Ingredients and cytotoxicity of MTA and 3 kinds of Portland cements

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ABSTRACT

The aim of this study was to compare the compositions and cytotoxicity of white ProRoot MTA (white mineral trioxide aggregate) and 3 kinds of Portland cements. The elements, simple oxides and phase compositions of white MTA (WMTA), gray Portland cement (GPC), white Portland cement (WPC) and fast setting cement (FSC) were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES), X-ray fluorescence spectrometry (XRF) and X-ray diffractometry (XRD). Agar diffusion test was carried out to evaluate the cytotoxicity of WMTA and 3 kinds of Portland cements.

The results showed that WMTA and WPC contained far less magnesium (Mg), iron (Fe), manganese (Mn), and zinc (Zn) than GPC and FSC. FSC contained far more aluminum oxide (Al_2O_3) than WMTA, GPC, and WPC. WMTA, GPC, WPC and FSC were composed of main phases, such as tricalcicium silicate ($3CaO \cdot SiO_2$), dicalcium silicate ($2CaO \cdot SiO_2$), tricalcium aluminate ($3CaO \cdot Al_2O_3$), and tetracalcium aluminoferrite ($4CaO \cdot Al_2O_3 \cdot Fe_2O_3$). The significance of the differences in cellular response between WMTA, GPC, WPC and FSC was statistically analyzed by Kruskal-Wallis Exact test with Bonferroni's correction. The result showed no statistically significant difference ($p > 0.05$) .

WMTA, GPC, WPC and FSC showed similar compositions. However there were notable differences in the content of minor elements, such as aluminum (Al), magnesium, iron, manganese , and zinc. These differences might influence the physical properties of cements. 〔J Kor Acad Cons Dent 33(4):369-376, 2008〕

Key words : White MTA (white mineral trioxide aggregate), Portland cement, ICP-AES (Inductively coupled plasma atomic emission spectrometry), XRF (X-ray fluorescence spectrometry), XRD (X-ray diffractometry), Agar diffusion test

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Ⅰ. INTRODUCTION

Mineral trioxide aggregate (MTA) was developed in 1993 and has expanded its application widely. Because of its good biocompatibility[1,2], physical property, and antibacterial effect[3], it has been

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used in root end filling[4-6], pulpotomy[7], perforation repair[8,9], coronal barrier[10], and root canal filling.

In spite of these superior characteristics, the high cost of MTA limited its use in Korea. Recently many studies have been done to compare the compositions[11-13], biocompatibility[14-16], and physical properties[17-19] of MTA and Portland cements. Many researchers suggested that Portland cement is as biocompatible and have as good physical properties as MTA.

But Dammaschke *et al.*[20] reported that MTA contained significantly less iron (Fe) and manganese (Mn) than Portland cement and MTA cannot be simply replaced by cheaper Portland cement. But so far, only a little information about chemical compositions of Portland cement can be found in the literature[20] and precise comparative study of the compositions of MTA and Portland cement is required.

The main drawback of MTA is its long setting time. The setting time of MTA was reported to be 2 h 45 min[21]. Experimentally, $CaCl_2$ was added to shorten the setting time and improve the mechanical properties of MTA[22,23]. In this experiment, fast setting cement was used as future possible alternative for MTA. White Portland cement was also used in this experiment, which is known to be the material used for the production of white MTA[24].

The purposes of this study were to investigate the compositions and evaluate the cytotoxicity of MTA (tooth colored formula, WMTA), gray Portland cement (GPC), white Portland cement (WPC), and fast setting cement (FSC).

Ⅱ. MATERIALS AND METHODS

We used GPC (Lafarge Halla cement Corp., Seoul, Korea), WPC (Union Corp., Seoul, Korea), FSC (SSangyong cement industrial Co., Seoul, Korea) and WMTA (ProRoot MTA , tooth colored formula, Dentsply Tulsa dental, Johnson city, TN, USA).

Analysis of element by ICP-AES (Inductively coupled plasma atomic emission spectrometry)

ICP-AES (ICPS-1000Ⅳ, Shimadzu, Kyoto, Japan) was used to investigate the elements that comprise WMTA, GPC, WPC and FSC. Argon Plasma (6000K) was used. Detection limit of ICP-AES was 1-10 ppb. Measurement was carried out in triplicate and the mean value was determined.

Analysis of simple oxide by XRF (X-ray fluorescence spectrometry)

XRF (XRF-1700, Shimadzu, Kyoto, Japan) was used to investigate the relative proportions of simple oxides that comprise WMTA, GPC, WPC and FSC. We used Rh (rhodium) target and selected 40Kv and 30 mA for X-ray generator. Measurement was carried out in triplicate and the mean value was determined.

Phase identification by XRD (X-ray diffraction)

To investigate the large compounds and phases, XRD analysis (D8-advance, Bruker, Madison, WI, USA) was carried out. Phase identification was accomplished by the use of search-match software (Eva Version 9.0 Diffrac plus, Bruker, Madison, WI, USA). Target material was Cu. System capacity was 40kV and 40 mA.

XRF analysis was carried out in NCIRF (National Center for Inter-University Research Facilities, Seoul, Korea). XRD analysis was carried out in SNU DRI (Seoul National University Dental Research Institute, Seoul, Korea). ICP-AES analysis was carried out in NICEM (National Instrumentation center for environmental management, Seoul, Korea).

Cytotoxicity test by Agar diffusion method

L929 mouse fibroblast cells were used. Cells were grown in minimum essential medium under standard cell culture conditions (37°C, 5 % CO_2). Experimental materials were sterilized with ethylene oxide gas. Cultured cells were seeded into 6-well plate at an initial density of 2×10^5 cells/well with 2 ㎖ of medium and incubated for 24 hours. After 24 hours, 2 ㎖ mixture of agar-medium was added into each well of 6-well plate and stained with Neutral Red solution. Freshly mixed MTA (water powder [w/p] ratio 1 : 1), GPC (w/p ratio 1 : 2), WPC (w/p ratio 1 : 2) and FSC (w/p ratio 1 : 2) were inserted in polyethylene tube (3 ㎜ in inner diameter and 5 ㎜ in height) and placed on cultured cells. For each material (WMTA, GPC, WPC, and FSC), agar diffusion test was carried out in triplicate and the mean value was determined.

6, 12, 18, and 24 hours after placing the samples, the width of decolorization zones was measured. Zinc oxide euginol (ZOE) paste and Teflon discs were used as positive and negative controls. The significance of the difference in cellular response between WMTA, GPC, WPC and FSC was statistically analyzed by Kruskal-Wallis Exact test with Bonferroni's correction.

Ⅲ. RESULTS

Compositions of elements

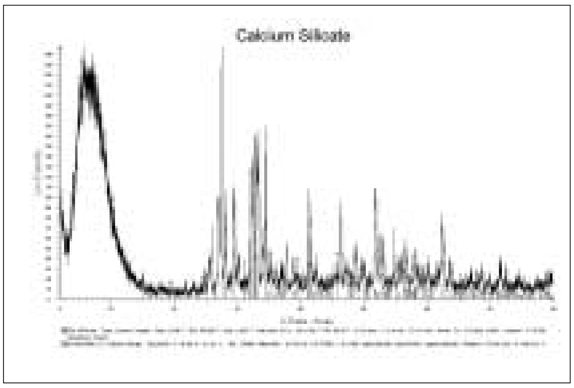
The compositions of main elements were shown in Table 1. FSC showed remarkably high concentrations of aluminum (Al), sulfur (S), and titanium (Ti). WPC showed high concentration of Fluorine (F). The concentrations of iron, magnesium (Mg), manganese(Mn) and zinc (Zn) in WMTA and WPC were lower than those in GPC and FSC.

Compositions of main oxides

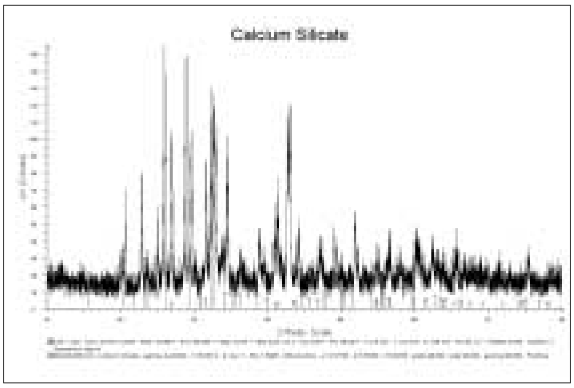
The results of XRF showed that calcium oxide (CaO) and silicate (SiO_2) were two main simple oxides that comprise WMTA, GPC, WPC and FSC. Aluminum oxide (Al_2O_3) content in FSC was higher than those in WMTA, GPC and WPC. The compositions of main oxides that comprise WMTA and Portland cements were shown in Table 1.

Mineral phase identification

The results of XRD analysis showed that main mineral phases comprising WMTA and Portland cements were similar (Figure 1). They were mainly tricalcicium silicate ($3CaO \cdot SiO_2$), dicalcium silicate ($2CaO \cdot SiO_2$), tricalcium aluminate ($3CaO \cdot Al_2O_3$) and tetracalcium aluminoferrite ($4CaO \cdot Al_2O_3 \cdot Fe_2O_3$). Other mineral phases such as calcium silicate ($3CaO \cdot SiO_2$), calcium aluminum oxide ($3CaO \cdot Al_2O_3$), calcium aluminum oxide sulfate ($3CaO \cdot 3Al_2O_3 \cdot CaSO_4$), magnesium oxide (MgO), cummingtonite (($Mg_{4.68}$ $Fe_{2.32}$) $Si_8O_{22}(OH)_2$), magnesiocummingtonite (($Fe_{3.17}$ $Mg_{3.83}$) ($Si_8O_{22}(OH)_2$)), gehlenite ($2CaO \cdot Al_2O_3 \cdot SiO_2$) were also found in WMTA and Portland cements.



(a) White MTA



(b) Portland cement

Figure 1. X-ray diffraction (XRD) analysis of white MTA (a) and Portland cement (b) showing the main phase ($2CaO \cdot SiO_2$) present in the cement. (MTA: mineral trioxide aggregate) (Black : peaks made by specimen, Red : peaks of reference)

Table 1. Compositions of elements, simple oxides, and phases that comprise WMTA, GPC, WPC and FSC

		MTA	GPC	WPC	FSC
Composition of main elements measured by ICP -AES(wt %)	Ca	25.23	29.33	29.36	23.47
	S	0.87	1.17	1.41	4.30
	Al	0.71	2.33	2.22	8.47
	Mg	0.25	1.88	0.65	1.01
	Fe	0.11	1.42	0.13	1.03
	K	0.09	0.72	0.10	0.42
	Sr	0.05	0.03	0.04	0.02
	F	0.0225	0.0379	0.1704	0.0954
	Ti	0.01	0.06	0.02	0.23
	Mn	0.006	0.043	0.005	0.035
	Zn	0.0006	0.069	0.0009	0.0157
Composition of simple oxide measured by XRF (wt %)	CaO	47.18	61.84	65.92	48.64
	SiO_2	19.42	21.00	21.39	11.62
	Al_2O_3	1.39	5.07	4.39	17.45
	Fe_2O_3	0.70	2.76	0.80	2.09
	MgO	0.34	3.28	1.16	1.50
	K_2O	0.04	1.11	-	0.61
Composition of phases (wt %) calculated by Bogue's method	$3CaO \cdot SiO_2$		54.07	75.09	
	$2CaO \cdot SiO_2$		19.50	4.77	
	$3CaO \cdot Al_2O_3$		8.77	10.28	
	$4CaO \cdot Al_2O_3 \cdot Fe_2O_3$		8.39	2.43	

Silicon (Si) was not measured because of technical difficulty. Main phases that comprise MTA and FSC were identified by XRD analysis. But the phase compositions of MTA and FSC could not be calculated.
(WMTA: white mineral trioxide aggregate, GPC: gray Portland cement, WPC: white Portland cement, FSC: fast setting cement, XRD: X-ray diffractometry, ICP-AES: Inductively coupled plasma atomic emission spectrometry, XRF: X-ray fluorescence spectrometry)

Table 2. The width of decolorization zone created at 6, 12, 18, and 24 hours after placement of samples (㎜)

	6H	12H	18H	24H
WMTA (n = 3)	1.23 (± 0.12)	1.37 (± 0.06)	1.43 (± 0.06)	1.5 (± 0.10)
GPC (n = 3)	1.23 (± 0.12)	1.47 (± 0.06)	1.47 (± 0.06)	1.47 (± 0.06)
WPC (n = 3)	1.23 (± 0.12)	1.43 (± 0.06)	1.47 (± 0.06)	1.47 (± 0.06)
FSC (n = 3)	1.23 (± 0.12)	1.3 (± 0.10)	1.33 (± 0.12)	1.43 (0.06)
ZOE (n = 3)	3.9	+	+	+
Teflon (n = 3)	-	-	-	-

(Mean ± SD, WMTA : White mineral trioxide aggregate, GPC : gray Portland cement, WPC : white Portland cement, FSC : fast setting cement, H : hours, + : complete decolorization, - : no discoloration)

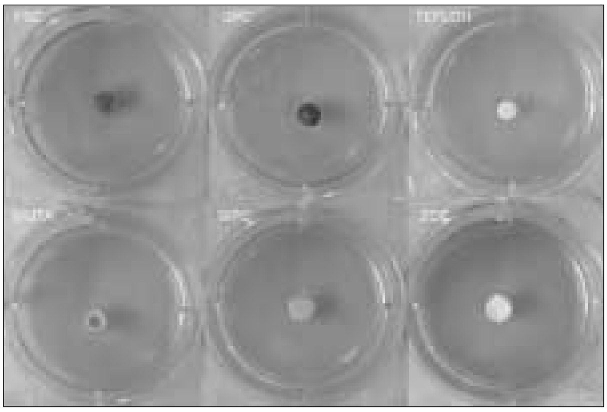


Figure 2. Cellular response to WMTA, GPC, WPC, and FSC, 6 hrs after placement of specimens.
(WMTA : white MTA, GPC : Gray Portland cement, WPC : white Portland cement, and FSC : Fast setting cement, ZOE : Zinc Oxide and Euginol)

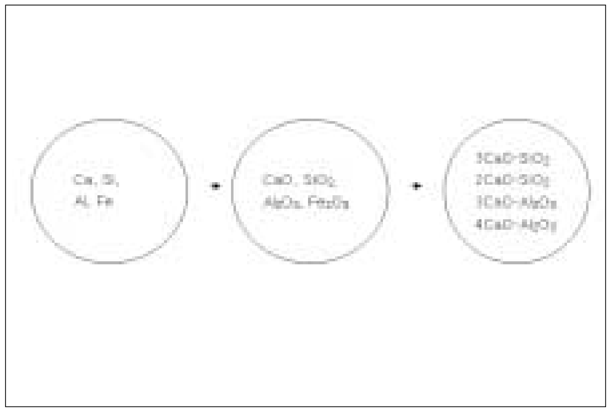


Figure 3. Schematic diagram showing the compositions of WMTA and Portland cements.
(WMTA : white mineral trioxide aggregate)

Measurement of decolorization zone

Decolorization zones were observed around the samples. Decolorization zones were smaller than 2 ㎜ in all groups except positive control (ZOE) group (Table 2) (Figure 2). The result showed no statistically significant difference ($p > 0.05$).

Ⅳ. DISCUSSION

Dammaschke *et al.*[20] analyzed the compositions of MTA and Portland cements by X-ray photoelectron spectroscopy (XPS), energy dispersive X-ray analysis (EDX), and inductively coupled plasma optical emission spectrometry (ICP-OES). Camilleri *et al.*[25,26] studied the chemical composition with EDX and XRD. These studies were mainly focused in elemental analysis and phase identification.

MTA and Portland cement are complex compounds and they are composed of mineral phases. These mineral phases are composed of simple oxides and these simple oxides are composed of elements. So in order to investigate the chemical compositions of WMTA and Portland cements, compositions of elements that comprise simple oxides were investigated. And then, compositions of simple oxides that comprise mineral phases were measured (Figure 3). And finally, compositions of large phases that comprise WMTA and Portland cements were calculated.

There has been no study that investigated the compositions of WMTA and Portland cements from elemental level to mineral phase level. Knowing the compositions of WMTA and Portland cement from elemental level to mineral phase level can be helpful in understanding the physical properties of WMTA and Portland cement.

ICP-AES analysis showed that the concentrations of magnesium, iron , manganese and zinc in WMTA and WPC were remarkably lower than those in GPC and FSC. This explains the color differences between WPC and GPC because iron and manganese are well-known chromophores[20]. It was interesting that WPC had the highest fluorine content among 4 samples. We might expect some anticariogenic effect of WPC when it is used clinically. Bismuth was found only in WMTA. Bismuth was reported to decrease the mechanical strength of MTA[27].

There has been few study that measured the compositions of simple oxides that comprise WMTA and Portland cements in unhydrated forms. By knowing the compositions of simple oxides, we can calculate the compositions of phases that comprise Portland cement. Two main oxides that compose WMTA and Portland cements were calcium oxide and silicate. Aluminum oxide content was remarkably high in fast setting cement, which might explain the short setting time of FSC. Bismuth oxide was known to be present in WMTA. However the amount of Bismuth oxide was not measured in this study due to technical difficulties.

The compositions of phases can be calculated from the compositions of CaO, SiO_2, Al_2O_3, Fe_2O_3, and SO_3 measured by XRF analysis. By Bogue's method[28], the compositions of tricalcium oxide, dicalcium oxide, tricalcium aluminate and tetracalcium aluminoferrite which comprise GPC and WPC were calculated. Bogue's method was not applicable to the calculation of mineral phase of WMTA and FSC, because Bogue's method is based on the chemical assumption that Portland cement is composed entirely of CaO, SiO_2, Al_2O_3, and Fe_2O_3 and this assumption does not apply to WMTA and FSC.

Min *et al.*[29] reported that Portland cement was biocompatible and had the potential to be used in pulp capping. Cellular responses to WMTA, GPC, WPC, and FSC showed no statistically significant differences ($p > 0.05$).

Ⅴ. CONCLUSION

The main elemental composition of WMTA and 3 Portland cement were similar. WMTA and WPC contained far less magnesium, iron, manganese, and zinc than GPC and FSC. WPC contained higher Fluorine than WMTA, GPC and FSC. FSC contained higher aluminum oxide than WMTA, GPC, and WPC. WMTA, GPC, WPC, and FSC were composed of tricalcicium silicate, dicalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite. The difference in cellular responses between WMTA, GPC, WPC and FSC was not statistically significant ($p > 0.05$). WPC showed remarkably lower heavy metal contents and higher fluorine content than GPC and FSC. This may contribute to good biocompatibility and anticariogenic effect.

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국문초록

MTA와 포틀랜드 시멘트의 구성성분분석과 세포독성에 관한 연구

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이 연구의 목적은 3 종의 포틀랜드 시멘트 (포틀랜드 시멘트, 백색 포틀랜드 시멘트, 초속경 시멘트)와 white MTA의 성분 및 세포독성을 비교하는 것이다. 성분비교를 위해서 X선 회절기 (XRD), X선 형광분석기 (XRF), 유도결합플라즈마 원자방출분광 분석기 (ICP-AES)를 사용하였으며, 세포독성비교를 위해서는 우무확산법 (agar diffusion test)을 사용하였다. 분석 결과, white MTA와 백색 포틀랜드 시멘트는 포틀랜드 시멘트나 초속경 시멘트에 비해 적은 양의 마그네슘 (mg), 철 (Fe), 아연 (Zn), 그리고 망간 (Mn)을 함유하고 있었다. 또한 초속경 시멘트는 다른 시멘트 및 white MTA에 비해 많은 산화 알루미늄 (Al_2O_3)을 함유하고 있었다. MTA와 포틀랜드 시멘트의 주된 성분은 tricalcicium silicate ($3CaO \cdot SiO_2$), dicalcium silicate ($2CaO \cdot SiO_2$), tricalcium aluminate ($3CaO \cdot Al_2O_3$), 그리고 tetracalcium aluminoferrite ($4CaO \cdot Al_2O_3 \cdot Fe_2O_3$)등이었다. 세포독성 실험결과를 Kruskal-Wallis Exact test와 Bonferroni 사후 검정법을 사용하여 분석한 결과 white MTA와 3 종의 포틀랜드 시멘트 군 사이에서 통계적으로 유의성 있는 차이를 보이지 않았다 ($p > 0.05$). White MTA와 3종의 포틀랜드 시멘트의 주성분은 유사하였으나 알루미늄 (Al), 마그네슘 (mg), 철 (Fe), 아연 (Zn), 그리고 망간 (Mn) 등의 함량에서는 차이를 보였으며 이러한 차이들은 물리적 성질에 영향을 미칠 것으로 보인다.

주요어: MTA (mineral trioxide aggregate), 포틀랜드 시멘트, XRD, XRF, ICP-AES, 우무확산법