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The Microcrystalline Structure and Elemental Analysis of Expired and Non-Expired Shelf-Life White MTA: A SEM and E-DAX analysis Study.

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Abstract:

Objectives: The aim of this study is to determine the microcrystalline structure and elemental analysis of expired and non-expired shelf-life white Mineral trioxide aggregate (MTA) through Energy Dispersive Analysis by X-ray (EDAX) and Scanning Electron Microscope (SEM).

Methods: Six different expired (after 6 months) and non-expired (Pro-Root) white MTA is used. A powder in a thin layer was dispersed over a slab made up of polymethyl- methacrylate mounted on an aluminium stub. Carbon coating of the specimen is done to get electrical conductivity. The prepared specimen was then viewed under the SEM, and EDAX.

Results: The microcrystalline structure of Expired white MTA showed that material is very similar to Non-Expired MTA, except for the absence of smooth elongated particles and the presence of angular rounded particles by using SEM. The elemental analysis of Expired and Non-expired white MTA also showed quite similar constituent except for slight decrease in calcium and aluminium and increase in oxygen and carbon in expired MTA through EDAX analysis.

Conclusion: Expiration date does not affect the structure but constituent of expired and non-expired shelf life of MTA differs. More studies are required to know the effect of expired MTA on vital permanent and primary teeth.

Keywords: Mineral trioxide aggregate, Energy Dispersive Analysis by X-ray, Scanning Electron Microscope.

Introduction:

In 1990 Torabinejad developed Mineral trioxide aggregate (MTA) as a root-end filling material[1]in Loma Linda University. It has excellent physical and biological properties, with its main ingredients including CaO, SiO_2, Al_2O_3, and Fe_2O_3 and is a bio-functional inorganic material. It is commercially available as Pro-Root® MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) after getting approval from United States Food and Drug Administration (FDA) in 1998. It is generally used as a standard dental repair material and possess properties such as dimensional stability, adequate adhesive ability, insolubility,

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biocompatibility, bioactivity in promoting hard tissue formation, marginal sealing ability and has unique antibacterial effects. MTA can be used for wide variety of applications in surgical and non–surgical procedures, including direct pulp capping, temporary filling material, perforation repair, retrograde and other types of root canal filling, vital pulpotomy, and apexification[2,3]. Earlier Gray MTA was produced but now recently white MTA is produced. Gray MTA contains iron which is responsible for the formation of tetra-calcium-alumino-ferrite phase.

MTA is a mechanical mixture of three powder ingredients[4]:

1) Portland cement (75%)
2) Bismuth oxide (20%)
3) Gypsum (5%).

According to standard patent MTA, it consists of calcium oxide and silicon oxide, which together constitute 75-95% of the cement. When these raw materials were blended; tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetra-calcium- alumino-ferrite are produced[5].

Composition	Percentage
Powder	
Tricalcium silicate	66.1
Dicalcium silicate	8.4
Tricalcium aluminate	2.0
Tetracalciumaluminoferrite	-
Calcium sulphate	-
Bismuth oxide	14
Calcium oxide	8
Silicon oxide	0.5
Aluminium oxide	1.0

Many studies reported that MTA is as good as Calcium hydroxide which has almost same action as that of MTA. There are still some drawbacks related to handling properties and setting time. However, manufacturers and Researchers are constantly making progress in overcoming these problems. Somehow MTA that are expired and outdated should be studied whether their setting time and handling property changes or not. No such studies have been carried out to determine the difference between expired and non-expired shelf-life white MTA. This study aims to determine the microcrystalline structure and elemental analysis of expired and non-expired shelf-life white MTA through Energy Dispersive Analysis by X-ray (EDAX) and Scanning Electron Microscope (SEM).

Materials and methods:

Analysis of powders:

The constitution of 6 Expired and Non-expired shelf-life white MTA(Pro-Root) was determined using Energy Dispersive Analysis by X- ray (EDAX) and their microcrystalline structure in a scanning electron microscope (SEM). EDAX reveals the presence of elements through generation of characteristic X-rays and is associated with electron microscopy. EDAX analysis shows both semi quantitative and semi qualitative data related to the constituents. A powder in a thin layer was dispersed over a slab made up of polymethyl-methacrylate which is mounted on an aluminium stub. Carbon coating of the specimen is done to get

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electrical conductivity. The prepared specimen was then viewed under the SEM, and EDAX. Secondary electron and back-scattered images were also taken at up to 25000, 50000, 100000, 200000 magnification. Two stubs were made for each powder and the micro-analysis was performed twice for each sample.

Results:
Powder analysis:

Scanning electron micrographs of Non-expired shelf-life white MTA gave different images in different magnifications.

Secondary electron images of Non expired shelf-life white MTA at 25000 and 50000 magnification respectively:

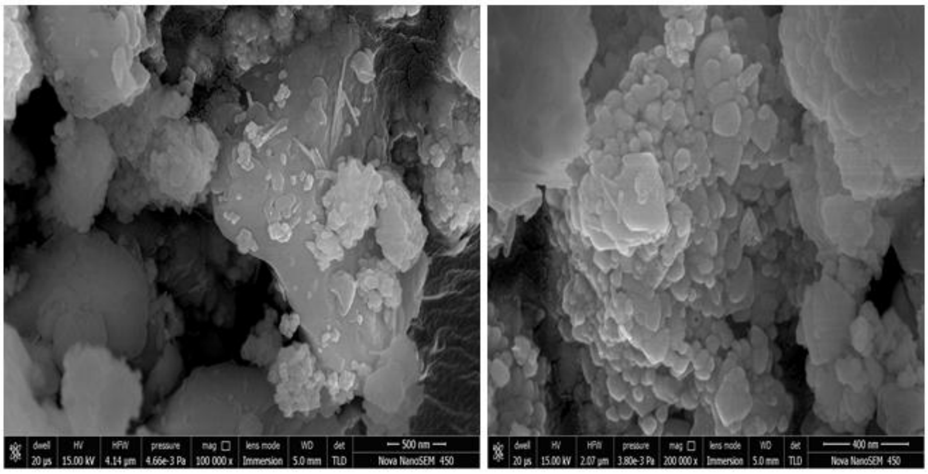

Figure 2: c) SEM image of Non expired shelf-life MTA at 100000 magnification.
d) SEM image of Non expired shelf-life MTA at 200000 magnification.

At higher magnifications it looks like the clustered structure or spiky-ball like clusters looked more evident lying under an amorphous layer with needle- like crystals projecting out on its periphery.
The EDAX analysis of Non-expired shelf-life white MTA showed the material to be composed of calcium, aluminium, and oxygen, and carbon. Calcium and Aluminium were the predominant elements.

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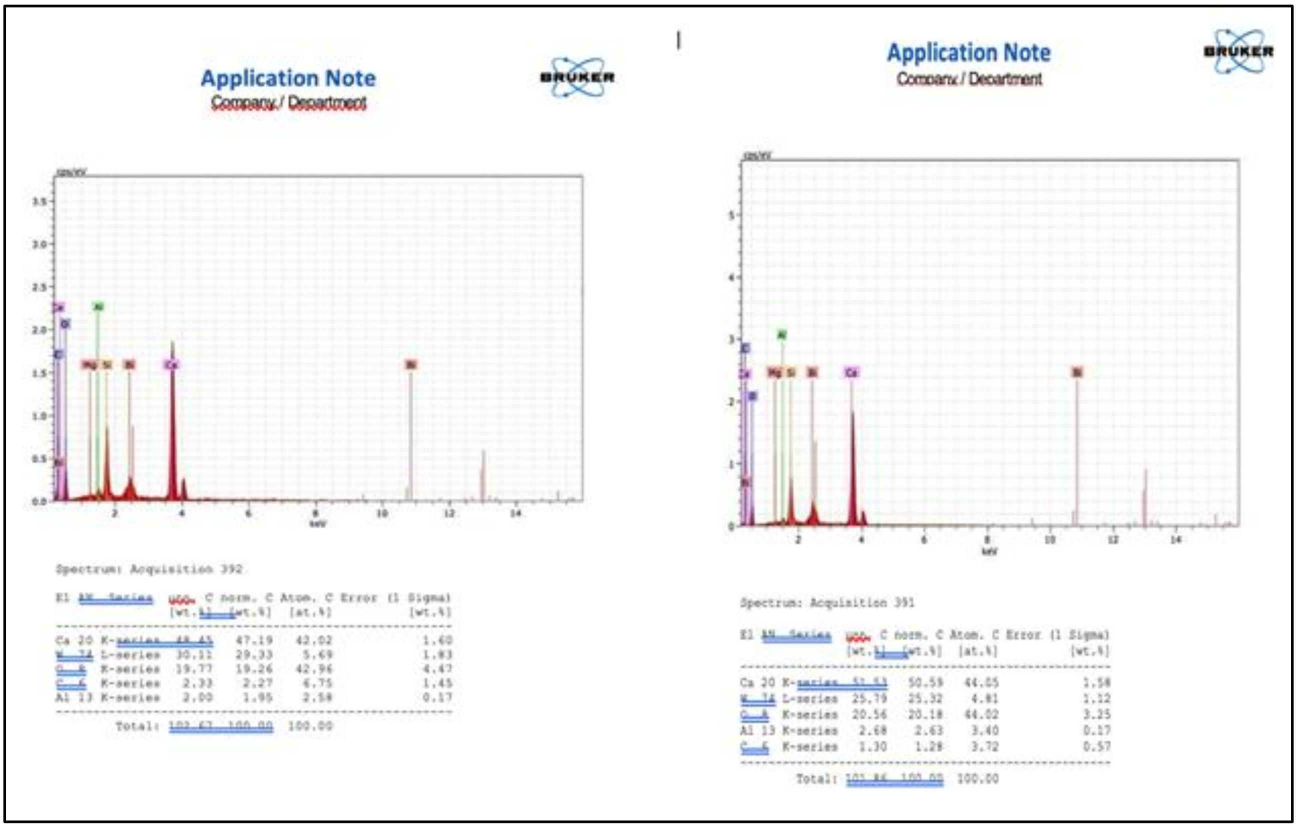

Figure 3: EDAX Analysis of Non expired shelf- life MTA

Scanning electron micrographs of Expired shelf- life white MTA gave different images in different magnifications.

Secondary electron images of Expired shelf- life white MTA at 25000 and 50000 magnification respectively:

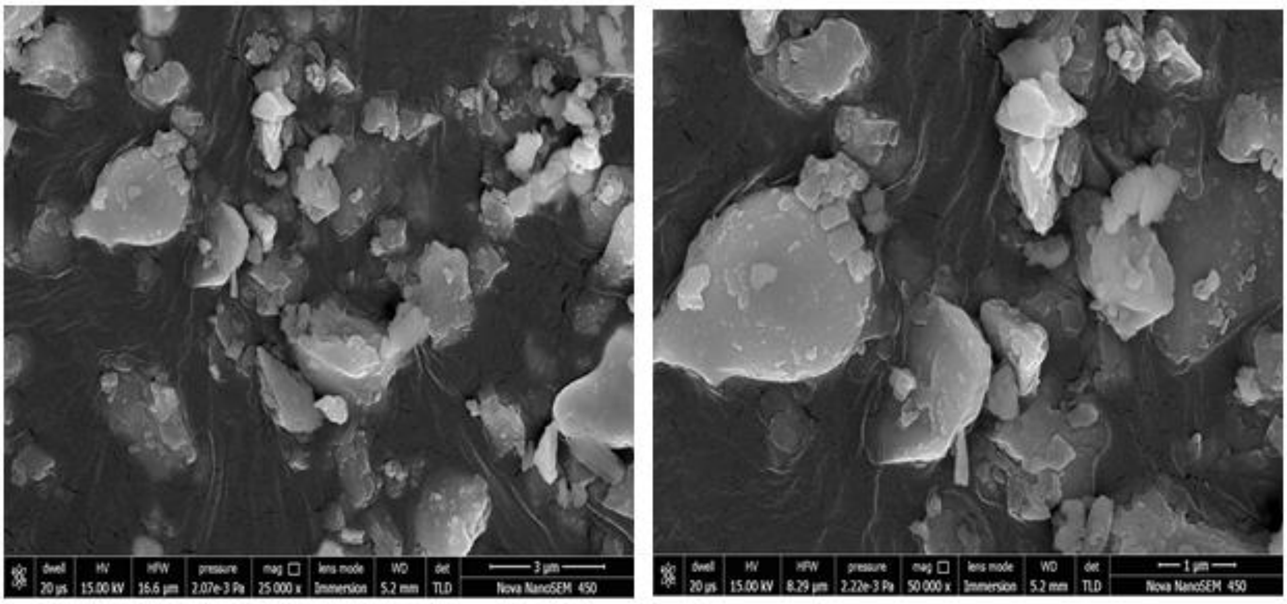

Figure 4: a) SEM of Expired shelf- life MTA at 25000 magnification.b) SEM of Expired shelf- life MTA at 50000 magnification.

Secondary electron images of Expired shelf- life MTA showed the presence of small irregular particles interspersed with some rounded and angular particles at 25000 and 50000 magnification.

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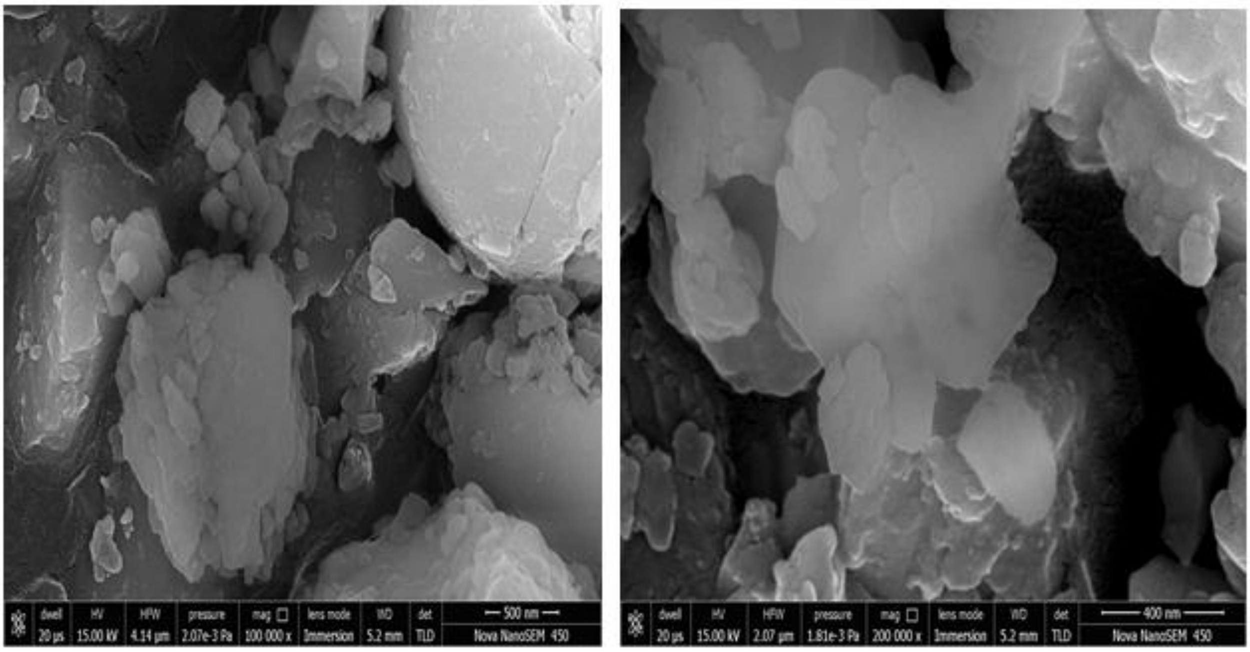

Figure 5: c) SEM image of Expired shelf-life MTA at 100000 magnification.d) SEM image of Expired shelf-life MTA at 200000 magnification

At 100000x some small crystalline plates look cuboidal or angular in shape while the larger ones look irregular in shape. At 200000x the cement surface looks rough with a more granular surface.

The EDAX analysis of Expired shelf-life white MTA showed that the material was composed of same constituent as Non-expired shelf-life white MTA. Calcium and aluminium were decreased and oxygen and carbon were increased.

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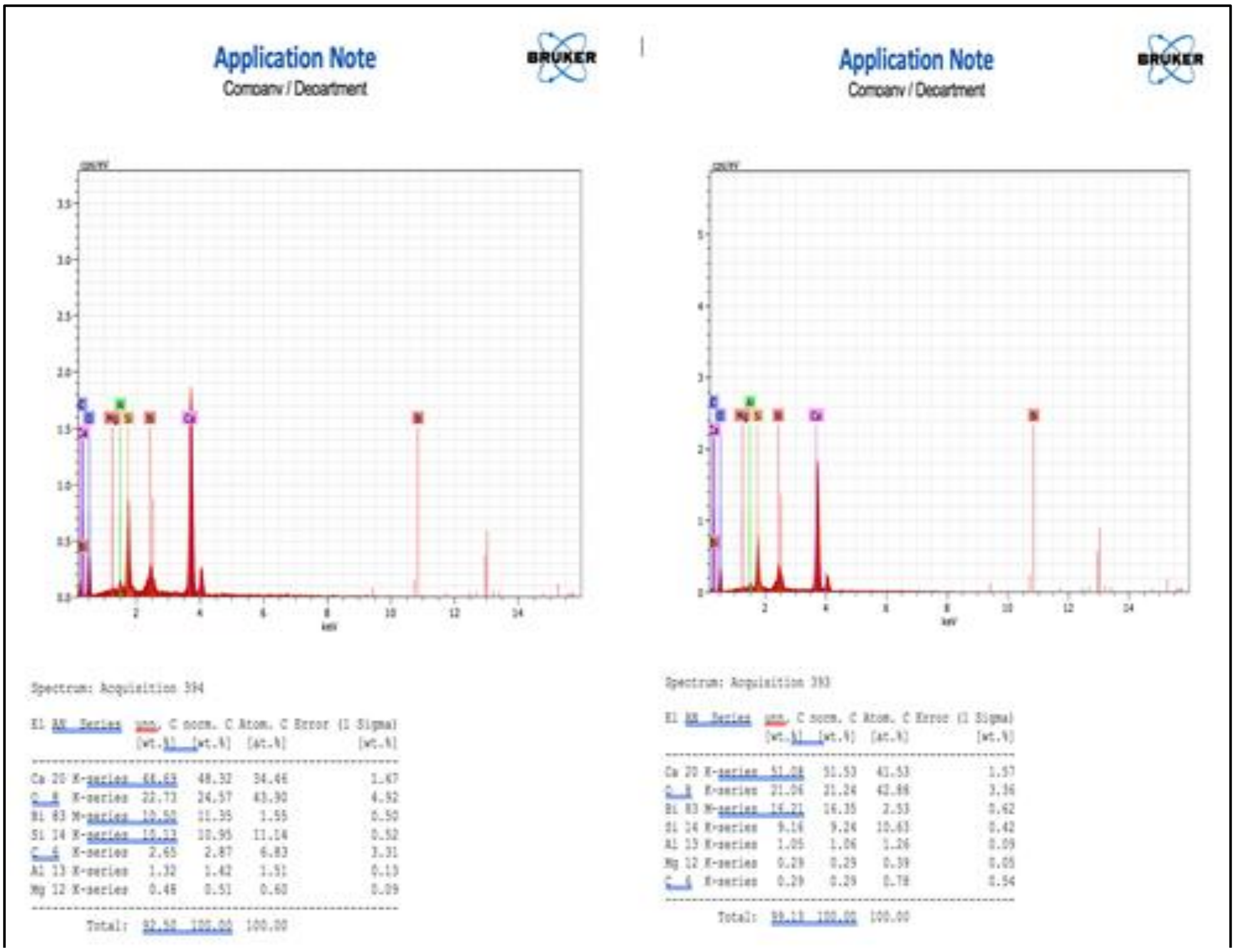


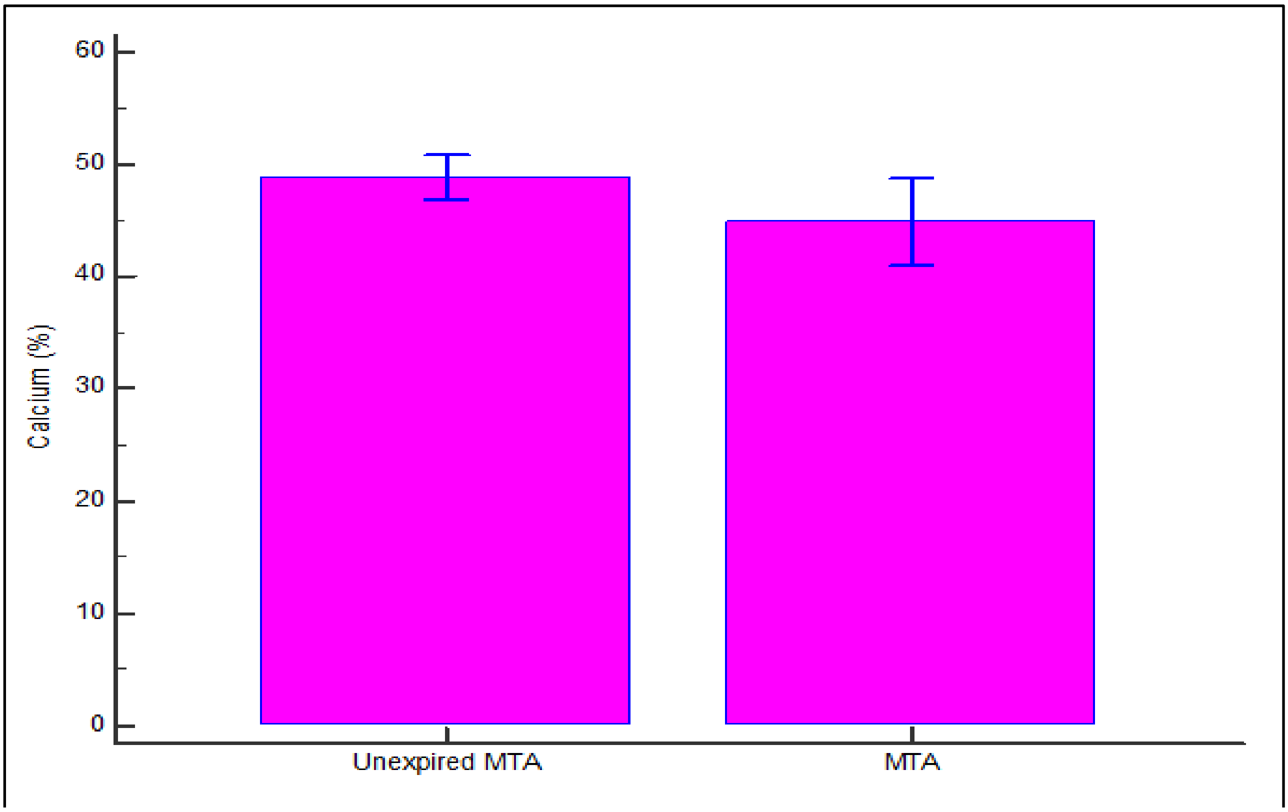
Figure 6: EDAX Analysis of Expired shelf-life white MTA

Calcium (%)

	Unexpired MTA	Expired MTA
Sample size	6	6
Arithmetic mean	48.8900	44.9250
95% CI for the mean	46.9357 to 50.8443	41.0221 to 48.8279
Variance	3.4680	13.8312
Standard deviation	1.8623	3.7190
Standard error of the mean	0.7603	1.5183
F-test for equal variances		P = 0.155

T-test (assuming equal variances)

Difference	-3.9650
Pooled Standard Deviation	2.9410
Standard Error	1.6980
95% CI of difference	-7.7484 to -0.1816
Test statistic t	-2.335
Degrees of Freedom (DF)	10
Two-tailed probability	P = 0.0417

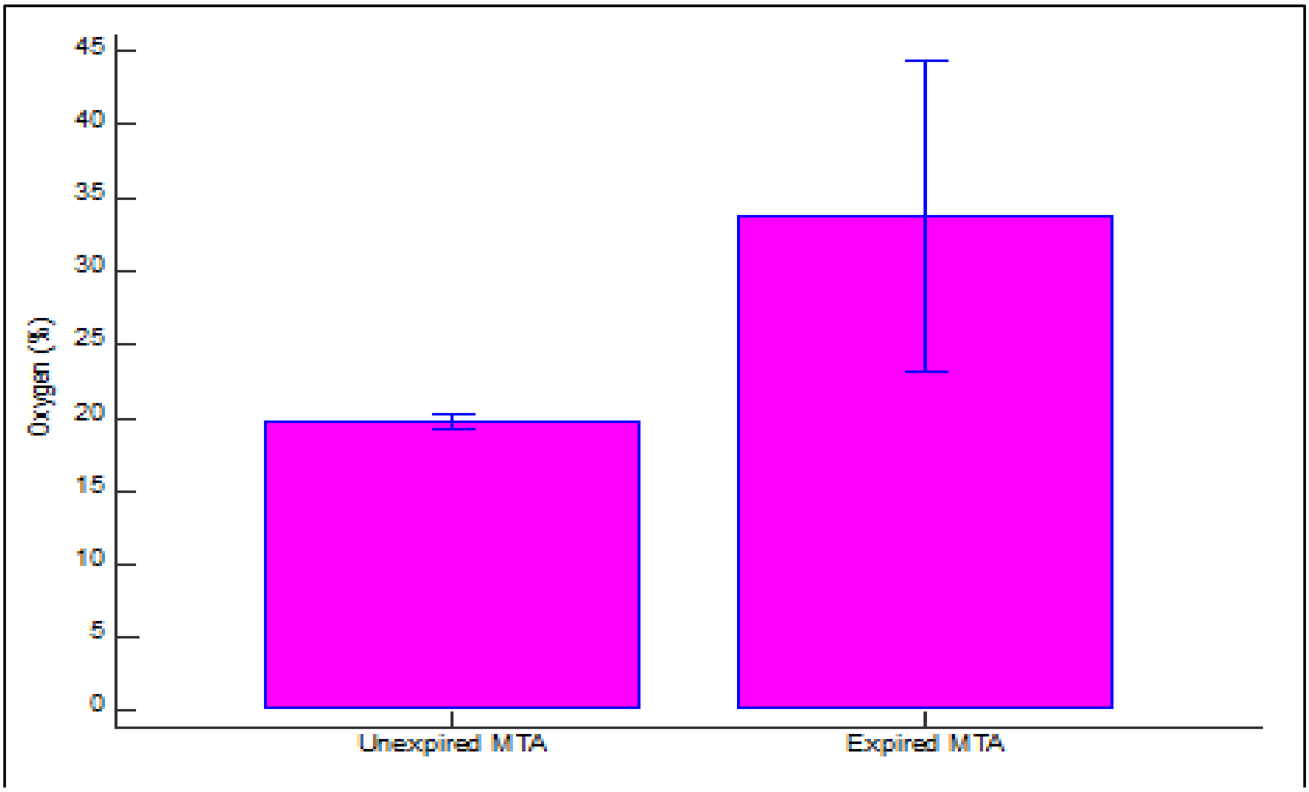


Oxygen (%)

	Unexpired MTA	Expired MTA
Sample size	6	6
Arithmetic mean	19.7200	33.7250
95% CI for the mean	19.1912 to 20.2488	23.2004 to 44.2496
Variance	0.2539	100.5768
Standard deviation	0.5039	10.0288
Standard error of the mean	0.2057	4.0942
F-test for equal variances		P < 0.001

T-test (assuming equal variances)

Difference	14.0050
Pooled Standard Deviation	7.1004
Standard Error	4.0994
95% CI of difference	4.8710 to 23.1390
Test statistic t	3.416
Degrees of Freedom (DF)	10
Two-tailed probability	P = 0.0066

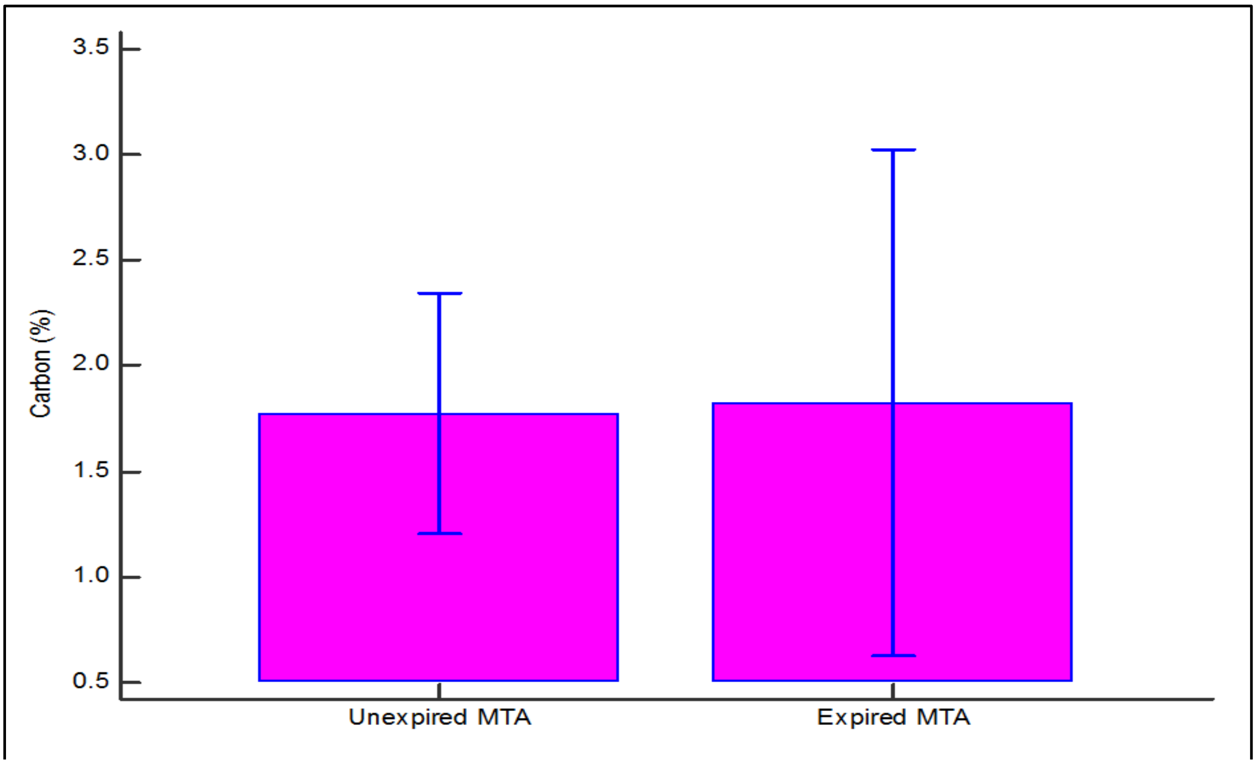


Carbon (%)

	Unexpired MTA	Expired MTA
Sample size	6	6
Arithmetic mean	1.7750	1.8250
95% CI for the mean	1.2059 to 2.3441	0.6237 to 3.0263
Variance	0.2940	1.3104
Standard deviation	0.5422	1.1447
Standard error of the mean	0.2214	0.4673
F-test for equal variances		P = 0.127

T-test (assuming equal variances)

Difference	0.05000
Pooled Standard Deviation	0.8957
Standard Error	0.5171
95% CI of difference	-1.1022 to 1.2022
Test statistic t	0.0967
Degrees of Freedom (DF)	10
Two-tailed probability	P = 0.9249



Aluminium (%)

	Unexpired MTA	Expired MTA
Sample size	6	6
Arithmetic mean	2.2900	1.3400
95% CI for the mean	1.8991 to 2.6809	1.2480 to 1.4320
Variance	0.1387	0.007680
Standard deviation	0.3725	0.08764
Standard error of the mean	0.1521	0.03578
F-test for equal variances		P = 0.006

T-test (assuming equal variances)

Difference	-0.9500
Pooled Standard Deviation	0.2706
Standard Error	0.1562
95% CI of difference	-1.2980 to -0.6020
Test statistic t	-6.082
Degrees of Freedom (DF)	10

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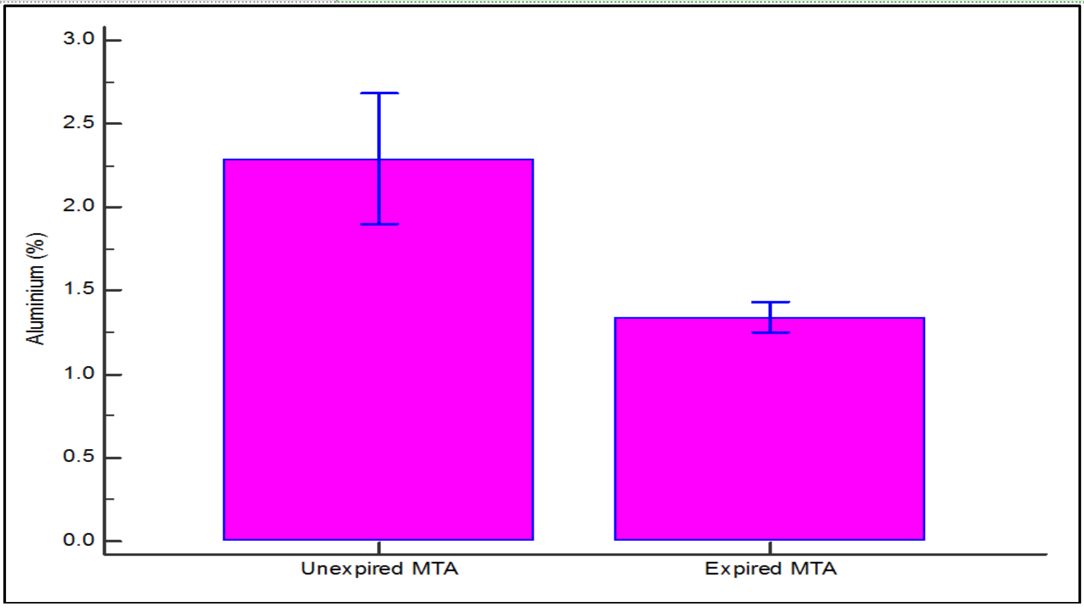
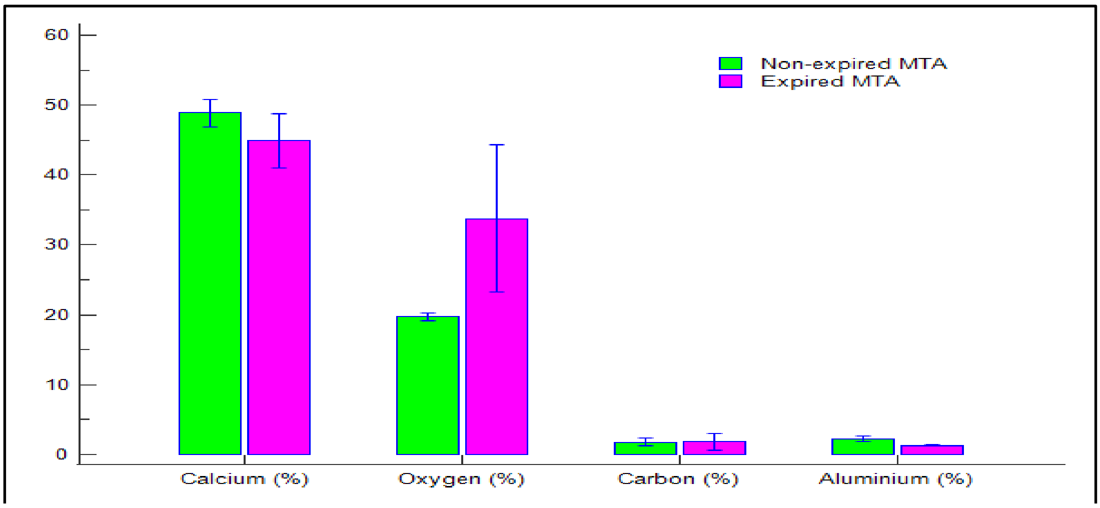
Two-tailed probability	P = 0.0001



Clustered mult



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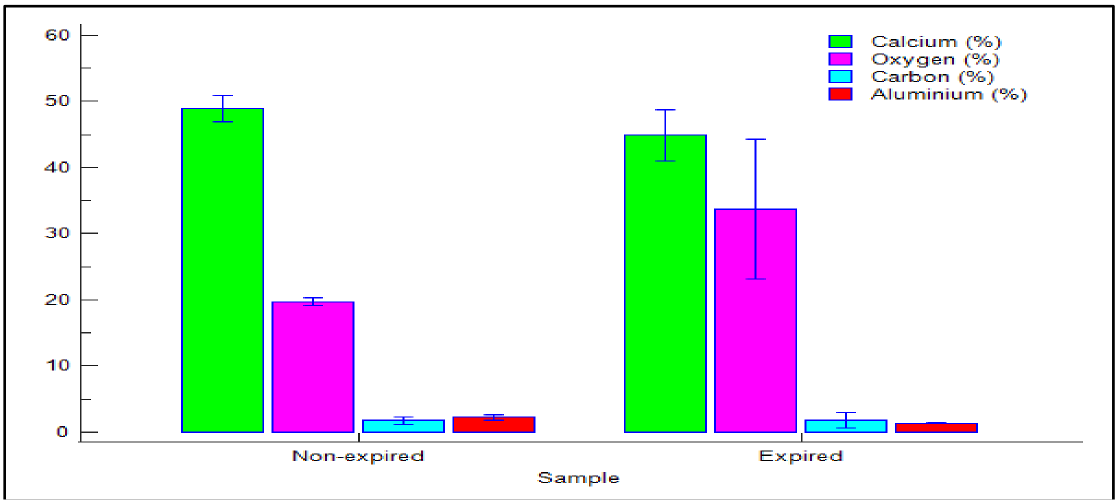

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Discussion

In the present study the microcrystalline structure and elemental analysis of expired and non- expired shelf-life white MTA is determined through SEM and EDAX analysis. The microcrystalline structure of Expired shelf-life white MTA showed the material to be very similar to Non-Expired shelf-life MTA, except for the absence of smooth elongated particles and the presence of angular rounded particles by using SEM. The elemental analysis of Expired and Non-expired shelf-life MTA also showed quite similar constituent except for slight decrease in calcium and aluminium and increase in oxygen and carbon content in expired shelf-life MTA through EDAX analysis. Biocompatibility and chemical constituents of MTA and Portland cement was done in a study using a fluorescence spectrometer and similar chemical constituents of these materials were reported[6]. Calcium oxide 50–75%, silicon dioxide 15–25% and aluminium oxide are the primary constituent[7].

In the field of endodontics, the most versatile material accepted with various clinical applications is MTA. The outstanding application of MTA is due to its excellent physical and biological properties. Various clinical application of MTA includes pulp capping, perforation repair (Furcal or root), pulpotomy, root end filling, root canal filling, resorption. Ca(OH)2 can easily be substituted by MTA as it exhibit greater sealing ability and greater quality and quantity of reparative dentin formation[8].MTA leads to the formation of dentine bride in pulpotomy by limiting the inflammation and maintaining the vitality of the pulp[9]. In primary and permanent teeth MTA promotes bone healing and eliminate inflammation so it can be used for repair for furcation and perforation[10]. MTA when used as root end filling showed better healing than orthograde GP filling[11]. In primary teeth MTA is used for obturation as it provides biocompatible seal of root canal system[12].MTA is considered as suitable material for treatment if internal resorption. It showed favourable result in treatment of pink tooth[13].

Dimensional changes and sealing ability of an MTA is related to its solubility, if space was left behind it will cause bacterial colonization and may result to periapical infection[14]. After setting MTA releases calcium and hydroxyl ions as soon as it contacts tissue fluids and pH raises to 12.5[15]. High pH levels and calcium and hydroxyl ions in MTA results in formation of hard tissues[16]. Many studies states that Biodentine also release calcium ions but in higher amount which can be related to the presence of calcium silicate and calcium chloride component in its composition[17,18].

Moisture also plays important role in determining the flow characteristics of MTA.

Expired MTA acquired more moisture than non-expired MTA from the humid environment. These moistures can alter its properties and may reduce the sealing ability of MTA. Therefore, it is always mandatory to use these endodontic materials under manufacturer recommendations.

A traditional SEM/EDX analysis generally requires three separate scans of powder particles:

1. To locate the position of particle
2. To determine size and shape of particle
3. To find out the constituent of particles.

The EDAX was performed with the specimens placed directly on aluminium stubs and held in place with carbon cement because placing the specimens on glass cover-slips could detect the elemental constitution of the cover-slips. All the data are collected using automated electron beam. Images at different magnifications such as 25000X, 50000X, 100000X, 200000X were taken to notice any significant changes.

The shelf-life of MTA is the length of time a product may be stored without becoming unsuitable for consumers from the date of manufacturing.It is strictly advised that MTA should be used according to the manufacturer recommendations only. The degree of degradation in the MTA is closely related to the chemical stability of the molecular groups in its composition. So, these materials may show a change in their constituent after expiration date but there is lack of significant changes seen in this study due to short time duration after expiration date. Longer time duration after expiration may result in certain changes. More studies are recommended to find out the effect of MTA on viable cells in root canal treatment and clinical use.

Conclusion

Within the limitations of this study, it can be can be concluded that expiration date does affect the structure variably but the constituent of non-expired and expired shelf life of MTA differs significantly. Decreased sealing ability and dimensional changes can be seen in expired MTA due to moisture acquired from environment. Hence, MTA should always be used according to manufacturer recommendations. More studies are required to know the effect of expired MTA on vital permanent and primary teeth.

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