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## Composition and microstructure of MTA and Aureoseal Plus: XRF, EDS, XRD and FESEM evaluation

### ABSTRACT

**Aim** The aim of this study was to determine the chemical composition and the phases' microstructure of Aureoseal Plus (OGNA, Italy) and ProRoot MTA (Dentsply Tulsa Dental, USA) and to compare their characteristics.

**Methods** Study Design: Comparing Aureoseal Plus and ProRoot MTA microstructure by means of several analyses type. The chemical analysis of the two cements was assessed following the UNI EN ISO 196-2 norm. X-Ray fluorescence (XRF) was used to determine the element composition. The crystalline structure was analysed quantitatively using x-ray diffraction (XRD). Powders morphology was evaluated using a scanning electron microscope (SEM) with backscattering detectors, and a field emission scanning electron microscope (FESEM). Elemental analysis was performed by energy dispersive x-ray analysis (EDS).

**Results** The semi-quantitative XRF analysis showed the presence of heavy metal oxides in both cements. The XRD spectra of the two cements reported the presence of dicalcium silicate, tricalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, bismuth oxide and gypsum. SEM analysis showed that ProRoot MTA powder is less coarse and more homogeneous than Aureoseal. Both powders are formed by particles of different shapes: round, prismatic and oblong. The EDS analysis showed that some ProRoot MTA particles, differently from Aureoseal, contain Ca, Si, Al and Fe. Oblong particles in ProRoot and Aureoseal are rich of bismuth.

**Conclusions** The strong interest in developing new Portland cement-based endodontic sealers will create

materials with increased handling characteristics and physicochemical properties. A thorough investigation on two cement powders was carried out by using XRF, XRD, SEM and EDS analysis. To date there was a lack of studies on Aureoseal Plus. This cement is similar in composition to ProRoot MTA. Despite that it has distinctive elements that could improve its characteristics, resulting in a good alternative to MTA.

**Keywords** ProRoot MTA, Aureoseal Plus, XRF, XRD, SEM, FESEM, EDS.

### Introduction

Mineral trioxide aggregate (MTA) (ProRoot; Dentsply Tulsa Dental, Tulsa, USA) was developed at Loma Linda University in the 1990s by Torabinejad and subsequently approved by the Food and Drug Administration [Torabinejad et al., 1995]. MTA consists of a fine powder of tricalcium silicate ( $3\text{CaO}\cdot\text{SiO}_2$ ), tricalcium aluminate ( $\text{Ca}_3\text{Al}_2\text{O}_6$ ), dicalcium silicate ( $2\text{CaO}\cdot\text{SiO}_2$ ), calcium sulphate dehydrate ( $\text{H}_4\text{CaO}_6\text{S}$ ) and bismuth oxide ( $\text{Bi}_2\text{O}_3$ ) [Torabinejad et al., 1995; Lee et al. 1993; Camilleri et al., 2005]. It is basically a Portland Cement (PC) with 4:1 proportion of bismuth oxide added for radiopacity [Camilleri et al., 2005; Camilleri et al., 2006; Mitchell et al., 1999]. When hydrated, the silicate phases of PCs undergo a series of physicochemical reactions resulting in the formation of a nanoporous matrix/gel of calcium silicate hydrates (C-S-H phases), and of a soluble fraction of calcium hydroxide  $\text{Ca}(\text{OH})_2$ , leading to an alkaline pH. MTA can set and develops its properties in a wet environment because of its hydrophilic nature. The hydration setting reaction reaches an initial set in 3–4 hours, but maturation and resistance to dislodgement increases with time [Torabinejad et al., 1995; Dammaschke et al., 2005]. Hydrated MTA's initial pH is 10.2 and it grows to a maximum value of 12.5 three hours after mixing [Torabinejad et al., 1995; Camilleri et al., 2005; Dammaschke et al., 2005; Asgary et al., 2005]. MTA's alkalinity can explain its induction of hard tissues formation and its antibacterial and antifungal activity [Roberts et al., 2008]. Its biocompatibility, hydrophilic nature, antibacterial and good sealing ability, good marginal adaptation and long-term stability [Torabinejad et al., 1995; Abdullah et al., 2002; Torabinejad et al., 1995b; Torabinejad et al., 1993; Briso et al., 2006; Pereira et al., 2004; Ribeiro et al., 2005], make it useful in several clinical situations as direct pulp capping [Camilleri et al., 2008], apexification, root-end filling and reparation of root perforation [Parirokh et al., 2010], internal root resorption [Lee et al., 1993; Torabinejad et al., 1999; Tittle et al., 1996; Shabahang et al., 1999; Pitt Ford et al., 1995]. However, MTA has some drawbacks including long setting time [Jang et al., 2013], initial looseness [Choi et al., 2013], poor handling characteristics

[Kogan et al., 2006], limited physical strength properties [Islam et al., 2006; Camilleri, 2008, 2010; Nekoofar et al., 2010], gum darkening and high cost [Jacobovitz et al., 2009]. These properties raised the interest of scientists worldwide to improve the handling characteristics and some physicochemical properties of MTA. Consequently, new MTA-based root end filling cements have been proposed, such as Aureoseal Plus (OGNA, Milano, Italy). It mainly consists of PC type I, with bismuth oxide, accelerator and plasticizing agents that are not specified by the manufacturer. Due to the lack of studies on Aureoseal Plus (OGNA), the aim of the study was to assess the chemical composition and the phases' microstructures of this cement, and to compare its characteristics to those of ProRoot MTA (Dentsply Tulsa Dental).

## Materials and methods

ProRoot MTA (Dentsply Tulsa Dental) and Aureoseal Plus (OGNA) were used in the study. The chemical analysis of the two cement powders followed the UNI EN ISO 196-2 norm. X-ray fluorescence (XRF) (EDXRF; Bruker S2 Ranger, Bruker Corporation, Madison, USA) was used to determine the elements composition. Powders were analysed quantitatively by X-ray diffraction analysis (XRD) using CuK $\alpha$  radiation (1.54439 Å) in order to investigate the crystalline structure of cements. 1.5 g of each specimen was placed into a sample holder and packed with a sterile glass slide to provide a uniform surface. The diffractometer (X'PERT PRO Alpha-1, PANalytical, Almelo, The Netherlands) was set to run at 40 kV and 40 mA in continuous mode. The scan range was 10-70°2 $\theta$  with a scan speed of 2°2 $\theta$  per minute. Peaks of diffraction patterns of each sample were matched with those of the standard data in the powder diffraction files (PDF) (X-PERT JCPDS DATABASE 32 PC PDF WIN). The morphology of the powders was evaluated using a scanning electron microscope (SEM) (JSM-66; Leol Ltd., Tokyo, Japan), also with backscattering detectors, and a field emission scanning electron microscope (FESEM) (FESEM LEO SUPRA 35, Oxford Instruments). Elemental analysis was performed by energy dispersive x-ray analysis (EDS), using INCA X-SIGHT detector, which was attached to the SEM. Cements were sprinkled on the top side of carbon double-sided tape attached to an aluminium stub. High magnifications were used to discern the chemical compositions of specific crystal types within a sample. Via this process a spectrum was obtained, and elements could be identified.

## Results

### XRF Analysis

The chemical composition of Aureoseal Plus was established with the UNI EN ISO 196-2 norm (Table 1). The semi-quantitative XRF analysis showed the presence

Chemical Formula	Weight percentage
CaO	38.00
RBC	36.00
SiO <sub>2</sub>	12.00
Al <sub>2</sub> O <sub>3</sub>	3.03
SO <sub>3</sub>	2.00
Bi <sub>2</sub> O <sub>3</sub>	1.72
Fe <sub>2</sub> O <sub>3</sub>	1.52
MgO	1.40
K <sub>2</sub> O	0.60
Na <sub>2</sub> O	0.20
TiO <sub>2</sub>	0.14
Ignition Loss	3.10

TABLE 1 Chemical analysis of Aureoseal Plus.

Chemical Formula	Weight percentage
CaO	48.00
RBC	16.38
SiO <sub>2</sub>	11.70
Al <sub>2</sub> O <sub>3</sub>	4.75
SO <sub>3</sub>	4.26
Bi <sub>2</sub> O <sub>3</sub>	3.24
Fe <sub>2</sub> O <sub>3</sub>	2.54
MgO	0.16
K <sub>2</sub> O	0.11
Na <sub>2</sub> O	0.06
TiO <sub>2</sub>	4.50
Ignition Loss	3.10

TABLE 2 Chemical analysis of ProRoot MTA.

of heavy metal oxides: P<sub>2</sub>O<sub>5</sub> (0,140%), ThO<sub>2</sub> (0,0944%), La<sub>2</sub>O<sub>3</sub> (0,0777%), SrO (0,0678%), MnO (0,0493%), BaO (0,0380%), V<sub>2</sub>O<sub>5</sub> (0,0247%), CuO (0,0211%), Cr<sub>2</sub>O<sub>3</sub> (0,0157%). The chemical composition of ProRoot MTA followed the UNI EN ISO 196-2 norm only for the main oxides (CaO, SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, MgO, Fe<sub>2</sub>O<sub>3</sub>). Other oxides were analysed with a semi-quantitative XRF analysis. Combining the two analyses ProRoot MTA composition was determined (Table 2). Similarly to the Aureoseal Plus the semi-quantitative analysis showed the presence of metals and metal oxides, as P<sub>2</sub>O<sub>5</sub> (0,0232%), ThO<sub>2</sub> (0,433%), La<sub>2</sub>O<sub>3</sub> (0,364%), SrO (0,0304%), MnO (0,0826%), BaO (0,0157%), V<sub>2</sub>O<sub>5</sub> (0,105%), Cr<sub>2</sub>O<sub>3</sub> (0,0518%), As<sub>2</sub>O<sub>3</sub> (0,0769%), Rh (0,0113%), I (0,0331%), CeO<sub>2</sub> (0,253%), Pr<sub>6</sub>O<sub>11</sub> (0,111%), Nd<sub>2</sub>O<sub>3</sub> (0,195%), Sm<sub>2</sub>O<sub>3</sub> (0,0279%), Eu<sub>2</sub>O<sub>3</sub> (0,0110%), Gd<sub>2</sub>O<sub>3</sub> (0,0565%), Hg (0,00383%).

### XRD Analysis

The XRD spectrum of the Aureoseal Plus powder is reported in Figure 1. The main peaks are related to dicalcium silicate, tricalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, bismuth oxide and gypsum. In Figure 1 only the main peaks are visible, while the peaks of the other phases are pointed by symbols, as shown in the legend. The curve visible at 2 $\theta$  = 18° - 21,5° means that the material has an amorphous component. The XRD spectrum of the ProRoot MTA powder is reported in Figure 2. The analyses revealed the presence of dicalcium silicate, tricalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, bismuth oxide and gypsum. Figure 2 shows only the peaks of the main phases, while the peaks of the other phases are indicated by symbols, as shown in the legend. In Table 3 and Table 4 crystalline phases of both cements are listed.

### SEM/EDS Analysis

Particles forming Aureoseal Plus powder (Fig. 3) are quite

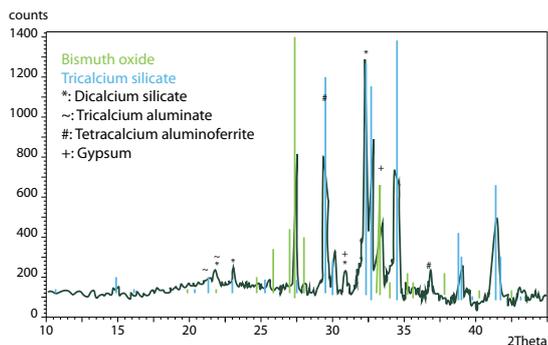
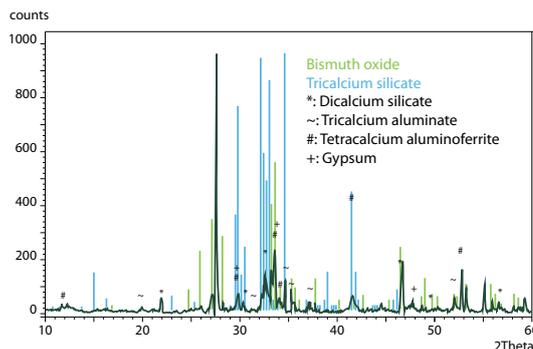


FIG. 1 XRD spectrum analysis of Aureoseal (peaks of main phases).  
 IG. 2 XRD spectrum analysis of ProRoot MTA (peaks of main phases).



Phase	Mineral name	Cement Nomencl.	Chemical Formula	PDF	Reference
Dicalcium Silicate	Belite	C2S	Ca <sub>2</sub> SiO <sub>4</sub>	24-0034	Mitchell 1999
Tricalcium Silicate	Alite	C3S	Ca <sub>3</sub> SiO <sub>5</sub>	73-0599	Nekoofar 2010
Tricalcium Aluminate	Aluminate	C3A	Ca <sub>3</sub> A <sub>2</sub> O <sub>6</sub>	38-1429	Parirokh 2010
Tetracalcium Aluminoferrite	Ferrite	C4AF	4CaO.Al <sub>2</sub> O <sub>3</sub> .F <sub>2</sub> O <sub>3</sub>	74-0803	
Bismuth Oxide	Bismite	Bi <sub>2</sub> O <sub>3</sub>	Bi <sub>2</sub> O <sub>3</sub>	76-1730	Pereira 2004
Calcium Sulphate Dihydrate	Gypsum	CSH <sub>2</sub>	CaSO <sub>4</sub> .2H <sub>2</sub> O	33-0311	

TABLE 3 Phases identified in Aureoseal Plus. PDF: powder diffraction files.

Phase	Mineral name	Cement Nomencl.	Chemical Formula	PDF	Reference
Dicalcium Silicate	Belite	C2S	Ca <sub>2</sub> SiO <sub>4</sub>	24-0034	Mitchell 1999
Tricalcium Silicate	Alite	C3S	Ca <sub>3</sub> SiO <sub>5</sub>	86-0402 85-1378	Nekoofar2010
Tricalcium Aluminate	Aluminate	C3A	Ca <sub>3</sub> A <sub>2</sub> O <sub>6</sub>	38-1429	Parirokh 2010
Tetracalcium Aluminoferrite	Ferrite	C4AF	4CaO.Al <sub>2</sub> O <sub>3</sub> .F <sub>2</sub> O <sub>3</sub>	74-0803	
Bismuth Oxide	Bismite	Bi <sub>2</sub> O <sub>3</sub>	Bi <sub>2</sub> O <sub>3</sub>	76-1730	Pereira 2004
Calcium Sulphate Dihydrate	Gypsum	CSH <sub>2</sub>	CaSO <sub>4</sub> .2H <sub>2</sub> O	28-0739	

TABLE 4 Phases identified in ProRoot MTA. PDF: powder diffraction files.

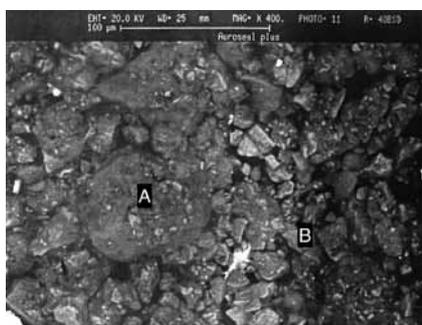


FIG. 3 Aureoseal's SEM micrograph (400x), overview. A = large roundish aggregate ; B = cubic particles

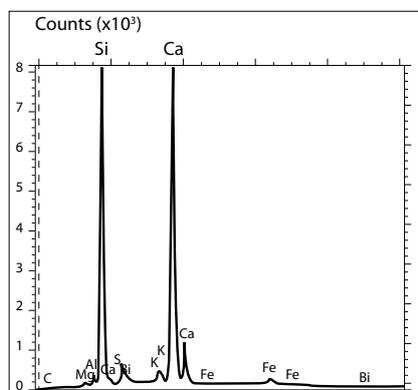


FIG. 4 Aureoseal's EDS analysis.

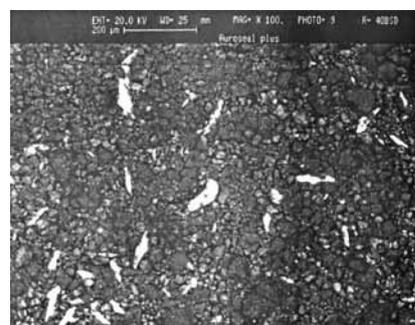


FIG. 5 Aureoseal's SEM Micrograph (100x). Detail: particles containing Bi<sub>2</sub>O<sub>3</sub>.

coarse, with different morphology and dimension ranging from 5 to 100 μm. The most common microstructures are large roundish aggregates, cubic particles and oblong particles. Examining the round particles with a greater magnification, 1-2 μm spheres are clearly visible.

The EDS analysis (Fig. 4) showed the presence of large amount of Ca and Si, while other materials, as Fe, Al, Bi, S, K and Mg are less represented. The EDS determined that the little spheres and the bigger particles are mainly composed by silica. Cubic particles are rich of all those elements that form the silicates, the aluminates and the ferrites of calcium aluminate. They also show a great amount of potassium. These particles are equally distributed in the sample, and with a greater magnification, a tight link with the round particles is noticeable. In long particles bismuth

is mainly represented. They are not equally distributed in the sample: they are abundant in some areas but missing in some others (Fig. 5). These particles are visible only in back-scattering mode, because they are completely covered by other particles and are not detectable with standard observations. ProRoot MTA powder is less coarse and more homogeneous than Aureoseal Plus. It is composed by particles of different shapes (round, prismatic, long), ranging from 1 to 10 μm in size. The EDS analysis (Fig. 6) showed an increased presence of Ca, Si and Bi and traces of Al, Fe, S and Mg. Specific EDS analysis on some round particles showed that 5-μm aggregates and 1-μm particles are rich of Ca, Si, Al and Fe. Moreover, differently from Aureoseal Plus, some particles contained Ca, Si and Al and some other contained Fe. The EDS analysis showed high

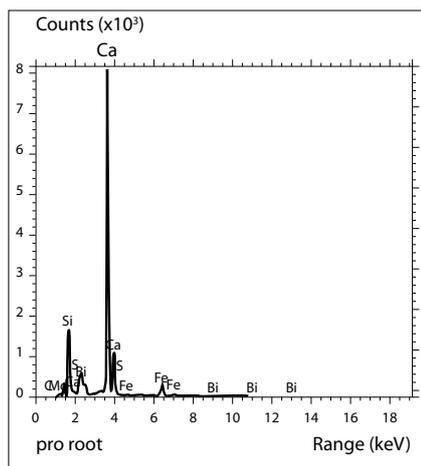


FIG. 6 ProRoot MTA's EDS analysis.

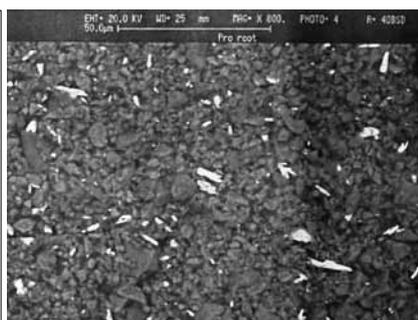


FIG. 7 ProRoot MTA's SEM Micrograph (800x). Detail: particles containing Bi<sub>2</sub>O<sub>3</sub>.

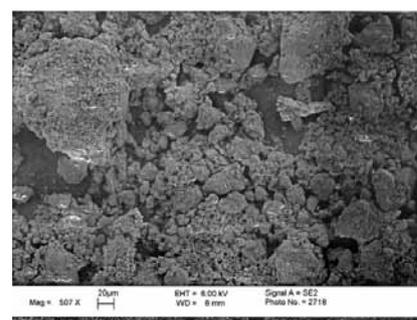


FIG. 8 FESEM Micrograph (800x). Detail: Silica Fume.

level of sulphur and calcium in the prismatic particles. The long particles in ProRoot MTA, similarly to Aureoseal Plus, are rich of bismuth, only visible in back-scattering mode. However, differently from Aureoseal Plus, these particles are equally distributed in the sample (Fig. 7).

## Discussion

New MTA-based sealers and cements were studied since the early investigation of Torabinejad et al. [Torabinejad et al., 1995; Torabinejad and White, 1995]. MTA contains a significant amount of PC, whose major components have been investigated and identified [Torabinejad et al., 1995; Asgary et al., 2005]. Earlier studies reported that it is mainly composed by tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide and bismuth oxide [Torabinejad et al., 1995; Asgary et al., 2005]. In the present study different techniques were used to analyse the characteristics of two endodontic Portland-based cements, ProRoot MTA and Aureoseal Plus. The chemical analysis showed that both cements contain the main oxides of PC (CaO, SiO<sub>2</sub>, Fe<sub>2</sub>O<sub>3</sub>, Al<sub>2</sub>O<sub>3</sub>, Bi<sub>2</sub>O<sub>3</sub>, SO<sub>3</sub>, MgO, Na<sub>2</sub>O, K<sub>2</sub>O), plus bismuth oxide (Bi<sub>2</sub>O<sub>3</sub>), added for radiopacity. The MTA patent [Torabinejad and White, 1995] reports a 20% bismuth oxide loading. This was confirmed for ProRoot MTA [Camilleri, 2008; Belio-Rejes et al., 2009]. In the present study Aureoseal Plus shows a percentage of Bi<sub>2</sub>O<sub>3</sub> lower than the one contained in ProRoot MTA (1,72% against 11,7%).

The strong interest in developing MTA-based endodontic materials originates from different characteristics: the excellent biocompatibility, bioactivity, and osteoconductivity of MTA [Torabinejad and Parirokh, 2010]. It is claimed that ProRoot MTA is produced specifically for Dentsply Tulsa Dental under clean and segregated conditions to prevent any contamination. The ingredients used are certified for their purity and the absence of heavy metal contamination [Dentsply data sheet, Accessed August 15, 2015]. Despite that, it was shown that MTA contains toxic heavy metals

and their compounds like arsenic (As), chromium (Cr), lead (Pb), aluminium (Al) and potassium (K) [Dammachke, 2005; Danesh, 2006; Achternbosch, 2003; Bramante, 2008]. According to Achternbosch et al. [2003], the heavy metals originate from the use of waste materials as inorganic raw material or from secondary fuels used to fire the kiln during cement manufacture. These wastes are used to reduce production costs. Trace elements found in wastes used in cement production may be transferred to the cement [Achternbosch et al., 2003]. Chromium and lead contamination were never reported for MTA [Schembri et al., 2010], while there has been a special concern regarding the presence of Arsenic. Arsenic reacts with protein thiols, and exposure to high concentrations of this element may induce genotoxicity [Salles et al., 2012]. Its toxicity can affect the skin and induce digestive, glandular, blood, and respiratory disorders. Arsenic exists in different stages of oxidation (-3, 0, +3, +5), where the trivalent and pentavalent stages are the most toxic [Hughes et al., 2002]. It is not known if the arsenic released by cements is harmful to the health of the host because the ISO 9917-1 (2007) standards only specify the limits for the total arsenic content, which is set at 2 mg kg<sup>-1</sup>, and not for the released species [Schembri et al., 2010]. The level of Arsenic detected in ProRoot MTA was reported to be higher than levels specified in ISO 9917 [British Standard Institution Dentistry, 2007], while other studies reported levels of Arsenic below the limit, with As III being the species mostly present in cements [De Deus et al., 2009; Matsunaga et al., 2010]. In root-end filling materials any toxic component could lead to irritation, degeneration or tissue necrosis [Huang et al., 2005]. Therefore, knowing the precise composition of materials used is important and it may assume a greater relevance in paediatric patients. In our study the semi-quantitative XRF analysis showed the presence of heavy metal oxides in both cements. Some of them could also be present as impurities of the bismuth oxide. The two cements differ for the content of metals and heavy metal oxides: Aureoseal Plus contains V<sub>2</sub>O<sub>5</sub>, Cr<sub>2</sub>O<sub>3</sub>, MnO and La<sub>2</sub>O<sub>3</sub>, while ProRoot MTA contains As<sub>2</sub>O<sub>3</sub>, CeO<sub>2</sub> and Hg. Aureoseal Plus contains also a remarkable percentage (36%) of silica fume, whose role is to improve the resistance to acids and the handling characteristic. The XRD analysis showed

that the main phases of the two cements are  $C_3S$ ,  $C_2S$ ,  $C_3A$ ,  $C_4AF$  and  $B_2O_3$ . PCs are composed by particles with a wide size range [Achternbosch et al., 2003]. Particle size and shape might influence the surface area and the biological quality of materials [Komabayashi et al., 2008]. Surface characteristics may influence biocompatibility because they affect cell adhesion and spreading [Hwang et al., 2009]. SEM and EDS were used in previous studies to evaluate morphology and composition of materials [Camilleri et al., 2005]. The microstructure of ProRoot MTA powder is more homogeneous than Aureoseal Plus and it is composed by particles ranging from 1 to 10  $\mu\text{m}$ . This may be explained by the different elements concentration, even though they have the same main components. EDS analysis showed that in both cements, small and bigger round particles are mainly composed by silica. As the silica fume is composed by 98% of  $SiO_2$  in 1- $\mu\text{m}$  microspheres, it can be hypothesized that it is always added in cements and it appears as big agglomerate (Fig. 8). Moreover, regarding the pozzolanic material detected during the chemical analysis, it can be considered not a generic pozzolanic material, but silica fume itself. EDS analyses of the oblong particles in Aureoseal Plus and XRD analysis within the same specimens, showed the presence of bismuth oxide, typically aggregated in such rectangular shapes.

## Conclusions

Results of our study showed that Aureoseal Plus and ProRoot MTA are made by similar elements but in different concentrations. Moreover Aureoseal Plus has distinctive elements in its composition. Differences in term of chemical composition and phases' microstructure between Aureoseal Plus and ProRoot MTA may not have significant effects on the characteristics of the two cements. As a result, Aureoseal Plus could be considered as a good alternative to ProRoot MTA. More *in vitro* and *in vivo* clinical studies are required to assess Aureoseal Plus chemical, physical and biological properties.

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