国際セッション

（E会場）

座長　九州大学大学院 歯学研究院 口腔機能修復学講座 歯周病学分野
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E会場（43+44会議室）

14：20～15：40

I-01
2199

Probiotic as bacteriotherapy candidate against Aggregatibacter actinomycetemcomitans

Norzawani Jaffar

Keywords: probiotic, Biofilm, co-culture, inhibition

This study aims to evaluate potential of probiotic bacteria as bacteriotherapy against the periodontal pathogen, Aggregatibacter actinomycetemcomitans. Probiotic bacteria screened for inhibition activity against A. actinomycetemcomitans via overlay agar method. Probiotic bacteria with inhibition activity were subjected to indirect co-culture with A. actinomycetemcomitans and inhibition was evaluated by measuring the growth reduction after 24 and 48 hour incubation under an anaerobic condition at 37C. Then, inhibition effect on A. actinomycetemcomitans was confirmed for morphological changes using SEM. Later, inhibition activity was tested for biofilm deformation of A. actinomycetemcomitans by co-culturing the probiotic bacteria with A. actinomycetemcomitans and evaluate the biofilm formation in comparison with A. actinomycetemcomitans without probiotic. Two out of six probiotic strains subjected for indirect co-culture showed bactericidal effect. They are Lactobacillus plantarum 15891 and Lactobacillus johnsonii 13952 with growth reduction value 4.18 and 4.77 (log CFU/ml) respectively. A. actinomycetemcomitans cells treated with indirect co-culture L. plantarum 15891 and L. johnsonii 13952 appeared collapsed and smaller in size compared to control. Biofilm deformation of A. actinomycetemcomitans evaluated up to 3-fold reduction compared to mono-culture. In conclusion, this probiotic strains owns great potential as a candidate for bacteriotherapy in controlling A. actinomycetemcomitans growth in-vitro.

I-02
3104

Pan-genome and comparative genome of 16 *Porphyromonas gingivalis* strains

Dali Liu

Keywords: *Porphyromonas gingivalis*, Pan-genome, Comparative genome

Objectives: *Porphyromonas gingivalis* is a major pathogen of chronic periodontitis, which leads to the destruction of periodontal tissues and finally to tooth loss. The aim of this study was to analyze the pan-genome and comparative genome of *P. gingivalis*.
Materials and methods: Five clinical strains of *P. gingivalis* (SJD2, SJD4, SJD5, SJD11, and SJD12) were isolated from subgingival plaque of patients with severe chronic periodontitis in China. The virulent properties of these strains were identified by using mouse subcutaneous soft tissue abscess model. Draft genome of these 5 *P. gingivalis* strains was sequenced by high-throughput Solexa sequence analyzer and protein-coding sequences of these strains were predicted. The predicted proteins of 11 reference strains and of the 5 newly sequenced strains, were compared against each other, by using the OrthoMCL 5 program. The pan-genome and core-genome analysis were performed with the pan-genomes analysis pipeline.
Results: Using mouse subcutaneous soft tissue abscess model, SJD2 and SJD12 strains showed typical highly-virulent properties, which are comparable to W83 strain. The function model of *P. gingivalis* pan-genome was established as $P = 1114.3\ N^{0.466} + 976.1$ ($R^2 = 0.999$), in which the N denotes the genome number. Further comparative genomic analysis showed that 7 genes appeared to be present in virulent strains, but absent from the less-virulent strains.
Conclusions: Based on these results, the pan-genome of *P. gingivalis* is defined as open, suggesting that this organism evolved actively with great expansions of the genome. In addition, several genes may directly involve in the virulent properties of *P. gingivalis*.

I-03
2504

The involvement of Wnt5a in sphingosine-1-phosphate-modulated mesenchymal stem cell differentiation into osteoblast

Yoko Hashimoto

Keywords: mesenchymal stem cell, osteoblast, sphingosine-1-phosphate, Wnt5a

Objective: Recent studies have shown that mesenchymal stem cells (MSC) in periodontal ligament play crucial role in periodontal tissue regeneration. Sphingosine-1-phosphate (S1P) is a signaling molecule which regulates many cellular responses, including cellular differentiation. We previously reported that S1P induces osteogenic differentiation, while inhibits adipogenic differentiation. In this study, we tried to understand the involvement of Wnt signaling in S1P-induced osteogenic differentiation, as it is essential for osteogenic differentiation.
Materials and Methods: C3H10T1/2 cells (mouse MSC cell line) were cultured in osteogenic or adipogenic differentiation medium with or without S1P. The expression levels of osteogenic differentiation-related genes (alkaline phosphatase (ALP), osteocalcin (OC), Wnt5a, low-density lipoprotein receptor-related protein (LRP) 5, LRP6) were examined. Adipogenic differentiation was also monitored.
Results: ALP, OC, Wnt5a, LRP5, LRP6 mRNA expression increased by S1P treatment in C3H10T1/2 cells. The expression of these genes was inhibited by anti-Wnt5a antibodies. Adipogenic differentiation was inhibited by S1P.
Conclusion: The results suggest that S1P up-regulates Wnt5a expression, leading to the induction of LRPs, thereby promoting MSC differentiation into osteoblast. Thus, S1P may be a favorable reagent for osteogenic induction.

I-04
2504

Prevalence and evaluation of bone loss pattern among patient with aggressive periodontitis

Mohd Faizal Hafez Hidayat

Keywords: Aggressive Periodontitis, Prevalence, Alveolar Bone Loss

Objectives: To determine the prevalence of Aggressive Periodontitis (AgP) and the alveolar bone loss (ABL) pattern.
Materials and methods: A retrospective study was done by examining dental records of patients referred to the specialist periodontal clinic at Faculty of Dentistry UiTM Shah Alam from January 2009 until December 2014 for AgP cases based on the 1999 Classification Workshop. A radiographic linear measurement procedure (Planmeca Romexis version 2.9.2 software) was used on their panoramic radiographs (OPGs).
Results: 2.5% of patients were diagnosed with AgP (13 male and 11 female). ABL% was demonstrated at mesial of maxillary second molar for both quadrant of male (right=18.50%, left=17.65%) and female (right=10.55%, left=10.24%). For mandibular tooth, ABL% is at the mesial of right mandibular first molar and distal of left mandibular first molar on both male (right=8.77%, left=10.08%) and female (right 11.13%, left=9.27%) patients. Significant correlation was observed between ABL% on both right and left quadrant of maxilla of male patients. However for female patients the correlation is weaker. Percentages of vertical bone defect were found higher at the mesial of maxillary second molar, distal and mesial of maxillary first molar and distal and mesial of mandibular first molar.
Conclusions: Pattern of alveolar bone loss in patients diagnosed with AgP in this study affected the first and second molars, similar to the findings in the literature. ABL showed bilateral or symmetrical pattern. Vertical bone defects was found affecting the molars than the premolars.

I-05
2504

Resveratrol inhibits NLRP3 inflammasome-derived IL-1 beta secretion induced by dental calculus in murine macrophages

Jorge Luis Montenegro Raudales

Keywords: Dental calculus, periodontitis, IL-1 beta, NLRP3, inflammasome, resveratrol

Objective: Our previous data showed that dental calculus could induce NLRP3 inflammasome-mediated interleukin 1β (IL-1β) secretion in murine macrophages. Recent studies have shown that the polyphenol, resveratrol, found in grape skin and red wine, can reduce NLRP3-derived IL-1β production. This study aimed to explore the inhibitory effect of resveratrol in IL-1β secretion induced by dental calculus.

Materials and methods: Macrophages from C57BL/6 mice were stimulated with dental calculus from periodontitis patients in the presence or absence of resveratrol. To further investigate the effect on crystal stimulation, cells were primed with lipid A to induce pro-IL-1β or left unprimed and stimulated with synthetic hydroxyapatite (HA), in the presence or absence of resveratrol. After 8 hours, IL-1β secretion levels were measured by ELISA.

Results: Dental calculus induced IL-1β in macrophages without priming, suggesting it can stimulate both pro- and mature forms of IL-1β. HA crystals induced IL-1β in lipid A-primed but not unprimed cells. Resveratrol significantly reduced IL-1β secretion in cells stimulated with either dental calculus or HA crystals.

Conclusion: These findings imply that resveratrol can suppress NLRP3 inflammasome-mediated IL-1β secretion at least partially by interfering in crystal stimulation, suggesting a potential anti-inflammatory effect of this natural compound in periodontal disease.

I-06
2609

Novel implant prosthetic system – Mechanical and Biological complication rates of the advanced lateral screw

Young-Taek Kim

Keywords: mechanical complication, biological complication, prosthesis failure, single-tooth dental implants

Objectives: The purpose of this retrospective study was to evaluate the mechanical and biological complication rates of the implant-supported single crowns (ISSCs) with the advanced lateral screw prosthetic (ALS) system in the posterior region and how these complication rates are affected by clinical factors.

Materials and methods: The mechanical complications (i.e., lateral screw loosening (LSL), abutment screw loosening (ASL), lateral screw fracture (LSF), and ceramic fracture (CF)) and biological complications (peri-implant mucotitis (PM), peri-implantitis (PI)) were identified by examining the patients' treatment records, clinical photographs, periapical, panoramic radiographs, and clinical indices. Statistical analyses were performed to identify the relationship between clinical factors and complication rates.

Results: Mechanical and biological complications were present in 27 (37%) of the 73 investigated ISSCs with the ALS system. LSL and PM were the most common complication (15.1%), followed in order by ASL (2.7%), LSF (1.4%), CF (1.4%), and PI (1.4%). The incidence of mechanical complication was significantly related with gender (P = 0.018). The other clinical factors showed no significant relation regarding the mechanical and biological complication rates.

Conclusions: Within the limitations of this study, the incidence of mechanical and biological complications for ISSCs with the ALS system in the posterior region is relatively low compared with other ISSCs. Also, the ALS system is effective in the prevention and treatment of mechanical and biological complications.

I-07
2205

A simple method to generate a large amount of developmentally selected mesenchymal stem cells

Takehito Ouchi

Keywords: mesenchymal stem cells

Aim: Recently, dental mesenchymal stem cells (MSCs) have been identified, and used for periodontal regenerative medicine. These MSCs defined by conventional criteria include a heterogeneous cell population; therefore, it is difficult to summarize the potential of them. We focused dental MSCs are derived from neural crest cells (NCCs). NCCs with multi-lineage potential survive as neural crest stem cells (NCSCs) even in adult. Some dental MSCs are with properties that overlapped with those of NCSCs. In this study, our purpose is to clarify the developmental black box of MSCs.

Methods: Human ES cells and iPS cells were induced into NCCs. To confirm the existence of highly potent purified human MSCs that expressed LNGFR and THY-1, we analyzed cell surface markers using flow cytometry. Sorted LNGFR$^+$THY-1$^+$ NCCs were analyzed *in vitro*.

Results: Human ES cells and iPS cells condensed and formed neuro-ectoderm spheres. After that, spheres attached spontaneously, and NCCs migrated out. We were able to selectively purify LNGFR$^+$THY-1$^+$ cells from NCCs, and they showed the features of both NCSCs and MSCs.

Discussion: To advance human periodontal stem cell research, additional clarification is needed. Previously, we reported highly potent purified human MSCs that express LNGFR and THY-1, providing purified MSCs from dental tissues. However, they are limited by their small population and minimal proliferative activity. Here, we demonstrate a method which provides a simple way to generate a large amount of developmentally selected MSCs that express LNGFR and THY-1. They will be promising candidates for periodontal regenerative medicine.

I-08
2207

Effects of wavelength-tunable nanosecond pulsed Cr:CdSe laser on dental hard tissues: examination in the spectral range of 2.76-3.00 μm

Taichen Lin

Keywords: laser, wavelength tunable, enamel, dentin, cementum, ablation, erbium lasers

Objectives: Er:YAG (2.94 μm) and Er,Cr:YSGG (2.78 μm) lasers has been increasingly used for various applications in periodontal therapy. Recently, a chromium-doped: cadmium-selenide (Cr:CdSe) laser system was developed, which enables laser oscillation around 2.9 μm. The aim of the study was to evaluate the effects of the Cr:CdSe laser on dental hard tissues in the range of 2.76 - 3.00 μm.

Materials and Methods: We used the wavelengths of 2.76-3.00 μm and energy output of 0.28-2.0 mJ (fluency: 1.6-11.2 J/cm2/pulse, pulse duration: approximately 250 ns, beam diameter: approximately 150 μm). Dental hard tissues such as enamel, dentin and cementum were irradiated with the Cr:CdSe laser at 10 Hz without water irrigation. After irradiation, morphological changes, ablation depth, and thickness of thermally affected layer of the irradiated surfaces were analyzed by using stereomicroscopy, SEM, and light microscopy of non-decalcified histological sections.

Results: The Cr:CdSe laser irradiation effectively ablated dental hard tissues with no visible thermal damage such as carbonization, major melting and cracks, and accompanied with approximately 20 μm width thermally affected layer. The efficacy of ablation gradually increased from 3.00 μm towards 2.76 μm and wavelength of 2.76 μm revealed the highest ablation efficacy on dentin.

Conclusions: These results demonstrated the excellent ablation effects of the nanosecond pulsed Cr:CdSe laser in dental hard tissue ablation, and clarified the remarkable wavelength dependence of its ablation effect on dentin in the range of 2.76-3.00 μm.