

# **19<sup>th</sup> Biennial Joint Meeting of the International Academy of Periodontology (IAP) and the Croatian Society of Periodontology (HPD)**

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## **ABSTRACTS**

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# Hyaluronic acid enhances cell migration, viability and mineralized tissue specific genes in cementoblasts

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## Abstract

**Objective:** Hyaluronic acid (HA) have been used in regenerative periodontal therapy, but the cellular effects of HA on the cementoblasts are still unknown. The purpose of this study was to investigate the effects of HA on cementoblasts functions, cell viability, migration, mineralization, differentiation and mineralized tissue-associated genes and cementoblast-specific markers of the cementoblasts were tested.

**Methods:** Cementoblasts (OCCM-30) were treated with various dilutions (from 1:2 to 1:128) of HA and examined for cell viability, migration, mineralization, and gene expressions. The mRNA expressions of osteocalcin (OCN), runt-related transcription factor 2 (Runx2), bone sialoprotein (BSP), collagen type I (COL-I), alkaline phosphatase (ALP), cementum protein-1 (CEMP-1), cementum attachment protein (CAP) and small mothers against decapentaplegic (Smad) -1,2,3,6,7,  $\beta$ -catenin (Ctnnb1) were performed with RT-PCR.

**Results:** HA enhanced cell viability and migration of the cells to the wound healing area. Increased mineralized nodules were determined at all HA dilutions. mRNA expressions of COL-I, BSP, RunX2, ALP, OCN, CEMP-1 and CAP significantly up-regulated by HA treatments, compared with control group on day 3 and day 8. Smad 2, Smad 3, Smad 7 and  $\beta$ -catenin mRNAs were increased, while Smad1 and Smad 6 were not affected by HA administration.

**Conclusion:** Positive effects of HA on the cementoblast functions demonstrated that HA application may play a key role in cementum regeneration.

**Funding source(s):** Selcuk University Research Coordination Office.

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# Complex treatment of gummy smile by periodontal subtractive surgery and esthetic zirconia crowns

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## Abstract

**Objective:** To provide healthy supracrestal tissue attachment before prosthetically restoring a patient with gummy smile (GS).

**Methods:** A young female patient diagnosed with GS, presenting etiology related to altered passive/active eruption, resulting in short tooth syndrome (STS). After facial, lips, and teeth analyses, a digital wax-up and an indirect mock-up was tried on. Surgical crown lengthening was performed by apically positioned flap, osteoplasty, and ostectomy using a surgical guide. The level of the ostectomy respected the distance of at least 2 mm from the apical ridge of the surgical guide on the maxilla. Three weeks later, early preparation of teeth was performed by using a supragingival knife edge technique and healed soft tissue as a margin. Temporary crowns were applied. Six months later, final zirconia crowns were cemented.

**Results:** Correction of GS was achieved and healthy periodontal tissues adapted nicely with zirconia crowns.

**Conclusion:** Analysis to determine the etiology of the GS must be done. The final position of restorations must lead the periodontist while performing the apically positioned flap along with osteoplasty and ostectomy (restorative approach) in order to establish healthy supracrestal tissues, even gingival margins, and proportioned teeth.

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# Vitamin C mesotherapy versus topical application for gingival hyperpigmentation: a clinical and histopathological study

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## Abstract

**Objective:** To compare the efficacy of vitamin C intra-mucosal injection (mesotherapy) versus topical gel, regarding clinical effect and patient satisfaction for managing gingival hyperpigmentation.

**Methods:** Twenty ASA (American Society of Anesthesiology) type 1 individuals of both genders, with age range 18-40 years old with increased pigmentation in gingivae, were included randomly allocated into two groups: Group 1 - vitamin C intra-mucosal injection (G1-Mesotherapy), in which intra-mucosal field injections of 1 ml Cevaryl were done, then repeated 3 times with 1-week interval; Group 2 - vitamin C topical gel (G2-Gel), in which ascorbic acid 2-glucoside gel was applied once daily, for 3 months. Clinical assessment was done at baseline, after 3 months and 6 months by including the following parameters: Dummett-Gupta Oral Pigmentation Index (DOPI: 0 = pink tissue; 1 = mild light brown tissue; 2 = medium brown and 3 = deep brown); Satisfaction questionnaire; and Melanin area fraction (MAF), detecting melanocytes.

**Results:** 1) DOPI Group 1 showed significant change in DOPI by time. 2) Patient satisfaction score: Group 1 showed higher cosmetic satisfaction. 3) MAF: The difference in MAF was insignificant between the two groups by the end of treatment period.

**Conclusion:** The topical vitamin C is a promising alternative technique to surgical melanin depigmentation techniques.

**Funding source(s):** Personal fund by all authors.

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# Digitally-assisted esthetic crown lengthening in the management of gummy smile: a randomized controlled trial

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## Abstract

**Introduction:** Surgical guides have been proposed in an attempt to reach more predictable outcomes for esthetic crown lengthening (ECL).

**Objective:** The objective of the present study was to evaluate the effectiveness of ECL using 3D-printed surgical guides, in the management of excessive gingival display due to altered passive eruption type 1B.

**Methods:** Sixteen patients diagnosed with altered passive eruption type 1B participated in this randomized clinical trial. The subjects were divided into two groups: In the control group (n=8), the surgical procedure was planned by manual measurements; and in the study group (n=8), the planning was digitally assisted and the procedure was carried out using a dual guide. The clinical parameters were assessed at 1 week, 2 weeks, 3 months and 6 months postoperatively.

**Results:** There was no statistically significant difference in terms of wound healing, pain scores and gingival margin stability between both groups at different time intervals ( $P = 1$ ); however, there was a statistical difference between both groups in terms of operating time, with the study group being significantly lower ( $P < 0.001$ ).

**Conclusion:** Digitally assisted ECL helps shorten the operating time and reduces the possibility of errors during measurements taking. This will be useful in helping practitioners achieve better results.

**Funding source(s):** Self funded.

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# Oral and gut microbial profiling in periodontitis and Parkinson's disease

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## Abstract

**Objective:** The current study aimed to test the hypothesis that Parkinson's disease (PA) alters the oral and gut microbiome in periodontitis.

**Methods:** Patients with Stage III, Grade B periodontitis with PA (PA+P) and without PA (P); and systemically and periodontally healthy individuals (HC) were enrolled. Clinical and periodontal parameters were recorded. Severity of motor functions of PA patients was scored. Unstimulated saliva samples and stool samples were collected. Next-generation sequencing of 16S ribosomal RNA (V1-V3 regions) was performed.

**Results:** PA patients had mild to moderate motor dysfunction, which indicated that oral hygiene was not inadequate in PA+P group. In saliva, there were statistically significant differences between HC and PA+P ( $p=0.001$ ), HC and P ( $p=0.012$ ), P and PA+P ( $p=0.013$ ) in beta diversity. The correlation between HC and PA+PD ( $p=0.001$ ), and P and PA+P ( $p=0.002$ ) was also detected. The microbial profiles of saliva and fecal samples were distinct. In stool test, Aitchison distance metric analysis showed statistically significant differences in PA+P and P groups, when compared to HC. However, there were no significant differences of differential abundance in the gut microbiome profiles of the study groups.

**Conclusion:** These data suggested that Parkinson's disease modifies the oral microbiome in periodontitis independent of the gut microbiome.

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# Parkinson's disease is positively associated with periodontal inflammation

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## Abstract

**Objective:** To test the hypothesis that Parkinson's disease (PA) is associated with periodontal tissue inflammation.

**Methods:** Patients with Stage III, Grade B periodontitis with PA (PA+P) and without PA (P); and systemically and periodontally healthy individuals (C) were enrolled. Clinical and periodontal parameters were recorded. GCF samples from deep and shallow sites were collected, to measure the inflammatory and neurodegenerative targets (YKL-40, fractalkine, S100B, alpha-synuclein, tau, VCAM-1, BDNF, NfL).

**Results:** Periodontal parameters and GCF volume were significantly higher in the P and P+PA groups than in the control group. PA was associated with significantly increased BOP, compared to P-alone ( $p < 0.05$ ), while other clinical parameters were similar between in P and P+PA groups. In GCF, YKL-40 levels from deep sites were significantly higher in the P+PA ( $p = 0.0105$ ) and P ( $p = 0.0011$ ) groups than in the C group. Alpha-synuclein levels were also higher in the P+PA group, compared to the C ( $p = 0.0052$ ) and P ( $p = 0.0012$ ) groups. S100B was higher in the P+PA group than in the C group ( $p = 0.0194$ ).

**Conclusion:** The data suggested that PA is highly associated with increased periodontal inflammatory burden—bleeding upon probing and inflammatory markers—, in parallel with PA-related neuroinflammation.

**Funding source(s):** This research was supported by a grant from the NIH/NIA (R01AG062496 to A. Kantarci).

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# The adjunctive effect of probiotics to nonsurgical treatment of chronic periodontitis: a randomized controlled trial

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## Abstract

**Objective:** This study was conducted to clinically and microbiologically evaluate the adjunctive effect of *Lactobacillus reuteri* (*L. reuteri*) to scaling and root planing (SRP) in the treatment of chronic periodontitis.

**Methods:** Clinical evaluation was performed in 20 sites of chronic periodontitis in 12 patients, and followed up at 3 and 6 months using clinical attachment level, probing pocket depth, plaque index (PI), bleeding on probing; in addition to microbiological evaluation for *Porphyromonas gingivalis* (*P. gingivalis*) load. Patients meeting the inclusion criteria were scheduled within 1 week for two sessions of SRP. After SRP, oral hygiene measures were reassured, and sites were randomly divided into two groups, with 10 sites each. Group I received SRP only, Group II received SRP and subgingival delivery of 1 ml of *L. reuteri* suspension at baseline and 1, 2, and 4 weeks, using blunt syringe. Periodontal pack was applied after the placement of the drug.

**Results:** We found noticeable variation between the two groups in all evaluation aspects, at 3 and 6-month follow-up, except PI at 6 months, with no significant difference.

**Conclusion:** Results proved the antimicrobial benefit of *L. reuteri* in improving periodontal parameters. However, further long-term studies with large sample size are needed to evaluate this added value.

**Funding source(s):** Self-funding, except the PCR kit, supplied by our institution.

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# Differences in bacteriome of dental plaque, saliva, and tissue biopsies in oral cancer

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## Abstract

**Introduction:** Oral microbial dysbiosis has been associated with oral carcinogenesis. Hence, it is necessary to comprehensively characterize key dental plaque microbial communities implicated in carcinogenesis, so that they can be preferentially targeted during therapy.

**Objective:** The aim of the present study was to determine whether there is an overlap between microbiota of the oral cavity and that pertaining to oral cancer lesions.

**Methods:** Samples of tumour tissue, adjacent tissue, dental plaque and saliva from cancer patients were subjected to microbial identification using MALDI-ToF-MS. Conventional PCR was used to investigate prevalence of *Porphyromonas gingivalis*. Selected samples were subjected to bacterial 16S rRNA gene Next Generation sequencing.

**Results:** *Veillonella*, *Haemophilus*, *Actinomyces* and *Streptococcus* species were found in abundance on identification using MALDI-ToF MS. *Pasteurella*, *Eikenella* and *Selenomonas* were prevalent in tumor and plaque samples, while being absent from adjacent tissue. *Fusobacterium pseudoperiodonticum*, *Fusobacterium nucleatum*, *Fusobacterium canifelinum*, and *Fusobacterium hwasookii* were found in high prevalence. *Desulfotomaculum* and *Clostridium* had a higher distribution in plaque. *Tenericutes* phylum was absent in plaque. *Fusobacterium nucleatum* was found in higher quantities as compared to *P. gingivalis*.

**Conclusion:** A significant microbiome-level overlap was noted between oral cavity and oral cancer lesions, thereby emphasizing the significant role of periodontal health and its maintenance.

**Funding source(s):** The study has been supported by grant from Indian Council of Medical Research, letter number NCD/Ad-hoc/87/2020-21 dated 17/3/2021(5/4/2021 Oral Health/ 2021- NCD-II).

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# Resolvin E1 and Maresin 1 differentially regulate porphyromonas gingivalis LPS-induced macrophage polarization

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## Abstract

**Introduction:** Lipopolysaccharide (LPS) activated macrophages can differentiate into M1 (pro-inflammatory and involved in bacterial killing and promoting inflammation) and M2 (pro-resolution of inflammation and tissue repair) phenotypes.

**Objective:** To test the hypothesis that macrophage polarization in response to *Porphyromonas gingivalis* LPS was reversed by two different specialized pro-resolution mediators of inflammation (SPMs).

**Methods:** Murine macrophage cells were cultured and treated with *P. gingivalis* LPS with or without RvE1 or MaR1. M1 and M2 phenotypes were characterized by flow cytometry using CD80, CD86, F480, and CD206. TNF- $\alpha$ , TGF- $\beta$ 1, IL-6, IL-10, IL-1 $\beta$ , and Nf $\kappa$ B expressions were measured by qPCR. Pro-inflammatory and anti-inflammatory cytokines were measured in cell culture supernatants.

**Results:** F4/80+ CD11b+ cells were significantly increased in response to *P. gingivalis* LPS; MaR1 significantly prevented this expression ( $p < 0.05$ ), while RvE1 did not have any significant impact. In parallel, RvE1 and MaR1 prevented the *P. gingivalis* LPS-induced increase in the expressions of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  ( $p < 0.05$ ), while the SPMs did not significantly impact the TGF- $\beta$ 1 and IL-10 expressions. MaR1 and RvE1 significantly prevented the TNF- $\alpha$  production induced by *P. gingivalis* LPS.

**Conclusion:** SPMs differentially prevented the M1 polarization induced by *P. gingivalis* LPS, while they did not impact the M2 phenotype, suggesting a reduced pro-inflammatory activation.

**Funding source(s):** Hacettepe University (Ankara, Turkey), Forsyth Institute (Cambridge, MA, USA).

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# Mean platelet volume, triglyceride/high-density lipoprotein cholesterol and salivary interleukin-1 $\beta$ as determinants for periodontitis grading

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## Abstract

**Objective:** This study aims to investigate the local and systemic effect of periodontal disease according to the current classification of periodontal and peri-implant diseases, and conditions of its possible impact on stage and grade differentiation.

**Methods:** Two-hundred and forty individuals were included in this study. Eight groups were constituted according to: gingival health and gingivitis (on intact and reduced periodontium) and periodontitis (stages 1-4). Clinical, sociodemographic and anthropometric parameters were recorded. Complete blood and lipid profile analysis were made in serum. Interleukin (IL)-1 $\beta$  and matrix metalloproteinase (MMP)-8 levels were analyzed in serum and salivary samples with Enzyme Linked Immunosorbent Assay (ELISA).

**Results:** According to the findings of this study, salivary and serum IL-1 $\beta$  and MMP-8 levels increased with disease severity; high density and very low density lipoprotein cholesterol levels showed significant differences among the groups ( $p < 0.01$ ). Mean platelet volume, salivary IL-1 $\beta$  and Triglyceride/High-Density Lipoprotein Cholesterol have shown high classification function coefficients in discriminating the grades of periodontitis.

**Conclusion:** Correlations between anthropometric, clinical, salivary and serum parameters and differences among groups could be taken into consideration, especially in grade determination. Considering the biomarkers, which are not reported yet as grade determinants in the current classification, the findings of this study are original and unique.

**Funding source(s):** This study was supported by the Coordination Unit of Scientific Investigations, Süleyman Demirel University, Isparta, Turkey (project number: TDK-2022-8700).

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# The effectiveness of enamel matrix derivative as an indication to treat isolated residual deep pockets in a non-surgical manner: randomized clinical trial

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## Abstract

**Objective:** To investigate the efficacy of EMD with laser therapy for the treatment of residual deep pockets measuring 5 to 8 mm in depth 3 months after initial periodontal therapy.

**Introduction:** Various agents were tried as an adjunct to periodontal therapy. Recently the effect enamel matrix derivative (EMD) on periodontal therapy has been studied, EMD is an enamel matrix proteins (EMPs), secreted by Hertwig's epithelial, derived from enamel layer of developing porcine teeth. EMD proved to establish regeneration in furcation, intrabony defect and has been proved to significantly increase the clinical attachment level when added as an adjunct to surgical periodontal therapy.

**Methods:** In this split-mouth clinical trial (NCT05823389), initial non-surgical periodontal therapy was administered to 25 adults with stage III periodontitis. Following a six-week reinforcement of oral hygiene; laser therapy was utilized for re-instrumentation of deep, isolated pockets (5-8 mm) with bleeding on probing in a 3-month visit. The test group was administered EMD, whereas the control group was not. After EMD application, Clinical Attachment Level (CAL), Periodontal Pocket depth (PPD), and Bleeding on Probing (BOP) were evaluated three months later.

**Results:** Compared to baseline, there was improvement in both test and control groups in pocket depth (PPD) ( $3.52 \pm 0.76$  mm and  $4.70 \pm 0.99$  mm respectively;  $p < 0.05$ ). However, using EMD in the re-treatment phase significantly decrease the PPD, CAL and BOP as compared to control group.

**Conclusion:** As an adjunct to non-surgical periodontal therapy, EMD in combination with laser therapy substantially improved periodontal health by decreasing CAL and PPD. EMD demonstrates a viable treatment option, minimizing the need for surgery in some cases.

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# Cytokine gene polymorphisms and socio-demographic parameters in patients with periodontal disease

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## Abstract

**Introduction:** Periodontal disease is an epigenetic multi-factorial disease resulting from both modifiable (such as socio-demographics etc) and non-modifiable (such as tumour necrosis factor alpha [TNF- $\alpha$ ], interleukin [IL]-1 beta [ $\beta$ ] and IL-6 gene polymorphism [PcGP]s) factors.

**Methods:** One hundred and forty four individuals with systemically healthy and periodontal disease were included in this cross-sectional study. Socio-demographic characteristics (education level, economic status, tooth brushing and interdental cleaning habits and body mass index [BMI] etc.) and clinical periodontal parameters were recorded. All of the individuals were categorized as periodontal status (gingivitis, periodontitis and periodontally healthy). The genotypes and allele frequencies of TNF- $\alpha$ -308, -238, IL1 $\beta$ -511, +3953, IL-6-174, -572 polymorphisms were performed using the PCR-RFLP technique.

**Results:** IL-6-174 (he risk allele 2 [G/T allele]-[CG/CT genotypes]) was associated with gingivitis. There were significantly positive correlations between PcGPs and clinical periodontal parameters ( $p < 0.05$ ). Considering the severity of periodontal disease, individuals with periodontitis had worse socio-demographic and clinical periodontal parameters. Furthermore, BMI was significantly correlated with poor oral hygiene, clinical periodontal parameters and PcGPs.

**Conclusion:** Further longitudinal studies with larger populations are needed to explain the association among PcGPs, socio-demographic and clinical parameters of periodontal disease.

**Funding source(s):** TÜBİTAK (project number, number: 107S506) Ankara-TURKEY.

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# Vertical bone augmentation: Khoury technique with computer-guided autogenous bone harvesting

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## Abstract

**Introduction:** Vertical bone augmentation is one of the most challenging things in oral surgery and Implantology. Posterior atrophic mandible is one the hardest areas to treat, due to limited access and poorer blood supply. There is a lot of critical elements to take into consideration, as bleeding and neurosensory disturbance. We have to think about planning, soft tissue management, bone harvesting and hard tissue management. Safe-Cut method is a method that uses digital technology for safely harvesting autologous bone block from the mandible

**Objective:** To report a clinical case of bone harvesting using a guide, in a scenario where nerve exposure was previously planned, due to lack of bone in the mandible. Utilizing Khoury technique and FGG, the ridge was completely regenerated.

**Results:** 8 mm of vertical bone augmentation was obtained. Three implants were inserted into the vital bone. Four-year follow-up has shown stable bone and no bone remodeling in the implant neck.

**Conclusion:** Vertical bone augmentation is a predictable method. Survival rate in augmented bone is similar to the native bone in loading conditions. Safe-Cut technique can offer a safe, predictable, planned and user-friendly technique to harvest a bone block.

**Funding source(s):** De Stavola et al. 2015, Jomi De Stavola et al. 2017, PRD De Stavola and Tunckel, 2013, Jomi

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# Electrolysed oxidising water as a novel mouthwash for viral transmission prevention: *in vitro* study

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## Abstract

**Introduction:** The role of mouthwashes in viral transmission prevention, particularly in the context of the COVID-19 crisis, has brought the necessity to assess their antiviral properties.

**Objective:** To evaluate the antiviral effectiveness of various mouthwash active ingredients and provide data to improve protocols for safety of patients and dental healthcare providers. This study examined the potential of electrolysed oxidising water (EOW) as a novel rinse for such approaches.

**Methods:** In order to evaluate the *in-vitro* virus infectivity potential, a cytopathic effect assay was conducted using a model system to examine both membrane-enveloped and non-enveloped viruses. Mouthwash active ingredients, including Chlorhexidine, Cetylpyridinium chloride, Ethanol (EtOH), Hydrogen peroxide, Sodium hypochlorite (NaOCl), and EOW were assessed. Additionally, cytotoxicity was evaluated on human oral keratinocytes.

**Results:** EOW outperformed other mouthwashes, by absolute prevention of infectiousness of both virus types within a clinically relevant 1-minute exposure time. Except for EtOH, all other mouthwashes effectively targeted enveloped viruses, whereas only chlorine-based mouthwashes (NaOCl and EOW) exhibited potency against more robust non-enveloped viruses. Notably, all mouthwashes except for EOW exhibited cell cytotoxicity.

**Conclusion:** EOW showed promising results for mitigating viral transmission in *in-vitro* conditions, while remaining safe to human cells. This novel rinse should be considered for clinical applications.

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# Association between stages of periodontal disease and different diagnostic biomarkers

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## Abstract

**Objective:** To investigate the significance of inflammatory biomarkers collected from serum, gingival crevicular fluid (GCF) and saliva, for diagnostic staging of periodontitis.

**Methods:** Periodontal examination was carried out on 245 patients. Serum, saliva and GCF samples were quantitatively analysed for inflammatory biomarkers (matrix metalloproteinase-8 (MMP-8), interleukin-8 (IL-8), prostaglandin E-2 (PGE2), surfactant protein D (SP-D), prior to active periodontal treatment. Staging was performed according to the 2018 classification. Univariate statistical analysis of differences of the expression of biomarkers in blood, saliva and GCF at various stages of periodontitis have been done with Analysis of Variance or Kruskal-Wallis test ( $\alpha = 0.05$ ).

**Results:** Patients presented with a mean number of  $24 \pm 5$  teeth. Four patients were diagnosed with periodontitis stage II (1.6%), 138 with stage III (56.3%) and 103 with stage IV (42.0%). Levels of MMP-8 in serum and GCF, of PGE2 in GCF, and of IL-8 in saliva showed significant differences between periodontal stages ( $p < 0.001$ ). On the other hand, serum levels of SP-D values were not differentially expressed among patients with different disease stages.

**Conclusion:** Levels of MMP-8 in serum and GCF, of PGE2 in GCF and of IL-8 in saliva showed significant association with the stage of periodontitis. Accordingly, these biomarkers reach relevance in the diagnosis of periodontitis.

**Funding source(s):** The study was self-funded by the authors and their institution.

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# Ceramic soft-tissue trimmer vs scalpel surgical technique in gingival depigmentation: a split-mouth randomised controlled trial

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## Abstract

**Introduction:** Gingival hyperpigmentation (GH) is an esthetic problem caused by excessive melanin deposition. The available techniques for gingival depigmentation (GD) are either bloody (e.g.: scalpel, bur abrasion) or require sophisticated equipment (e.g.: LASERs, Radiotherapy, electrosurgery). Ceramic soft-tissue trimmers (Ceratip) were primarily launched to be used in gingivoplasty, but have recently been used in GD. They have the advantage of good hemostasis, because of blood coagulation by frictional heat.

**Objective:** The objective of this study was to compare GD by Ceratip vs the gold standard Scalpel technique.

**Methods:** Eight patients who had GH in both arches were randomized into two groups, so that each patient had GD by scalpel in one arch and Ceratip in the opposing. The patients were followed up for three months. The assessed parameters were pain levels, Treatment time, clinical outcomes, patient satisfaction, and re-pigmentation rates.

**Results:** There was no statistically significant difference in all the measured parameters at different assessment timepoints. However, the scalpel had better initial clinical outcomes, while Ceratip had less visible re-pigmentation, pain scores, treatment time, along with more patient satisfaction.

**Conclusion:** Ceratip and scalpel had comparable results. Thus, Ceratip is an alternative technique for gingival depigmentation, due to its simplicity, efficacy and its bloodless nature.

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# Impact of physical exercise on local inflammatory profile in humans and rats

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## Abstract

**Introduction:** Physical inactivity is a risk factor for periodontitis, and consistent evidence suggests that engaging in physical exercise (PE) may be beneficial to treatment.

**Objective:** To evaluate the local inflammatory profile of humans and rats with periodontitis submitted to PE.

**Methods:** Seventeen periodontitis patients (according to Classification) were randomly allocated to receive either subgingival instrumentation (SI) or PE (high-intensity interval training, 7min/day, 3x/week). Periodontal parameters and gingival crevicular samples were collected at the worst site (PPD  $\geq$  4mm, with attachment loss and bleeding on probing) at baseline and after 45 days. Also, 16 male Wistar rats received cotton ligatures around the lower first molars, which remained for 7 days. Then, either ligatures were removed for periodontal repair (n=8) or rats started PE (swimming 30 min/day, 5x/week; n=8) for 28 days, when gingival samples and mandibles were collected. For both studies, samples were evaluated using an inflammatory panel (Luminex).

**Results:** In humans, mean PPD reduction was  $0.24 \pm 0.2$  for SI and  $0.14 \pm 0.2$  for PE ( $p > 0.05$ ). In rats, bone loss was not significantly different between groups. In both studies, IL-1 $\beta$  and VEGF were increased after PE (although only significant for rats). IL-4, -10, -1- $\alpha$ , TNF and EGF were comparable.

**Conclusion:** In conclusion, VEGF seems to play a key role on the beneficial impact of PE on periodontitis.

**Funding source(s):** Araucária Foundation (Curitiba/PR, Brazil) and National Council for Scientific and Technological Development (CNPq, Brasília, DF, Brazil) (PPSUS).

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# Novel approach of Interdental Guided Creeping Therapy for augmentation of interdental papillae: case report

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## Abstract

**Objective:** To present the treatment of interdental papillary recession with minimally invasive interdental guided creeping technique (IGCT), aiming to provide less traumatic therapy, with less morbidity and high patient satisfaction.

**Methods:** Papillary recession was treated using IGCT. Horizontal incision was performed beneath the apical end of interdental junctional epithelium, followed by creating a horizontal blind tunnel at the level of palatal papilla. Then the tunnel was augmented, to support the papillary level at its ultimate coronal reposition. Interdental papillary fill and visual analogue scale for pain and swelling were measured.

**Results:** Papillary fill was obtained with minimal post-operative pain.

**Conclusion:** Interdental Guided Creeping Therapy can be a promising novel minimally invasive technique for treating papillary recession with minimal trauma.

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# Case report of periimplantitis treatment with the help of new techniques and materials

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## Abstract

**Introduction:** Periimplantitis is defined as a pathological condition characterized by inflammation in the peri-implant mucosa and progressive resorption of supporting bone. A variety of protocols have been proposed to treat periimplantitis.

**Objective:** To present a case report of a patient with periimplantitis treated using new techniques and materials.

**Methods:** After full thickness flap elevation and granulation tissue removal, the implant surface was disinfected with Galvano-surge unit. The exposed implant surface was augmented with the GBR technique. A composite bone graft was used to graft the periimplant bone defects. The bone graft was covered with a resorbable form stable membrane, secured with resorbable pins. After two weeks of healing, a small flap dehiscence occurred, which was left to heal by secondary intention. Two months before implant uncovering procedure, a free gingival graft was used to correct the scars resulting from the secondary healing, improve the soft tissue thickness and widen the keratinized periimplant mucosa. One year after the surgical treatment, the case was finalized with a definitive screw retained crown.

**Results:** At six-month recall appointment, improvements in the clinical and radiological periimplant parameters were evident.

**Conclusion:** The combination of implant surface disinfection performed with the Galvanosugre unit, and GBR procedure soft tissue augmentation resulted in a successful outcome of the periimplantitis treatment.

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# Localized fibrous gingival hyperplasia: a case report

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## Abstract

**Introduction:** Gingival tumours are very rare clinical conditions. It is important to recognize and treat them according to histopathologic examination.

**Objective:** To report the clinical case of a patient with localized fibrous gingival hyperplasia.

**Methods:** A 46-year-old male complained on aesthetics compromised by single mass in the upper left canine region that has been present for 7 years. There was no systemic disease or long-term medication use in the patient's medical history. He had a car accident and facial injury 15 years ago. Clinical examination showed a solitary fibrous pedunculated nodular growth (1.8cm × 1.5cm × 0.8cm) that was attached with the buccal gingiva, extending from distal of tooth #23 to mesial of tooth #25. On palpation, it was firm and insensitive, but bleeding on probing was present. The growth was surgically removed and sent for histopathologic examination.

**Results:** Histological evaluation showed moderately cellular, collagenized fibrous connective tissue with chronic inflammatory infiltrate. Overlying stratified squamous epithelium is partly keratinized, with variable thickness, without cytological atypia and focally infiltrated with neutrophils and lymphocytes.

**Conclusion:** A normal anatomy of attached gingiva was restored, in order to improve oral hygiene maintenance and aesthetics outcome in 1, 3, and 6 months follow-up.

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# Specialized pro-resolving mediators reverse transcriptomic changes in oral squamous cell carcinoma cells mediated by periodontopathobionts

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## Abstract

**Introduction:** Resolvins and maresins are active regulators of the resolution of inflammatory processes in several non-communicable diseases, including periodontitis and systemic pathologies.

**Objective:** To assess whether these active and specialized mediators of inflammation (SPMs) impact the functional and transcriptional characteristics of oral squamous cell carcinoma cells (OSCC) modified by periodontal pathobionts.

**Methods:** Oral squamous cell carcinoma cells (SCC-25) were infected with *Porphyromonas gingivalis* (P.g.), and resolvin E1 (RvE1), maresin 1 (MaR1), or their combination at 10nM were tested, and the full transcriptomic profile was analyzed.

**Results:** There was an upregulation of 43 and a downregulation of 27 genes in response to MaR1, while RvE1 upregulated 25 and downregulated 15 genes, compared to the control group. The combined use of the SPMs led to upregulating of 104 genes and downregulating of 37 genes. Among several pathways the SPMs impacted, the tumor necrosis factor receptor family was a major target.

**Conclusion:** These findings suggest that SPMs reverse some transcriptomic responses of OSCC following P.g. infection.

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# Titanium chip preparation for fluorescence *in-situ* hybridization (FISH) analysis of oral samples

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## Abstract

**Introduction:** The accumulation of a bacterial biofilm on the coronal surfaces of implants and/or the abutments is the main reason for peri-implantitis.

**Objective:** To investigate the possibility to process *in-vivo* samples with biofilms from periodontal pockets to a remaining thickness of 30 µm, using a non-destructive grinding technique.

**Methods:** 68 T-formed titanium chips were inserted into untreated periodontal/peri-implant pockets of 10 patients with periodontitis. After different resting times (1, 2, 7, 14 days), chips were removed and embedded in Technovit (Kulzer). The thickness of the chips was measured by a laser triangulation displacement sensor (optoNCDT 1420, Micro Epsilon, Ortenburg, Germany) and the most prominent part was assigned the value zero for the subsequent measurements. Multiple measurements were performed until a residual thickness of 30 µm was reached.

**Results:** The residual thickness of the chips ranged between 25 and 35 µm. After further processing, an oral biofilm was detectable on all titanium chips (n = 65) by microscopic analysis. Three chips were lost because of crooked embedding.

**Conclusion:** A grinding and measurement technique could be established, that provides the possibility to reduce the thickness of titanium-chips to 30 µm.

**Funding source(s):** This study was supported by BMBF "PROCEED"(ref. no. 13GW0414D)

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# Salivary C-reactive protein following non-surgical periodontal therapy in patients with stage III and IV periodontitis

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## Abstract

**Objective:** The aim was to determine the values of salivary C-reactive protein (CRP) in a standardized sample three months after two types of periodontal therapy in subjects matched by sex, age and periodontal status.

**Methods:** This study included 30 patients with generalized periodontitis stage III and IV with a minimum of 20 teeth. The study group (n=15) underwent supragingival and subgingival full-mouth therapy with oral hygiene instructions, while the control group (n=15) received only supragingival therapy with oral hygiene instructions. Salivary CRP was determined at the baseline and three months after the non-surgical periodontal therapy.

**Results:** Statistical analysis revealed a reduction in the values of salivary CRP in the test group after therapy, with a statistically significant reduction ( $p < 0,01$ ), and in the control group ( $p < 0,01$ ) at 3 months, compared to baseline. Salivary CRP was statistically significantly lower in the study group compared to the control group 3 months after therapy ( $p = 0.035$ ).

**Conclusion:** The study revealed decreased values of salivary CRP following the non-surgical periodontal therapy. Additional studies are needed to establish the consistency of the values of salivary CRP and possible use as a biomarker for periodontitis.

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# Protein binding specificity predicts initial oral bacteria attachment on the implant and tooth surface

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## Abstract

**Introduction:** Although dental implants are exposed to the same environment as dental surfaces, physical-chemical surface properties modulate the salivary protein binding and, consequently, microbial accumulation.

**Methods:** Protein layer was formed (2h) using human saliva, and the proteomic profile was evaluated by mass spectrometry. Bacterial adhesion (2h) and initial biofilm formation (24h) were evaluated in an *in vitro* polymicrobial model using human saliva as microbial inoculum and analyzed by 16s RNA sequencing.

**Results:** Among the 349 proteins adsorbed, some were unique to titanium (12), dentin (42), or enamel (3). Linear discriminant analysis (LDA) showed that specific proteins drive toward a unique profile for each substrate. Differences ( $p < 0.05$ ) in abundance levels of 28 bacterial species among the substrates at 2h were found. Higher alpha diversity was found for enamel and titanium at 24h, with increased levels of *Fusobacterium*, *Prevotella*, and *Streptococcus* species for all substrates. LDA analysis showed that enamel and dentin are more closely related in terms of the microbiome. Canonical correlation analysis showed that specific proteins are correlated to higher abundances of specific bacterial species, as prediction analysis.

**Conclusion:** Dental implants and tooth surfaces differ in terms of salivary protein adsorption, which predicts higher accumulation of specific bacterial species.

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# A novel pH-sensitive MAA-based film coating antimicrobial LbL system for enhancing soft tissue seal

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## Abstract

**Objective:** Our preliminary studies validated the drug incorporation method into our layer-by-layer (LbL) system on titanium (Ti) substrate. Here, we developed an acidic pH-sensitive MAA-based film onto the multilayers, to improve the therapeutic effect of the LbL system and soft tissue seal on abutment surfaces.

**Methods:** Detailed multilayer coating characterization was performed by different approaches. Coating stability of MAA-based film was confirmed under neutral and acidic pH. Microbiology experiments were performed to uncover the broad-spectrum of drug against different bacteria. The effect of MAA-based film on cell behavior was evaluated by cells in monolayer and collagen matrix.

**Results:** MAA-based film instability in acidic environment over time was identified through changes in physical-chemical properties. The protective capacity of MAA-based film was also identified with a higher concentration of drug released at acidic pH up to 15 days. Quantitative and qualitative assessments demonstrated a strong antimicrobial efficacy of MAA-based film against polymicrobial biofilm. Similarly, the non-cytotoxic effect was confirmed over a sufficient period to measure the hazard potential.

**Conclusion:** MAA-based film is a dual-function transparent coating that promotes soft tissue seal and holds antibacterial activity. Conveniently, the film discloses a slight capacity of controlling the drug release under neutral condition.

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# Clinical evaluation of sodium hypochlorite/ amino acids and cross-linked hyaluronic acid adjunctive to non-surgical periodontal treatment

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## Abstract

**Objective:** To compare the clinical outcomes obtained with either mechanical subgingival debridement (SD) in conjunction with a sodium hypochlorite and amino acids containing gel, followed by subsequent application of a cross-linked hyaluronic acid gel (xHyA) gel, or with SD alone.

**Methods:** Forty-eight patients diagnosed with stages II-III periodontitis were randomly treated with either SD (control) or SD plus adjunctive sodium hypochlorite/amino acid and xHyA gels (test). Primary outcome variable was probing depth (PD) reduction. Clinical attachment level (CAL), bleeding on probing (BOP) and plaque index (PI) were secondary outcomes. The outcomes were assessed at baseline, at 3 and 6 months following therapy.

**Results:** At 6 months, the test group showed statistically significantly better results in terms of mean PD reduction ( $2.9 \pm 0.4$  vs  $1.8 \pm 0.6$  mm,  $p < 0.001$ ). Mean CAL gain was statistically higher in the test, compared to the control group (test:  $2.6 \pm 0.5$  vs control:  $1.6 \pm 0.6$  mm,  $p < 0.001$ ). Mean BOP and PI scores decreased with statistically significant difference favouring the test group ( $p < 0.001$ ).

**Conclusions:** Both treatments resulted in statistically significant improvements in all evaluated clinical parameters. The adjunctive subgingival application of sodium hypochlorite/amino acid and xHyA to SD yielded statistically significantly higher improvements, compared to SD alone.

**Funding source(s):** Lithuanian University of Health Sciences, Regedent.

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# Hyaluronic acid impact on periodontal condition of second molars after third molars extraction: retrospective study

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## Abstract

**Objective:** Evaluate clinical changes at the distal aspect of the second molars after removal of non-impacted third molars and application of cross-linked hyaluronic acid (xHyA) in patients with periodontitis.

**Methods:** Twenty-one patients diagnosed with stages II-III periodontitis were referred for third molar extractions before periodontal treatment. Patients were randomly allocated into test (the post-extractional alveolus filled with xHyA) and control (after third molar extraction, a suture was affixed) groups. Clinical measurements: probing depth (PD), clinical attachment level (CAL), bleeding on probing (BoP) and plaque index (PI) were obtained before extraction, after 3 and 6 months.

**Results:** No statistically significant differences were observed between groups at baseline. After 3 and 6 months, statistically significant differences were found in PD and CAL changes in both groups, compared to baseline ( $p < 0.001$ ). When a comparison between test and control groups was made, statistically significant differences were detected in changes of PD and CAL at 6 months, in favour for test group ( $p = 0.012$  and  $p = 0.006$ , respectively). BOP and PI decreased significantly in both groups at both timepoints ( $p < 0.05$ ), however, no statistically significant difference was observed between the groups.

**Conclusion:** Adjunctive application of xHyA after third molar extraction lead to significantly better clinical improvements at the distal aspect of second molars.

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# Guided oral biofilm recolonization: the spray cryotherapy approach

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## Abstract

**Introduction:** Cryotherapy is used in dental practice as a safe, easy to perform, and relatively inexpensive treatment for various oral lesions, and to improve the healing process. However, its effect on oral biofilms is not known yet.

**Objective:** This study aimed at evaluating the consequences of applying cryotherapy to in-vitro oral biofilms.

**Methods:** Multispecies oral biofilms were grown on hydroxyapatite discs, then treated with CryoPen® X+ for 1, 2, or 3 freeze/thaw cycles. Biofilms without treatment served as control. Biofilms were split into two groups, one for collection and analysis directly after the cryotherapy, and the other for reincubation for 24hr, to allow biofilm recovery. Biofilms then were analysed with v-qPCR, scanning electron microscopy (SEM) and a confocal laser microscopy (CLSM), to follow possible changes.

**Results:** Cryotreatment decreased the biofilm bacterial load with more treatment cycles. The v-qPCR results showed significantly better ecology of the recovered biofilms than the non-treated ones. Also, CLSM showed changes in the layers of the biofilms after applying the cryotherapy, and SEM revealed dominance of the commensal species in the biofilms after recovery.

**Conclusion:** Cryotherapy represent a totally new approach in guiding oral biofilm recolonization after treatment, with selective eradication of pathobionts and maintaining oral commensals, without antimicrobial use.

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# Kynurenine pathway, hypoxia and oxidative stress in periodontitis

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## Abstract

**Introduction:** The kynurenine pathway is involved in the oxidative breakdown of tryptophan, and is essential in immune function and, like hypoxia (HIF-1 $\alpha$ ) and oxidative stress (8-OHdG), associated with periodontitis.

**Objective:** The aim of this study was to evaluate the levels of these factors and relationship between KP, hypoxia and oxidative stress in periodontal inflammation.

**Methods:** Saliva and serum samples were collected from 23 systemically healthy stage III periodontitis patients and 22 periodontally healthy control individuals. Salivary and serum TNF- $\alpha$ , HIF-1 $\alpha$ , 8-OHdG levels were evaluated with ELISA, and salivary and serum KP metabolites were evaluated with Tandem Mass Spectrometry (LC-MS/MS) method.

**Results:** Salivary TNF- $\alpha$ , HIF-1 $\alpha$ , 8-OHdG levels were significantly higher in periodontitis group ( $p < 0.05$ ); serum levels did not differ among groups. Serum and saliva TRP levels were significantly lower in the periodontitis group than in the control group ( $p < 0.001$ ). Salivary and serum KYN/TRP ratio were significantly higher in the periodontitis group than in the control group ( $p < 0.012$  and  $p < 0.001$ ; respectively). Salivary HIF-1 $\alpha$  and 8-OHdG were significantly positively correlated with serum KYN/TRP ratio.

**Conclusion:** The present results support that periodontal inflammation might play a role in local and systemic tryptophan-kynurenine metabolism and local hypoxia and oxidative stress levels are related with the systemic kynurenine metabolism.

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# Healing patterns in a smoker and a non-smoker patients undergoing CAF operations with CTG

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## Abstract

**Objective:** To evaluate the healing patterns in smoker and non-smoker patients who have undergone coronally advanced flap (CAF) surgery with connective tissue graft (CTG).

**Methods:** Two healthy patients, with an insignificant medical history and a close age gap, presenting gingival recession on their tooth number 23 (FDI). A CAF using Zuchelli's technique with connective tissue graft was utilized to amend the recession areas. The healing patterns were clinically monitored and recorded.

**Results:** The non-smoker patient experienced faster and proper healing. However, in the smoker patient, healing in the operation zone took longer. Additionally, tooth #24 (FDI) remained uncovered, and a line of fibrotic tissue formed distally, likely due to prolonged healing time and/or inflammatory response.

**Conclusion:** It is important to emphasize postoperative patient education, particularly focusing on the effects of smoking on healing tissues. In non-smoker patients, the healing stage tends to be faster and more proper. However, in smoker patients, a longer healing time in the operation zone is observed, due to the known factors associated with smoking. These factors can significantly hinder the healing process and increase the risk of complications.

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# The osteogenic differentiation of human gingival fibroblasts affects osteoclast formation

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## Abstract

**Objective:** The purpose of this study was to investigate the effect of osteogenic differentiation of gingival fibroblasts (GF) on their osteoclast-inducing capacity.

**Methods:** GF (n=12) were cultured for one, two or three weeks with osteogenic medium. After three weeks, peripheral blood mononuclear cells (PMBCs) were added to each condition and co-cultured for more three weeks. To assess the mineralization, Alizarin Red S staining, Alkaline phosphatase (ALP) activity, Calcium (Ca), and scanning electron microscopy (SEM) were performed (day 21), followed by the determination of the gene expression of TRAcP, IL-1 $\beta$ , TNF- $\alpha$ , RANKL, and OPG by quantitative PCR (day 21 and 35). Further, protein levels of TNF- $\alpha$  were measured using ELISA at days 28 and 35.

**Results:** ALP/DNA and Ca concentrations increased after three weeks of culturing GF in mineralization medium ( $p<0.05$  and  $p<0.01$ , respectively). Mean area covered by mineral nodules was assessed by SEM and increased gradually per time duration ( $p<0.05$ ), which was confirmed by Alizarin Red S staining. All investigated gene expression levels significantly increased over time, except for OPG, which decreased ( $p<0.001$ ).

**Conclusion:** GF are able to differentiate into osteoblast-like cells and produce mineral nodules. Their degree of differentiation seems to reduce their osteoclast inducing capacity. This further indicates their potential to participate in the development, differentiation, remodeling, and repair of the periodontium.

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# The influence of increased body mass on periodontitis: a preliminary, cross-sectional study

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## Abstract

**Objective:** The main objective of this research was to determine whether there is a relationship between overweight and periodontitis and whether increased body mass index (BMI) and waist-hip ratio (WHR) affect the severity of the periodontal disease.

**Methods:** A detailed medical history was obtained from each subject (n = 40), including age, gender, smoking habits, oral hygiene. A detailed periodontal clinical examination and radiographic analysis was performed. The body mass was assessed using the BMI and WHR. The test group consisted of 20 subjects with a diagnosis of periodontitis, while the control group consisted of 20 periodontally-healthy subjects.

**Results:** This study showed that the large proportion of subjects with periodontitis had an increased BMI (80%), and 50% of subjects had an increased WHR. In the control group, only 25% of the subjects had an increased BMI and 20% of them had an increased WHR. The median BMI in the test group was higher by 3.75, and the WHR median was higher by 0.05, when compared to the control group. The quality of life regarding oral cavity health is higher in healthy individuals than in those with periodontitis.

**Conclusion:** BMI and WHR were increased in subjects with periodontitis, compared with subjects in the control group.

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# Treatment of a localized gingival recession with different consecutive mucogingival procedures: a case report

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## Abstract

**Introduction:** Covering the exposed root surface is one of the goals of mucogingival surgery.

**Objective:** The aim of this case report was to share clinical results of multiple root coverage procedures on a patient with an excessive localized gingival recession.

**Methods:** A 43-year-old systemically healthy male patient was referred to our clinic with the complaint of gingival recession. Clinical examination revealed a 7 mm Cairo RT-II type of recession in the vestibule of upper left canine, with lack of keratinized tissue. Following non-surgical therapy, recession was treated with the Envelope technique using de-epithelialized gingival graft (GG). Patient brushed the operation area in the early healing period, resulting in 2-mm recession. Four months later, a semilunar coronally advanced flap surgery was performed, but because of complications during the healing process, the recession increased to 3 mm. A third operation was performed two months later, using enamel matrix proteins in combination with the Envelope technique using de-epithelialized GG again.

**Results:** Root coverage was achieved on this challenging case. One month after the last surgical procedure, the recession depth was 0.5 mm, and keratinized tissue width was 5 mm.

**Conclusion:** Among the different bilaminar techniques, the envelope technique appears to be a safe and predictable method in treating localized gingival recessions.

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# Oral hypersensitivity reactions: clinical and histopathological manifestations

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## Abstract

**Introduction:** Oral hypersensitivity reactions (OHRs) clinically and histologically differ from skin lesions. Decreased barrier functions of mucosal epithelium, intraepithelial edema, and increased permeability lead to facilitation of possible agents and antigenic penetration. These lesions can be seen as major aphthous ulcers, mucosal exfoliation, erythematous, lichenoid, and vesiculobullous lesions.

**Objective:** The purpose of this case series is to report clinical and histopathological features of oral hypersensitivity reaction cases with known contacts and to review these conditions.

**Methods:** Six cases of oral hypersensitivity reactions with known contacts that exhibited discrete clinical and histopathologic patterns were identified.

**Results:** Major histocompatibility groups, differences in TCR and TLR organizations, and allergy tendencies are effective in the formation of the reactions. As histological general findings: edema in the epithelium, inflammatory cell exocytosis, interface inflammation, perivascular inflammation in the deep layers of the connective tissue, lymphoid follicle formations and sometimes granulomatous reaction can be found.

**Conclusion:** Clinical presentation of OHR is varied and often overlaps with other oral conditions, complicating their diagnosis and management. OHRs may affect the patient's quality of life and should be diagnosed and treated by clinicians.

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# Matrix metalloproteinase-8 in the gingival tissue of patients with arrested periodontitis following implant placement

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## Abstract

**Objective:** To detect the presence of matrix metalloproteinase (MMP)-8 in the gingival tissue (GT) harvested during the two stages of implant surgery, and compare its expression between healthy controls and test group with arrested periodontitis.

**Methods:** GT at the implant site were harvested during the first (pre-operative) and second stage (post-operative) of implant surgery, along with clinical data and gingival crevicular fluid (GCF) of the adjacent tooth. GT were formalin-fixed and paraffin-embedded prior to the immunohistochemical analysis. GCF MMP-8 level were quantified using commercially available enzyme-linked immunosorbent assay kit.

**Results:** 29 subjects were recruited (15 control, 14 test; mean age: 48.41 years; 12 female) but only 24 post-operative tissue were available for analysis. MMP-8 immunostaining was positive in all samples. No significant difference was seen on the pre-operative and post-operative GCF MMP-8 level and the GT immunoreactive score (IRS) between groups. However, the IRS showed increment in both groups post-operatively. Intense staining of MMP-8 was also seen in submucosa region of 7 samples, which were not detected pre-operatively. There was significant correlation between the IRS of gingival epithelium and lamina propria at both time-points.

**Conclusion:** Increased intensity of MMP-8 staining in gingival tissue following implant placement may have a role in determining the onset of peri-implantitis.

**Funding source(s):** Malaysian Ministry of Higher Education's Fundamental Research Grant Scheme (grant no. FP011-2019A).

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# Treatment of gingival recessions by modified tunnel technique with de-epithelialized gingival graft: a case report

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## Abstract

**Introduction:** Connective tissue graft is a golden standard in order to obtain and maintain superior clinical results in root coverage procedures.

**Objective:** The aim of this case report was to evaluate the 1-year clinical results of de-epithelialized gingival graft (GG) using Modified Coronally Advanced Tunnel (MCAT) technique in the treatment of multiple gingival recessions.

**Methods:** A 19-year-old systemically healthy female patient who referred to our clinic complaining of gingival recessions and dentin hypersensitivity revealed multiple gingival recessions in different areas of her mouth. Thin gingival biotype and incorrect/excessive brushing were considered as possible etiologic factors. Following correction of oral hygiene habits and non-surgical periodontal therapy, multiple mucogingival operations were planned. A total of four consecutive modified tunnel technique with de-epithelialized GG were performed, and the patient was precisely followed during whole treatment process. No complications were encountered in post-operative controls.

**Results:** In total, 10 gingival recessions in all quadrants were treated and completely covered. The mean recession depth was 1.6mm and keratinized tissue width was 1.4mm at baseline. The mean keratinized tissue width was 5.05 mm at 12 months.

**Conclusion:** De-epithelialized GG with MCAT technique is a predictable treatment method for covering multiple gingival recessions. De-epithelialized GG is a successful alternative in stabilizing quality and dimension of the tissue obtained in the recession area.

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# Neopterin levels in the serum and saliva of patients with periodontitis

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## Abstract

**Objective:** The aim of this study was to measure the levels of neopterin in saliva and serum in periodontitis patients, comparing them with periodontally healthy subjects.

**Methods:** Saliva and serum samples were collected from 23 systemically healthy periodontitis patients and 23 periodontally healthy control individuals. Clinical periodontal parameters (plaque index (PI), probing pocket depth (PPD), gingival recession (GR), clinical attachment loss (CAL), and bleeding on probing (BOP)) and saliva flow rates were recorded. Salivary and serum neopterin and 7-di-hydroneopterin (7NP) levels were evaluated with Tandem Mass Spectrometry (LC-MS/MS) method.

**Results:** The concentrations of free and total salivary neopterin were statistically significantly higher in the patients with periodontitis, compared with control group ( $p < 0.001$ ). In periodontitis group, salivary 7NP was also significantly higher than control ( $p < 0.001$ ). There was no significant difference in serum between periodontitis and control groups. 7NP levels in the saliva were significantly and positively correlated with all the clinical periodontal parameters (PI, PPD, BOP, and CAL;  $p < 0.01$ ).

**Conclusion:** Our results demonstrated that salivary concentrations of neopterin (free, total and 7NP) were associated with periodontitis. This study suggests that salivary neopterin concentrations may reflect local immune activation even in situations where no systemic activation can be detected.

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# Continuous vs. intermittent maintenance: a 12-year clinical review

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## Abstract

**Objective:** The study aimed to determine the results of 12 years of periodontal therapy in correlation with the regularity of patients' visits during supportive periodontal therapy (SPT).

**Methods:** The study examined 16 patients with adult periodontal disease who underwent a full-mouth radiographic examination before and 12 years after the initial examination. Tooth loss, probing pocket depth (PPD) change, and mean alveolar bone loss (MBL) over 12 years were evaluated. Based on the regularity of patients' visits in the phase of SPT, the patients were divided into two groups: continuous maintenance (n=8) and intermittent maintenance (n=8).

**Results:** In the group of continuous maintenance, the results showed a 63,4% decrease in sites with PPD>4mm. The average PPD has decreased by 1.16mm, MBL regarding the initial exam was 1.23mm, and 2.1% of teeth were lost. In the group of intermittent maintenance, the results showed 1.4x more sites with PPD>4mm. The average PPD has increased by 0.31mm, MBL regarding the initial exam was 2.41mm, and 11.8% of teeth were lost.

**Conclusion:** The results showed a significant difference in disease progression between the two groups. The group of continuous maintenance had a significantly better outcome.

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# Identification of dental implant failure risks: an overview of systematic reviews

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## Abstract

**Objective:** To identify risk factors for dental implant failure (DIF) from available systematic reviews (SR).

**Methods:** Searches were performed using Epistemonikos, Cochrane Database of Systematic Reviews and PubMed. Authors (TPP, JZ, NB, MM) independently screened search results using Covidence. The same authors independently extracted data from included SRs. Authors (TPP, JZ, NB, TG) working in pairs independently evaluated the quality of SRs using AMSTAR 2. Size and direction of DIF risk for population, exposure, and outcome measures were summarized.

**Results:** After deduplication, 299 records were screened, with 31 SRs preliminarily included. The following risk factors were assessed: loading protocol (9 SRs), HIV infection (1SR), implant surface (2 SR), presence of cantilevers (1SR), screw- or cement-retained prosthesis (1 SR), splinted vs non-splinted implant-supported restorations (1 SR), diabetes (3 SRs), use of bisphosphonates (3 SRs), history of periodontitis (3 SRs), implant length (1 SR), osteoporosis (1 SR), polymorphisms in IL-1 and TNFα (1 SR), bone augmentation (1 SR), sex (1 SR), and bruxism (1 SR). One SR included smoking, radiotherapy, diabetes, and osteoporosis.

**Conclusion:** Understanding the likelihood of DIF regarding different risk factors and performing individual risk assessment before dental implant placement is crucial for the long-term success of implant rehabilitation.

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# The effect of medical Manuka honey in the treatment of periodontitis: randomized clinical trial

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## Abstract

**Introduction:** Initial non-surgical therapy (NST) is the first step in the treatment of periodontitis. Besides that, adjuncts can be applied and one of these could be Manuka honey.

**Objective:** The aim of the study is to compare outcomes of the non-surgical initial therapy with and without additional topical application of Manuka honey.

**Methods:** In this randomized clinical trial, a total of 45 participants with stage III periodontitis underwent full-mouth non-surgical therapy. Manuka honey was applied after randomization to 23 participants, and 22 participants received placebo. The effectiveness of Manuka honey was investigated by measuring the clinical parameters of periodontal disease (bleeding on probing, probing depth, plaque index, recession) at two time-points, T0 and after 3 months. The microbiological analysis was performed at 3 time-points: T0, after one week and after 3 months.

**Results:** Percentage of pocket closure: Manuka (M) = 78.8%; Placebo (P) = 69.0%. CAL gain (mm): M =  $0.87 \pm 1.47$ , P =  $0.67 \pm 1.35$ . PPD reduction (mm): M =  $1.07 \pm 1.4$ , P =  $0.87 \pm 1.28$ .

**Conclusion:** The use of Manuka honey showed a promising potential for being used as an adjunctive therapy to NST. The improvements in outcomes were statistically significant for the participants treated with Manuka in terms of pocket closure, PPD reduction, CAL gain and reduction of bacteria associated with periodontitis after each of the follow-up time points.

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# Epulis: clinical and morphological features

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## Abstract

**Introduction:** Epulis is a term often applied to exophytic processes originating from the gingiva. Several of these processes are gingival reactive lesions to limited local irritation or trauma. Very often epulis occurs in women with hormonal imbalance.

**Objective:** The aim of this study was to determine the frequency, anatomic location and morphological signs of epulis in persons of different ages.

**Methods:** Clinical examination and surgical removal of tumor-like gingival lesions was carried out in 17 patients aged 16 to 84 years. Histological study of all biopsy specimens was performed.

**Results:** Various forms of epulis were identified in 17 women, most of whom (70.6%) were young (up to 44 years). Fibrous epulis was diagnosed in 9 cases (52.9%). In 6 women, of whom 4 were pregnant, vascular epulis, or pyogenic granuloma, was observed. Also, 2 women (61 and 84 years old) presented inflammatory fibrous hyperplasia (epulis fissuratum) that result from chronic trauma induced by a denture flange. Epulis is more common on the gingiva and alveolar mucosa of the maxilla (64.7%). Fibrous and vascular epulis were located near incisors and canines in 11 patients, premolars in 2 women, and molars also in 2 women.

**Conclusion:** To clarify the diagnosis of epulis, histological study of biopsy specimens is mandatory.

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# Non-invasive periodontal examinations using ultrasonography and artificial intelligence automated processed images

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## Abstract

**Objective:** This feasibility study aimed to evaluate the efficiency and utility of ultrasonographic periodontal examinations, as well as highlight the drawbacks of this method. This study also aimed at reviewing the high-frequency ultrasonography identification of the periodontium, and lowering the operator-dependency of this imaging technique, using 3D modeling and artificial intelligence.

**Methods:** Examinations of pig mandibles, but also of periodontally healthy human patients or gingivitis and periodontitis patients having or not calculus deposits were performed using 20 – 40 MHz standard ultrasound machines with the transducer placed intra-orally or extraoral. 3D ultrasound reconstruction of larger areas of the maxillary and mandibular alveolar process were performed using a 3D ultrasound scanner prototype, for lowering operator dependence.

**Results:** 3,400 annotated periodontal ultrasound images were used for training artificial intelligence models for faster imaging diagnosis. The obtained IoU ranged between 40 and 80% correlated with the size of the masked tissue and the number of annotated images in which it appears.

**Conclusion:** 3D periodontal ultrasonography may help a less experienced operator to generate higher quality datasets in the future. To achieve a higher quality of the results for automatic segmentation, further studies are necessary using larger datasets for training AI models.

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# Influence of *Streptococcus salivarius* on *Aggregatibacter actinomycetemcomitans* in mixed biofilm on dense polytetrafluoroethylene (d-PTFE) membranes

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## Abstract

**Objective:** Analyze the influence of *Streptococcus salivarius* K12 on *Aggregatibacter actinomycetemcomitans* colonization in mixed biofilm on two dense polytetrafluoroethylene (d-PTFE) membrane: Permamem® (Botiss biomaterials, Germany) and Cytoplast® (Osteogenics Biomedical, USA).

**Methods:** An early mixed streptococcal biofilm (*Streptococcus mutans* and *Streptococcus oralis*) with and without *Streptococcus salivarius* K 12 was tested for its inclusion of *Aggregatibacter actinomycetemcomitans* in the mature mixed biofilm by determination of the number of bacteria on both tested membranes in salivarius and non-salivarius mixed biofilm. At the same time, the presence of biofilm matrix production in the mixed biofilm was monitored by using the fluorescent staining method.

**Results:** The total number of bacteria was greater in the salivarius mixed biofilm, with a significant difference on the Permamem® membrane, but the number and the proportion of *Aggregatibacter actinomycetemcomitans* in the mature 72-h biofilm was significantly lower in the salivarius mixed biofilm, regardless of the membrane tested. The presence of *Streptococcus salivarius* increased the biofilm's biomass due to the creation of an EPS-rich matrix and the development of a complex biofilm structure.

**Conclusion:** *Streptococcus salivarius* inhibited the growth of *Aggregatibacter actinomycetemcomitans* in the mixed mature biofilm and helped maintain the physiological balance in the oral biofilm.

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# Impact of hyaluronic acid on free gingival graft shrinkage and keratinized tissue gain: pilot study

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## Abstract

**Objective:** This randomized controlled pilot study aimed to evaluate the impact of hyaluronic acid (HA) on free gingival graft shrinkage and keratinized tissue gain after augmentation procedure.

**Methods:** 14 patients with keratinized tissue width less than 2 mm underwent the surgical procedure. The patients were randomly, examiner-blind divided in two groups: HA and control. Difference between the groups were analysed (ANOVA, *post-hoc* Bonferroni, Mann-Whitney U test).

**Results:** The graft areas were measured in horizontal and vertical dimensions on baseline, 14 days, 1 month, 3 months and 6 months postoperatively. Shrinkage of graft was higher in control group during time ( $19.10 \pm 5.46$ ,  $p < 0.1$ ). There was no statistically significant difference between groups at the same time point. Keratinized tissue gain was statistically significant between the groups 3 months postoperatively ( $p = 0.01$ ). In HA group there was significantly better result of keratinized tissue gain during the whole study period. Changes in gingival recession did not present statistically relevant results in this study.

**Conclusion:** Seems that HA might have positive effects on graft area dimensional changes and keratinized tissue gain pointing to the potential clinical relevance in keratinized tissue augmentation procedures.

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# Lack of physical activity as a risk factor for severe periodontitis: A cross-sectional pilot study

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## Abstract

**Introduction:** Severe periodontitis (SP) is associated with the most common chronic diseases sharing similar lifestyle risk factors, such as poor plaque control, smoking and obesity.

**Objective:** This study evaluated the lack of physical activity (LPA) as a risk factor of SP.

**Methods:** SP patients (stages III, IV) were considered as cases and mild periodontitis (MP) patients (stages I, II) were considered as controls. Along with anamnesis and periodontal examination, patients were given GPAQ questionnaire and underwent Six Minute Walk test (6MWT) for assessment of physical activity. The object of this test was to walk as far as possible for six minutes and total distance was recorded.

**Results:** Out of 50 patients, 38 were diagnosed as SP and 12 had MP. SP patients were less physically active, according to GPAQ (58.3% vs. 72.7%), whereas they had similar scores of 6MWT; both results did not show statistically significant difference ( $P=0.613$ ,  $P=0.674$ ). Logistic regression analysis adjusted for age, gender, smoking and body mass index showed that LPA might be associated with increased risk for SP. This finding did not reach statistical significance based on this sample size.

**Conclusion:** In this pilot study, LPA was not statistically significant predictor of SP. These results should be confirmed on the larger sample size.

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# Papillon-Lefèvre Syndrome in an 8-year-old patient: a case report

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## Abstract

**Objective:** This report aims to present a rare case of Papillon-Lefèvre Syndrome (PLS) in an 8-year-old patient, focusing on clinical manifestations, treatment, and outcomes. Its purpose is to raise awareness among dental professionals to enable early recognition and management of PLS.

**Methods:** An 8-year-old patient with PLS was evaluated at a university clinic. The examination involved a detailed medical and dental history, intraoral and extraoral assessments, radiographic examination, and genetic testing. The patient showed extensive periodontal destruction, including deep pockets, bleeding, and significant loss of clinical attachment. Radiographs confirmed alveolar bone loss. Treatment included periodontal therapy, consisting of oral hygiene instruction, scaling and root planing for bacterial infection control, and systemic antibiotics for inflammation.

**Results:** After treatment, there was a reduction in inflammation, with improved gingival health and reduced bleeding. However, due to the advanced disease stage at diagnosis, the patient experienced premature loss of several primary teeth.

**Conclusion:** Early diagnosis and periodontal therapy are crucial for managing PLS in pediatric patients. The condition can lead to severe periodontal destruction and early tooth loss, necessitating prompt and tailored interventions. Collaboration between dentists and medical specialists is vital to provide comprehensive care and enhance long-term oral health outcomes for affected individuals.

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# MIF promotes periodontal disease associated to pregnancy in a murine model

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## Abstract

**Objective:** Investigate the role of macrophage migration inhibitory factor (MIF) in the exacerbation of pregestational periodontal disease (PGPD).

**Methods:** In this work, we evaluated in a murine model of pregestational PD in BALB/c females WT and MIF<sup>-/-</sup> mice, histological changes and histometric analysis of the clinical attachment loss (CAL), relative expression of metalloproteinase (MMP)-2 and -13 by immunofluorescence and the relative expression of MIF, *tnf-α*, *ifn-γ* and *il-17* by qPCR.

**Results:** Elevated levels of MMP-13, MIF and *tnf-α* were found. These correlated with tissue destruction and increased depth of the CAL in PGPD WT mice, compared with control. Importantly, over-expression of MMP-2 and -13 and *il-17*, abated levels of *tnf-α*, *ifn-γ* and a severe tissue remodeling were found, and no CAL modification in PGPD Mif<sup>-/-</sup> mice.

**Conclusion:** These results suggest that MIF plays an important role in exacerbating the inflammatory pathology of preexisting periodontitis. The absence of MIF down-regulates inflammatory transcripts and decreases insertion loss in PD.

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# How does periodontal treatment affect quality of life: a case series

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## Abstract

**Introduction:** Quality of life is a concept that defines the state of well-being of individuals within society. Oral health, as an integral part of health, have significant impact on individual's quality of life. Various scales have been used to determine the quality of life after treatments. The most comprehensive, accessible, and commonly used scales are the Oral Health Impact Profile OHIP-49 and the short version of OHIP-14.

**Objective:** Evaluate the individuals' initial periodontal conditions using OHIP-49 and the change during the first-month follow-up after the non-surgical periodontal therapy (NSPT) on their quality of life, according to sociodemographic variables.

**Methods:** At the initial clinical and radiographic examinations, four patients diagnosed with Stage 3, Grade B/C periodontitis were selected. Patients were asked information about demographic, social-economic status, and oral hygiene practice. Clinical periodontal parameters were evaluated before and fourweeks after NSPT. OHIP-49 TR questionnaire was answered by patients to assess their oral health quality of life before and four weeks after NSPT.

**Results:** Clinical periodontal parameters and OHIP-49 TR scores have shown a significant change four weeks after NSPT.

**Conclusion:** NSPT lead to a significant enhancement in periodontal health and overall quality of life.

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# Different porosity sizes of titanium meshes influence bone neoformation in GBR: microtomographic and histomorphometric study

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## Abstract

**Objective:** The aim of this study was to evaluate the influence of different sizes of porosity of titanium meshes on the bone neoformation process in defects surgically created in rat calvaria, by means of microtomographic and histomorphometric analyses.

**Methods:** Five millimeters in diameter defects were created in calvaria of 36 rats, that were randomly divided into six groups (6 animals per group): NCOG (negative control), TEMG (Teflon membrane), SPTMG (Small pore titanium mesh), SPMMG (Small pore mesh +Teflon), LPTMG (Large pore titanium mesh), LPMMG (Large pore mesh +Teflon). After 60 days, the animals were sacrificed, and the bone tissue formed was evaluated. The data were compared by ANOVA followed by Tukey post-test ( $p \leq 0.05$ ).

**Results:** Microtomographic results showed that the GMMG group presented the highest value for BV/TV ( $22.24 \pm 8.97$ ), with statistically significant differences for all the other groups except GMMG. Considering the histomorphometric evaluation, groups with only porous titanium meshes showed higher values compared to the groups that used the Teflon membrane and the negative control. SPTMG presented higher values in the parameters of area ( $0.44 \pm 0.06 \text{ mm}^2$ ), extension ( $1.19 \pm 0.12 \text{ mm}^2$ ), and percentage ( $7.56 \pm 1.45\%$ ) of neoformed bone.

**Conclusion:** Titanium mesh with smaller pores showed better results, suggesting a correlation between mesh porosity and underlying bone repair.

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# Interdisciplinary treatment of endo-perio lesions

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## Abstract

**Introduction:** Endo-perio lesions refer to pathological conditions that involve both pulpal and periodontal tissues of a tooth, presenting complex challenges in diagnosis and treatment.

**Objective:** This case report details a successful interdisciplinary treatment of a premolar initially planned for extraction.

**Methods:** Medical history of a 24-year-old male revealed the presence of a deep narrow pocket on the tooth #25, on which root canal treatment (RCT) was previously performed. The patient was referred to an endodontist for a second opinion due to suspected vertical fracture. Clinical and radiographic examination confirmed the diagnosis of endo-perio lesion. Comprehensive treatment included non-surgical RCT, followed by root scaling and planning of the pocket, to eliminate infection and inflammation.

**Results:** After one week, the narrow deep pocket closed. The success of treatment was monitored clinically by the presence of the pocket and radiographically by the size and presence of a periapical lesion. A 6-month follow-up showed no signs of pathology.

**Conclusion:** Non-surgical periodontal therapy combined with RCT successfully resolved the endo-perio lesion of the tooth #25, avoiding its extraction. This case emphasizes the significance of a multidisciplinary approach in managing such cases.

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# Pink tooth: cervical external root resorption

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## Abstract

**Introduction:** Cervical root resorption (CRR) is a type of external root resorption, predominantly in the cervical root region. Although commonly associated with orthodontic treatment, CRR can also result from trauma or inflammation.

**Objective:** To present a CRR clinical case successfully treated with minimally invasive methods due to early detection and diagnosis.

**Methods:** Medical history of a 20-year-old male undergoing orthodontic treatment revealed a pink coloration in the cervical region of the tooth #11. The patient was referred to an endodontist. Clinical and radiographical examination confirmed CRR. Considering the tooth's vitality, it was opted for a minimally invasive treatment. Papilla-preserving flap was performed under local anesthesia, followed by removal of granulating tissue using diamond and carbon steel burs. The cavity was thoroughly disinfected, followed by adhesive bonded restoration with composite. After polishing of the restoration, the flap was fixed with suturing.

**Results:** After one year, the patient remained asymptomatic, with a vital tooth showing no tenderness to percussion and no increased probing depth. Radiographically, no evidence of radiolucency was observed.

**Conclusion:** Regular monitoring of orthodontic patients is crucial for early detection of CRR. Despite its asymptomatic nature, early detection and treatment are essential to avoid endodontic treatment or more severe complications such as increased tooth mobility or tooth loss.

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# Experience in the use of hyaluronic acid in the treatment of periodontal pockets

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## Abstract

**Introduction:** Hyaluronic acid (HA) has an anti-inflammatory, anti-edematous and antibacterial effect in the treatment of gingivitis and periodontitis.

**Methods:** 22 patients with periodontitis II degree aged 34-45 participated in this clinical studies: 12 patients made up the experimental group using hyaluronic acid Hyadeny BG (BioScience, Germany), and 10 patients composed the control group. In each group, conservative treatment was performed (scaling root planning, SRP). Pocket depth (PD) was re-measured after 3 weeks. In 12 patients of the experimental group, after SRP, 0.1 ml of Hyadent BG was injected into PD of 5-7 mm. In the comparison group, only repeated SRP was performed.

**Results:** The PD in patients of the experimental group before treatment was  $5.82 \pm 0.97$  mm, and in the control group it was  $5.78 \pm 0.86$  mm. Three months after treatment, PD in the experimental group was  $5.02 \pm 1.09$  mm. PD after treatment in the control group was  $5.47 \pm 0.94$  mm.

**Conclusion:** The use of the drug Hyadent BG is promising in the treatment of periodontal pockets in patients with generalized periodontitis, which is due to the pronounced regenerative properties of hyaluronic acid.

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# Effectiveness of minimally invasive non-surgical approach in the treatment of severe periodontitis: a prospective study

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## Abstract

**Introduction:** Minimally invasive periodontal approaches can enhance clinical outcomes and reduce morbidity. Minimally invasive non-surgical periodontal therapy (MINST) has not been clearly evaluated as an alternative modality to surgical approaches.

**Objective:** The present study aimed to evaluate the effectiveness of MINST in the treatment of severe periodontitis.

**Methods:** Thirteen patients with 31 defects were included in the study. The teeth were treated by sub-papillary access to the defect, with the aid of magnification loupes. Thorough debridement of the root surfaces was conducted with Gracey minicurettes under local anesthetic. Following debridement, a stable clot was stimulated by natural filling of the defect with blood. Chlorhexidine digluconate was used twice a day for two weeks, and regular supportive periodontal therapy was performed monthly.

**Results:** At 6 months post-treatment, PPD reduction was  $4.37 \pm 0.76$  mm, with all the patients achieving a PPD of  $\leq 5$  mm in the treated site. CAL gain was  $3.34 \pm 2.39$  mm. A statistically significant bone gain of  $2.91 \pm 2.31$  mm was reported.

**Conclusion:** Treatment of periodontally compromised teeth with MINST with subsequent application of a meticulous maintenance protocol can be a viable alternative for invasive procedures. Further studies are needed to evaluate the long-term outcomes achievable with MINST.

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# Development and *in vitro* evaluation of doxycycline and atorvastatin loaded alginate nanoparticles for periodontal pockets

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## Abstract

**Objective:** The aim of the present study was to develop an atorvastatin (AS) and doxycycline hydrochloride (DS) loaded alginate delivery system for local treatment of periodontal disease.

**Methods:** To develop this dual drug delivery system, ionic gelation method was used to synthesized AS and DS loaded alginate nanoparticles (AS/DS-ALG) at various drug weights, such as DS:AS ratio percent w/w, 100/0, 0/100, and 50/50. FTIR spectroscopy and scanning electron microscopy (SEM) were used to determine the chemical structure and surface morphology of the nanoparticles, respectively. The amount of AS and DS from *in vitro* release studies were determined by HPLC method in artificial saliva medium at 37 °C. *In vitro* antimicrobial studies were performed against *Porhyromonas gingivalis*, *Eschericha coli*, and *Staphylococcus aureus*.

**Results:** The chemical structure of AS/DS-ALG was confirmed by FTIR. The distribution of particle size was narrow and uniform, which was measured by SEM. Inhibition zones for AS/DS nanoparticles were measured as 11±1, 15±2 and 37±4 mm for *E. coli*, *S. aureus* and *P. gingivalis*, respectively.

**Conclusion:** The AS- and DS-loaded alginate drug delivery system has been developed as a promising adjunctive therapeutic candidate for periodontal pockets, exhibiting noteworthy *in vitro* antimicrobial efficacy.

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# Smoking thirties: how tobacco & BMI shape the subgingival microbiome

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## Abstract

**Objective:** The aim was to test the hypothesis that both obesity and smoking increase the risk for periodontitis, by disrupting the oral microbiome, albeit in different ways.

**Methods:** 183 subjects were recruited following informed consent, and divided into groups based on body mass index and smoking status. Bacterial 16S rDNA genes were sequenced from subgingival plaque samples. Sequence analyses were conducted using QIIME and PhyloToAST. Metabolomic analysis was performed on saliva samples using NMR spectroscopy. Levels of selected cytokines and adipokines in gingival crevicular fluid were determined by multiplexed bead-based assay.

**Results:** Linear Discriminant Analysis revealed significant group separation between obese smokers and normal-weight non-smokers ( $p = 0.001$ ). Obese smokers were enriched for putative pathogens belonging to the genera *Atopobium*, *Bacteroidaceae*, *Bifidobacterium*, *Dialister*, *Lactobacillus*, *Mycoplasma*, *Prevotella*, *Treponema* and *Veillonellaceae*. Salivary metabolic profiles also differed significantly between obese smokers and normal-weight nonsmokers. Obese smokers presented with higher levels of lactate acid and lower levels of methanol, compared to normal-weight nonsmokers. Significant correlations were observed between these metabolites and bacterial community networks.

**Conclusion:** Obesity and smoking impact the subgingival microbiome in significant and distinct ways, leading to enrichment of specific bacterial species, as well as the metabolites produced by them.

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# Predicting periodontitis severity and complexity from periapical x-rays using deep learning networks

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## Abstract

**Objectives:** Developing a deep learning model for determining severity of periodontitis by measuring the percentage of radiographic bone loss (RBL) on periapical x-rays; and determining complexity of periodontitis by furcation involvement (FI) detection of maxillary and mandibular molars, and type of bone loss (horizontal vs vertical) through periapical x-rays.

**Methods:** Standardized periapical radiographs were taken for periodontitis patients at Jordan University Hospital. Total of 2,643 radiographs were collected from 143 patients, excluding poor quality radiographs, resulting in 2,432 radiographs. Radiographs were categorized into; training set, validation set, and testing. Annotation for the training set was done by labeling bone, root, and crown for the segmentation model. Image analysis model was used to calculate RBL% and determine type of BL. For FI, object detection models were used. 124,389 radiographs were collected, and radiographs with no FI were excluded resulting in 1,500 radiographs.

**Results:** Accuracy for segmentation model was 92%. Accuracy for detection of FI was 90% (mandibular) and 60% (maxillary). Accuracy of bone loss differentiation was 88%. Model can accurately determine the stage of periodontitis according to the amount of RBL.

**Conclusion:** The deep learning model can accurately predict the severity (RBL%) and complexity (FI and type of bony defect) using periapical x-rays for periodontitis patients.

**Funding source(s):** University of Jordan research fund.

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# Non-surgical periodontal therapy in a patient with “burnout syndrome”

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## Abstract

**Introduction:** Periodontitis is a chronic multifactorial inflammatory disease associated with a dysbiotic biofilm and systemic risk factors. Stress may increase susceptibility to periodontal disease, by changing the host's immune response and negative behavior change.

**Objective:** This case report aims to present non-surgical treatment results and positive behavior change in a burnout syndrome patient.

**Methods:** A 49-year-old systemically healthy female, smoker (15 cigarettes/day), was referred due to teeth mobility and significant gingival hyperplasia. The patient reported subjective depression and burnout syndrome-related symptoms, which were not professionally managed or treated. The patient was diagnosed with generalized periodontitis, stage IV, grade C. Treatment followed the evidence-based guidelines. After steps I and II, the patient was prescribed systemic antibiotics. She was also further referred to a restorative dentistry specialist.

**Results:** A significant improvement in clinical presentation and all periodontal indices was observed at reevaluation. The patient's oral hygiene habits significantly improved, and she reported reduced work-related stress. She received further localized corrective treatment (step III) and was enrolled in supportive therapy (step IV).

**Conclusion:** A successful long-term outcome relies on cause-related treatment and systemic risk factor control, including stress. The complex interactions of stress and depression in inflammatory periodontal diseases remain to be further studied and defined.

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# Three free gingival graft techniques for lower anterior incisor recessions

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## Abstract

**Introduction:** FGG as a treatment option for single or multiple teeth recession is generally associated with a significant increase in KT width, but inferior root coverage potential and poor aesthetic appearance.

**Objective:** This case report aims to present three different free gingival graft (FGG) techniques and their outcomes.

**Methods:** Patients were systemically healthy non-smokers. The surgical techniques use were: 1-stage conventional FGG, 2-stage FGG + later CAF, and a novel, modified FGG. After the respective recipient site preparation, a graft was harvested from the palate region, averaging 1.5 mm in thickness and sutured at the recipient site with 5-0 and 6-0 sutures. In the case of the 2-stage approach, the coronal advancement was performed three months following the first surgery.

**Results:** The sutures were removed two weeks after the surgery. The healing was appropriate in all patients at two weeks and at three months follow-up. An increase in KT was observed with all three techniques, however, root coverage varied. All sites presented with chromatic and tissue texture changes.

**Conclusion:** FGG remains one of the surgical treatment options, particularly in the lower anterior regions. Modified FGG may improve root coverage and, thus, decrease the need for second-stage surgery.

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# Short implants as an alternative treatment for areas of implant explantation

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## Abstract

**Objective:** The current case series aims to demonstrate the use of short implant as an alternative treatment to avoid complicated surgical interventions in patients needing explantation.

**Methods:** Three patients complaining of implant related problems referred to Ege University, Faculty of Dentistry, department of Periodontology, were clinically and radiologically examined (All male were in their 50's, systemically healthy, with no allergies or other medical conditions). The implants were in function for more than 10 years and patients didn't comply to recalls. Three implants placed in the patient had bone loss — two implants because of periimplantitis and one implant because of an implant breakage. All the implants were explanted and, after a healing period of 6 week, one short implant for each explanted implant was placed.

**Results:** The healing was uneventful, with no early or delayed complications. The implants were loaded using a standard loading protocol with porcelain fused to metal crowns. The patients were placed on a six-month regular recall program. There was no complaint during the recall period of two years.

**Conclusion:** Within the limits of the current case series, it can be concluded that short implants might be an alternative in cases needing explantation, at least for the short term of two years.

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# Gingival enlargement following cardiac transplant surgery in 44-year-old male patient

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## Abstract

**Objective:** Present a case of 44-year-old male patient with gingival enlargement.

**Case report:** On 2/2/2023, he had a cardiac transplant due to genetic cardiomyopathy. Problem with gingival enlargement appeared three months after surgery. His continued oral therapy was as follows: Amlopin 5mg, Fursemid 40mg, Sandimmun Neoral 250mg, Decortin 20mg, Cellcept 500mg, Valcyte 450mg, Controlloc 40mg, Sinersul F 960mg, Rojazol, Andol 100mg, Heferol te Folacin. The probable cause of gingival enlargement was synergistic action of Ca antagonist (Amlopin) and cyclosporin (immunosuppressive-Sandimmun Neoral).

**Treatment:** Patient underwent non-surgical periodontal therapy. The treatment included scaling, root planing and oral hygiene instruction. Patient was nonsmoker.

**Results:** After 3-months of follow-up, the results were satisfactory and stable. There was no further gingival enlargement visible.

**Conclusion:** Clinical approach should focus on eliminating infection sources and creating an oral environment that facilitates easy plaque control. Through proper diagnosis and treatment combined with good patient cooperation, there was no need for surgical therapy. As additional therapy, patient used antiseptic mouthwash (chlorhexidine 0.12 %) and Amlopine substitution was recommended with similar action and no gingival growth stimulation.

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# Evaluation of gingival crevicular fluid Annexin-A1 levels in periodontal diseases

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## Abstract

**Introduction:** Inflammation is a critical determinant of effective host defense. Annexin A1 (AnxA1) has multifunctional roles in the regulation of inflammatory cells and resolution of inflammation.

**Objective:** The present study aimed to investigate gingival crevicular fluid (GCF) AnxA1 levels in health and periodontal diseases.

**Methods:** In total, 80 systemically healthy individuals were included in this study: 20 with periodontitis stage 3 grade B (P-Stage III/B), 20 with periodontitis stage 3 grade C (P-Stage III/C), 19 with gingivitis, and 21 with clinically healthy periodontium (H). Probing depth, clinical attachment level, plaque index and papillary bleeding index were recorded. GCF AnxA1 levels were analyzed by enzyme-linked immunosorbent assay. Non-parametrical tests were used for statistical comparisons.

**Results:** All clinical parameters were significantly higher in periodontitis patients, compared to healthy individuals ( $p < 0.008$ ). P-Stage III/B, P-Stage III/C, gingivitis and healthy groups had similar GCF AnxA1 total amount ( $p < 0.008$ ). GCF AnxA1 negatively correlated with the all clinical parameters ( $p < 0.05$ ).

**Conclusion:** Low GCF AnxA1 levels in periodontitis might point to the failure of AnxA1 in controlling the local inflammatory response and resolving inflammation and to the possible role of AnxA1 in the pathogenesis of periodontitis.

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# Gene expression in implants: nano-hydroxyapatite and L-PRF impact on osseointegration in osteoporotic rat model

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## Abstract

**Objective:** The present study evaluated the effect of leukocyte- and platelet-rich fibrin (L-PRF) presence, in sham and osteoporotic female rats that received different implant surfaces (nano-hydroxyapatite (Nano) or dual acid-etched surface (DAE)), on gene expression involved in bone metabolism.

**Methods:** One implant of each surface was surgically placed in the right tibia of sham and osteoporotic female rats. Both groups received L-PRF. They were euthanized at 7 and 30 days. The peri-implant bone was harvested to investigate the mRNA expression (Alp, Runx2, Opn, Rank) by real-time PCR.

**Results:** The presence of L-PRF in sham-Nano group increased Alp mRNA at 30 days (Alp (sham-Nano + L-PRF):  $10.11 \pm 2.66$  versus Alp (sham-Nano):  $4.01 \pm 2.66$ ). The L-PRF also increased Alp, Runx2, Opn and Rank mRNA at 30 days only in the osteoporotic-Nano group (Alp (osteoporotic-Nano + L-PRF):  $11.69 \pm 3.90$  versus Alp (osteoporotic-Nano):  $3.25 \pm 3.56$ , Runx2 (osteoporotic-Nano + L-PRF):  $4.49 \pm 2.79$  versus Runx2 (osteoporotic-Nano):  $0.08 \pm 2.79$ ; Opn (osteoporotic-Nano + L-PRF):  $6.76 \pm 2.31$  versus Opn (osteoporotic-Nano):  $1.90 \pm 2.54$ ; Rank (osteoporotic-Nano + L-PRF):  $9.06 \pm 4.12$  versus Rank (osteoporotic-Nano):  $0.01 \pm 2.91$ ).

**Conclusion:** These findings suggest that combining nano-hydroxyapatite and L-PRF may improve healing and osseointegration, enhancing dental implant rehabilitation outcomes.

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# Free gingival graft after excision of gingival papilloma

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## Abstract

**Objective:** To report the procedure of transplanting a free gingival graft into the area of morphologically changed gingiva after removal of a hyperplastic formation, intending to recreate the lost band of attached gingiva.

**Methods:** The clinical examination of a 12-year-old female patient revealed the existence of a clearly limited formation, with a wide base connected to the attached gingiva in the area of tooth #11. At the control examination after supragingival instrumentation, the measured PPD was 4 mm at vestibular site, with negative BOP and plaque index. The operation was performed under local anesthesia, during which excision and fenulectomy were immediately performed. A free gingival graft was taken from the hard palate area and sewn to the place of the removed formation. Instructions were given regarding the proper maintenance of oral hygiene.

**Results:** After the procedure, healing was successfully. The sample was sent for pathohistological diagnosis, which showed that it was part of a papilloma.

**Conclusion:** By transplanting a free gingival graft at the site of removed formation, the existing gingival recession was partially covered and thus created the band of lost attached gingiva, with satisfactory aesthetic result.

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# Covering of gingival recessions with coronally advanced flap surgical technique by Zucchelli, using hyaluronic acid

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## Abstract

**Introduction:** The procedure of coronally advanced flap using the technique first described by Zucchelli achieves a very effective covering of non-inflammatory multiple gingival recessions.

**Objective:** To report a clinical case in which hyaluronic acid was used in addition to the mentioned technique.

**Methods:** Analysis of the patient's orthopantomogram, as well as the measured indexes, showed no signs of periodontitis. The recessions were 3 mm on teeth #21 and #22, and 4 mm on tooth #23. The width of the attached gingiva was 4 mm on tooth #21, 5 mm on tooth #22, and 2.5 mm on tooth #23. The operation was performed under local anesthesia, exposed parts of root surfaces were instrumented with a curette and treated for a period of two minutes with EDTA gel, and hyaluronic acid was applied before suturing.

**Results:** At the follow-up examination after 14 days, healing was successfully, without subjective disturbances. A control examination after 3 and 6 months showed clinical stability and expansion of the attached gingiva zone.

**Conclusion:** With this procedure, recessions can be successfully covered on all teeth in upper and lower jaws, as the technique shows excellent long-term aesthetic results.

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# Expanding the band of attached gingiva around the implant using a free gingival graft transplant

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## Abstract

**Introduction:** The procedure of expanding the band of attached gingiva in an area around the implant is performed with the aim of stabilizing the peri-implant soft tissues, and thus facilitating the maintenance of oral hygiene, which will prevent plaque retention and the possible development of peri-implantitis.

**Methods:** In a 76-year-old patient, the clinical examination revealed the absence of a band of attached gingiva around the implant in the area of the extracted tooth #44. The analysis of the retroalveolar dental image as well as the measured indices did not show any signs of periimplantitis. After supragingival instrumentation was performed, the surgery was performed under local anesthesia, during which the free gingival graft was taken from the area of the hard palate and fixed with non-absorbable monofilament sutures. Postoperative care included rinsing with 0.2% chlorhexidine solution, while avoiding brushing the operated region.

**Results:** At the clinical examination 14 days after procedure, healing was successfully, with no signs of inflammation.

**Conclusion:** At the follow-up examination three months after the procedure, the width of the band of attached gingiva was 3 mm, and the peri-implant mucosa showed great stability.

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# Periodontal status of IQOS tobacco heating device smokers and cigarette smokers

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## Abstract

**Objective:** Determine and compare periodontal status through periodontal indices PPD, GR, TM, FD, CAL, FMPS and FMBS between non-smokers, cigarette smokers and smokeless cigarette smokers (IQOS).

**Methods:** The research was conducted on 66 patients of the KBC Rijeka, Clinic of dental medicine. Patients were divided into three groups: non-smokers, classic cigarettes smokers and IQOS smokers. The procedure involved gathering of the anamnestic data, clinical examination and measurement of periodontal indices: PPD, GR, TM, FD, CAL, FMPS and FMBS.

**Results:** There was a difference in CAL and average PPD between cigarette smokers and non-smokers, as well as between IQOS smokers and cigarette smokers in CAL, but not in PPD. No difference was observed in any of the periodontal indices between the non-smokers and IQOS users. Non-smokers and IQOS smokers had a lower number and lower proportion of teeth with deep periodontal pockets ( $\geq 6$  mm), compared to the group of cigarette smokers.

**Conclusion:** IQOS use demonstrated a less harmful effect on the periodontal status measured by the periodontal indices PPD and CAL, compared to classic cigarettes. No significant difference has been found for PPD and CAL values between non-smokers and IQOS smokers.

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# Salivary mediators involved in the regulation of transendothelial migration of neutrophils in grade C periodontitis

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## Abstract

**Objective:** Salivary levels of Developmental endothelial locus-1(Del-1) Del-1, Growth Differentiation Factor-15(GDF-15), Pentraxin-3(PTX-3) which play a role in the inhibition of transendothelial migration, and salivary levels of Leukocyte function-associated molecule 1(LFA-1), ICAM-1, VCAM-1, P-Selectin, which provide transendothelial migration, were examined to understand the roles of these mediators in the acute inflammatory response and their effects on periodontal pathogenesis.

**Methods:** A total of 40 systemically healthy non-smokers (Clinical Health n=20, Grade C Periodontitis n=20) were included in the study. Clinical periodontal records and unstimulated saliva samples were collected. Cytokine levels were evaluated by ELISA method. A Mann-Whitney U test (unpaired Wilcoxon test) was run to determine whether there was a statistically significant difference between groups, according to the biochemical parameters.

**Results:** Considering the biochemical parameters, the LFA-1 level was significantly higher in the Grade C Periodontitis group, compared to the Clinical Health (p=0.009). On the other hand, it was not observed statistically significant difference between groups of salivary PTX-3, GDF-15, P-Selectin, VCAM, ICAM, and Del-1 levels.

**Conclusion:** According to this study, LFA-1 is more active player during transendothelial migration. Increased inflammatory response might be related to the higher LFA-1 levels in Grade C periodontitis. Further studies with higher number of participants with different grading and staging are required to clarify the pathogenesis of periodontitis.

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# Stackable guides: a predictable way for immediate function

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## Abstract

**Introduction:** Advancements in digital technology and workflow in dentistry aim to provide better and safer solutions for the dentist and patient. Stackable guides are the preferred new method for solving full arch implant cases.

**Methods:** Preparation is key for full arch therapy. The dentist must work closely with the laboratory technician to ensure perfect surgical and prosthetic planning. CBCT scan, intraoral scans and digital photography are needed to start the process. From the available data, the dentist can start the implant position planning. Then, the laboratory technician designs the prosthetic provisional bridge from the implant positions. After combining the two, changes are applied if necessary. In the end, the laboratory technician customises the stackable guide. The guide consists of: positioning guide, implant guide and prosthetic guide.

**Results:** Implants placed with stackable guides are better positioned, have more stability and are more predictable in the long term, compared with those placed freehand. The prosthetic part is done very precisely and individualised for each patient.

**Conclusion:** Stackable guides provide ultimate control of the surgery and speed for the dentist and comfort for the patient. Prosthodontics are much more predictable, functionally and aesthetically better. The downsides are preparation time and learning curve.

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