1. **A New Model to Study Neuronal Mechanisms Underlying Functional Development of Jaw and Tongue Movements**

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Transition from suckling to mastication occurs during one month after birth in rats. However, developmental changes of the neuronal mechanisms controlling feeding behavior are unclear, because the electrophysiological techniques for studying neuronal circuits have been difficult to be applied during postnatal development. To overcome the difficulties, we have adapted a decorticate, artificially-perfused *in situ* preparation which was modified from the preparation developed by Pickering and Paton (J. Neurosci. Meth., 2006).

Using Wistar rats aged between postnatal day 9 and 24, extracellular recordings from the phrenic, hypoglossal and mylohyoid nerves were performed to investigate the interplay of neural discharge patterns involved in respiration, tongue movement and jaw movement in response to various perfusion flow rates at room temperature. When the flow rate was set at approximately six times the total blood volume per minute, the phrenic nerve discharge showed an incrementing pattern and regular respiratory rhythm. The hypoglossal and mylohyoid nerve discharges were synchronized with the phrenic nerve discharges. The frequencies of neuronal discharges from all the three nerves increased with increases in the flow rates. The discharge patterns of the three nerves were similar at all ages we examined. These results indicate that the jaw opening movement occurs in the inspiratory phases in the decorticate, artificially-perfused *in situ* rat preparation. This preparation will provide a system to explore the neuronal mechanisms underlying functional development of the jaw and tongue movements.

Key words: jaw movements, respiratory, rat

2. **The Effects of Sleep-wake Cycles on Masseter Muscle Activity in Mice**

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Motor systems are generally inactive or suppressed during sleep, while jaw muscles are found to be activated during the normal sleep of mammals. However, it is not clear how masseter muscle activity changes over 24 hours in mice. In this study, we examined state-specific activity of the masseter muscle compared with the activity of the neck muscle during wakefulness, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep using recordings of electroencephalography (EEG), electro-oculography (EOG) and electromyography (EMG) of neck and masseter muscles. The total time of wakefulness during the dark period was longer than the time during the light period, whereas those of NREM sleep and REM sleep during the dark period were shorter than during the light period. The mean EMG activities of both neck and masseter muscles during wakefulness were significantly larger than...
those during NREM sleep and REM sleep irrespective of
dark or light periods. There were no significant differences
in activities of both neck and masseter muscles during
wakefulness, NREM sleep and REM sleep between dark
and light periods. Sequential plots of the EMG activity of
the neck and masseter muscles in each 10-s epoch showed
that the basal level of neck muscle activity decreased in
the transition from NREM sleep to REM sleep, whereas that
of masseter muscle activity remained unchanged. These
results suggest that neck and masseter muscle activities in
mice are likely to be differentially modulated by the neural
mechanisms controlling sleep-wake regulation but do not
appear to be affected by the circadian rhythm.

Key words: sleep-wake cycles, masseter, neck muscle,
EMG, circadian rhythm

3. Clinical Features of Pediatric Patients with
Eating and Swallowing Disorders at the Center
for Special Needs Dentistry at the University
Dental Hospital — 2012 to 2013 —

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Introduction
Bisphosphonate-related osteonecrosis of the jaw (BRONJ) develops as a side effect of long-term administra-
tion of bisphosphonates, especially intravenous

Results
1. In total, there were 861 patients. Of these, 60 were in
the pediatric age group (mean age, 4 years 1 month) at the
initial visit, with most patients being less than one year old.
2. Most patients lived near the hospital, and most cases
were referred by medical facilities outside of our clinic.
3. Diseases of the central nervous system and muscular
system accounted for 60% of cases.
4. The most common chief complaint was regarding
eating from the mouth. Feeding tubes were used in 47% of
patients.
5. A problem with swallowing was the most common
finding in the initial clinical evaluation (47%).

Conclusion
Because diseases of the central nervous system and
muscular system are often associated with eating and
swallowing disorders soon after birth, many young
patients had problems with swallowing. Many parents
seemed to be concerned about eating function, a common
chief complaint. Our center provided effective support to
pediatric patients with eating and swallowing disorders
from neighboring areas.

Key words: pediatric patients, eating and swallowing
disorders, eating and swallowing function
therapy

4. Case Study of a Team Approach to Treatment
of Bisphosphonate-related Osteonecrosis of the
Jaw

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preparation for the treatment of multiple myeloma, bone metastases of breast cancer and prostate cancer, and hypercalcemia associated with malignancy. Based on experience of cases involving medical cooperation with other departments this study reports the dental treatment for BRONJ of a breast cancer patient.

Case
The patient was a 69-year-old female initially diagnosed in September, 2013. Her chief complaint was bleeding from the gums of the left upper jaw and she had a medical history of breast cancer and bone metastasis. She was clinically diagnosed as having osteonecrosis of the left upper jaw, maxillary sinusitis of the left; BRONJ stage 3.

Examination
Maintaining a healthy oral environment and uninterrupted administration of bisphosphonate drugs in all osteoporosis patients would reduce the risk of osteonecrosis of the jaw and systemic fractures, and these measures would improve the quality of life through maintenance of ADL and mastication capability. Prescribing orthopedic surgeons and dentists should collaborate to protect bone health throughout the body as it is thought that these professions contribute most to healthy life in the elderly. We should proudly take part in the prevention and treatment of osteonecrosis of the jaw induced by bisphosphonate drugs.

Key words: bisphosphonate drugs, bisphosphonate-related osteonecrosis of the jaws (BRONJ), breast cancer

5. The Present and Future of Medical Cooperation at the Dental Clinic of Showa University Fujigaoka Hospital

The purpose of this presentation is to introduce the present situation of medical cooperation at the dental clinic of Showa University Fujigaoka Hospital and future challenges.

In April 2008, when Showa University Oral Health Care Center was founded, inpatient oral care started at the Dental Clinic of Showa University Fujigaoka Hospital. At the beginning, oral examination, oral health care and oral hygiene instruction were given.

In September 2008, oral care using clinical path started for the cardiovascular surgery patients. This system has influenced other professionals such as medical doctors, nurses, pharmacists and nutritionists. As a result, they recognize that the role of dentistry is not limited to the treatment of dental caries or periodontal diseases because they came to know that oral health care is essential, especially in hospital dentistry.

In October 2010, dental hygienists and dentists started to take part in the nutrition support team (NST). New participants contribute to the inpatients with malnutrition by cleaning the mouth, checking the mastication and swallowing and so on. Dental hygienists and dentists can define the problem even when other professions and patients themselves do not notice. In this way, the participation of dental hygienists/dentists in the NST seems valuable as a team approach to health care at Showa University Fujigaoka Hospital.

In April 2012, the oral health care at the perioperative period started. Medical cooperation is required to keep good oral bacterial flora for the patients with esophageal cancer, gastric cancer, head and neck cancer, childhood cancer and so on. The oral health care at the perioperative period seems important for almost all operations under general anesthesia. From April 2012 to March 2013, the number of new patients at the dental clinic of Showa University Fujigaoka Hospital was 1,436, and 298 (21%) were referred for oral health care. These included patients with diabetes, patients treated with bisphosphonate (BP) and patients at the perioperative period. Moreover, there are many inpatients that need oral health care besides these patients such as those with pneumonia,
cerebrovascular disease and so on. In addition, medical cooperation with private practices became important after the patients were discharged from the hospital.

As mentioned above, medical cooperation has brought beneficial results to Fujigaoka Hospital. However, the challenge has been a lack of human resources. If we aim to achieve a higher level, we need sufficient human resources, as well as an integrating section and a facility to efficiently control the medical cooperation.

Key words: Fujigaoka Hospital, role of the dental clinic, medical cooperation

6. Data on Endodontic Treatment Performed between April 2012 and March 2013 in the Department of Endodontics at Showa University Dental Hospital

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This study collected data on endodontic treatment (total number of treatments, number of root canal-filled teeth, prevalence of pulpitis, and prevalence of periapical periodontitis) performed between April 2012 and March 2013 on outpatients in the Department of Endodontics at Showa University Dental Hospital for comparison with data from a previous study on endodontic treatment performed between April 2009 and March 2010.

The following results were obtained:

(1) In total, 863 outpatients received endodontic treatment.

(2) Of the total of 953 root canal-filled teeth, 372 had pulpitis and 581 had periapical periodontitis. Of the root canal-filled teeth with pulpitis, 69 were lower molars, 83 were maxillary molars, 46 were lower premolars, 53 were maxillary premolars, 66 were lower anteriors and 55 were maxillary anteriors. Of the root canal-filled teeth with periapical periodontitis, 154 were lower molars, 112 were maxillary molars, 68 were lower premolars, 89 were maxillary premolars, 49 were lower anteriors and 109 were maxillary anteriors.

(3) The mean number of treatments was 2.8 for root canal-filled teeth with pulpitis and 3.5 for teeth with periapical periodontitis.

(4) Comparison of the present data with data from the previous study revealed that the total number of outpatients increased (419 to 863) and the total number of root canal-filled teeth increased (348 to 953) between the two time periods. In contrast, the mean number of treatments decreased for both teeth with pulpitis (3.9 to 2.8) and teeth with periapical periodontitis (5.7 to 3.5).

Key words: pulpitis, periapical periodontitis, root canal-filled teeth

7. Volunteer Activities by Students of Showa University after the Great East Japan Earthquake

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First-year Student Undergraduate Student, Showa University School of Dentistry
Ryouta Kawai

The earthquake triggered the response of students to start volunteer activities. Immediately after it occurred, students participated in relief efforts as part of a medical team from Showa University. Students who took part in logistics support and volunteer activities did so out of a desire to do something to help in the affected areas.

On the first visit, students went to Yamada-cho and a further three hours one way to Morioka-shi. Activities involved sorting of relief supplies and dismantling the rubble. On the second visit, student went to Yamada-cho from Miyako-shi and took part in debris removal and making sandbags. On the third and fourth visit, students went to Kamaishi-shi, in Otsuchi-cho, and took part in weeding of graves, ground leveling, and debris/sand removal. On the fifth visit, students performed health consultations at the temporary housing in Miyako-shi. We visited the community center in Orikasa Yamada-cho. On the sixth visit, we conducted cleaning such as weeding and health consultation, while helping as volunteers.
Participants were increased using a portal site to collectively contact the students via computers and mobile phones.

We strongly hope for a quick recovery from the disaster.

Key words: Great East Japan Earthquake, volunteer activities, Iwate prefecture

8. Assessment of Swallowing Function using a Non-contact Measurement System to Determine Swallowing Time

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Purpose
Assessment of the swallowing function is currently conducted in various medical facilities, and many are accompanied by invasive procedures. In many cases, it is difficult to quantitatively analyze the decline in swallowing function using qualitative criteria alone. Therefore, the purpose of this study was to determine the reflection and reaction times using a fiber grating vision (FG) sensor. We conducted a study to quantitatively analyze the decline in feeding function with increase in age.

Materials and Methods
This study comprised 26 healthy adult men (average age 57.50 ± 4.52 years).

The volunteers were asked to swallow 3 mL of water, and we measured the reflection and reaction times using a FG sensor. Two additional screening tests were also conducted; i.e., MWST and RSST. The data obtained from the above tests were then used for statistical analysis.

Results
Average values of the above tests conducted were as follows: reaction time: 0.49 ± 0.55 s; reflection time: 1.02 ± 0.51 s; RSST: 4.77 ± 0.43 times; MWST: 4.27 ± 0.16 times.

A correlation was observed between the volunteer’s age and the other values.

Correlations were also observed between the reaction time and MWST value as well as the reflection time and MWST value. However, no correlation was observed with the RSST value.

Conclusion
The findings of this study suggest that swallowing function decreases with age. However, a few postural problems were detected. In spite of this limitation, we conclude that this system can potentially be used as a screening test.

Key words: swallowing, dysphagia, fiber grating vision sensor, aging, screening test

9. Study of Oral Pathogens in Children with Respiratory Disease

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Purpose
Respiratory diseases occur frequently in children. The common infection routes include the nasal cavity, mouth and trachea. The present study aims to determine the different oral pathogens present in the mouth of children with respiratory disease. The relationship between Hellman’s dental age and oral pathogens was studied along with a comparison between pathogens found in the mouth and those in the nasopharynx.

Materials and Methods
This study included 32 children with respiratory diseases, who were classified according to Hellman’s dental age, and 32 healthy children who served as the control group. Mouth samples were collected by swabbing the deepest part of the palate twice, once upon admission and then during discharge. The culture results of the nasopharyngeal swabs taken at the same time were collected from medical records and compared with those of the palate.

Results
Cultures of both the palate and nasopharynx revealed six bacterial species: α-Streptococcus sp., Corynebacterium sp., Haemophilus-influenzae, Haemophilus-parainfluenzae, MRSA and Neisseria sp.

The detection rates of the Neisseria sp. in the palate and MRSA in the nasopharynx were significantly different. A significant difference was observed in the IA stage of the α-Streptococcus sp. after comparing the changes in the
palate and nasopharynx. The detection rate in the palate increased, whereas that in the nasopharynx decreased.

**Conclusion**

The detection rate of certain bacteria in the palate increased with an increase in Hellman’s dental age. These findings suggest the number of oral pathogens increased, even during the IA stage before tooth eruption, indicating the need for oral care.

Key words: respiratory diseases, oral pathogens, nasopharynx, dental age, children

**10. Evaluation of Chitosan-Citrate Solution as a Root Canal Irrigant**

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**Introduction**

The purpose of this study was to determine whether chitosan-citrate solution shows antibacterial properties against *Enterococcus faecalis* and removes the smear layer when used as a root canal irrigant.

**Methods**

*Enterococcus faecalis* (4532D) was incubated in brain-heart infusion broth. Chitosan-citrate solution was then added to the bacterial inoculum for 5, 15, 30 and 60 min. Survival rates of *E. faecalis* were statistically analyzed using one-way repeated measures ANOVA and Tukey test. Extracted teeth were used for this experiment. The middle third of the root was cut longitudinally, and all specimens were immersed in chitosan-citrate solution until an antibacterial effect was obtained. All specimens were examined under scanning electron microscopy. The condition of remaining smear layer was statistically analyzed using Kruskal-Wallis and Steel-Dwass multiple-comparison tests.

**Results**

The antibacterial effect of chitosan-citrate solution was achieved at 5 min. Furthermore chitosan-citrate solution was indicated in a significantly greater reduction of the survival rate than 10% citric acid after 5 min of application (*p*<0.01). The smear layer was also removed after immersion in chitosan-citrate solution for 5 min. SEM analysis revealed that chitosan-citrate solution removed significantly more of the smear layer than 10% citric acid (*p*<0.05).

**Conclusions**

Chitosan-citrate solution showed antibacterial activity and enabled removal of smear layer. As this ability depended on chitosan, it is considered that the action was enhanced by chitosan. Chitosan-citrate solution has been indicated as a possible root canal irrigant.

Key words: chitosan oligosaccharide, citric acid, *Enterococcus faecalis*, smear layer

**11. LIPUS and Nanohydroxyapatite Enhances Osteogenesis of Buccal-fat-pad-derived Stem Cells**

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**Objectives**

Osteogenic induction of adipose-derived stem cells (ADSCs) has been regarded as an important step in bone tissue engineering. In the present study, we focused on the buccal-fat-pad as a source of adipose tissue. Since the buccal-fat-pad is encapsulated by adipose tissue and is often co-extripated during an oral operation, it is easily available as a source of auto-transplantation. On the other hand, it has been well demonstrated that low intensity pulsed ultrasound (LIPUS) is effective in the treatment of fractures. In addition, nanohydroxyapatite is known as a bone substitute material. Thus, in the present study, we investigated the synergistic effects of LIPUS and nanohydroxyapatite in osteogenesis *in vitro*.

**Methods**

ADSCs were cultured in an osteogenic differentiation medium in the absence or presence of nanohydroxyapatite as a scaffold. The cells were subjected to LIPUS at the intensity of 100 mW/cm² for 10 min everyday. On days 7, 14 and 21, expression of osteogenic
genes and histomorphometric were analyzed.

**Results**

Stimulation of LIPUS significantly increased the expression of osteogenic gene of the stem cells and the mineralization of extra-cellular matrix. With application of nanohydroxyapatite as a scaffold, these observations were enhanced concordantly.

**Conclusions**

In the present study, it is demonstrated that synergetic effects of LIPUS and nanohydroxyapatite are capable of driving buccal-fat-pad stem cells into differentiation of osteoblasts. These results potentially suggest a novel therapy for bone regeneration by transplantation of ADSCs.

Key words: LIPUS, nanohydroxyapatite, buccal-fat-pad-derived stem cells

12. Aberrant Expression of EZH2 Associated with Pathological Findings and p53 Alteration

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EZH2 and BMI1 are members of the polycomb group of proteins, which function as transcriptional repressors through chromatin modification. EZH2 composes polycomb repressive complex (PRC) 2 with other members, while BMI1 is a component of PRC1. Previous studies have shown that EZH2 is highly expressed in various cancers.

We immunohistochemically investigated the expression of EZH2 and BMI1 in 99 surgically resected oral squamous cell carcinoma (OSCC) and 34 epithelial dysplasia. We also analyzed associations between aberrant expression of EZH2 and BMI1, and both clinicopathological findings and outcomes. p53 expression was examined and analyzed in relation to EZH2 and BMI1 expression.

The EZH2 protein and BMI1 protein were upregulated in OSCC tissue in comparison with epithelial dysplasia and normal epithelium. Aberrant EZH2 and BMI1 protein expression was observed in 32 and 59, respectively, of the 99 OSCC. Aberrant EZH2 and BMI1 expression was significantly associated with invasive modes, but not associated with lymph node metastasis and survival of the patients. Expression of EZH2 has been reported to be regulated by the p53-RB-E2F pathway and a recent study showed a correlation between p53 mutation and EZH2 expression in breast cancer tissue. Therefore, we examined the relationship between p53 and EZH2. Aberrant EZH2 expression was associated with p53 alteration in OSCC tissue. Expression of EZH2 mRNA in SAS/neo cells that have a wtp53 was significantly lower than SAS/mp53 cells that have an mp53 gene.

p53 alteration may be involved in dysregulated EZH2 expression, and expression of EZH2 may play a role in OSCC carcinogenesis.

Key words: oral squamous cell carcinoma, EZH2, BMI1, p53

13. Contrivance of the Individual Tray for Preventing Fracture of Isolated Abutment Tooth in Working Cast when Separating the Impression

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**Introduction**

When the working casts for removable partial denture that have isolated abutment teeth with advanced bone resorption separate from impressions, breakage of casts may occur. However, if the tray was divided in advance, fracture of isolated abutment tooth on the working cast could be prevented when separating the impression.

This paper shows the method to set a dividing line to an individual tray beforehand.

**Procedures**

1. Mark the location of stoppers on the study cast with black ink.
2. Place paraffin wax on the study cast for the spacer.
3. Perforate the wax with a sharp hand instrument at the location of the stopper.
4. Place the ready casting wax for the study model at the dividing line.
5. Mix the individual tray material and put a small amount of the resin at the locations of the stoppers.
6. Place the residual resin on the study cast and fabricate the handle. Note that the ready casting wax can be seen through the tray.
7. Remove the set tray from the cast.
8. After the final impression, form the boxing of impression
and pour the dental stone.

9. Cut the individual tray with a round bar at the dividing line. The dividing line can be seen through the tray.

10. Separate the impression from the cast.

**Discussion**

Because the dividing line, as the mark of the cutting of the individual tray, was provided to the individual tray, removal of the master cast becomes possible without fracture of the isolated tooth.

Key words: isolated abutment tooth, individual tray, working cast, impression

**14. Evaluation of the Effect of Surface Modifications for the Bonding Strength of CAD/CAM Ceramics**

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**Objectives**

The aim of this study was to evaluate the bonding strength of zirconia ceramics (PNZR) to lithium disilicate ceramics (LDC) using resin cement with surface modification and connective glass ceramic.

**Methods**

1. We evaluated the effect of several surface modifications for PNZR and LDC plate by shear bond test with titanium cylindrical specimen bonded with resin cement.

2. PNZR plate and LDC cylindrical specimens were prepared. The specimens were grouped into two groups. (CE): PNZR and LDC was also applied surface modification. The cylindrical LDC was bonded to PNZR by a resin cement. (CO): The cylindrical LDC was bonded to PNZR by crystallization in the furnace with connective glass ceramic.

Specimens were stored in deionized water for 24 hours and suffered thermal cycling. Shear bond tests were performed by a universal testing machine at 1 mm/min. Shear bond strengths (SBSs) were analyzed statistically using Tukey’s multiple comparison test (p<0.05).

**Results**

SBSs of the CO significantly decreased after thermal cycling but those of the CE did not decrease after thermal cycling. Since the backscattered electron images of the fracture surface and quantitative analysis, the bonding durability of CE was superior to CO.

**Conclusions**

The surface modifications for CAD/CAM ceramic enhanced bonding strength and durability of the resin cement. It was suggested the methods of fabrication of the ceramic bonded zirconia bridge with resin cement has many clinical advantages for repair of veneering ceramics. Further study is needed to research more clinical models.

Key words: zirconia, lithium disilicate ceramics, surface modification

**15. Nanomechanical Properties and Molecular Structures of In vitro Mineralized Tissues on Anodically Oxidized Titanium Surfaces**

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The anatase phase TiO2 in amorphous matrix accompanied with micro-submicron surface texture on anodically oxidized titanium surfaces results in enhanced expression of osteogenic genes and increased mineralization of adherent primary osteoblasts on its surface. These effects are due to the superhydrophilicity of COO- and OH- oxidation products of reactive oxygen species (ROS) on these surfaces. Preparation in a solution containing chloride generates surface hypochloride, whereas preparation in Na2HPO4 solution generates surface hydroxyl radicals. The surface hypochloride destroyed the cell wall membrane of adherent microorganisms, thus demonstrating strong antimicrobial activity. The biomechanical stability of mineralized tissues at the interface between the implant surface and bone tissue is of critical importance. We evaluated the nanomechanical properties and molecular structures of the in vitro mineralized tissues developing around anodically oxidized titanium surfaces with and without preparation in chloride solution. Anodically oxidized titanium surfaces showed superior osteogenic gene expressions than those of thermally oxidized and bare titanium surfaces. Preparation of anodically oxidized titanium in chloride enhanced the production of mineralized tissue around it. However, the
mineralized tissue around anodically oxidized titanium prepared without chloride had increased mineral : matrix and cross-linking ratios, resulting in higher hardness and inelasticity. The type and amount of ROS and the expression of bone matrix proteins on anodically oxidized titanium surfaces influenced the amount of mineralized tissue after cell culture. The ROS generated from hydroxyl radicals on anodically oxidized titanium without a chloride solution was responsible for the higher hardness and inelastic properties of the resultant mineralized tissue.

Key words: titanium, osteoblast, reactive oxygen species, nanoindentation, raman spectroscopy

16. Enhanced In vitro Biological Activities Generated by the Surface Characteristics of Anodically Oxidized Titanium

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Anodically oxidized titanium surfaces, prepared by spark discharge, are commonly noted to have micro-submicron topography and hydrophilic functional groups that enhance osteogenic gene expression and the mineralization of adherent osteoblasts on the surface. If these anodically oxidized surfaces are to be used in future bone therapeutics, it will be important to assess the influence of reactive oxygen species (ROS) that are spontaneously generated on the anodically oxidized titanium surface, besides the effects of the topographic profile and hydrophilicity. This study examined the in vitro biological changes induced by various surface characteristics of anodically oxidized titanium with or without release of ROS onto the surface. Anodically oxidized titanium enhanced the expression of genes associated with differentiating osteoblasts and increased the degree of matrix mineralization by these cells in vitro. The phenotypes of cells on the anodically oxidized titanium were the same with or without ROS release; however, the nanomechanical properties of this in vitro mineralized tissue were significantly enhanced on ROS-released surfaces. In addition, the mineralized tissue produced in the presence of bone morphogenetic protein 2 alone had significantly weaker integrity, despite there being a higher overall volume of mineralized tissue and a concomitant increase in gene expression levels. Therefore, we show that enhanced osteogenic gene expression is not a sufficient indicator of bone formation, since mineralized tissues did not achieve proper mechanical properties without either being cultured on a ROS-generating surface, or supplementation with lysyl oxidase-like 2. This study thus reveals the importance of nanomechanical testing to fully evaluate the biological integrity of tissue formed by cells on modified titanium implants.

Key words: titanium, reactive oxygen species, osteoblast, gene expression, bone morphogenetic protein 2, nanoindentation

17. Immunohistochemical Localization of PACAP Receptor in Developing Mouse Salivary Glands

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The control of saliva secretion is mainly under autonomic nervous control. Pituitary adenylate cyclase-activating polypeptide (PACAP) is now recognized as the multifunctional neuropeptide in various organs. Our previous study indicated the localization of PAC1R in adult mouse major salivary glands. In this study, we examined the ontogenic expression of PAC1R in mouse salivary glands. Salivary glands at 0, 3, 5, 7, 14 and 21 days postnatal male C57BL/6 mice were used for the immunohistochemical detection of PAC1R. In the parotid gland, the acinus was not clearly formed and PAC1R was not detected until 14 days after birth. At 14 days, PAC1R was detected in the striated duct. In the sublingual gland, the acinus and duct were formed at 0 day after birth. PAC1R was constantly expressed in the striated duct from the 0 day postnatal stage. In the case of the submandibular gland, the granular duct was not clearly identified and PAC1R was localized in the striated duct and first detected in the striated duct until 21 days after birth. After 21 days, PAC1R was clearly detected at pillar cells in the granular duct. Some of cells in the duct showed intense immunoreactions of PAC1R. These results indicated that PAC1R was expressed in the striated duct of parotid and sublingual glands and that the localization of PAC1R was changed with the formation of granular duct. A precise study may be necessary to clarify
the function of pillar cells by examining the shift of PAC1R-positive cells in the submandibular gland.

Key words: salivary glands, PAC1R, mouse

18. Resorption Analysis of Deproteinized Cancellous Bone
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Deproteinized cancellous bovine bone (DPBB) has been reported as non-absorbed bone filling material and used for the induction of new bone formation in the osseous defect and oral surgery. Hydroxyapatite (HA) in bone is calcium-deficient and carbonate-rich apatite. Our previous study indicated that chemical equivalent HA was not resorbed by osteoclasts and that octacalcium-derived apatite was calcium-deficient and carbonate-rich apatite and resorbed by osteoclasts. In this study, we first analyzed the chemical composition of DPBB and then compared the tissue reactions of DPBB and ODA after the implantation into mouse bone marrow. X-ray diffraction analysis (XDA) and Fourier transform infrared spectroscopy (FTIS) were performed to analyse the chemical composition of DPBB. DPBB and ODA were implanted into the bone marrow of mouse tibiae and examined using micro-CT, histological and ultrastructural methods. XDA and FTIS showed that DPBB was carbonate-rich apatite. Micro-CT analysis indicated that massive newly formed bone was detected on DPBB and ODA at two weeks after the implantation. The volume of the bone was gradually decreased to 12 weeks after the implantation. Histological examination indicated many columnar osteoblasts were aligned along the newly formed bone surface at 2 weeks after the implantation. DPBB and ODA were removed. GFP-positive cells and -negative cells were separated using a flow cytometer and GFP-positive cells expressed high levels of Sox10. To identify the marker genes of NCDCs in adult tissues, we performed DNA microarray analysis of GFP-positive cells and -negative cells isolated from P0 mice, and then selected some genes that had different gene expression patterns between them. Real-time PCR analysis also revealed that GFP-positive cells expressed Gp130 and Ednrb at higher levels, but Pdgfra and Pdgfrb at lower levels compared to GFP-negative cells. These results indicated that NCDCs have a characteristic gene expression profile in cell surface molecules. The cell sorting with combination of these specific cell surface proteins might be a useful strategy for isolation of high-purity NCDCs.

Key words: deproteinized cancellous bovine bone, osteoclast, calcium-deficient and carbonate-rich apatite, mouse

19. Characteristic Gene Expression Profile of Neural Crest-derived Cells in Adult Mouse Tissues
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Neural crest cells (NCCs) are induced at the dorsolateral edge of the neural plate during early embryogenesis. They migrate in the embryo to reach target sites where they differentiate into a variety of derivatives as neural crest-derived cells (NCDCs). Some NCDCs are maintained in an undifferentiated state throughout the life of the animal. Hence, NCDCs are considered to be useful cell sources for regenerative medicine. To analyze the characteristic of NCDCs within adult tissues, we utilized double transgenic mice, P0-Cre/CAG-CAT-EGFP transgenic mice (P0 mice), in which NCDCs expressed EGFP and we can recognize NCDCs as GFP positive cells. GFP-positive cells were found in the submandibular gland of P0 mice. We surgically removed the submandibular gland from the adult mice, and digested the samples into a single cell suspension. GFP-positive cells and -negative cells were separated using a flow cytometer and GFP-positive cells expressed high levels of Sox10. To identify the marker genes of NCDCs in adult tissues, we performed DNA microarray analysis of GFP-positive cells and -negative cells isolated from P0 mice, and then selected some genes that had different gene expression patterns between them. Real-time PCR analysis also revealed that GFP-positive cells expressed Gp130 and Ednrb at higher levels, but Pdgfra and Pdgfrb at lower levels compared to GFP-negative cells. These results indicated that NCDCs have a characteristic gene expression profile in cell surface molecules. The cell sorting with combination of these specific cell surface proteins might be a useful strategy for isolation of high-purity NCDCs.

Key words: neural crest-derived cell, submandibular gland, DNA microarray, cell surface marker genes
20. Nanoindentation Tests to Assess Polymerization of Resin-based Luting Cement

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The optimal polymerization of resin-based luting cements plays a critical role in the long-term clinical success of dental prosthesis and indirect restorations. With increasing demand for aesthetic properties and adequate marginal seal, the use of resin-based luting cement has become dominant. Despite their superior aesthetic and mechanical properties, conventional resin-based cements require pre-application of a total etch system or acid-functionalized monomer such as 10-methacryloxydecyl dihydrogen phosphate (MDP), because the resin substrate lacks chemical adhesive properties. Acidic monomers may compromise the curing mechanism of resin-based cements and hence optimal polymerization of the cements with MDP application is uncertain. Inferior polymerization of resin-based materials may negatively affect their mechanical properties, causing significant deterioration of clinical performance. This study investigated the degree of polymerization of a dimethacrylate resin-based luting cement with and without pre-application of the acidic monomer MDP. Conformational changes in the luting cement were measured using conventional infrared spectrophotometry and quasi-static and dynamic nanoindentation tests. The results of infrared spectrophotometry and nanoindentation testing were proportional in samples without acidic monomer pretreatment. When considerable residual monomer persists within the final products, the superior mechanical properties of the resin-based luting cements might be impaired. As a viscoelastic material, resin-based luting cements demonstrated time-dependent behaviors such as large creep rate during constant load and tangent of phase lag with dynamic indentation. The time-dependent behaviors found in the nanoindentation tests likely resulted from the residual monomer, so that the degree of polymerization of the resin-based materials could be assessed using this technique in conjunction with infrared spectrophotometry.

Key words: dimethacrylate, 10-methacryloxydecyl dihydrogen phosphate, residual monomer, FTIR, mechanical testing, time-dependent behavior

21. Effect of the Excimer UV Irradiation to the Titanium Surface

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Background

Recently there have been a number of reports of surface treatments with ultraviolet lamp irradiation to promote cellular attachment and proliferation by their radiation effect to titanium. The purpose of this study was to compare the ability of excimer UV lamp (EX-UV) and the low-pressure Hg-UV lamp (LP-UV) to the titanium surfaces.

Methods

For the measuring contact angle on titanium plates, Ti plates were prepared from JIS grade 2 Ti block. Ti plates were wet-abraded with SiC abrasive papers and cleaned in acetone, ethanol and sterile water using an ultrasonic washing machine. After radiation of UV, EX-UV and LP-UV, the angle was analyzed at various radiation times, ranging from 0 to 120 min. Furthermore, in vitro mRNA expression levels of oxidative stress genes in osteoblastic cell cultured on titanium with EX-UV irradiation were analyzed via real-time PCR as compared to cells cultured on plates with LP-UV.

Results

Only the peak at 175 nm was observed on EX-UV. LP-UV showed a main peak at 250 nm and several small peaks with different wavelengths. The contact angle of titanium plates with EX-UV after 1 min radiation was significantly lower than LP-UV. The expression of oxidative stress genes was not significantly different between EX-UV and LP-UV three and five days later.

Conclusions

The excimer UV lamp enabled a shorter irradiation time to obtain the same effect of LP-UV. Further analyses of surface chemical change before and after the UV radiation, and cellular responses are necessary to identify the effect of UV irradiation on osseointegration of titanium implants.

Key words: titanium, ultraviolet lamp, osseointegration, contact-osteogenesis
22. Optical Coherence Tomography for Occlusal Caries

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Introduction

Optical Coherence Tomography (OCT) uses near-infrared laser to acquire images. It provides tomographic images with the highest spatial resolution available through current medical imaging modalities. A recently developed small OCT probe enables its use in the oral cavity. In this in vitro experiment, we studied the characteristics of OCT images of occlusal caries with reference to the possible application of OCT to caries diagnosis.

Methods

We used seven extracted human molar teeth, five with occlusal pit or fissure-type caries lesions, and two with no caries lesions. The imaging device was a prototype OCT device (The Yoshida Dental MFG). Its central wavelength was 1310 nm, and the range of swept wavelength was 100 nm. OCT image obtained perpendicular to an occlusal surface of each tooth. Estimated resolution to the depth direction was 13 μm in air and 8 μm in enamel. Acquired OCT images were transferred to ImageJ software (National Institutes of Health) and two radiologists evaluated the characteristics of the OCT images in the caries regions.

Results and Conclusions

OCT images of non-caries tooth surfaces were characterized by strongly reflected smooth white lines. In the case of fissure-type caries reflection of the tooth surface at the caries region appeared as irregular lines. The highly reflective areas under the surfaces of caries extended into deeper regions. In the case of subsurface demineralization, low reflectivity black bands could be seen underneath the tooth surfaces. These results suggest that OCT can be useful as a diagnostic method for occlusal caries.

Key words: optical coherence, tomography, SS-OCT, occlusal caries

23. Analysis of Implant Cases at the Implant Center of Showa University Dental Hospital between April 2012 and September 2013

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Purpose

The purpose of this study was to determine the information regarding trends in new patients and dental implant placement performed at the Implant Center of Showa University Dental Hospital in 2012.

Patients

Between April 2012 and March 2013, there were 359 new patients consisting of 135 men and 224 women at the Implant Center of Showa University Dental Hospital. Between April 2012 and March 2013, there were 266 cases of dental implant placement surgeries, 84 on men and 182 on women.

Results

Of the 359 new patients, 189 patients were introduced by other departments of the Dental Hospital, 102 patients were introduced from outside the hospital and 68 patients were not introduced. In addition, 81% of new patients visited for consultation regarding implant treatment and 19% visited
because of some other kind of trouble.

A total of 532 dental implants, including 301 Bränemark system implants, 139 Straumann system implants, and 68 BIOMET 3i system implants, were placed. The cases of dental implants in the posterior region of the mandible were most frequent, accounting for 48% of the total.

**Conclusion**

Between April 2012 and March 2013, there were 266 cases of dental implant placement surgery performed at the Implant Center of Showa University Dental Hospital. A total of 532 dental implants were placed, an increase of 95 from the previous year. Dental implant placement in the posterior region of the mandible was most frequent.

Keywords: statistical analysis, dental implants, new patients, reason for visit