PHYSICAL PROPERTIES OF CALCIUM SILICATE CEMENT WAS INFLUENCED BY RADIOPACITY AGENT AND/OR ANTI-WASHOUT ADMIXTURE

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Summary

Introduction: The goal of the present study was to improve the poor handling of calcium silicate cement (CSC) and to develop materials that can be used as root canal sealing materials by adding a radiopacity agent (RA), Bi_2O_3 or Ta_2O_5, and/or an anti-washout admixture (methylcellulose, MC) with different molecular weight of MC (MC4000, 400, 25). We examined the radiopacity, flow, and setting time according to ISO 6876-2012.

Methods: Samples for the radiopacity test were prepared by adding RA to Portland cement (PC) up to final concentrations (%) of 0, 5, 10, 15, 20, and 25, respectively. Samples for the flow and setting time tests were prepared using mineral trioxide aggregate as a control group. In test groups, final concentrations of 18% RA and 2% MC, or 20% RA alone were added to PC. All of the methods in the present study conform to ISO 6876-2012.

Results: A radiopacity test revealed that the radiopacity of PC containing over 10% volume of each RA was higher than or equal to standard radiopacity. PC containing Ta_2O_5 and the MC25 group passed a flow and setting time test.

Conclusions: Bi_2O_3 and Ta_2O_5 can be used similarly in radiopacity, flow value and setting time test. The flow and setting time of CSC varied significantly with the

molecular weight of the added MC.

Key word

mineral trioxide aggregate; tantalum oxide; methylcellulose; radiopacity; flow; setting time; anti-washout admixture

Introduction

Root canal filling is able to seal the content of the root canal system, thereby preventing the egress of microorganisms or byproducts into periradicular tissues. [1] An ideal root canal filling material should be biocompatible, antibacterial, non-toxic, and radiopaque and should not be resorbable or soluble in an oral environment. In addition to these characteristics, the material should be cost-effective, easy to handle, and closely adaptable to the cavity walls. [1]

Mineral trioxide aggregate (MTA, ProRoot MTA; Dentsply-Maillefer, Switzerland), which is a calcium silicate cement (CSC), was first developed by Torabinejad *et al*.. [2, 3] CSC is composed primarily of Portland cement (PC). MTA is used primarily for root canal filling, perforation repair, and retrofilling because MTA has unique biocompatibility, [4, 5] antibacterial properties, [6, 7] sealability, [8] and promotes hard tissue formation. [9, 10] In the case of the retrofilling by MTA, microscope is usually used for keeping brightness and magnification on operation area (Fig 1). MTA is difficult to use because of its granular consistency, slow setting time, and initial looseness. [11]

In previous reports on the use of a radiopacity agent (RA) as an additive to CSC, Bi_2O_3 (BO) has been reported to affect a variety of physical properties. For example, BO has been reported to reduce the compressive strength [12] and extend the setting time [13] of CSC.

BO is an RA, and MTA contains 20% BO, which increases the radiopacity of MTA. [14] Furthermore, BO affects $Ca(OH)_2$ precipitation after MTA hydration. [14] Since BO dissolves in an acidic environment, it has been suggested that placing MTA in an acidic environment, such as inflammatory tissues, might result in the release of BO, [14] which might decrease the biocompatibility of MTA because BO does not encourage cell proliferation in a cell culture. [15]

Bioaggregate (Innovative BioCeramix, Canada) has been introduced as a modified version of MTA. This cement contains Ta_2O_5 (TO), which has excellent biocompatibility, for radiopacity. [16, 17] In addition, compared with BO, TO is smooth and has a small particle size. The addition of TO as an RA may affect the physical properties of the CSC.

On the other hand, numerous attempts have been made to improve the handling properties of MTA by adding calcium compounds as a setting accelerator or other materials to enhance viscosity. [11, 18] Ber *et al.* [18] reported that all of the methylcellulose (MC), which is an anti-washout admixture, and MC/$CaCl_2$ concentrations greatly improved the handling of CSC. The molecular weight of MC is adjustable. When MC with various molecular weights is added to CSC, further variations in the properties of CSC may occur (although this has not yet been reported).

Therefore, improvement of the handling characteristics of CSC was examined in the present study. We examined the change in the physical properties of CSC caused by the addition of RA and/or the MC with various molecular weights. In the present study, CSC was investigated for use as a dental root sealing material. The examination was performed in accordance with ISO 6876-2012 (Dental root sealing materials) of the International Organization for Standardization. [19]

Materials and methods

1. Examination of radiopacity

The following experimental groups were established according to the materials to be tested: RA (BO or TO) (Wako, Japan) was added to PC (TAIHEIYO CEMENT, Japan) to final concentrations (%) of 0, 5, 10, 15, 20, and 25, respectively. Samples were mixed with pure water using a 1:0.35 powder/liquid ratio. The shape of the surface and the particle size of the RAs (BO and TO) were observed using a scanning electron microscope (S-2150, HITACHI, Japan) before the experiment (Fig 2).

The radiopacity test was conducted according to ISO 6876-2012. After mixing, each sample was packed into a stainless steel ring mold with an internal diameter of 10 mm and a depth of 1 mm. The mold was placed on a glass slab before inserting the material. The mold was then covered with a glass slide and allowed to set for 3 hr. Radiographs were taken with a focus-film distance of 300 mm. The dental X-ray unit (DCX-100N, ASAHI, Japan) was set at 70 kV, 7 mA, and an exposure time of 0.25 sec in order to provide a radiographic density reading for the exposed and processed film under a 1-mm-thick section of the aluminum stepwedge. Radiographs were taken for each sample. The exposed film was processed in an automatic developing machine. The photographic densitometer (PDA-15, Konica, Japan) was used to take readings of the

radiographic images of the samples, each step of the stepwedge, and the unexposed part of the film. Three readings were taken for each film, and the mean was calculated. The radiographic density values of the materials were transformed into a radiopacity expressed as the equivalent thickness of aluminum.

2. Examination of flow value and setting time due to the addition of RA and/or MCs with various molecular weights to CSC

The following experimental groups were established according to the materials to be tested: 1) MTA (control group) and 2) final concentrations of 18% or 20% RA (BO or TO) and 2% MC (MC 4000, 400, 25, Wako, Japan) contained in PC (Table 1). MC 4000, 400 and 25 was the abbreviation of the product name (Table 2). Samples were mixed with pure water using a 1:0.35 powder/liquid ratio. The conditions of PC for each molecular weight of MC are shown in Fig 3.

3. Flow value

The flow value test was conducted according to ISO 6876-2012. A total of 0.05 ml of each sample was placed on a glass plate (40 mm × 40 mm × 5 mm). At 180±5 sec after mixing was started, another plate with a mass of 20±2 g and a load of 100 g was placed

on top of the material. Ten minutes after mixing was started, the load was removed, and the major and minor diameters of the compressed material were measured. If both measurements were within 1 mm of each other, the results were recorded. If the major and minor diameter discs were not uniformly circular or did not fall within 1 mm of each other, the test was repeated. The test was conducted three times for each experimental group, and the mean value was recorded.

4. Setting time

The setting time test was conducted according to ISO 6876-2012. Each sample was placed in the mold (internal diameter: 10 mm, height: 1 mm) 120 sec after the start of mixing. Samples were maintained at a temperature of 37°C and a relative humidity of not less than 95%. After 30 min, the indenter as described in ISO 6876-2012 (mass: 100 g, diameter of flat end: 2 mm) was carefully lowered vertically onto the horizontal surfaces of the samples. The indenter tip was cleaned, and the operation was repeated at 5 min intervals until the needle failed to make a complete circular indentation in the sample. The time from the start of mixing at which this occurs was recorded. Setting times were determined as the mean of three test results for each experimental group.

5. Statistical analysis

The data obtained in experiment 2 were analyzed by a one-way ANOVA test for global comparison and by a Tukey's test for individual comparisons, and *p*-values of less than 0.05 were considered to be statistically significant.

Result

1. Examination of radiopacity

The radiopacity of the PC samples containing over 10% volume of BO or TO was higher than or equal to 3.00 mm Al (equivalent to a 3.00-mm-thick Al plate) as determined by ISO 6876-2012 (Fig 4).

2. Examination of flow value and setting time due to the addition of RA and/or MCs with various molecular weights to CSC

The results of the flow value test indicated that there was no significant difference among MTA, PCBO, and PCTO when MC was not added. The flow value increased when MC of lower molecular weight was added to PC in PCBO and PCTO. And experimental groups with the addition of low- molecular weight MC (MC25) exhibited a significantly higher flow value compared with other groups. A flow value of approximately 17.00 mm, as determined according to ISO 6876-2012, was only obtained in the PCTO+MC25 group, and the flow values of other groups were lower than 17.00 mm (Fig 5).

The setting time test revealed that all samples had a setting time of from 30 min to 72 hr, as determined by ISO 6876-2012. There was no significant difference in setting time

between PCBO groups and PCTO groups under the same MC condition. On the other hand, there was a significant difference between the MC-added groups and the MTA group. The setting time was extended by the addition of MC. In the case of MC addition, the setting time decreased in the order of MC25 > MC400 > MC4000 (Fig 6).

Discussion

We examined the physical properties of CSC by varying the RA and adding MC with various molecular weights. To date, the majority of the RA contained in CSC has been BO. However, problems such as adverse effects on physical properties and biocompatibility have been reported as a result of the addition of BO to CSC. [13] Therefore, TO was added to CSC in place of BO. [16]

There has been no comparative report on the radiopacity of TO and BO added to CSC. The results of experiment 1 reveal that the addition of approximately 10% of either TO and BO provided sufficient radiopacity to PC. Furthermore, BO and TO have approximately the same radiopacity, regardless of particle size. In the results of experiment 2, no significant difference in the flow value or the setting time was observed between the PCBO group and the PCTO group. Therefore, it is suggested that BO and TO can be used in the same manner. In the groups in which only RA was added to PC, no samples exhibited a flow value higher than 17.00 mm, as determined by ISO 6876-2012. Changing the RA alone seemed insufficient if CSC is to be used as a dental root sealing material.

In improving the flow value of CSC by MC addition, the flow value increased depending on the molecular weight of the MC, regardless of the type of RA.

Experimental groups with the addition of low- molecular weight MC (MC25) exhibited a significantly higher flow value compared with other groups ($p < 0.05$). Moreover, a flow value of approximately 17.00 mm, as determined according to ISO 6876-2012, was only obtained in the PCTO+MC25 group. Although the mean flow value of the PCTO+MC25 group satisfied the ISO standard, some of the samples did not fulfill the standard (Fig 5). In order to use of CSC as a dental root sealing material, all samples should be satisfy the ISO standard. Therefore, the flow value of CSC should be improved further. We have to investigate that improvement of existing additives, such as MC and propylene glycol, [11, 18, 20, 21] and search for new additives.

The results of experiment 2 revealed there was no significant difference in setting time between PCBO groups and PCTO groups under the same MC condition (MC4000, 400, and 25). On the other hand, the setting time was extended when lower molecular weight MC was added to PC. Therefore, the molecular weight of the MC is believed to affect the setting time, whereas the RA does not affect the setting time. The setting time test revealed that the setting times of all samples were within 30 min to 72 hr, as determined by ISO 6876-2012 (Fig 6). However, Torabinejad *et al*. [22] reported that MTA had a long setting time, which is a major drawback for use as a root canal sealing material. Moreover, Hsieh *et al*. [11] reported that the slow setting time of CSC made it a difficult

material to use. In the present study, the setting time of CSC was significantly extended by the addition of MC. Furthermore, a tendency for the setting time to be extended was observed as the flow value increased. In the future, it will be necessary to examine additives that reduce the setting time while maintaining the flow value.

Conclusion

1. TO and BO can be used in the same way as an RA in ISO 6876-2012.
2. PC containing TO as an RA and low- molecular weight MC (MC25) obtained a flow value more over 17.00 mm, as determined according to ISO 6876-2012.
3. The molecular weight of the MC affects the setting time of PC, whereas the RA does not affect the setting time of PC.

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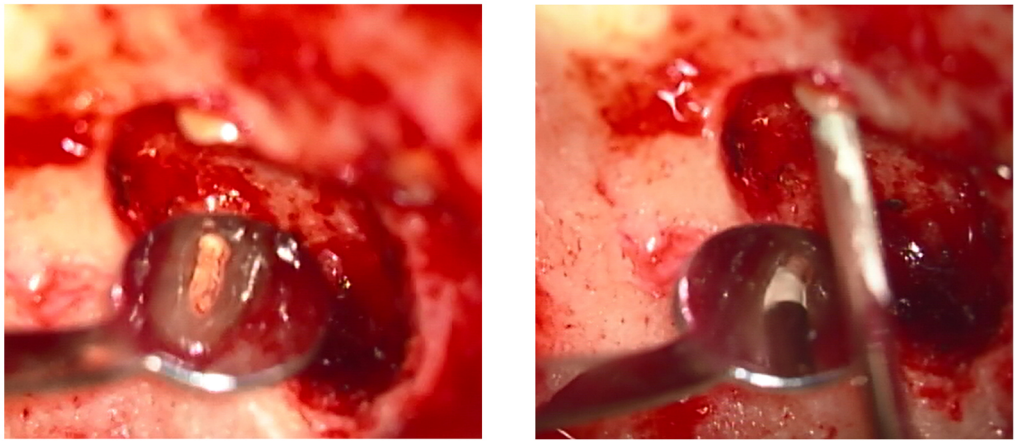


A B

Fig 1 After the keeping brightness and magnification by microscope (A), MTA is filled by exclusive instruments (B).

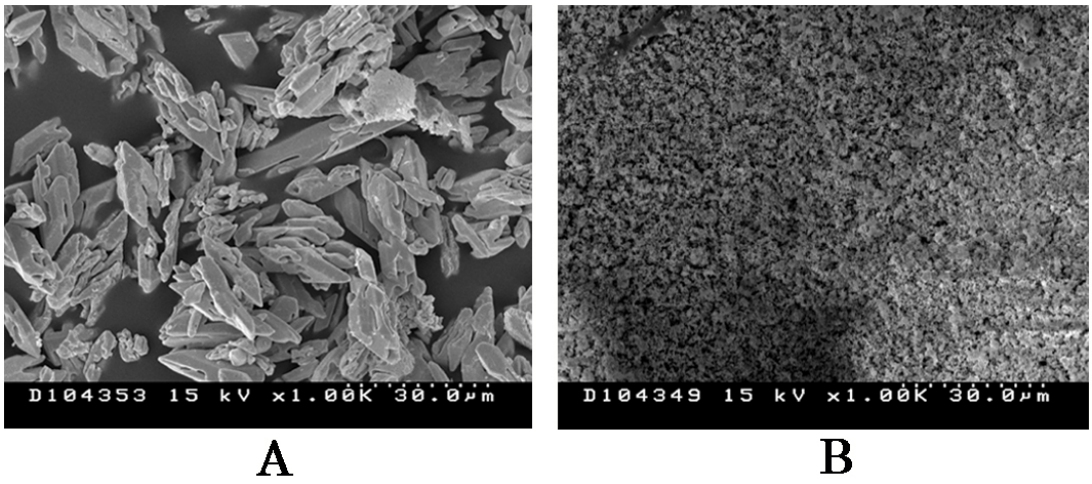
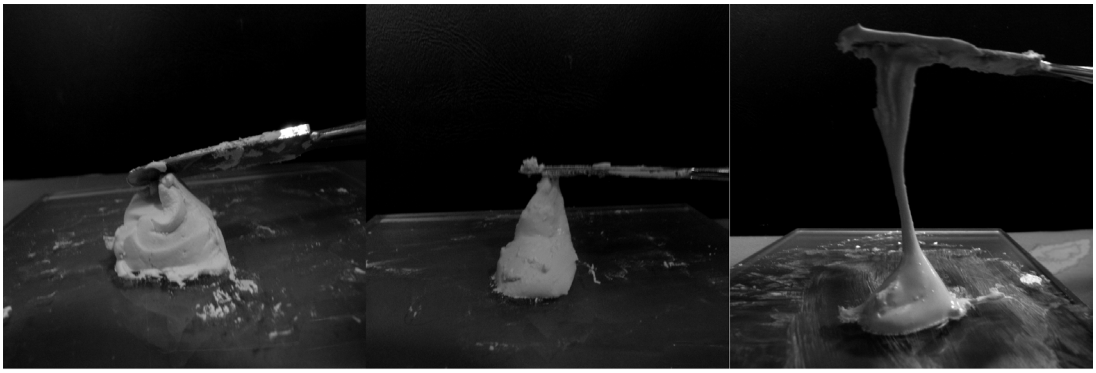


Fig 2 (A) BO: Shape of surface: rough, Particle size: approximately 30 μm.

(B) TO: Shape of surface: smooth, Particle size: less than 1 μm.



A B C

Fig 3

(A) 2%MC with a weight-average molecular weight of 140,000 was added to PC.

(B) 2%MC with a weight-average molecular weight of 84,000 was added to PC.

(C) 2%MC with a weight-average molecular weight of 40,000 was added to PC.

Table 1　Samples used in the experimental 2

	MTA	PC	BO	TO	MC
MTA	100%				
PCBO		80%	20%		
PCBO+MC4000		80%	18%		2%
PCBO+MC400		80%	18%		2%
PCBO+MC25		80%	18%		2%
PCTO		80%		20%	
PCTO+MC4000		80%		18%	2%
PCTO+MC400		80%		18%	2%
PCTO+MC25		80%		18%	2%

Samples for the flow and setting time tests were prepared using MTA as a control group. In test groups, final concentrations of 18% RA and 2% MC, or 20% RA alone were added to PC. Samples were mixed with pure water using a 1:0.35 powder/liquid ratio.

Table 2　The details of methylcellulose in present study.

	weight-average molecular weight	viscosity (2%, 20℃)	mean degree of polymerization
methylcellulose 4000	140, 000	3,500~5,600 mPa·s	740
methylcellulose 400	84, 000	350~550 mPa·s	440
methylcellulose 25	40, 000	20~30 mPa·s	200

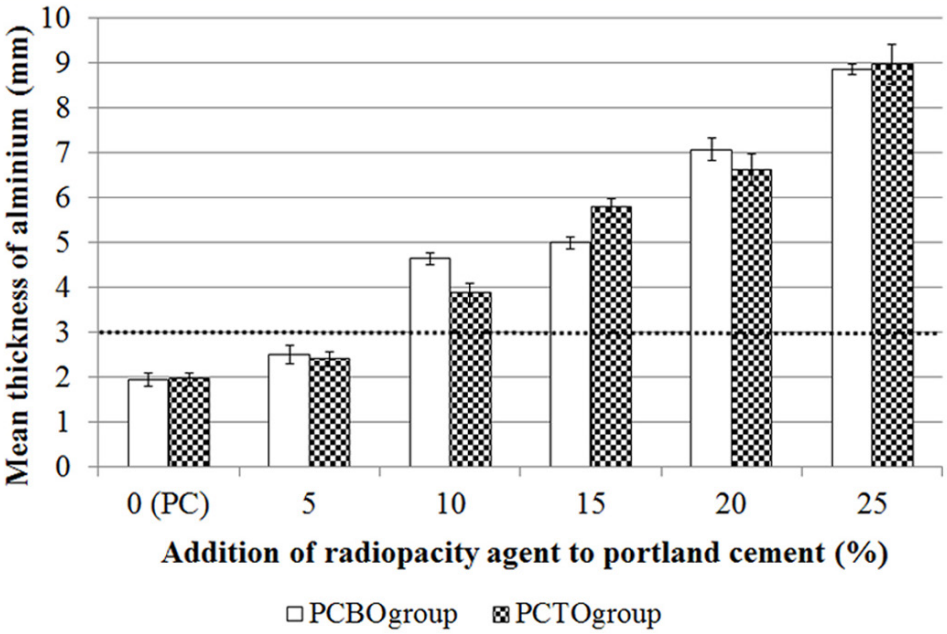
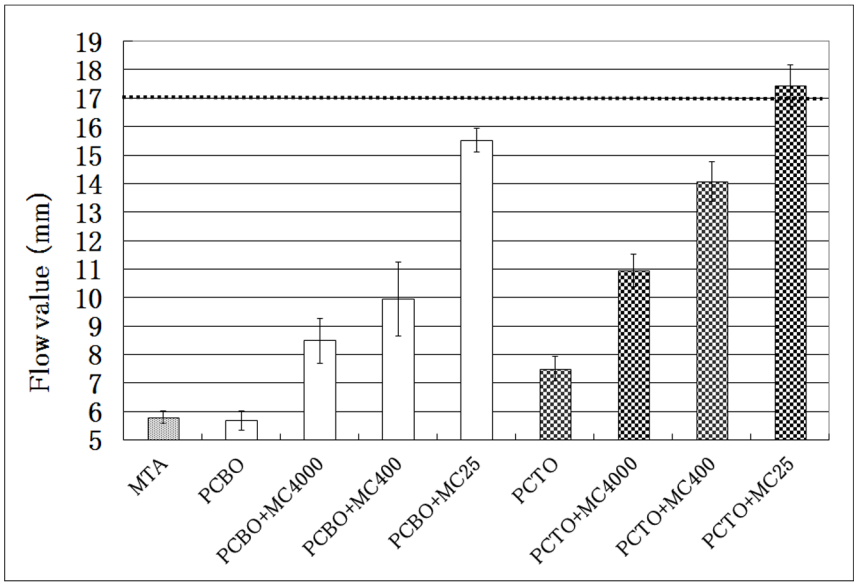


Fig 4 Radiopacity of the samples as determined according to ISO 6876-2012.

A radiopacity agent (BO or TO) that was added to PC to final concentrations (%) of 0, 5, 10, 15, 20, and 25, respectively, has been examined. Samples were mixed with pure water using a 1:0.35 powder/liquid ratio.

Reference value of radiopacity : greater than 3.00 mm Al (equivalent to a 3.00-mm-thick Al plate).



	MTA	PCBO	BO+4000	BO+400	BO+25	PCTO	TO+4000	TO+400	TO+25
MTA			●	●	●		●	●	●
PCBO			●	●	●		●	●	●
BO+4000					●		●	●	●
BO+400					●	●		●	●
BO+25						●	●		
PCTO							●	●	●
TO+4000								●	●
TO+400									●
TO+25									

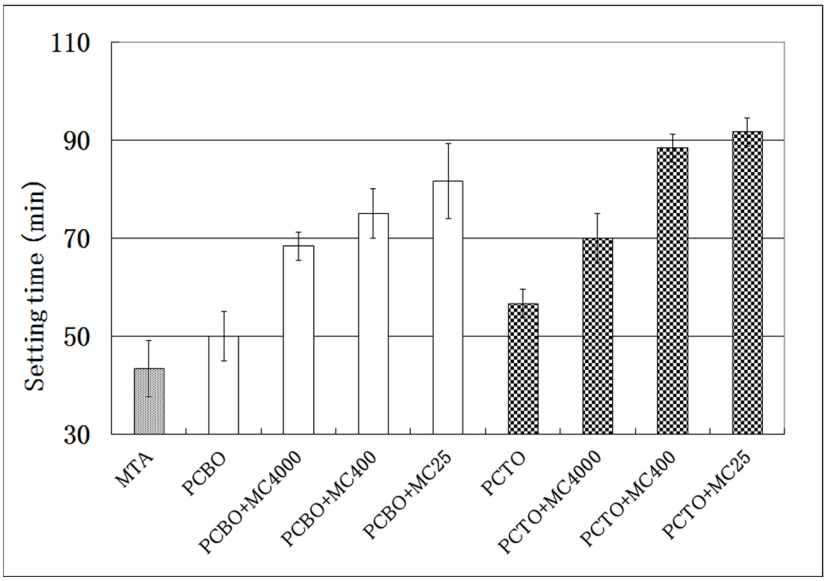
Fig 5 Flow value of the samples determined according to ISO 6876-2012.

All of the methods in the present study conform to ISO 6876-2012.

Reference value of flow : greater than 17.00 mm.

● showed significant differences by Tukey's multiple comparison

($n = 3$, $p < 0.05$)



	MTA	PCBO	BO+4000	BO+400	BO+25	PCTO	TO+4000	TO+400	TO+25
MTA			*	*	*		*	*	*
PCBO			*	*	*		*	*	*
BO+4000								*	*
BO+400						*			*
BO+25						*			
PCTO								*	*
TO+4000								*	*
TO+400									
TO+25									

Fig 6　Setting time of the samples determined according to ISO 6876-2012.

All of the methods in the present study conform to ISO 6876-2012.

Reference value of setting time : the setting time shall be within the range of 30 min to 72 hr.

＊ showed significant differences by Tukey's multiple comparison ($n = 3$, $p < 0.05$)

論文の内容の要旨

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専攻分野の名称：博士（歯学）

論文題名：PHYSICAL PROPERTIES OF CALCIUM SILICATE CEMENT WAS INFLUENCED BY RADIOPACITY AGENT AND/OR ANTI-WASHOUT ADMIXTURE
（エックス線不透過性重金属と水中不分離剤がケイ酸カルシウム系セメントの物理的性質に与える影響）

根管充填は，歯内療法分野における操作のひとつであり，根管拡大，洗浄によって無菌となった根管を緊密に充填する事により二次感染を防止し，歯の機能を維持するものである。現在までに，様々な根管充填材料が開発，応用されてきた中で，ケイ酸カルシウム系セメント（CSC）の市販品である Mineral trioxide aggregate（MTA，ProRoot MTA; Dentsply-Maillefer，Switzerland）は，良好な生体親和性などの，根管充填材料の要件を多く満たし，歯内療法分野において非常に有用な材料である。しかしながら，MTA をはじめとする CSC はいずれも，granular consistency,，slow setting time,，および initial looseness などの性質を持つため，操作性が悪い材料であると報告されており，根管充填材料としての使用には，いくつかの物理的性質の改善を要する材料である。

CSC には，治療状態等を確認するためにエックス線不透過性材料（RA）が 20%程度添加されている。MTA に添加されている RA は Bi_2O_3（BO）であるが，セメントの生体親和性および物理的性質に影響を与える可能性が指摘されたため，Ta_2O_5（TO） を添加している CSC も存在する。TO の粒径は BO よりも小さく，表面が滑沢であるため，CSC に添加することによって物理的性質に変化を与える可能性が考えられる。

一方，水中不分離剤である methylcellulose（MC）の添加によって CSC の操作性を改善したという報告があるが，添加した MC の詳細について記載はなく，どのような性質をもつ MC を添加したのかが不明瞭である。特に，MC の分子量は調節することが可能であり，添加する MC の

分子量の違いによって，CSC の物理的性質が多様に変化する可能性が考えられるが，現在までにその報告はない。

そこで本研究の目的は，RA および，分子量の異なる MC の添加による，CSC の物理的性質への影響を検討し，臨床における操作性改善の一助とするものである。

エックス線不透過性試験：CSC に添加した際の，BO および TO のエックス線不透過性について検討した。CSC として，本実験では Portland cement（PC）を使用した。PC に BO または TO を 0，5，10，15，20，25%添加した群を用意した。試料は pure water にて練和し，粉液比 1 : 0.35 とした。ISO 規格 6876-2012 に記載される方法に準じて，エックス線不透過性試験を行った結果，TO を添加した群は，BO を添加した群と同程度のエックス線不透過性を有していた。

フロー値測定試験および凝結時間測定試験：RA および，水中不分離剤である MC の添加が，CSC のフロー値および凝結時間に与える影響について検討した。コントロール群として MTA 群，実験群として PC に RA（BO または TO）を 20%添加した 2 つの群，および PC に RA を 18%，MC（分子量 140,000，84,000 および 40,000）を 2%添加した 6 つの群（計 9 群）を用意した。試料は pure water にて練和し，粉液比 1 : 0.35 とした。ISO6876-2012 に記載された方法に順じて，フロー値測定試験，および凝結時間測定試験を行った。実測値は全て，一元配置の分散分析後，Tukey 検定（$p < 0.05$）で統計処理を行った。

結果として，PC に RA を 20%添加した 2 つの群のフロー値および凝結時間に有意な差は認められず，RA の違いは CSC の物理的性質に影響しなかった。また，この 2 つの群には，フロー値試験の基準値を満たす試料は得られず，そのため，根管充填材料として用いるならば，RA の置換のみでは不十分である事が示唆された。

次に，MC の添加による CSC のフロー値向上について，RA の種類に関わらず，低分子量の MC を PC に添加した群は，より高い分子量の MC を添加した群と比較して有意に高いフロー値を得た。また，PC に TO と低分子量の MC を添加した群では，本実験で検討した群のうち最大のフロー値が得られ，その群の平均値が，ISO 規格 6876-2012 に記載されるフロー値測定試験の基準

値である 17.00 mm を超える値であった。

凝結時間測定試験の結果から，BO を添加した 4 つの群（PC に BO のみ添加した 1 群，および BO と各分子量の MC と添加した 3 群）と，TO を添加した 4 つの群（PC に TO のみ添加した 1 群，および TO と各分子量の MC と添加した 3 群）の間に有意な差を認めなかった。また，添加された MC の分子量が低い程，凝結時間は延長した。よって，CSC の凝結時間には，RA の違いよりも MC の分子量が強く影響する事が示唆された。

結論として，本研究は以下の事柄を明らかとした。

1. 本研究において，RA の違いは CSC の物理的性質に影響を与えなかった。

2. TO および低分子量の MC 添加が，ISO 規格の基準値を満たすフロー値を CSC に与えた。

3. CSC の凝結時間には，RA の違いよりも MC の分子量が強く影響した。