TRPV1 and ANO1 involved in the acute pain during orthodontic treatment

○Rosa Baldeon-Gutierrez[1], Naoto Ohkura[1], Nagako Yoshiba[2], Shintaro Takahara[1], Susan Gomez-Kasimoto[1], Naoki Edanami[1], Takako Ida[1], Shoji Takenaka[1], Yuichiro Noiri[1]

[1]Division of Cariology, Operative Dentistry and Endodontics, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.
[2]Division of Oral Science for Health Promotion, Department of Oral Health and Welfare, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.

I. Objective

We aimed to elucidate the localization of prostaglandin I_2 synthase (PGIS), transient receptor potential vanilloid (TRPV)1, and anoctamin (ANO)1 during the orthodontic procedure in the periodontal, pulp tissue, and nerve fibers.

II. Materials & Methods

A Ni-Ti closed coil spring was attached between the maxillary incisors and the first molar on the right side of the maxilla, and a force of about 150 gf was applied for 24 hours, while the contralateral homonymous tooth was observed as a control. After 24 hours, a 3-dimensional stress analysis was performed to predict tooth movement in the actual rat mouth. Specific antibodies for PGIS, TRPV1 and ANO1 were used to analyze their localization in periodontal ligament tissue, and pulp tissue after normal and orthodontic force application by immunohistochemistry and immunofluorescence.

III. Results

Finite element analysis (3D stress analysis) predicted that the distal root of rat maxillary molars would move with a proximal tilt centered on the apical one-third of the root and, the stress concentration would be concentrated on the centrifugal side of the buccal distal root of the maxillary first molar. This prediction was consistent with the histological findings since some periodontal ligament cells in the apex of the buccal distal root of the first molar showed strong PGIS positive reaction. In addition, PGIS immunopositive reactions were also observed in odontoblasts and peripheral nerves in both the control and orthodontic model rats. Double immunofluorescence localized TRPV1 and ANO1 in odontoblast cells, nerve fibers and periodontal ligament in both and orthodontic model rats, with no significant differences observed in both.

IV. Conclusion

PGIS activity was observed at the site of stress concentration site, resulting in increased PGI_2 production, which may be responsible for the pain. TRPV1 and ANO1 were also immunolabeled in the stress zone, which may be related to pain sensation.

Contact information of the authors

Name: Rosa Baldeon Gutierrez, Affiliation: Division of Cariology, Operative Dentistry and Endodontics, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan, e-mail address: rosabaldeong@dent.niigata-u.ac.jp

Phase 1 and 2 clinical trials of CPNE7-derived peptide (Selcopintide) for dentin hypersensitivity: safety, tolerability, efficacy and pharmacokinetics studies

○**Myung Jin Lee[1], Won Jun Shon[2]**

[1]Department of Conservative Dentistry, School of Dentistry and Institute of Oral Bioscience, Jeonbuk National University, Jeonju, Republic of Korea

[2]Department of Conservative Dentistry, Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Republic of Korea

I. Objective

Copine7 (CPNE7) is known to induce odontoblast differentiation and tertiary dentin formation, and thus has the potential to be used as a therapeutic agent for dentin hypersensitivity. CPNE7-derived functional peptide (Selcopintide), which replicates the function of CPNE7 has been developed for better stability and manipulation. Randomized, double-blind and dose escalation phase 1 and 2 clinical trials were carried out to assess the safety, tolerability, efficacy and pharmacokinetics of Selcopintide solutions in dentin hypersensitivity patients.

II. Materials & Methods

In Part A, 24 subjects were topically applied with Selcopintide at their exposed dentins once, and in Part B, 16 subjects were applied with Selcopintide multiple times. Safety was evaluated with physical and oral examinations, vital sign and ECG check, clinical laboratory test and adverse event collection. Efficacy was assessed by evaluating change from baseline in ice-cold water measured by visual analogue scale (VAS), in evaporative air sensitivity as measured by Schiff sensitivity score, and in tactile threshold. The subjects were also hospitalized for pharmacokinetic blood analysis.

III. Results

All adverse events were transient and recovered, without any alterations or withdrawals of the drug, and no serious adverse events occurred. The efficacy results showed that although Selcopintide in Part A reduced VAS score, it did not show superiority compared to the placebo group. However, when the drug was applied multiple times in Part B, VAS showed greater reduction in the study group compared to the placebo group. No Selcopintide concentrations were detected at any point of pharmacokinetic blood samplings of all subjects.

IV. Conclusion

It can be concluded that Selcopintide is safe and well tolerated when administered to exposed dentin with the dose up to 10ug/tooth. When applied multiple times, it shows tendency to reduce dentin hypersensitivity. However, to verify its efficacy larger scale clinical trials are needed with greater number of patients. A Phase 2a and 2b clinical trial involving 171 subjects is currently underway at a multicenter base, and the efficacy of Selcopintide is anticipated to be verified through the analysis of its results.

Contact information of the authors

Name: Myung Jin Lee, Affiliation: Department of Conservative Dentistry, School of Dentistry and Institute of Oral Bioscience, Jeonbuk National University, Jeonju, Republic of Korea, e-mail address: eem4323@jbnu.ac.kr

Name: Won Jun Shon, Affiliation: Department of Conservative Dentistry, Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Republic of Korea, e-mail address: endoson@snu.ac.kr

Retrospective Study of Factors Affecting on Longevity for Resin-based Composite Restorations

○Akimasa Tsujimoto[1,2,3], J. C. Thomas,[4] Chandler Pendleton,[4] Daniel Caplan[5]

[1]*Department of Operative Dentistry, School of Dentistry, Aichi Gakuin University, Nagoya, Japan.*
[2]*Department of Operative Dentistry, College of Dentistry, University of Iowa, Iowa City, IA, USA*
[3]*Department of General Dentistry, School of Dentistry, Creighton University, Omaha, NE, USA*
[4]*Division of Biostatistics and Computational Biology, College of Dentistry, University of Iowa, Iowa City, IA, USA*
[5]*Department of Preventive & Community Dentistry, College of Dentistry, University of Iowa, Iowa City, IA, USA*

I. Objective

To determine which patient and restoration factors significantly influence restoration longevity, while comparing resin-based composites (RBC) using supra-nano spherical fillers to conventional RBC materials.

II. Materials & Methods

Anonymized data was extracted from the Axium database at the University of Iowa College of Dentistry, covering all treatments given to patients since 2016. After removing cases with missing data, 6,111 cases remained for analysis. A Cox proportional hazards model was fitted to determine the hazard ratios of the factors recorded in the database.

III. Results

The number of surfaces covered by the restoration has a strong negative influence on longevity, increasing with increasing number of surfaces ($p<0.001$, HR 1.56 for 2 surfaces, 2.14 for 3, 2.73 for 4 or 5). Most patient factors do not show a significant influence, including gender and age. The factors that do show a significant influence are insurance type (Medicare etc., HR 1.33, $p=0.016$), eating disorder (HR 1.78, $p=0.01$), and dental fear (HR 1.38, $p=0.002$). Notably, the presence of bruxism does not show a significant influence. Resin-based composites using supra-nano spherical fillers had significantly better longevity than conventional RBCs (HR 0.64, $p=0.005$).

IV. Conclusion

Few patient factors have a large influence on restoration longevity. On the other hand, the size of the restoration has a strong influence, and material differences are also important.

Contact information of the authors

Name: Akimasa Tsujimoto, Affiliation: Aichi Gakuin University School of Dentistry, e-mail address: aki-tj@dpc.agu.ac.jp

The influence of dimethyl sulfoxide on the flow behavior of calcium silicate cement in root canal obturation

○**Bokyung Shin[1], Won-Jun Shon[2]**

[1]Department of Conservative Dentistry, Seoul National University Bundang Hospital, Gumi-ro 173 bun-gil 82, Bundang-gu Seongnam-si, Gyeonggi-do, South Korea
[2]Department of Conservative Dentistry, Dental Research Institute, Seoul National University School of Dentistry, Daehak-ro 101, Jongro-gu, Seoul, South Korea

I. Objective

To investigate a calcium silicate based cement (CSC) filling technique, and to evaluate the flow behavior, microleakage, and quality of root canal filling when using CSC containing DMSO.

II. Materials & Methods

Two types of CSC, one containing DMSO (CSC-DMSO) and the other containing polyethylene glycol (PEG) (CSC-PEG), were prepared, and the chemical composition of the mixtures was analyzed using gas chromatography-mass spectrometry (GC-MS). The flow characteristics of these cements were compared in gypsum and resin channels using a high-speed camera. Eight root canals were filled with either CSC-DMSO or CSC-PEG, using a cement delivery device, and the quality of the root canal fillings was evaluated by measuring the filling length on periapical radiographs. The filling length was assessed in relation to the apical constriction, referred to as the 'apico-coronal extension.' Microleakage was assessed in thirty human molars, which were randomly filled with CSC-DMSO, CSC-PEG, or a combination of gutta-percha and AH Plus sealer. Additionally, in a preliminary study, the use of CSC-DMSO with a cement delivery device in human teeth was evaluated in terms of filling length and the presence of voids, using periapical radiographs. For statistical analysis, the Kruskal-Wallis test was used for simulated root canal fillings, and one-way ANOVA was applied for the leakage test ($\alpha = 0.05$).

III. Results

In GC-MS analysis, DMSO was detected in CSC-DMSO, and the monomer of PEG was found in CSC-PEG. The flow rate of CSC-DMSO reduced in gypsum channels compared to resin channels, whereas CSC-PEG showed no significant difference between the channels ($p > 0.05$). The median absolute value of apico-coronal extension was significantly lower in CSC-DMSO in comparison to CSC-PEG ($p < 0.05$). There were no statistically significant differences in microleakage among the groups ($p > 0.05$). In the preliminary obturation study, the mean apico-coronal extension of CSC-DMSO measured -0.297 ± 0.724 mm, while CSC-PEG showed excessive apical extrusion.

IV. Conclusion

CSC-DMSO could be considered as an alternative filling material for root canal obturation.

Contact information of the authors

Name: Won-Jun Shon (Corresponding author), Affiliation: Department of Conservative Dentistry, Dental Research Institute, Seoul National University School of Dentistry, Daehak-ro 101, Jongro-gu, Seoul, South Korea, e-mail address:endoson@snu.ac.kr

Name: Bokyung Shin, Affiliation: Department of Conservative Dentistry, Seoul National University Bundang Hospital, Gumi-ro 173 bun-gil 82, Bundang-gu Seongnam-si, Gyeonggi-do, South Korea, e-mail address: sbkendo@snubh.org, pppppori@gmail.com

Comparisons of the smear layer removal efficacy of dual-action irrigants and different activation techniques

○Yanyan Chen, Satoshi Watanabe, Phu Yadanar Aung Myint, Sota Mochizuki, Takashi Okiji

Department of Pulp Biology and Endodontics, Division of Oral Health Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU)

I. Objective

Dual-action root canal irrigants, which combine disinfectants and chelators, are expected to improve canal cleanliness efficiency. This study aims to evaluate the effectiveness of these irrigants and various activation techniques in removing the smear layer.

II. Materials & Methods

Seventy-six extracted human mandibular premolars were prepared for root canal treatment up to an apical size of #40/0.06 with the approval of the Institutional Review Board of Tokyo Medical and Dental University (No. D2023-029). These teeth were grouped and irrigated as follows, and the smear layer on each third of the canal wall was evaluated under scanning electron microscopy. In Experiment 1, syringe irrigation (SI) was performed utilizing either a dual-action irrigant (Triton or SmearOFF) or a combination of 17% ethylenediaminetetraacetic acid (EDTA) with 6% sodium hypochlorite (NaOCl) (n = 12, each group). In Experiment 2, Triton was activated using different techniques: laser-activated irrigation (LAI) with a novel Er:YAG laser equipment (Adverl SH, Morita Manufacturing), a LAI protocol called shockwave-enhanced emission photoacoustic streaming (SWEEPS), or ultrasonic-activated irrigation (UAI). These methods were compared with SI (n = 10, each group).

III. Results

In Experiment 1, the Triton group demonstrated significantly superior smear layer removal than the EDTA and NaOCl combination groups across all canal thirds ($P < 0.05$). In Experiment 2, with Triton as the irrigant, both the LAI and SWEEPS groups showed significantly lower smear layer scores compared to the UAI and SI groups across all canal thirds ($P < 0.05$).

IV. Conclusion

Under the conditions evaluated in this study, Triton was more effective at removing the smear layer than the combination of EDTA and NaOCl across all canal thirds. SmearOFF also showed superior smear layer removal in specific areas compared to the EDTA and NaOCl combination. Additionally, activating Triton with LAI and SWEEPS enhanced the cleaning efficacy and simplified the root canal irrigation procedures.

Contact information of the authors

Name: Satoshi Watanabe, Affiliation: Tokyo Medical and Dental University Pulp Biology and Endodontics, e-mail address: s.watanabe.endo@tmd.ac.jp

Implementing microfluidic flow device model in utilizing dural substitutes as pulp capping materials for vital pulp therapy

○**Hi-Won Yoon, Min-Yong Lee , Sun-Il Kim, Jae-Sung Kwon, Jeong-Won Park, Su-Jung Shin**

Department of Conservative Dentistry, Gangnam Severance Dental Hospital, Yonsei University, College of Dentistry Seoul, South Korea

I. Objective

Although vital pulp therapy (VPT) has shown high success rates in long-term follow-up, adverse effects have been reported due to the calcification of tooth canals by mineral trioxide aggregates (MTAs), which commonly used in VPT. To address this issue, this study evaluated the mechanical properties of dural substitutes intended to alleviate intra-pulpal pressure caused by inflammation, along with assessing the biological responses of human dental pulp stem cells (hDPSCs) and human umbilical vein endothelial cells (HUVECs), both of which play crucial roles in dental pulp. The study examined the application of dural substitutes as pulp capping materials, replacing MTA.

II. Materials & Methods

The microfluidic flow device was fabricated using polydimethylsiloxane (PDMS), with fluid flow velocity set to 2.65 cm/s. Computational fluid dynamics (CFD) were utilized to assess the fluid flow velocity at the bottom of the microfluidic flow chip, influencing attached cells through mechanical stimuli. Following this, MTA or dural substitutes (Biodesign; BD and Neuro-patch; NP) were placed in the microfluidic flow device, simulating vital pulp therapy procedure. Evaluating pressure release ability of each dural substitutes, and 20 mM 2-hydroxypropyl methacrylate (HEMA) solution was applied to the pulp cover materials and the penetration resistance of each materials was assessed. Subsequently, the response of HUVECs was analyzed through tube formation assay and assessment of angiogenesis-related gene expression upon exposure to different pulp cover materials. Additionally, hDPSCs were cultured, stemness marker, angiogenesis-related, and hard tissue-related gene were evaluated for each group.

III. Results

Computational fluid dynamics simulations were employed to ensure that the fluid flow velocity within the microfluidic flow device matched the actual blood flow velocity within the dental pulp. Additionally, the dural substitutes demonstrated effective intra-canal pressure relief and resistance to penetration HEMA leached from upper restorative materials and bonding agents. Finally, while MTA increased the expression of angiogenesis-related and hard tissue-related genes in HUVECs and hDPSCs, respectively, BD and NP did not alter gene expression and preserved the original characteristics of both cell types.

IV. Conclusion

Dural substitutes have emerged as promising alternatives for VPT due to their stress-relieving elastic properties, resistance to HEMA penetration, and ability to maintain stemness. Furthermore, the microfluidic flow device model closely replicated the cellular responses observed in live pulp chambers, thereby indicating its potential use as an *in vivo* testing platform.

Contact information of the authors

Name: Hi-Won Yoon, Min-Yong Lee , Sun-Il Kim, Jae-Sung Kwon, Jeong-Won Park, Su-Jung Shin, Affiliation: Department of Conservative Dentistry, Gangnam Severance Dental Hospital, Yonsei University, College of Dentistry, e-mail address: Seoul, South Korea won2362@yuhs.ac sujungshin@yuhs.ac

Anti-biofilm effect of on-demand aqueous chlorite dioxide solution on endodontic bacteria

○MAEZONO Hazuki[1], SHIMAOKA Tsuyoshi[1], ONO Shunka[1], ASAHI Yoko[1], TAKAHASHI Yusuke[1], SAKAI Takayoshi[2], HAYASHI Mikako[1]

[1] Department of Restorative Dentistry and Endodontology,
Osaka University Graduate School of Dentistry, Suita, Osaka, Japan
[2] Department of Rehabilitation for Orofacial Disorders,
Osaka University Graduate School of Dentistry,
Suita, Osaka, Japan

I. Objective

Apical periodontitis, which subsequently occurs from dental caries is a biofilm-caused infection. Sterility in a root canal is necessary for successful treatment, and not only mechanical cleaning but also irrigation using chemical solutions plays an important role. Sodium hypochlorite (NaOCl) solution, which is commonly used for root canal treatment, has strong bactericidal effect. However, it is also highly cytotoxic, therefore extreme caution is required to avoid the damages of oral tissues. A novel agent, on-demand aqueous chlorine dioxide solution (matching transformation system®; MA-T), which has both biosafety and bactericidal effects demonstrates potentials to utilize as a root canal irrigant. This study was aimed to investigate basic anti-biofilm effects of MA-T when applied on endodontically pathogenic biofilms.

II. Materials & Methods

This study was approved by the Ethics Committee Review Boards of Osaka University Graduate School of Dentistry (certificate R6-E3). To assess the potency of MA-T on biofilms, biofilms prepared from *Enterococcus faecalis, Parvimonas micra, Fusobacterium nucleatum* and human supragingival plaque obtained from healthy volunteers were exposed to MA-T and control agents. Viable cell count, ATP assay, crystal violet (CV) staining and confocal laser scanning microscopy (CLSM) observations were conducted to investigate its efficacy. Statistical analysis was performed using one-way ANOVA with Tukey's HSD post-hoc test, and significance level was set at 5%.

III. Results

MA-T significantly reduced the number of biofilm bacteria investigated in this study. Longer application with higher concentration of MA-T showed higher anti-biofilm effect.

IV. Conclusion

MA-T was found to inhibit endodontically pathogenic biofilm bacteria, indicating its potential for future application as a root canal irrigant.

Contact information of the authors

MAEZONO Hazuki, Department of Restorative Dentistry and Endodontology, Osaka University Graduate School of Dentistry, maezono.hazuki.dent@osaka-u.ac.jp

In Vitro Study on Biological Properties of a Novel Dual-cure Resin-Modified Calcium Silicate-Based Cement

○**Minjung Kim[1], Sung-Hoon Lee[2], Dong-Hoon Shin[1] and Yong-Bum Cho[1]**

[1] Department of Conservative Dentistry, College of Dentistry, Dankook University
[2] Department of Oral Microbiology and Immunology, College of Dentistry, Dankook University

I. Objective

Vital pulp therapy is a widely recognized treatment modality within the field of endodontics. The properties of materials that directly interact with vital pulpal tissue are crucial for treatment success. Calcium silicate-based cements, such as ProRoot MTA®, Retro MTA®, and Biodentine™ (BD), are well-established and highly effective materials for pulpal tissue coverage. Theracal® LC (TL) is another commonly used material; however, recent studies have reported negative biological properties associated with TL, including cytotoxicity, induction of pulpal inflammation, and inadequate dentin bridge formation. These adverse effects are thought to be related to the release of unpolymerized monomers. A novel dual-cured resin-modified calcium silicate material, Theracal PT® (TP), was recently introduced, and it is recommended by the manufacturer for direct application on vital pulpal tissue, with the assumption that it may offer superior performance compared to TL. This study aims to evaluate the biological properties of TP in comparison with TL and BD.

II. Materials & Methods

To assess biological properties, a Cell Counting Kit-8 (CCK-8) assay was employed on human dental pulp cells (hDPCs) to evaluate cell viability across the three materials at 24- and 48-hour time point. Antibacterial activity against *Enterococcus faecalis* was examined under anaerobic conditions. The odontogenic differentiation potential of the materials was assessed by measuring the relative gene expression levels of osteocalcin (OCN), osteopontin (OPN), and collagen type I (ColI) using real-time polymerase chain reaction (PCR) at 12- and 48-hour time point.

III. Results

At the 24-hour mark, cell viability was no significant different between the control, TL, and TP, but BD showed significantly lower viability than TL and TP. By 48 hours, BD's cell viability greatly increased, matching the control and surpassing TL and TP levels seen at 24 hours. In contrast, TL and TP had the lowest viability at 48 hours, significantly lower than both the control and BD. TP demonstrated superior antibacterial activity compared to the other materials. At the 12-hour time point, all materials exhibited significantly elevated expression levels of ColI, OCN, and OPN compared to the control group. BD and TP showed similar levels of ColI and OCN, whereas TP displayed notably higher OPN expression. At 48 hours, TP demonstrated reduced expression of ColI and OCN relative to the control, while OPN expression was the highest among all groups. Conversely, BD and TL maintained elevated expression levels of ColI, OCN, and OPN compared to the control, reflecting continued odontogenic activity.

IV. Conclusion

BD exhibited the most favorable biological characteristics for use in vital pulpal therapy. TP demonstrated improved properties over TL, including enhanced antibacterial effects and odontogenic differentiation potential.

Contact information of the authors

Name: **Minjung Kim**, Affiliation: *Department of Conservative Dentistry, College of Dentistry, Dankook University*, e-mail address: 12180285@dankook.ac.kr