Special Lecture

Space experiment using regenerating fish scales of goldfish
- The natural face of osteoclast under microgravity -

Makoto J. Tabata

Graduate School of Tokyo Medical and Dental University, Section of Biostructural Science, Tokyo, Japan

In the space, where the gravity is extremely small, osteoporosis-like syndrome occur in astronauts. Thus, the astronauts who stayed in the international space station (ISS) for long time cannot stand alone for a while after the return to the earth. To study this phenomenon, our team focused on the cell morphology observed on the surface of regenerating fish scales under microgravity. So, we had established several special methods for the experiments in the ISS and set up Uroko-Laboratory in our university to prepare the materials. STS-132 (space shuttle Atlantis) was launched successfully on May 14, 2010 and our space experiments i.e., culture of fish scales under microgravity, were carried out. As a result, the activation of osteoclast was demonstrated by the bone absorption and the morphological changes.

Award Lecture

Chronic changes in the atrophied submandibular gland after long-term ligation of the main excretory duct in mice

Kazuyoshi Murayama¹, Miyuki Kawakami¹,², Akira Tanaka¹,²

¹Department of Oral and Maxillofacial Surgery, School of Life Dentistry at Niigata, The Nippon Dental University, Niigata, Japan
²Division of Cell Regeneration and Transplantation, Advanced Research Center, School of Life Dentistry at Niigata, The Nippon Dental University, Niigata, Japan

To examine the pathology of salivary glands that have undergone atrophy or hypofunction due to old age or disease, we investigated long-term ligation of the salivary gland in mice and examined the chronic changes in atrophied glands. The ligation periods were 1, 2 and 3 months, and resected glands (each period) were examined by histologic exam and RT-PCR. Results, disappearance of acinar cells in histologically, but acinar cell markers were still observed after 3 months of ligation. In addition, specific duct-like structures increased over time in the tissue of salivary glands that atrophied due to long-term ligation, and markers for stem cell and progenitor cells were expressed in these areas. These results suggest that the atrophied glands have residual repair capacity.
Oral Presentation

Reconstruction of the jaw bone using guided bone regeneration with selective laser melting titanium mesh membrane: A case report

Kazuya Inoue, Yoichiro Nakajima, Nahoko-kato-Kogoe, Nozomu Fukui, Hiroyuki Nakano, Takaaki Ueno

Department of Dentistry and Oral Surgery, Division of Medicine for Function and Morphology of Sensory Organs, Faculty of Medicine, Osaka Medical College

Selective laser melting titanium mesh method can be used to pre-adapt Ti mesh based on preoperative computed tomography data considering bone defect morphology and the length, diameter number of the dental implant. This method enables shorter surgery times compared with conventional titanium mesh methods, as well as regeneration of an ideal alveolar bone shape. We present a case of bone augmentation using the selective laser melting titanium method. The patient was a forty-six years old man who had left-side tooth loss from the maxillary canines to the premolars due to severe periodontitis associated with extensive resorption of the surrounding alveolar bone. Thick porous Ti mesh (0.3 mm) was prefabricated using the SLM method. We trial-fitted the SLM Ti mesh membrane to the defect with sufficient result. There was adequate bone formation observed immediately under the Ti mesh membrane sufficient bone regeneration was observed on the buccal side postoperatively on CT imaging.

The effect of ozone ointment on bone matrix production by human osteosarcoma cell line Saos-2

Pao-Li Wang¹, Kazuya Masuno¹, Nobutaka Okusa¹, Tetsunari Nishikawa¹, Yasuhiro Imaamura²

¹Department of Innovation on Dental Education and Forensic Dentistry, Osaka Dental University, ²Department of Dental Pharmacology, Matsumoto Dental University

In this study, we demonstrated that an optimal dosage of ozone ointment enhanced the proliferation, type 1 collagen production, and alkaline phosphatase (ALP) secretion of Saos-2 cells in vitro. Proliferation of Saos-2 cells was assessed by MTT and DNA synthesis assays. Type 1 collagen production and ALP secretion were evaluated using enzyme-linked immunosorbent assay and ALP assays. The cells were treated with/without ozone ointment for 24 h. Ozone ointment significantly induced the proliferation of Saos-2 cells. Ozone ointment enhanced type 1 collagen production and ALP secretion. The results indicated that ozone ointment controls the cellular metabolism of osteoblasts, resulting in the secretion of early bone-related biomarkers.

Animal model for non-surgical alveolar bone regeneration therapy by BMP-2/7 gene expression vector

Mariko Kawai¹, Kiyoshi Ohura²,²

¹Department of Pharmacology, Osaka Dental University, ²Faculty of Nursing, Taiseigakuin University

It is aimed at the development if a new animal model for non-surgical alveolar bone regeneration therapy by BMP-2/7 gene expression vector. Non-viral plasmid vector pCAGGS-BMP-2/7 or pCAGGS control was injected into palatal periodontal tissue of the first molar of the rat maxilla and immediately electroporated with 32 pulses of 50 V for 50 msec. Over the following two weeks, we analyzed histological changes and measured mineral apposition rates (MAR) by double bone staining. We observed additional alveolar bone tissues like mini-modeling. Moreover, MARs were significantly higher than those of control groups. We could constructed a new model for alveolar bone regeneration therapy.

A Clinical Study of Short Implants for the patients with insufficient bone height of the posterior mandible

Hiroyuki Nakano, Yoichiro Nakajima, Nahoko-kato-Kogoe, Nozomu Fukui, Kazuya Inoue, Takaaki Ueno

Department of Dentistry and Oral Surgery, Division of Medicine for Function and Morphology of Sensory Organs, Faculty of Medicine, Osaka Medical College

Recently, several studies reported the usefulness of short implants for the patients with insufficient bone height of the posterior. In this study, we report clinical evaluation of short implants placed in the posterior mandibular region. The six of short implants were placed in the insufficient bone height of the posterior mandibular regions. The follow-up period ranged 2 years 8 months to 5 years 3 months. The overall survival rate of the 7 implants was 100%. These results demonstrated the short implants were as useful as implants longer than 10mm.

The relationship between the oral functions and the occlusal bite force in the elderly

Naoko Imagawa¹, Nozomu Fukui¹, Yoshifumi Suwa¹, Hiroyuki Nakano¹, Nahoko Kato-Kogoe¹, Yoichiro Nakajima¹, Kazuya Inoue¹, Michi Omori¹, Kayoko Yamamoto¹, Kei Suzuki¹, Haruhiko Terai¹, Satoyo Ikehara¹, Hirohiko Kakihana¹, Akemi Nitta¹, Mizuho Nagata¹, Kuniyasu Kamiya¹, Masaaki Hoshiga¹, Jyunko Tamaki¹, Takaaki Ueno¹

¹Department of Oral and Maxillofacial Reconstructive Surgery, ²Department of Hygiene and Public Health, ³Department of Cardiology, Osaka Medical College

In the recent treatment of oral cancer the hard tissue reconstruction is required. Recently, bone graft with vascularized free flap has been developed, and this technique enables sufficient recover of oral function. Although there have been a lot of reports about bone reconstruction technique, the reports regarding oral function standardization for jaw reconstruction is limited. In this study, we report the relationship between the oral functions and the occlusal bite force in the elderly.

The relationship between the oral function and the cognition function in the elderly

Kei Suzuki¹, Haruhiko Terai¹, Hiroyuki Nakano¹, Nahoko Kato-Kogoe¹, Yoichiro Nakajima¹, Kazuya Inoue¹, Michi Omori¹, Kayoko Yamamoto¹, Naoko Imagawa¹, Satoyo Ikehara¹, Hirohobu

J.Hard Tissue Biology Vol. 27(4): 363-368, 2018
Shimizu Health 2

Tetsuya Togashi Medicine, Dentistry, and Pharmaceutical Sciences, Regenerative Medicine, Okayama University Graduate Dental Research Center, Nihon University School of Dentistry, Neuropsychiatry, Osaka Medical College

It is the most important factor of preventing the development of dementia in the aging society. Although there have been a lot of reports about the relationship between the loss of teeth and the development of dementia, the reports regarding the relationship between the oral function and the development of dementia, although there was no significant effect on the cell proliferation of bone marrow cells. In this study, we report the relationship between the oral function and cognition function in the elderly.

Continuous application of compressive force induces osteoclast differentiation

Rieko Matsuike1, Hideki Tanaka1,2, Kumiko Nakai1,2, Kotoe Mayahara1,2, Takayuki Kawato1,2, Masao Maeno1, Noriyoshi Shimizu1, Mituru Motoyoshi1,4

1Nihon University Graduate School of Dentistry, 2Department of Oral Health Sciences, 1Division of Functional Morphology Dental Research Center, 4Department of Orthodontics, 1Division of Clinical Research, Dental Research Center, Nihon University School of Dentistry, 2Nihon University School of Dentistry

We investigated the effect of compressive force (CF) on osteoclasts differentiation. RAW264.7 cells were continuously stimulated with CF, which was generated by increasing the volume of culture medium in the wells. Osteoclast-like cell formation, mRNA expression, and NFATc1 localization was evaluated by TRAP staining, real-time PCR, and immunostaining, respectively. Osteoclast-like cells, the expression of DC-c and OC-STAMP, and cells with nuclear translocation of NFATc1 were increased by CF. RANK expression was upregulated whereas LGR4 expression, which inhibits osteoclast differentiation, was downregulated by CF. Our study demonstrated that continuous application of CF induced osteoclast differentiation by enhancement of RANL-RANKL signaling via upregulation of RANK and downregulation of LGR4.

Effect of anti-Dickkopf1 (DKK-1) antibodies on osteogenic differentiation of bone marrow cells

Resmi Raju1, Miho Inoue1, Mayu Miyagi1, Kentaro Akiyama1, Masamitsu Oshima1, Masahisa Inoue1, Yoshizo Matsuka1

1Department of Stomatognathic Function and Occlusal Reconstruction, Tokushima University, 2Department of Oral Rehabilitation and Regenerative Medicine, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 3Laboratories for Structure and Function Research, Faculty of Pharmaceutical Sciences, Tokushima Bunri University

Dickkopf1 (DKK-1) produced from osteoblasts or osteocytes is an inhibitor of Wnt/β-catenin signaling pathway in bone metabolism, and it has been reported that DKK-1 inhibits the differentiation of osteoblasts. Anti-Dkk1 antibody is considered to relieve the inhibition of Wnt/β-catenin signaling pathway, and accelerate bone formation, but these mechanisms are yet to be explored. Therefore, the aim of the present study was to elucidate the effect of anti-DKK-1 antibodies in the bone marrow cells. The anti-DKK-1 antibody improved the osteogenic differentiation of the bone marrow cells, although there was no significant effect on the cell proliferation of bone marrow cells.

Effect of bone formation using TNF-α treated carbonate apatite and hydroxyapatite

Miho Inoue1, Resmi Raju1, Masamitsu Oshima1, Noboru Kajimoto2, Kanji Tsuru2, Kunio Ishikawa1, Masahisa Inoue1, Yoshizo Matsuka1

1Department of Stomatognathic Function and Occlusal Reconstruction, Tokushima University, 2Department of Dental Engineering, Bioengineering, Fukuoka Dental College, 3Department of Biomaterials, Faculty of Dental Science, Kyushu University, 4Laboratories for Structure and Function Research, Faculty of Pharmaceutical Sciences, Tokushima Bunri University

Tumor necrosis factor (TNF)-α is found to be involved in wound healing and tissue regeneration. Composition of Hydroxyapatite and carbonate apatite is similar to bone, it is widely used as bone regeneration materials in vivo, following implantation it has been found to be absorbed into the surrounding tissue. In this study, we investigated the bone regeneration potential ofapatite with and without TNF-α treatment. Following 2 weeks of implantation in rat tibia, TNF-α treated apatite showed bone regeneration and it was not observed in control group. In addition, apatite and bone marrow cell transplantation experiments to the kidney induced calcification.

Effect of parenchyma-stromal interactions on bone tissue in ameloblastoma

Ayaka Sumi1, Takaki Sakaue1, Mei Hamada2, Takabatake Kiyofumi1, Hitoshi Nagatsu3, Hidetsugu Tsujigiwa1,2

1Department of Life Science Graduate School of Science, Okayama University of Science, 2Department of Oral Pathology and Medicine Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University

In recent years, it has been reported that the microenvironment around the tumor affects tumor development. In this study, we investigated how the tumor parenchyma-stromal interaction of ameloblastoma influence on the differentiation of osteoblast and osteoclast and invasive capacity of fibroblast. Osteoblast differentiation was suppressed by ameloblastoma-derived cell lines (AM-1), however, the reactivity was different in case of squamous cell carcinoma cell lines (HSC-2). Activation of osteoclast by AM-1 was lower than by HSC-2. The invasive capacity of fibroblasts by AM-1 tended to be higher than by HSC-2. These results suggest that ameloblastoma inhibit osteoblasts and induce bone resorption by activating fibroblasts in a mechanism different from squamous cell carcinoma.

Control of tumor biological character by tumor stroma in oral squamous cell carcinoma

Kiyofumi Takabatake, Hotaka Kawai, Saori Yoshida, Hiroyuki Matsuda, Masae Fujii, Keisuke Nakano, Hitoshi Nagatsu

The 27th Annual Meeting of the Society for Hard Tissue Regenerative Biology

365
Tumor parenchyma-stromal interactions affect the properties of tumors. However, there is no report that stroma controls parenchyma. In this study, we investigated how tumor stromal influence on parenchyma.

We collected stroma derived from invasive squamous cell carcinoma (SCC-st) and verrucous carcinoma (VSCC-st). Each stroma and HSC-2 were co-cultured, and we investigated activity of tumor proliferation and invasion, and histological findings. When SCC-st and HSC-2 were co-cultured, HSC-2 showed small tumor nest formation and high invasiveness. On the other hand, when VSCC-st and HSC-2 were co-cultured, HSC-2 showed verrucous form showing a marked keratinization tendency and poor infiltration. These results suggest that tumor stroma controls tumor biological character.

**Histological analysis of the scute structure and the forming cells of black-spotted boxfish**

Momoko Sakaguchi, Makoto Sugiura-Nakazato, Eri Ushimura, Takafumi Nakano, Makoto Tabata

Black-spotted boxfish (Ostracion immaclatus) has armored skin consisting of dermal scute. We were interested in this specialized structure, so we investigated it histologically to reveal the forming cells. The scute was covered with mucosal epithelium. Almost all of the scute was non-mineralized and the “roof” and “floor” of the scute were mineralized. The joint region between scutes was non-mineralized. From the transmission electron microscopy analysis, fibroblasts were in a row of the scute outline. These cells bundled collagen fibrils to make collagen fiber of the scute.

**Morphological study of tooth germ of Tiger shark, Galeocerdo cuvier: Formation process of enamaloid and serration**

Eri Ushimura, Makoto Sugiura-Nakazato, Momoko Sakaguchi, Takafumi Nakano, Makoto Tabata

The formation process of enameloid and serration structure of the tooth germs of Tiger shark larva (total length 12-15cm) was examined histologically. The dental lamina invaginated with bending but not branching. And secondly tooth germ was appeared when the firstly one become early bell stage. Epithelium cells are simple cuboidal to columnar epithelium, but the localization of nucleus was opposite site of one of ameloblast suggesting that the function is different with that of ameloblast.

**Cell identification in enamel organ of rat incisor: reexamination of differentiation markers using frozen sections**

Takafumi Nakano, Momoko Sakaguchi, Eri Ushimura, Makoto Sugiura, Yoshiyuki Mori, Makoto Tabata

The aim of this study is to elucidate the outermost surface of human enamel by analysis for crystal morphology and composition, especially focusing on Mg. We observed human enamel (post-eruptive enamel without caries and pre-eruptive original enamel) by optical-microscope, EPMA and TEM. As results, it was revealed that a 1-2μm-thick layer containing much acidic calcium phosphate exists on the high calcified area of the outermost enamel.

**Acidic calcium phosphate crystal layer at interface observed in outermost enamel**

Yuka Asada, Toshie Chiba, Shinji Shimoda, Yasuko Momoi, Takatsugu Yamamoto

The aim of this study is to elucidate the outermost surface of human enamel by analysis for crystal morphology and composition, especially focusing on Mg. We observed human enamel (post-eruptive enamel without caries and pre-eruptive original enamel) by optical-microscope, EPMA and TEM. As results, it was revealed that a 1-2μm-thick layer containing much acidic calcium phosphate exists on the high calcified area of the outermost enamel.

**Poster Presentation**

**Differences of foreign body reactions to absorbable suture threads Vicryl® and Vicryl Rapide® - Examination using GFP bone marrow transplantation model rats**

Yoshikazu Nakayasu, Saeka Aoki, Masahito Shoumura, Naoto Osuga, Norimasa Okafuji, Hitodugu Tsujigiwa, Hitoshi Nagatsuka, Toshiyuki Kawakami

Using the GFP (Green florescence Protein) bone marrow transplanted rats, we examined the tissue reactions to two absorbable suture threads Vicryl (V) and Vicryl Rapide (VR) in subcutaneous tissues, using histopathological and immunohistochemical (IHC) techniques. Histologically, the disintegrated both suture threads were observed as a circular in void surrounded by the proliferation of macrophages and foreign
body giant cells. Regarding the V specimens, even after 3 and 6 months, some clusters considered to be residues of macrophages that grew on the suture were still remained, although almost disappeared Vr specimens. IHC showed that all proliferated macrophages and foreign body giant cells were GFP-positive.

Effects of collagen membrane containing osteogenic protein-1 on bone regeneration

Manami Ozaki1,2, Tadahiro Takayama3, Takanobu Yamamoto1, Yasumasa Ozawa2, Hideki Tanaka1,2, Kenmiku Nakai1,2, Masao Maeno2, Shuichi Sato3,4, Takayuki Kawato1,3

1Department of Oral Health Sciences, Nihon University School of Dentistry, 2Department of Periodontology, Nihon University School of Dentistry, 3Division of Functional Morphology, Dental Research Center, Nihon University School of Dentistry, 4Division of Advanced Dental Treatment, Dental Research Center, Nihon University School of Dentistry, 5Nihon University

Previously, we reported that collagen membrane containing osteogenic protein-1 (OP-1), a growth factor, promoted bone regeneration in the rat mandibular bone defect model. In the current study, we investigated the effects of collagen membrane containing OP-1 on cell viability and alkaline phosphatase (ALP) activity in MC3T3-E1 cells. According to methyl thiazolyl tetrazozium assay results, the collagen membrane containing OP-1 did not alter cell viability. ALPase staining revealed that OP-1 in collagen membrane increased ALPase activity in a dose-dependent manner. The results of this study and the previous in vivo study suggest that collagen membrane containing OP-1 may induce guided bone regeneration in clinical applications.

Effects of the geometrical structure of a honeycomb TCP structure on bone and cartilage formation and angiogenesis

Hiroyuki Matsuda1, Kiyofumi Takabatake1, Hidetsugu Tsujigawa1, Hotaka Kawai1, Saori Yoshida1, Keisuke Nakano1, Hitoshi Nagatsuka1

1Department of Oral Pathology and Medicine Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 2Department of Life Science Graduate School of Science, Okayama University of Science

We have reported on the influence of the geometry of honeycomb TCP (HTCP) on hard tissue formation, however, the detail is unknown. Recently, there are reports that angiogenesis plays an important role in hard tissue formation. In this study, we examined the influence of HTCP geometry on angiogenesis. HTCPs with pore diameters of 75, 300 and 500μm with BMP-2 were transplanted into rat femoral muscle. At 75μm, a small number of thin blood vessels penetrated into the HTCP and condrogenesis were observed. At 300, 500μm, linear and thick blood vessel invasion and vigorous osteogenesis in HTCP were observed. These results suggest that the geometric structure of HTCP controls vascular invasion and selective hard formation.

Human fresh fibrin (CGF) / BMP-2 membrane induces bone in back and head skin

Keiko Onji1, Kenji Yokozeki2, Mamata Shakra3, Bowen Zhu4, Toshiyuki Akawasa1, Takashi Saito1, Masaru Murata2

1Division of Clinical Cariology and Endodontology, 2Division of Oral Regenerative Medicine, Health Sciences University of Hokkaido, 3Department of Materials Technology, Hokkaido Research Organization

The aim of this study was to observe fresh fibrin (concentrated growth factors: CGF) as a delivery glue of BMP-2 in ectopic sites. Human venous blood was centrifuged, and the middle layer (fibrin buffy coat layer) was taken. The fibrin layer was compressed to convert to fibrin membrane (CGF). Forty microliter of BMP-2 solution (0.025g /L) was added into the CGF membrane (5x5x2mm). CGF/BMP-2 (1μg) and CGF alone were grafted into subcutaneous tissues of back skin and supra-periosteal tissues in nude mice (5 week-old), and explanted at 2 weeks. CGF/BMP-2 induced bone and marrow at 2 weeks, while CGF alone was absorbed in the skin. Fresh fibrin might be a delivery auto-glue for BMP-2 molecule in bone regeneration.

Effect of radiopacifiers on the biological properties of calcium silicate cements

I-Ting Wu1, Chung-Cheng Chen4, Shinn-Jyh Ding2

1Department of Periodontology, China Medical University Hospital, Taichung, Taiwan, 2Institute of Oral Science and 3School of Dentistry, Chung Shan Medical University, Taiwan, 4Department of Dentistry, Chung Shan Medical University Hospital, Taichung, Taiwan

To improve the radiopacity of calcium silicate cements (CSCs), the oxide dopants (Bi2O3, SrO, ZnO, ZrO2) were used as radiopacifiers. Effects of dopants on in vitro osteogenic activity of CSCs were investigated. As a result, the greater the oxide amount, the higher radiopacity was found. The effect of the dopants on radiopacity followed the order Bi2O3 > ZrO2 > SrO > ZnO, which were greater than 3 mm of Al recommended by ISO 6876 standards. The adverse effect of Bi2O3 and ZrO2 on cell functions was found, while SrO supported MG63 cell growth and differentiation.

Methylene blue-based photodynamic therapy on titanium alloy in vitro

Tsun-Chin Huang1, Kojun Setsu2, Chun-Cheng Chen3,4

1Institute of Oral Science, Chung Shan Medical University, Taichung, Taiwan, 2Faculty of Pharmaceutical Sciences, Tokushima Bunri University, 3School of Dentistry, Chung Shan Medical University, Taichung, Taiwan, 4Department of Dentistry, Chung Shan Medical University Hospital, Taichung, Taiwan

The present in vitro study evaluated antimicrobial photodynamic therapy (PDT) of methylene blue (MB) at eliminating Gram-negative (P. gingivalis) and Gram-positive (S. mutans) bacteria on sandblasted Ti alloy. The results indicated that PDT exhibited better antibacterial efficacy with increasing treatment time. As expected, the decontamination efficacy increased with increase in the MB concentration. The PDT effectively reduced the number of bacterial species from the results of morphology when compared with the control without MB, which is consistent with the alamarBlue assay. It is concluded that the use of
A basic study of laser irradiation for organ cultured articular cartilage

Yoshihiko Sugita\textsuperscript{1,2}, Ryoko Kawai\textsuperscript{1}, Waka Yoshida\textsuperscript{1,2}, Katsutoshi Kubo\textsuperscript{1,2}, Madoka Isomura\textsuperscript{1}, Nobuaki Sato\textsuperscript{1}, Hatsuhiro Maeda\textsuperscript{1,2}

\textsuperscript{1}Department of Oral Pathology, School of Dentistry, Aichi Gakuin University, \textsuperscript{2}Center of Advanced Oral Science, Aichi Gakuin University.

The purpose of this study was to evaluate the effects of the low power diode laser to mandibular condyle. The rat mandibular condyles were divided four groups, control group, F+L- group, F-L+ group, and F+L+ group. The laser irradiation was performed to cultured condyle every 24 hours for 5 days. The mandibular condyles were investigated histologically after 8 day cultures. The cultured mandibular condyle was bigger than the control group in the F+L+ group, and the cell number of the differentiation layer and the hypertrophy layer increased, and it was big in order of a control group, F+L- group, F-L+ group, F+L+ group. This study suggested that the irradiation of the low power diode laser promoted the proliferation and differentiation of chondrocyte.

Influence of chronic periodontal disease caused by \textit{Fusobacterium nucleatum} on immune response of intestinal mucosal system.

Watanabe Arata\textsuperscript{1}, Tetsuro Kono\textsuperscript{1}, Ryoki Kobayashi\textsuperscript{2}, Ryo Tamamura\textsuperscript{1}, Tomoko Ochiai\textsuperscript{1}, Hiroyuki Okada\textsuperscript{1}

\textsuperscript{1}Department of Histology, \textsuperscript{2}Departments of Infection and Immunology, Nihon University School of Dentistry at Matsudo

In late years, there are several reports that \textit{Fusobacterium nucleatum} (\textit{F.n}) which is one of the periodontal pathogenic bacteria was detected in the lesion mucous membrane part of colitis ulcerosa and large intestine cancer. We examined of influence a chronic periodontal disease by \textit{F.n} ATCC23726 strain (1×10\textsuperscript{9}cfu) were mixed with 2% carboxymethyl cellulose (CMC), and administered to BALB/c mice oral cavity of age 8-10 weeks. In this study, we observed immunohistochemically the development and growth of laryngeal cartilage using embryonic mouse. At embryonic day 13.5, SOX 9 - positive mesenchymal cells agglutinated in the larynx. At embryonic day 15.5, these cells differentiated into chondrocytes and formed early laryngeal cartilage. At embryonic day 16.5, ligament formation was observed between the cartilage, developing a joint. These results suggested that SOX9 positive cell group may develop both laryngeal cartilage and ligament by 13.5 days of embryogenesis.

Acid resistance study of the remineralization enamel using S-PRG filler-containing toothpaste

Yasuho Miate\textsuperscript{1}, Hiroyuki Mishima\textsuperscript{2}

\textsuperscript{1}Department of Histology and Developmental Biology, Tokyo Dental College, \textsuperscript{2}Department of Dental Engineering, Tsurumi University School of Dental Medicine,

This study examined the effects of toothpaste containing S-PRG filler on the remineralization of enamel and acid-resistance of remineralized enamel. Bovine enamel was immersed in solutions in the following order: lactic acid demineralizing solution, S-PRG solution, remineralizing solution, and demineralizing solution. Specimens were sectioned and observed under SEM, EPMA, CMR and TEM. As a result, the acid-resistant region under investigation displayed progressive demineralization on the surface enamel rod sheath layer, however remineralization resistance was evident in the middle layer. Remineralization was greater in S-PRG specimens where associated ions led to greater resistance during the second demineralization.