C+SMx and vs O. Tb.Th was lower in O+SMx (125,4±3,07), vs O (142,7±1,31) and vs C+SMx (158,1±1,83). LP space was higher in O+SMx (120±4,17) vs C+SMx (81,94±3,59). At day 7, BVp was lower in C+SMx (1,13±0,03) and O

(1,12±0,02), vs C (1,35±0,04). Also O+SMx (0,71±0,03) was lower vs C+SMx and vs O. Tb.Th was lower in O+SMx (121,6±2,86), vs O (161,9±5,05) and vs C+SMx (159±1,95). Tb.Sp was increased in O+SMx (180,1±2,03) vs O (167,9±5,5). LP space was higher in O (183,7±3,87) vs O+SMx (149,1±5,11).
Conclusions Submandibulectomy exacerbates the effects induced by orthodontics on the interradicular bone. In the first stage, the decrease in bone volume can be seen as lower trabecular thickness and increased LP space. In longer periods of time, it would also increase the medular space between trabeculae.

Coverage Analysis of the "GES 60": Still Not Enough

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Objectives To estimate the coverage of the program for the year 2019 of the FONASA beneficiaries and their territorial variability disaggregated by Health Service (HS), sex and type of provider (public or purchase of services).
Methods An observational, ecological, cross-sectional study was carried out, using secondary data from public sources (DEIS, FONASA). Coverage, understood as the ratio between the population that receives the guarantee and the population that should receive it, expressed as percentage, was estimated using the "dental discharges" as the numerator and the 60-year-old population affiliated to FONASA as the denominator. Total national coverage and by sex was estimated, stratified for each SS.
Results The coverage of the program in the public health sector was 22.8% in 2019. The lowest coverage was observed in Arica HS (5.3%) and the highest in Arauco HS (37.9%). National coverage was significantly higher (p-value = 0.001) in women (27.1%) than in men (17.9%). Purchase of services from external providers totaled 12.2% of the dental discharges, this proportion being heterogeneous between SS with an inverse relationship between "Purchase of services" and "Coverage".
Conclusions Despite its universal character and being implemented for more than 12 years, the GES 60 program has very low coverage throughout the country, being insufficient to solve the high burden of morbidity of elderly Chileans. There is wide territorial variability of the coverage, presenting differences by sex, being greater in women, and with a tendency to decrease as the proportion of purchase of services increases. Thus, the scope and objectives should be reconsidered and, if possible, adjusted according to the current challenges and expectations of this group.

Effect of a new bleaching agent formulation containing hexametaphosphate and fluoride on enamel esthetic efficacy and microhardness

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Objectives
The aim of the study was to evaluate in vitro the addition of sodium hexametaphosphate (HMP) in the presence or not of sodium fluoride (F) to 35% hydrogen peroxide (PH) on the esthetic efficacy and hardness enamel.
Methods Bovine enamel blocks were selected by initial surface hardness (SHi) and divided into 5 experimental groups (n = 10): 1) Gel based on 35% PH (PH); 2) Gel PH + 0.1% F (PH/F); 3) Gel PH + 1% HMP (PH/HMP); 4) Gel PH + 0.1% F + 1% HMP (PH/F/HMP) and 5) Gel HP Blue 35% [FGM - Dental Products (HP Blue)]. The gels were applied once, during 3 sessions of 40 minutes/session, every 7 days. Then, the color change was measured and the final surface hardness (SHF), percentage of surface hardness loss (%SH) were determined. Data were submitted to ANOVA and followed by Student's t test ($p < 0.001$).
Results The gels had similar whitening efficacy. The PH/F/HMP bleaching agent reduced %SH by 60%, 56.7% and 54% when compared to treatment with the HP Blue, PH and PH/HMP gels, respectively ($p < 0.001$).
Conclusions It was concluded that the addition of HMP and F in a conventional bleaching gel reduced enamel demineralization when compared to its counterpart, promoting a similar bleaching effect.

Cp-Ti or Ti6Al4V Implants With or Without Laser Modified Surface

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Objectives This study aimed to evaluate the biological and mechanical behavior of peri-implant bone tissue in implants manufactured in cp-Ti or Ti6Al4V with machined surfaces (CPMS or ALLOYMS) and modified by LASER beam (CPLS or ALLOYLS).
Methods The surfaces were analyzed using scanning electron microscopy coupled with X-ray dispersive energy spectroscopy (SEM-EDX) prior to experimental surgery and after removal of the implants. Ninety-six (2x4mm) implants were installed in surgical beds milled in the right and

left tibias of 48 male *Wistar* rats, one implant of each material (metal) or surface installed in each tibia. Biomechanical analysis was performed using the implant removal torque in all groups in the periods of 14, 21 and 42 days postoperatively and the tibiae were processed for histological analysis. The data obtained in the biomechanical analysis were submitted to statistical analysis ($p<0.05$).
Results The SEM-EDX before the installation of the implants showed a difference between the surfaces machined and modified by LASER beam, regardless of the metal of the implant. The removal torque of ALLOYLS was statistically higher than CPLS, CPMS and ALLOYMS in 14 and 21 days ($p<0.05$), as well as CPLS was statistically higher than CPMS and ALLOYMS. In 42 days CPLS and ALLOYLS showed statistical differences when compared to CPMS and ALLOYMS ($p<0.05$). The SEM-EDX of the removed implants showed total bone coverage on the surfaces of CPLS and ALLOYLS and presented a more physiological distribution of Ca and P when compared to CPMS and ALLOYMS. In the qualitative histological analysis, a mature bone can be observed for CPLS and ALLOYLS.
Conclusions The implants with surface modification by LASER beam (CPLS or ALLOYLS), regardless of the material manufactured, provided important physical-chemical modifications on the surface, allowing better mechanical resistance with bone tissue when compared to implants with machined surface (CPMS or ALLOYMS).

Posters 2

Neutrophils N1 and N2 subsets and its role in periodontal diseases

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Objectives Periodontitis is a chronic inflammatory disease caused by a dysbiotic subgingival microbiota which enhance an inflammatory and pro-bone-resorptive response by triggering the polarization of pro-inflammatory immune cell phenotypes that finally activate osteoclastogenic precursors and induce bone resorption. Neutrophils are the first innate immunity cells that recognize the bacteria and are found in high concentration in tissues affected by periodontitis. Although several studies define different roles of them during health or periodontitis, it is unknown if they can polarize to an inflammatory or modulatory phenotypes. Recently, two neutrophil phenotypes have been described: pro-inflammatory or N1, and modulatory or N2. The aim of this systematic review was to evaluate the presence of neutrophil phenotypes in periodontal tissues in health or disease.
Methods A systematic review was carried out following the indications of the PRISMA

guideline, via an electronic search in the Medline database using Mesh terms “neutrophil”, “bacteria”, “periodontitis”, “subsets”, and “phenotypes” up to January 30th 2021. We include human cross-sectional, case-control and cohort studies. To determine agreement in the studies selection, data extraction and risk of bias the Cohen's κ coefficient was determined (k>0.8 in every step of the review).
Results From 3,658, 16 articles were included for qualitative analysis after apply eligibility criteria. Different studies identify the presence of different neutrophil phenotypes: pro- and para-inflammatory, hyper-reactive and hyper-active, and high or low responder phenotypes were identified.
Conclusions There is no evidence of N1 or N2 phenotypes detection in periodontal tissues. Although the evidence suggests that during periodontitis the presence of N1 neutrophil could plays an important role in the onset of the immune-inflammatory response, the induction of N1 neutrophils by oral bacteria must be exploited.

Microfiltration Of Immediate Sealing Of Universal Adhesive And Resin Flow

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UNAB

Objectives Compare the percentage of marginal microleakage of immediate dentin sealing (IDS) with universal adhesive+resin flow and Optidbond FL adhesive in indirect resin luting with self-adhesive cement.
Methods 56 human molars were used after disinfection and preservation. On each tooth two-cavity preparations (occlusal-mesial and occlusal-distal) standardized 3x3x3 mm in depth, mesiodistal width, and length. Immediately after the preparations were randomly divided into 3 groups. Group SS: Negative control (without IDS), Group OBF: (Optibond FL- Kerr Orange, USA) and Group AU+F: Single Bond Universal (3M-Espe, USA) + layer of resin flow (Revolution Formula 2, Kerr-USA). In each tooth, the SS group was used in one cavity and the OBF or AU+F group in the other. The teeth were stored in distilled water for 48 hours and to perfomed the indirect resins with Filtek Z250 (3M-ESPE, USA). Were cemented with Relyx U200 (3M-ESPE, USA). All procedures were performed according to the manufacturer's instructions and using a light-curing unit calibrated at 1000 Mw/cm2 (Bluephase G2, Ivoclar-Vivadent, Liechtenstein). The samples were stored in water for 72 hours and subjected to 500 cycles between 5-50 C, stained with methylene blue for 24 hours and cut in half to evaluate the percentage of microfiltration. The samples were evaluated with a 4x magnifying glass (Micrometrics SE Premium, Accu-Scope-USA). Data were analyzed through Kruskal Wallis and Mann-Whitney tests with for significant differences at a 95% confidence level.
Results The mean, median, and standard deviation (±) respectively for the groups were; SS: 72.8, 100.0 (± 35.3); OBF: 48.3, 33.0 (± 33.0) and AU + F: 23.8, 13.5 (± 31.6). There were statistically significant differences in the 3 groups ($p < 0.05$).

Conclusions There was less marginal microfiltration in the IDS group with AU + F compared to OBF and SS in the cementation of indirect resins with self-adhesive cement.

Performance of a copper-containing adhesive in a cariogenic oral environment

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Objectives To evaluate the effect of the incorporation of copper nanoparticles (CuNp) into a universal adhesive on the antimicrobial activity (AMA), bond strength (µTBS), nanoleakage (NL), elastic modulus (EM) and nanohardness (NH) of the resin-dentin interfaces, at 24 h (24h) and after *in situ* cariogenic challenge (CC).
Methods CuNp (0% [control] and 0.1 wt%) were added in a universal adhesive. After enamel removal, the adhesives were applied to dentine surfaces. Each restored tooth was longitudinally sectioned to obtain two hemi-teeth; one of them was evaluated after 24h and the other was included in an intra-oral palatal device placed in the mouth of ten volunteers for 14 days in CC. After that, each hemi-tooth was removed and oral biofilm formed was collected. The AMA was evaluated against *Streptococcus mutans*. For 24h and CC groups, each hemi-tooth was sectioned in "x" direction to obtain one slice for each EM/NH evaluation. The remains of each hemi-tooth were sectioned in "x" and "y" direction to obtain resin-dentin beams for µTBS and NL evaluation (24h and CC). ANOVA and Tukey's test were applied (α=0.05).
Results The presence of CuNp significantly improved AMA as well as all properties evaluated (24h; $p<0.05$). Although a decrease in the adhesive properties (µTBS/NL) for all groups after CC ($p<0.05$) occurred, the incorporation of CuNp maintained NH/EM values after CC ($p<0.05$).
Conclusions The addition of 0.1% CuNp in an adhesive may be an alternative to provide antimicrobial activity and increase its bonding and mechanical properties, even under a cariogenic challenge. This is the first *in situ* study proving that the incorporation of CuNp in a universal adhesive is a achievable alternative to provide antimicrobial properties and improved integrity of the hybrid layer under *in situ* cariogenic challenge.

Universal adhesives MDP-containing improve bonding effectiveness associated with shortening etching time.

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Objectives The effect of different etching times using four commercial phosphoric acids on calcium (Ca) content into demineralized dentin matrix, the resin-dentine microtensile bond strength (µTBS) and nanoleakage (NL) using an MDP-containing or a MDP-free universal adhesive.
Methods The phosphoric acid were used: Ultra-etch [ULE], Scotchbond Universal Etchant [SUE]; Dentsply Dental Conditioner [DDC] and Total Etch [TTE]. For Ca-content into demineralized dentin matrix, mid-coronal human dentine slices were etched for 3 s or 15 s and evaluated by energy-dispersive X-ray (EDX). Slices with no etching procedure (0 s) were used such control. The phosphoric acids (1 ml) were titrated by adding successive 1 ml drops of NaOH (0,08N). For µTBS and NL, flat dentine surfaces were etched for 3 s or 15 s. After that, an MDP-containing universal adhesive (Scotchbond Universal [SBU] and a MDP-free universal adhesive (Peak Universal Bond [PUB] were applied to flat dentine surfaces, composite resin build-ups and specimens were sectioned to obtain resin–dentine sticks. µTBS and NL were evaluated after 24h of water storage. ANOVA and Tukey's test were applied ($\alpha = 0.05$).
Results Ca content decreased significantly for all acids at times 3 and 15 s respectively when compared with the control time (0 s). Higher µTBS values were observed for ULE and SUE; the nanoleakage regarding to the acid type, lower NL values were observed for ULE and SUE mainly when compared to DDC and TTE , except for 3 s etching time for PUB adhesive, where DDC was similar to ULE and SUE. Regarding to the adhesive type, lower NL values were observed for SBU when compared to PUB.
Conclusions The ULE and SUE acids were less aggressive avoiding overreaching dentin mainly with the shortening etching time associated with MDP-containing adhesive improved bonding effectiveness.

The use of mouthwash as a complementary therapy to toothpaste reduces the demineralization of dental enamel

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Objectives The aim of the present study was to evaluate *in vitro* the ability of the association of media between toothpastes (TP) and mouthwashes (MW) supplemented or not with sodium trimetaphosphate (TMP), in reducing the demineralization of dental enamel.
Methods Bovine blocks (n = 60) were selected by initial surface hardness (SHi) and divided into 5 experimental groups (n = 12): 1) Placebo dentifrice (without F/TMP); 2) TP 1100 ppm F (1100F), 3) TP 1100 ppm + MW 100 ppm F (1100F-100F), 4) TP 1100 ppm + MW 225 ppm F (1100F-225F) and 5) TP 1100 ppm F + MW 100 ppm F supplemented with 0.4% TMP (1100F-100F-TMP). The blocks were treated twice a day with TP and MW, being submitted to 5 pH cycling for 7 days. After pH cycling, the final surface hardness (SHf), the percentage of surface hardness loss (%SH) and the hardness in longitudinal section (ΔKHN) were determined. The

data were submitted to ANOVA and Student's t ($p < 0.001$).
Results The blocks treated with 1100F-225F and 1100F-100F-TMP showed significantly lower %SH when compared to the other groups ($p < 0.001$). 1100F-100F-TMP had the highest ΔKHN ($p<0.001$). The 1100F group differed statistically from the groups associated with MW ($p<0.001$).
Conclusions It was concluded that the association of TP and MW produced a greater protective effect in inhibiting enamel demineralization when compared to TP, and that TMP supplementation in the MW with 100F had a greater effect than a MW with 225F. Clinical Significance: The combination of treatments may be an alternative for patients at high risk of caries.

Social Determinants and Oral Health Knowledge in Migrants

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Objectives The objective of this study is to determine the relationship between social determinants of health and the level of knowledge associated with oral hygiene habits among migrants in the V Region 2021, Chile.
Methods A descriptive, cross-sectional study was carried out through the application of a previously validated survey. This instrument allowed us to identify social determinants, level of knowledge and oral hygiene habits of immigrants in Chile. The sample consisted of 101 subjects between 18 and 35 years of age, who consented to participate in the study.
Results Three social determinants were identified that are statistically related ($p<0.05$) to hygiene habits and knowledge associated with oral health. These determinants are: Education level, income range and social security system. There is evidence of a detriment in hygiene habits and level of knowledge, in relation to a lower number of years of study and economic income of the migrant. Likewise, it is observed that those who are affiliated to a social security system have better habits and level of knowledge than the population without coverage.
Conclusions The multifactorial characteristics related to the causes of migratory movement in Latin America promote diminished social determinants. Although there is scientific evidence that relates social determinants to the health status of an individual, the literature associated with the level of knowledge and hygiene habits is limited. From the results obtained, it is concluded that the particularities of the migrants in Chile are conducive to a lower quality of oral health, which should be discussed as a health policy at a national level.

Performance of Noninvasive Therapies in Treatment of Temporomandibular Joint Osteoarthrosis.

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Objectives To determine the performance of noninvasive therapies in the treatment of osteoarthrosis (OA) of the temporomandibular joint (TMJ).
Methods Seventy patients (average=30.32 years old ±12.32), who consulted for the first time for internal TMJ disorders, were assessed clinically (k=0.82) and imaging (k=0.86). They were evaluated at baseline and one year later according to DC-TMD and Ahmad-2009 criteria. Inclusion criteria were: patients of both sexes, 13-62 years old, complete clinical record, clinical/imaging diagnosis of TMJ OA, with non-invasive treatment, with computed tomography and magnetic resonance examinations at baseline and one year later. Exclusion criteria: history of TMJ fractures/surgeries, history of the oncologic disease, corticoid intake, facial malformations. The therapeutic objectives were pain control, return to normal functional range, limit the degenerative process, control its progression and perform prospective screening of axis 2 (HADS Test). Data were analyzed descriptively, compared with the Wilcoxon test, and correlated with the Spearman test (SPSS v15.0).
Results Comparison at baseline and post-treatment clinical/imaging data determined significant improvements by non-invasive treatment of degenerative bone alterations: pain (p=0.0001), headache (p=0.0001), joint synovitis (p=0.01), condylar erosion (p=0.083) and condylar bone sclerosis (p=0.001). Adherence to treatment was 76.7% Joint pain was associated at baseline with anxiety: Rho 0.883, self-reported stress: Rho=0.717, female sex: Rho=0.849 and headache: Rho=0.780.
Conclusions The non-invasive treatment of TMJ OA, presents high performance in modifying the variables joint pain and joint synovitis, after one year of treatment returning to normal functional range, limiting the degenerative process and controlling its progression. HADS test supported the diagnostic evaluation of axis 2.

Remineralizing and antiproteolytic effect of fluoride / trimetaphosphate on dentin tissue

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Objectives the study evaluated the ability of solutions containing fluoride (F) and/or sodium trimetaphosphate (TMP) and F/TMP on the inhibition of MMP-2 and MMP-9, and on dentin remineralization *in vitro*.

Methods Bovine root dentin blocks (6 × 4 × 2 mm, *n* = 130) were prepared, and caries-like lesions were induced in two thirds of the surface (each block served as its own control). Blocks were then were randomly divided into 13 groups/solutions: Placebo; 0.3%, 1% and 3% NaOH-hydrolyzed TMP; 0.3%, 1% and 3% TMP; 250 ppm F; 500 ppm F; 1100 ppm F; 250 ppm F + 0.3% TMP; 500 ppm F + 1% TMP and 1100 ppm F + 3% TMP. One third of each specimen was treated for one minute, twice a day with the respective solutions, and subjected to a pH-cycling regime for 7 days. The mineral concentration ($g_{HAp} \times cm^{-3} \times \mu m$) was determined by computed X-ray microtomography, and data submitted to ANOVA and Student-Newman-Keuls' test ($p<0.05$). The ability of the solutions on the inhibition of MMP-2 and MMP-9 activity was assessed by zymograpy.
Results Subsurface lesions presented no mineral gain after treatment with F-free solutions ($p<0.001$). The mineral gain in the outermost region of the carious lesion showed dose-response relationship as a function of the F concentrations in the solutions ($p<0.001$), with no significant differences between their counterparts containing TMP ($p>0.05$). However, coadministration of F and TMP led to higher mineral gain in deeper regions of the lesion compared to counterparts without TMP ($p<0.001$). Solutions containing 3% TMP (hydrolyzed or not), 500 ppm F and 1100 ppm F completely inhibited MMP-2 activity, while for MMP-9 such effects were only achieved by treatment with 1100 ppm F + 3% TMP.
Conclusions Treatment with 1100 ppm F + 3% TMP promoted the highest capacity to promote dentin remineralization, especially at deeper regions of the lesion, and to inhibit dentinal matrix metalloproteinases.

Prevalence And Main Cause Of Premature Loss Of Temporary Tooth

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Objectives This investigation consists of determining the prevalence and main cause of tooth loss before their physiological replacement in the Korkhaus support zone in children between 6 and 9 years old who attend the CAS-UDD Clinic, La Florida
Methods For this purpose, we examined children whose ages ranged are from 6 to 9 years of age, who attended the CAS-UDD Clinic in the city of Santiago during the months of August to October 2016 to receive comprehensive treatment.
For the calibration by the tutor, the examiners evaluated 10 children at random who were between 6 and 9 years old and it was evaluated together with the teacher whether or not they presented loss of constituent pieces of the ZSK.
All guardians and children signed an informed consent. Then, the clinical file was completed and the evaluation was carried out.
Results 31.5% of the children in the sample have premature loss of primary teeth and the main cause of loss of primary teeth in children between 6 and 9 years of age is the presence of caries
Conclusions For the analysis, the prevalences of the causes of premature tooth loss were

estimated, and as expected, the most prevalent cause of tooth loss corresponds to dental caries It is suggested to implement prevention measures, such as oral hygiene instruction for children from an early age. It is also important to make timely referrals to reduce the intake of a cariogenic diet

Prevention Methods for Medication-Related Osteonecrosis of the Jaw: Microtomographic Evaluation

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Objectives To evaluate the effect of preventive therapies on medication-related osteonecrosis of the jaw in the alveolar repair process.
Methods Seventy-two Wistar rats were used and treated with 0.035 mg/kg of zoledronic acid and subsequently submitted to the extraction of the lower right molars. After extraction, the animals were divided into 9 groups (n=8), GS (Sham - did not receive zoledronate), GC (Clot), Gβ (β-tricalcium-phosphate), GD (10% doxycycline gel), GP (photo-dynamic therapy), GDβ, GPβ, GPD, and GPDβ. After 28 days of tooth extraction, the pieces were scanned in a Skyscan microtomographic to obtain bone volume (BV/TV), trabecular thickness (Tb.Th), separation (TB.Sp), and number (Tb.N) of trabeculae. Data were subjected to statistical analysis (one-way ANOVA, followed by tukey's post hoc, $p < 0.05$).
Results All groups present higher means of BV/TV when compared to GS (42.17% ± 2.65) and the best result was GPDB, 69.85% ± 6.25, followed by GPD and GDB (64.62 ±0.13 and 64.69 ± 4.40) when compared to GC, GS, GP, and GPB ($P < 0.05$). In Tb.Th, GD presented 0.163 mm ± 0.015 ($P = 0.043$) when compared to GP, 0.111 mm ± 0.0070. As for Tb.N, GPD 5.53 mm ± 5.53 had a greater number of trabeculae, compared to GS, 3.68 mm ± 3.48 ($P < 0.05$). There was no difference for Tb.Sp ($P=0.199$). Qualitatively, GS demonstrated bone sequestration, while therapies allowed the repair process.
Conclusions The therapies associated or not demonstrated to be effective in preventing osteonecrosis and the use of biomaterial and doxycycline demonstrated maintenance of alveolar volume.