International Joint Symposium: The University of Tokushima, Universitas Gadjah Mada, Niigata University

Denpasar, Bali, December 17-18, 2010

PROGRAM BOOK

PROGRAM BOOK

International Joint Symposium on Oral Science
Held by Niigata University – Universitas Gadjah Mada – The University of Tokushima

Featuring:
Oral Health-related Improvements Promote Quality of Life

Scientific topics:

1. Biomaterials and Tissue Engineering: Challenges in Oral Sciences
2. Oral Physiological Problems: Mastication and Swallowing
3. Oral Health Improvement towards Better Quality of Life: Current Status and Challenges
4. Dental nursing and educational system

The Patra Bali, Kuta
December 17th – 18th, 2010
Bali, Indonesia

PREFACE

It is our great pleasure to have you in our International Joint Symposium on Oral Science featuring “Oral Health-related Improvements Promote Quality of Life”. Excellent lectures and poster presentations on in dental tissue engineering, oral physiology, and oral health will be presented during the symposium. The symposium would be great opportunity for researchers from Indonesia, Japan, and other Asia and world to discuss their current knowledge on oral science.

Hopefully, the symposium would be the starting point to strengthen and expand scientific collaborations between Niigata University, Universitas Gadjah Mada, and The University of Tokushima and in general all participants contribute in the symposium.

We hope you to have fruitful symposium.

Takeyasu Maeda, Dean
Faculty of Dentistry
Niigata University, Japan

Iwa Sutardjo, Dean
Faculty of Dentistry
Gadjah Mada University, Indonesia

Yoshio Hayashi, Dean
Faculty of Dentistry
University of Tokushima, Japan

SYMPOSIUM SCHEDULE

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<tr><td>08.50-09.20</td><td colspan="2">Keynote Speaker:
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Siti Sunarintyas, Universitas Gadjah Mada
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<tr><td>10.20-10.40</td><td colspan="2">Coffee Break</td></tr>
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Makoto Inoue, Niigata University</td></tr>
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Munakhir Mudjosemedi, Universitas Gadjah Mada
Kensuke Yamamura, Niigata University
Nobuhiko Kawai, The University of Tokushima</td></tr>
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Workshop and Dental Nursing and Educational System</td></tr>
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December 18th, 2010	
08.00-08.40	**Special Lecture on Clinical Science: Oral Biofilms and Dentistry** Lakshman P. Samaranayake, Hongkong University
Session 3: Oral Health Improvements	
08.40-09.10	**Keynote Speaker:** Frank Abbas, Rijks University Groningen, The Netherlands
09.10-10.10	**Speakers:** Hendri Susanto, Universitas Gadjah Mada Yumoto Hiromichi, The University of Tokushima Hideo Miyazaki, Niigata University
10.10-10.30	**Coffee Break**
10.30-12.00	**Communication Forum Among Deans Affiliated in AFDOKGI**
12.00-14.00	**Lunch**

Workshop on Dental Nursing and Dental Education:

Purpose:
The purpose of this workshop is to enrich the qualities requisite for a dental educator. This workshop focuses on the goal of the oral health education of dental hygienist. The group-work will be carried out and discussed about the skills and acknowledgments acquired of a dental hygienist.

Detail Workshop Schedule:
13:00-13:30 Mini lecture and Icebreaking
13:30-14:30 Group discussion
14:30-15:00 Plenary discussion

Participation Capacity: 12-18

Communication Forum Among Deans Affiliated in AFDOKGI

No.	Time	Sub-theme and Speaker
1.	10.30-11.00	**Managing Dental School in Indonesia: Problems and A Way Forward** Chairperson of AFDOKGI **European Higher Education After Bologna Process and Dental Education in European Countries** Dean of the Faculty of Dentistry, Rijks University Groningen, The Netherlands
2.	11.00-11.30	**Managing Research and Community Services in Dental School of Developing Country** Dean of the Faculty of Dentistry, Universitas Gadjah Mada **Managing Research in Dental School: Lesson Learned from the University of Tokushima, Japan** Dean of the Faculty of Dentistry, The University of Tokushima
3.	11.30-12.00	**Dental Education Management: Lesson Learned from Niigata University, Japan** Dean of the Faculty of Dentistry, Niigata University **Learning System in Dental School: Lesson Learned from Hongkong University, Hongkong** Dean of the Faculty of Dentistry, Hongkong University
4.	12.00-12.10	**Conclusion Remarks (Prof. Samaranayake) Followed by Closing Remarks from The Committee (Moderator)**

P-01

Effect of Allicin for Reepithelialization of Wound Healing in Oral Ulcer Model

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Subagyo, G.

Oral Medicine;
Faculty of Dentistry, Gadjah Mada University,
Yogyakarta – Indonesia

Abstract

Allicin (*diallyl thiosulfinate*) is the main component of fresh garlic with multi-bioactivities as therapeutic agent. Recently, *allicin* is being developed as alternative medication for oral ulcer. The aim of this study was to determine the topical effect of *allicin* in aqueous garlic extract for *re-epithelialization* of wound healing process on acetic acid induced oral ulcer *Sprague Dawley* (SD) rats. Thirty male SD rats aged 2 months were divided into 2 groups as treatment and control group, each group consisted of 15 rats. Ulcer model on buccal mucosa was made by induction of 99.5% glacial acetic acid after anesthesized with 0.2 ml intramuscular Ketamine injection. In treated group, the rats were applied twice daily 1 drop of allicin for 1 minute and observation was carried out. Three rats each group were sacrificed on the 2^{nd}, 3^{rd}, 6^{th}, 9^{th} and 12^{th} day after induction and noted as H_0, H_1, H_4, H_7 and H_{10} group, respectively the tissues were prepared for HE staining. *Re-epithelialization* was assessed by measuring the epithelial thickness using ocular micrometer under light microscope. The data were statistically analyzed by t-test with significant p-values of <0.05 (95%). The result showed significant differences of epithelial thickness between each groups. Histological findings and graphical pattern showed a faster and better *re-epithelialization* on the treatment groups. It conclude that topical application of *allicin* can accelerate *re-epithelialization* of ulcer healing process.

Keywords: Allicin, oral ulcer model, reepithelialization, ulcer healing process

P02

Expression of p45^{Skp2} and p38^{Jab1} as a Target Of Mutant Type p27^{Kip1} in Human Head and Neck Cancer Cells

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1. Department of Oral Medicine, Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia.
2. Private Dental Clinic, Surakarta, Central Java, Indonesia.
3. UMS Medical Centre, Surakarta, Central Java, Indonesia.
4. Intregated Research Laboratory, Faculty of Dentistry, GMU, Yogyakarta.

Abstract

It has been reported that p27^{Kip1} to be an important diagnostic and prognostic marker in various human malignancies. Loss of p27^{Kip1} is associated with disease progression and an unfavorable outcome in several malignancies. Because p27^{Kip1} mutation is extremely rare in human tumor, expression of p27^{Kip1} protein is though to be controlled by post-transcriptional mechanism involved in in-activator, S-phase kinase associated protein 2 (p45^{Skp2}) and Jun activation domain-binding protein 1 (p38^{Jab1}). In the present study, an expression vector expressing mutant type *p27^{Kip1}* gene (pcDNA3.1-p27^{Kip1} mt), with mutation of Thr-187/Pro-188 (ACGCCC) to Met-187/Ile-188 (ATGATC), which is not influenced by ubiquitin-mediated degradation was constructed. The usefulness of mutant type of *p27^{Kip1}* gene therapy targeting down regulation of p45^{Skp2} and p38^{Jab1} was investigated. To determine the function of p27^{Kip1}, p45^{Skp2} and p38^{Jab1}, human head and neck cancer cells (B88 and HSY cells) were transfected with pcDNA3.1-*p27^{Kip1}* wild type (wt), pcDNA3.1-*p27^{Kip1}* mutant type (mt), or pcDNA3.1-empty vector. The growth inhibition of B88 and HSY cells mediated by pcDNA3.1-*p27^{Kip1}* mt was specifically due to a significant induction of apoptosis characterized by an increased activation of caspase-3 and caspase-9. Up-regulation of p27^{Kip1} was detected in pcDNA3.1-*p27^{Kip1}* wt and pcDNA3.1-*p27^{Kip1}* mt-treated cells, but an inverse correlation was appeared in p45^{Skp2} and p38^{Jab1} expression. Interestingly, the amount of p27^{Kip1} protein was slightly higher in *p27^{Kip1}* mt than in *p27^{Kip1}* wt. Furthermore, the transfection of pcDNA3.1-*p27^{Kip1}* mt induced a strong growth inhibition of xenograft tumors, and during the experimental period, no loss of body weight was observed in each group of the mice. In conclusion, pcDNA3.1-*p27^{Kip1}* mt-treated cells may have the potential to become a novel and powerful gene therapy tool through down regulation of p45^{Skp2} and p38^{Jab1}.

Keywords: Head and neck cancers, *p27^{Kip1}* mt, *p27^{Kip1}* wt, p45^{Skp2}, and p38^{Jab1}.

P03

Expression of MRP 8 mRNA in Human Monocytes of Rapidly Progressive Periodontitis Patients

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Gadjah Mada University, Yogyakarta, Indonesia

Abstract

It is well known that monocytes increased calprotectin release in inflammatory tissues and detected in gingival crevicular fluid (GCF)of periodontitis patients at high levels. In previous study we had reported that lipopolisaccharide of *Porphyromonas gingivalis* (P-LPS) and cytokines induce the release of calprotectin by monocytes which were isolated from healthy human peripheral blood and identified that P-LPS and cytokines is a significant etiologic factor in the production of calprotectin. In this study, we investigated the basal level of calprotectin mRNA from human monocytes of rapidly progressive periodontitis patient by examining of MRP8 and MRP14mRNA expression. Monocytes were isolated from the peripheral blood of healthy donors and early onset periodontitis subjects. Total RNA were isolated from both resting monocytes, and they were assessed by RT-PCR methods using MRP8, MRP14, and GAPDH primers. Expression of basal level of MRP14 mRNA is relatively stable in monocytes that were isolated from healthy and early onset periodontitis subject, but MRP8 mRNA was decreased in monocytes of early onset periodontitis. MRP8 significantly decreased to about 2.5-fold that of the control level. These results demonstrated that monocytes that were isolated from rapidly progressive periodontitis subject has low basal level of MRP8 mRNA indicate that calprotectin play role in the immunopathogenesis of rapidly progressive periodontitis

Keywords: Calprotectin, MRP-8, Monocytes, RPP

P04

Anti-Inflammatory Effect Of Ethanol Extract Of Avocado Seeds (*Persea Americana mill*) In Wistar Rats

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Muhammadiyah University of Yogyakarta, Yogyakarta, Indonesia

Abstract

Inflammation is a local protective response caused by injury or tissue damage. Avocado seeds (*Persea americana* Mill) that contain flavonoid can be utilized as a substance suspected to accelerate the wound healing process. The purpose of this study was to determine the effect of ethanol extract of avocado seeds as antiinflammatory agent in wistar rats. A number of 30 rats were injected with 0.1 ml 1% carrageenan subcutaneously on the right hind paw an hour after oral administration of ethanol extract of avocado seeds with various concentrations : 2.5%; 5%; 10%; and 20%. Positive control using dose diclofenac sodium 40 mg / kg body weight of rats and 0.5% Carboxymethylcellulose (CMC) of sodium as a negative control. Applications per oral dose of 2 ml/200 g body weight of rats. Edema volume measurements performed with an interval of one hour for six hours using plethysmometer. Data analysis using ANOVA and LSD. Results showed that significant effects of ethanol extract of avocado seeds toward decreasing the volume of rat foot edema. The result obtained was not significant in comparison with the positive control avocado seeds. Increasing the concentration of avocado seeds yield increased percentage inhibition of edema. Concluded that ethanol extract of avocado seeds is equivalent to a positive control and increasing concentrations of ethanol extract of avocado seeds can increase the percentage inhibition of edema.

Keywords: Avocado seeds (*Persea americana* Mill), anti-inflammatory, Wistar rats

P05

Streptococcus α Haemolytic Growth in Moderate Gingivitis Patient Dental Plaque After Gargle Clove Essential Oil (*Syzygium aromaticum* (L.))

Keshia Dinda Widowati, Alma Linggar Jonarta, Regina TC. Tandelilin

Department of Oral Biology, Faculty of Dentistry, Gadjah Mada University

Abstract

Moderate gingivitis is one of high prevalent periodontal disease in the community. Gingivitis occurs due to the accumulation of mature plaque. Streptococcus α haemolytic plays important roles in the formation of dental plaque. One of potential natural substance that may able to heal gingivitis is essential oil of clove. The aim of this study was to determine the effect concentration 3%, 4%, 5% or 6% of clove essential oil (*Syzygium aromaticum* (L.)) gargling in the growth of Streptococcus α haemolytic on plaque of moderate gingivitis patients. Twenty five subjects were randomly divided into two groups, control (chlorhexidine gluconate 0.12%) and treatment (clove essential oil) groups. Five subjects of each group (control and 3%, 4%, 5%, or 6% of subtreatment concentrations) were asked to gargle 10 ml of solution twice a day for 5 consecutive days. Dental plaque, collected in the morning before teeth brushing on the first and sixth day, were cultured. The number of Streptococcus α hemolytic before and after serial gargling were then compared. The result of total number of Streptococcus α haemolytic, based on proportional value, were analyzed using one-way ANOVA ($p<0.05$) and LSD (Post Hoc) tests. The result of this study showed that concentration 3%, 4%, 5%, and 6% of clove essential oil significantly suppress the growth of Streptococcus α haemolytic. It can be concluded that the 4% of clove essential oil indicates a better suppression in the growth of Streptococcus α haemolytic in moderate gingivitis patients.

Keywords: Clove essential oil, Streptococcus α haemolytic, gingivitis

P06

Antibacterial Effect of Holy Basil (*Ocimum sanctum L.*) Leaf Extract towards The Growth of *Streptococcus viridans.*

Esty Riyanda Astuti

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Abstract

Holy basil *(Ocimum sanctum)* is one kind of medicinal plant that performs antibacterial effects. Basil leaf contains Phenolic compound (1-hidroxy-2-methoxy-4-allylbenzene) with Nerolidol, Spatuleno , cis-Ocimen, Hexadecadienol , α–Bergamoten, Nerolidol, Octodecano*,* Bisabolo, Heptadecan–1–ol, methyl cinnamate, linalool. The compounds have obvious antimicrobial effects regarding their tested antibacterial activity toward both aerob and anaerob Oral bacteria. They're also succesfully tested as antifungal. The aim of this study is to know if Basil leaf extract has antimicrobial activity toward the growth of *Streptococcus viridans* Isolat Str.V/09/XI. The study was considered to be *in vitro* Laboratory Experimental Research. The research was conducted in Microbiology Laboratory of Faculty of Medicine of Muhammadiyah University of Yogyakarta. Antibacterial activity of Basil leaf extract was evaluated by dilution method. The concentrations used were 50% bv, 25% b/v, 12,5% b/v, 6,25% b/v, 3,12% b/v, 1,56% b/v, 0,78% b/v, 0,38% b/v dan 0,19% b/v. Antibacterial effect was determined by Minimal Inhibition Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of Holy basil leaf extract. MBC is started from the smallest concentration that has antibacterial effect toward the growth of *Streptococcus viridans* Isolate Str.V/09/XI in both dilution tube and blood agar. The research was conducted 3 times for the reliability. The result performed that holy basil leaf extract has antibacterial effect toward the growth of *Streptococcus viridans* Isolate Str.V/09/XI and the Minimal Bactericidal Concentration was on 25%. The conclusion of this study is holy basil leaf extract has antibacterial effect toward the growth of *Streptococcus viridans* Isolate Str.V/09/XI*.*

Keywords: Holy basil leaf, antibacterial, S*treptococcus viridans.*

P07

Treatment Of Class II Division 1 Malocclusion with Temporo Mandibular Disorder (Case Report)

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3. Staff Member of Paedodontic Department, Faculty of Dentistry, Padjadjaran University

Abstract

A 13 years-old girl presented with a Class II division 1 incisor relationship on a mild skeletal Class II base. The patient complained TMD symptoms, such as clicking, pain in opening, protrusive, and lateral movement. Treatment was commenced using Twin Blocks appliance, and will followed by fixed appliance. The basic Twin Blocks technique achieves Class II correction by taking advantage of the inclined occlusal planes to hold the mandible in the forward position. The appliance was combined with upper tongue crib to eliminate bad oral habit and lower expansion screw for lateral development.

Keywords: Twin Blocks, TMD, Class II division 1 malocclusion.

P08

Chemical Effectiveness of *Salvadora persica* and Commercial Whitening Toothpaste at Preventing Tea and Chlorhexidine Stain (in vitro study)

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4. Final-year dental student, School of Dental Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia

Salvadora persica, a plant which contains wide range of healthy components has been used as chewing stick for ages in maintaining good oral hygiene and currently had been approved to remove stain. However, it's preventing effect still under research. The objective of this study was to reflect the effectiveness of *Salvadora persica* and commercial whitening toothpastes in preventing tea and chlorhexidine stain. Three group studies were conducted; group A was drinking water, group B was Commercial whitening toothpaste and group C was Whitening toothpaste with *Salvadora persica* extract. Sixty clear acrylic blocks were used; 20 for each group. A baseline measurement by spectrophotometer was taken before starting the procedure. All specimens were immersed in the artificial saliva (2 minutes), rinsed in distilled water, and exposed in 0.2% chlorhexidine (2 minutes). Then, blocks were carefully removed, washed and placed in standard tea solution (2 minutes). These cycles were performed 8 times a day. Intervention with whitening toothpaste was done for 2 minutes; twice a day. Eventually, all blocks were removed, washed and dried. Stain was assessed by spectrophotometer and visual assessment using Lobene stain index (1968). The blocks remained in the artificial saliva when not in used. This procedure was performed for 5 days. Records show significant results (Kruskal-Wallis test, $p<0.001$); group C (10% of heavy stain), group B (95%), group A (100%). Chemically of whitening toothpaste with *Salvadora persica* extract is more effective than commercial whitening toothpaste in preventing stain formation.

Keywords: *Salvadora persica*, preventing stain, whitening,

P09

Increased Gingival Epithelial Cell Maturity of Moderate Gingivitis Subjects After Clove Essential Oil Gargling

Lidya N. Arfiadi, Regina TC. Tandelilin, Alma Linggar Jonarta

Abstract

Gingivitis is a high prevalence oral problem which occurs due to the accumulation of dental plaque. Dental plaque bacteria and its products are capable of irritating to the extent of gingival inflammation. Inflammation can alter the maturity of gingival epithelial cells due to the decreased degree of keratinization resulting in a constricted number of superficial cells. Eugenol contained clove essential oil (*Syzygium aromaticum* (L.)) is known to have the ability to heal gum inflammation. The aim of this study was to determine the alteration of gingival epithelial cell maturity in moderate gingivitis subjects as a response to gargling clove essential oil solutions with various concentrations. The subjects involved in this research were 35 subjects suffering moderate gingivitis. These subjects were randomly divided into 2 groups: treated and controlled. Seven controlled group subjects were treated with 0.12% chlorhexidine gluconate and subjects of treated sub groups were treated with 3%, 4%, 5% or 6% clove essential oil solutions. All subjects gargled the solutions every morning and night within 5 consecutive days. Gingival swabs were taken before and after the treatment. The swab were stained using papanicolaou technique and observed under light microscope. Observation was done within 100 gingival epithelial cells and cell types (basal, intermediate, superficial cells) were identified and counted. The result of various gingival epithelial cells identification were then analysed with one way ANOVA ($p<0.05$). The obtained Maturation Index (MI) after gargling 0.12% chlorhexidine gluconate, and 3%, 4%, 5% and 6% concentrations of clove essential oil solutions were : 0:38:62; 0:41:59; 0:36:64; 0:34:66; and 0:38:62. Therefore, this research shows that gargling 3%, 4%, 5% and 6% clove essential oil solutions significantly increased the maturity of gingival epithelial cells in moderate gingivitis subjects which is equivalent to the ability of 0.12% chlorhexidine gluconate.

Keywords: Epithelial cell maturity, gingivitis, *Syzygium aromaticum* (L.), essential oil

P10

The Difference of Effectiveness between Miswak (*Salvadora persica*) Extract and Carbamid Peroxide 10% in tooth bleaching

Mega Aprilia
Any Setyawati

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Abstract

Miswak contains chloride that can be used as an alternative tooth bleaching material. The aim of the present study was to assess the hypothesis that there was difference of effectiveness between miswak extract and carbamid peroxide 10% in tooth bleaching process. A number of 10 samples of post extracted teeth were discolorized in black tea solution. They were divided into two sample groups i.e. group 1: five discolorized post extracted teeth soaked in miswak extract during 56 hours, group 2: five discolorized post extracted teeth soaked in carbamid peroxide 10% during 56 hours. Colour change was measured by spectrophotometer and matched by shade guide. Color change comparison was tested using Paired Sample t-test and Dependent t-test. There was no significance difference (p>0,05) between miswak extract (*salvadora persica*) and carbamid peroxide 10% in tooth bleaching process. It means that miswak extract can be used as an alternative tooth bleaching material as effective as carbamid peroxide 10%

Keywords: Miswak extract, carbamid peroxide 10%, tooth bleaching.

P11

Increased Glutathione Level in Saliva of Moderate Gingivitis Patients after Lemongrass (*Cymbopogon citratus*) Essential Oil Gargling

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2. Department of Oral Biology, Gadjah Mada University, Indonesia

To determine the glutathione level of moderate gingivitis patients' saliva after gargling lemongrass essential oil. This study involved 50 moderate gingivitis subjects that were randomly divided into 2 groups, treated and controlled group. The treated group gargled with 0.5% lemongrass essential oil (10 subjects), 1% (10 subjects), 2% (10 subjects) and 4% (10 subjects). In controlled group, 10 subjects gargled using hexetidine 0,1%. Each subject gargled twice a day, morning and night, for 5 days consecutively. On the first day before the serial treatment as well as the next day after the serial treatment, samples of saliva were taken and the GSH level was measured using a spectrophotometer at 412 nm wave length and analyzed using ANOVA. The glutathione level in saliva of moderate gingivitis subjects increased after the treatment, along with the increase of lemongrass essential oil concentrations. The data was analyzed using ANOVA (p=0.00) showed that lemongrass essential oil was significantly effective to enhance glutathione level in subjects' saliva. Moreover, Post hoc LSD test was conducted and suggested that 2% and 4% lemongrass essential oil had the similar potent with 0.1% hexetidine. Gargling lemongrass essential oil with 2% concentration isbest to enhance the GSH level in saliva of moderate gingivitis patients than compared to the other level of essential oil concentrations and 0.1% hexetidine. Thus, lemongrass essential oil solution can accelerate gingivitis healing process. However, the optimum concentration has not yet been determined.

Keywords: Gluthatione level, moderate gingivitis, *Cymbopogon citratus*

P12

Expression of MRP8/MRP14 mRNA in Monocytes of Periodontitis: Comparison between Diabetic and Non Diabetic Patients

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2. Department of Prosthodontic, Faculty of Dentistry, Gadjah Mada University
3. Department of Immunology, Faculty of Medicine, Gadjah Mada University

The severity of periodontitis on patients with type 2 Diabetes Mellitus patients was strongly thought caused by decreasing of leukocytes function such as monoctyes and neutrophils. In my previous research found that calprotectin (MRP8/MRP14) level in leukocytes of periodontitis patients with type 2 DM is higher than periodontitis non DM. The aim of this study was to determine calprotectin (MRP8/MRP14) mRNA expression in human monocytes of periodontitis patients with type 2 DM and without DM. Monocytes were isolated from the peripheral blood of periodontitis patients with uncontrolled type 2 DM, controlled type 2 DM, and non DM. The expression of total RNA calprotectin (MRP8 and MRP14) were detected by RT-PCR using GAPDH as the innate control. The value of MRP8/MRP14 mRNA expression in DM patients were higher than non DM, and the highly significant increase expression ($p<0.05$) was on the uncontrolled type 2 DM. The basal level of MRP8/MRP14 expression increased in monocyte of periodontitis and type 2 DM patients compared with non diabetes subjects. It was suggested that high basal level MRP8/MRP14 has role in the regulation of severity periodontitis with diabetes mellitus.

Keywords : MRP8/MRP14, monocytes, periodontitis, type 2 DM

P13

The Effect Topical Application 10% Cashew Fruit (Anacardium occidentale L) on Secretory Immunoglobulin A (sIgA) OF Saliva Woman with Recurrent Apthous Stomatitis (RAS)

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Abstract

Cashew fruit has anti-inflammatory effect to stomatitis. Flavonoids and vitamin C of cashew fruit can heal stomatitis. Level of sIgA was elevated in patients with Reccurent Apthous Stomatitis (RAS). The aim of this study was to evaluate the effect topical application 10% cashew fruit extract (Anacardium occidentale L) on secretory immunoglobulin A (sIgA) saliva of women with RAS. Twenty women with RAS, 20-30 years old, with the approval of ethical clearance from Ethic Comittee Medical Faculty of Gadjah Mada University, Yogyakarta. Subjects were divided into treatment and control groups, each group consist 10 women. 10% cashew fruit extract was used topically as treatment and Kenalog was applied as control. Aplication was done in the morning and evening for 7 days. Saliva samples were collected at afternoon (16.00-18.00 pm) for 1 minute using unstimulating method. The measurement of sIgA level by ELISA kit (Salimetrics LLC). Optical Density was read on standard plate at 450 nm. Data for level sIgA were analyzed using ANOVA ($p<0,05$). The results showed significant differences between before and after treatmet using topical application of cashew fruit extract. This study indicating that cashew fruit extract can increase the level sIgA in saliva woman with RAS.

Keywords: Cashew fruit (*Anacardium occidentale* L), Reccurent Apthous Stomatitis (RAS), secretory immunoglobulin A (sIgA), saliva.

P14

An *In Vitro* Genotoxicity Study Of Silver Amalgam on Ames Test

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3. School of Dental Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

Abstract

Silver amalgam/Silverfil Argentum® is a 'Malaysian made amalgam' has already been approved to be free from cytotoxicity, however its genotoxic effect has not been explored yet as biocompatible material. The objective of this study was to identify the genotoxic characteristic of silver amalgam by using Bacterial Reverse Mutation Assay (Ames test). This was a descriptive experimental study involving one strain of mutated *Salmonella*. The test material was evaluated in one mutated strain of *Salmonella typhimurium* TA1538 with and without an external metabolic activation system (S9 Mix). The bacteria were incubated for 48 hours at 37±0.5ºC before the colony growth or revertant colonies were counted. Data obtained was analyzed by using non-statistical method. The investigation of the genotoxic reaction on the test material revealed that the number of revertant colonies in both strains with and without S9 Mix were less than twice of the negative control even in the presence of high silver amalgam concentrations (5.0µg/ml). This study demonstrated that the test material did not exhibit any mutagenic activity under the chosen conditions. Thus, silver amalgam could be considered to have no genotoxicity effect.

Keywords: Silver amalgam, genotoxicity, Ames test

P15

Gender Dependence in Mouth Opening Dimensions in Normal Adult Malaysian Population

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Abstract

While measurement of mouth opening is an important clinical examination to assist in diagnosis and management of oral disease, data on non-Western populations are limited. This study was therefore conducted to determine the range of mouth opening in normal Malaysian adults, with a comparison of males and females. A total of 34 dental students of Universiti Sains Malaysia (USM) Kubang Kerian were chosen at random and their maximum mouth opening were measured after being asked to open their mouth sufficiently to accommodate three fingers. Measurement was performed from the edge of the upper incisor to the lower incisor using a caliper divider. The median values were 47.6 mm in males 40.8 mm in females, the difference being significant ($p<0.05$). Thus the width of mouth opening in our Malaysian student population was gender dependence although further study with a larger sample size and with other ethnic groups should be carried out, focusing on age and size.

Keywords: Gender dependence, mouth opening

P16

Marsupialization, Enucleation to the Mandibular Dentigerous Cyst, A Case Report

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Abstract

Marsupialization is a surgical treatment undertaken to remove the fluid in the cyst cavity. It aims to eliminate the pressure of the cyst fluid, which generates pain and aggravates the cyst to grow bigger. Eliminated cyst fluid is expected to relieve pain and prevent the growing cyst. Marsupialization is done to the cyst embedded to risky structures or the one which likely generates massive damage prior to enucleation. Enucleation refers to the cyst-treating surgery in which the cyst along with the cyst capsule is completely removed. The surgery aims to prevent the cyst to recur and to get perfect recovery. Dentigerous cyst is caused by the epithelial rests of tooth-forming Malassez which develops to be a cyst. The dentigerous cyst is commonly followed by impacted teeth, and mostly found in the mandible with impacted mandibular third molar tooth. Marsupialization and enucleation were performed to the dentigerous cyst with impacted mandibular third molar tooth of a 25-year old female patient. The surgery was undertaken because the cyst was massively growing and leading to mandibular fracture.

Keywords: Marsupialization, enucleation, dentigerous cyst

P17

Antigen Fractions of *Porphyromonas gingivalis* Induce the Activation of Neutrophil MMP-9

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Abstract

Our previous study demonstrated that some antigen fractions of *Porphyromonas gingivalis* were positively reactive with antiserum of ACS (acute coronary syndrome) patients. This evident was emerging hypothesis the involvement of *P. gingivalis* antigen fractions in vascular destruction. One biomarker of vascular destruction is matrix metalloproteinase 9 (MMP-9), which was known as an enzyme with the substrate specificity is vascular type IV collagen, the main structure of sub endothelial basal membrane. Because the main source of MMPs is netrophil, therefore it was suggested that *P. gingivalis* antigen induce vascular destruction through the activation of neutrophil MMP-9. This study purposed to demonstrate the potency of *P. gingivalis* antigen in neutrophil MMP-9 activation. This experimental in vitro study utilized isolated neutrophil from human pheripheral blood donor, which was exposed to *P. gingivalis* antigen. The filtrate medium were then analyze for MMP-9 by means of gelatin-zymography method. The results showed that *P. gingivalis* antigen induce the activation of neutrophil MMP-9 that was demonstrated by the present of gelatinolytic zones. This mechanism may explain the role of *P. gingivalis* antigen on vascular destruction leading to ACS.

Keywords: *P. gingivalis*, MMP-9, neutrophil.

P18

Porphyromonas gingivalis Induce Neutrophil Collagenolitic Activity Leading to Vascular Collagen Degradation

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Abstract

Vascular collagen degradation is the basis molecular process for atherosclerotic plaque rupture (APR) leading to coronary thrombosis and ACS (acute coronary syndrome). *Porphyromonas gingivalis* was identified in human coronary atherosclerotic plaque (AP) and supposed to be involved in vascular collagen degradation on AP leading to APR. This experimental study purposed to demonstrate in vitro the potency of *P. gingivalis* to stimulate neutrophil collagenolytic activity which caused vascular collagen degradation. Collagenolytic activity was demonstrated by means of SDS-PAGE (Sodium Dodecyl Sulphate Polyacryamide Gel Electrophoresis) and SBA (Soluble Biotinylated Assay). The involvement of some collagenolytic components such as *P. gingivalis* proteases, neutrophil MMP-9 and ROS (reactive oxygen species) were analyzed by means of SDS-PAGE, Western blotting and NBT (Nitrobluetetrazolium) assay, respectively. The results showed that *P. gingivalis* stimulated neutrophil to produce collagenolytic components and its interaction generated collagenolytic activity leading to vascular collagen degradation. This mechanism may explain the role of *P. gingivalis* on APR and ACS.

Keywords : *Porphyromonas gingivalis*, neutrophil, collagen, MMP, ROS.

P19

Comparison of Shear and Microtensile Bond Strengths of Composite Repair

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Abstract

The objective of this study is to compare the shear bond strength (SBS) and microtensile bond strength (MTBS) testing methodologies for non aged and aged compsite-to-composite bond strength in two types of composites employed two surface conditioning methods. Composite disks for SBS and composites blocks for MTBE were fabricated and randomly divided into two groups, non aged and aged by thermocycling (5000x, 5-55 ºC, abbreviated TC). Two surface conditioning methods were evaluated : (1) intermediate adhesive resin application (IAR), (2) Chair side silica coating (30 µm SiO_2) and silanization + IAR (Visio^{TM}-Bond) (SC). Resin composites, of the same kind as the substrate, were adhered onto the substrates. Shear and microtensile force was applied to the adhesive interface and failure types were evaluated under optical microscopy. Significant differences between the two test methods, aging conditions and composite types were observed ($p<0.05$). The mean of SBS values (7.3 ± 2 – 27.4 ± 5.6 MPa) ware lower than MTBS (32.3 ± 8.9 – 53.7 ± 19.3). However, survival analysis showed that the Weibull modulus between SBS (2.2 – 4.4) and MTBS (2.1 – 4.7) was insgigniffican different. In addition, SBS test resulted more cohesive failures (0% - 100%) than those of MTBS test (8 – 43%), regardless of the compositetypes, aging method and surface conditioning. The results of this study that MTBS resulted higher value and less cohesve failure than SBS, but insignificant different in Weibull moduli. MTBS was capable of detecting smaller differences by using survival analysis.

P20

The Use of Maxillofacial Protheses on Nose Amputated Patient

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Abstract

Maxillofacial prosthetics is a branch of dentistry that deals with congenital and acquired defects of the head and neck. Acquired defects can be devided into intraoral and extraoral. Intraoral defects may involve the mandible, tongue, soft palate, or hard palate, while extraoral defects may involve any other area of the head or neck. The aim of making maxillofacial prosthetics is to make better aesthetics and functional so the post amputated nose patient will not ashamed with her appearance. A woman age 60 years old came to the prosthodontics clinic of RSGMP Prof. Soedomo, bringing reference letter from ENT specialist. She wants to make a nose prosthesis because her nose was amputated since 5 years ago. This patient had nasopharyx cancer since 15 years ago and have been through operation for 3 times and on the last operation she had her nose amputated. Nose prosthesis has been made for this patient using silicon material which have almost the same texture as the original one. This prosthesis was attached on eye glasses so the patient can use and remove it easily. The result of using maxillofacial prosthetics for post amputated nose patient is the patient have better aesthetics. Beside that the function of speech and breathing can also be aided. So the conclusion is that with the use of maxillofacial prosthetics on post amputated nose patient, can aid both aesthetics and functional.

Keywords : Maxillofacial prosthetics, post amputated nose

P21

The Use of Overdenture Base Root Type for Aesthetic Correction

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Abstract

Overdenture is a full and partial denture that supported by mucoperiosteum and teeth or root teeth that underwent endodontic treatments. Base root is the simplest support type, after root canal treatment, the teeth were prepared and shortened about 2-3 mm above the gingival. The teeth were restorated uing composite resin, compomer or amalgam. The purpose of using overdenture base root type was to repair aesthetics. The patient came with central left insisivus, lateral upper left insisivus, upper left caninus and upper left second premolar were in oblique position, extrution and the midline were moved. The teeth must underwent root canal treatment and will be made a new denture with better aesthetics. The patient had systemic disease, high blood pressure and diabetes mellitus so teeth extraction can't be done. Therefore, the patient was made overdenture base root type for the central left insisivus, lateral upper left insisivus, upper left caninus and upper left second premolar. The used of partial overdenture showed good retention and stabilization, mastication function, aesthetics and fonetics. After evaluating the treatment for two weeks, the examination showed no more pain and teeth able to function normally.

Keywords : Overdenture, base root, aesthetics.

P22

The Influence of Chitosan Hydrogel Concentration to Neutrophil and Macrophage in Gingiva Ulcer Healing Process in Sprague Dawley Rat

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Abstract

Chitosan is a natural polysaccharide taken from crustacean that has been widely used due to its low cost, large scale availability, high biocompatibility, biodegradability and wound healing properties. The aims of this study is to evaluate the effect of chitosan gel concentration toward the neutrophil and macrophage in gingival ulcer healing process of Sprague Dawley rats. Twenty subjects were divided into four groups. Group A was given 1% gel chitosan, group B (2% gel chitosan), group C (3% gel chitosan) and group D as control group without application. The ulcer was made by applicating the 2X2 mm2 Whatmann number 1 filter paper which had been soaked into the 98% acetic acid for 5 minutes on the gingival surface below the interdental of the two anterior mandibular teeth of the rats for 40 seconds. Topical application was twice a day on ulcer by using pipette during three days. The subjects then being sacrificed and its gingival tissue was taken for histologically processed and stained with hematoxylin eosin. Neutrophil and macrophage were counted and analyzed using one way ANOVA with significance level at 95%. The result of one–way ANOVA test showed that significant difference neutrophil and macrophage among group ($p<0,05$) for 1%, 2% and 3% gel chitosan. The result of Pearson correlation test showed that there is a positive and strong correlation (0,979) between concentration chitosan and macrophage. Chitosan gels with 1%, 2%, and 3% concentration influenced neutrophil and macrophage density. The higher concentration of gel chitosan decreased the neutrophil but increased the macrophage because they have antimicrobial activity. These data indicate that the 3% chitosan gel can accelerate wound healing in gingival ulcer than 1% and 2% concentration.

Keywords: Chitosan gel, wound healing, gingival ulcer, neutrophil, macrophage

P23

Oral Health Status among School Children: Does Partnership with School of Dentistry Make a Difference?

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Abstract

The School of Dentistry, Gadjah Mada University, has been involved in school dental health programs in 9 primary schools for the past 12 years. The purpose of the study is to evaluate whether the partnership with the School of Dentistry improved oral health status of the schoolchildren. A sample survey was carried out, 106 fifth and sixth graders from the schools participating in the partnership and 90 schoolchildren from the non-participating schools were examined by trained dental students. Oral health status was represented by OHIS and DMFt measurement. Knowledge, attitude and practices of oral health were measured using structured questionnaires. The differences in the results of the measurements between children and the participating and non-participating schools were tested using t-test. Causal modeling was developed using path analyses. The study indicated that the means of OHIS and DMFt among schoolchildren participating in the partnership were 0.11 and 0.01 lower respectively compared to those of their counterparts, although the differences were not significant statistically. The knowledge, attitude and practices among schoolchildren in the partnership were 2.49, 4.18 and 3.86 higher, all were highly significant ($p < 0.001$). Path analyses showed that the partnership reduced OHIS and DMFt with an overall path coefficients – 0.086 and -0.076 respectively. The effects of partnership between the School of Dentistry and primary school were significant only on the knowledge, attitude and practices of the schoolchildren. Although knowledge was associated with DMFt and attitude with OHIS, there were other unmeasured variables which were more strongly associated with oral health status of the schoolchildren.

Keywords: Partnership with School of Dentistry, oral health status and KAP survey

P24

The Effect of Stolephorus Insularis to the Mandibular Growth of Novergicus strain Wistar Rat

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Stolephorus Insularis or have known with Teri Jengki Fish easily found in Indonesia. This fish contain calcium and fluor that give much benefit to human body especially for mandibular growth. The aim of this study was to investigate the role of stolephorus insularis and calcium tablets to increasing mandibular growth of novergicus strain wistar rat. This study devides in 3 groups of diet. The first group is the control group or standart diet group, the second group is a standart diet that have mixed with stolephorus insularis powder, and the third group is a standart diet that have mixed with calcium tablet. The 3 kinds of diet formed in pellet. 24 ratus novergica strain wistar with weight amount 150-200grams were selected as the subject of the study. The 24 ratus novergica strain wistar divide in 3 groups and feed with each 3 kind of diet for 60 days. After 60 days, the rats being sacrified and take the mandibular and examine the length of mandible. All data experiment were analyzed by Univariat Anova and LSD test. The result of this experiment that there was a significant difference in increasing the length of mandible after being fed with stolephorus insularis and calcium tablets for 60 days. Adding stolephorus insularis in food is effective to increasing the length of mandible in its growth of novergica strain wistar rat.

Keywords: Stolephorus Insularis, calcium tablets, bone, mandibular growth.

P25

The effect of Cu concentration in the Cu-natural zeolite on its antifungal activity towards Candida albicans

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Abstract

Copper has ability to inhibits microorganism growth. Antibacterial material
can be prepared by adding copper ions into porous materials such as zeolite. The aim of this research was to investigate the effect of Cu concentration in the Cu-natural zeolite on its antifungal activity towards C.albicans. Natural zeolite (Zeoprima,Yogyakarta), $CuCl_2.2H_2O$ (Merck, Germany), C. albicans culture, BHI and Sabouraud agar were used in this research. Five groups of Cu-zeolite (were made. Zeolite powder (100 mesh) was activated (200^oC/1hour). Cu-zeolite was made by reacting 2 mg zeolite with 80 mL $CuCl_2$ solutions (0.05M, 0.1M, 0.15M, 0.2M, and 0.25M) 1 hours at 100^oC, filtered, washed, and dried (100^oC/24 hours). C.albicans (0.1 mL x108 CFU/mL) were inoculated at Saboraud agar, than 30 mg Cu-zeolite were put into agar wells and the agar were incubated (n=5). Translucent diameter zones were measured by digital sliding calipers. The data were analyzed by one-way anova and HSD (p= 0.05). The results showed that the means of translucent zones were 16.15 + 1.54 mm (0.05M group), 17.75 + 0.71 mm (0.10M group), 17.44 + 1.01 mm (0.15M group), 22.23 + 0.67 mm (0.20M group), and 20.75 + 1.18 mm (0.25M group). One-way anova test showed that Cu concentration in the Cu-zeolite influenced significantly on the translucent zones of C.albicans ($p< 0.01$). HSD analysis showed significant differents between 0.05M to 0.15M groups and 0.20M to 0.25M groups ($p<0.05$). Conclusion: the Cu concentration in the Cu-natural zeolite influenced the antifungal activity towards C.albicans. 0.20M Cu concentration had the highest antifungal activity.

Keywords: Cu concentration, Cu-natural zeolite, antifungal, *C.albicans.*

P26

Effect of Zirconia-Filler in Hydroxyapatite on the Growth of *Staphylococcus epidermidis*

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Abstract

Synthetic hydroxyapatite has been extensively developed in medical applications as material for bone substitute. Previous study showed hydroxyapatite did not have the capacity to induce acute toxicity in animal research. The addition of zirconia is needed to improve the strength of hydroxyapatite. One of the drawbacks in the use of biomedical materials is the occurrence of biomaterial-centred-infections. Recent method of limiting the presence of micro organism on biomaterials is by providing biomaterial-bound metal-containing compositions. It was also stated that S. epidermidis is the most common infecting organism in biomedical-centred- infection. The aim of this study was to evaluate the effect of zirconia as filler in hydroxyapatite on the growth of S.epidermidis. The subjects were hydroxyapatite discs, with the diameter of 10 mm and 3 mm in thickness. Ten discs were divided into one control and one treatment group. Zirconia powder with the concentration of 40% was added into the treatment group. All discs were immersed into S.epidermidis culture for 24 hours and soaked into a medium of PBS. The cultured medium was spread on manitol salt agar. After the incubation for 24 hours at 37oC, the numbers of colonies were measured with colony counter. Data obtained were analyzed by Student's t-test. Statistical analysis proved that zirconia-filler showed a significant influence on the number of S.epidermidis colony ($p < 0.05$). It can be concluded that the addition of zirconia-filler in hydroxyapatite showed minimum number of colony.

Keywords: Hydroxyapatite, zirconia, *S.epidermidis*

P27

Inhibitory Effects of *Punica granatum linn* peel extract against *Streptococcus mutans* and *Candida albicans* and its toxicity on fibroblast cell line

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Abstract

Pomegranate rind (*Punica granatum linn*) had been used as traditional medicine for long time, however its efficacy and safety is still obscure. Phytochemistry screening revealed that the peel of pomegranate rind (*Punica granatum linn*) contained alkaloids and flavonoids and therefore supposed to have antibacterial and antifungal activity. This study purposed to examine antibacterial and antifungal activity of *Punica granatum linn* peel aqueous extract against oral microorganism namely *S. mutans* and *C. albicans* respectively and analyzed its tocixity. Antibacterial and antifungal activity were demonstrated by means of inhibitory assay, while cytotoxic activity was analyzed on Fibroblast Cell Line BHK-21. Serial dilutions of extract *Punica granatum linn* (3,125%; 3,00%; 2,75%; 2,50%; 2,25%; 2,00%; 1,75%; 1,50%; 1,25% ; 1,00%; 0,75%; 0,50% and 0,25%) were tested. Results showed that all *Punica granatum linn* peel extract concentration tested were significantly ($p<0,05$) inhibit the growth of both *S. mutans* and *C.albicans*. The extract concentration of 0,50% and 0,25% were not toxic, while extract concentration of 3,125%; 3,00%; 2,75%; 2,50%; 2,25%; 2,00%; 1,75%; 1,50%; 1,25% ; 1,00% were toxic for BHK cell culture. Considering its toxicity, further studies were needed before using its traditional medicine plant for human tissues.

Keywords: *Punica granatum linn,* fibroblast cell line, *S. mutans, C. albicans*

P28

The Effect of Toothbrushing Period on Nickel Chromium Alloy Wear

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Abstract

Nickel chromium alloy is one of prefered materials which is used in fixed partial denture because it presents low cost, hardness, good physical and mechanical properties. Toothbrushing using toothpaste may cause a restoration abrasion especially for a long time period. The objective of this study was to observe the effect of toothbrushing period relating to nickel chromium alloy wear. This study used 24 nickel chromium alloy specimens of 30 mm long, 15 mm wide, and 1 mm thick. Alloy surfaces were treated by toothpaste which had been placed on toothbrushing machine (wear test machine, pin on plate unidirectional movement type). Wear was observed by measurement of surface roughness using Surfcorder SE 1700 and weight loss by Mettler Toledo AG 285. Toothbrushing periods were 30,9, 77,25, 123,6, 154,5 hours simulated of 2, 5, 8, 10 years. The data were analyzed by one way ANOVA followed by LSD. The result showed that toothbrushing period for 2, 5, 8, 10 years increasing surface roughness (Ra) 0,16 µm, 0,39 µm, 0,43 µm, 0,5 µm, 0,56 µm and weight loss about 8%, 15%, 23%, 32 %. The result of Anova showed that there was significant influence of toothbrushing period towards nickel chromium alloy wear which was proved by increasing of surface roughness ($p<0,05$) and weight loss ($p<0,05$). The result of LSD showed that there were significant influence ($p<0,05$) between groups of toothbrushing periods. The conclusion of this research is that toothbrushing period for 10 years increasing the wear of nickel chromium alloy which could be detected from increasing in surface roughness and weight loss. The increasing of surface roughness increase wear volume of nickel chromium alloy which showed with R=0,03 for 3 years toothbrushing period.

Keywords: Nickel chromium alloy, wear, abrasion, weight loss, surface roughness

P29

The Optimum Concentration of Anacardium occidentale bark as Mouthwash on Streptococcus mutans Inhibition Growth

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Abstract

Streptococcus mutans is an oral bacterium that can cause dental caries and gingivitis. The infection can be prevented by depressing the amount of existing microorganisms using mouthwash that contains therapeutic compounds such. *Anacardium occidentale bark* contains cardol, anacardic acid and tanin compounds which have anti bacterial and antiinflamation. The aim of this research is to find out optimum concentration of the *Anacardium occidentale* extract's concentration in the mouthwash on Streptococcus mutans inhibition growth. *Anacardium occidentale bark* was extracted with maceration method and using ethanol as solvent. *Anacardium occidentale* mouthwash was made in standard mouthwash composition and added by the extract of anacardium occidentale with this following concentration; 1%, 2%, 3%, 4%, 5%, 6%,7%,8%,9%and 10%. Inhibition growth was tested by filled as much as 50µl mouthwash to a 6mm hole which have been made on MHA media and been planted with *Streptococcus mutans*. The media was incubated for 24 hours in 37 C. The inhibition zone that occurred around the hole then measured with 0.01mm sliding calipers. The data then analyzed with one-way ANOVA and continued with LSD0.05. The result of this research showed the etanolik extract of *Anacardium occidentale* in the mouthwash influence to the *Streptococcus mutans* inhibitions growth. There were significant mean differences between the mouthwash 1% until 5% and there were not significant between 5% and 10%. The Result showed that optimum consentration of ethanolik exstract of annacardium occidentale bark as mouthwash on *Streptococcus mutans i*nhibition growth was 5%.

Keywords: *Streptococcus mutans* - Ethanol extracted anacardium occidentale bark – mouthwash-inhibition growth

P30

Antiinflammation Potential of Bali Grape (*Vitis vinifera* L. varietas Alphonso lavalle) Extract in Carragenan-induced Rat Paw Edema.

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Abstract

Bali grape (Vitis vinifera L. varietas Alphonso lavalle)is a natural product which contains tannin and flavonoid which are reported have anti-inflammatory activity. Inflammation is a complex tissues reaction as a consequence of tissue injury. Edema and increasing of neutrophil number on the peripheral blood are two of five cardinal inflammation signs. The purpose of this study is to investigate the effect of Bali grape extract on the artificial edema in the paw of Wistar rats and neutrophil number. The Bali grape extract with 12,5%, 25%, 50% concentration were used. Aspirin 350mg/kg body weight and aquabides 2ml/100g body weight were used as positive and negative control, respectively. Treatment were given with oral administration. Thirty minutes after treatment, the artificial edema was induced by carrageenin in the right paw. Right paw volume was measured every 30 minute from 30 until 180 minute after carragenan injection. Neutrophil count was done at 180 minute after carragenan injection. The results showed Bali grape extract inhibited edema of rat paw. There no significant different in the paw edema inhibition between 50% Bali grape extract concentration and aspirin. Aspirin and 50% Bali grape extract reduce neutrophil number. The neutrophil number no significant different between Aspirin and 50% Bali grape extract.

Keywords: Bali grape, inflammation, rat paw edema, neuutrophil

*All authors have equal contributions to this work

P31

The Estrogenic Effect Of Soy Powder Rich-In-Isoflavone On The Density Level Of The Mandibular Bone (Experimental Study On Sprague Dawley Hipoestrogenic Rats)

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Abstract

Various researches stated that there is a relationship between osteoporosis in the skeletal structure with the quality of the jaw bone. Some opinions stated that a long-term use of HRT (Hormone Replacement Therapy) might increase the risk of breast cancer and endometriosis. Therefore, it is necessary to find a replacement for estrogen. Soy isoflavones such as genistein and daidzein, are non-steroid elements whose molecular structure is similar to 17β-estradiol. Even though its estrogenic effect is weak, high intake ofsoy isoflavones from various soy products gives a sounding effect with problems related to menopause, including osteoporosis. In this research, there are four groups, with eight rats in each group as follows: the no ovx group is the normal bone density group used for standard reference, the ovx group is the negative control group, the ovx+17β-estradiol group is the positive control group, and the ovx+soy powder rich in isoflavone group as the experimental group. A contrast test on the density level of alveolar mandible, humerus and femur bones indicated that the ovx group is lower compared to the density level in the ovx+isoflavone group and the ovx+estradiol group. Result of the Kruskal-Wallis test indicated the following: the alveolar mandibular bone (p:0.002), humerus (p:0.004), femoris column (p:0.129), trochanter major (p:0.016), and the triangle Ward (p:0.129). The conclusion is: soy powder rich in isoflavones may decrease the rate or prevent a drop of the alveolar mandible bone density in particular, and the skeletal structure in general. If compared to the 17β-estradiol, the soy powder rich in isoflavones has the same effect towards the density level of mandibular bone, humerus, and femur on the hipoestrogenic rats.

Keywords: Soy powder, isoflavones, density level

P32

Root Coverage by Flap Surgery With and Without amnion Membrane for the Treatment of Gingival Recession

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Abstract

Gingival recession is progressively dental root denudation caused by apically displacement of gingival margin from cemento enamel junction. In this study, we conducted root coverage method using flap surgery with or without amnion membrane application. Human amnion membrane (HAM) is an allogenic graft material containing mesenchym, fibroblast, and growth factor. Thus, amnion membrane may induces re-epithelization, decreases inflammation, fibrosis, controls angiogenesis and blocks apical migration of epithelium. This research aimed to define the efficacy difference of flap surgery with or without amnion membran application for gingival recession treatment as assessed by root coverage. Research samples were 20 mandibulary and maxillary anterior gingival recession cases Class I and II Miller. Samples were devided into two groups; 10 samples flap surgery with amnion membrane and 10 samples without amnion membrane application. Measurement of recession depth (RD) conducted in month 0 and 3 month after surgery. Independent T-test analyses showed statistically difference were not significant. Root coverage by flap surgery with amnion membrane is 63% and without amnion membrane is 55 %. It may concluded that flap surgery with amnion membrane as effective root covered as if the surgery conducted without amnion membrane .

Keywords: Gingival recession, flap surgery, amnion membrane

P33

The Effect of *Chlorhexidine gluconate* 0.05% on Post Extraction Bacteremia

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Abstract

The aim of this study is to examine the effect of preoperative rinsing with *Chlorhexidine gluconate* 0.05% on post extraction bacteremia. The study protocol was approved by the institution's ethics committee, and the patients gave their informed consent in writing. Twenty respondents participated as the subjects on the clinical trial ,conducting treatment as follow : ten as treatment group rinsing with CHX 0.05% and control group rinsing with Na Cl 0.9%. Blood vein samples were taken directly after extraction and put on the tube containing Brain Heart Infusion broth. The blood samples were incubated in anaerobic condition. Data obtained of Colony Forming Units of the bacteria growth was done in a descriptive method. Showed that there was not Colony Forming Units of the bacteria growth using *Chlorhexidine gluconate* 0.05% ,while there was found Colony Forming Units of the bacteria growth using Na Cl 0.9%**.** We concluded that preoperative rinsing with *Chlorhexidine gluconate* 0.05 % is effective in suppressing post extraction bacteremia. It can be used as preoperative disinfectant to prevent the risk of post extraction bacteremia.

Keywords: *Chlorhexidine gluconate* 0.05%, Post Extraction Bacteremia

P34

Topical application of osteoprotegerin inhibits alveolar bone loss in rat experimental periodontitis

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Abstract

Periodontitis is characterized by progression of alveolar bone loss. It is reported that RANKL/RANK signaling controls osteoclast number and its maturation to regulate bone resorption. The purpose of this study is to investigate the effects of topical application of osteoprotejerin (OPG), decoy receptor of RANKL, on alveolar bone loss in rat experimental periodontitis. Eight-week-old male Wistar rats were used. The cervical area of the right second molar of the maxilla was ligatured with nylon thread for 14 days (n=6). After ligaturing, OPG (2.5μg/side/day) was injected on palatal cervical gingiva every 2 days, Same volume of saline was injected in non-OPG-treated and non-ligatured groups. On days 5 and 14, their maxilla were collected and prepared for micro-CT analysis, followed by histological analysis such as HE staining, TRAP staining, and immunohistochemical staining of IL-β. On days 5 and 14, micro-CT images showed that alveolar bone resorption (the distance between alveolar bone crest and cement-enamel junction) was significantly inhibited by OPG treatment. Osteoclasts were observed on the alveolar bone surface and the number was decreased by OPG treatment on day 5, whereas there were no osteoclasts on the surface on day 14. On day 5, the expression level of IL-β in periodontal tissue was similar between OPG-treated and non-treated groups. The number of polymorphonuclear leukocyte and blood vessel in the subepithelical connective tissue (per 0.05mm ×0.1mm) was not affected by OPG treatment. Topical application of OPG inhibited alveolar bone loss through a decrease of osteoclast number. These results show that OPG may be useful as a medication drug in periodontal therapy.

P35

Characterization of *Sp6* Promoter Activity

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Abstract

SP6 is a member of the SP/KLF transcription factor family and plays a key role in tooth development and morphogenesis. To investigate the temporal and spatial regulation of *Sp6* gene expression, we first isolated the mouse *Sp6* promoter region and characterized its activity by luciferase reporter assay. The 15 kb 5'-flanking region of mouse *Sp6* gene was isolated from mouse genomic DNA by PCR. After confirming the sequence of the isolated DNA, serial deletion reporter constructs were prepared, and dual luciferase reporter assay was performed using various cells, including rat dental derived cells. Since BMP and Wnt molecules are detected during tooth development, we further examined the effects of these two cytokines on the luciferase activity. The reporter analysis revealed positive and negative regulatory domains in the *Sp6* promoter region. Unexpectedly, we detected the potential 3rd promoter activity in the intron 2 region of the *Sp6* gene. The luciferase analysis also revealed the enhancing effects of Wnt and BMP signals on the *Sp6* promoter activity, suggesting the regulatory roles of two cytokines in the *Sp6* gene expression during tooth development. We demonstrated the structural organization of *Sp6* gene and potential regulatory regions of promoter activity in cell specific manner. Our findings demonstrated the functional signaling link from cytokines, BMP and Wnt, to *Sp6* gene expression. Further analysis is required to understand the precise molecular basis for the tooth development through the regulation of *Sp6* gene expression.

P36

Analysis of Osteoclast Activation in the Pathogenesis of Rheumatoid Arthritis

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Abstract

Although osteoclasts (OCs) are known to play a key role in the pathogenesis of rheumatoid arthritis (RA), the precise mechanism how OCs are activated in RA has been still obscured. In this study, we investigated functions of OCs using MRL/*lpr* mice, one of animal models for human RA. Bone marrow-derived OCs (BMOCs) were generated by the culture with M-CSF and RANKL, and their phenotypes or functions were analyzed by flow cytometry, pit formation assay, and real-time RT-PCR. *In vivo* bone dynamics was scanned using a micro CT system, and investigated by pathological and biochemical analyses. The bone findings of MRL/*lpr* mice showed the decreased bone density like an osteoporosis. The number of mature BMOCs from MRL/*lpr* mice was significantly increased compared with those from control MRL+/+ mice. Pit formation assay revealed that function of BMOCs from MRL/*lpr* mice was more enhanced than that from control mice. The mRNA expressions of OC markers such as cathepsin K and tartrate-resistant acid phosphatase (TRAP) from MRL/*lpr* mice were increased compared with those from control mice. In addition, the mRNA expression of apoptosis-related genes including second mitochondria-derived activator of caspase (Smac)/ direct inhibitor of apoptosis-binding protein with low pl (DIABLO) of BMOCs from MRL/*lpr* mice was significantly decreased in contrast to control BMOCs. Enhanced activation of OCs in MRL/*lpr* mice might be associated the pathogenesis for RA with bone destruction.

P37

ATF6 Links Endoplasmic Reticulum (ER) Stress to Intestinal Inflammation In Mice

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Abstract

The accumulation of unfolded proteins in the endoplasmic reticulum (ER) initiates a cellular stress induced by various physiological and pathological conditions. The transcription factor ATF6α and ATF6β are a key molecular in sensing and responding to ER stress. However, the pathological role of ATF6s is poorly understood. Our aim was to obtain insight in the role of ATF6s in inflammation. ATF6αknockout (ATF6αKO) and ATF6βknockout (ATF6βKO) mice were characterized and challenged by a colitis-inducing agent, dextran sulfated sodium (DSS). We monitored clinical symptoms, intestinal morphology and histology, and expression of ER stress related genes. Induction of ER stress related genes was observed in DSS-treated colonic tissues. After administration of 3% DSS for 3 days, the ATF6αKO and ATF6β KO mice developed more severe colitis compared with wild-type mice. Further, we identified the genes mediated by ATF6αand/or ATF6β in DSS-inducible genes by DNA microarray analysis. This study shows that ATF6s deficiency leads to inflammation of the colon in the experimental colitis model. Our data suggests substantial differences between the target genes of ATF6αand ATF6β.

P38

Functional Analysis of a Novel Adipokine, D-Dopachrome Tautomerase, In Preadipocytes

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Abstract

Adipose tissue (AT) is not only an energy storage organ, but also a source of various secreted functional factors (adipokines). Obese AT secretes various inflammatory cytokines or free fatty acids, leading to systemic diseases, such as type 2 diabetes, hypertension, hyperlipidemia, and atherosclerosis. Furthermore, obesity is reported to be associated with periodontitis. We identified novel adipokine-candidates from cultured medium of human adipocytes using proteome analysis. Out of these candidates, we focused on D-dopachrome tautomerase (DDT), because its gene expression levels in adipocytes were negatively correlated with obesity-related clinical parameters. DDT has been identified as an enzyme converting D-dopachrome into a tautomer and is similar to macrophage migration inhibitory factor (MIF) in primary and tertiary structures. MIF is well known as a pleiotropic cytokine, whereas it has an enzyme activity converting D-dopachrome into another tautomer. These similarities with MIF suggest a possibility that DDT also acts as a cytokine. In this study, we investigated effects of DDT on the expression of inflammatory cytokines and adipogenesis in human preadipocytes. An SGBS cell line derived from human preadipocytes was used in this study. Gene expression and secretion of inflammatory cytokines in the cells treated with or without recombinant DDT (rDDT) were measured by real-time RT-PCR and ELISA, respectively. Involvement of MAPK family in the response to rDDT was investigated by western blot analysis using each MAPK antibody. Degree of differentiation into adipocytes was evaluated by Oil red O or Sudan□ staining and adipogenic markers mRNA levels in the cells subjected to adipogenic induction for 9 days. Gene expression and secretion of inflammatory cytokines such as IL-6 or MCP-1 were increased in preadipocytes treated with rDDT. Phosphorylation of ERK1⁄2, but not p38, was observed in the cells treated with rDDT and rDDT-induced IL-6 mRNA expression was attenuated by pretreatment with an ERK inhibitor, U0126. Furthermore, accumulation of triacylglycerol, number of cells with oil droplets, and mRNA levels of adipogenic markers, PPAR□, C/EBP□, aP2, and adiponectin, in adipocytes differentiated from SGBS cells treated with rDDT were lower than those in untreated cells, indicating the inhibitory effect of rDDT on adipogenesis. DDT induces inflammatory cytokines expression via ERK-MAPK signaling pathway and inhibits adipogenesis in preadipocytes.

P39

Analysis of Molecular Mechanism for Pathogenesis of Metal Allergy

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Abstract

Along the various kinds of metal alloys used in dentistry, nickel-containing metal alloys are extensively used for dental prostheses and orthodontic appliances. Although nickel is known as the most common allergenic metal, the precise pathogenesis of nickel allergy has been still unclear. In this study we analyzed the cellular and the molecular mechanism for allergic reaction using a mouse model for nickel allergy. Immune cells infiltrating in the inflammatory lesion in the skin from nickel allergy model were detected by flow cytometric and immunohistochemical analysis. In addition, regulatory T (Treg) cells purified from normal mice were transferred into nickel allergy mice to prevent allergic immune response. The allergic lesions were investigated by pathological and immunological analysis. Titanium was used as a control metal. CD3+ T cells (40 %) and CD19+ B cells (15 %) were infiltrated into the inflammatory lesion of nickel allergy model. In addition, the number of Foxp3+ Treg cells and CD11c+ dendritic cells were significantly increased compared with those of control mice. T cells including Treg cells play a key role in the pathogenesis of nickel allergy. Understanding the cellular and molecular mechanism of metal allergy may be useful for the clinical application.

P40

Cytokine Profile In Human Saliva And Its Relationship With That In Blood

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Abstract

Recently, it has been reported that various bioactive substances such as cytokines are present in saliva, as well as digestive enzymes or secretory IgA. The use of saliva as an examination sample is expected because its collection is noninvasive and easier compared with blood. The aim of this study was to clarify the relationship between cytokines in saliva and in blood for the application of saliva sample use in health examinations. Saliva and blood samples were obtained from 17 Japanese adults without diagnosed diseases. Concentrations of 14 cytokines (IL-1-beta, IL-8, IL-10, IL-12(p70), IFN-gamma, IP-10, TNF-alpha, VEGF, IL-1-alpha, IL-18, HGF, MIF, M-CSF, and VCAM-1) in saliva and blood were measured using the Bio-Plex suspension array system (Bio-Rad, Hercules, CA, USA). The relationships between levels of cytokines in saliva and blood were analyzed employing Spearman's rank correlation test using SPSS 15.0J. Among the 14 cytokines we measured, all cytokines in saliva and 13 in blood except VCAM-1, which was beyond the upper limit of the measurement range, were quantifiable. Although a significant ($p<0.05$) positive correlation between the levels in saliva and blood for IP-10 and a negative correlation for VEGF were indicated, there were no significant correlations regarding other cytokines. It was shown that many cytokines were present in saliva, and the levels of many cytokines in saliva were higher than in blood. In addition, 11 of the 13 quantifiable cytokines in this study showed no correlation between the levels in saliva and blood, thereby suggesting that cytokines in saliva may be controlled by a mechanism differing from that in blood.

P41

Differential Regulation of *Sp6* Silencing in Dental Epithelial Cells

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Abstract

SP6 is one of SP/KLF transcription factor family members and indispensable for tooth development. There are several reports on the SP6 function based on the experimental data obtained by both gain-of-function and loss-of-function analyses. However, there are a quite limited information on the basic biochemical characteristic of SP6 molecule. To investigate the precise biological roles of SP6 in ameloblast differentiation, we analyzed the *in vivo* regulation of its expression as a first step. We prepared *Sp6* stable transformant, CHA9, using ameloblast-lineage G5 cells. *Sp6* mRNA and its protein expression were examined by RT-PCR, real time PCR analyses and western blot analysis. Protein stability and epigenetic regulation of *Sp6* expression were also analyzed with several inhibitors against proteasomal protease, DNA methyltransferases and histone deacetylase. Long term culture of CHA9 cells reduced SP6 expression, although *Sp6* mRNA expression was kept at the similar level at the starting time of the culture. SP6 expression was detected in CHA9 cells at passage 7 (P7), but it was disappeared at P28. However, *Sp6* mRNA expression was not changed until P28, and decreased at P50. Treatment with proteasome inhibitors dramatically enhanced the SP6 expression at P7 and P28, and induced at P50. SP6 expression was also enhanced when CHA9 cells were treated with inhibitors of either DNA methyltransferases or histone deacetylase, although both inhibitors had no effects on the *Sp6* mRNA expression at P7 and P28 except for P50, at which significant enhancement of exogenously transduced *Sp6* expression was observed. *Sp6* gene expression is regulated not only posttranslationally but also epigenetically in CHA9 cells, suggesting that SP6 may have a unique regulation of its expression to play the biological roles during ameloblast differentiation.

P42

Analysis of Microrna In Saliva and Submandibular Gland Cell Lines

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Abstract

MicroRNAs (miRNAs), small RNA molecules of approximately 22 nucleotides, have been shown to be up- or down-regulated in specific cell types. These molecules have been recognized as one of the major regulatory gatekeepers of coding genes in the human genome. miRNAs are produced in a tissue-specific manner, and changes in miRNA levels within a tissue type can be correlated with disease status. As miRNAs can be secreted in exosomes, they are highly stable in body fluid such as plasma, urine, milk, and saliva. The aim of this study was to access miRNA expression in saliva and submandibular gland (SMG) cell lines and to define their regulation by TNF-α. Saliva samples were collected from healthy Japanese (The study protocols were approved by Ethics Committee of the Tokushima University Hospital). Immortalized human SMG acinar and ductal cell lines were named as NS-SV-AC and NS-SV-DC, respectively. Exosomes were obtained by ultracentrifugation of saliva. Total RNA from exosomes was isolated using mirVana isolation kit (Ambion). miRNA in saliva and the SMG cell lines were profiled by using the Agilent Human microRNA Microarray V2, and output text files were loaded into GeneSpring GX. Profiling data was further validated by RT-PCR. To analyze the effect of a transient inflammatory signal on miRNA levels, the SMG cell lines were treated with TNF-α. miRNAs were detected in exosomes isolated from saliva. Out of 723 human miRNAs analyzed by microarray, 198, 164, and 92 miRNA were detected in NS-SV-AC, NS-SV-DC, and saliva, respectively. The 63 miRNAs were commonly detected in NS-SV-AC, NS-SV-DC, and saliva. Most of the miRNAs detected in saliva were frequently observed in the SMG cell lines. miRNAs expressed differentially in the two SMG cell lines were confirmed in saliva. TNF-α treatment up-regulated miR-21 only in NS-SV-AC whiles no changes of several miRNA levels were observed in either NS-SV-AC or NS-SV-DC cells. Salivary miRNA profile was highly similar to those in the SMG cell lines. Therefore, most of salivary miRNAs may be derived at least from the SMG. Several miRNAs detected only in saliva may be derived from another salivary glands or oral tissues. As induction of miR-21 by TNF-α was observed, it may be one of the inflammatory biomarkers in saliva. In conclusion, salivary miRNAs expression profilling creates a new paradigm in the emerging field for noninvasive molecular diagnosis.

P43

Advanced Glycation End-product Affects Calcification in Cultured Rat Dental Pulp Cells

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Abstract

Amorphous calcification frequently appears in the dental pulp of diabetic patients, but its pathogenesis is poorly understood. We previously reported that pathologic pulp calcifications such as pulp stone and thickened predentin actually occurred in a rat diabetic model. Recent research has demonstrated that the accumulation of advanced glycation end-products (AGEs), final products in the Maillard reaction, correlates with the progression of vascular calcification in diabetes patients. The aim of this study is to investigate whether or not AGEs induce calcification in cultured rat dental pulp cells *in vitro*. Dental pulp cells were prepared from rat maxillary incisors as described by Kasugai *et al*. (*Arch Oral Biol* 1993). AGE-BSA was prepared by a modification method of Makita *et al*. (*J Biol Chem* 1991). Firstly, mRNA expression of AGE receptor (RAGE) in rat diabetic pulp tissues was determined and compared to that in healthy pulp by RT-PCR. Secondly, the effect of AGE-BSA (200 μg/ml and 1 mg/ml) on alkaline phosphatase activity and calcium deposition in culture was investigated by enzyme assay and alizarin red staining, respectively. RAGE mRNA in dental pulp tissues expressed both in diabetic and nondiabetic rats. AGE-BSA significantly increased alkaline phosphatase activity in cultured rat dental pulp cells. A high concentration of AGE-BSA (1 mg/ml) increased calcium deposition, whereas a low concentration of AGE-BSA (200 μg/ml) did not show any changes. The present study demonstrates that AGE may be a potent factor affecting pathologic pulp calcification in diabetic rats.

Key words: Advanced glycation end-products, Dental pulp, Pathologic calcification, Diabetes

P44

Histochemical Examination on Collagen Fibrils In Periodontal Ligaments of Ascorbic Acid Insufficient Od/Od Rats

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Abstract

Collagen fibers in periodontal ligaments play important roles in bearing mechanical force and physiological movement of teeth. Od/od rats lack L-gulonolactone oxidase, and therefore, are not able to synthesize ascorbic acid which is essential for collagen synthesis. In order to provide clues for better understanding the remodeling of collagen fibers in the periodontal ligaments, we have attempted to inhibit collagen synthesis, and examined histological alteration of the collagen fibers in periodontal ligaments by using od/od rats. Thirteen week-old od/od male rats were fed with a normal diet (control group, ascorbic acid 200mg/kg) or an ascorbic acid-insufficient diet (insufficient group, ascorbic acid 0.3mg/kg), and then their mandibles were histochemically examined. The od/od rats in the insufficient group showed a less amount of collagen fibers in the periodontal ligament of the first molar, which was demonstrated by Azan staining and type I collagen immunostaining. The number of ED1-positive cells (macrophages and osteoclasts) and tartrate resistant acid phosphatase (TRAP)-positive cells (osteoclasts) was similar between the control and the insufficient groups. Therefore, the reduced synthesis of mature collagen fibers did not seem to affect the number and localization of macrophages and osteoclasts. However, silver impregnation demonstrated the presence of reticular fiber-like fibrils associated with collagen fibers in the periodontal ligaments of the insufficient group. In contrast, the control group revealed silver impregnation-positive reticular fibrils merely around blood vessels, but not in the periodontal ligaments. Interestingly, matrix metallo-proteinase (MMP) 2-immunoreactivity was intense in the region of reticular fiber-like fibrils in the ascorbic acid-insufficient periodontal ligaments, but not in the control specimens. Under transmission electron microscopy, fibroblastic cells in the control periodontal ligaments engulfed fibrillar structures associated with lysosomes. Taken together, the insufficiency of ascorbic acid appears to induce an immature form of collagen fibrils, *i.e.,* reticular fibrils, which subsequently permit fibroblastic cells of periodontal ligaments to digest such immature reticular fibrils. Collagen fibers in the periodontal ligaments appear to be continuously remodeled in a physiological state, probably by fibroblastic cells but not by macrophages.

P45

A Reference In Evaluating The Length of Complete Lower Denture Borders.

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The borders of complete denture extent between attached and unattached mucosa, therefore an impression area of the complete denture is difficult to decide. Functional form of mouth floor changes by tongue movements, so making impressions for lower complete dentures is especially difficult. The reference for lengths of lower denture borders will be useful for inexperienced dentists. The purpose of this study was to measure the distance between the cervical line of the lower molars and the external oblique as well as the mylohyoid lines of the mandibular dry specimens and to seek a reference in evaluating the length of complete lower denture borders. One hundred and four mandibular dry specimens of Southeast Asian with complete dentition were examined. These were divided into two groups, namely 7-7 group (n=30) and 8-8 group (n=74). The 7-7 group has 14 teeth from right second molar to left second molar and the 8-8group has 16 teeth from right third molar to left third molar. The distances from the middle point of the buccal cervical lines in the first molar (M1) and the second molar (M2) to the external oblique line at the intersection points on perpendicular line to the occlusal plane (referred to as BAD: Buccal Alveolar Distance) were measured by digital caliper. Similarly, the distances from the middle point of the lingual cervical lines in the M1 and M2 to the Mylohyoid line (referred to as LAD: Lingual Alveolar Distance) were measured. In 7-7 group, Mean±1SD (mm) of the BAD at M1 and M2 were 13.3 ± 2.0 and 7.8 ± 1.0, respectively. Also, Mean±1SD (mm) of the LAD at M1 and M2 were 14.2 ± 2.0 and 9.5 ± 1.4, respectively. In 8-8 group, the Mean±1SD (mm) of BAD at M1 and M2 were 15.8 ± 2.4 and 9.8 ± 1.6, respectively. Mean±1SD (mm) of the LAD at M1 and M2 were 16.6 ± 2.5 and 11.0 ± 2.1, respectively. There were no statistically significant differences on the right and left sides in all measurements. The standard deviation of BAD and LAD was 13~19% of the mean value. These results suggest that the measurements of BAD and LAD may become a concrete reference for lengths from the cervical line of the arranged artificial molar teeth to the buccal and lingual borders of the complete lower denture.

P46

DGGE Analysis of Subgingival Bacterial Communities Under Mechanical Debridement With Different Primers

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Abstract

Denaturing gradient gel electrophoresis (DGGE) of 16S ribosomal DNA (16S rDNA) is one of the most frequently used methods to study microbial communities. In this study, we investigated the DGGE profiles of different 16S rDNA regions of periodontal pathogens, *Porphyromonas gingivalis, Fusobacterium nucleatum*, *Prevotella nigrescens*. The results evidenced that V3-V5 and V6-V8 fragments may suitable for community analysis of subgingival bacteria.Further analysis of subgingival samples with V3-V5 and V6-V8 regions as target fragments suggested that the re-colonization of the periodontal bacteria may happen at 6 weeks after mechanical debridement in chronic periodontitis with high population similarity compared to baseline.

Keywords: DGGE, subgingival bacterial community, 16S rDNA, mechanical debridement

P47

Characterization of A Unique Subpopulation Of Oral Mucosa Keratinocytes Produced From A Monolayer Culture: the omPUK Cultured Cell Strain

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Abstract

By simply transferring culture supernatant into a new culture vessel, omPUK (oral mucosa Pop-Up Keratinocyte) cells that are "pop up" a monolayer culture can generate a new subculture. This novel technology has the potential to expand a greater number of cells than using traditional trypsin/EDTA subcultures. The aim of this study was to characterize the omPUK cell population. Primary oral mucosa keratinocytes were serially cultured in a defined medium (EpiLife®) without serum, pituitary extract or use of a feeder layer. Once cells in a T-150 flask reached 60-70% confluence, a 15mL of EpiLife® was added into the flask every day. Three days after the cells became confluent, the omPUK cells were collected by centrifuging the supernatant. The 100% confluent cells (Adh cells) were detached in trypsin/EDTA. The expression of α6 integrin and CD71 and aldehyde dehydrogenase (ALDH) activity between both populations was compared by flow cytometry. Regenerative capability of oral mucosa using an organotypic culture system was also assessed. The Adh cells were relatively smaller in size and the major populations were $\alpha6^{bri}/CD71^{dim}$ and $\alpha6^{bri}$ /CD71 ^{dim} while the omPUK had a variety of cell sizes and the majority were $\alpha6^{dim}$ /CD71 ^{dim}, implying "post-mitotic" keratinocytes. In native oral mucosa, ALDH expression lacked in basal layer but was present in lower suprabasal layer. The percentage of $ALDH^{bri}$ cells was higher in omPUK than in Adh cells, suggesting a more differentiated cell subpopulation. Nontheless, the omPUK was able to develop a more organized and thicker epithelial layer in vitro. The omPUK may provide a novel insight into oral mucosa keratinocyte biology and be attractive for use in regenerative medicine.

P48

Mechanical Loading Induces the MMP-13 induction at Epithelial Rests of Malassez.

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Abstract

Clinically, it is well known that the periodontal ligament (PDL) is highly responsive to mechanical loading and that stimulates tissue remodeling. The matrix metalloproteinases (MMPs) have been shown to play a central role in the breakdown of extracellular matrix (ECM), which is essential for tissue development and remodeling. MMP-13 is a collagenase which is known to be expressed in periodontal tissue in response to mechanical stimuli and inflammation, suggesting its participation in tissue remodeling. Since PDL cells are consist of variety of cells, such as fibroblast, osteoblast, cementoblast and epithelial cells , mechano-responsive cells are still not well characterized. Among the PDL cells, epithelial rests of Malassez (ERM) have been proposed to maintain the space for PDL as a mechano-sensing cell. Even though the presence of ERM in PDL has been described for decades, their active function has not been well characterized. The purpose of this study was to investigate the expression of MMP-13 on ERM. The induction of MMP-13 upon mechanical loading on ERM was further analyzed. Maxillary molars with surrounding tissue were dissected from 4 weeks old male SD rats, fixed with 4% formaldehyde and decalcified by 10% EDTA. The samples were embedded in paraffin according to the standard protocol. Five-μm thickness serial-sections were prepared along with the sagittal plane. To detect the distribution of MMP-13, immunohistochemistry was carried out with anti-MMP-13 antibody (R&D systems, USA). Human PDL cells were obtained from healthy extracted human premolar under the permission of Niigata University ethical committee. The PDL cells were subjected to 0.5g/cm^2 of compressive mechanical loading for 24 hours. The localization of MMP-13 on ERM were analyzed by immunofluoresence with anti- MMP-13 and cytokeratin antibody(AE1/AE3, DAKO, Denmark). On 4 weeks old rats periodontal tissue, MMP-13 positive cells were detected at alveolar bone, ERM and epithelial root sheath that were still localized at the tip of root apex. The distribution of MMP-13 and cytokeratin positive cells were completely overlapped within PDL. The anti- cytokeratin and anti-MMP-13 double positive human PDL cells were increased after the compressive mechanical loading. The preferential expression of MMP-13 on ERM and further induction by mechanical loading was shown here. These results suggest that the epithelial rests of Malassez may contribute to the PDL tissue homeostasis through the production of specific MMPs in response to mechanical loading.

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Type XII Collagen Modulates Matrix Formation and Mineralization on Human PDL Cells

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Abstract

Type XII collagen is a FACIT (Fibril Associated Collagens with Interrupted Triple helices) type collagen which originally identified in periodontal ligament (PDL). The FACIT type collagen are known to interact with the surface of fibrillar collagen (i.e. collagen type I) and act as a bridge between collagen and other extra cellular protein. Recently, possible genetical polymorophism on COL12A1 gene, encoding type XII collagen, has reported on patients with connective tissue disorders, that is indicating the critical role of this molecule. The purpose of this study was to investigate the effect of type XII collagen gene silencing on matrix formation and mineralization by using human PDL cells. Methods: Human PDL cells were obtained from healthy human premolar by enzymatic treatment under the permission of Niigata University ethical committee. The vector based shRNA was used for COL12A1 gene silencing. Four different siRNA sequences for COL12A1 gene was separately inserted to pGeneClip hMGFP vector (SA Biosciences, MD, USA). A scramble siRNA sequence inserted vector was served as control. The hMGFP-COL12A1-shRNA plasmid was transfected to the human PDL cells and gene silencing was confirmed by realtime PCR. The Cell proliferation, Osteoblastic and PDL related gene expression, ALP activity and Collagen matrices formation were analyzed on the vector transfected human PDL cells. Results: Forty eight hours after the transfection of hMGFP-COL12A1-shRNA constructs, the expression of COL12A1 gene was suppressed ~20% while no effect was observed on the cell proliferation. By the application of hMGFP-COL12A1-shRNA constructs, up-regulation of osteoblastic gene expression, enhancement of ALP activity and sparse distribution of collagen matrices were observed. Conclusion: Our results clearly showed that the type XII collagen is a negative regulator for the osteoblastic differentiation on human PDL cells. These results may indicate the importance of type XII collagen on the PDL tissue maintenance, especially for its no-mineralizing feature.

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Effects of Zoledronic Acid on Primary Human Oral Mucosa Keratinocytes and Fibroblasts

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Abstract

Objective: Bisphosphonates (BPs) constitute an effective treatment for various bone diseases such as osteoporosis and bone metastases in cancers. However, BP-related osteonecrosis of the jaws (BRONJ) has drawn attention as one severe side effect for patients receiving BPs. Although the primary target of BRONJ is the bone because due to the inhibitory effects on osteoclasts, we hypothesized that changes in oral mucosa might also contribute to its pathogenesis. We therefore examined the primary effects of a nitrogen-containing BP, Zoledronic acid (ZA), in vitro, on two major cellular components, keratinocytes and fibroblasts, in oral mucosa using a monolayer culture and a 3D construct of an ex vivo-produced oral mucosa equivalent (EVPOME). Methods: The tissue samples were procured from discarded oral mucosa (n=14, 2 males and 12 females) after the consent form was obtained and soaked in 0.04% trypsin solution overnight. The epithelial layer was scraped off and primary oral keratinocytes were cultured in a chemically-defined serum-free medium. The underlying connective tissue was minced and an explant culture technique was used for oral fibroblast culture by feeding DMEM containing 10% FBS. Monolayer cultures of oral keratinocytes and fibroblasts were exposed to concentrations of ZA (0(control), 3, 10μmol/L).

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Comparison of different experimental techniques for visualizing viability of biofilm bacteria

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Abstract

Confocal laser scanning microscopy (CLSM) is a standard strategy for fluorescent imaging of biofilm bacteria. However, penetration of the excitation beam can be a limiting factor in thick and/or very dense biofilms. This study aimed to compare the performance of two imaging techniques (CLSM direct visualization and CLSM after longitudinal sectioning) and two fluorescence staining techniques in estimating bacterial viability of artificial thick biofilms developed on a resin composite material. The rotating-disc biofilm reactor was used to develop standardized 3-day *Streptococcus mutans ATCC 25175* biofilms on resin composite (Beautifil Flow, Shofu, Japan) discs in one-tenth BHI broth. The biofilms were then treated for 30 s with a mouthwash (Listerine, J&J), and then stained with either a Live/Dead kit (SYTO9/propidium iodide (PI) staining) or calcein-AM (CAM). The samples were assigned to either direct observation or cryosectioning. For direct observation,stacks of image were collected in the z-dimension, and then three-dimensional reconstruction was carried out using MetaMorph software. For cryosectining, specimens were covered with an embedding medium, frozen, and then cut longitudinal to the biofilm layer at ten µm using a tungsten carbide blade with the aid of an adhesive tape attached to the frozen specimen. The viability of biofilms with and without the mouthwash treatment was also quantified by culture. Direct CLSM of SYTO9/PI-stained, mouthwash-treated biofilms showed high percentages of PI-positive membrane damaged bacteria (80.0 to 99.9% in each stack; calculated total ratio of 97.5 ± 3.4%; $p < 0.01$ vs non-treated controls), whereas this method caused loss of fluorescent signals in the deeper area. Cryosectioned specimens yielded entire visualization of the biofilm without detachment from the resin surface, and confirmed that the majority of bacterial cell were stained with PI after the mouthwash treatment. However, CAM-stained biofilm sections revealed that bacteria in the deeper area remained CAM-positive after the mouthwash treatment, indicating that they retained esterase activity. Furthermore, direct plate counting of the mouthwash-treated biofilms yielded > 10^7 viable cells. The mouthwash caused a pronounced increase in the ratio of membrane damaged bacteria in the artificial biofilm. PI-staining seemed sensitive in detecting "dead" cells, although it may cause a certain degree of "false-positive" results. The cryosectioning technique used in this study yielded improved fluorescent visualization of the deeper area of biofilm structure.

This work was supported by the JSPS Institutional Program for Young Researcher Overseas Visits.

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Analysis Of Patients Visiting Niigata University Medical And Dental Hospital With Chief Complaints Of Dental Metal Allergy And/Or Dental Focal Infection In The Previous 8 Years

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Abstract

Dental metal allergy and dental focal infection are possible causes of dermatological diseases, but have been the subjects of few reports to date. We have been treating such patients in our special clinic for more than 20 years. Objective: The purpose of the present study was to investigate the mouths of patients visiting our dental hospital over an 8-year period, with the aim of clarifying whether dental metal allergy and/or dental focal infection affects their dermatologic conditions. We surveyed all clinical records of the 185 patients who visited Niigata University Medical and Dental Hospital with chief complaints of dental metal allergy since 2002. Diagnostics of skin diseases, periodontal records, periapical lesions, dental caries, dental metal series patch test results and Electron Probed Micro-Analysis (EPMA) data were investigated. Results: The number of patients who were suspected of having a dental metal allergy was one-hundred and eighty five (63 male, 122 female), and the male/female ratio was about 1:2. Ninety-two (49%) patients were suffering from pustulosis palmaris et plantaris (PPP). The male/female ratio among Ninety-two PPP patients was 1:2 and the number of patients and 20 (11%) patients had lichen planus. Eighty-two (49%) patients showed positive reactions on patch testing. Based on the result of patch tests, Ni showed the highest positivity rate (62%, 51 patients), but on EPMA, the number of patients with Ni as an allergen was fourteen (27%). On the other hand, more than 90% of patients who showed positive reactions on patch test to Pd and Au, and had these metals in their dental prostheses. In addition, one hundred twelve (60%) patients showed the possibility of dental focal infections.

Keywords: Dental metal allergy, dental focal infection

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Effect of Saliva Occult Blood Test to Behavioral Change in Daily Interproximal Plaque Control

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The objective of this study was to ascertain whether daily oral hygiene behavior was change in community-dwelling people when saliva test was introduced in mass screening on periodontal disease. Subjects were 185 mothers (mean age=32.3±4.2), who took their children fo 1-year dental check-ups, employed at health centers in Niigata City. Subjects were randomly divided into two groups such as test group (n=98, mean age=32.8±4.1) and control (n=87, mean age=31.7±4.3). For the test group, saliva occult blood test was used for their periodontal assessment instead of CPI. Inaddition, oral health instruction was performed about importance of interproximal plaque control using dental floss or interdental brushes and regular professional dental check-ups. We used a jaw model, dental floss and booklets to support the personal instruction. For the control group, oral hygiene instruction as same as done for test group was perform after clinical examination using a CPI was conducted. A follow-up survey was performed 6 months later to evaluate changes in oral health behavior. The results showed that the rate of subjects who used dental floss or interdental brushes during six months increased from 38.4% (baseline) to 48.8% (after 6 months) in the test group although statistically difference was not found. While, the rate was likely found no change during 6 months with 38.8% (baseline) and 37.3% (after 6 months) in the control group. In the test group, on the other hand, subjects were classified into 3 subgroups according to the categories by the result of saliva occult blood test; strongly positive (++), positive (+) and negative (-). The rate of subjects who were confirmed to start use of dental floss or interdental brushes at 6 months later was found statistically significant (44%, $p<0.05$: Scheffe's multiple comparison) in ++ group when compared among the 3 subgroups categorized by saliva test. These results suggested that saliva occult blood test might be effective to behavioral change in daily interproximal plaque control. Saliva test might be better tool to give impact to community-dwelling people in terms of easy self-recognition because result of test was visible.

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Evaluation of Ecological Shift During Radiotherapy For Head And Neck Cancer

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To evaluate the biodiversity changes in oral microflora of patients with head and neck cancer treated with postoperative intensity-modulated radiotherapy (IMRT) or conventional radiotherapy (CRT). And to investigate the microbiota shift of these subjects. Pooled dental plaque samples were collected during the radiotherapy from patients receving IMRT (n=5) and CRT (n=4) respectively. The denaturing gradient gel electrophoresis was used to analyze the temporal variation of these plaque samples. The stimulated and unstimulated salivary flow rates were also measured and compared between IMRT and CRT. There are reductions in the severity of hyposalivation during IMRT compared with CRT. Both the stimulated and unstimulated salivary flow rate of IMRT group was significantly higher than the CRT group. We also observed that the community temporal stability was higher in the IMRT group (70.56±9.07%) than in the CRT group (52.81±12.76%); the difference was statistically significant. Sequence analysis of excised DGGE bands disclosed that all genuses belong to normal commensal microfora. And the increases of bacteria abundance have no significance among different subjects. The findings of the present study suggest that, IMRT is conducive to maintain the oral ecosystem relatively stable comparted with CRT. During the radiotherapy, the infection control should focus on maintaining ecology equivalence, not specific "pathogen" as well.

P55

The Prevalence of Fibroma In Oral Mucosa Among Patient Attending USM Dental Clinic Year 2006-2010

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The purpose of this preliminary retrospective study is to determine the prevalence of fibroma in oral mucosa among patient attending USM dental clinic from 1/6/2006-1/6/2010. Fibroma of the oral mucosa is the most common benign tumor of the oral cavity. It is a reactive hyperplasia of fibrous connective tissue in response to local irritation or trauma. A lesion on any part of the oral mucosa have a broad differential diagnosis ranging from traumatic lesions (mucocele), neurogenic lesions (neurofibromatosis), lipoma , epithelial tumors (squamous papilloma) and inflammatory/reactive hyperplasia of soft tissue (pulp polyp). A total number of 192 patients (82 male and 110 female) who are registered in the Oral Medicine and Oral Pathology Log Book are included in this study regardless of their age. 16 % of them are diagnosed to have fibroma and out of that, 29% of them are males and 79% are females. The peak incidence of the lesion was in the 3rd decade of life. The lesions occurred in the tongue, lip mucosa, sulcus region and buccal mucosa are each to be 12.9%, 12.9% , 32.2 % and 41.9%. This study shows that fibroma is one of the common oral mucosal lesion and it occurred mostly in the 3rd decade of life where the prevalence is higher in female patients.

P56

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Abstract

Periodontal disease, a common inflammatory oral disease involved periodontal tissues, has been linked with the evidence of some systemic disorders. Recently, periodontal disease has been suspected as a trigger of systemic disorders. Penetration of bacterial products, such as lipopolysaccharide (LPS) may reach into deeper periodontal tissues. Therefore there may affect systemic blood and cytokines production. Interleukin-1β (IL-1β) and Tumour Nuclear Factor-α (TNF-α) are known as pro-inflammatory cytokines. The production of systemic IL-1β and TNF-α of *E. coli* lipopolysaccharide-induced periodontitis model on rats was investigated in this research. Fifteen male Wistar rats, aged 6-8 weeks used for this study were divided into 3 groups. For group 1 and 2, silk ligature 3/0 were inserted in interdental area between upper right molar 1 and 2. First and second group received solution containing 10μg/ml and 1mg/ml *E. coli* lipopolysaccharide, respectively, mixed with 2% carboxymethylcellulose (CMC) diluted in 100μl of phosphate buffer saline (PBS). The solution was topically applied on gingival tissues around the gingival sulcus, a single topical application of solution once per 2 days for 14 days. Untreated subjects were used as negative control. On day 15, the blood was collected from vena orbitalis, and rats were sacrificed. The blood serum of each group was divided into 2 groups and cultured for 4 hours with or without 20μl of 100ng/ml of *E. coli* LPS. ELISA techniques were used to measure the cytokine productions of the supernatant. The data was analysed using Repeated Measure ANOVA. This study showed that there was a significant increase of IL-1β production on low dose of LPS compared to control and high dose of LPS groups ($p<0.05$). Whereas TNF-α not significantly showed increasing trend. The increasing trend of pro-inflammatory cytokine productions, such as IL-1β and TNF-α, on LPS-induced periodontitis model in this experiment supports the previous studies about the contribution of periodontal disease in the pathogenesis of systemic diseases.

Keywords: IL-1β, TNF-α , E. coli LPS, rat's periodontitis model

P57

The Effect of Visible Light Cure (VLC) Exposure to Gingival Tissue's *Sprague dawley* Rats

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Abstract

Visible Light Cure (VLC) is a blue light used in dentistry as an activator restorative material and bonding fixed orthodontic. The wavelength between 400-500 nm is non-ionizing radiation that can produce free radicals. According to previous research, the light at wavelength < 500 nm could inhibit cells mitosis, caused cells damage, reduced cells growth, and inflammation. The purpose of this study was to know the effect of VLC exposure on gingival epithelial thickness, total neutrophil and macrophage count of gingival connective tissue of Sprague dawley rats.
The subjects of this study consisted of 20 Sprague dawley rats, in 2-3 months of age and divided into 4 groups (group A, B, C, D). Each group was 5 rats. The rats in each group were sacrified before (0 day, as group A) and after 1st (group B), 3rd (group C), 5th (group D) day of VLC exposure, respectively. The exposure of VLC was done in labial aspect of cervical anterior teeth of mandible. The distance of exposure was as thick as 2 layers of celluloid strip from it. The histologic specimens were stained by Hematoxylin Eosin. Each specimen was measured its gingival epithelium thickness by using a micrometer and counted the number of neutrophil and macrophage. They were calculated from 10 fields of view and taken the average as the data. The data of the thickness of gingival epithelium from 4 groups were analyzed by Kruskal Wallis. For the neutrophil and macrophage data were analyzed by using one way ANOVA.
The results of this study showed that there were significant differences among groups in the thickness of gingival epithelium, the number of neutrophil and macrophage in the gingival connective tissue of Sprague dawley rats. The conclusion of this study indicated that VLC exposure could decreasing the thickness of gingival epithelium but increasing the number of neutrophil and macrophage of gingival connective tissue of Sprague dawley rats.

Keywords: Visible Ligth Cure, Radiation, Epithelial thickness, Neutrophil, Macrophage

P58

The Effect of Anchovy *Stolephorus baganensis* on Salivary Mutans Streptococci

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Abstract

A clinical trial was carried out to investigate the effect of Anchovy of *Stolephorus baganensis* on mutans streptococci inhibiting the growth of the salivary mutans streptococci for a period one week consumption. Before enrolled in the study, respondents fill and signature the informed consent. Twenty respondents participated as the subjects on the clinical trial , conducting two times of treatment as follows: twenty as treatment groups before and after consuming anchovy of *Stolephorus baganensis* and the twenty subjects as control groups before and after consuming non-anchovy of *Stolephorus baganensis*. Saliva samples were collected before and after consuming anchovy of *Stolephorus baganensis* and with a non-anchovy of *Stolephorus baganensis* . A serial dilution was made, followed by inoculating on TYS20B medium (Shaeken,M.J.M ,van der Hoeven , J.S and Franken , H.C.M , 1986). Data which were obtained from colony forming units of salivary mutans streptococci grew on the TYS20B medium before and after consuming anchovy of *Stolephorus baganensis* were analyzed in a descriptive and "t" test**.** Results howed that there is no significance in the average amount of *Streptococcus mutans* colonies between before and after consuming non-anchovy of *Stolephorus baganensis.* However, a significant difference was found respectively as results before and after consuming anchovy of *Stolephorus baganensis*. We concluded that Anchovy of *Stolephorus baganensis* has anti microbial activity against local strains of *Streptococcus mutans* isolated from human harbouring species. Therefore in a long term of consuming Anchovy fish of *Stolephorus baganensis*, caries can be prevented

Keywords: Anchovy of *Stolephorus baganensis*, Salivary Mutans Streptococci**.**

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Relationship Between Oral Condition And Quality of Life In Educated Elderly

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Abstract

Quality of life assessment mostly is based on general health of people. In fact, oral condition might play a significant role in determining the quality of life of someone. Deterioration of physiologic condition, polypharmacy and the high occurrence of chronic disease in elderly may manifest in oral cavity that can affect oral function. This study aimed to correlate between the oral condition and quality of life in educated elderly. Data of this study were obtained from anamnesis, oral and general health questioners, quality of life questioner modified from Dental Impact of Daily Living Index and from intra-and extra-oral examinations. Results of this study showed that DMFT index was 13.75, and more 50% subjects were found with gingivitis. Most subjects had moderate oral hygiene and were denture wearer. Oral mucosal lesions occurred in 70% of subjects. Dental attrition was the most prominent dental lesion. Hypertension and *Diabetes mellitus* were the first and the second most common disease suffered by elderly in this study. Mean score of quality of life was 25.7 and classified as satisfying. It can be concluded from this study that no significant relationship between oral condition and quality of life in educated elderly from population used.

Keywords: Oral condition, quality of life, elderly

Special Lecture 1:

Periodontal Disease and Systemic Health

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Abstract

The relationship between poor oral health and systemic diseases has been increasingly recognized over the past two decades. Indeed, the clichés "You cannot have good general health without good oral health", "The mouth is part of the body" and "Floss or die", are gaining an increasing momentum. There is considerable epidemiological evidence to support the concept that poor oral health, especially the extent and severity of periodontal disease, may put patients at a significant risk for a variety of systemic conditions. The majority have shown an association, although not always strong. As a result, a number of meta-analyses have been conducted and have confirmed the associations and at the same time cautioned that further studies are required, particularly with regard to the effect of periodontal treatment in reducing risk. A number of biologically plausible mechanisms have been put forward to explain the association and there is accumulating evidence in support of them, although at this stage, insufficient to establish causality. Nevertheless, the relationship between poor oral health and systemic diseases has become a significant issue, such that adult oral health can no longer be ignored in overall health strategies. Therefore, better understanding of this correlation will help both dental and medical professionals to determine the best approach to patient care. In this symposium, an update on current understanding of the contribution of poor oral health to systemic diseases, the possible mechanisms involved and the relevance of this for the members of dental professions will be provided.

Special Lecture 2:

Brief Introduction to College of Stomatology, Shanghai Jiao Tong University

Zheng Jia Wei, MD, DDS, FICD

Professor of Oral and Maxillofacial Surgery Vice Dean College of Stomatology Ninth People's Hospital Shanghai Jiao Tong University School of Medicine

Special Lecture 3:

Can Oral Health Affect Systemic Health and Life Quality?

Lakshman Samaranayake, Hon DSc, Hon FDSRCSE, BDS, DDS, FRCPath, FCDSHK, FHKCPath, FHKAM (Dent Surg)

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Tam Wah-Ching Professor of Dental Science
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Abstract

The two commonest human diseases, caries and periodontal disease are found in the oral cavity and, are caused by oral biofilms. There is a growing body of data that oral biofilm related diseases, can have profound effects on total health and the eventual life quality. The systemic disease that have proven associations with oral health comprise cardiovascular disease including stroke, adverse pregnancy outcomes, diabetes, and pulmonary disease. New data indicate that kidney disease and pancreatic cancer too may have associations with oral biofilms related focal infection.This presentation will provide an overview of how improvements in oral health may improve systemic health, and the eventual quality of life.

Keynote Lecture Session 1:

Perspective for the Future Regeneration Therapy

Takafumi Noma

Department of Molecular Biology, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima, 770-8504, Japan. I would like to introduce the recent topics of stem cell therapy. The induced pluripotent stem (iPS) cells can self-renew and maintain the developmental potential to differentiate into any kinds of cell-types. Therefore, they are one of the potential cell sources for the regenerative medicine. The best way to apply the stem cells to the personalized medicine is using the patients' own cells since there are no ethical concerns and immunological rejection problems. Taking advantage of this autologous system, we established the way to generate the iPS cells from individuals (*J Biosci Bioeng*. 110:345-350, 2010). Here I would like to propose that oral mucosal fibroblasts are the good cell source for personalized iPS cell production and discuss the perspective for the regeneration therapy using stem cells.

Keynote Lecture Session 2:

Functional interaction between orofacial behaviors

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Abstract

Basic patterns of chewing and swallowing are both programmed by the lower brainstem and adapted to peripheral conditions in order to complete mastication in natural situation. Those functions may also affect the orofacial movements including an elementary reflex to avoid unnecessary movements in functions. The aim of this study was to examine the effects of swallowing on the jaw opening reflex, i.e, one of the simplest oral reflexes in the body. Experiments were carried out on 11 anesthetized rabbits. The jaw opening reflex (JOR) evoked by electrical stimulation of either the inferior alveolar (IAN) or lingual nerve (LN) was recorded from electromyogram (EMG) of digastric muslce. Stimulus intensity was determined as 1.5 (innoxious) or 4 (noxious) T the threshold for evoking the reflex. As a conditioning stimulation, the superior laryngeal nerve (SLN) was repetitively stimulated to evoke swallowing. The peak-to-peak of EMG amplitude was measured and compared with and without SLN stimulation, as well as with and without swallowing. Comparisons were also made between low- and high-threshold afferent evoked JORs. The JOR was strongly suppressed during SLN stimulation. The degree of suppression increased and the latency for the JOR were delayed when the stimulus current applied to the SLN was increased. Such modulation was apparent when the low-threshold afferent evoked JOR was recorded. Effects of motor outputs of swallowing events and those of single-pulse stimulation of SLN on the inhibition of the JOR were not noted. These results suggest that the JOR evoked by both the low- and high-threshold afferents was inhibited during pharyngeal sensory input and following swallowing, probably to prevent opposing jaw movements evoked by oral sensory input during swallowing.

O1-1

Properties of Porous Titanium Using Moldless Process

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Abstract

There are a few disadvantages of titanium for bone substitution material; for example, a high elastic modulus and low bone-bonding strength. A popular approach to improve these problems is introducing pores into titanium. Major processes of producing porous metal require a mold, and therefore they are not appropriate for tailor-made biomedical-devices due to the cost. In this study, a mixture of titanium-powder and an inlay-wax binder was developed for moldless forming and moldless sintering of porous titanium. Titanium-powder (diameter < 150 □m) was mixed into a medium-type inlay-casting-wax (mixing rates: 90 mass%). Specimens were formed manually in the same manner using a wax-up technique. Debindering was performed in air at 380ºC for 2 h and sintering was performed in Ar at 1100ºC for 1, 5 and 10 h. Sintering progress was confirmed by scanning electron microscopy (SEM). The dimensional changes and porosity after sintering were measured. The mechanical properties of the sintered porous titanium were evaluated using bending and compression tests. No macroscopic transformation was observed after sintering, indicating that the moldless process in this study was appropriate for forming porous titanium devices. The porosity of 1-, 5- and 10-h sintering specimens were 14, 11 and 8%, respectively, indicating no significant difference due to the limited amount of data. SEM, however, clearly showed a decrease in porosity and sintering progress. The bending strengths of them were 135±11, 245±13 and 356±34 MPa, and the compression strengths were 178±41, 588±97 and 1226±38 MPa, respectively. The strengths of 5- and 10-h sintering specimens differed significantly from those of 1-h sintering specimen (T-test, $p<0.05$). These effects of sintering time on the strengths were mainly due to the decrease in porosity. Since the strengths of 5- and 10-h sintering specimens showed no inferiority to cortical bone, the most appropriate sintering time in this study is therefore concluded to be 5 h. The contraction rates of them were 4.0±1.2, 6.2±0.8 and 6.4±0.4 %, respectively. The relatively large contraction rates of the specimens are a possible problem for fitting porous device into the prepared cavity. An effective solution would be to control the titanium-powder diameter distribution and adjust the other parameters of the mixture and debindering and sintering processes. The moldless process in this research showed a clear advantage for formability and the production of porous titanium devices for biomedical applications.

O-1-2

Biocompatibility Evaluation of Biomaterials

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Abstract

Biomaterial refers to any non vital material intended to interact with biological system within the human body. Biocompatibility is the interaction between a biomaterial and the tissues and physiologic systems of the patient treated with the biomaterial. The biocompatibility evaluation of a biomaterial starts with simple in vitro tests mostly based on cell cultures as is generally done in toxicology. If these experiments and investigations of a biomaterial's efficiency deliver promising findings, then more comprehensive studies on experimental animals and usages test (in vivo evaluation) will be performed. Clinical studies are the final step of this evaluation process. Biocompatibility of a biomaterial cannot be evaluated by using a single test rather than a group of various techniques. The primary purpose of biocompatibility evaluation of biomaterials is to protect patient safety.

Key words: Biocompatibility-evaluation-biomaterial

O1-3

Histrogical Investigation on the Bone Surrounding Dental Implant upon Occlusal Load Using a Novel Rat Model

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Abstract

Dental implant is one of important prosthodontic options and seems to be more likely to improve patient's QOL. However, causes of implant failures at various stages are still unknown. It is very important to know the mechanisms of late implant failures to improve the success rate. Purposes of the present study were to establish appropriate animal implant occlusion model and to investigate on bone tissue changes including degenerative ones under occulsal loading. Upper first and second molars on both sides of four-week-old male Wister rats were extracted. One month after tooth extraction, the bone cavities for implantation were prepared and custom-made pure titanium implants were installed. At 2 or 4 weeks after implantation, two types of abutment that were designed to generate relatively normal load (round type) or overload (cantilever type) to the implant were set respectively. Then rats were sacrificed after 5, 10, 15 days. Specimens were brought to decalcified or undecalcified sections for histological observations under light microscopy. Attritions on occluding opposite teeth and shining spots on the abutments indicated that this model was useful for histological investigation on the remodeling and changes of bone around implants. Specimens with 2 weeks healing periods with round type abutment showed degenerative changes of oseointegration and bone volume was reduced as a result of active bone resorption, while 4 weeks ones showed relatively stable bone tissue around the implants even after the initiation of occlusion. Re-establishment of osseointegration was rarely observed, even though bone formation was observed at implant adjacent area for 15 days. Specimens with cantilever type abutment (applying excessive load) showed remarkable bone loss and degradation of osseointegration even after 4 weeks healing period. In cases of 4 weeks. healing period with cantilever type abutment, even though bone resorption at the interface was limited, there was active bone resorption at remote area of the implants even after 15 days of occulusion. We successfully established implant occlusion model using rat. With this model, degenerative changes of osseointegration or bone around implants upon excessive occlusal load were shown. These results indicated the risk of immediate and over loading. This also suggested that it is important to select healing period, loading degree and the site of implantation according to the specific conditions of each patient. More detailed histological observations will be needed to know the precise mechanisms of maintenance or loss of osseointegration.

O2-1

A Study Dentition and Mastication of The Familly *Hominidae*: An Anthrophological Approach

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Abstract:

Familly *Hominidae* is biological classification that includes all groups of genus *Homo* and in common name is man. Usually they need food for growth and development. The dentition and mastication has some correlation with food variations. When a group eating of a hard food, their mastication will be hard work. Beside that, when the part of the body has no function or less of physiological function, it becomes atrophied. The purpose of the study will be known on anthroplogical aspect especially on evolution of the dentition, and the mastication of family *Hominidae*. The study was done by literature approach. Data collection of dentition was adopted from any population on many literatures and analyzis by quantitative and qualitative approach. The sizes some dentition on mesio-distal measurement (mm) of M^1 (maxillary one) of some populations are 11,7 (Wajak); 11,5 (Sampung); 11,0 (Australoid), and 10,8 (Javanese); M_1 (mandibular one) are 13,6 (Wajak); 12,3 (Sampung); 11,8 (Australoid), and 11,5 (Javanese). The food of that population has any differently, and it has corelation with their mastication. The sizes of dentition on modern man less then the sizes of dental on *Pithecanthropus* group for example on Wajak fosil (*Pithecanthropus Wajakensis*), and they mastication has corresponding of their food.

Key words: Dentition, mastication, *Hominidea*, anthropology

O2-2

Recent Insights into Swallowing Initiation

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Abstract

Swallowing involves several motor processes such as bolus formation and intraoral transport of a food bolus (oral stage) and a series of visceral events that occur in a relatively fixed timed sequence but are to some degree modifiable (pharyngeal stage or swallow reflex). Reflecting the progressive aging of society, patients with swallowing disorders (i.e., dysphagia) are increasing. Therefore, there is expanding social demand for the development of better rehabilitation treatment of dysphagic patients. To date, many dysphagia diets have been developed and are available commercially to help bring back the pleasure of mealtimes to dysphagia patients. Texture modification of food to make the food bolus easier to swallow with less risk of aspiration is one of the important elements in dysphagia diets from the viewpoint of safety assurance. However, for the further development of dysphagia diets, new attempts based on new concepts are needed. One of the possible approaches is to develop dysphagia diets that facilitate swallow initiation. For this approach, an understanding of the mechanisms of swallow initiation and identification of factors that facilitate or suppress swallow initiation are important. In this lecture, I first summarize the effects of various inputs from higher brain and/or sensory receptors on swallow initiation based on data mainly obtained from experimental animals. Then I introduce a recently established technique in our laboratory for eliciting swallowing using electrical stimulation in humans and the findings obtained from our ongoing study in humans.

O2-3

Influences of The Mastication On The Development of Jaw Muscles In Rats

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Abstract

The craniofacial system develops as a response to functional needs, while, the deficiency of proper masticatory stimuli affects its growth and development. The purpose of this study is to relate alterations of muscle activity during postnatal development to adaptational changes in the muscle fibers. Fourteen 21-day-old Wistar strain male rats were randomly divided into 2 groups and fed on either a solid (hard-diet group) or a powder (soft-diet group) diet for 63 days. A radio-telemetric device was implanted to record muscle activity continuously from the superficial masseter, anterior belly of digastric, and anterior temporalis muscles. The degree of daily muscle use was quantified by the total duration of muscle activity per day (duty time) exceeding specified levels of the peak-activity (5, 20, and 50%). Fiber type composition of the muscles was examined by the myosin heavy chain (MyHC) content of fibers by means of immunohistochemical staining. At lower activity levels (exceeding 5% of the peak activity), the duty time of the anterior belly of digastric muscle was significantly higher in the soft-diet group than in the hard-diet group ($p<0.05$). At higher activity levels (exceeding 20 and 50% of the peak-activity), the duty time of the superficial masster muscle in the soft-diet group was significantly lower than that in the hard-diet group ($p<0.05$). There was no difference in the duty time of the anterior temporalis muscle at any muscle activity levels. All muscle fibers were identified as slow type I, and fast type IIA, IIX, or IIB. The percentage of type IIA fiber in the superficial masseter muscle was significantly lower in the soft-diet group than in the hard-diet group ($p<0.01$) and with regard to type IIB fiber, the opposite was true ($p<0.05$). There was no difference in the muscle fiber composition of the anterior belly of the digastric and anterior temporalis muscles. The slow-to-fast transition of muscle fiber was shown in only the superficial masseter muscle. Therefore, the reduction in the amount of powerful muscle contractions could be important for the slow-to-fast transition of MyHC isoform in muscle fibers.

O3-2

The Relationship between Periodontitis and Cardiovascular Diseases

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Abstract

Atherosclerosis, one of the cardiovascular diseases, is related to over 50% of death in USA, results in complications including coronary thrombosis and myocardial infarction, and is a multifactorial disease. The possible causes of this disease include cigarette smoking, genetic alterations, hyperlipidemia, hypertension, diet and exercise. However, approximately 50% of patients with atherosclerosis lack classic risk factors such as smoking, hypertension and hypercholesteremia. In these years, the association between the atherosclerotic risk and infectious reagent/inflammatory response has been proposed. A variety of pathogens, including *Chlamydia pneumonia*, *Helicobacter pylori*, Herpes simplex virus, Cytomegalovirus, and *Porphyromonas gingivalis*, have been also detected in human atheromas. *P. gingivalis* is a primary etiological agent of periodontal disease, a local chronic inflammatory disease that results in oral inflammatory bone destruction. In addition to local chronic inflammation at the initial site of infection, a growing body of evidence has accumulated supporting a role for *P. gingivalis*-mediated periodontal disease as a risk factor for several systemic diseases at sites distant from oral infection including, diabetes, preterm birth, and stroke as well as atherosclerotic cardiovascular disease. A more recent study also demonstrated a strong association between pathogen burden, not specific bacteria, and cardiovascular disease, independent of classic risk factors, and suggested that the pathogen burden could also be a predictor of coronary complications. Recently, Toll-like receptor (TLR) family has been identified as the recognition molecules, innate immune receptors for pathogen-associated molecular patterns (PAMPs), on the defense mechanism against infection and the TLRs ligands have been also identified. The intracellular signaling pathways through MyD88 and IRAK are activated by binding of PAMPs to TLRs. Through these pathways, NF-□B is activated and lots of genes regarding inflammatory cytokines and T-cell activation are up-regulated to lead to inflammation. After 2002, there are lots of reports suggesting the relationship between atherosclerosis and TLRs/inflammation/infection. It has also been reported that TLR2 and TLR4 are preferentially expressed by macrophages in human lipid-rich atherosclerotic plaque lesions. Considering the relationship between periodontal infection and cardiovascular diseases, continued investigations should focus on the mechanistic links among innate immune system, oral infection, inflammation and atherosclerosis.

O3-3

Oral Gases as Bio Markers can Predict Future Periodontal Destruction

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Abstract

More than 200 different volatile organic compounds are detected from breath air. Recently, breath microanalysis has been attracted attention as non-invasive examination tool in medical field. For example, microanalysis of C4-C20 alkanes and monomethylated alkanes in patients' breath air can make it possible to diagnose early stages of lung cancer, breast cancer or heart transplant rejection. As far as dental science concerned, we have much longer history to analyze breath air including oral air in order to diagnose oral disease or to assess oral health condition. Concentration of volatile sulfur compounds (VSCs) such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide [$(CH_3)_2S$] are good indicator for halitosis. Experimental studies have investigated that anaerobic microorganisms, especially periodontitis-related microorganisms produce VSCs in the process of their protein metabolism. Many clinical studies have also demonstrated that high levels of VSCs are detected from oral air in halitosis patients and that relatively higher level of CH_3SH is found in patients with active periodontal disease. Our previous study showed that persons with more than 0.35 of CH_3SH/ (H_2S +CH_3SH) ratio had 3 times higher risk for having at least one site of periodontal pockets 6+ mm. On the other hand, clinicians prefer to have a risk predictor rather than a risk indicator for periodontal disease. Because, we already have accurate clinical examination like measurements of bleeding on probing, periodontal pocket depths and attachment levels although gas analysis has advantage in terms of easy and non-invasive. One study reported that risk for increasing 20 % or more sites showing 4+ mm periodontal pocket depth during 2 years was about 10 times higher in persons having VSC of 150≤ ppb at baseline examination when compared with persons having VSC of 150> ppb. In recent study, VSCs were measured in community-dwelling elderly people with 20+ teeth and their periodontal disease progressions were observed for 4 years. When divided into 4 groups according to the components of VSC, multiple comparison tests showed that number of teeth recorded 3+ mm additional attachment loss during 4 years was significantly higher ($p=0.006$) in group with (CH_3SH/H_2S) ratio of 0.5≤ at baseline than group with only H_2S. These studies suggest that oral gas measurements as clinical examination may be useful not only to assess patients' present periodontal disease status but also to predict their future periodontal disease progression or occurrence.