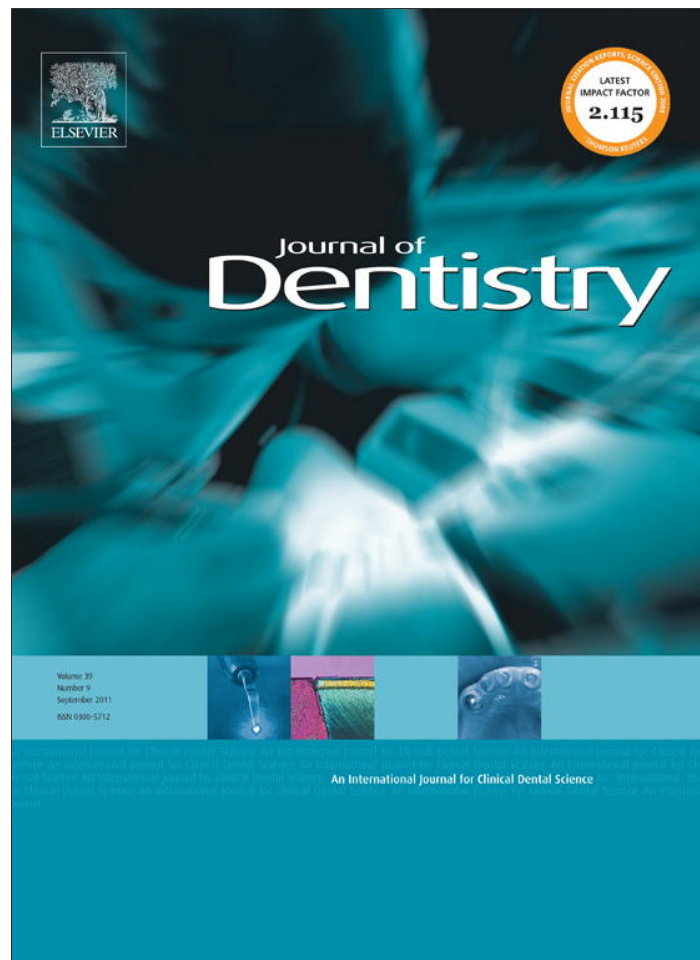


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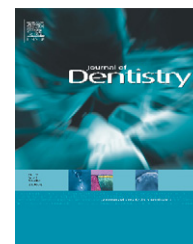


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## Cytotoxicity of 45S5 bioglass paste used for dentine hypersensitivity treatment

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### ABSTRACT

**Objectives:** 45S5 bioglass mixed with 50% phosphoric acid has been suggested to treat dentine hypersensitivity and incipient enamel caries. This study is going to evaluate the biocompatibility of using the aforementioned technique with the rat pulpal cells.

**Methods:** The relative cytotoxicity of 45S5 bioglass on rat dental pulp cells was compared to the cytotoxicity of a temporary filling material (Cavition; GC, Japan), Type 1 glass ionomer cement (Fuji I; GC, Tokyo, Japan) and commercial desensitising agent (SuperSeal; Phoenix Dental, Fenton, MI, USA) using a transwell insert model. Cell viability was measured by means of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The number of viable cell counts were compared using one way ANOVA ( $p < 0.05$ ). The morphological alterations of the pulp cells were observed directly by phase contrast microscope.

**Results:** The results of this study indicated that cell viability recorded by the 45S5 bioglass paste group did not differ significantly from those of the Cavition, glass ionomer or superseal, moreover pulpal cells microscopic analysis revealed that 45S5 bioglass elicited minimal toxic effect.

**Conclusions:** 45S5 bioglass paste can serve as a biocompatible material that can potentially be used safely on dentine.

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## 1. Introduction

Dentine hypersensitivity is one of the major challenges in dental practice.<sup>1</sup> The prevalence of dentine hypersensitivity varies from 4% to 57%<sup>2</sup> whilst the prevalence of dentine hypersensitivity is between 60% and 98% in patients with

suffering from periodontitis.<sup>3</sup> The hydrodynamic theory<sup>4,5</sup> explains the phenomenon of dentine hypersensitivity as an increase in the flow of the fluids present in dentinal-tubules that have patent orifices, thereby activating nerves situated in the outer layers of the pulp. Exposure of dentinal-tubules orifices may be caused by many factors, such as acid erosion,<sup>6–10</sup> attrition, abrasion,<sup>11</sup> parafunctional habits or gingival

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recession.<sup>12</sup> These dentinal-tubule orifices require permanent blocking as a treatment approach.<sup>1,13–15</sup>

Agents employed for treating dentine hypersensitivity show only temporary effect clinically because they are gradually removed by daily brushing, food friction, and change in the pH in the oral cavity.<sup>10</sup>

Bioactive glasses such as 45S5 bioglass can interact with the hard tissues by forming a calcium phosphate-rich layer which can bond chemically to these hard tissues.<sup>16–18</sup> We have previously reported that a 45S5 bioglass–50% phosphoric acid paste can form a crystallised calcium phosphate rich layer that can penetrate within the dentinal tubules' orifices; this layer was reported to be durable to brushing-abrasion wear challenge which suggests the possibility of using this technique for treating dentine hypersensitivity effectively.<sup>19,20</sup> However, there is no report about the biocompatibility of this technique.

The objective of this study was to compare the cytotoxicity of 45S5 bioglass on rat colonal dental pulp cells to the cytotoxicity of a temporary filling material containing zinc oxide (Cavition; GC, Japan), Type 1 glass ionomer cement (Fuji I; GC, Tokyo, Japan) and an oxalic acid containing desensitising agent (SuperSeal; Phoenix Dental, Fenton, MI, USA) using a transwell insert model by means of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

The hypothesis in this study was that 45S5 bioglass paste will show an acceptable biocompatibility when compared to the other tested materials.

## 2. Materials and methods

### 2.1. Culture of (RPC-C2A) dental pulp cells

The colonal cell line (RPC-C2A) established from dental pulp of rat incisor<sup>21</sup> were used in this study. The culture medium was Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% foetal bovine serum (FBS) and antibiotic solution (60 µg/ml of kanamycin), under a humidified atmosphere of 95% air, 5% CO<sub>2</sub> and maintained at 37 °C.

### 2.2. Cytotoxicity evaluation with the transwell insert model

The tested materials included a temporary filling material (Cavition; GC, Japan), Type 1 glass ionomer cement (Fuji I; GC, Tokyo, Japan) and a commercially available desensitising agent (SuperSeal; Phoenix Dental, Fenton, MI, USA), and 45S5 bioglass (NovaMin<sup>®</sup>, 5 µm average particle, NovaMin Technology, Florida, USA), whilst a number of empty inserts served

as the control group. All materials were sterilized by gas sterilization before cytotoxicity testing. The transwells used in this study were 6.5 mm in diameter, with a pore size of 0.4 µm (Costar Transwell-Clear, Corning Costa, Cambridge, MA). The transwells were transferred into 24-well culture plates that were seeded with pulp cells ( $5 \times 10^3$  cells/well) and kept for 24 h.

### 2.3. Application of the tested materials in the transwells

For the 45S5 bioglass, one tenth of a gram of 45S5 bioglass powder composed of (Na<sub>2</sub>O, CaO, P<sub>2</sub>O<sub>5</sub>, SiO<sub>2</sub>) was mixed on a glass slab for 1 min by spatula with 0.2 ml of 50% phosphoric acid that was prepared by diluting 85% phosphoric acid (Wako Chemicals, Osaka, Japan) in distilled water to form a gel (pH 2.2). The three other dental materials were manipulated according to their manufacturers' instructions and were applied as well as the 45S5 bioglass paste on the base of the transwells to form a 2 mm height of each material.<sup>22</sup>

Pulp cells in all groups exposed to the respective dental material and controls were then incubated for 3 days under a humidified CO<sub>2</sub> incubator at 37 °C. All of the transwells were then removed from the incubator, and the cell viability was measured using an MTT assay. For the MTT assay, the cells in each well were incubated with a culture medium containing 100 µL MTT (Roche) solutions for a period of 3 h at 37 °C. Only viable cells that feature functional mitochondria are able to reduce MTT to insoluble purple formazan crystals. Subsequently to this incubation, the medium was aspirated and 200 µl dimethyl sulfoxide (DMSO) was added to dissolve the reduced formazan crystals. The resultant solution was then lightly shaken for 15 min by a microplate shaker. The optical density (OD<sub>570</sub>) of the formazan solution, which is directly proportional to the number of viable cells present in the solution, was measured with a microplate reader (Model 450, Bio-Rad [Bio-Rad Laboratories, CA]). A blank well was regularly used for data subtraction by placing the same volume of culture medium with MTT into culture wells that contained a transwell but no dental materials. Results of the MTT assay were analysed using one-way analysis of variance (ANOVA) followed by a Tukey test ( $p < 0.05$ ) in order to determine whether any significant difference existed between the tested dental materials as regards relative toxicity to cultured dental-pulp cells.

### 2.4. Morphological observation of the cultured pulp cells

Morphological observations of the pulp cells were conducted using phase contrast microscope and then photographed using an Olympus camera.

**Table 1 – Materials used in this study.**

Materials	Composition	Procedures
45S5 bioglass (NovaMin <sup>®</sup> Technology, USA)	SiO <sub>2</sub> (45 wt%), Na <sub>2</sub> O (24.5 wt%), CaO (24.4 wt%) and P <sub>2</sub> O <sub>5</sub> (6 wt%)	Mix 0.1 g of 45S5 bioglass to 0.2 ml of 50% phosphoric acid
Cavition (GC Corporation, Tokyo, Japan)	Zinc oxide, plaster of Paris, vinyl acetate, ethanol	Apply directly
Fuji I (GC Corporation, Tokyo, Japan)	Powder: alumino-silicate glass Liquid: polyacrylic acid, water	Apply one scoop of powder to one drop of liquid
SuperSeal (Phoenix Dental, Fenton, MI, USA)	Oxalic acid, potassium salt	Apply directly

### 2.5. pH measurements of tested materials

The 4 tested materials were manipulated as described in Table 1 and then placed in a mould of 4 mm diameter and 6 mm height.<sup>23,24</sup> The materials were immediately placed in a falcon tube and 5 ml of deionised water were added to the tested materials. pH measurements were recorded using a pH metre (Sartorius PB-11, Melsungen, Germany) at 0, 2, 30, 60 and 1440 minutes after placing the materials in the test tubes.<sup>23,24</sup> Five separate trials were conducted for each material and the means of results were recorded for each material.

## 3. Results

### 3.1. Cytotoxicity evaluation with the transwell insert model results

Fig. 1 shows the MTT results of tested materials. The largest mean count value to the least mean count value is corresponding to the following materials: 45S5 bioglass paste, Caviton, SuperSeal, and Fuji I glass ionomer cement. There were no significant differences ( $p < 0.05$ ) between the recorded values of the MTT counts for all tested materials.

### 3.2. Morphological observation of the cultured pulp cells

The cultured RPC-C2A cells appeared spindle-shape before confluent; however after confluent the cells became polygonal in shape (Fig. 2A). 45S5 bioglass paste elicited little cytotoxic effects on the morphology of the cultured cells however the size of the cells became slightly smaller in size. The density of the cultured cells decreased in the glass ionomer cement group and then in the Caviton group respectively with marked

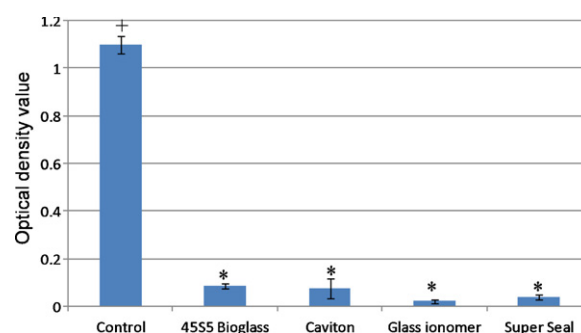


Fig. 1 – Cytotoxicity of four dental materials with culture medium on rat dental pulp cells. MTT results ( $n = 5$ ) of tested materials. One-way ANOVA revealed a significant difference ( $p < 0.05$ ). The same symbols (+, \*) represented that there were no significant differences.

increase in the intercellular spaces. Moreover, some of the pulp cells became retracted or rounded, with loss of functional organisation. The SuperSeal group showed marked increase of cell degeneration, cell debris and signs of pulpal cells necrosis.

### 3.3. pH measurements of tested materials

The mean of the pH values are shown in Fig. 3. The initial pH measurements of the 45S5 bioglass mixture were initially acidic i.e. 2.2, however there was a slightly steady increase in the pH along the period of observation till it reached 4 after 24 h. The SuperSeal maintained its acidic behaviour along the experimental period. The glass ionomer and the Caviton showed higher pH values that remained relatively stable throughout the experiment.

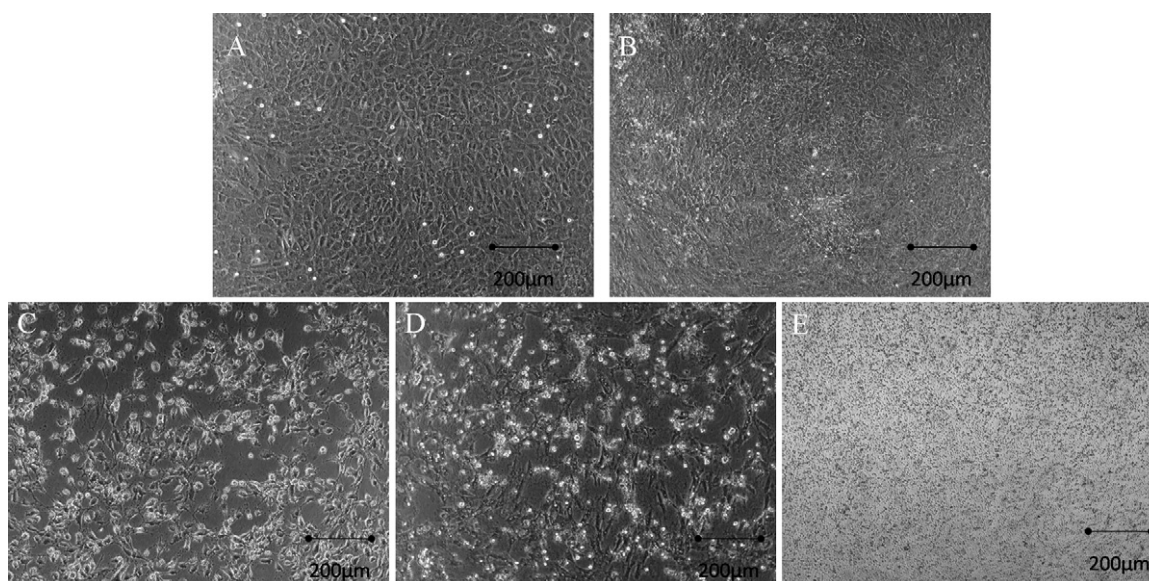
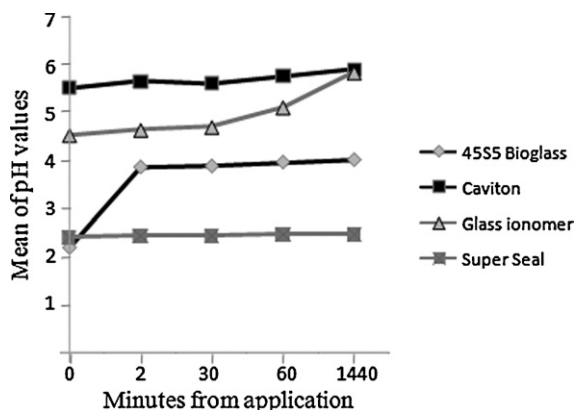


Fig. 2 – Morphological changes of pulp cells following exposure to the various tested materials for 3 days. (A) Control pulp cells: polygonal-shaped cells can be observed and (B) 45S5 bioglass paste group: size of the cells became slightly smaller. (C) Caviton group: decrease in pulp cells density and increase in intercellular spaces. (D) Glass ionomer group: decrease in pulp cells and marked increase in intercellular spaces. (E) SuperSeal group: marked observation of cell degeneration, cell debris and signs of pulpal cells necrosis (100 $\times$ , original magnification).



**Fig. 3 – Mean of the pH values recorded for the tested materials at the experimental durations.**

#### 4. Discussion

45S5 bioglass is a highly biocompatible material<sup>25</sup> that has an antibacterial effect.<sup>26,27</sup> It was previously reported that the combination of 45S5 bioglass with phosphoric acid will form a calcium phosphate rich layer that can bond to dentine.<sup>19</sup> Moreover, this layer showed good abrasion resistance and acceptable mechanical properties.<sup>20</sup>

The viability of the cells observed by the MTT experiment showed that the 45S5 bioglass paste did not exert any significant cytotoxic effects on the cultured pulp cells when compared to the results obtained from the other tested commercially available materials. Moreover, 45S5 bioglass showed the least cytotoxic effects on the morphology of the cultured cells when observed under the microscope.

The slight difference between the MTT experiment results and the microscopic examination results may be attributed to the low metabolic activity of the cultured cells caused by the application of the dental materials which may have caused difficulty in obtaining significant differences in the MTT counts when comparing the tested materials.<sup>28</sup>

The highest cytotoxicity was associated with the SuperSeal which is mainly composed of oxalic acid and potassium salts with pH 2.7. This acidic solution depends on attacking the peritubular dentine and using the dentine's calcium to form insoluble calcium oxalate crystals capable of blocking the dentinal tubules orifices.<sup>29</sup> Thus, it is speculated that this solution caused the drop of the pH of the culture media which had a negative effect on the cultured cells.

On the other hand, there were some cytotoxic effects associated with the 45S5 bioglass paste because it is initially acidic after mixing (i.e. pH 2.2) however, due to the rich calcium and phosphate contents of the powder the pH of the paste was gradually increasing to be 4 after 24 h. Thus it is expected that the bioglass mixture exhibited some cytotoxic effects on the cells initially after mixing due to its initial acidity, however these effects were rapidly diminished. It was suggested that the mechanism of calcium-phosphate crystals formation by the 45S5 bioglass paste on tooth surfaces using our technique is as follows: when the 45S5

bioglass powder was mixed with the aqueous solution of the 50% phosphoric acid the calcium, phosphate and sodium crystals leached into the aqueous acidic media.<sup>19,20,30</sup> The phosphate ions released from 45S5 bioglass and those abundant in the phosphoric acid solution reacted with the calcium ions from bioglass and dentine to form calcium-phosphate salts. These inorganic salts precipitated on top of the dentinal-surface with smaller crystals penetrating the dentinal-tubules.<sup>19,20</sup>

There were some cytotoxic effects observed for the glass ionomer group and the Caviton group which may be attributed to the leaching of some cytotoxic components from these materials. In case of glass ionomer it is speculated that some unreacted polyacrylic acid<sup>31</sup> might have permeated from the cellulose acetate filter of the transwell and affected the viability of the cultured cells. The cytotoxic effects observed in the Caviton group may be attributed to its calcium sulphate content which was previously reported to exhibit a moderate cytotoxic effect on the pulp cells.<sup>32</sup>

This in vitro cytotoxicity testing may offer some advantages when compared to in vivo testing as it is rather quick to perform, less expensive, able to be standardised, able to provide large-scale screening and more sensitive method for detecting any slight negative effects on the pulp cells.<sup>33</sup> However, the In vivo studies using animals permit the analysis of biocompatibility under conditions that allow for cell-to-cell interactions that more closely mimic the clinical situation.<sup>34</sup>

However, conducting a direct correlation between the results obtained from the current study to corresponding results derived from in vivo study or any clinical trial should be cautioned against, because in the clinical situation it is expected to place these tested materials on dentine which will act as barrier between the dental materials and the pulp cells, this dentine barrier can alleviate the cytotoxicity of many dental materials and acts as a good pH buffering structure.<sup>35</sup>

Moreover, the dentinal fluid and proteins present within dentinal tubules are able to effectively neutralize the toxic effects of many components released from a variety of restorative dental materials.<sup>36</sup> Thus, it is expected that the current tested materials will exhibit better biocompatibility to the dental pulp cells clinically.

The durability of using the 45S5 bioglass<sup>20</sup> paste and its high biocompatibility suggest that this paste can serve as an efficient aid in the treatment of dentine hypersensitivity.

#### 5. Conclusion

The 45S5 paste showed good biocompatibility to pulp cells when compared to three commercially available dental materials suggesting the safety of using this paste as an aid in treatment of dentine hypersensitivity.

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