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Micro-CT evaluation of mineral trioxide aggregate and bioactive glass as apical seal

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Abstract

Purpose:

The purpose of this study was to determine and compare the ability of bioactive glass (BAG) with mineral trioxide aggregate (MTA) as a potential apical sealer.

Methods:

Recently extracted sixty human premolars single-rooted teeth for orthodontic reasons were selected. Matched pairs were separated into two groups (n=30). Teeth of the first group were obturated with Thermafil whereas the second group obturated with BAG. Micro CT was performed and reconstruction of the specimen image was done by using the cone-beam technique.

Results:

Bioactive glass (BAGs) when compared to the mineral trioxide aggregate (MTA) as an apical seal found to be less porous. There were virtually no marginal linear gaps when analyzed by Micro CT. The apical thirds filled with MTA had a significantly lower 3D void volume (P< 0.05) when compared with apical thirds filled with BAG.

Clinical significance:

The results of this in-vitro study suggest potentiality of bioactive glass as a root canal sealer.

Keywords: apical sealing, bioactive glass, micro-CT, Mineral trioxide aggregate, obturation

Introduction:

An impeccable apical seal is required to minimize and eventually stop the bacterial contamination and their toxins from approaching the root apex in endodontic treatment.[1]Apical leakage is therefore supposed to be the most possible cause of failure of endodontic treatment, further affected by filling procedures along with the chemical and physical properties of a sealer and the smear layer.[2,3]

The ability of any sealing material is affected by the presence of voids between its particles.[1] There are many commercially available sealing materials for the apical area of the roots of the teeth. Mineral trioxide aggregate (MTA) has favorable physical, chemical, and biologic properties.[4-6] An aqueous mixture of MTA in the form a slurry paste is condensed into the root canals with extreme care, not to create any voids. Among many techniques, the use of a lentulo spiral filler technique has been suggested for obturation of root canals to make MTA reach up to the root apex[7] and sometimes manual files and pluggers in a hand compaction-method are used.[8,9]

Bioactive Glass (BAG) is another material commercially available for applications in dentistry. Bioglass™ also known as 45S5, is commonly used for bone grafts,[10] in repair of hard tissues,[11] for preparation of scaffolds,[12] as a coating material for implants,[13] to decrease dentine hypersensitivity[14] has antibacterial effect,[15] as a root canal filling and sealing material.[16]

As a characteristic when BAGs are brought into interaction with body fluids a immediate release

of Na+ and congruent dissolution of Ca2+, PO and Si 4+ occurs at its glass surface. A poly-condensed silica-rich (Si-gel) layer is formed, which then serves as a prototype for the formation of a calcium phosphate (Ca/P)layer at its external surface. Eventually, the Ca/P crystallizes into HCA, the composition of which corresponds to that of bone.[17]

An endodontic treatment is still evaluated by radiographic imagesto see the obturation of root canals clinically. When on a radiograph, a tooth is seen with a nonhomogeneous filled canalspace, and this is also combined with a periapical lesion orclinical symptoms, it is easily decided to revise the treatment.[18] Cone-beam is also an option to have a 3D visualization and analysis of the endodontic treatment done in clinical studies.[19,20]

In laboratory investigations, micro-computed tomography (micro-CT) is considered to be a non-destructive 3D imaging technique[21] to evaluate the micro-morphology and to compare the densities of mineralized tissues and the internal structure and porosity of biomaterials and scaffolds.[22] BAGs also have an application in root canal therapy, providing a biological seal in the form of mineral deposition inducing materials in the root canal and at the apex.[16] Therefore its re-mineralizing property led us to design this study to analyze and compare the presence of voids and porosities between the material particles and to judge the quality of apical seal using MTA and BAG as materials, respectively. We hypothesized that BAG would effectively seal the apex compared to MTA.

Methods:

Selection and preparation of samples:
Recently extracted sixty human premolars single-rooted teeth for orthodontic reasons were selected from a pool of extracted teeth and approved from the Research Ethics Committee, KAUFD. A verbal consent is always taken from the patients whose teeth are extracted for orthodontic purpose and then these teeth are sent to the collective pool of extracted teeth from where the teeth are distributed after written ethical approval. The ethical approval King Abdul Aziz University Faculty of Dentistry Research Ethics Committee (KAUFDREC) 091-13 (2013). This study has been conducted in full accordance with the Declaration of Helsinki. Teeth were immersed in 10% formalin solution for 7 days to disinfect internal and external structure of the teeth effectively. Bucco-lingual and mesio-distal radiographs of the teeth were taken to exclude the presence of any second canal. The teeth were decoronated at the cement-enamel junction by using water cooled, high speed diamond bur (Hi-Di, Dentsply MEA) to enable them to be fixed onto MicroCT stage for proper positioning.

Canal was accessed and working length was determined by using #15 K-file (DentsplyMaillefer, Switzerland)by keeping it 1 mm short of the apex. Biomechanical Crown down preparation technique was carried out with 6Nickel Titanium ProTaper rotary files (Dentsply, MEA) in the sequence approved by the manufacturer. ISO size 35 was followed for canal preparation. The debris was irrigated with 3% sodium hypochlorite solution by using 27G monoject needle (Tycohealthcare, Gosport, UK) between each instrumentation. Pair of teeth selected according to the size, flatness, and curvature of the root canals by seeing in a set of three-dimensional micro-CT images. Matched pairs were separated into two groups of 30 teeth each. Both groups were cleansed with 2ml 3% NaOCl, and flushed with 10% of polyacrylic acid solution and finally bathed with saline solution. Sterile paper points(Detrey, Dentsply, UK) were used for drying the canals.

Obturation and sealing of canal:
Teeth of first group were obturated with Thermafil (Maillefer, Dentsply; Switzerland) and apically sealed with commercially available Mineral Trioxide Aggregate (MTA Angelus, UK)by using the manufacturer's instructions.

Teeth of the second group were obturated with Thermafil (Maillefer, Dentsply; Switzerland) and apically sealed with Bioactive Glass which

Table 1: Showing the mean and standard deviation of porosities % in materials used

	Mean	Minimum	Maximum	Standard deviation	P-Value*
BAG porosity	1.40	1.27	1.63	0.085	<0.001
MTA porosity	2.05	2.03	2.07	0.008	

*One Sample T Test
**p value <0.05 = significant, <0.01 = Very Significant, <0.001 = Highly Significant

Table 2: Showing the mean and standard deviation of voids % in materials used

	Mean	Minimum	Maximum	Standard deviation	P-Value*
BAG voids	0.83	0.81	0.92	0.025	<0.001**
MTA voids	2.15	2.03	2.46	0.145	

*One Sample T Test
**p value <0.05 = significant, <0.01 = Very Significant, <0.001 = Highly Significant

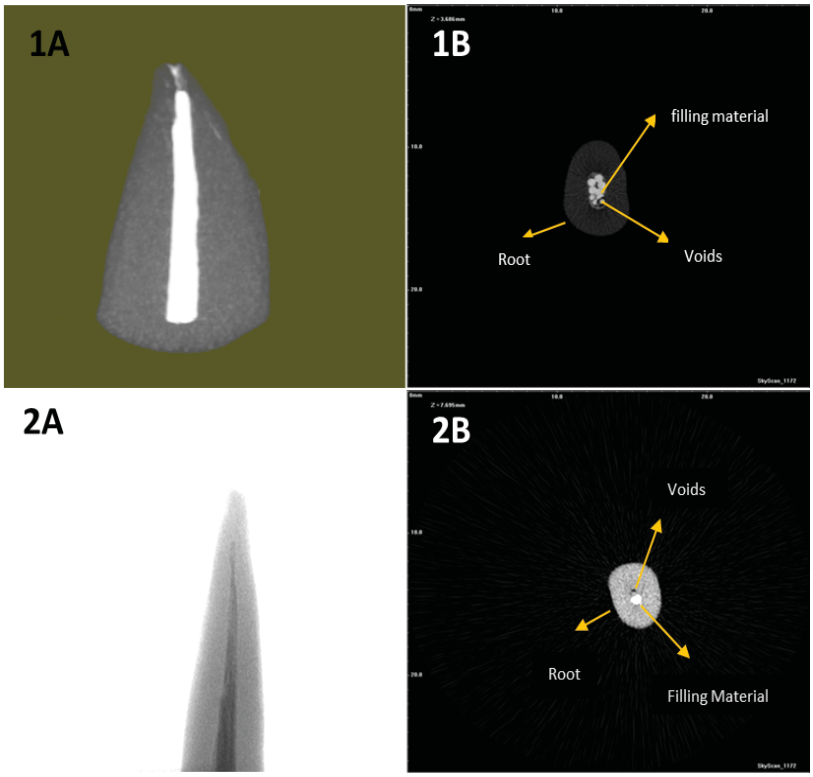

Figure 1A: *Apical region showing the thinning of the MTA material endorsing the probability of the gaps and voids. 1B: Cross sectional Micro CT Image showing clear gaps and spaces between the filled MTA material. 2A: Apical region showing the BAGs material intact almost no gap and void. 2B: Cross sectional micro-CT Image showing fewer gaps and voids between the filled BAG material*

is available commercially by the name Sylc (Osspray, UK). Manufacturer's instructions were strictly followed for handling, mixing and manipulation of the material. The specimens were stored at 37oC in an incubator (INE 200-800, MemmertGmbH+Co. KG, Schwabach, Germany) with deionized water for one week before micro CT evaluation.

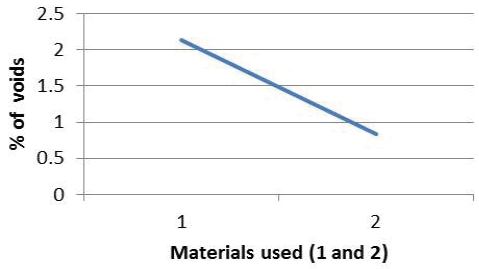

Figure 2: *Graphical representation of the voids percentage 1) MTA and 2) BAG*

Micro-CT data & imaging:

The hardware device we used in this study was commercially available micro-CT (Skscan 1172, Brüker, Aartselaar, Belgium). The x-ray tube was operated at 90 kV and 100 mA (0.5 mm Al+Cu filter), and the scanning was performed by 3600 rotation around the vertical axis and with rotation step of 0.30. By this, acquisition of 1100-1200 transverse cross sections per tooth was resulted at a pixel size of 7-13 um. Data was stored for later use.

The reconstruction of the specimen image was done by using the cone-beam reconstruction application (NRecon software 1.6.7.2). The advantage of this software includes beam-hardening correction, optimized alignment, correction of ring artifact, reconstruction in a restricted volume of interest, reconstruction of objects larger than the field of view, external and internal calibration into Hounsfield units, defect pixel masking, interactive density window selection and many other options. The output file is 'trimmed' to a reduced size according to the sample size or region of interest. Reconstructed slices were saved in .jpg format for viewing.

The reconstructed set of slices can be flexibly viewed in Skyscan's Dataviewer software 1.5 (build 13); with the option to view images axially, coronally, and sagittally. It is a program which allowed the visualization of every single slice and as well as allowing to view of a sample from any angle.

For image analysis and visualization of the re-

sults from micro-CT system scans, CTan and CTvol programs were used. CT Analyzer, Version: 1.12.10.3 was used for 2D/3D analysis whereas CTvol Version 2.0 was used for realistic 3D visualization.

Results:

Bioactive glass (BAG) sealer on comparison with mineral trioxide aggregate (MTA) found to be less porous. Virtually no marginal linear gaps and voids were observed in BAG sealer when scanned under Micro-CT. A highly significant statistical difference was observed in One-sample t-test ($p< 0.001$). The 3D images obtained with NRecon software revealed higher number of voids in MTA sealer (Figure 1) as compared to BAG sealer (Figure 2).

However, the apical thirds of teeth filled with MTA had a significantly lower 3D void volume percentage ($P < 0.05$) when compared with apical thirds filled with BAG (Table 1).

Discussion:

The present study evaluated the sealing ability of a bioactive glass, Sylc (Osspray, UK) as a potential apical sealing material. A quantification of number and volume of voids were computed with micro-CT analysis. For this empirical study, the micro-CT was desired over scanning electron microscope (SEM) because of it successful performance of volumetric measurements of root canal fillings and other dental materials[23,24] at much lower magnification. The non-destructive 3D technique for precise and speedy observation of the internal structure of the specimens[3] make this machine a highly sophisticated tool for both qualitative and quantitative analysis. On the other hand, SEM analysis needs sectioning or destruction of the specimens for the observation of internal structure. The possibility of inaccuracies are high in the form of artifacts creation or loss of filling material during sectioning.[25]

The success of endodontic therapy relies on several contributing factors namely effective micro-organisms control, well-prepared and filled canals. Guttapercha is a widely used and acceptable solid canal filling material in combination with different canal sealing materials.[26] In this study, Thermafil was selected because of its simplicity, ease of use and efficiency in obturating the canal.[27] Although both MTA based sealer and the experimental bioglass based sealer exhibited gaps and voids in the canal obturation with Thermafil yet the marked differences in the volume % of voids data indicates the two tested sealers behave differently in their interaction with the exposed dentine surface.

Predominately, the teeth obturated with MTA sealer showed the higher volume percentage of voids and gaps compared to Sylcbioglass sealer (Table 1 and 2). Comparison of the tested sealers can also be seen in Figure 1 and 2. However, the apical thirds filled with MTA had significantly lower 3D void volume ($P < 0.05$) when compared with apical thirds filled with BAG. This can be attributed to small particle size which allows complete wetting during mixing of the MTA material.[28] Due to the lower viscosity, the apical thirds of roots were wetted in a better way as compared to the experimental bioglass sealer. On the contrary, Sylcbioglass was composed of bigger particle size that increased its viscosity and could not wet the apical one-thirds of the root effectively because of the apical constriction. Abdulla et al. study justify this claim, the mean diameter of apical foramina of all the single rooted teeth was 0.35 mm ± 10 mm in their study.[29] The results of this study showed lesser % of voids in the apical two-thirds with Syclbioglass. The reason could be the elements the bioactive glass composed of i.e., calcium sodium phosphosilicate. These elements occur naturally in the body's hard tissues. This could be the reason that when Sylc exposed to exposed dentine, it underwent a surface reaction over several hours, allowing it to physically adhere to dentine surface. Within a short period of time, essentially all of the bioactive glass particles react to form hydroxycarbonate apatite (HCA), which is chemically and structurally similar to natural tooth mineral.

In near future, studies related to consistency of the tested sealers and obturation techniques

would be useful in determining the effectiveness of the tested sealers. The grounding of a bio-glass powder into nano scale levels would be interesting in evaluating the effectiveness of this bioactive material in apical one-thirds. The effect of long term artificial water aging on the sealing capability would also be interesting to evaluate.

Conclusion:

As conclusions, within the limitations of this study we may summarize that:

- None of the tested sealers showed void free sealing.
- The 3D analysis demonstrated virtually no marginal linear gaps with Sylcbioglass experimental sealer.
- The hypothesis of this study is partially accepted. The tested Sylcbioglass material as a sealer showed comparatively lower void percentage in the apical one-thirds of the root filling.

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Role and contribution of authors:

Dr Jamaluddin Syed, collected the data and did the initial writeup

Dr Aftab Ahmed Khan, helped in introduction, methodology, discussion and conclusion writing and gave the final touchups.

Dr Erum S. Khan, helped in collecting the references and helped in the introduction and discussion writing.

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