

**H001****Radiographic assessment of condylar position after Le Fort I osteotomy in asymptomatic temporomandibular joints**

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The research aimed to carry out a prospective radiographic study of condylar position, in eleven randomly selected patients who were referred to the Division of Oral and Maxillofacial Surgery of Piracicaba Dental School – Unicamp, for surgical correction of dentofacial deformity. All patients had the same diagnoses of anterior-posterior deficiency of the maxilla, in which the final surgical goal was the isolated Le Fort I osteotomy to advance the maxilla. The radiographic images were taken at the immediate pre-operative, immediate post-operative (1-2 weeks) and late post-operative (minimum of 6 months) period. Tracings on acetate paper were done for the submento-vertex radiograph, and for the tomographic images, in maximal intercuspation, rest position and maximal opening, for the three periods. Linear measurements were made in the tomographic images, over the posterior, superior and anterior articular spaces. Tomographic images with the tracings were digitized and measured by means of computer software (UTHSCSA Image Tool 3.0), after its adequate calibration. Data analysis was submitted to statistical analysis (ANOVA – 5% of significance). The results were not statistically significant for the linear measurements of the articular spaces in any of the periods, or for the axial angular measurements of the condyles, with significant difference only for the maximal opening, in both sides.

In conclusion, Le Fort I osteotomy for maxillary advancement did not cause any significant changes in this specific group of patients evaluated.

**H002****The influence of bisphosphonates on induced tooth movement and root resorption**

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Bisphosphonates are a class of drugs acknowledged for their ability to prevent bone resorption. The presented study tested the following hypothesis: Could bisphosphonates prevent root resorption once incorporated into dentin or cementum? Eighty-nine Wistar rats (*Rattus norvegicus*, albinus) with and without bisphosphonate medication – alendronate, 1 mg/Kg twice a week – were submitted to induced tooth movement for 0, 3, 5 and 7 days. The orthodontic appliance consisted of a coil spring moving the first left molar forward, supported by the incisor. The rats were divided into 4 groups and submitted to different protocols of medication. The Control Group received no medication whatsoever; Experimental Group 1 was submitted to alendronate intake since intrauterine life until the end of the experiment; Experimental Group 2 received alendronate during intrauterine life until 60<sup>th</sup> day of life. A month after medication interruption, the molars were moved prior to the rats' sacrifice. Experimental Group 3 consisted of a group of adult rats treated with alendronate after the third month of life until the seventh, when dental movement took place. Microscopic and statistical analysis revealed that animals submitted to alendronate therapy presented significantly reduced amounts of root resorption. There was no further influence of alendronate on bone structure during induced tooth movement.

These results allow us to conclude that the use of bisphosphonates, alendronate, to be more specific, may increase the resistance to root resorption during induced tooth movement or even due to traumatism.

**H003****Periodontal effects of rapid maxillary expansion: a computed tomography evaluation**

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This study aimed at evaluating rapid maxillary expansion (RME) periodontal effects by means of computed tomography (CT). The sample comprised 8 girls aged 11 to 14 years, presenting with posterior crossbite. All maxillary expanders were activated up to the full 7-mm capacity of the expansion screw. The patients were submitted to spiral CT scan before expansion and after a 3-month retention period, when the expander was removed. One-millimeter thick axial sections were performed parallel to the palatal plane, comprising the dentoalveolar and basal areas of the maxilla. Multiplanar reconstruction was used to measure buccal and lingual bone plate thickness and buccal alveolar bone crest level by means of the computerized method. Paired *t*-test was used to compare the pre-expansion and post-expansion measurements ( $p < 0.05$ ). Pearson's index evaluated the relationship between initial buccal bone plate thickness and buccal alveolar crest level changes. Results showed that RME significantly reduced the buccal bone plate thickness of supporting teeth and conversely increased the lingual bone plate thickness. RME induced significant bone dehiscences on the anchorage teeth buccal aspect ( $7.1 \pm 4.6$  mm in first premolars and  $3.8 \pm 4.4$  mm in the mesiobuccal area of first molars), mainly in subjects with initial thinner buccal bone plates ( $r = -0.733$ ;  $p = 0.0039$ ).

During RME, the supporting teeth were moved through the alveolar bone and not concurrently with it, leading to buccal bone dehiscences. For this reason, the quality of the keratinized mucosa and the toothbrushing technique should be strictly controlled in patients submitted to RME in order to prevent gingival recession in the long run.

**H004****Orthodontic movement in bone defects filled with xenogenic graft: an experimental study in minipigs**

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This study investigated if it was possible to orthodontically move teeth into a bone defect previously filled with xenogenic graft, with emphasis on the reactions occurring on the teeth and adjacent tissues. Six minipigs (Minipig BR-1) were used. The defect was created at the mesial aspect of the 1<sup>st</sup> permanent molar and was filled with the xenogenic graft at the test side, whereas the opposite side was allowed to heal spontaneously. Three months later, an orthodontic appliance was placed in each quadrant to allow mesial movement of the 1<sup>st</sup> molars. When the experimental teeth were moved into nearly half the defect site, the animals were killed and biopsies of the areas of interest were performed. The mesial roots of the 1<sup>st</sup> molar and adjacent tissues were histologically and morphometrically evaluated. Morphometric analysis to determine the bone densitometry and percentage of root resorption, and the bone loss in height were evaluated with the aid of the image analysis software KS300 (Zeiss®). Data analysis revealed that: 1) the extension of tooth movement was similar for both groups; 2) the percentage of root resorption was lower for the test group (4.16%) compared to the control (6.52%); 3) there was no statistically significant difference between groups as to the bone density; 4) the bovine bone matrix was almost totally replaced by structured bone tissue; 5) the test group revealed a significantly lower loss of bone (2.18 mm) compared to the control group (3.26 mm).

It was concluded that the biomaterial allowed tooth movement, avoided bone loss in height, did not cause significant damage to the tooth roots and was almost entirely replaced by new bone tissue after tooth movement.

**H005****The relationship between dentinogenesis and pulp superficial blood vessels**

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This study aimed to analyze the pulp microcirculation of rats' incisors, continuous growing teeth, in different thirds, and relate it to dentinogenesis. Adult male Wistar rats were used. Mercox® resin was perfused through their ascending aorta. After resin polymerization, the rats' mandibles were decalcified in (10%) HCl, the soft tissues were corroded with (1%) NaOH and the pulp tissue was carefully pulled out from the tooth through an apical aperture. The remaining vascular cast was coated (carbon/gold) and observed in SEM. Descriptive and quantitative analyses were performed to evaluate the diameter and percentage of area occupied by capillaries in the surface pulp vessels network. Data were analyzed by ANOVA and Tukey's test,  $p < 0.05$ . The arrangement of the pulp vascular network showed a fish-net-like configuration with different spaces between the capillaries. In the apical third of the rats' incisors, the superficial vascular network arrangement had three layers (terminal capillary network, capillary network and venular network) and the vessels that were the largest of superficial vessels (5.62 µm) occupied 54.15% of the total area. In the incisal third, the vessels were the smallest (4.26 µm), the area occupied was 39.55% and they were organized as a monolayer of vessels.

In rats' incisors, the percentage of vessels and their diameter are closely related to their activity regarding dentinogenesis.

**H006****Microbial leakage and apical response in dogs' teeth filled with different sealers and exposed to the oral environment**

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This study evaluated the microbial leakage (ML) and apical inflammatory response (AIR) in dogs' teeth filled with different sealers, prepared for post and exposed to the oral environment, testing the hypothesis that there is a positive correlation between these two histological parameters. Sixty-four root canals of 8 mongrel dogs were used. After dogs' general anesthesia and rubber dam isolation of the teeth, canals were cleaned, shaped and randomly distributed into the following groups, according to the sealer used: S26 - Sealer 26 (n = 18); AP - AH Plus (n = 18); RS - RoekoSeal (n = 19); and C - Control, no sealer (n = 9). Root canals were filled by lateral condensation technique. Post space preparation was done leaving 4 mm of filling material in the apical third of the root and let them exposed to the oral environment for 90 days. The dogs were killed and jaw blocks containing each root were histologically processed, using both Brown & Brenn and H.E. staining techniques. ML and AIR were scored from 1 to 4. Results were statistically analyzed using ANOVA, Duncan's *post-hoc* and Spearman correlation. ML and AIR average score values were as follows: S26 (2.44 and 2.50); AP (2.50 and 2.22); RS (1.84 and 2.63); C (2.56 and 3.11). Statistical differences were found between Groups AP and C for AIR ( $p < 0.05$ ).

Although RS showed the lower ML and AP the lower AIR average values after 90-day oral environment exposure, no statistical differences were found among the three sealers tested and no correlation was found between ML and AIR, rejecting the tested hypothesis.

**H007****Sucrose-induced changes in dental biofilm fluid and in whole plaque**

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Dental biofilm formed in the presence of sucrose presents in its matrix high concentration of insoluble polysaccharides (IP) and low concentration of fluoride (F), calcium (Ca) and inorganic phosphorus (Pi). However, the concentration of these ions or their mobilization soon after sugar ingestion or after the interruption of sugar exposure is still unknown. Sixteen volunteers wore, during 3 phases of 15 days, a palatal appliance with 8 enamel blocks, which were exposed 8 times/day to deionized water (DW), 10% glucose + 10% fructose (GF) or 20% sucrose (S) solutions. At the 14th day, treatments with DW or the carbohydrates were inverted. Variables analysed were biofilm acidogenicity (pH), and F, Ca and Pi in the biofilm fluid (after 10 h fasting or 5 min after an acidogenic challenge with 20% glucose), and F, Ca, Pi and IP in whole biofilm. Biofilm pH was significantly lower ( $p < 0.05$ ) for GF and S groups in comparison with DW group, before and after the acidogenic challenge. F, Ca and Pi in the whole biofilm were lower in the GF and S groups than in the DW one ( $p < 0.05$ ), but this effect was not observed in the fluid. After the cariogenic challenge, Ca in the fluid increased and Pi decreased significantly ( $p < 0.05$ ), but F did not change ( $p > 0.05$ ). Ca and Pi in whole biofilm increased significantly 24 h after suspending the carbohydrate treatments ( $p < 0.05$ ), but this was not observed in the fluid ( $p > 0.05$ ). IP were significantly higher in the S group than in the GF and DW groups ( $p < 0.05$ ).

The findings suggest that the changes induced by sucrose in the whole plaque do not reflect in the biofilm fluid. (Support: FAPESP 02/00261-4, 03/07926-4, 04/06624-7.)

**H008****Susceptibility of *Streptococcus mutans* biofilms to photodynamic therapy using a HeNe laser or a LED light**

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The accumulation of bacterial biofilm on tooth surfaces may result in dental caries, one of the most prevalent bacteria-induced human diseases. Previous studies have shown that photodynamic therapy using conventional lasers is able to kill gram positive oral bacteria. However, the antimicrobial effect of toluidine blue O (TBO) associated to a red Light Emission Diode (LED) has not been previously studied. The purpose of this study was to evaluate the antimicrobial effect of toluidine blue O, in combination with either a Helium/Neon (HeNe) laser or a LED light, on the viability and architecture of *Streptococcus mutans* biofilms. Biofilms were grown on hydroxyapatite discs in a constant depth film fermenter fed with artificial saliva, which was supplemented with 2% sucrose 4 times a day, thus producing a typical 'Stephan pH curve'. Photodynamic therapy was subsequently carried out on biofilms of various ages with light from either the HeNe laser or LED using energy densities between 49 Jcm<sup>-2</sup> and 294 Jcm<sup>-2</sup>. Significant decreases in the viability of *S. mutans* biofilms were only observed when biofilms were exposed to both TBO and light, and reductions in viability of up to 99.99% were observed with both light sources. In general, the results showed that the bactericidal effect was light dose-dependent and that older biofilms were less susceptible to photodynamic therapy. Confocal laser scanning microscopy images suggested that lethal photosensitization occurred predominantly in the outermost layers of the biofilms.

In conclusion, photodynamic therapy may be a useful approach in the treatment of dental plaque-related diseases.

## H009 *In situ* evaluation of the effects of CO<sub>2</sub> laser and fluoride dentifrice on caries development in human enamel

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Previous *in vitro* studies have shown that the irradiation of dental enamel by CO<sub>2</sub> laser combined with fluoride reduces the acid reactivity of enamel. However, no *in situ* studies have been performed on this subject. Thus, this study aimed to evaluate the physical and chemical changes promoted by a TEA (transversely excited atmospheric pressure) CO<sub>2</sub> laser, at 9.6 µm, and the combined effects of this laser and fluoride dentifrice use on *in situ* enamel demineralization. Enamel surface changes after irradiation were monitored using FT Raman Spectroscopy and Scanning Electron Microscopy (SEM). In this cross-over study, 17 volunteers wore palatal appliances during two phases of 14 days each, and were submitted to 4 treatment groups, as follows: 1) non-fluoride dentifrice; 2) fluoride dentifrice; 3) laser irradiation plus non-fluoride dentifrice and 4) laser irradiation plus fluoride dentifrice. A 20% sucrose solution was dripped on the slabs 8 X/day and the dentifrices were used 3 X/day. After the experimental period, mineral loss was assessed by enamel cross-sectional microhardness. The Raman spectrum of the irradiated slabs showed a decrease in the organic matrix/phosphate ratio. SEM analyses evidenced that fusion and melting occurred in the enamel. The results analyzed by two-way ANOVA and Tukey's test showed that the mean mineral loss for groups 1 to 4 were 1523.1a ± 939.8, 801.7b ± 533.4, 982.7b ± 445.5 and 235.8c ± 164.3 respectively. Percentage of caries inhibition for groups 2 to 4 was 47, 35 and 84% respectively.

The CO<sub>2</sub> laser treatment inhibits caries lesion in the human mouth, being more effective when combined with fluoride dentifrice.

## H010 Plaque F and Ca concentrations are correlated in areas with different water F levels after placebo or F dentifrice use

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This double-blind, crossover study determined the relationships between plaque and salivary concentrations of fluoride (F) and calcium ([Ca]) in three communities with drinking water with [F] of 0.05 (A), 0.8 (B) and 3.5 (C) ppm during a two-week period. The subjects (aged 8-10; n = 16-20) brushed their teeth with a F dentifrice (FD; 1030 ppm as NaF) during one week and a placebo dentifrice (PD) during the other week. On the 6<sup>th</sup> day of each week the subjects brushed only the occlusal surfaces to allow accumulation of smooth-surface plaque which was collected (half-mouth) the next morning about 12 h after brushing. Then they then brushed the occlusal surfaces for 1.0 min and plaque was collected from the opposite side 1 h later. F in plaque was extracted with 0.5 N HClO<sub>4</sub>, buffered with Total Ionic Strength Adjustment Buffer and analyzed with the electrode. Salivary F was analyzed after hexamethyldisiloxane diffusion. Ca was analyzed by atomic absorption spectrometry. The results were analyzed using repeated measures ANOVA and linear regression (p < 0.05). The average [F] in plaque (± SE; mg/Kg dry wt) for PD and 1 h and 12 h FD were 27.9 ± 4.3<sup>a</sup>, 144 ± 27<sup>b</sup> and 75.9 ± 15.0<sup>c</sup> for A; 41.4 ± 6.2<sup>a</sup>, 172 ± 36<sup>b</sup> and 70.1 ± 12.5<sup>c</sup> for B; and 107 ± 15<sup>a</sup>, 231 ± 43<sup>b</sup> and 115 ± 17<sup>c</sup> for C, respectively. In saliva, a similar trend was observed, except that [F] obtained 12 h after the use of FD were significantly higher than with the use of PD for all communities. Positive correlations (p < 0.01) were found between [F] and [Ca] in plaque in all the situations.

The results confirm our previous conclusion that plaque [F] are dependent on plaque [Ca].

## H011 Biom mineralization of polyanionic collagen-elastin matrices and bone remodeling during calvarial bone repair

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Polyanionic collagen-elastin matrices (PCEMs) are osteoconductive scaffolds that present high biocompatibility and efficacy in the regeneration of bone defects. In this study, the objective was to determine if these matrices are directly mineralized during the osteogenesis process and their influence in the organization of the new bone extracellular matrix. Samples of three matrices, with different negative charge density, were implanted into critical-sized calvarial bone defects created in rats and retrieved 3, 7, 15, 30, 60 days and one year after implantation. Biom mineralization was assessed by the detection of alkaline phosphatase activity, macrophage activity, tracking the elastin, high resolution optical microscopy and transmission electron microscopy. The organization of the new bone matrix was evaluated by image texture analysis techniques, using the Shannon's entropy and the fractal dimension of digital images. This combination of methods revealed that PCEMs were directly biom mineralized by osteoblasts and incorporated into the new bone, and that the eventual removal of PCEMs occurred during the bone remodeling process. The changes in entropy in the different periods of analysis were exponential, and the fractal dimension followed a power law. The evolution of these values revealed that PCEMs were quickly remodeled, showing values increasingly similar to the original bone structure.

These results show that PCEMs have improved biological properties, skipping the biomaterial resorption phase, allowing the faster formation of new bone.

## H012 Analysis of Hunter-Schreger Bands as a Biometric Method of Human Identification

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Dental enamel is characterized by layers of prisms of regularly alternating directions. These successive layers form Hunter-Schreger Bands (HSB), which appear as dark and light bands when viewed with a powerful source of light. In the present study, we evaluated the HSB singularity in human teeth as a biometric-based method for personal identification, since differences in the pattern of HSB in teeth have never been studied. The sample was composed of 265 lower central incisors. The following procedures were performed: the teeth were photographed at low magnification. The contrast of the captured images was increased and then they were analyzed in automated biometric-based identification software (Verifinger Demo 1.4 SDK/Fingersec). The software generated a list of biometric data comparisons with a similarity measurement (minutiae). The storage of database comparisons could be represented with a similarity matrix. We also analyzed the thickness of the bands, since this parameter is very variable and could be used to confirm the identification. The results demonstrated that the pattern of HSB is highly variable, being unique for each tooth. HSB could be observed in 96.7% of the teeth examined. Teeth with 0 or 1 minutiae comprised 4.1% of our sample. In these cases, the distinction could be made by simple visual comparison.

Thus, the biometric measurements of HSB proved to be a valuable method for personal identification, since enamel can resist extreme environmental conditions and the images could be easily obtained. These characteristics make HSB a potentially useful model for personal physical or biological measurements to give a correct description of an individual.

## H013 Radiodensity and hardness of enamel and dentin of human and bovine teeth, varying the tooth's age

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Studies have evaluated the characteristics of hard dental tissues of animal species in order to substitute human teeth usage. This study evaluated the radiodensity and hardness of enamel and dentin of bovine teeth varying the age of animals, and compared them with human teeth. Five samples (1 mm thick) were obtained from bovine incisors, from animals with 20 (G1), 30 (G2), 38 (G3) and 48 (G4) months of age, and from human third molars (G5). The radiographic images were taken with a phosphor plate digital system (Digora). The radiodensity was obtained and Knoop hardness (KHN) was recorded (100 g for 15 s - 5 indentations per sample). Data were analyzed by Kruskal-Wallis test, ANOVA following Tukey's test and Pearson correlation (p < 0.05). The results showed a strong correlation between hardness and radiodensity (r = 0.832; p = 0.000). Radiodensity on enamel was similar for all the groups (G1 = 78.37a; G2 = 77.71a; G3 = 79.38a; G4 = 81.74a; G5 = 75.79a), but bovine dentine presented higher radiodensity than human dentin (G1 = 56.8a; G2 = 57.07a; G3 = 56.55a; G4 = 53.87a; G5 = 44.13b). Enamel was always more radiodense than dentine and also presented greater KHN (p = 0.000). Enamel-KHN and Dentine-KHN showed differences just between G2 and G4/G5 (G1 = 256.88ab; G2 = 228.68b; G3 = 261.04ab; G4 = 274.76a; G5 = 261.6a), and G2 and G4 (G1 = 56.87ab; G2 = 50.85b; G3 = 51.55ab; G4 = 57.44a; G5 = 55.17ab), respectively.

In spite of the strong correlation between radiodensity and KHN, a standard variation behavior when these factors are analyzed in separate was not observed. Bovine teeth characteristics seem to follow a particular randomized variation in radiodensity and hardness, making it difficult to establish a similarity to human teeth when varying the animals' age.

## H014 Biosecurity in public and private offices

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Biosecurity is currently a concern for all health-related services, including dentistry, since infection control has considerable importance. In dental practice, health-related occupations have contact with a great number of individuals who are potentially capable of transmitting pathogens. This study comprised a descriptive evaluation of the universal precaution measures for infection control adopted by dental practitioners working in public and private offices in the city of Araçatuba, SP. Data collection was performed by a quiz with questions about individual and collective protection equipments. The results showed that the use of caps was reported by 55% of the professionals working in the public sector and 90% of those working in the private sector. The use of masks and gloves was reported by all professionals surveyed; nevertheless, glove change between patients was not reported by 40% of the professionals working in the public sector.

There were more flaws in public offices as to the use of protective barriers, since except for the use of gloves, gowns and masks, the frequency of use of those barriers was smaller than at private offices. (Support: Capes.)

## H015 Effect of microwave irradiation on *Candida albicans* cell suspensions

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Microwaves have been used in the disinfection of acrylic resin dentures. However, controversy exists about the lethal effects of microwave radiation on microorganisms. The presence of differences in cell counts and substances released between irradiated and non-irradiated *C. albicans* cell suspensions was evaluated. *C. albicans* (ATCC 10231) was cultured in tryptic soy broth. The cells were centrifuged, resuspended in 400 ml distilled water (10<sup>8</sup> cell/mL) and divided into experimental (irradiated for 6 min at 650 W) and control suspensions (non-irradiated). Cell counts were carried out in Agar Sabouraud dextrose (ASD) plates, Neubauer chamber and with optical density (OD) at 550 nm. The intracellular substances released were quantified by: Microprobe and Sensiprote systems for protein; potentiometry for sodium and potassium ions; Liquiform for calcium ion; OD at 260 nm for DNA. Experiments were carried out in duplicate and repeated 8 times. Data were analyzed by Student's *t*-test or Wilcoxon's *z* test, at a 5% level of significance, comparing coupling data of means. No differences in OD at 550 nm were observed (p = 0.272). All control cells presented membrane integrity whereas all experimental cells seemed ruptured. Viable *C. albicans* were not detected in the experimental ASD plates. The protein-released contents of the experimental suspension were higher than those of the control for Microprobe (p < 0.001) and Sensiprote (p = 0.005) systems. The release of potassium and calcium ions and DNA from the experimental suspensions was higher than that from the control (p < 0.001, p = 0.012 and p = 0.046).

The experimental suspension presented lower values of cell counts and higher values of substance release.

## H016 Distribution of *Streptococcus mutans* genotypes in caries-free and caries-active children and evaluation of acid susceptibility

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*S. mutans* is associated to dental caries. Several genotypes in the same subject could increase the ability of the microorganism to stand environmental stress. This study aimed to evaluate if the specificity of colonization by *S. mutans* genotypes exists, and to evaluate biofilm formation and sensitivity to acids of different genotypes. Eleven caries-free (CF) and 10 caries-active (CA) children were evaluated considering SM levels and number of *S. mutans* genotypes. *S. mutans* isolates were obtained from 4 distinct sites. A total of 339 samples were genotyped with RAPD-PCR. The survival in pH 2.8 and biofilm formation were evaluated. A total of 51 genotypes were identified. Fourteen individuals were colonized by more than one clone. Three genotypes were exclusive to white spot lesion and 4 to biofilm over sound surface. Average susceptibility to acid shock in CA genotypes was 90.63 ± 14.58% and, in CF, 97.01 ± 6.6%. Biofilm formation was 0.96 ± 3.41 for CA and 0.36 ± 0.38 for CF genotypes. No significant differences were observed between the groups, but a high diversity was observed in the CA group. In the CA group, 20% of the genotypes exhibited higher acid resistance, and 17% of the genotypes exhibited high formation of biofilm, whereas these characteristics were rarely found among genotypes from CF subjects (4.5%). No isolate revealed high values for both analyses.

This study revealed that strains exhibiting higher resistance to acids or higher biofilm formation can be more often found among genotypes of *S. mutans* harbored by caries-active than by caries-free subjects. Thus, the greater fitness of these genotypes in a sucrose rich environment may lead to a more cariogenic challenge.

## H017 Osteoclastogenesis induction by a novel *Porphyromonas gingivalis* phospholipid

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*Porphyromonas gingivalis* (Pg) synthesizes several classes of complex phospholipids (PLs) in addition to LPS. However, little is known about the biological effects of these PLs on bone resorption. Mouse osteoclast precursors, including RAW264.7 and bone marrow cells, were stimulated with a novel PL isolated from Pg. Pg LPS, RANKL and a mammalian structural isomer of the PL (iso-PL). Tartrate-resistant acid phosphatase (TRAP) staining and a dentine-pit formation assay were used for the identification of activated mature osteoclasts. PL's counter-ligand expressed on RAW264.7 cells was identified by affinity purification and mass spectrometry-based proteomics, and counter-examined by confocal microscopy. Using Western-blot, signaling pathways triggered by PL during osteoclastogenesis were examined with pharmaceutical drugs and RNAi technology. PL, but not LPS or iso-PL, induced significantly higher number of TRAP+ multinuclear cells from osteoclast precursors than RANKL, along with dentine-pit formation, which was not inhibited by RANKL decoy receptor OPG. Pg LPS, but not PL, induced TNF- $\alpha$  and IL-1 $\beta$  by RAW264.7 cells. Proteomic analyses identified a 110-kDa protein as a ligand for PL. Confocal microscopy revealed the co-localization of PL and its ligand on the surface of RAW264.7 cells. PL induced stronger phosphorylation of p38 MAP kinase than RANKL, supported by the inhibition of PL-mediated osteoclastogenesis with p38 specific blocker SB202190.

A novel P. gingivalis phospholipid that is distinct from LPS represents a new class of RANKL-independent osteoclast differentiation factor.

## H018 Expression of the CLA and alpha4beta1 integrin antigens by T cell lymphocytes in gingival tissues

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This work tests the hypothesis that TCR  $\alpha\beta$  T cells that home to the oral cavity are closely aligned with TCR  $\alpha\beta$  T cells in the cutaneous immune compartment and become activated in response to oral pathogens such as *Porphyromonas gingivalis* (Pg). Using flow cytometry, ELISPOT, lymphocyte stimulation with peptides of Pg, cultural data, clinical assessment and confocal microscopy of biopsy tissue, expression of antigens was assessed. Adult patients with and without chronic periodontal disease were recruited. Peripheral blood and tissue samples from the same patient were collected. Pg was cultured from the site where the gingival sample was taken. Patients with clinical periodontitis were culture positive for Pg. Pg53-kDa peptides, Pg381 strain and staphylococcal enterotoxin B were used to stimulate *ex vivo* derived lymphocytes. Periodontitis patients responded to the peptides and a trend for a strong IFN gamma expression in lymphocytes was observed. We observed that 93.2% were CD4/CD45RO and 92.51% were CD8/CD45RO. These trends were strongest in positive culture patients ( $p < 0.05$ ). The subsets of CD4/CLA, CD8/CLA, CD8/CD29, CLA/CD29, CD4/CD49d, CD8/CD49d and CLA/CD49d cells were elevated in the periodontitis and in the positive culture groups ( $p < 0.05$ ). VCAM-1 and E-selectin were upregulated in the periodontitis group ( $p < 0.05$ ), confirming the hypothesis tested. Confocal microscopy supported the hypothesis that CLA and  $\alpha 4\beta 1$  integrin were predominant in tissue samples.

These results demonstrate that T lymphocytes home to the oral site of mucosal infection are activated by antigens such as Pg. These findings add new information to mucosal immunity. (Support: Fogarty.)

## H019 Dental scientific research in Brazil: quantitative aspects of production

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Scientific research has experienced considerable growth in recent years, expressed by a larger dissemination of specialized journals. This paper aims at finding out the current stage of scientific production in Odontology through the researches presented in the 20<sup>th</sup> and 21<sup>st</sup> Annual Meetings of SBPqO (Brazilian Society for Oral Research). The methodology was based on the mailing of 1,350 questionnaires via electronic mail to the presenters of scientific papers of the last two editions of the SBPqO Annual Meeting. According to the questionnaires, the researches in 2003 were mainly concentrated on the following areas: Periodontology (15.67%), Public Health (14.2%) and Pediatric Dentistry (13.5%). Of the papers presented, 65.15% were quantitative and 34.85% qualitative. The most common methodological illustrations found were related to clinical research (34.1%), followed those related to laboratory research (33.1%). In 2004, the most cited areas were still Periodontology and Public Health, with 19.72% and 14.73% of the total answers. Quantitative researches continued to outnumber the qualitative ones (64.6% over 35.4%). With regard to the answers to the questionnaires sent out that year, the illustrations related to clinical research outdid those related to laboratory research with 40.81% against 25.39%.

From these results, one could claim that a large number of the researches presented in the SBPqO meetings have been predominantly quantitative, with a clear focus on clinical and laboratory studies, and that the areas of concentration most commonly found were Periodontology and Public Health.

## H020 Analysis of Fluoride Concentration in Mother's Milk Substitutes and Dental Fluorosis Risk

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Mother's milk is the ideal food for children's first months of life. However, there are situations that make its consumption impossible and substitutes like cow's milk, infant formula and soy milk are employed. This research aimed to determine the fluoride content of some powder brands of these products and evaluate the possibility of dental fluorosis development by its consumption. Three cans from different batches of 7 brands of infant formula, 8 of cow's milk and 3 of soy milk were obtained. Powder samples were reconstituted in deionized water and analyzed with the specific electrode method, after HMDS facilitated diffusion. Estimations of fluoride daily intake with the consumption of the products were calculated. Infant formulas, cow's milk and soy milk presented respectively  $0.044 \pm 0.038$  to  $0.326 \pm 0.169$  mg F/L,  $0.014 \pm 0.003$  to  $0.045 \pm 0.022$  mg F/L and  $0.253 \pm 0.035$  to  $0.702 \pm 0.071$  mg F/L. Significant statistical difference ( $p < 0.05$ ) was found among cans of different batches in the majority of the analyzed brands. The reconstitution of all products in water with optimal fluoride concentration for consumption during the mineralization phase of primary teeth results in daily fluoride intake above 0.07 mg F/kg body weight/day. The same can be said in regards to one of the types of soy milk analyzed, when children still forming their permanent anterior teeth consumed it.

Therefore, the consumption of these products may cause development of dental fluorosis, depending on the fluoride content of the water used for reconstitution and the fluoride bioavailability. (Support: FUNDUNESP - 87503/2003.)

## H021 In vitro wear of deciduous and permanent teeth - simultaneous abrasion and erosion

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The aim of this study was to evaluate the wear performance differences between deciduous and permanent teeth caused by the association of abrasion and erosion. Fragments of forty human teeth (20 deciduous and 20 permanent teeth) were cast in acrylic rings, polished to expose a flat enamel area, and profiled with MTS 3D Profiler using Capture software. The antagonists (enamel cusps) were made from deciduous and permanent human molars. The specimens were distributed into 4 groups ( $n = 10$ ), according to the type of slurry (Neutral - PMMA, poppy seeds, water; and Acidic - PMMA, poppy seeds, 0.3% citric acid, pH 3.2) and the type of substrate: DN - deciduous neutral; DA - deciduous acidic; PN - permanent neutral; PA - permanent acidic. The specimens were cycled 100,000 times in the OHSU Oral Wear Simulator (1.9 Hz), cleaned and re-profiled. Volume loss ( $\text{mm}^3$ ) was determined using the profiler and Ansur 3D software. The area of the wear facet on the antagonist was also measured. ANOVA and Tukey's test were used for data analysis ( $p < 0.05$ ). Deciduous enamel (DN = 0.027; DA = 0.057  $\text{mm}^3$ ) showed more wear than permanent enamel (PN = 0.016; PA = 0.023  $\text{mm}^3$ ), regardless of the slurry type used. The acidic environment increased the wear rate compared to the neutral environment ( $p < 0.05$ ). The association of abrasion and erosion increased the wear rate for deciduous teeth only ( $p < 0.05$ ). There was no significant difference among the groups for cusp wear. There was no correlation between cusp wear and the wear of the samples.

Deciduous teeth showed greater wear than permanent teeth, and the association of erosion and abrasion increased the wear of deciduous teeth.

## H022 In vitro abrasive wear and microhardness analysis of two dental composites filled with nanoparticles

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One of the most important properties of dental composites is their wear resistance. The presence of nanoparticles in their composition appears to improve this property. The aim of this research was to compare the wear resistance and hardness of two dental composites (EsthetX - Dentsply and Filtek Supreme - 3M), considering the presence of nanometric particles (they present high specific surface area) in their composition. The influence of artificial saliva storage in their properties was also evaluated. Eighteen samples of each material (dimensions  $4 \times 8 \times 30$  mm) were fabricated according to the manufacturers' instructions. Microhardness was measured with a Vickers hardness tester through twelve indentations on three samples of each material (10, 25 and 50 g). The wear test of six samples of each material was performed using a three-body abrasion wear machine (cycles of 50, 200, 500, 1,000 and 1,500, weight-100 g / alumina-1  $\mu\text{m}$ -abrasive agent). Half the samples were stored in artificial saliva during 62 days at 37°C, and the tests were performed again. The data were analyzed by ANOVA and Student's *t*-test ( $p \leq 0.05$ ). EsthetX's hardness, before and after storage in saliva, was 64.3 and 52.2 HV, respectively. Filtek Supreme's hardness was 60.8 and 53.1 HV. EsthetX's wear rate, before and after storage in saliva, was  $3\text{E}^{-7}$  and  $2\text{E}^{-7}$   $\text{mm}^3/\text{N}\cdot\text{mm}$ , respectively. Filtek Supreme's wear rate was  $6\text{E}^{-7}$  and  $5\text{E}^{-7}$   $\text{mm}^3/\text{N}\cdot\text{mm}$ .

For the two tests, EsthetX presented better results than Filtek Supreme. Saliva caused a fall in the superficial hardness of the two materials, although it increased their wear resistance, suggesting that post-cure took place with both materials.

## H023 Effects of potassium oxalate gel/adhesive agent combined application on dentin permeability: an in vitro study

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The treatment of dentin hypersensitivity with precipitation of insoluble salts or application of adhesive agents is usually not successful because precipitates are removed by brushing and current simplified adhesives are permeable membranes. We tested a novel approach to reduce water permeation across dentin treated with combined application of potassium oxalate followed by adhesive resins. Full crown preparations were cut on extracted human molars to expose deep dentin. Roots were sectioned and the pulp was removed. Crown segments were connected to an automatic flow-recording device. Minimum and maximum permeability were recorded after smear layer removal and phosphoric acid treatment, respectively. New smear layer was created and the permeability was measured after the crowns were bonded with Single Bond-3M-ESPE, One UP Bond F-Tokuyama and AdheSe-Ivoclar, either according to the manufacturers' instructions or after treating the acid-etched dentin with a potassium oxalate gel. The results were expressed as a percentage of maximum permeability values. Impressions and epoxy resin replicas of the crown segments were produced for SEM examination. Results showed that no adhesive was able to eliminate the fluid flow through dentin. Two-way ANOVA revealed that for the three bonding systems, the application of potassium oxalate prior to the bonding procedures was the most effective technique in reducing the permeability of the crowns ( $p < 0.05$ ). SEM micrographs showed that transudation of dentinal fluid could be identified on the surfaces of all replicas examined, regardless of the treatment.

The new technique tested was effective in reducing the permeability of bonded dentin.

## H024 Water sorption and solubility of dental adhesive resins

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It has been speculated that the incorporation of more hydrophilic and ionic functional groups into dental adhesives may lead to increased water absorption by such materials. This study evaluated the water sorption, solubility and kinetics of water diffusion in commercial and experimental methacrylate-based adhesives, formulated to be used as dentin and enamel bonding agents. Four commercial adhesives were selected along with their solvent-monomer combination: the bonding resins of Adper Scotchbond Multi-Purpose (MP) and Clearfil SE Bond (SE) systems, and the "one-bottle" systems Adper Single Bond (SB) and Excite (EX). Five experimental methacrylate-based resins of known hydrophilicity (R1-R5) were used as reference materials. Specimen disks were prepared by dispensing the uncured resin into a mould (5.8 mm x 0.8 mm). After desiccation, the cured specimens were weighed and then stored in distilled water for evaluation of the water diffusion kinetics over a 28-day period. Resin composition and hydrophilicity (ranked by their Hoy's solubility parameters) influenced water sorption, solubility and water diffusion in both commercial and experimental dental resins. The most hydrophilic experimental resin, R5, showed the highest water sorption, solubility and water diffusion coefficients. Among the commercial adhesives, the solvated systems, SB and EX, showed water sorption, solubility and water diffusion coefficients that were significantly greater than those observed for the non-solvated systems, MP and SE ( $p < 0.05$ ).

The kinetics of water diffusion in methacrylate-based resins was positively dependent on adhesives' hydrophilicity.

## H025 Antibacterial activity of resin cements with antibacterial agents incorporated

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Nowadays, indirect aesthetic restorations for posterior teeth have been more used due to our patient's requests. One way to decrease the leakage at the teeth/restoration interface could be to incorporate antibacterial agents to resin cements. The aim of this study is to evaluate the antibacterial activity of resin cements with antibacterial agents incorporation by agar diffusion test using *Streptococcus mutans*. Triclosan Irgasan DP-300 and chlorhexidine diacetate were incorporated in different ratios (1 and 3% w/w) into C&B - Bisco® and Fill Magic Dual Cement - Vigodent® resin cements. The incorporation was performed manually using metallic spatula and a glass slab. The components were weighed using an analytical balance. After incorporation, the samples were manipulated according to the manufacturers' instructions and placed on the agar plates inoculated with the bacteria. After 48 hours, the growth inhibition zone was observed and measured. All assays were carried out in triplicate under aseptic technique. Control groups with the resin cements without incorporation and the groups with triclosan incorporation did not show antibacterial activity. The groups with chlorhexidine incorporation presented antimicrobial activity observed by the presence of an inhibition halo. Positive control plates showed inhibition growth in all agar plates. The antibacterial effectiveness was influenced by the chlorhexidine concentration (ANOVA -  $p < 0.05$ ). The groups with 3%(w/w) were more effective than those with 1% (w/w) in both resin cements ( $p = 0.03$ , Student's *t*-test).

C&B and Fill Magic cements showed better antibacterial activity with chlorhexidine incorporation at 3% (w/w) concentration.

## H026 Influence of restorative materials and endodontic treatment on stress distribution in maxillary premolar – 2D Finite Element Analysis

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The aim of this study was to investigate the effect of four restorative materials and presence of endodontic treatment on stress distribution in mathematical models of maxillary premolar. To develop a 2-D mathematical finite element analysis model, an intact normal extracted human maxillary second premolar was selected. The premolar embedded resin block was sectioned on the long axis of the tooth (buccal-lingual direction). An image processing system (Matlab) was employed to detect the boundaries of enamel, dentin and pulp from the section images. Surrounding tooth tissues such as periodontium ligament and alveolar bone were generated by growing the outer surface of the tooth model from 2 mm below the CEJ, processed in CAD design (MechanicalV14). These images were exported to Ansys 7.1. A 2D mathematical linear, elastic and isotropic model was created. The wireframe model was automated step-by-step according to boundaries and mechanical properties (Young's modulus and Poisson ratio) of materials and structures, using 8-node element PLANE183 and 90 N-load. Thirteen models were constructed: one intact, six MOD preparations (restored with amalgam, composite resin, preparation only, and 3 endodontically treated), six inlay preparations (restored with ceromer, ceramic, preparation only, and 3 endodontically treated). Qualitative and comparative analysis of the stress distribution of models was performed.

Within the limits of this study, the authors concluded that reduction of the remaining structure influences the concentration of stress distribution. The material properties and endodontic treatment promoted alteration and concentration of stresses in the subjacent dentin, respectively.

## H027 Influence of light-curing sources on *in vitro* interfacial gap formation of resin composite restorations

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The influence of different light sources on the presence of an interfacial gap in bonded restorations was evaluated using fluorescence microscopy. The combination bonding agent (Adper Single Bond (AD)) / composite (Filtek Z250 (RC)) was used to restore Class I preparations (6.0 x 4.5, 2.5 mm deep) in extracted human third molars, according to the manufacturers' instructions. Restoratives were light-cured using XL3000 (QTH: 540 mW/cm<sup>2</sup>); Elipar FreeLight 2 (LED: 750 mW/cm<sup>2</sup>); and Arc Light II (PAC: 2,130 mW/cm<sup>2</sup>). Nine groups were formed with light combinations to cure AD/RC: QTH/QTH; QTH/LED; QTH/PAC; LED/LED; LED/QTH; LED/PAC; PAC/PAC; PAC/QTH; PAC/LED. Fluorescent agent (Rhodamine B) was added to AD, highlighting the bonded interface. A different color dye (Fluorescein) was placed in the pulp chamber (4 hs), diffusing toward the interface. The teeth were then sectioned and microscopically analyzed (Two-Photon Laser). Qualitative and quantitative evaluations were gap location (scores 1 to 7) and extension (in  $\mu\text{m}$ ). Data were statistically analyzed. No significant difference was found in gap location (Kruskal-Wallis,  $p > 0.05$ ). PAC/QTH and PAC/PAC presented significantly lower gap extension and LED/LED, the highest (two-way ANOVA,  $p < 0.05$ ). PAC/QTH presented more gap-free interfaces than other combinations. Using PAC for AD provided lower gap extension and best scores for gap location. PAC/PAC presented superior results compared to QTH/QTH and LED/LED.

Different light sources influence gap extension. Gap site and extension are more dependent on the polymerization technique used for the adhesive than on the technique used for the resin composite.

## H028 Resin-dentin interface degradation: ultramorphological, physico-mechanical and bond strength evidences

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The aims of this study were: to evaluate the ability of adhesive systems to prevent nanoleakage in resin-dentin interfaces; and to analyze dentin bond strength, water sorption and solubility, ultimate tensile strength, elastic modulus and ultrastructural characteristics of the bonding agents. Adhesive systems that use different etching and infiltration strategies were used: two etch-and-rinse systems (Single Bond/SB and Prime&Bond NT/ PB); two two-step self-etching primers (Clearfil SE Bond/CF and Clearfil Protect Bond/CP); and a one-step self-etching adhesive (One-up Bond F/OB). Tests were performed after 6 or 12 months of storage in water or mineral oil. Ammoniacal AgNO<sub>3</sub> was used as a tracer for the nanoleakage test. Specimens were prepared for Transmission Electron Microscopy (TEM). Bond strengths were analyzed by means of the microtensile test and failure modes were observed. For measurements of water sorption and solubility, the mass of specimens was measured before and immediately after water-immersion, and after desiccation. The volume of specimens was measured, and sorption and solubility values were calculated and expressed in  $\mu\text{g}/\text{mm}^3$ . Ultimate tensile strength and elastic modulus were analyzed in a universal testing machine. Ultramorphological characteristics of bonding agents were observed with TEM. Results showed that no adhesive system was able to prevent nanoleakage. SB and OB were the most susceptible to degradation after storage in water.

Based on the tests performed and parameters analyzed, it can be concluded that degradation of resin-dentin bonds is related to the hydrophilicity of the materials tested.

## H029 *In vivo* study of pulpal healing after pulpotomy using three pulp-capping agents and variable methodologies

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This *in vivo* study was carried out to evaluate the reparative potential of pulps mechanically exposed following pulpotomy with three different capping agents. Calcium hydroxide, Mineral Trioxide Aggregate (MTA), and PROROOT™ MTA were evaluated considering the morphology, location, and extension of the reparative hard-barrier deposition. Three methods were used to evaluate the images from the reparative area (scanning electron microscopy - SEM, stereomicroscopy - Lupe, and optical microscopy). Five 12 month-old Beagle dogs were used. The exposed pulps were capped with the selected experimental or control materials and the cavities were restored with amalgam. After 90 to 106 days, the animals were sacrificed and the teeth surgically removed "en bloc" were processed for SEM assessment. An image analyzer was used to measure the total area of the exposition and compare it to the area occupied by the hard-barrier. Data from SEM were used to evaluate morphology and location of the hard-barrier formation as well as the percentage of pulpal wound obliteration, which was also measured by stereomicroscopy and optical microscopy.

PROROOT™ exhibited the highest number of samples with complete hard-barrier formation when compared to the tested materials. Calcium hydroxide samples presented the lowest number of total repairs and the hard-barrier observed in a few samples exhibited amorphous histological characteristics. SEM evaluation did not allow detailed assessment of the hard-barrier formation such as its extension and location, therefore additional methods are necessary to determine the capacity of pulp tissue to respond to pulp therapy by deposition of a hard-barrier.

## H030 Image Scanner to Acquire Customized Prosthetic Components of Dental Implants

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Recent developments in image-processing techniques allow for some exciting new medical and dental applications, from CT enhancements that greatly increased diagnostic precision to the innovative new use of optical scanners to deliver affordable customized prosthetic goods expeditiously. The downside so far is the high cost of this new equipment. This study proposes a new method for acquiring full 3D reconstructions of prosthetic parts from simple-to-obtain digital images of wax models. Furthermore, data extracted from these images by computer-vision algorithms can later be used to mill final components in compatible CAD/CAM systems. Briefly, a collection of cross-section images is processed to obtain sets of frontier points. They are later combined using proper software to form the finished virtual 3D component, which may then be viewed and edited on compatible CAD program, and subsequently sent for production.

Lab tests have shown great fidelity of digital models to their real models. Successful clinical applications also reinforce our convictions that this system is ready for everyday use. Lastly, given the low cost of this solution, it is hoped that dental labs will use it to provide better care to a larger segment of the population.

## H031 Effect of relining, cyclic loading and water storage on the flexural strength of a denture base acrylic resin

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This study investigated the effect of relining, cyclic loading and water storage on the ultimate flexural strength (FS) and on the flexural strength at the proportional limit (FSpl) of a denture base acrylic resin (Lucitone 550-L). Rectangular bars of L acrylic resin were made (64 x 10 x 2 mm) and relined (1.3 mm) with four relining resins (Kooliner-K, Ufi Gel Hard-U, Tokuso Rebase Fast-T and New Truliner-N). In addition, intact L specimens and specimens relined with L acrylic resin were made (64 x 10 x 3.3 mm). Forty specimens were made for each material, divided into four groups (n = 10) and tested after 1) polymerization; 2) water storage for 30 days; 3) cyclic loading (10,000 cycles at 5 Hz) and 4) water storage for 30 days followed by cyclic loading. Data (MPa) were analyzed with 3-way ANOVA and Tukey's HSD tests ( $p = 0.05$ ). After water storage, L-U and L-T demonstrated an increased FS (41.49 to 50.64 MPa and 49.95 to 57.36 MPa, respectively) ( $p < 0.05$ ). Only L-T demonstrated an increased FSpl (20.58 to 24.21 MPa) after water storage ( $p < 0.05$ ). After water storage, L-T demonstrated higher FS and FSpl than L-K, L-U and L-N. L-L specimens presented the highest FS (between 78.57 and 85.09 MPa) and FSpl (between 31.30 and 34.17 MPa) ( $p < 0.05$ ). Cyclic loading decreased the FS and FSpl of all materials ( $p < 0.05$ ).

Water storage improved the FS of L-U and L-T and the FSpl of L-T. L-L presented the highest FS and FSpl. The FS and FSpl of all materials were detrimentally influenced by cyclic loading.

## H032 Oral lichen planus: extra-hepatic manifestation of hepatitis C

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Studies of prevalence of the association between oral lichen planus (OLP) and hepatitis C conducted in different countries show conflicting results, and some authors consider OLP an extra-hepatic manifestation of the virus C infection. Thus, the aim of this work was to investigate the possible association between OLP and hepatitis C. Following approval by the Ethics Committee of the Federal University of Minas Gerais (UFMG), 50 OLP patients (Group 1) and 215 chronic hepatitis C patients (Group 2) were evaluated. Group 1 patients were submitted to anti-HCV (hepatitis C virus) serological exams for investigation of infection by the hepatitis C virus and Group 2 patients were examined for OLP lesions, which were histologically confirmed. Data collected were analyzed by the Fisher's exact test at 5% significance level. One case of chronic hepatitis C was diagnosed in Group 1 (2.00%), with no significant difference ( $p = 0.464$ ). In Group 2, OLP was diagnosed in five patients (2.33%), which was statistically significant ( $p = 0.002$ ).

In conclusion, our results strongly suggest OLP as an extra-hepatic manifestation of hepatitis C. Thus, we believe that HCV carriers should be periodically and systematically submitted to intra-oral examination for OLP investigation. Moreover, OLP patients in special should be submitted to hepatitis C tests, due to the chronic, asymptomatic and high morbidity character of this liver disease.

**H033****Is daily chewing gum really effective to increase stimulated whole saliva?**

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Subjects with low saliva output can suffer from dry mouth, present increased risk of oral infections, as well as disturbed sleep. The effect of daily chewing gum on the unstimulated whole saliva has been demonstrated. However, it remains inconclusive with regards to stimulated saliva. The aim of this experiment was to evaluate the effect of chewing gum on whole stimulated saliva. The salivary flow rate of a sample of 30 women, with mean age of 57.16 years, was measured. This was done before and immediately after the experiment with chewing gum and at the first day of this within-subject study. The chewing gum was used daily for 15 min after breakfast, lunch and dinner, during three weeks. The data were analysed by descriptive statistic and using paired Student's *t*-test ( $p$ -value  $< 0.05$  was considered significant). The dose-response curve showed that an increase in the saliva output was observed at the moment that the chewing gum was used, for all participants ( $p < 0.05$ ). However, for only 15 of the subjects with initial salivary flow rate up to 0.7 ml/min a statistically significant increase ( $p < 0.05$ ) of 75 % was observed immediately after three weeks of the experiment.

The authors suggest that there might be a persistent increase in the whole stimulated saliva only for subjects with hyposalivation, and an increase can occur for all subjects at the moment of use of chewing gum.

**H034****Comparison of hard tissue density changes around implants by conventional radiographs and digital subtraction images**

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The aim of this *in vivo* study was to compare peri-implant bone density assessed by the mean gray value of the histogram in digitized radiographs and two digital subtraction radiography methods, linear and logarithmic. Thirty-four patients were monitored by standardized periapical radiographs one week after surgery and four months later. The radiographs were digitized and manipulated by means of EMAGO® software. Linear and logarithmic digital subtraction radiographs were obtained, and a filter was added to the logarithmic image. Control and test regions were selected and the mean value of the gray level of the histogram of these selected areas was obtained. This process was carried out in the digitized conventional radiographs (DCR) and the two methods of digital subtraction radiography (DSR). After that, the images were divided into two groups, with and without bone loss, and statistical analysis was carried out. The results indicate that differences between the two groups with and without bone loss reached significance, in all the images and in both jaws, which was expected. But no statistically significant difference was observed when assessing radiographic density between the digitized radiographs and the two methods of subtracted images.

Monitoring of peri-implant bone density by the mean gray value of the histogram in a selected area can be assessed either by linear and logarithmic digital subtraction images or by conventional digitized radiographs.

**H035****Analysis of tooth/restoration interface in dental enamel by using optical coherence tomography**

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The integrity of the tooth/restoration interface is very important for the success of dental treatment. The evaluation of this site can be done using visual and tactile inspection, which can be helped by the use of radiographic exams. These diagnosis methods, however, may not show all the information needed for a final and correct diagnosis. For this reason, alternative techniques have been proposed as auxiliary diagnosis methods in Odontology. This presentation describes the use of Optical Coherence Tomography (OCT) as a method to evaluate the tooth/restoration interface in dental enamel. Five extracted human molars were used in the research. One tooth was restored by the standard way, while in the other samples a gap of ~ 50 µm was purposely left at the tooth/restoration interface. The OCT system built for this research was based on Michelson Interferometer and on a wideband light source. A Titanium-sapphire laser was the light source that was employed operating at 800 nm, allowing 10 µm axial resolution and 32 µm lateral resolution. The results were depicted as bidimensional images.

By using the OCT technique in the mentioned dental evaluation, it was possible to identify the exact location of the restoration failure in the tooth, and also make a quantitative analysis. This was not possible using the conventional methods. The research showed that OCT is a high-resolution non-invasive optical imaging technique and can become an important diagnosis method for Odontology.

**H036****A protocol standardization to be used in photodynamic therapy in animals**

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Photodynamic therapy (PDT) was applied mainly in neoplasia treatments in mucosa and skin surface neoplasia. It is also indicated in cases where surgery and clinical procedures do not respond to conventional treatments. Thus, PDT limited itself to very specific indications and poor prognostic cases, due to a lack of clinical trial studies and its high costs. Nowadays, multidisciplinary studies are being conducted looking for new drugs and light sources that should be able to decrease the side effects of PDT and improve its specificity. With the aim of introducing a PDT standardization protocol for animals, 25 golden syrian hamsters were used in association with a hematophorphyrin photosensitizer drug (HpD), together with a diode compact laser device (630 nm). After establishing an adequate drug dose and a correct pathway of administration, PDT was applied on the left side surface of hamsters' tongues after 12 hours of intraperitoneal administration of 10 mg/kg of HpD in 50, 100, 150 and 200 J/cm<sup>2</sup> fluencies. Histological analyses were performed 6 days later and showed epithelial alterations only at 50 J/cm<sup>2</sup> protocols. Positive correlations were also observed between tissue alterations and fluencies related to necrosis deepness and vascular penetration at 100, 150 and 200 J/cm<sup>2</sup>.

Therefore, with these results, we may give support to clinical trials in animal models of carcinogenesis, analysing the behavior of new drugs and new sources of lights, which should be more effective and economically accessible.

**H037****E-cadherin abnormalities resulting from CpG methylation promoter in metastatic and non-metastatic oral cancer**

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Loss of E-cadherin expression resulting from CpG methylation promoter has not been investigated in non-metastatic oral cancers such as verrucous carcinoma (VC). To further understand the alterations in the methylation profiles of E-cadherin in oral cancer, we analyzed nine cases of VC, twenty well differentiated squamous cell carcinoma without lymph node involvement (SCC-pN0) and seventeen with lymph node involvement (SCC-pN+) by methylation-specific polymerase chain reaction. Immunohistochemical expression of E-cadherin was also performed. The frequency of E-cadherin gene methylation was 55.5% for VC, 60% for SCC-pN0 and 59% for SCC-pN+, and no statistically significant differences ( $p = 0.975$ ) were found among the groups. The immunohistochemical expression of E-cadherin in oral VC was significantly higher ( $p = 0.016$ ) when compared with SCC-pN0 and SCC-pN+ groups. E-cadherin gene methylation was not correlated with its abnormal immunohistochemical expression in oral VC and SCC-pN0 groups. All tumors of the SCC-pN+ group with unmethylated E-cadherin gene showed significant loss of E-cadherin immunoreactivity ( $p = 0.044$ ).

In conclusion, the alteration in immunohistochemical expression of E-cadherin resulting from CpG methylation promoter is common and heterogeneous in metastatic and non-metastatic well-differentiated squamous cell carcinomas. The presence of E-cadherin gene methylation in tumors with lower invasive and metastatic potential as oral verrucous carcinoma suggests the early involvement of this epigenetic event in the multistep progression of oral carcinogenesis.

**H038****Nitric oxide deficiency inducing bone loss *in vivo* via imbalance of cytokines and osteoclastic factor expression**

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The inducible NO synthase (iNOS) derived NO plays important role in inflammation and bone metabolism. The aim of this study was to evaluate the role of NO in the pathogenesis of periapical lesions in mice. Wild-type (WT, C57/BL6) and iNOS knockout (iNOSKO) mice had the pulp of first molars exposed and inoculated with 4 bacterial strains (*P. gingivalis*, *P. nigrescens*, *A. viscosus*, *F. nucleatum*). Contralateral molars were used as control. After 21 days, bone loss was evaluated by histomorphometry and inflammatory mediators expression by real-time PCR and ELISA assay. WT and iNOSKO mice infected showed periapical lesions with intense inflammatory infiltration accompanied by significant bone loss, while the contralateral periodontal ligament was intact. However, in iNOSKO mice, the lesion size was approximately 75% larger than in WT (WT:  $0.12 \pm 0.02$  mm<sup>2</sup>; iNOSKO:  $0.21 \pm 0.06$  mm<sup>2</sup>). In 25% of the iNOSKO mice with larger lesion, abscess in the jaw was present. The increased bone loss in iNOSKO mice was associated with upregulation of osteoclast differentiation factor expression, like RANKL (receptor activator of NfκB ligand) and SDF-1 (stromal cell derived factor-1), and reduction of OPG (osteoprotegerin), an inhibitor of resorption. Furthermore, iNOSKO mice also presented low levels of IL-1β, TNFα and IFN compared with WT.

These results show an important role of NO in the pathogenesis of pulp infection, since NO deficiency was associated with downregulation of pro-inflammatory cytokine production, important to the infection control, and with an imbalance of bone resorption modulator factors, leading to a severe infection-stimulated bone loss.

**H039****Association between p21waf1/cip1 cyclin dependent kinase inhibitor gene polymorphism and oral squamous cell carcinomas**

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P21 is a potential tumor suppressor and has been identified as a p53 mediator and an inhibitor for cyclin-dependent kinases (Cdks) and proliferating cell nuclear antigen (PCNA). Changes in p21 conformation could adversely affect regulation of cellular proliferation and increase the susceptibility to cancer. This study was conducted to investigate p21waf1/cip1 gene alterations and a possible association with immunohistochemical expression of p21 in human oral squamous cell carcinomas (OSCC). Besides, p21waf1/cip1 genetic alterations and p21 immunoreactivity were associated with clinicopathological parameters of oral cancer patients. Matched normal and cancer tissues from 31 patients with oral carcinomas and 50 samples of saliva from healthy individuals were screened for p21waf1/cip1 alterations by PCR (polymerase chain reaction) and SSCP (single strand conformation polymorphism). The PCR-SSCP technique suggested the presence of two polymorphisms in exon 2 and one in exon 3. Sequence analysis confirmed the presence of the AGC (Ser), AGA (Arg) polymorphism in codon 31 of exon 2. This substitution was more frequent in cancer patients (23.3%) than in healthy individuals (12%), which was considered statistically significant. No significant association was observed between p21waf1/cip1 variant at codon 31 and immunohistochemical expression of p21. There was no significant association between p21 expression, the presence of codon 31 polymorphism and clinicopathological parameters of OSCC.

Our results suggest that the polymorphism involving codon 31 of p21waf1/cip1 gene may be directly involved in development and/or progression of oral squamous cell carcinomas.

**H040****Hoxb13 transcripts in oral squamous cell carcinoma**

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The aim of this study was to verify the HOXB13 gene expression in oral squamous cell carcinoma (OSCC) using *in situ* hybridization technique (ISH). Tumoral tissues and adjacent non-tumoral oral mucosa specimens were obtained from 25 patients with OSCC. The samples were cDNA extracted, submitted to the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) technique; direct sequence analysis was performed on Polymerase Chain Reaction (PCR) products to confirm cDNA sequence. Digoxigenin-labeled riboprobes were confectioned for *in situ* hybridization analysis. ISH technique exhibited a consistent and invariable pattern of expression for HOXB13 transcript on both tumoral and non-tumoral tissue samples.

The present results point out to a correlation between HOXB13 expression and cell differentiation, and this may represent a valuable tool to understand the biological behavior of OSCC.

#### H041 Detection of PITX1, PMX1 and TGIF transcripts in oral squamous cell carcinoma by *in situ* hybridization

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Oral squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide. Homeobox gene family encodes transcriptional factors during development; hence it could be involved in carcinogenesis. The purpose of this study was to verify the presence of 3 homeobox gene transcripts (PITX1, PMX1, TGIF) in OSCC and adjacent non-tumoral tissues (NT). Twelve OSCC samples and 8 NT were obtained at HC-FMUSP. For *in situ* hybridization (ISH), serial sections were stained with digoxigenin-labeled sense and antisense riboprobes. Signal was always located at the cell cytoplasm. For NT samples, PITX1 signal was seen at all epithelium layers in 33% of the cases, only at basal layers in 50% and in dispersed cells of the epithelium in 17%. PMX1 stained at basal and supra basal layers in 80% of the samples and at all epithelium layers in 20%. Transcripts of TGIF were stained in 50% of the samples at basal and suprabasal layers, while in 50% of the cases they were disperse, being more intense in the spinous cell layer. In OSCC, all genes were expressed in 100% of the cases. PITX1 staining was weak, although well-differentiated areas expressed more transcripts than poorly differentiated ones. PMX1 showed a spread signal, though more intense in areas with isolated carcinoma cells. TGIF transcripts were visualized in all tumor cells, but in poorly differentiated areas the signal was less intense.

*These data show that PITX1 and TGIF have lower expression in OSCC compared with NT, while PMX1 has a more intense signal. Our results strongly suggest that these genes might be involved in OSCC carcinogenesis.*

#### H042 Stress analysis around implants' external hexagon, internal hexagon and internal conical connectors, and one piece implants using photoelasticity

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This work aims to analyze quantitatively stress distribution around implants. Implants were fabricated with the same external design, differing only at the connectors: external hexagon (EH), internal hexagon (IH), internal conical (IC) and no connection to the abutment (one piece, OP). The samples were embedded in photoelastic models and submitted to two compressive loads: (1) axial, load I; and (2) 6.5 mm away from the center, load II. Sixty-one points around the implants were analyzed. The maximum shear stress values were determined by fringe orders, thickness of the models and optical constant of the photoelastic resin. Graphs were made using the analyzed points, and their areas (distributed stress) were calculated in the two load situations, for the implant body and at the platform. No statistical differences were found for load I (Kruskal-Wallis,  $p < 0.05$ ). For load II, when analyzing the body of the implant, IH presented significant difference (Mann-Whitney U,  $p < 0.05$ ), with the lowest values. The analysis of the platform region under load II revealed that IH presented significant differences when compared with EH and OP, with the lowest values, but with IC did not present difference (Mann-Whitney U,  $p < 0.05$ ). The IH group presented the best results, with the lowest values, and the EH the worst, with 12.9% more stress than IH for the implant body and 17.4% more stress at the platform region.

*It could be concluded that for axial load the connectors did not influence stress distribution. For the load away from the center, the IH implants presented the best stress distribution, whereas at the platform region the IC also presented favorable results.*

#### H043 Evaluation of hexagon integrity of internal torque implants after simulating surgical placement under different torque levels

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In the controversial discussion about external and internal hexagon implants, the prosthetic predictability of the first and the easiness of surgical placement of the latter have been shown. A product available in the market with both qualities can mean an important technique improvement. The aim of this study was to evaluate the integrity of the external hexagon of a conventional implant (EH) and of a new implant (Internal Torque – IT, Neodent, Curitiba, Brazil), which is inserted by internal torque, without the need of a mount. A device was made to measure the rotational freedom degrees between hexagons of implant and abutment. Measures were obtained for EH and IT with the intact implant and after simulating surgical placement under 45, 60 and 80 Ncm torque levels. Data were submitted to Student's *t*-test, showing no significant difference ( $p < 0.05$ ) for the degrees of the intact implant: EH ( $3.31 \pm 0.41^\circ$ ), and IT ( $3.14 \pm 0.17^\circ$ ); and after 45 Ncm torque: EH ( $3.27 \pm 0.38^\circ$ ), and IT ( $3.20 \pm 0.26^\circ$ ). However, after 60 Ncm torque there were significant differences ( $p < 0.05$ ): IT ( $3.53 \pm 0.30^\circ$ ), and EH ( $4.03 \pm 0.54^\circ$ ). After 80 Ncm torque, IT showed values of  $3.59 \pm 0.38^\circ$ , whereas EH did not support the highest torque.

*It could be concluded that the IT implant reacted similarly to the EH implant when intact and after 45 Ncm torque, and better than the EH implant after 60 and 80 Ncm torque. Furthermore, the external hexagon of the EH implant became deformed after 80 Ncm torque, showing that the surgical placement of the IT implant does not influence the integrity of the junction implant/abutment.*

#### H044 Fc gamma receptors IIa and IIb polymorphism in Brazilians with generalized aggressive periodontitis

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This study aimed to determine the frequency of the Fcγ<sub>2</sub>RIIa and Fcγ<sub>2</sub>RIIb haplotypes in Brazilians with generalized aggressive periodontitis (GAP) and periodontal health (PH). Thirty-one GAP subjects and 49 individuals with PH were selected. Pocket depth, clinical attachment level, bleeding on probing (BOP) and supragingival biofilm (SB) were recorded at 6 sites/tooth for all subjects. Mouthwash samples were collected for human DNA isolation. The genetic polymorphism was detected by PCR and hybridization with oligonucleotide probes. Differences in clinical parameters and frequency of allotypes/haplotypes between the groups were analyzed by Mann-Whitney, Chi-squared, and Configurational frequency analysis. GAP patients presented significantly more attachment loss as well as BOP and SB ( $p < 0.001$ ) than healthy individuals. The alleles H/131 - Fcγ<sub>2</sub>RIIa and NA1 - Fcγ<sub>2</sub>RIIb were the most prevalent ones in this population. There was an over-representation of NA2 in GAP patients, whereas NA1 was more detected in PH individuals (OR: 32.5; 95% CI: 10.6 – 99.8;  $p < 0.001$ ). No significant differences in the distribution of the H/H-131, H/R-131 and R/R-131 haplotypes were observed between the groups. The prevalence of NA2/NA2 was significantly higher in GAP patients, while NA1/NA1 was predominant in the PH group ( $\chi^2 = 45.1$ ;  $p < 0.001$ ). The NA2/NA2-H/H-131 genotype was more frequently observed in GAP patients than expected from marginal frequencies ( $\chi^2 = 12.5$ ;  $p < 0.001$ ; configurational frequency analysis).

*The data suggest that the NA2 allele and/or NA2/NA2 haplotype may be associated with GAP, and the NA1 and/or NA1/NA1 haplotype with PH in Brazilians.*