

## Poster

### P-01

#### Effect of Adhesive System Application for Cavity Prepared with Erbium, Chromium: Yttrium Scandium Gallium Garnet Laser on Rat Dental Pulp

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We examined the effects of experimental adhesive systems applied for a laser-cut cavity using an Er,Cr:YSGG laser on rat dental pulp at 24 h and 14 days postoperatively. Laser-cut cavities were treated with a self-etching primer and bonding agent (group 1); pretreated with a phosphoric acid, and then treated with a self-etching primer and bonding agent (group 2); pretreated with a phosphoric acid and sodium hypochlorite, and then treated with a self-etching primer and bonding agent (group 3); and treated with an all-in-one adhesive (group 4). A flowable resin composite was used as filling material for each cavity treated with each experimental adhesive system. A glass ionomer cement was used as a control. The following items were evaluated: pulp tissue disorganization, inflammatory cell infiltration, tertiary dentin formation, and bacterial penetration. The results were statistically analyzed using the Kruskal-Wallis test for differences among the experimental groups at each postoperative period and Mann-Whitney U test for differences between the two postoperative periods. No significant differences were observed among the experimental groups for all parameters at 24 h and 14 days postoperatively ( $P > 0.05$ ). The pretreatment on the cavity prepared with the laser using phosphoric acid or sodium hypochlorite did not affect the dental pulp healing of rat tooth. On the parameter of tertiary dentin formation, all experimental groups showed significant differences between the two postoperative periods ( $P < 0.05$ ). The majority of the specimens showed disorganization of the odontoblastic layer with edema formation at 24 h postoperatively; however, all the specimens demonstrated pulpal healing with tertiary dentin formation at 14 days postoperatively.

### P-02

#### Change of Dentin Surface by Low-power Irradiation of Er:YAG Laser

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Er:YAG laser is suitable for removing both intact and carious dental hard tissues. Recently, low level laser therapy (LLLT) is introduced for dental medicine. For LLLT, diode laser, carbon dioxide laser and Nd:YAG lasers have been often used. However, there are very few research about LLLT using Er:YAG laser. The purpose of this study was to observe the morphological change of dentin surface by low-power irradiation of Er:YAG laser *in vitro*. The roots of extracted human molars were slightly ground to obtain flat surfaces and polished by silicon carbide papers and diamond pastes. An Er:YAG laser (Erwin AdvErl Evo, J.Morita, Kyoto, Japan) was irradiated using a quartz tip (C600F, J.Morita) with 30mJ, 10pps without water spray coolant. The power density of the laser irradiation was controlled by means of changing the distances between the dentin surface and tip keeping from 0 to 40mm at every 5mm. The irradiated specimens were trimmed, dried and gold coated. The irradiated surface and the cross cut surface of each specimen were observed using a scanning electron microscope. In the specimens of distance of less than 15mm, ablation of dentin was observed on irradiate surfaces and cross cut surfaces. At the distance from 20mm to 30mm, the dentin surface was degenerated and partially melted without ablation, and dentinal tubules were occluded. At the distance of more than 35mm, any morphological change was not observed on the irradiated surfaces and cross cut surfaces. If the dentinal tubules were occluded without ablation by Er:YAG laser irradiation, the dentin may be able to be protected from the irritation which cause dentinal hypersensitivity. Therefore, this treatment by Er:YAG laser is expected to reduce dentinal hypersensitivity. Further study is necessary for establishing the procedure. It was concluded that the power of Er:YAG laser affected the morphological change of dentin surface irradiated with low power.

### P-03

#### Disinfectant Effect of Antimicrobial Photo Dynamic Therapy (a-PDT) on Infected Dentin Model

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Antimicrobial photodynamic therapy (a-PDT) utilizes ac-

tive oxygen (singlet oxygen), generated by a photosensitizer with irradiation of a diode laser or an LED. The purpose of this study was to evaluate the sterilization effect of a-PDT on infected dentin model.

Thirty-three extracted human molar teeth, coronal part, were sliced into 66 quadrangle-shaped dentin plates (3.0 mm width, 1.0 mm thickness) then sterilized by autoclave. All dentin plates were immersed in *S. mutans* solution (ATCC27175, optical density 0.3), incubated in 37°C, 5% CO<sub>2</sub> atmosphere for 3 h, then centrifuged for 10 min at 2,000 rpm to provided bacteria into dentinal tubule. All specimens were divided to 6 groups (n = 11), different sterilizations were performed as following procedures; group 1, laser irradiation (eZlase 940, Biolase, CA, US) for 60 s; group 2, 0.01% Methylene blue (MB) application for 5 min; group 3, 0.01% Azulenocyanine (Azc) application for 5 min; group 4, a-PDT using 0.01% MB (laser irradiation for 60 s after MB application for 5 min); group 5, a-PDT using 0.01% Azc (laser irradiation for 60 s after Azc application for 5 min); and control (no sterilization). After accomplished each treatment, the specimens were transferred to the solution of 10mM sterilized phosphate buffered saline (sPBS), and then *S. mutans* were detached from the dentin plates into the solution of sPBS using ultrasonic homogenizer (UR-200P, TOMY SEIKO). The solution of sPBS was diluted by ten-fold serial dilution method, and 0.1 ml aliquots of each dilution were plated onto brain heart infusion (BHI) agar. After the BHI agar was incubated for 48 h at 37°C and 5% CO<sub>2</sub> atmosphere, the colony-forming units per milliliter (CFU/ml) was determined.

Statistical analyses were performed by Kruskal-Wallis test and the Steel-Dwass test as a post-hoc test for differences among the experimental groups at a significant level 0.05. There were significant differences among the experimental groups except between group 2 and 5 ( $p < 0.05$ ). The number of colonies of each group 2, 3, 4 and 5 was less than that of control ( $p < 0.01$ ). The number of colonies of group 4 was significantly less than that of other experimental groups ( $p < 0.01$ ). Every treatment showed the sterilization effect for the infected dentin plates.

However, the degree of the effect was different each other. The a-PDT using MB was the most effective in sterilizing the infected dentin model.

#### P-04

##### Study on Sealability of Dentinal Tubules With CO<sub>2</sub> Laser Using a Model of Hypersensitive Dentin

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**Purpose:** In recent years, the number of patients with tran-

sient dentin hypersensitivity to cold water and abrasion pain without dental caries has been increasing. In the treatment of dentin hypersensitivity, the laser is used for a purpose of LLLT with the low power laser and the sealability of dentinal tubules. The purpose of this study was to evaluate laser irradiation conditions suitable for the treatment of dentin hypersensitivity on the sealability of dentinal tubules using a model of hypersensitive dentin.

**Methods:** The lasers used in this experiment were Bel Laser and Panalas C05Σ as a CO<sub>2</sub> laser. The dentin discs were prepared from extracted bovine anterior teeth as hypersensitivity model samples. The samples were applied to a device produced by modifying that reported by Pashley, and the pulpal pressure was determined to be 25 mmHg. The motor driven XY stage was moved at speed of 1.0mm/s. The laser was irradiated with BP mode 2.0 W (Bel Laser) or Σ mode 2.0 W (Panalas C05Σ) to the sample surface from a distance of 0 or 2 mm, and dentin permeability was measured. Statistical analysis was performed by two-way analysis of variance and Scheffe's method.

**Results:** The dentin permeability inhibition rate was 77.2 ± 10.3 % and 51.6 ± 10.8 % at the irradiation distance of 0 mm and 2 mm about Bel Laser. The dentin permeability inhibition rate was 57.5 ± 14.0 % and 50.5 ± 8.1 % at the irradiation distance of 0 mm and 2 mm about Panalas C05Σ. The dentin permeability inhibition rate of the irradiation distance of 0 mm with Bel Laser was significantly higher than the irradiation distance of 2 mm with Bel Laser and 2 mm with Panalas C05Σ ( $p < 0.05$ ).

**Conclusion:** These results suggest that symptoms of dentin hypersensitivity may be relieved by the near contact irradiation of CO<sub>2</sub> laser.

#### P-05

##### Evaluation of the Effects of Er: YAG and Er,Cr: YSGG Dental Laser Systems on Human Dentin Tissue

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**Purpose:** The aim of this study was to evaluate the dentin surface after application of different surface modification techniques (37% phosphoric acid etching, Er:YAG laser, Er:YAG laser with X Runner handpiece, Er,Cr:YSGG laser).

**Materials and Methods:** Two hundred and forty human molar teeth with similar dimensions were used in this study. Occlusal third of the crowns were sectioned under water cooling. All specimens were randomly divided into five groups of 24 teeth each according to the surface modifica-

tion techniques tested. Group 1 (Control): no etching applied, group 2: 37% orthophosphoric acid applied, group 3: Er:YAG laser irradiation with H02 handpiece (15 sec, 120 mJ/pulse, repetition rate 10 Hz, 100  $\mu$ s pulse duration), group 4: Er:YAG laser irradiation with scanning handpiece (X Runner, Fotona) group 5: Er,Cr:YSGG laser irradiation (Waterlase IPlus, Biolase) 15 sec, 130 mJ/pulse, repetition rate 30 Hz., 60  $\mu$ s pulse duration. After surface modifications, self etch adhesive (SE Bond, Kuraray) and composite resin (Majesty Posterior, Kuraray) were applied. One part of the specimens were thermocycled for 5000 cycles, the other part of the specimens were aged in chewing simulator for 100.000 cycles. The surface roughness measurement, surface analysis with Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and  $\mu$ tensile bond strength of composite resin to dentin surfaces were evaluated. The obtained data were evaluated by analysis of variance and paired samples t test.

**Results:** The surface roughness values were increased in all groups except control group ( $p < 0.05$ ). The highest roughness increase was detected in laser etched groups. The etching pattern affected the  $\mu$ tensile bond strength ( $p < 0.05$ ). The highest  $\mu$ tensile bond strength values were achieved in Er:YAG laser irradiated specimens. There were no significant differences between 37% phosphoric acid etched and Er,Cr:YSGG laser irradiated specimens. There were no significant differences between the aging methods ( $p > 0.05$ ).

**Conclusion:** After Er:YAG Laser irradiation with scanning handpiece, a more homogenous surface pattern was detected with SEM and AFM evaluation than manual Er:YAG laser irradiation, however there were no significant differences between  $\mu$ tensile bond strength values.

## P-06

### A Comparative *in vitro* Study of Heating Intrapulpal Applying Blue and Violet Light in Tooth Whitening

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The application of light tooth whitening has been widely studied in modern dentistry. One of the concerns when applying light tooth whitening is the intrapulpal heating vitalized tooth can cause damage to dental pulp. The aim of this

*in vitro* study is to quantify the intrapulpal heating during tooth whitening using LED violet (410nm) and blue LED (450nm). Three premolars extracted for orthodontic treatment purposes were selected, were sectioned their crowns, inserted a digital thermometer (Termopar ET-2082C; Mini-pa, São Paulo, SP, Brazil) into the pulp chamber, the teeth were irradiated with violet LED devices (200mW/cm<sup>2</sup>) (Bright Max Whitening-MMOptics, São Carlos, SP, Brazil) for 30 minutes fractionated (30 sec, light – 1 min, standby) and Blue LED (80mW/cm<sup>2</sup>) (Bright Max-MMOptics, São Carlos, SP Brazil) and 35% hydrogen peroxide gel for 10 continuous minutes. The results show an intrapulpal heating 8.5°C after 10 minutes by applying blue LED and 2°C after 30 minutes with violet LED. We can conclude that the violet LED when used in the protocol of this experiment does not heat the inside of the pulp chamber and not may cause damage to dental pulp.

## P-07

### Laser Teeth Whitening

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**Purpose:** To discuss the application of laser on teeth-whitening.

**Materials and methods:** A 26 year-old female who had 11 discolored due to its dead pulp. Irradiate diode laser onto the bleaching gel on the basis of general peroxide bleaching.

**Result:** The whiteness of 11 improves remarkably.

**Conclusion:** Laser bleaching improves the whiteness and perceived aesthetic appearance of tooth tissue.

## P-08

### The Application of Laser In Dental Trauma

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**Purpose:** To research the application of laser in pulp capping and adhesion of fractured crown fragments.

**Materials and methods:** An 11 years old male child who broke his 11, satisfies the definition of complex crown fracture. Diode laser irradiate onto the pulp, then capping the pulp with MTA. Irradiate The erbium laser onto the exsomatized crown. Bond the exsomatized crown onto onto the tooth.

**Result:** Achieved remarkable success in pulp capping and shape recovering of traumatic tooth.

**Conclusion:** Diode laser is not only hemostatic but also an-

tiseptic. Erbium laser energy over 200mJ could drill obvious holes on enamel and dentin. Laser could increase indications, simplify treatment courses, shorten treatment time, reduce the use of medicine, give the patient relief from pain. Finely processes, no need to anesthetize. It's good application future in dentistry shows us the potency to replace the tooth drill someday.

#### P-09

##### Apicocurretage Using Er:YAG Laser

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This paper reports six cases who were recurrent after root canal therapy and received Er:YAG laser on fistula.

**Methods:** Six subjects who were diagnosed as chronic apical periodontitis with fistula and did not wish to have apicectomy were divided into Group A; four cases recurrent after root canal therapy and Group B; two cases recurrent after apicectomy.

Under infiltration anesthesia, the distance between the fistula and the lesion was determined with gutta-percha point, and then laser was irradiated at 80 mJ, 25 pps for 30 seconds with an Er:YAG (Osada E1 fine 400) laser .

**Results:** Group A; In three of four cases recurrent after root canal therapy, several irradiation eradicated fistula. One case with no improvement had root fracture.

Group B; In two cases recurrent after apicectomy, fistula was disappeared after one or two irradiations.

**Discussion:** Er:YAG laser eradicated fistula with several irradiations on periapical lesion. As the case with no improvement had root fracture, preoperative detailed examination would be necessary. From those results we consider laser irradiation on fistula reached periapical biofilm and affected periapical lesions. We will report results of long-term follow-up of those cases.

#### P-10

##### Kinetics of Cavitation Bubbles during Diode Laser-activated Root Canal Irrigation

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**Purpose:** To investigate the kinetics of vaporized cavitation bubbles during laser-activated irrigation by diode lasers.

**Materials and Methods:** Thirty five standardized plastic canal models (Thermafil TrainingBloc, Dentsply) filled with distilled water were used. The lasers used were; (1) an 810 nm diode laser (LIGHTSURGE 3000, Osada, 2W, 40 mJ/50 pps) with a normal tip (SAT09, Osada; LS group) or the normal tip coated with carbonized porcine soft tissue (LS-heat tip group) ; and (2) a 980 nm diode laser (Alta MLS, DPI, 2W, 45 mJ/44 pps) with a normal tip (DS1-200, DPI; AL group) or with a computer-controlled carbonized tip (TOP TipTM; AL-heat tip (TOP) group). The tip was positioned at 9 mm from the bottom of the model, and irradiation was repeated 7 times for 5 seconds each (n = 35). Non-irradiated canals served as control. The kinetics of vaporized bubbles in distilled water was recorded with a high-speed camera (VW-9000, Keyence), and the number and diameter of the bubbles were analyzed. Data were statistically analyzed using one-way ANOVA and Tukey-Kramer test at a significance level of 0.05.

**Results:** The mean number of bubbles in the LS-heat tip and AL-heat tip (TOP) groups was significantly larger than that in the control, LS and AL groups ( $p < 0.05$ ). The mean diameter of the bubbles in the AL-heat tip (TOP) groups was significantly smaller than that in the AL group ( $p < 0.05$ ).

**Conclusion:** Under the condition of this preliminary study, diode laser-activated irrigation with heat tips generated larger number of vaporized bubbles than that with normal tips. Further investigation is necessary on the effect of the number and diameter of vaporized bubbles on the cleaning efficacy.

## P-11

### Visualization of Substructures of Resected Apical Root Surface by Swept-source Optical Coherence Tomography

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**Purpose:** In endodontics, apicoectomy is performed for the management of apical periodontitis when orthograde root canal treatment is not feasible. At the surgery, the resected surface of the apex is usually evaluated using dental operating microscope (DOM), although the subsurface structure cannot be observed. Recently, a new diagnostic imaging technique, optical coherence tomography (OCT), has been developed to depict subsurface anatomical structures. The purpose of this study was to investigate whether subsurface structures of the resected apical root surface of the maxillary premolar can be visualized with OCT.

**Methods:** Five extracted human maxillary premolars were used. Three millimeters of the apex was removed by a low speed saw perpendicular to the long axis of the tooth. The resected surface (baseline) was treated with EDTA, irrigated with saline solution and scanned with a swept-source OCT system at a 1330-nm center wavelength and a 20-kHz sweep rate. Then, the resected apex was further cut twice from the baseline surface in an approximately 0.5-mm increment, and images of the methylene blue dye-stained surface after each cut were obtained with DOM. For evaluation with OCT, the cross sectional images were reconstructed by 3D image processing software and horizontal slice images were drawn at the same level as the section with DOM. Finally, the images obtained from OCT and DOM were compared and evaluated.

**Results:** The resected dentin thickness after the first and second cuts was 0.58-0.68 mm and 0.91-1.19 mm, respectively, from the baseline. The OCT images at the first cut level clearly depicted the substructures, such as the root canal and isthmus, that were seen in the corresponding DOM images. In the OCT images at the second cut level, however, the substructures were indistinct, especially in specimens in which 1.17-1.19 mm had been cut from the baseline.

**Conclusion:** OCT enabled to generate clear horizontal tomographic images of the substructures that were present approximately 0.5 mm inside the resected apical root surface, which suggests that OCT is useful for the real-time examination during apicoectomy. However, clear OCT images were not obtained for substructures that exist at approximately > 1-mm deep.

## P-12

### The Effect of Low-level Laser Therapy on Bone Regeneration —Evaluation by Micro Ct and Histological Assessments—

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**Purpose:** Because of its pleiotropic effects, such as anti-inflammation, pain relief, acceleration of wound healing, low-level laser therapy (LLLT) has gained enormous attentions and been applied to many clinical applications. Among them, LLLT has intensely used for the bone fracture treatment. However, the precise basic mechanisms of these effects remain unknown. In the field of endodontic treatment, apical root amputation has been applied to refractory infected root canals. In the present study, the alveolar bone defect model was established and the effect of LLLT on the bone regeneration process was examined.

**Materials and Methods:** Eight-week-old Wistar rats weighing 180 to 200 g were used. Under a systemic anesthesia with pentobarbital, the oral mucosa was surgically cut and the surface of alveolar bone was exposed. An artificial bone defect was generated at the apical region between the first and second lower molars. All experimental animals were divided into LLLT treated and untreated groups. This region was treated with or without laser (0.1 W) for 40 sec according to the protocol of Khadra. LLLT was applied once a day up to day 6. After treatment, regenerated bone volume and bone density was measured by *in vivo* X-ray micro computed tomography at day 7, 14, 21 and 28 and analyzed qualitatively and quantitatively with i-View (Rigaku). Histological sections of the treated areas on each experimental day were prepared from both groups, and stained with hematoxylin for histology and with anti-vWF for immunohistochemistry.

**Results:** As results, early bone regeneration was observed in LLLT treated group at day 7, but no differences were observed between LLLT treated and untreated groups. Bone regeneration was clearly observed between day 7 and day 14. After this time point, the accelerated bone regeneration was observed in LLLT treated group based on bone volume and bone density evaluation. Histological examination confirmed this difference clearly. At day 7, the defect was filled with granulation tissue with bleeding in the untreated group. On the other hand, newly generated micro vessels and thin bone trabeculae were observed in LLLT treated group. After day 14, thick bone trabeculae were observed comparably in both groups. Immunohistochemically, the number of vWF-positive micro vessel was much abundantly observed in LLLT treated group compared to the untreated group.

**Conclusion:** These findings suggested that LLLT could ac-

celerate bone regeneration on the initial healing stage of the apical root amputation.

### P-13

#### Hydroxyl Radical Generated from Hydrogen Peroxide and Blue Light Suppressed *Porphyromonas Gingivalis* in Biofilms

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**Purpose:** Periodontal diseases are inflammatory diseases caused by groups of selected bacteria such as *P.gingivalis* and *P.intermedia*. These bacterias contain black pigment that is sensitive to blue light (BL). The hydroxyl radical ( $\cdot\text{OH}$ ) produced from BL and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) can cause cellular damage. This study aims to evaluate the use of BL and  $\text{H}_2\text{O}_2$  in the treatment of *P. gingivalis* biofilms. The  $\cdot\text{OH}$  produced from BL and  $\text{H}_2\text{O}_2$  were also studied by using the fluorescence probe technique.

**Materials and Methods:** *P.gingivalis* biofilms were prepared from a subject with chronic periodontitis. A BL source laser with the wavelength of 405 nm and power density of 50 mW/cm<sup>2</sup> was used. 1%  $\text{H}_2\text{O}_2$  (v/v) was used to inhibit the *P. gingivalis* in comparison to BL treatment and also the combined treatment of BL and  $\text{H}_2\text{O}_2$  on *P. gingivalis* were assessed. The survival fractions of *P. gingivalis* were then determined at 0, 3 and 6 h after processing.

**Results:** All groups resulted to reduction in CFU count of *P. gingivalis* biofilms. BL +  $\text{H}_2\text{O}_2$  exhibited the best long-term effect. Fluorescence probe revealed that more  $\cdot\text{OH}$  group was produced when  $\text{H}_2\text{O}_2$  + BL than  $\text{H}_2\text{O}_2$  alone.

**Conclusion:** The BL +  $\text{H}_2\text{O}_2$  is more effective against *P. gingivalis* biofilm than the individual treatments done in solitary.

### P-14

#### Antimicrobial Photodynamic Therapy Using a Laser Transmitted Through Soft Tissue

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**Purpose:** Recently, growing attention has been paid to antimicrobial photodynamic therapy (aPDT) in periodontal treatment. aPDT is a therapeutic method for the disinfection of bacteria by singlet oxygen which was generated from a photosensitizer (PS) excited to a light with a specific wavelength. Our group has reported on the fundamental research of aPDT with an 810nm diode laser and indocyanine green-loaded nanosphere coated with chitosan (ICGnano/c) for periodontal treatment. One of the problems in the use of aPDT for periodontal treatment is difficulty of access to lesions via periodontal pocket due to the morphological complexity of bone defect and furcation. Because the diode laser with a wavelength of 810nm used in our research has a high tissue permeability, it might be possible to use for treatment of lesions difficult to access by irradiation from the outside of periodontal pocket through gingiva. Therefore, in this study, we designed a gingival model for the irradiation from outside of the periodontal pocket and examined its suppression effect on periodontal bacteria.

**Materials and Methods:** We prepared four Bacterial solutions of *Porphyromonas gingivalis* ( $2 \times 10^8$ CFU/ml). Two were mixed with ICG-Nano/c (10mg/ml) which was set in the gingival model and the other two were irradiated with the 810nm diode laser (RPT mode, output power: 2W, duty cycle: 50%, pulse width: 100ms, exposure duration: 3minutes). The CFU were subsequently counted after 7 days of anaerobic culture.

**Results:** The aPDT treatment (ICGnano/c + laser) group compared with other groups showed a significant decrease in CFU. And the aPDT treatment group showed a CFU reduction 99% against the control group.

**Conclusion:** A suppression effect against bacterial culture of aPDT through an energy transmitting tissue model was demonstrated and thus validity of outer irradiation in aPDT for periodontal disease was suggested.

**P-15****Fundamental Assessment of Irradiation Method for Anti-microbial Photodynamic Therapy (aPDT) Using a Laser Transmitted Through Soft Tissue***Yuki Iwamura<sup>1</sup> (Japan)**Jun-ichiro Hayashi<sup>1</sup>, Yasuyuki Sasaki<sup>1</sup>, Kousuke Okada<sup>1</sup>, Takeki Fujimura<sup>1</sup>, Eisaku Nishida<sup>1</sup>, Makoto Aino<sup>1</sup>, Takeshi Kikuchi<sup>1</sup>, Hiromitsu Yamamoto<sup>2</sup>, Akio Mitani<sup>1</sup>, Mitsuo Fukuda<sup>1</sup>*<sup>1</sup>Department of Periodontology, Aichi Gakuin University<sup>2</sup>School of Pharmacy, Aichi Gakuin University

**Purpose:** Previously, we had reported *in vitro* and clinical study of antimicrobial photodynamic therapy (aPDT) with an 810nm diode laser and indocyanine green-loaded nanosphere coated with chitosan (ICGnano/c) for periodontal treatment. Because the diode laser with a wavelength of 810nm used in our study has a high tissue permeability, it might be possible to use for treating furcation involvement which is difficult to access via the periodontal pocket. Therefore, to confirm that the permeable energy of this laser irradiated from outside periodontal pocket through gingiva is suitable for aPDT, we designed the gingival model, and investigated irradiation conditions and cooling method.

**Materials and Methods:** In this study, we used the 810nm diode laser: OSADA LIGHTSURGE SQUARE.

At first, for the design of the gingival model, we compared the permeable energy through 3mm slices of beef, pork and chicken .

Secondly, we investigated suitable irradiation conditions through 3mm slices of beef for aPDT.

Third, for the investigation into the possibility of whether or not aPDT using permeable energy is possible, we examined whether ICG-Nano/c solution absorbed permeable energy through 3mm slices of beef.

Finally, we compared the four cooling methods: 1) the control group: 2) the intermittent illumination (10 seconds rest after 60 seconds laser irradiation) group: 3) the air irradiation group and 4) the air + intermittent irradiation group.

**Results:** The permeable energy of beef was significantly small when compared with pork and chicken.

Irradiation conditions where permeable energy becomes suitable for aPDT in periodontal treatment was 1W (output power: 2W , duty cycle: 50%).

Further more, we found that ICG-Nano/c solutions significant decreased permeable energy.

The air + intermittent irradiation group showed less than a 10°C temperature rise which was more than 20°C less than the control group.

**Conclusion:** It was suggested that aPDT by irradiation from outside of the periodontal pocket through the gingiva is possible.

**P-16****A Short-term Evaluation of Nd:YAG Laser as an Adjunct to Scaling and Root Planing in Treatment of Drug-influenced Gingival Enlargement***Jing Su<sup>1</sup> (China)**Dongqing Wang<sup>2</sup>, Xinlin Wang<sup>2</sup>*<sup>1</sup>Municipal Center for Quality Control & Improvement on Dental Healthcare, Beijing Stomatological Hospital, Capital Medical University<sup>2</sup>Department of Periodontics, Beijing Stomatological Hospital, Capital Medical University

**Objective:** The aim of this study was to investigate the efficacy of Nd:YAG in adjunct to scaling-root planning (SRP) for the treatment of Drug-influenced gingival enlargement.

**Methods:** Twelve patients were recruited. Mandibular left or right side was randomly assigned as the test- (SRP with laser treatment) and control-side (SRP alone). The water-cooled Nd:YAG laser was used at four Watt, 80 millijoule per pulse, 50 Hertz. Clinical parameters, such as probing depth (PD), plaque index (PLI), bleeding index (BI), were recorded before and 3 months after treatment.

**Results:** At the three-month follow-up, both sides showed significant improvement in clinical parameters. The test side showed greater reduction in PD than control side.

**Conclusion:** In short-term, SRP in combination with a single application of a water cooled Nd:YAG laser significantly improves clinical signs compared to treatment by SRP alone in the in treatment of Drug-influenced gingival enlargement.

**P-17****The Effect of Er: YAG laser for Periodontal Regenerative Therapy Using the Enamel Matrix Protein***Toshiaki Shibutani (Japan)**Tadashi Yasuda, Atsuhiko Yamamoto*

Department of Periodontology, Asahi University, School of Dentistry

**Purpose:** The purpose of this was to study the effects of Er: YAG laser treatment assists for the periodontal regenerative therapy using the enamel matrix protein.

**Materials and methods:** The patients visited in Asahi University Hospital with complain for periodontal treatment. Patients were received initial periodontal treatment including the plaque control, scaling, root planning, and occlusal treatments. After reevaluation, the patients were agree with the periodontal surgery and regenerative therapy using the enamel matrix protein. The experimental lesion were reflected the gingival flaps and removed the soft tissue. For control group were treated the root surface with ultrasonic scaler. The experimental group were treated the root surface with Er: YAG laser. Then, both groups were treated for root surface with tetracycline. The alveolar bone defects and root surface were added the enamel matrix protein. The gingival

flaps were sutured. After 6 month, the surgical lesion were evaluated the clinical conditions.

**Results:** The clinical conditions of both groups were improved. The soft tissue conditions were not different significantly in both groups. The ratio of alveolar bone regeneration in the experimental group were higher than that in the control group.

**Discussions and Conclusions:** The Er: YAG laser treatment of root surface may be effective for periodontal regeneration using the enamel matrix protein.

#### P-18

##### Ten Year Non Surgical Periodontal Treatment Protocol with the Adjunctive Use of Diode Laser Monitoring Clinical Outcomes in $\geq 6$ mm Pockets: a Retrospective Controlled Case Series

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**Purpose:** The clinical effects of treatment with 808 and 980 nm diode lasers as an adjunct to a uniform and consistent nonsurgical periodontal treatment protocol (NSPTP) for initial probing pockets  $\geq 6$  mm, with a 10-year follow-up were evaluated.

**Materials and Methods:** In total, 24 patients (16 test cases and 8 control cases) were treated with the NSPTP. The control group (CG) was treated with only nonsurgical conventional therapy, while the test group (TG) received treatment with thermal diode lasers (wavelength of 808 or 980 nm). All patients had recall visits every 3 months and were re-evaluated 1-year post-NSPTP. Following the 1-year follow-up, patients were monitored at 4-month intervals for the remaining 9 years.

**Results:** In total, 646 teeth (433 in TG [125 multi-rooted teeth, 309 single-rooted teeth] and 213 in CG [64 multi-rooted teeth, 149 single-rooted teeth]) were included. The initial probing pocket depth (PPD) in single-rooted teeth from TG patients decreased from an average of 6.7 mm to 3.3 mm after 12 months, and to 2.3 mm after 10 years. The mean increase in clinical attachment level (CAL) was 3.8 mm. The initial PPD of single-rooted teeth in CG patients decreased from an average of 6.4 mm at baseline to 4 mm after 12 months and 3.3 mm after 10 years. The mean increase in CAL was 2.5 mm in CG. In TG, the PPDs of multi-rooted teeth decreased from 6.2 mm to 3.3 mm after 12 months, where they remained stable for 10 years. The mean increase in CAL was 2.2 mm in this group. In CG, the PPDs for multi-rooted teeth decreased from 7 mm to 5.8 mm after 12 months and were 5.1 mm after 10 years. The mean in-

crease in CAL was 1.9 mm. TG had significantly better clinical parameters than CG, including reduced tooth loss.

**Conclusions:** Compared to conventional debridement alone, the use of thermal diode lasers with NSPTP improves PPD and increases the CAL in periodontal pockets ( $\geq 6$  mm) over the long term.

#### P-19

##### Clinical Investigation of Carbon Dioxide Laser Treatment for Lingual Leukoplakia

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**Purpose:** Because leukoplakia is often clinically diagnosed without any histopathological examination, current opinion regarding treatment methods and preoperative histopathological diagnosis is inconsistent and no standard site-specific treatment guidelines exist for oral leukoplakia. The purpose of this study is to clinically evaluate carbon dioxide (CO<sub>2</sub>) laser treatment for leukoplakia of the tongue.

**Materials and Methods:** We examined 38 lesions in 35 patients of lingual leukoplakia who underwent CO<sub>2</sub> laser treatment with using Lugol staining at our hospital over a 10-year period, i.e., from 2001 to 2010. We investigated gender-related differences, age distribution, left and right differences, clinical classification, histopathological diagnosis (presence or absence of epithelial dysplasia), treatment technique (excision or evaporation), recurrence rate, residual rate, and malignant transformation among these cases.

**Results:** Regarding the breakdown of the 35 patients, 21 were males and 14 were females. The age range was 27-88 years, with an average age of 61 years. Approximately 80% of the patients were aged 50 years. All 38 lesions in the 35 patients occurred on the tongue, with 13 lesions involving the left lingual margin, 22 in the right lingual margin, and 3 in the center of the dorsum. Following the clinical classification according to Amagasa et al., 23 lesions were clinically classified as the white-spotted type, 11 as the hillock type, 3 as the erythroleukoplakia type, and 1 as the unknown type. All lesions were histopathologically examined by total excision or local biopsy before treatment. Epithelial dysplasia was observed in 22/38 lesions (57.9%); dysplasia was absent in 15 lesions and presence or absence of dysplasia could not be confirmed for 1.

With regard to the relationship between the clinical type and the presence of epithelial dysplasia, 15/23 white-spotted lesions (65.2%), 5/11 hillock-type lesions (45.5%), and 1 erythroleukoplakia-type lesion (33.3%) showed epi-



thelial dysplasia.

CO<sub>2</sub> laser was applied during excision of 35 lesions and evaporation of 3. All patients who underwent evaporation also performed local biopsy before surgery. Recurrence occurred in 4/38 lesions (10.5%), all of which were excision cases. Three of the 4 recurrence lesions showed no dysplasia. In contrast, 3 lesions (7.9%) were residual. Two of these were excision cases, and 1 was an evaporation case.

**Conclusion:** Our results suggest that the CO<sub>2</sub> laser excision method with using Lugol staining, as performed at our hospital, is useful for the treatment of lingual leukoplakia.

## P-20

### Clinical Investigation of Oral Vascular Malformations by Multiple Photocoagulation with Nd:YAG Laser

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**Purpose:** The aim of the present study was to report the effectiveness of oral vascular malformations treated with Nd:YAG (neodymium-doped yttrium aluminium garnet) laser photocoagulation. The Nd:YAG laser, with a wavelength of 1064 nm in the near-infrared part of the electromagnetic spectrum, has unique characteristics, such as absorption by oxygenated hemoglobin, negligible absorption by water, and deep depth of penetration, all of which are ideal for performing the photocoagulation of vascular malformations. The majority of vascular malformations have a diameter 15 mm, and treatment is completed with a single application in many cases. For the small number of lesions with diameter 15mm, multiple applications are performed. However, there are no clear guidelines for the number of applications or application intervals, and there have been few reports regarding this from other medical facilities. We hereby report the clinical investigation of 7 patients who underwent multiple photocoagulation for the treatment of a vascular malformation.

**Materials and methods:** 7 patients diagnosed with oral vascular malformations were treated with a noncontact Nd:YAG laser. The subjects comprised 2 males and 5 females, aged between 23 and 75 years (mean age: 53 years). They were examined at our department from 2007 to 2016. The 7 lesions were 15 mm in diameter and included 3 tongue lesions and 4 lower lip lesions. The equipment used was an Nd:YAG laser (InPulse Dental laser; Incisive Japan Co., Ltd., Tokyo, Japan). The procedure involved performing local anesthesia on the area surrounding the lesion followed by laser photocoagulation with an output of 7-15 W, while maintaining a distance of 10mm between the fiber tip and the lesion.

**Results:** Lesion size was 23-45 mm (mean: 35.4 mm). The number of applications was 2-6 (mean: 3.8). The treatment period was 2-26 months (mean: 14 months). All lesions were successfully treated with multiple irradiation exposure. No patient developed serious complications from this treatment.

**Conclusions:** We were able to obtain satisfactory results without clinical complications, using only external irradiation. These results suggest that multiple photocoagulation with Nd:YAG laser is effective for the treatment of oral vascular malformation.

## P-21

### Reduction of the Postoperative Infection Using Biore-sorbable Osteosynthetic Materials Considering of Influence of Bacterial Adhesion and Effective with 405nm Blue-violet Laser Irradiation

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**Purpose:** Bioresorbable osteosynthetic materials composed of poly-L-lactic acid (PLLA) have been widely used and its benefit in the clinical practice is substantial. However, some cases of postoperative infection or abscess formation after surgery have been also reported. The mechanism underlying this postoperative infection has not been elucidated in detail, nor have an effective prevention been established yet. The purposes of this study are to make the mechanism of the postoperative abscess formation clear from the viewpoint of adhesion of bacteria onto the material surfaces of PLLA alone (PLLA) and forged composites of unsintered hydroxyapatite particles and PLLA (HA-PLLA), and to evaluate the usefulness of the 405nm blue-violet laser irradiation as an effective method to prevent the postoperative infection.

**Materials and Methods:** Suspension of *Staphylococcus aureus* FDA 209P (106 ~ 8 colony forming units/mL) were inoculated onto the surface of discs of PLLA or HA-PLLA. After culturing, the disks were washed under various conditions. Then, bacterial adhesion was observed by scanning electron microscope.

**Results:** SEM observation showed the difference in surface condition between the two materials, that is, the surface of HA-PLLA was much smoother than that of PLLA. Besides, greater colony formation was noted in the PLLA group compared with the HA-PLLA group. Next, to examine the sterilization effect of 405nm blue-violet laser light, the surface of HA-PLLA discs was irradiated at 0.2 W for 300 or 600 seconds. The irradiation with a 405 nm laser had a significant

bactericidal effect.

**Conclusion:** From these findings, it was confirmed that once the surface of material composed of PLLA or HA-PLLA has been contaminated with bacteria, a certain amount of the bacteria will remain fixed onto the surface even after washing, followed by the bacterial colony formation on the material surfaces. The results also strongly imply the possibility that sterilizing and disinfecting material surfaces just before closing the wound and endowing material surfaces with antibacterial properties may lead to effective prevention of postoperative infections. The present study clearly demonstrates that 405 nm blue-violet laser irradiation is an alternative management strategy for prevention of postoperative infection using bioresorbable osteosynthetic materials.

## P-22

### Treatment of Oral Vascular Malformations Using a Long Pulsed Nd:YAG Laser

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**Purpose:** Over the past 30 years, laser therapy has found its place among treatment strategies for vascular anomalies, and several types of laser, including Argon, Nd:YAG, KTP, and dye lasers, have been used for treating vascular lesions. The Nd:YAG laser is known for its high tissue penetration and scattering of laser light with a blood vessel coagulation effect.

Recently the devices and oscillator of the laser has been innovated for aiming effective and less-invasive treatment. A long pulsed Nd:YAG laser has excellent properties. A pulse duration is limited about 30-50ms to avoid heat accumulation and its high peak power can induce the high tissue penetration effect.

We reported three cases of vascular malformation of oral cavity using laser photocoagulation of a long pulsed Nd:YAG laser with a contact cooling device which was treated with a long pulsed Nd:YAG laser.

**Materials and Method:** Two cases of venous malformation and a case of capillary malformation in the oral cavity were treated with a long pulsed Nd:YAG laser under local anesthesia. The lesions were located at sites including the lip, tongue, buccal mucosa. All lesions was treated by non-contact superficial transmucosal irradiation in single-pulsed wave mode, in which the laser energy was delivered in a multiple spot fashion.

**Results:** All cases showed regression and treatment outcomes were satisfactory without serious complications, such

as deep ulcer formation, scarring, bleeding, severe swelling, and paresthesia.

**Conclusion:** These results suggest that the approach using the Long Pulsed Nd:YAG laser is a promising technique for an effective and less invasive treatment of vascular malformation in the oral cavity.

## P-23

### Management of Primary Squamous Cell Carcinoma of the Lower Lip with Autofluorescence Detection and Quantic Molecular Resonance Scalpel

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**Purpose:** Oral squamous cell carcinoma (SCC) is the sixth most common malignant tumour, with an incidence of more than 500.000 cases per year.

In most cases, radical resection of SCC with free margins at histological evaluation is still considered the best therapeutic approach.

Auto fluorescence (AF) uses natural fluochromes which are located within the epithelium and the submucosa and which are excited when irradiated with specific wavelengths. Such a property may be useful in guiding the surgeon during the resection of epithelial malignant tumours.

The quantic molecular resonance (QMR) scalpel is an innovative and versatile instrument which can safely be used in oral surgery, also for removal of SCC.

Here we report an example of management of a patient with SCC of the lower lip through perilesional a AF detection and quantic molecular resonance (QMR) scalpel.

**Material and methods:** A 78 year-old man with a no-healing dishomogeneous red and white lesion on the lower-lip of approximately 1 cm in its larger size was referred to the Center of Oral Medicine, Pathology and Laser surgery of the University of Parma, Italy. The lesion was present since the last 6 months. Working diagnosis included SCC of the lower lip. The patient was otherwise healthy. Further work-up excluded the presece of locoregional or distant metastases (Stage I).

The lesion was excised under local anesthesia through a wedge excision. Clinical margins were determined through the use of the AF detection. Removal of the lesion was performed with the sole use of a RQM scalpel either for cutting and coagulating.

**Results:** Histopathologic evaluation confirmed the presence

of a SCC of the lower lip completely removed. The patient is alive and well after an admittedly short follow-up of one year.

**Conclusion:** The method described here (AF and QMR scalpel) is a safe procedure for the management of selected cases of Stage I SCC of the oral cavity.

## P-24

### Single Application of Photobiomodulation Therapy after Lower Third Molars Surgery: Randomized Controlled Clinical Trial

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**Purpose:** Photobiomodulation therapy (PBM) has shown benefits in multiple or everyday protocols for controlling postoperative inflammatory distress after third molars surgery. However, there are not established protocols of single applications. This trial aimed to verify the outcomes of a single intra or extra oral PBM application in controlling edema, trismus and pain after lower third molars surgery.

**Material and Methods:** Twenty healthy patients of both genders, 18 to 35 years old underwent bilateral lower third molar removal. The same surgeon performed the surgeries under local anaesthesia within 50 minutes. For pain-memory-washout the second surgery was performed with a month interval. Each side to be operated was randomly allocated to intra oral (IL) or extra oral (EL) group. Immediately after tooth removal the IL group received PBM with GaAlAs laser (660nm, 100 mW, 0.028cm<sup>2</sup> spot size, 107J/cm<sup>2</sup>, 30s, 3J per point), on 4 intra oral points (occlusal, lingual and buccal tooth socket surfaces and the retro molar region). EL group received PBM with same laser irradiation parameters, except by the wavelength that was 808nm on four extra oral points (insertion, origin and two midpoints of Masseter muscle). Controls (placebo) were the contra lateral side of each group where the laser device was kept off. Postoperatively, all patients received the same analgesic medication. Edema was evaluated by measuring the distances between standard anatomical facial points using millimetre tape. Mouth opening was obtained by measuring the distance between the incisors using a calibrated calliper in three moments: preoperatively, 24 hours and 7 days after surgery. A visual analogical scale (VAS) measured pain after 4, 6, 8, 24, and 48 hours after surgery. Data were submitted to one way-ANOVA- with a significant *p* value (*p* < 0.05).

**Results:** Sixteen out of 20 patients completed bilateral sur-

geries and postoperative controls. There were no differences in postoperative pain level and edema. The mouth opening after 7 days was significant higher in EL group (*p* = 0.034).

**Conclusion:** Single application of photobiomodulation therapy after lower third molars surgery has no effect on edema and pain control. However, a single extra oral PBM application improves mouth opening limitation.

## P-25

### Soft Tissue Management with the Er:YAG Laser in Implant Therapy

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**Purpose:** With the expansion of implant dentistry and laser application in dental practice, Er:YAG laser has been used in a variety of treatments in implant dentistry such as gingivectomy during stage II surgery, treatment of peri-implant-mucositis and peri-implantitis, and preparation in osteotomy for implant placement. In particular, during soft tissue surgery with Er:YAG laser, most of cases can be done without using local anesthesia. This cases report addresses the characteristic of clinical effects of Er:YAG laser in soft tissue treatment.

**Clinical case 1:** A 34-years-old female patient requested implant treatment on the left mandible second premolar site. Two months after implant placement, gingivectomy at stage II surgery was performed using an Er:YAG laser (Dentlite 30, HOYA, Japan). During the procedure, firstly as the pre-irradiation, defocused laser was irradiated at an energy setting of 75 mJ/pulse with 20 pps without water spray in non-contact mode until the surface of gingival tissue changed white or brown color. After the pre-irradiation, gingivectomy was performed with water spray in contact mode without applying local anesthesia and then healing abutment was placed. Wound healing of the laser-treated gingival tissue was favorable and then zirconia crown was fabricated and delivered.

**Clinical case 2:** A 64-years-old female presented a chief complaint of gingival tissue swelling around implant at maxillary left molar site, which was diagnosed as peri-implantmucositis due to bleeding on probing, suppuration, and no change of bone level after delivery of final prosthesis. The peri-implantmucositis was treated by using an Er:YAG laser with the same procedure used in the clinical case 1 to evaporate inflamed granulation tissue around the implant. After surgery, the wound healing was uneventful without pain and was almost completed 7 days of post-

treatment. The peri-implant soft tissue was maintained without any sign of inflammation 6 months after treatment.

**Discussions and conclusions:** There are many clinical advantages in soft tissue management with Er:YAG laser, such as reduction of pain occurrence during treatment and enhancement of subsequent wound healing. The pre-irradiation technique applying defocused laser without water spray is useful to reduce pain occurrence in soft tissue surgery. The great advantages of utilizing Er:YAG laser irradiation for soft tissue management seems to be able to induce analgesic effect and avoid local anesthesia during treatment, as well as to achieve uneventful and favorable wound healing.

## P-26

### Comparison the Implant Wound Healing Condition between Conventional Blade & Er:YAG Laser Incision 1 Month Follow up Case report

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In this case, a 70-year-old man received implant surgery over bilateral mandible, molar area in the same day with healing abutment exposed (2 implants in one side, total number: 4 implants). The case highlights on comparing the wound healing between conventional flap surgery (sutured) and flapless surgery (non-sutured) with Er:YAG laser (Lite Touch, 2W) incision, not only from intraoral area, but also from extraoral profile and x-ray image in the same patient. We collected the data mentioned above and compared wound healing condition between bilateral surgical site before and post the surgery on 1st, 8th, 27th, and 39th day.

## P-27

### Roughness Evaluation after Diode Laser Irradiation for Peri-implantitis Treatment at Zirconia and Titanium Surface

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**Objective:** This study investigated the effects of diode laser (gallium, aluminium, arsenide, GaAlAs) irradiation with decontamination parameter on temperature of yttrium-stabilized tetragonal zirconia polycrystal (Y-TZP), titanium (Ti) and sandblasted large grit acid-etched titanium (SLA).

**Materials and methods:** Three groups (n = 10) of standardized disks with 5 mm diameter and 2 mm high were produced with Y-TZP obtained from CAD-CAM (computer-aided design e computer-aided manufacturing), machined titanium (Ti) and sandblasted large grit acid-etched titanium (SLA). The diode laser single application (808 nm, 20 s, 1 W, 50 Hz, ton = 100 ms, toff = 100 ms, energy density = 28.29 J/cm<sup>2</sup>) was performed in contact mode, on each disk. The parameters Ra (in µm) and Sa (in µm<sup>2</sup>) were measured by white light confocal laser microscopy to express the surface roughness. Roughness data was analyzed by t-test ( $\alpha = 0.05$ ).

**Results:** For both roughness parameters (Ra and Sa), data did not show statistical significant differences to "irradiation" factor ( $p > .05$ ) to Y-TZP and SLA. The Ra results (in µm) were: Y-TZP (control) = 0.73 (0.55); Y-TZP (irradiated) = 0.45 (0.27); SLA (control) = 0.74 (0.23); SLA (irradiated) = 0.99 (0.33). The Sa results (in µm<sup>2</sup>) were: Y-TZP (control) = 1.39 (1.05); Y-TZP (irradiated) = 0.73 (0.41); SLA (control) = 0.85 (0.08); SLA (irradiated) = 1.27 (0.44).

**Conclusions:** There was no surface alteration after diode laser irradiation for peri-implantitis treatment.

**P-28****Usefulness of an Er:YAG Laser for Frenotomy in Children****Takahisa Toyama (Japan)****Youki Ou, Ryo Ishizaka, Shintaro Komoto, Susumu Matsumoto, Yumi Furukawa, Takamitsu Iwai, Hideyuki Niwa, Mie Hirayama, Toshiaki Ono, Osamu Fukuta***Department of Pediatric Dentistry, School of Dentistry, Aichi Gakuin University*

**Purpose:** Lasers are relatively safe cutting instruments as their cutting ability disappears when irradiation is stopped. For this reason, an Er:YAG laser is often used for surgical treatment of ankyloglossia in our pediatric dental clinic. Here, we studied the usefulness of the Er:YAG laser for frenotomy in children.

**Participants and methods:** Participants were 29 children (aged 3 years 1 month to 14 years 7 months) who came our pediatric dental clinic at Aichi Gakuin University Dental Hospital. An Er:YAG laser (Erwin and Erwin Adverl, Morita, Japan) was used as the laser device. Irradiation conditions were as follows: energy, 3050 mJ/pulse at the contact tip; pulse repetition rate, 10 pps; and no water mist. The procedure was performed with local anesthesia. Hemostasis and pain were evaluated postoperatively.

**Results:** A small amount of bleeding was observed intraoperatively when the tongue was lifted in most patients. Postoperative hemostasis was achieved naturally or by applying pressure in 23 cases. Bleeding was not observed in 6 cases. No sutures were required in any case. On postoperative day 1, there was no pain in 14 cases, while 6 cases had pain when the tongue was in the lift position but not the rest position, and 9 cases had pain in both the rest and lift positions. At 1 week postoperatively, 25 cases had no pain, and 4 cases had pain in the lift position only.

**Conclusion:** No sutures were required when frenotomy was performed using the Er:YAG laser. Therefore, the treatment time was short and the burden on patients was low. Moreover, by 1 week postoperatively, pain had improved in all cases. In conclusion, the Er:YAG laser is useful for frenotomy in children.

**P-29****Detection of Oral SCC Using Autofluorescence-based Examination in Combination with 5-ALA in Mice****Mitsuhiko Ohta<sup>1,3</sup> (Japan)****Hayao Nakanishi<sup>2</sup>, Kenichi Kurita<sup>3</sup>, Kenji Yoshida<sup>3</sup>**<sup>1</sup>*Department of Oral Maxillofacial Surgery, Tosei General Hospital*<sup>2</sup>*Laboratory of Pathology and Clinical Research, Aichi Cancer Center Aichi Hospital*<sup>3</sup>*Department of Oral and Maxillofacial Surgery, Aichi-Gakuin University*

The prognosis of oral squamous cell carcinoma (OSCC) still remains poor with 5-year survival rates of less than 50%. To improve the survival rates of OSCC, the development of a new optical imaging method capable of early diagnosis leading to the treatment of OSCC at an early stage is crucial. Conventional gross inspection remains the gold standard for the screening and diagnosis of oral neoplasms. Recently, however, light-induced tissue autofluorescence-based examination (Velscope, for example) has been introduced to assist conventional OSCC screening. This convenient autofluorescence visual imaging is a widely used and safe method approved by the U.S. Food and Drug Administration (FDA), but its sensitivity and specificity for detecting malignant lesions still remain to be evaluated. In the present study, we examined the effect of 5-Aminolevulinic acid (5-ALA) administration on the imaging with Velscope to improve the sensitivity and specificity for detection of OSCC in mouse model. We found that 3 out of 4 human OSCC intracutaneously transplanted tumors (SAS, UMSCC81, HSC-2 and CA9-22 cells) in the back of nude mice were detected as a low density area with clear margin in Velscope imaging alone. On the other hand, in the fluorescence image with 5-ALA administration alone, detection rate was high (100% = 4/4), but the margin was obscure because of the high background fluorescence in the skin. Therefore, we conducted Velscope imaging after addition of 5-ALA intraperitoneal administration into mice. The results demonstrate that all 4 OSCC tumors in mice were successfully detected with clear margin probably due to the decrease in background fluorescence by Velscope-based imaging. These results suggest that combination of Velscope and 5-ALA administration would be potentially useful for the sensitive detection and more accurate estimation of tumor margin of OSCC in the clinical setting.

**P-30****Diode Laser Therapy in the Treatment of Major Recurrent Aphthous Stomatitis and Aphthous -like for Chemotherapy Drugs. A Preliminary Study****Maria Garcia-Pola<sup>1</sup> (Spain)****Santiago Llorente-Pendas<sup>2</sup>, Sandra Rubn-Milla<sup>3</sup>, Jos Mil-la-Sitges<sup>3</sup>, Jose Garca-Martin<sup>4</sup>**<sup>1</sup>CLUO (Clinic University of Odontology), Oviedo University<sup>2</sup>Oral and Maxillofacial Surgeon. Private practice of Oral and Maxillofacial Surgery. Medical Director of Head and Neck Center. Pérez de la Sala 37. 33007.<sup>3</sup>Oviedo University<sup>4</sup>Clinic Univeristy of Odontology. Oviedo University

Recurrent aphthous stomatitis (RAS) is one of the most oral inflammatory disorders. RAS is characterized by painful and recurrent single or multiple ulcerations of the oral mucosa. Furthermore, numerous chemotherapy agents cause oral ulcerative mucositis, leading to a poor oral health and quality of life to patients with active oral ulcer compared to ulcer-free patients. The main goal of current therapeutic approaches includes the management of pain.

**Purpose:** Our aim was to evaluate the effect of diode laser therapy as a treatment in pain of ulcerative lesion in major RAS and chemotherapy-induced oral ulcerative mucositis to minimize the symptoms.

**Materials and methods:** Eight major RASs and eight erosive mucositis triggered by chemotherapy were included in the present study. A diode laser (GaAs laser; ARC, Fox, Germany) was used as the light to irradiate the areas of the lesions. The laser parameters were as follows: wave length of 810 nm, fluence 1.5 J/cm<sup>2</sup>, continuous-wave mode. The laser beam was applied to the patient anaesthetized, at a probe distance of approximately 2-3 mm from the oral mucosa, in a continuous circular motion, covering entire ulcer surface. The treatment consisted of one appointment with three sequential sessions of laser applications, each lasting 30 seconds, with a gap of 30 seconds between each session, for a total laser application of 1.5minutes.

Pain intensity was determined by using a visual analog scale (VAS) of 0-10 with 10 divisions, being 0 no pain and 10 is the worst possible pain.

**Results:** A total of 16 patients, with age ranging from 35 to 59 (average age 52.56 years) were recruited in the study, among them 12 (75%) were women and 6 (25%) were men. The average pain score before treatment was 9.12 (ranging from 7 to 10). After the third application, the mean level of pain decreased to 7. No side-effects were observed after treatment.

**Conclusions:** According to the results of this study, diode laser therapy demonstrated analgesic effects with regard to major ulceration oral mucosa right after its application.

**P-31****Low-level Laser Therapy to Treat Cutaneous Radionecrosis Induced by 125I Seed in a Murine Model****Rodrigo C Mosca (Brazil)****Martha S Ribeiro, Carla Daruich de Souza,****Carlos A Zeituni**

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**Background:** The malignant cancer incidence has increased significantly in recent years due to population growth and aging. The cancer treatment usually consists in individual or combined use of chemotherapy, surgery and radiotherapy depending on the etiology of the tumor. In cases where radiotherapy is used, and considering the therapeutic effects of radiation, specific severe complications can occur, ranging from erythema to radionecrosis. Studies showed that low-level laser therapy (LLLT) can be successfully used for tissue repair, depending on the light parameters and characteristics of the target tissue.

**Methods:** Eight Swiss mice were divided into two groups: control and intervention. 125I seed (5 mCi) is inserted subcutaneously with a needle into the back of the mouse. Each animal was anesthetized using a dilution of xylazine and ketamine before any procedure. Just after the radiodermatitis wound emerge, the intervention group was submitted a low-level laser therapy, wavelength of 660 nm, 40 mW power with of 30 s (4 J/cm<sup>2</sup>) exposure in a single dose with no wound contact. All animals were photographed every 5 days and the wound sized were calculated by the Image J software.

**Results:** Ulceration appeared on the skin after 22 days after seed implantation. No systemic or lethal sequelae occurred in any animal. All animals were accompanied for 60 days and no significant differences were observed between groups regarding the repair process.

**Conclusion:** Under the parameters used in this study, the iodine-125 seed was effective in causing radionecrosis in the back of the test animals. LLLT in was unsuccessful in repairing radionecrosis. We are focusing our efforts in using higher energy laser and other parameters.

**P-32****Histological Bone Healing Process of Bone Defects Performed with Er:YAG Laser and Steel Bur***Hiroyuki Makihara (Japan)**Kenji Yoshida, Hidenori Nakayama, Hiroki Inoue, Akihiko Goto, Hiroshi Horibe, Tsubasa Yamamoto, Mitsuhiro Ohta, Kohta Fukuta, Yuichiro Kuroiwa, Kenichi Kurita**Department of Oral and Maxillofacial Surgery, Aichi Gakuin University*

**Purpose:** Osteotomy and corticotomy are essential surgical techniques in the field of oral and maxillofacial surgery and orthopedic surgery. The purpose of this study is to test different instruments and to evaluate the healing process of bone tissue after bone cutting using either Er:YAG laser hard mode or round steel bur.

**Materials and Methods:** Femoral bone osteotomy was performed in 40 male Wistar rats using either Er:YAG laser (n=20) or round steel bur (n=20). Osteotomies were performed with a steel bur at 20,000 rpm or Er:YAG laser (Syneron, Lite Touch, Israel) with the following parameters in hard mode (power, 3 W; pulse energy, 100mJ; repetition rate, 30Hz). Each laser or steel bur group was classified into five subgroups with equal numbers (n=4); rats were sacrificed at 0, 1, 2, 4 and 12 weeks postoperatively. Histological analysis was performed to compare the course of bone healing between both methods.

**Results:** Both laser and steel bur osteotomies of the rat femoral bone did not cause thermal damage and osteogenesis took place in both instruments. Histological evaluation 1 week after treatment showed primary gap healing with callus formation at both manners. Bleeding and cell infiltration surrounded with injured bone were prolonged in steel bur method compared with the laser at earlier stage of bone healing. Temporary bone increment after surgery was also augmented using the laser compared to the steel bur. Furthermore laser osteotomy showed earlier recovery of the continuity of the injured bone at 2 weeks after treatment while it took 4 weeks with steel bur.

**Conclusion:** Our study suggested that the use of Er:YAG laser enabled faster healing than that of steel bur. It might have been caused by LLLT (Low level laser therapy) effect. Hemodynamic stability, bleeding control, decrease of operative time and enhancement of surgical results might be the advantages of using laser instruments. Further scientific study is needed to optimize surgical conditions.

**P-33****Effect of Diode Laser Irradiation on Bone Healing in Rat Tibiae***Takahide Yamazaki (Japan)**Hiroshi Kadokura, Takayuki Ueda, Satoshi Yokose**Division of Endodontics and Operative Dentistry, Department of Restorative and Biomaterials Sciences, Meikai University School of Dentistry*

Acceleration of the bone healing period is important in clinical situations such as implant and periodontal treatments. Recently, it has been demonstrated that mechanical stimuli including ultrasound and microwaves are potentially powerful treatments for bone regeneration and the acceleration of bone healing. Moreover, laser irradiation has been shown to stimulate bone formation. However, there are few reports describing the histological changes in bone following laser irradiation. The aim of this study was to examine the effect of diode laser (910 nm) irradiation on histological changes during bone healing in rats. Two groups of rats with bone defects in the tibiae were subjected to laser irradiation at 0, 40, 80, and 120 J; group 1 was irradiated daily with each dose for a total of 14 days, and group 2 was irradiated at 120 J for 7 days and subsequently evaluated for up to 14 days post-irradiation. Tibiae were removed at 3, 7, 14, and 21 days, and subjected to serial sectioning. Morphological examination of bone formation was conducted using hematoxylin-eosin staining and calcein labeling of sections. In group 1, bone formation was stimulated by laser irradiation, and at day 7 the effect was found to be energy-dependent. However, at day 14, bone volume was decreased in an energy-dependent manner. In group 2, the laser-irradiated tibiae showed a greater volume of bone formation than that of the control on day 14. However, no differences in bone volume were observed in the treatment groups on day 21. These results indicated that diode laser irradiation induced marked bone formation in the early phase of the bone healing process and the effects depended on the irradiation energy; however, a longer period of high-power laser irradiation inhibited bone formation. This study suggests that diode lasers can be utilized for bone regeneration, taking into consideration the irradiation period and energy.

**P-34****Effects of Low Reactive Level Laser Therapy in Promoting Bone Healing****Yoshihiko Sugita**<sup>1,3</sup> (Japan)**Nobuaki Sato**<sup>1</sup>, **Katsutoshi Kubo**<sup>1,3</sup>, **Morioki Fujitani**<sup>2,3</sup>, **Akira Senda**<sup>2,3</sup>, **Hatsuhiko Maeda**<sup>1,3</sup><sup>1</sup>Department of Oral Pathology, School of Dentistry, Aichi Gakuin University<sup>2</sup>Department of Operative Dentistry, School of Dentistry, Aichi Gakuin University<sup>3</sup>Center of Advanced Oral Science, Aichi Gakuin University

**Purpose:** Low reactive level laser therapy (LLLT) is known to promote bone defect healing. This study was designed to investigate the effects of LLLT on bone defect healing in a rat model.

**Material and Methods:** Eight-week-old male Sprague Dawley rats were used in this study. Bone defects reaching into the bone marrow located 11 mm from the femur articular surface on either side were created using a low-speed round bur (1.6 mm) under sterile saline. After hemostatic wound closure was provided, one side was irradiated with He-Ne laser (Soft Laser 632, wavelength: 632.8 nm) and the other was left unirradiated (Laser group and control group, respectively). The laser irradiation was performed transcutaneously to the bone defects for 5 minutes (Output power: 25 mW, Distance: 25 mm) every 24 hours for 5 days soon after the operation (6 times in all). The rats were sacrificed after 7 and 14 days, and the resected femurs were subjected to the radiological evaluation. All experiments were performed according to the Ethics Committee of the School of Dentistry, Aichi Gakuin University (approval No. 266).

**Results:** Micro-CT examination demonstrated that both Trabecular Separation (Tb. Sp) and Trabecular Spacing (Tb. Spac) in laser group were lower than those in control group at 7 days but were higher at 14 days. The laser group exhibited more Trabecular Number (Tb. N) at 7 days but less at 14 days. The progress of bone healing was observed at early stage in laser group.

**Conclusion:** This particular study revealed the LLLT effects of He-Ne laser in promoting bone healing at experimental bone defects in a rat model.

**P-35****Dentine is Changed with Osteoporosis Induced in Rats: a Study of Optical Spectroscopy****Fernanda Rossi Paolillo**<sup>1</sup> (Brazil)**Renan Arnon Romano**<sup>1</sup>, **Luciana de Matos**<sup>1</sup>,**Vitor Hugo Panhóca**<sup>1</sup>, **Fátima Zanin**<sup>2</sup>,**Airton Abrahão Martin**<sup>3</sup>, **Jarbas Caiado de Castro Neto**<sup>1</sup>, **Vanderlei Salvador Bagnato**<sup>1</sup><sup>1</sup>Optics Group from São Carlos Institute of Physics (IFSC), University of São Paulo (USP)<sup>2</sup>Director of Biophotonics Center at Institute Brugnara and Zanin, R. Groenlândia<sup>3</sup>Laboratory of Biomedical Spectroscopy (LEVB), University of Vale do Paraíba (UNIVAP)

Osteoporosis is a systemic disease characterized by low bone mass and microarchitectural deterioration of bone tissue. However, the effects of osteoporosis on the teeth, to our knowledge, has not been investigated. The aim of this study was to investigate the effects of demineralization on teeth evaluated by Raman spectroscopy, fluorescence spectroscopy, and energy dispersive X-ray (EDX) in rats ovariectomized. Ten female Wistar rats were randomized into 2 groups: Control Group (C): non-ovariectomized rats and; Ovariectomy Group (OV): ovariectomized rats to induce osteoporosis. The optical spectroscopies and EDX of lower incisor tooth were performed for long-term (6 months post-ovariectomy). Raman spectra was obtained with excitation laser at 785 nm (infrared). Fluorescence spectra was obtained with diodo laser and a doubled-frequency of a Nd:YAG laser consecutively for excitation at 532 nm (green). The atomic concentrations of phosphate (P) and calcium (Ca) were measured using EDX. One-way ANOVA and Tukey's post-hoc test were performed at a significance level of 5% ( $p < 0.05$ ). The phosphate/amide I ratio was significantly greater ( $p < 0.05$ ) in OV compared with C for dentine ( $86 \pm 34$  vs.  $53 \pm 27$ ). There were changes in fluorescence spectra with laser at 532 nm which conduced to significant increase ( $p < 0.05$ ) of band area in OV compared with C for teeth ( $80989 \pm 4512$  vs.  $97222 \pm 704$ ). The concentrations of P for dentine ( $11.15 \pm 0.09$  vs.  $12.05 \pm 0.07$ ) were significantly reduced ( $p < 0.05$ ) in OV compared with C. There were negative correlations between fluorescence band area and phosphorus ( $r = -0.71$ ,  $p = 0.02$ ) for teeth. The aging process and osteoporosis may lead to alterations in mineral crystallites and reduced mineral content of teeth. Moreover, osteoporosis leading to loss of dentine quality may be assessed by optical spectroscopies. These findings corroborate those obtained by EDX.



**P-36****Effect of Laserphototherapy Associated or Not to Vitamin C in the Induction of Cell Sheets of Human Dental Pulp Stem Cells***Ana Clara Pedroni<sup>1</sup> (Brazil)**Maria Stella Moreira<sup>1,2</sup>, Ivana Márcia Alves Diniz<sup>1</sup>, Gabriela Abe<sup>1</sup>, Carla Sipert<sup>1</sup>, Márcia Martins Marques<sup>1</sup>*<sup>1</sup>Department of Dentistry, School of Dentistry, University of Sao Paulo<sup>2</sup>Post Graduate Program, Ibirapuera University

**Purpose:** Cell Sheets, consisting of stem cells (SCs) are self-detachable from the cultivation plate, and with no subcultivation can generate large amount of cells, which keeps the cell connections and the extracellular matrix produced in culture. Vitamin C (VC) has inductive effect on cell sheet formation, increasing the longevity and the stemness of the cells for long period. These biological responses are similar to those of Laserphototherapy (LPT) on cells and tissues, thus, these therapies could improve the prognosis of future clinical application of these cell sheets in regeneration of dental tissues.

**Material and Methods:** LPT and VC were applied, associated or not, to induce human dental pulp stem cells (hDPSCs). The experimental groups were: Control: Cells in P3 grown on regular medium (RM); Senescent: P27 grown in RM; VC: P3 on regular medium supplemented with VC (20 µg/ml); Laser: P3 in RM and submitted to LPT (punctual and contact mode-5 points / well, 660 nm, 20 mW, 0.028 cm<sup>2</sup>, 0.71 W/cm<sup>2</sup>, 7 s, 5 J/cm<sup>2</sup>, 0.14 J by point, 48 hours interval) and Laser+VC. Within 24 hours, 7 and 13 days cultures were observed macroscopically and microscopically. Telomerase enzyme activity was assessed by PCR-TRAP and ELISA. RT-qPCR was used for evaluating the expression of genes related to the nature (mitofilin) and differentiation (Oct 4), longevity (catalytic phase of telomerase-hTERT enzyme), and the senescence of the senescent group cells ( $\beta$ -galactosidase). The data were compared by ANOVA and Tukey's test ( $p < 0.05$ ).

**Results:** The cell sheets were formed only in the VC and Laser+VC groups (100%) The cell sheets of the Laser+VC group presented easier handling. Telomerase activity in hDPSCs was observed only in 24 hours (Control and Laser) and seven days (VC and Laser + VC). The Oct 4 and mitofilin, as well as hTERT were expressed in hDPSCs of all groups. Oct4 and hTERT presented expressions significantly higher at 7 days in VC and Laser+VC groups than in all other groups ( $p < 0.0001$ ;  $p = 0.0009$ , respectively). The expression of mitofilin was significantly higher in the Laser+VC group ( $p = 0.0338$ ).

**Conclusion:** The LPT when combined with VC did not interfere with the formation of the cell sheets, neither with the maintenance of longevity and undifferentiating status of hDPSCs. Moreover, LPT improved the handling of the cell

sheets. Thus, the association of VC and LPT in the induction of cell sheets seems promising for future use in regenerative dentistry.

**P-37****Oral Mucositis Treated with Low Level Laser Therapy: Clinical Outcomes in Elderly Patients Undergoing Hematopoietic Stem Cell Transplantation***Fernanda de Paula Eduardo<sup>1</sup> (Brazil)**Leticia Mello Bezinelli<sup>1</sup>, Giuliana Gadoni Borges<sup>2</sup>, Carlos de Paula Eduardo<sup>2</sup>, Nelson Hamerschlak<sup>1</sup>, Luciana Correa<sup>2</sup>*<sup>1</sup>Centro de Oncologia e Hematologia do Hospital Israelita Albert Einstein, SP-Brazil<sup>2</sup>School of Dentistry, University of São Paulo, Brazil

**Purpose:** Oral mucositis (OM) is a painful oral condition with high impact in nutritional status and quality of life in patients undergoing hematopoietic stem cell transplantation (HSCT). In HSCT elderly patients, the risk and severity of OM is high, and the impact of LLLT on the OM morbidity was not investigated yet. The aim of this study is to describe a LLLT protocol for OM prevention and treatment in HSCT elderly patients, and to compare the OM clinical outcomes to those described in the literature.

**Patients and Methods:** HSCT patients (n=60) with age  $\geq$  60y were retrospectively analyzed regarding type of HSCT transplantation, chemotherapy regimen, OM degree, and LLLT protocol and days number of laser treatment.

**Results:** All the analyzed patients were submitted to LLLT with a protocol using 660nm, 100mW, 1J, 35J/cm<sup>2</sup>. The laser treatment was adopted every day from the first day of chemotherapy to the period of marrow recovery (mean of  $15 \pm 2$  days). All the patients undergone chemotherapy regimen of high risk for OM (containing busulfan, melphalan, cyclophosphamide, and total body irradiation). Both autologous and allogeneic HSCT patients exhibited high frequency of discrete OM (grade 0 - 5.0%; grade 1 - 51.7%; and grade 2 - 33.3%) and low frequency of severe OM (grade 3 - 8.3%; and grade 4 - 0.0%). None reported discomfort during and after LLLT. In the comparison with the literature, the frequency of severe OM in the present study was reduced.

**Conclusion:** The use of LLLT in HSCT elderly patients was safe and attenuates the expected risk of severe OM. Prospective clinical studies must be conducted for the confirmation of its efficacy in the prevention and treatment of OM in elderly patients.

**P-38****Fungicidal Effect of Combined Nano TiO<sub>2</sub> With Erythrosine and Anthocyanin for Mediated Photodynamic Therapy on Candida Albicans: An *in vitro* Study**

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**Purposes:** The purposes of this *in vitro*-study were to evaluate the fungicidal effect of photodynamic reaction using erythrosine gel, anthocyanin (cyanidin) gel and -combined erythrosine with cyanidin gel as photosensitizers stimulated by blue LED ( $\lambda$  420 - 480 nm) and supplemented nano-titanium dioxide (TiO<sub>2</sub>) as a catalyst on *Candida albicans* biofilms.

**Materials and Methods:** *Candida albicans* (ATCC 10231) biofilms were prepared by adjusted *C. albicans* suspension to an optical density of 0.381 at 530 nm (10<sup>7</sup> cells/ml). The plate was incubated at 37°C in an orbital shaker incubator at 75 rpm. The biofilm samples were randomly divided into 10 test groups namely; erythrosine gel 220  $\mu$ M, 440  $\mu$ M, cyanidin gel 202  $\mu$ M, 404  $\mu$ M, erythrosine gel 440  $\mu$ M + cyanidin gel 404  $\mu$ M in the presence and absence of 1% TiO<sub>2</sub> and 23 control groups namely; the 10 groups of photosensitizers without irradiation, 0.12% chlorhexidine gel, water, irradiated water, 1% TiO<sub>2</sub> suspended in water gel (by volume corresponding to molecular weight of test groups) and the irradiated 1% TiO<sub>2</sub> in water gel. All test groups were irradiated by blue light power 250 mW/cm<sup>2</sup> for 1 minute (energy 15 J/cm<sup>2</sup>). After 1, 3 and 6 hours, the biofilms were disrupted and seeded onto Sabouraud dextrose agar, incubated at 37°C. The numbers of colony forming unit (CFU/mL) were counted after incubated for 48 hours.

**Results:** The erythrosine 220  $\mu$ M, 440  $\mu$ M, cyanidin 404  $\mu$ M, erythrosine 440  $\mu$ M + cyanidin 404  $\mu$ M in the presence and absence of 1% TiO<sub>2</sub> groups showed significantly decrease *Candida albicans* number compared with all control groups ( $p < 0.001$ ). Erythrosine (220, 440  $\mu$ M) and cyanidin (404  $\mu$ M) + TiO<sub>2</sub> stimulated with blue light significantly diminished *C. albicans* when compared with erythrosine 220 M, 440 M ( $p = 0.014$ ,  $p < 0.001$ ) and cyanidin 404  $\mu$ M ( $p < 0.001$ ) without TiO<sub>2</sub> stimulated with blue light. Whereas, there is no statistically significant difference in number of CFU/mL of *C. albicans* in cyanidin 202  $\mu$ M and erythrosine 440  $\mu$ M + cyanidin 404  $\mu$ M combined with TiO<sub>2</sub> stimulated with blue light when compared with

those groups without TiO<sub>2</sub> ( $p = 0.583$ ,  $p = 0.402$ ).

**Conclusion:** Erythrosine gel 440  $\mu$ M + 1% TiO<sub>2</sub> is the most effective photosensitizer on killing *C. albicans* when stimulated by blue light in photodynamic therapy.

**P-39****New Strategies for the Treatment of Oropharynx**

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Upper respiratory tract diseases caused by acute, chronic or recurrent infections. The most common bacteria present in this disease are *Streptococcus pyogenes* and *Staphylococcus aureus*. Bacterial pharyngitis symptoms include fever, cough and sore throat which symptoms are common in hospital emergency departments. The antibiotic resistance has been attributed to drug overuse. These factors associated to high incidence, adverse effects of used drugs and disease complications show the importance of new therapy options. Photodynamic therapy is a new treatment option providing local treatment and fast results. The procedure consists in 1) Drug application containing photosensitizer (curcumin, 0.75 mg/mL; PDT Pharma, Cravinhos, São Paulo, Brazil) and 2) illumination of infection site (450 nm and 6.57 J/cm<sup>2</sup>) with a device developed (MMOptics, São Carlos, SP, Brazil). The clinical protocol has compared pharmaceutical formulations, prototypes, and illumination parameters. This study demonstrated a effectiveness treatment with 94.73% of clinical cure in children and adults patients.

**P-40****Evaluation of the Antimicrobial Effect of Photodynamic Antimicrobial Therapy on *Streptococcus Mutans* in Orthodontic Accessories and Patients**

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Demineralization and dental caries are commonly present in patients with orthodontic braces due to increased accumulation of dental plaque containing *Streptococcus mutans*.

Chlorhexidine is currently regarded as the gold standard in oral decontamination, however has side effects such as tooth staining and taste alterations in their continued use. Photodynamic therapy (aPDT) has been used as an alternative treatment to inactivate *S mutans*. This work, carried out both *in vitro* and *in vivo*, aims to evaluate the antimicrobial effect of aPDT on *S mutans* present on the metal surface of orthodontic appliances and in patients undergoing orthodontic treatment. The *in vitro* study evaluated the effect of aPDT induced Curcumin diluted surfactant (SDS) and blue light-emitting diode (LED). The samples were treated with Curcumin (1 g/L, 0.1%), surfactant and LED blue (455 ± 10 nm, 46mW/cm<sup>2</sup>), fluence of 30 J/cm<sup>2</sup>. The experimental groups studied were: control group (P-L-S-); light (P-L+S-), surfactant (P-L-S+) and photosensitizer (P+L-S-) groups; surfactant-light (P-L+S+), photosensitizer-surfactant (P+L-S+) and PDT (P+L+S-) groups; and the PDT-surfactant (P+L+S+) group. The results were analyzed by ANOVA and the post hoc Tukey test (p < 0.05). The results showed that in this research protocol, the group P+L+S+ was able to promote significant decrease (p < 0.001) on the population of *S. mutans* of 99.999% compared to the control group. In the *in vivo* study, 24 volunteers were randomly selected in order to evaluate the clinical utility of aPDT. associated with the SDS as oral decontamination agents in orthodontic patients. The selected patients (n = 24) were randomly divided into seven groups: G1 - Negative control, G2 - Curcumin mouthwash, G3 - Curcumin mouthwash+SDS, G4 - light irradiation, G5 - aPDT, G6 - aPDT+SDS and G7 - Positive Control (chlorhexidine 0.12% moutwash). Saliva samples were collected from unstimulated way of each patient in three steps (S) as follows: S1 - initial condition, S2 - Treatment with rinsing (water, Curcumin or chlorhexidine) and S3 - After aPDT. The Curcumin with concentration of 1 g/L. Two types of blue LED light source emitting in 450 ± 10nm were used in the present study: one emitting with an intensity of 33.54 mW/cm<sup>2</sup> and other with 212 mW/cm<sup>2</sup>. Evaluation of microbial reduction, and survival fraction in each of the studied levels, was performed using the Kruskal-Wallis ANOVA test. Statistical analysis showed that only G6 and G7 presented results of bacterial inactivation that showed statistically significant differences (p < 0.05) compared to those observed in G1. These results indicated that aPDT, when combined with SDS, may be used as an adjunct agent for convenient oral decontamination.

## P-41

### Effects of a-PDT with Curcumin and Blue Light: *In vitro* and *in vivo* Studies

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The mouth has a large number of bacteria. It results in increased risk of infection, mainly in periodontal disease. However, curcumin-mediated antimicrobial photodynamic therapy (a-PDT) can be used at low doses in combination with light exposure, with considerable antibacterial effect. The aim of this study was evaluate the effects of the PDT on *in vitro* and *in vitro* disinfection. For the *in vitro* study, dental plaque samples were obtained from 10 subjects with chronic periodontitis. Human dental plaque bacteria in planktonic and biofilm phase were evaluated. Suspensions of plaque microorganisms were sensitized with curcumin (1.5 mg/mL and 3 mg/mL) during 10 minutes then exposed to 48 blue LEDs (450 ± 10 nm) at 30 J/cm<sup>2</sup>. After PDT, survival fractions were calculated by counting the number of colony-forming units (CFU). For the *in vitro* study, 27 adults were randomly divided into 3 groups (1) the PDT group, which was treated with the curcumin and blue light; (2) the light group, which was treated only with the blue light and; (3) the curcumin group, which was treated only with the curcumin. A curcumin concentration of 30 mg/L was used. The irradiation parameters were: blue LED (450 ± 10 nm), 400 mW, 5 minutes of application on the oral cavity, 600 mW/cm<sup>2</sup>, and 200 J/cm<sup>2</sup>. The saliva samples were collected for bacterial counts at baseline and after the experimental procedure. The data were analyzed by one-way ANOVA with Tukey's post-hoc test. The significance level was set at 5% (p < 0.05). For the *in vitro* study, a-PDT showed significant reduction in survival of CFU for suspension and biofilm at 1.5 and 3.0 mg/mL, particularly, 3.0 mg/mL reduced significantly the bacterial colonization at 75% for suspension and 55 % for biofilm (p < 0.01). For the *in vitro* study, the PDT group showed a significant reduction of CFU (from 6.6 ± 0.8 to 5.1 ± 0.9, p < 0.01). The parameters of irradiation and curcumin concentration were higher in clinical trial to those applied in *in vitro* study because *in vitro* studies show complexity regarding variety of biological tissues in the oral cavity and in immunological response. Then, blue light for PDT using curcumin may be used for reduction of microorganisms in dentistry.

## P-42

**Evaluation of Photodynamic Therapy on Fibroblast Viability and Cytokine Production**

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**Objectives:** The photodynamic therapy (PDT) is an aggregate of physics, chemical and biological procedures, that activate the photosensitizer (PS) with light (Laser or Led) to destroy the target cells. The aim of this study was to evaluate *in vitro* cytotoxicity of PDT, comparing it to different irrigating solutions.

**Methods:** Mouse fibroblasts L-929 were used, held on standard conditions of culture cells (37°C with 5% of CO<sub>2</sub>) which were distributed on: G1-Sodium Hypochlorite 5%; G2-Sodium Hypochlorite 2.5%; G3-Chlorhexidine 2%; G4-Sodium Chloride 0.9%; G5-PDT; G6-Culture Medium (Control). PDT was realized with PS curcumin 500 mg/L and Led  $\lambda$  480 nm for 4 minutes. Were diluted in culture medium DMEM (1x10<sup>4</sup> cells), 0,001ml of the solutions to be tested for 6h, 24h and 48h. Three wells were used for each solution containing the fibroblasts which remained incubated for 3h. Cell viability was obtained by colorimetric method MTT. The absorbance was realized with espectrophotometer of  $\lambda$  570 nm.

**Results:** The results were analysed by ANOVA test with correction of BonFerroni ( $p < 0.05$ ). PDT and sodium chloride 0.9% presented light cytotoxic effect no significant difference statistics compared to the control. There was a significant difference statistics of PDT compared to sodium hypochlorite 5%, 2.5% and chlorhexidine 2% in the period of 6h, 24h and 48h.

**Conclusions:** PDT with curcumin in the concentration used was not cytotoxic unlike the solutions of sodium hypochlorite 5%, 2.5% and chlorhexidine 2%.

## P-43

**Photodynamic Effects of Methylene Blue Associated to Sodium Nitrate Against *Streptococcus Mutans* in Planktonic Culture**

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The aim of this study was to evaluate the *in vitro* effect of antimicrobial photodynamic therapy on the viability of *Streptococcus mutans* (ATCC 25175<sup>TM</sup>) in planktonic culture mediated by methylene blue associated or not to sodium nitrate irradiated with red Laser at 660nm, 40mW of power and 30J/cm<sup>2</sup> of energy density). The bacterial suspension containing *Streptococcus mutans* 1 × 10<sup>8</sup> CFU/mL was prepared and 250µL pipetted according to the evaluated Groups: L–P– (negative control), L–P+ (photosensitizer associated or not to sodium nitrate at 6 concentrations: 0.625, 1.25, 2.5, 5, 10 and 20µM), L+P– (Laser 30J/cm<sup>2</sup>), chlorhexidine (positive control), sodium nitrate and Group L+P+ (methylene blue associated or not to sodium nitrate and irradiated with Laser). For the dark cytotoxicity test, 250µL of methylene blue associated or not, were added and incubated in the dark for 5 minutes. For the antimicrobial photodynamic therapy Groups, they were incubated in the dark for 5 minutes and irradiated by red Laser (Twin Flex, MMOptics Optical Equipments, Sao Carlos, Sao Paulo, Brazil) during 30 seconds. After treatment, the strains were seeded onto BHI agar at 37°C during 48h for subsequent visual counting of CFU/mL. Data were transformed into log<sub>10</sub>, analyzed by one and two-way ANOVA and Tukey's test at  $p < 0.05$ . The results showed that there was no cytotoxicity in the dark for the different concentrations of methylene blue associated or not. The photodynamic therapy promoted complete reduction in the viability of *Streptococcus mutans* at concentrations of 0.625 and 5µM for associated methylene blue showing to be more effective than chlorhexidine. Antimicrobial photodynamic therapy may be a viable technique to eliminate or reduce these bacteria in the oral cavity.

**P-44*****In vitro* Evaluation of The Efficacy of Lethal Photosensibilization of Oral Microorganisms With Photogem<sup>®</sup> Associated to Red LED ( $\lambda 640\text{nm} \pm 5\text{nm}$ )****Aldo Brugnera Júnior<sup>5</sup> (Brazil)****Fátima A. A. Zanin<sup>3</sup>, Gustavo M. Pires Santos<sup>1</sup>,  
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The aim of this study is to evaluate the lethal photosensibilization of microorganisms mediated by red LED (MMOptics, São Carlos, SP,  $\lambda 640\text{nm} \pm 5\text{nm}$ , output of 120mW, spot of  $0,785\text{cm}^2$ ) associated with Photogem<sup>®</sup>. Microorganisms of the posterior dorsal region of the tongue and oral mucosa were collected and inoculated in 8mL of TSB medium overnight followed by inverse homogenization. Culture plates with 24 wells were used for the irradiated and non irradiated species. Each well received 400 $\mu\text{L}$  of the suspension containing the microorganisms. In eight wells no Photogem<sup>®</sup> was used and they were the irradiated and non irradiated control. The remain of the wells had Photogem<sup>®</sup> applied with a pre-irradiation time of 5 min in concentrations of 40, 20, 10, 5 e 2.5 $\mu\text{g}/\text{mL}$ . LED was applied for 5 min and 45 sec. After agitation, 200 $\mu\text{L}$  were withdrawn from each well and turbidity measurements were immediately taken. Other 200 $\mu\text{L}$  were withdrawn from the wells after 1 hour in bacteriological incubator for a second measurement. The best results found were for 40 $\mu\text{g}/\text{mL}$  Photogem<sup>®</sup> associated with LED irradiation and immediate measurement (36.7%) and for 5 $\mu\text{g}/\text{mL}$  with LED irradiation with measurements one hour after incubation (42.8%). This study suggests that lethal photosensibilization with Photogem<sup>®</sup> associated to red LED can be a potencial mechanism of control of oral microorganisms.

**P-45****Fabrication Titanium Denture Base by 3D Laser Printing and Evaluation Its Fitness with Laser Scan Technique****Jiang Wu (China)****Li-juan Shen, Xiao-bo Wang, Bo Gao**

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**Purpose:** to integrate the technologies of computer-aided design (CAD) and 3D laser printing (3DLP) for the fabrication of the titanium (Ti) base of a complete denture. And to evaluate its fitness by applying laser scan technique.

**Materials and Methods:** Initially, a patient's upper edentulous cast was made and the cast was scanned and reconstructed as digital one. And based on it, 3D denture base was designed and Ti base was fabricated with 3DLP method. To evaluate its fitness, the Ti base was fixed on the original plaster cast and sealed the fringe by wax. Then the original plaster cast was removed through flask. Another plaster cast which duplicated the surface of the Ti base was then made on the same plane in the flask. Both of the plaster casts were slightly trimmed and were located in the same place of the laser scan worktable and then the tissue surface of both casts was recorded as point clouds data by laser scan method. 2 groups of data were automatically compared to calculate the discrepancies between those of 3DLP Ti base and plaster cast by applying reverse engineering software.

**Results:** After CAD/3DLP method, the Ti denture base plate was designed and successfully fabricated layer-by-layer. By laser scan method and 3-D computing, the average adaptation errors of 3DLP made base were about 0.107mm and the mean deviation was found to be 0.037 mm.

**Conclusion:** The CAD/3DLP method is a potential candidate to replace the traditional lost-wax-casting technique and provides a new platform for the design and manufacturing of custom-made Ti denture plates and other restorations, and this new method equipped with high reliability and fast speed can meet the need of the study of quality of fit.

**P-46****Three-dimensional Morphological Analysis of Irradiation Tip Alteration with Er:YAG Laser Using Microfocus X-RAY Computed Tomography****Kazunori Takamori (Japan)**

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**Introduction:** Er:YAG laser is useful for not only removal of dental caries, but also for oral surgery, endodontics, and periodontal therapies, and has lots advantages compared with traditional treatments. The irradiation conditions of

wavelength, output power, and pulse repetition are important for treatment efficacy. Otherwise, morphological change to an irradiated tip is a major factor impacting adjustment of laser power to specific tissues. However, there is insufficient data regarding tip alterations during treatment. The aim of this study was to evaluate morphological changes to tips altered by irradiation using microfocus X-ray computed tomography (micro-CT).

**Materials and Methods:** Quartz contact tips for cavity preparation or for soft tissue surgery were analyzed. Micro-CT was performed on five tips both before and after use. The irradiated tips were used for 3 month, with constant output power, and repetition pulses at 100-200 mJ for cavity preparation or 50-100 mJ for soft tissue surgery, at 10 or 20 pps, respectively. Micro-CT (R\_mCT, Rigaku, Tokyo, Japan) were obtained under the following conditions: tube voltage, 90 or 100 kV; Current 100 or 200  $\mu$ A; and slice width, 0.16 or 0.08 mm, respectively. The obtained 3D re-constructions were analyzed using the software (i-VIEW, Morita, Kyoto, Japan).

**Results:** The 3D data correctly obtained from unused contact tips shape by micro-CT. The used tips changed to irregular shape, and it clearly demonstrated alterations to with tips for soft tissue surgery compared with those of cavity preparation.

**Conclusion:** These results revealed that micro-CT was useful for 3D morphological analysis alteration to irradiated tips.

## P-47

### Processing Ceramic Blocks Using Femtosecond Laser

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**Purpose:** Well-known zirconia crowns are produced either by machining a completely-sintered zirconia, or by machining semi-sintered-bodies and then sintering them completely. The latter method is more popular because of its easy processing; however, it is necessary to design the shape considering the anticipated shrinkage during sintering. The drilling method for completely-sintered zirconia blocks using a nanosecond Nd:YVO<sub>4</sub> laser was reported in 2014. It was also found that femtosecond lasers, with a shorter pulse width, are extremely accurate and efficient in processing compared to nanosecond lasers.

The purpose of this study was to develop a method of processing completely-sintered zirconia using femtosecond Ti:sapphire laser, and also examine the possible use of hydroxyapatite and alumina for ceramic crown.

**Materials and Methods:** Completely-sintered and semi-sintered zirconia blocks, dense and porous blocks of hydroxyapatite pellets, and alumina blocks; used in this study were prepared in dry-state as well as wet-state. We used two femtosecond Ti:sapphire lasers with different pulse widths of 35, and 150 fs (central wavelength: 800 nm, repetition rate: 1 kHz). The samples were set on an automatic stage and moved with a typical speed of 100  $\mu$ m/s. Laser pulses were incident vertically on the sample and focused with a lens of 20-mm focal length. After processing, the shape and depth of the laser irradiation trace were measured with a confocal microscope. Samples were processed at varying wavelength, irradiation energy, distance between the focal point of the lens and the sample surface, sample movement speed, with or without air flow for removing debris.

**Results:** The depth of drilling traces was found to increase, depending on the irradiation energy of laser pulses. The distance between the lens and the sample surface greatly affected the depth of drilling traces. It was the largest when the surface of samples was a few hundred micrometers ahead of the focal point. Also, the size of the drilling trace depended on the wavelength of laser pulses. Water was found to enhance the drilling efficiency depending on the sample structure. The cutting trace was also dependent on the sample movement speed. The size of cutting traces decreased with increasing moving speed of samples. We found the removal of the debris using air flow improved the drilling efficiency.

**Conclusions:** Femtosecond Ti:sapphire lasers with appropriate conditions were found to drill all the samples: blocks of completely-sintered zirconia, semi-sintered zirconia, hydroxyapatite pellet and alumina.

## P-48

### Direct Deposition of Apatite Film on Dentin Using Er:YAG Laser-ablation Method

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**Introduction:** To create a thin layer of Hydroxyapatite (HAp), we have developed a novel process using a pulsed Laser deposition (PLD) method for some substrates. Based on the concept of the PLD method, a tooth restoration technique with intraoral laser ablation has been newly proposed using an Er:YAG laser which is normally used for the removal of dental calculus. Previously, it was confirmed that the HAp thin layer is formed on dentin surface in the atmosphere at room temperature by the laser ablation method

using the Er:YAG Laser. In the present Study, properties of an HAp film deposited on dentin using the YAG laser ablation method was evaluated by the tensile and permeability tests.

**Materials and Methods:** A thin HAp film was created on the dentin surface of an extracted human tooth by the laser deposition method using an Er:YAG laser (Erwin AdvErL Morita Co.) and a tri-calcium phosphate bulk target. The Laser energy, pulse-repetition rate, and irradiation time in the present experiment were 200 mJ, 10 pulse/s and 20 min, respectively. The dentin substrate was kept wet with a calcium phosphate aqueous solution during the ablation. After  $\alpha$ -TCP was deposited on the dentin surface, the deposited layer was hydrolyzed by dipping pure water on the surface in order to create the HAp film. By means of such hydrolytic process, the  $\alpha$ -TCP layer changed into the HAp films for about 3 hours, and the thickness of the film was 100-200  $\mu$ m. Tensile tests were conducted to evaluate the adhesion between the HAp film and dentin. (diameter of 3 mm) Moreover, dentinal tubules were sealed with HAp particles. We evaluated the blockade effect of dentinal tubules using Pashley's method.

The interface structure between the HAp coating and the dentin surface was observed by scanning electron microscope (SEM).

**Results:** The adhesive strength of HAp films deposited on dentin was evaluated by quasi-static tensile tests. On mechanical evaluation, the adhesive strength was greater than 3.8 MPa. The measured average value for the sealing rate of the dentinal tubules was  $85.6 \pm 8.6\%$ . Electron micrographs showed that the HAp layer was formed on the dentin surface.

**Conclusion:** The present results suggest that this technique will be useful for the repair of dentin and the treatment of hyperaesthesia.

## P-49

### Irradiation of CO<sub>2</sub> Laser to An Orthodontic Adhesive Containing Heat-expandable Microcapsules Facilitates the Debonding of Orthodontic Brackets

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**Purpose:** In conventional removal methods for orthodontic brackets, a strong load must be applied physically to the bracket and tooth; this can sometimes cause fractures of the enamel and intense tooth pain. In particular, ceramic brackets offer excellent esthetic results compared with metal

brackets, but they are brittle, and it is time consuming to remove the bracket base when the bracket wings are broken. Our department reported using an orthodontic adhesive containing thermal expansion microcapsules, which expand 70-fold at 80 °C, that the bond strength of metal brackets bonded with the adhesive containing 30 or 40 % thermal expansion microcapsules decreased to 1/3 (7 MPa) or 1/5 (3MPa) with heating versus the unheated groups. However, heating with a high-temperature heater brings obvious risks of burns around the oral cavity. Thus, we examined safer and more effective bracket debonding methods.

The purpose of this *in vitro* study was to examine the reduction in debonding strength and the time taken using a ceramic bracket bonded with an orthodontic adhesive containing thermal expansion microcapsules and a CO<sub>2</sub> laser as the heating method while maintaining safety.

**Materials and Methods:** We examined the temperature of a bracket base during laser irradiation to decide the optimum in distance (0, 3, 5, 8 and 10 mm) from the tip of the laser to a ceramic bracket. Subsequently, ceramic brackets were bonded to bovine permanent mandibular incisors using bonding materials containing various microcapsule contents (0, 30, and 40 %), and the bond strengths were measured after laser irradiation for 4, 5, and 6 s with the condition of the above indicated irradiation distance and compared with nonlaser-treated groups. Also, the temperature in the pulp chamber during laser irradiation was measured.

**Results:** After laser irradiation for 5 or 6 s, the bond strengths of the adhesive containing 40 % microcapsules were significantly decreased to 0.40–0.48-fold (4.65.5 MPa) compared with the nonlaser groups. The mean temperature rise of the pulp chamber was 4.3 °C with laser irradiation for 6 s, which was less than that required to induce pulp damage.

**Conclusion:** Based on these results, we conclude that the combined use of a CO<sub>2</sub> laser and an orthodontic adhesive containing thermal expansion microcapsules can be effective and safe for debonding ceramic brackets with less enamel damage or tooth pain.

## P-50

### Photobiomodulation (PBM) Therapy Induced Gingival Papilla Tissue Regeneration- a Clinical Study

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**Purpose:** The interdental papilla has huge significance from

an esthetic point of view, especially in the anterior dentition, because it is almost universally exhibited during smile. The loss of interdental papillae leads to the so-called “black triangle”, which has a negative impact on patients self-esteem. Thus, periodontists are still looking for regenerating this lost tissue. In this context the tissue engineering (stem cells, scaffolds and growth factors) could be useful. As an adjunctive therapy to the tissue engineering the photobiomodulation (PBM) therapy has shown positive effects. PBM improves survival, proliferation, migration and differentiation of stem cells. Thus, the purpose of this retrospective clinical study was to present the outcomes of the reconstruction of black triangles in anterior dentition by using the PBM induced gingival papilla tissue regeneration.

**Material and Methods:** Under given consent 3 patients of a private dental clinic, who presented black triangles, participated of the study. They underwent to the PBM induced gingival papilla tissue regeneration approach, as follows: after occlusal adjustments and polishing of the esthetic veneers of the anterior teeth clinical photographs were taken for evidencing the black triangle. Then, the PBM therapy was applied with an InGaAlP diode laser (660 nm) in contact and punctual mode. (Laser Duo- MMO-São Carlos, Brazil). The parameters were: 100 mW, 3 mm<sup>2</sup> spot size, 20 s and 2 J per point in three points (one in the central portion of the marginal gingival of each affected teeth and other on the center of the defective papilla). After that, bleeding was provoked by using a multilaminar drill with 30 blades applied to the marginal gingiva in the teeth contour. Immediately after filling the black triangle with the blood clot, a new session of PBM therapy was applied. New clinical photographs were taken. These same procedures were applied twice with one-week interval. After the second and last session new clinical photographs were taken in order to qualitatively compare the size of the papilla before and after treatment.

**Results:** All patients presented any kind of improvement in the size of the interdental papilla. In most cases until 15 days the interdental papilla space was completely filled by the newly formed tissue.

**Conclusion:** The PBM induced gingival papilla tissue regeneration showed to be feasible. We propose that PBM therapy stimulated homing of gingival stem cells and supported their survival and differentiation in the blood clot favoring the regeneration of the interdental papilla.