English 学内発表会

An Opportunity to Improve Your Oral Presentation Skills

日　時：平成 26年 3月 7日（金）　午後5時～

会　場：日本歯科大学生命歯学部　第２会議室

日 本 歯 科 大 学 歯 学 会

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会　場：日本歯科大学生命歯学部　第２会議室
発　表：10分、質疑応答：5分

【Opening address】17:00~17:05

【Oral presentation session 1】17:05~17:25

Chairperson：Prof. KARIBE Hiroyuki

1. Cyclophosphamide Inhibits Molar Root Formation in Growing Mice

Kawakami T
Department of Pediatric Dentistry, The Nippon Dental University School of Life Dentistry at Tokyo

【Oral presentation session 2】17:30~17:50

2. Difference in marginal sealing between flowable and universal resin composites applied to cervical restoration

Maruyama S, Maseki T and Nara Y
Department of Adhesive Dentistry, The Nippon Dental University School of Life Dentistry at Tokyo

【Oral presentation session 3】17:55~18:15

Chairperson：Prof. IMAI Kazushi

3. Comparison of cellular aging and senescence in serially passaged long-term cultures of mesenchymal stem cells derived from extracted human teeth and bone marrow

Tamaki Y [1)], Nakahara T [1)], Ishikawa H [2)], Sato S [3,4)]
Department of Developmental and Regenerative Dentistry[1)], Department of NDU Life Sciences[2)], School of Life Dentistry at Tokyo, Department of Periodontology[3)], Division of Cell Regeneration and Transplantation, Advanced Research Center[4)], School of Life Dentistry at Niigata, The Nippon Dental University

【Oral presentation session 4】18:20~18:40

4. Isolation and characterization of human periodontal microvascular endothelial cells

Maruyama K, Tsubokawa M, Sato S
Department of Periodontology, The Nippon Dental University School of Life Dentistry at Niigata

【Closing address】18:40~18:45

1. Cyclophosphamide Inhibits Molar Root Formation in Growing Mice

Kawakami T
The Nippon Dental University School of Life Dentistry at Tokyo, Department of Pediatric Dentistry

Introduction:

It is well known that root development of permanent teeth is perturbed, such as microdontia and V-shaped short root, in patients who have survived childhood cancer. However, the effect of chemotherapeutic drugs on cells that initiate the growth of tooth root is not certain yet. Cyclophosphamide (CY) is one of the cytostatic drugs commonly used for the treatment of cancer in children. This study aimed to evaluate the effect of CY on development of molar teeth in growing mice.

Materials and Methods:

Twenty 12-day-old ICR mice were treated with CY (100 mg/kg, i.p.) and 20 control mice with saline. At 16, 20, 24, and 27 days of age (PN16, PN20, PN24, PN27), the mandibular molars were removed. Right sides of the mandibular molars were scanned using micro-computed tomography, and the distal root length and the area of apical foramen of the first molar was measured. The other side of the mandibular molars was fixed, demineralized, dehydrated, and embedded in wax in that order. Serial sagittal sections were prepared from the first molars and histomorphological (hematoxylin and eosin staining) and immunohistochemical (cytokeratin) studies were performed.

Results:

The root length in the experimental group (770μm) was shorter than that of the control group (1213μm) at PN27 ($p<0.001$, t-test). In addition, the area of root apical foramen in the CY group was smaller than that of the control group. Although all roots developed further after CY injection, microscopic examination showed that roots of the first molars of mice in the experimental group seemed to develop more slowly and were shorter than those in the control mice. Furthermore, experimental mice showed apical closure of the roots with dentine. The Hertwig's epithelial root sheath (HERS) disappeared from PN20, and only the epithelial rest of Malassez was detected on the root surface in the experimental mice.

Discussion:

HERS plays an important role in formation of the tooth root. These results suggest that CY can induce a defect in HERS and would alter the function of HERS. Inhibition by CY of root elongation and subsequent early closure of the apical foramen induce V-shaped short root of the molars.

2. Difference in marginal sealing between flowable and universal resin composites applied to cervical restoration

Maruyama S, Maseki T, Nara Y.
Department of Adhesive Dentistry,
School of Life Dentistry at Tokyo, The Nippon Dental University

Introduction :

Recently well-handling flowable resin composite has been widely applied to various clinical cases, instead of universal resin composite. On the other hand, cervical region is one of well-known predilection sites of dental diseases, i.e., dental caries and abrasion lesion, and is usually restored with a pair of resin composite restorative and resin adhesive system. Previous study reported that cervical resin composite restoration formed 35% of the total number of composite restorations. There is no doubt that intra-oral environment influences the marginal sealing of cervical resin composite restoration. The purpose of this study was to examine the difference between flowable and universal resin composites applied to cervical restoration under thermo-mechanical cyclic stress simulating intra-oral environment.

Materials and methods :

30 standardized V-shaped cavities with occlusal margin on enamel and gingival margin on dentin were prepared in the buccocervical region of extracted human lower premolars. A flowable resin composite, Supreme Ultra Flowable Restorative (**F**; 3M ESPE, USA), and a universal resin composite, Supreme Ultra Universal Restorative (**U**; 3M ESPE, USA), were used as restorative. An etch and rinse adhesive system, Adper Single Bond Plus (3M ESPE, USA) was applied to the cavities according to manufacturer's instruction, and two resin composites, **F** and **U**, were filled into the cavities and light-cured. All specimens were stored in a moisture box at 37 ºC for 24hrs, and then polished. Restored specimens were thermocyclyed (5ºC/55ºC, 200cycles) and cyclic loaded (118N $\times 10^4$) simultaneously. After dyeing by 1% methylene blue solution for an hour, microleakage of cervical cavities restored with **F** and **U** were evaluated by a graded criterion, and analyzed using Mann-Whitney U and Wilcoxon signed-rank tests.

Results :

Occlusal microleakage was not recognized in **F** restoration, and there was a significant difference in the leakage between **F** and **U** restorations at $p<0.05$. On the other hand, the gingival microleakage of **F** restoration showed less than a quarter of cavity wall length, and there was no difference in the leakage between **F** and **U** restorations. However, the occlusal leakage was significantly better than the gingival leakage at $p<0.05$, regardless of resin composite.

Discussion :

It could be considered that the marginal sealing of cervical restoration under thermo-mechanical cyclic stress was influenced by the material properties of resin composite applied to the restoration, such as coefficient of polymerization shrinkage, coefficient of thermal expansion and elastic modulus. In addition, it seemed that the phosphoric acid etching procedure was effective to get excellent sealing on enamel margin.

Conclusions :

The sealing of occlusal margin on enamel of flowable resin composites applied to cervical restoration, under thermo-mechanical cyclic stress simulating intra-oral environment, was significantly superior to that of universal resin composite. The sealing of gingival margin on dentin of flowable resin composites was similar to that of universal resin composite.

3. Comparison of cellular aging and senescence in serially passaged long-term cultures of mesenchymal stem cells derived from extracted human teeth and bone marrow.

Tamaki Y [1)], Nakahara T [1)], Ishikawa H [2)], Sato S [3,4)]

Department of Developmental and Regenerative Dentistry[1)], Department of NDU Life Sciences[2)], School of Life Dentistry at Tokyo, Department of Periodontology[3)], Division of Cell Regeneration and Transplantation, Advanced Research Center[4)], School of Life Dentistry at Niigata, The Nippon Dental University

Introduction:

In a previous report, we isolated and characterized the following four types of dental stem cells (DSCs) from mature and immature human teeth: dental pulp (DPSCs); periodontal ligament (PDLSCs); apical papilla (APSCs); and dental follicle (DFSCs). These DSCs were also compared with bone marrow stem cells (BMSCs) obtained from the human iliac crest. DSCs showed greater proliferative potential than BMSCs at passage 3. We therefore hypothesized that DSCs and BMSCs exhibit different properties, such as cell senescence, when serially passaged in vitro culture.

Materials and Methods:

DSCs and BMSCs were cultured in growth medium consisting of Dulbecco's modified Eagle's medium/Ham's nutrient mixture F12 containing 15% fetal bovine serum, and maintained for 20 passages at a 1:3 split ratio. The population doubling level (PDL) and population doubling time (PDT) were calculated at each passage. Flow cytometry was used to analyze cell cycle status at passages 3 and 15. Telomerase activity, ß-galactosidase assay (ß-gal; cell senescence marker), and cell death were examined using the respective analysis kits according to each manufacturer's protocol.

Results:

DSCs showed higher PDL and shorter PDT than BMSCs. Cell cycle analysis indicated that the DSC population contained a higher percentage of cells in the G2/M phase; DFSCs (26.3%) > APSCs (26.0%) > PDLSCs(25.0%) > DPSCs (17.0%), as compared with BMSCs (15.0%) at passage 15. Moreover, telomerase activity was higher in DSCs than in BMSCs at passage 15, ranging from 2.1 fold to 7.5 fold. DSCs showed less intense ß-gal staining than BMSCs; however, apoptosis and necrosis were not detected in any stem cells at passages 3 and 15.

Discussion and Conclusion:

We found that telomerase activity appears to be closely correlated with both cell proliferation and cellular senescence. Furthermore, apoptotic or necrotic cell death did not occur in any stem cells during the prolonged study period. These results suggest that cultured stem cells undergo cellular senescence and eventually die due to the loss of their innate lifespan, which is different from other potential cell arrest events such as apoptosis and necrosis. In this study, we demonstrated that DSCs possess and maintain high proliferative ability and retarded cellular senescence through long-term culture when compared with BMSC. Further studies are required to distinguish between DSC characteristics and properties.

4. Isolation and characterization of human periodontal microvascular endothelial cells

Kosuke Maruyama, Mizuki Tsubokawa, Soh Sato
Department of Periodontology, The Nippon Dental University School of Life Dentistry at Niigata

Introduction:

Endothelial cells (ECs) participate in key aspects of vascular biology, such as maintenance of capillary permeability, initiation of coagulation, and regulation of inflammation. According to previous reports, ECs have revealed highly specific characteristics depending on the organs and tissues. However, few reports have described the characteristics of the capillaries formed by human periodontal ECs. Therefore, the aim of the present study is to examine the functional characteristics of the periodontal microvascular ECs *in vitro*.

Methods:

We isolated human periodontal ligament-endothelial cells (HPDL-ECs) and human gingival-endothelial cells (HG-ECs) by immunoprecipitation with magnetic beads conjugated to a monoclonal anti-CD31 antibody. The isolated HPDL-ECs and HG-ECs were characterized to definitively demonstrate that these cell cultures represented pure ECs. Human umbilical vein endothelial cells (HUVECs) and human dermal microvascular endothelial cells (HDMECs) were used for comparison. Isolated cells were identified by expression of specific markers assessed by immunofluorescence staining and flow cytometry. The abilities of these cells to form tubes in basement membrane matrix and to take up acetylated low-density lipoprotein (Ac-LDL) were also examined.

Results:

HPDL-ECs and HG-ECs with a characteristic cobblestone monolayer morphology were obtained, as determined by light microscopy at confluence. Furthermore, the HPDL-ECs and HG-ECs expressed the EC markers platelet endothelial cell adhesion molecule-1 (also known as CD31), von Willbrand factor, and Ulex europaeus agglutinin 1, and the cells stained strongly positive for CD31 and CD309. In addition, the HPDL-ECs and HG-ECs were observed to form capillary-like tubes, and they demonstrated uptake of Ac-LDL.

Conclusion:

The cells isolated using the methodology described in this study are vascular ECs. This report demonstrates that the isolation method used in this study makes it possible to isolate vascular ECs from periodontal tissues, a type of periodontal tissue that is in extremely limited supply. Therefore, the methods described in this study may be used to provide a new source of cells for research into the pathophysiology of periodontal disease and immune function, as well as for research on periodontal tissue regenerative therapies.