様式３

論　文　内　容　の　要　旨

氏　名　（MANAHIL ALI SAEED ALI）	
論文題名	Lithium-containing S-PRG fillers promoted wound healing process of pulp tissues through activation of Wnt/β-catenin signaling pathway (リチウム含有S-PRGフィラーによるWnt/β-カテニンシグナル経路の活性化を経た歯髄創傷治癒に関する研究)

論文内容の要旨

[Background]

Ultimate goal of direct pulp capping is to maintain tooth vitality after being exposed due to bacterial infection, trauma or iatrogenic injury. Direct pulp capping is conducted through covering the exposed pulp using bio-active substance that aids the healing process. Although the pulp capping materials currently used such as calcium hydroxide and mineral trioxide aggregate (MTA) are widely accepted, they have been modified to compensate the shortage when they use them. Recently, new cement has been introduced called “Giomer”. Giomer is a terminology refer to any product that contains surface pre-reacted glass (S-PRG) fillers. They are formed by acid base reaction of fluoro-boro-aluminosilicate glass and polyacrylic acid in hydrated environment. They can release different types of ions such as Al^{+3}, BO^{-3}, Na^{+}, SiO_3^{-2}, Sr^{+2} and F^{-} that can show various biological effects. It has been previously shown that topical application of S-PRG cement as a direct pulp capping material and it could induce reparative tertiary dentin formation. Another study reported that lithium chloride (LiCl) could induce tertiary dentin formation in pulpotomized rat teeth. The results suggested the activation of the canonical Wnt/β-catenin pathway only from additional *in vitro* results. Therefore, the hypothesis of this study was that incorporation of LiCl into S-PRG fillers might promote reparative dentin-pulp complex healing and regeneration through activating Wnt/β-catenin pathway in animal models.

The objective of this study was to investigate the effects of S-PRG /LiCl combination by evaluation of tertiary dentin formation in a rat pulp capping model using human dental pulp stem cells (hDPSCS) and by assessment of activation of Wnt/β-catenin signaling pathway *in vivo*.

[Materials and Methods]

S-PRG fillers used in this study were provided by Shofu Inc. (Kyoto, Japan).

1. Pulp capping experiment: Animal studies were conducted under the approval of the Institutional Animal Care and Use Committee of Osaka University Graduate School of Dentistry (No. 28-013-0). Eight-week-old male Wistar rats were used in this study. After anesthetizing, a bowl shaped cavity was drilled on the maxillary first molar using a steel round bur to expose the pulp. The exposed pulp was capped using one of different cements (S-PRG, S-PRG/LiCl-10, 100 or 1000 mM) or MTA as a control. Then, the cavity was sealed with glass ionomer cement. After 4 weeks, the prepared teeth were collected to assess tertiary dentin formation at the site of injury using a micro-computed tomography (μCT) followed by histopathological assay using hematoxylin and eosin staining.

2. *In vitro* studies:

(1) Mechanical properties: Compressive strength, shear bond strength to dentin and the amount of the released lithium and other ions were measured using the extracted solutions from four types of the S-PRG cements (S-PRG, S-PRG/Li-10, 100 or 1000 mM).

(2) Cell studies: Human dental pulp stem cells (hDPSCs) were cultured in proliferative or differentiation inductive media containing the extracted solutions of S-PRG/LiCl-10, 100, 1000

mM or S-PRG without lithium. Cell proliferation was evaluated by trypan blue solution. Cytotoxicity of the cements were assessed by measuring the lactate dehydrogenase enzyme activity. Wound healing assay was performed to evaluate the cell migration ability. Differentiation and mineralization were investigated via alkaline phosphatase staining and alizarin red staining using the extracts from different cements.

3. Assessment of Wnt/β-catenin signaling activation *in vivo*: The rats were sacrificed at 7 or 14 days after pulp capping and teeth were processed for immunofluorescence staining against Wnt signaling markers (β-catenin and Axin2 antibodies) using S-PRG, S-PRG/Li-100 mM or tooth without pulp capping treatment as a control.

4. Comparative studies with MTA: To compare the properties of the S-PRG containing cements with MTA, following assays were conducted. Hydroxy-apatite (HA) formation on the materials surface was observed under a scanning electron microscope. To evaluate the sealing ability of the S-PRG cements, bovine root canals were filled with the three different cements and depth of penetration of rhodamine-B solution was measured. Subcutaneous tissue implantation test was also conducted to evaluate the biocompatibility *in vivo* (approval No. 29-028-0). Polytetra-fluoroethylene (PTFE) tubes were filled with each specimen or empty tubes were dorsally implanted using rats for 1 or 2 weeks. The tissue was evaluated by hematoxylin and eosin staining. For biosafety assessment, the concentration of lithium in peripheral serum of rats was measured using the flame method of atomic absorption spectroscopy.

5. Statistical analysis: All assays were statistically analyzed using one-way ANOVA with Tukey-Kramer post hoc test. P-values< 0.05 was considered to be significantly different.

[Results and Discussion]

The results of μCT and histopathological images showed that S-PRG/Li-10 and 100 mM could induce complete tertiary dentin structure continuous with the primary dentin and they were similar to that formed by MTA, while S-PRG and S-PRG/Li-1000 mM demonstrated defect or incomplete tertiary dentin formation. There was no significant difference in shear bond strength to dentin between S-PRG and S-PRG/Li specimens; while significant decrease in the compressive strength was observed in S-PRG/Li-100 and 1000 mM compared with the other groups. Functional assays using hDPSCs showed positive results using S-PRG/Li-10 and 100 mM combinations; S-PRG/Li-10 mM promoted cell migration compared with the control, while S-PRG/Li-100 mM significantly enhanced the migration, differentiation and mineralization of hDPSCs. S-PRG/Li-1000 mM inhibited all of the cell functions. The *in vitro* outcomes may justify the behavior of the formed complete tertiary dentin by S-PRG/Li-10 and 100 mM. Elevation in lithium concentration to 1000 mM led to inhibition of the cell profiles. The results of immuno-fluorescence staining revealed significant expression of β-catenin at the odontoblastic cell layer by S-PRG/Li-100 mM at 7-day post-operative samples and sharply declined to the minimum like the other experimental groups in 14-day samples. Axin2 expression showed significant elevation in S-PRG/Li-100 mM group at 7 and 14-day post-operative samples, while S-PRG and control groups remained low. The continuous expression of Axin2 suggested it could be potentially involved in another signaling pathways such as transforming growth factor-β. S-PRG showed good sealing ability, HA formation *in vitro* and displayed high biocompatibility *in vivo*. The level of serum lithium in experimental rat was lower than 0.01 mmol/L at all the different time points indicated the bio-safety of S-PRG/Li-100 mM as a direct pulp capping material.

[Conclusions] These results proved this study's hypothesis where S-PRG/Li-100 mM showed high bio-compatibility, promoted migration, differentiation, and mineralization of hDPSCs *in vitro*, and induced reparative dentin formation in rat models *in vivo*. These effects were due in part to the activation of Wnt/β-catenin signaling pathway.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は、リチウム含有 Surface Pre-reacted Glass (S-PRG)フィラーが歯髄創傷治癒に与える影響を動物実験モデルにて検討し、さらに同フィラー含有セメントが歯髄細胞に与える影響や、物理的性質、生体親和性や安全性について包括的に解析をおこなったものである。

その結果、リチウム含有 S-PRG セメントは歯髄の創傷治癒を促進することが明らかとなり、Wnt/β カテニンシグナル経路の活性化の関与が示唆された。さらに、同セメントは歯髄細胞の生物活性を賦活化し、高い物理的特性、生体親和性や安全性を有していることより、覆髄材として有用であることが示唆された。

以上の研究成果は、歯髄創傷治癒メカニズムを明らかにする上で新たな知見を提供するものであり、本研究は博士（歯学）の学位に値するものと認める。