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学位論文の題目	Functionalized Graphene Oxide Shields Tooth Dentin from Decalcification (官能化グラフェン酸化物は象牙質を脱灰から保護する)
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### 学位論文内容の要旨

Tooth decay and erosion are the critical clinical situations of complicated management because its multifaceted etiology has not yet been clearly understood. Several biomaterials, agents, and therapies have been proposed for the treatment of pain and hypersensitivity, however, none of them has been proven completely efficient, and the development of new desensitizing agents is the obligation now for an aging society.

Recently, significant efforts have been focused on 2-dimensional carbons due to their excellent chemical, physical, and electronic properties. Graphene oxide (GO) is a graphene analog mainly composed of sp<sup>3</sup>-bonded carbon atoms, possessing extraordinary physical and chemical properties. This *in vitro* study assessed the efficacy of graphene oxide (GO) and its nanocomposite with metal ions were in protecting dentin decalcification of dentin surface and sealing the orifice of dentinal tubules.

Five different GO-nanocomposites were synthesized by a one-pot method and a 1:1 weight % composite preparation with GO and nanoparticles. All the samples were characterized by scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and thermogravimetric analysis (TGA). The conditions of the surface of the hydroxyapatite plate (HAp) and dentin slice were observed by SEM before and after treatment with GO-nanocomposites and Saforide<sup>®</sup> as a positive control after they were incubated at 37°C in EDTA and citrate buffer for 24 hours. The antimicrobial test and the cytotoxicity test were performed for biological characterization by comparing to 0.1% povidone iodine as a positive control. Colony-forming unit (CFU) of *S. mutans* was counted after treating with GO-nanocomposites for 24 and 48 hours. MTS assay was performed to observe the viability of human epithelial HeLa cell line after treating with GO-nanocomposites for 48 hours.

Almost all GO-nanocomposites were effective to reduce decalcification of hydroxyapatite plate under decalcification conditions. GO-Ag-CaF<sub>2</sub> sealed the orifice of dentinal tubules completely (100%), and GO-CaF<sub>2</sub>, GO-Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, and GO-Ag were moderately (70-80%) or as Saforide<sup>®</sup> even under decalcification conditions. In addition, no color change was noticed on the dentin surface by treating with GO-nanocomposites. GO-nanocomposites were effective for reducing *S. mutans* CFU, especially GO-Ag-CaF<sub>2</sub> was stronger (60% more) than the positive control. GO-nanocomposites were not cytotoxic to HeLa cells except a high concentration of GO-Ag-CaF<sub>2</sub>(0.1%).

The ability of the GO-nanocomposites was to reduce dentin decalcification and to seal the orifice of dentinal tubules without discoloration. These results suggest that GO-nanocomposites may be potentially useful to protect dentin decalcification. However, further *in vivo* studies are needed before clinical studies.

## 論文審査結果の要旨

Human tooth dentin is the main component of the tooth, which consists of approximately 70% inorganic material and 30% organic material and water. Its inorganic part is calcium and phosphate ions that form hydroxyapatite (HAp) crystals. This HAp is easily dissolved by acids, resulting in tooth decay. Several biomaterials, agents, and therapies have been proposed for the treatment of pain and hypersensitivity, however, none of them has been proven completely efficient, and the development of new desensitizing agents is the obligation now for an aging society. This *in vitro* study assessed the efficacy of graphene oxide (GO) and its nanocomposite in protecting the decalcification of dentin surface and sealing the orifice of dentinal tubules.

GO-nanocomposites were synthesized by a one-pot synthesis technique and characterized by Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-Ray Diffraction, X-Ray Photoelectron Spectroscopy, and Thermogravimetric Analysis. The conditions of the surface of the HAp and dentin slice were observed by SEM before and after treatment with GO-nanocomposites and Saforide® as a positive control after they were incubated at 37°C in EDTA and citrate buffer for 24 hours. The antimicrobial test and the cytotoxicity test were performed by comparing to 0.1% povidone iodine as a positive control. The colony-forming unit (CFU) of *Streptococcus mutans* (*S. mutans*) was counted after treating with GO-nanocomposites for 24 and 48 hours. MTS assay was performed to observe the viability of the human epithelial HeLa cell line after treating with GO-nanocomposites for 48 hours.

This is the first study to test the series of f-GO nanocomposites to cover the dentin surface and to seal orifice of dentinal tubule under demineralization conditions. The important findings are below.

- 1) Almost all GO-nanocomposites were effective to reduce the decalcification of the HAp plate.
- 2) GO-Silver Calcium Fluoride (GO-Ag-CaF<sub>2</sub>) sealed the orifice of dentinal tubules completely (100%), and GO-Calcium Fluoride (GO-CaF<sub>2</sub>), GO-Tricalcium Phosphate (GO-Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>), and GO-Silver (GO-Ag) were moderately (70-80%) or as Saforide®. In addition, no color change was noticed on the dentin surface by treating with GO-nanocomposites.
- 3) GO-nanocomposites were effective for reducing *S. mutans* CFU, especially GO-Ag-CaF<sub>2</sub> was stronger (60% more) than the positive control.
- 4) GO-nanocomposites were not cytotoxic to HeLa cells except for a high concentration of GO-Ag-CaF<sub>2</sub> (0.1%).

The ability of the GO-nanocomposites was to reduce dentin decalcification and to seal the orifice of dentinal tubules without discoloration. It may be potentially useful as a clinical dentin mask, which must be established in future clinical studies.

Therefore, this study is of significant scientific value to the development of a novel dentin sealing and anti-demineralizing agent. The study has been already published in an international journal, Journal of Dental Research and recognized globally. The examination committee approves this study as a Ph.D. thesis in dentistry.