国際セッション口演

（A会場）

6月1日（金） A会場　8：50～10：10

IO-01
3104

Interaction between bone marrow derived macrophages and mesenchymal stem cells

Bin Chen

Keywords: Macrophages, mesenchymal stem cells, stem cell based therapy, oxidative stress

Objectives: The interaction between stem cells and immune cells is of great importance for improving the effects of stem cell based therapy. Therefore, we extracted bone marrow-derived mesenchymal stem cells and macrophages of SD rats and studied their interaction.

Materials and methods: Macrophages and mesenchymal stem cells of rats originated from the femur and tibia. Macrophages were induced by M-CSF at 50μg/ml for 7 days. The purity of the macrophages was over 95% after the surface markers were identified for subsequent experiments. The effects of mesenchymal stem cell on proliferation/polarization and apoptosis of macrophages were detected by CCK-8, flow cytometry and Western-blot. Finally, we evaluate the effects of different macrophage subtypes on mesenchymal stem cells against oxidative stress by CCK-8.

Results: (1) MSC had no significant effect on the proliferation of macrophages; MSC could promote the apoptosis of M1 and inhibit the apoptosis of M2, at the same time, MSC can promote the transformation of M1 to M2. (2) The proliferation of MSCs decreased in M1 conditioned medium, however, there was no obvious change in the proliferation of MSCs treated with M1 conditioned medium when exposed to oxidative stress. However, the proliferative capacity of MSCs in M2 conditioned medium was significantly increased, however, the proliferative capacity of MSCs in M2 conditioned medium was significantly reduced when exposed to oxidative stress.

Conclusions: (1) MSC can reduce tissue inflammation by changing the ratio of Ml/M2 in the tissue in the above manner. (2)For macrophages, M1, which plays a major role in the early healing of trauma, and M2, which plays a major role in the late and mid-stage of tissue damage, appear to be of very important timing, which is important for immunotherapeutic-based regenerative therapy studies.

IO-02
2203

Peptide 19 of *Porphyromonas gingivalis* heat shock protein is a potent inducer of low-density lipoprotein oxidation

Ji-Young Joo

Keywords: Periodontitis, Atherosclerosis, *Porphyromonas gingivalis*, Cardiovascular diseases

Objectives: Although periodontal pathogens show a strong association with development of atherosclerosis, little is known about how a microorganism contributes to disease onset and progression. Oxidation of low-density lipoprotein (LDL) is a major risk factor of atherogenesis. The principal objective of this study is to evaluate the ability of peptide 19 (Pep19) of *Porphyromonas gingivalis* (Pg) heat shock protein (HSP) as a potent inducer of LDL oxidation, and a secondary objective is to compare this ability with that of Pep19 from different bacteria.

Materials and methods: HSP60, Pep14, and Pep19 from Pg and THP-1 monocytes were cultured, and the extent of LDL oxidation induced by each peptide was evaluated by an assay for thiobarbituric acid-reactive substances (TBARS). Pep19 and HSP60 from *Chlamydia pneumoniae* and *Mycobacterium tuberculosis* were also cultured with THP-1 monocytes and evaluated by the TBARS assay. After incubation of macrophages with LDL and peptides from Pg, Oil Red O staining was performed for microscopic examination of foam cells, macrophages that took up the oxidized LDL.

Results: Monocyte-mediated native-LDL oxidation under the influence of Pep19 or HSP60 from Pg was significantly stronger than oxidation induced by the counterpart Pep19 or HSP60 from *C. pneumoniae* or *M. tuberculosis*. Pep19 from Pg HSP60 showed a stronger ability to induce LDL oxidation than did Pep14 from Pg HSP60

Conclusions: These results suggest Pep19 from Pg HSP60 has a distinct ability to induce native-LDL oxidation as a plausible mechanism by which this peptide may drive epitope spreading to the neoantigen, i.e., oxidized LDL, in the pathogenesis of atherosclerosis.

IO-03
2504

Identification of periodonto-pathogens and inflammatory cytokines in adults with chronic periodontitis

Rathna Devi Vaithilingam

Keywords: Periodonto-pathogens, Inflammatory cytokines, chronic periodontitis, qPCR, Elisa

Objectives: To identify and quantify periodonto-pathogens and inflammatory cytokines in moderate to severe chronic periodontitis (CP) subjects and correlate their presence with clinical parameters.

Materials and methods: Total of 167 CP and 134 healthy subjects were recruited. Visible plaque index (VPI), Gingival bleeding index (GBI), Probing pocket depth (PPD) and Clinical attachment loss (CAL) was recorded. Subgingival plaque and blood was sampled. Quantification of *Porphyromonas gingivalis* (*Pg*), *Tannerella forsythia* (*Tf*), *Prevotella intermedia* (*Pi*) and *Aggregatibacter actinomycetemcomitans* (*Aa*) using qPCR and resistin, Tumour necrosis factor alpha (TNF-α), Interleukin (IL)-6 and IL-17 using ELISA was performed.

Results: CP group had higher detection of *Pg* (24.66×10^5cells/μl), *Tf* (7.70×10^5cells/μl), *Pi* (6.61×10^5cells/μl) and *Aa* (0.14×10^5cells/μl) as compared to healthy group (8.32×10^5, 4.75×10^5, 5.17×10^5, 0.00 respectively) ($p<0.05$). Detection of IL-17 (597.77pg/ml), IL-6 (15.67pg/ml) and TNF-α (19.01pg/ml) was higher in CP compared to healthy group (83.36pg/ml, 6.12pg/ml, 0.00pg/ml respectively) ($p<0.001$). For CP group, weak correlation existed between *Pg* and *Tf* with PPD and CAL, *Pi* with CAL and IL-6 with VPI, PPD and CAL.

Conclusions: CP subjects had higher mean counts of *Pg*, *Tf*, Pi, Aa and serum IL-17, TNF-α and IL-6 levels than healthy subjects.

IO-04
2504

Subgingival glycine powder air-polishing as an additional approach to non-surgical periodontal therapy in subjects with untreated chronic periodontitis

Elvis Yiu Cheung Tsang

Keywords: Chronic periodontitis, Non-surgical periodontal therapy, Glycine powder air polishing, Gingival crevicular fluid

Objective: This study aimed to investigate the effect of subgingival glycine powder air-polishing (GPAP) as an additional approach to non-surgical periodontal treatment in subjects with chronic periodontitis.

Material and Methods: 27 non-smoking subjects were recruited. Two quadrants in each subject were randomly assigned according to a split-mouth design to receive scaling and root debridement (SRP) and GPAP (Test group) or SRP and air flushing with water (Control group) at sites with probing depth (PD) ≥5mm. Clinical parameters, gingival crevicular fluid (GCF) volumes and concentrations of IL-1β and IL-1ra in GCF were measured at baseline and 1, 3 and 6 months after the treatments.

Results: At baseline, no statistically significant difference in periodontal and GCF parameters was found between the Test and Control groups. Overall, the periodontal conditions of all subjects significantly improved after the treatments. Notably, the Test group showed greater reduction in GCF volume (0.37 ± 0.26 μl) than the Control group (0.23 ± 0.30 μl) at 3 months ($p<0.05$). The GCF levels of IL-1β and IL-1ra significantly decreased in both groups at 6 months, and no significant difference was found between the groups.

Conclusions: These preliminary results suggest that GPAP as an additional approach to nonsurgical periodontal treatment may be beneficial to short-term improvement of subclinical level of inflammation measured by GCF volume. Further longitudinal studies with larger sample sizes are required to clarify the exact benefits of GPAP treatment for controlling inflammation and maintaining long-term periodontal health.

IO-05
2609

Retrospective analysis of implant success rates and bone volumetric changes after lateral window sinus augmentation with β-tricalcium phosphate

Takaaki Kishimoto

Keywords: Dental Implants, Retrospective study, Maxillary sinus, Beta-tricalcium phosphate, Cone-beam computed tomography

Purpose: To analyze 1) implant success rates after lateral window sinus lift (LWS) using β-TCP, 2) marginal bone loss around the implants, 3) residual bone height (RBH) changes, 4) grafted bone volumetric changes using CBCT data, software, and radiographs, and 5) if changes in implant stability quotient (ISQ) values were correlated to implant success rates after LWS.

Materials & Methods: Patients had implant therapy after LWS using β-TCP were reviewed. CBCT was taken at different periods, and grafted bone volume changes before the implant placement were calculated using CBCT data and the software. ISQ values obtained at the time of implant placement (T_3) and second stage surgery (T_4) were compared. Implant success rate was assessed based on success criteria by Misch et al. (2008).

Results: A total 11 subjects with 29 implants after 14 LWS using β-TCP were included. LWS was successfully performed to place implants, and mean RBH was significantly increased 2.81 to 12.47mm after the LWS. The mean obtained bone volume at immediate after LWS using β-TCP and before implant placement was 1444.90mm^3 and 1327.37mm^3, respectively, and the percentage of grafted bone volume loss was 7.56% during 4.40 months. The implant success rate was identified 100% and mean marginal bone loss was 0.58mm during 3.85 ± 1.71 years follow-up. Mean ISQ values obtained at T_3 was 60.20 despite relatively short healing after LWS, and the ISQ value at T_3 was significantly increased to 69.79 at T_4 during 6.54 months.

Conclusion: Implant therapy after LWS using β-TCP was clinically successful, and β-TCP was stable as a bone graft material for LWS during the healing.

IO-06
2402

The effect of high glucose on primary human gingival fibroblasts

Prima Buranasin

Keywords: High glucose, Gingival fibroblasts, Diabetes, Wound healing, Oxidative stress

Molecular mechanisms of impaired gingival wound healing in diabetes have remained elusive. This study was to investigate the biological changes of human gingival fibroblasts (HGFs) under high glucose conditions.

Healthy gingiva-derived HGFs were cultured under high (25, 50 and 75 mM) and normal (5.5 mM) glucose conditions. Cell viability was examined by using WST-8 and Lactase dehydrogenase (LDH) assays. *In vitro* wound healing assay, EdU (5-ethynyl-2'-deoxyuridine) and Ki67 staining were performed to evaluate the cell migration and proliferation. mRNA expression of oxidative stress markers was quantified by real-time PCR. Antioxidant, *N*-acetylcysteine (NAC) was added to evaluate the involvement of oxidative stress by high glucose conditions.

Significantly lower cell viability and LDH elevation were observed in higher glucose conditions. Fibroblast migration and proliferation were decreased in *in vitro* wound healing assay. Positive cell numbers for EdU and Ki67 staining confirmed the impairment of HGF proliferation by high glucose. The mRNA levels of Heme oxygenase-1 and Superoxide dismutase-1 were significantly upregulated. NAC treatment diminished the inhibitory effect of high glucose conditions.

This study showed that high glucose impaired proliferation and migration of HGFs, via the induction of oxidative stress, and may explain the delayed wound healing in diabetic patients.

IO-07
2504

The effects of theaflavins on tissue inflammation and bone resorption on experimental periodontitis in rats, part 2

Ya-Hsin WU

Keywords: Theaflavins, Experimental periodontitis, Alveolar bone loss, Inflammation

Background: Theaflavins (TF), the major polyphenol in black tea, has shown the ability to reduce inflammation and bone resorption. The aim of this study was to evaluate the effect of TF on experimental periodontitis in rats.

Material and methods: Thirty rats were divided into 5 groups: Control (vehicle application without ligation), Ligature (vehicle application with ligation), TF1 (1mg/ml TF application with ligation), TF10 (10mg/ml TF application with ligation), and TF100 (100mg/ml TF application with ligation). To induce experimental periodontitis, ligatures were placed around maxillary first molars bilaterally. After ligature placement, rats were topically applied with 100μl of vehicle or TF every day. Rats were euthanized at 1 week after ligature placement.

The left side of maxilla was measured for bone resorption by micro-CT, and the gingival tissue was examined to investigate mRNA expression by RT-PCR. The right side was histologically analyzed by H-E and tartrate-resistant acid phosphatase staining.

Results: The alveolar bone loss was significantly inhibited by TF groups in a dose-dependent manner. Ligature group showed greater numbers of inflammatory cells in the gingival tissues and osteoclasts on the alveolar bone when compared with Control group, but these numbers decreased in TF10 and TF100 groups. Furthermore, TF10 and TF100 groups significantly downregulated the gene expression of IL-6 and RANKL, but not that of OPG.

Conclusion: The present study suggested that topical application of theaflavins had potential treatment effect on the experimental periodontitis in rats.

IO-08
2599

Dental calculus alters the permeability of HSC-2 oral epithelial cell monolayer

SM Ziauddin

Keywords: Dental calculus, Epithelial cell, Cell death

Objective: Previously, we found that dental calculus could induce cell death in HSC-2 oral epithelial cells and its crystalline structures play an important role in it. However, the effect of dental calculus on the barrier function of HSC-2 cell monolayer has not been investigated. This study aimed to examine if dental calculus could alter the permeability of HSC-2 cell monolayers.

Materials and methods: HSC-2 cells derived from human oral squamous cell carcinoma were exposed with dental calculus or synthetic hydroxyapatite (HA) crystals. After 24 hours, cytotoxicity was quantified by measuring lactate dehydrogenase release in the culture supernatants. For permeability assay, HSC-2 cell monolayer in the upper insert of a transwell membrane was exposed to dental calculus or HA crystals. After 24 hours, the medium in the upper chamber was replaced with Opti-MEM containing trypan blue and permeability was measured by detecting trypan blue pigments in the lower chamber.

Results: Dental calculus and HA crystals induced cell death in HSC-2 cells. The amount of pigments passing through HSC-2 cell monolayer significantly increased following exposure to dental calculus or HA crystals.

Conclusion: Dental calculus and HA crystals alter the permeability of the HSC-2 cell monolayers. Crystalline structure of dental calculus may deteriorate the barrier function of crevicular/pocket epithelium.