ASSESSMENT OF PUSH-OUT BOND STRENGTH AND INTERFACIAL ADAPTATION OF TWO NEW ENDODONTIC SEALERS; ENDOSEQUENCE BC AND MTA FILLAPEX TO ROOT CANAL DENTINE (AN IN VITRO STUDY).

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Abstract

Introduction: the aim of this in vitro study was to evaluate push-out bond strength and interfacial adaptation to dentine wall of EndoSequence BC (BC) and MTA Fillapex sealers compared to AH Plus sealer (AH+). Methods: sixty-six human extracted single rooted teeth with single root canal were selected and the crowns were removed at the level of CEJ to standardized the roots length (16 mm). Samples were instrumented using ProTaper universal NiTi rotary system up to size F5. After irrigation and smear layer removal sample s were randomly divided according to tested sealer used into three groups of 22 sample each, and then obturated using lateral compaction technique and stored for 1 week before testing. Each group was subdivided according to test into two subgroups of 11 sample each, push-out test or interfacial adaptation. Data were statistically analyzed by one-way ANOVA and Tukay’s post-hoc test (P≤0.05). Results: AH Plus has a statistically significant higher bond strength value than each of EndoSequence BC group and MTA Fillapex group. Regarding interfacial adaptation there was no statistically significant difference between the three sealers. Conclusion: EndoSequence BC bioceramic sealer, MTA Fillapex MTA based sealer showed their inferiority regarding push-out bond strength when compared to AH Plus resin based sealer. All of the three sealers showed different grades of gap width along the whole length of root canal wall.

Introduction:-
The main objective of root canal obturation is preventing reinfection of the canal space through providing three-dimensional seal against ingress of bacteria and their toxins (1). Guttapercha is the most commonly used root canal filling material to achieve this goal; however, it is criticized by lack of bonding to dentine wall resulting in incomplete seal of root canal space. So, it is coupled with root canal sealers to eliminate the interface with dentine walls and prevent leakage of any fluid or bacteria from coronal to periapical area and hence prevent development of apical periodontitis. Studies have shown that the sealing ability of root canal filling system depends largely on the sealer performance and mainly to its bond to dentine wall (2,3). The inadequate bond of sealers has contributed to
most of leakage occurring at the cement/canal wall interface (4) that can cause failure of endodontic treatment and hence adversely affect the longevity of the tooth and patient’s oral health. Today, there are many types of sealers available and can be categorized based on their main components: zinc oxide eugenol, calcium hydroxide, resin, glass ionomer, silicone, and bioceramic based root canal sealers (5). Previous investigations have shown that, using different sealers with gutta-percha is not capable of preventing migration of bacteria (2,5). Therefore, new modifications of sealers have been introduced in an attempt to optimize the root canal space seal. EndoSequence BC sealer is bioceramic material composed of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, and various filling and thickening agents. It has nano sized particles and according to manufacturer it forms a chemical bond with dentine wall and allow excellent adhesion. Additionally, it is a hydrophilic (reacts with fluid inside the canal) and it does not shrink on setting (6). MTA Fillapex is a new MTA based sealer with the main components of salicylate resin, bismuth trioxide, fumed silica, base resin titanium dioxide and MTA. According to manufacturer (7), MTA Fillapex is not sensitive to moisture and blood contamination and sets in its presence, forming a hydroxyapatite layer that provides a biological seal. Additionally, it is bioactive material that induces the regeneration of cementum and the periodontal ligament (7).

Material and methods:-
Selection of teeth:-
A total of sixty-six human extracted single rooted teeth with single root canal were selected for this study. The defected teeth were excluded. Teeth were disinfected using 5.25% NaOCl for 4 hours then stored in saline solution until needed.

Root canal preparation:-
The crowns of the teeth were removed at the level of CEJ and the roots length was standardized to 16 mm. The working length and patency of the root canal were determined by inserting a #10 K-file until it reaches the apical foramen, then subtracting 1 mm from this measurement. All samples were instrumented using ProTaper universal NiTi rotary system according to manufacturer instructions up to size F5. EDTA cream lubricant was used during root canal preparation and 3 ml NaOCl (5.25%) irrigation was used after each file during instrumentation. Smear layer was removed using 3ml 17% EDTA for 1min followed by 3 ml NaOCl (5.25%) after the completion of the preparation. Finally, the canals were flushed with 3 ml saline for 1 min and dried with paper points until last one appeared dry before obturation.

Samples grouping:-
The samples were randomly allocated into three groups of 22 teeth according to the sealer used for root canal obturation into BC group, MTA Fillapex group and AH Plus group (control).

Root canal obturation:-
All samples were obturated using cold lateral compaction technique. F5 protaper gutta-percha point that match master file was used as master cone and lateral compaction was completed with a size 30 finger spreader and size 25 accessory gutta-percha cones. Each group was obturated by one of tested sealers. In all groups, sealer was introduced into the canal on the master cone. Excess gutta-percha was removed with a heated instrument to leave 3mm empty entrance that was sealed with temporary filling. Each sample was wrapped in saline moistened gauze in closed plastic cones for 7 days at 37°C to allow sealer to set completely.

Evaluation of the push-out bond strength:-
A total of 11 samples of each group were randomly selected for push-out bond strength evaluation. The samples were embedded in chemical cured acrylic resin block and then cross-sectioned using high speed 0.6mm thickness diamond disk at speed 2500 rpm under water cooling to obtain 2mm thickness section. One section was obtained from each root third. The sections that contained filling material of a noncircular shape were discarded, as this would result in no uniform force distributions during push-out testing and inaccurate measurements. The push-out test was carried out on universal testing machine at a cross head speed of 0.5mm/min using a 500N load cell. The filling materials were loaded with a (0.5,0.7 and 1.0 mm) diameter stainless steel plunger that provided the most extended coverage over the filling material without touching the canal wall. The plunger tip was sized and positioned to touch only the filling without stressing the surrounding dentine. The highest value of loading force needed to dislodge the filing material was recorded. The push-out bond strength expressed in MPa at failure was calculated by dividing the loading force in newton by the area under the load. The area under load was calculated.
with the formula: Area = 2 \pi rh, where r= root canal radius in mm, \pi= 3.14 and h is the thickness of the sample in millimeters.

After push-out test, each root section was examined and photographed under stereomicroscope at 50X magnification to assess the mode of failure.

Assessment of interfacial adaptation:-
The remaining 11 sample from each group were used to assess the interfacial adaptation of the tested sealer using environmental scanning microscope (ESEM). Each sample was grooved longitudinally with a low-speed diamond disc without touching the root canal filling then split with metal spatula into two halves. The samples were mounted on metal tray and scanned by ESEM without any special treatment. Apical, middle and coronal sections of each sample was examined at 3,6,9 mm form the apex for presence of gaps in sealer/dentine interface and three measures gap width between the sealer and root canal dentine per root section was measured in micrometer (um) directly at \times 2000 magnification. (Fig 1) The mean of the three measures was calculated for each root section and considered as gap width and used in statistical analysis.

Statistical analysis:-
One Way ANOVA and Tukay’s post-hoc test were used for the analyses of the data. The significance level was set at P \leq 0.05.

Results:-

![Figure 1](image1.png)

**Figure 1:** ESEM photograph shows sealer dentine interface of representative sections of EndoSequence BC (A), MTA Fillapex (B) and AH Plus (C) sealers. The arrows point to the gaps. (GP: guttapercha, S: sealer, D: dentine). X2000 magnification.

![Figure 2](image2.png)

**Figure 2:** Histogram of the mean push-out bond strength values (MPa) for each root section of EndoSequence BC, MTA Fillapex and AH Plus sealers groups.
Results of Push-out strength test:-
Regardless of root sections, AH Plus group showed a significantly higher bond strength value than each of BC group and MTA Fillapex group. On the other hand, there was no significant difference between the BC group and MTA Fillapex group. Comparing root sections within the three sealers: In both BC group and MTA Fillapex group, there was no significant difference among the root sections. While in AH Plus group, the middle sections showed a significantly higher mean bond strength value than coronal and apical sections. While there was no significant difference between the coronal and apical sections. Comparing results root sections between the three sealers. In both coronal sections and apical sections, there was no significant difference in the bond strength among the three sealers. In the middle section, AH Plus group showed a significantly higher bond strength value than each of BC and MTA Fillapex. On the other hand, there was no significant difference between the EndoSequence BC and MTA Fillapex sealers.

Results of failure mode:-
For all tested sealer groups, no samples showed occurrence of complete cohesive failure, there was no significant difference in the percentage of adhesive and mixed failure in all three groups.

Table 1:- Mean ± SD of push-out bond strength values (MPa) of EndoSequence BC, MTA Fillapex and AH Plus sealers groups.

| Sealers | EndoSequence BC | MTA Fillapex | AH Plus | P- value |
|---------|----------------|--------------|---------|----------|
|         | Mean SD        | Mean SD      | Mean SD |          |
| Coronal | 2.31 0.91      | 2.25 1.07    | 2.81 0.55 | 0.285 NS |
| Middle  | 2.66a 0.67    | 2.44e 0.84   | 4.41e 0.99 | ≤0.001*  |
| Apical  | 2.80 1.25      | 3.04 1.07    | 3.32 1.21 | 0.633 NS |
| Overall | 2.60 0.97      | 2.56e 1.01   | 3.53e 1.14 | 0.001a   |

The means with the same letter within each row are not significantly different at p=0.05. *= Significant, NS=Non-significant.

Results of interfacial adaptation assessment:-
Regardless of root sections, there was no significant difference among the three groups. Comparing the root sections within three sealers: In BC group, there was no significant difference among the three sections. In MTA Fillapex group and AH Plus group, the apical section showed a significantly wider gaps than the coronal section. On the other hand, there was no significant difference between coronal and middle sections nor middle and apical sections. Comparing results of root sections between the three sealers, in the coronal, middle and apical sections, there was no significant difference between three groups.

Discussion:-
Combination of core material and sealer is the standard protocol used in root canal filling nowadays. Because of the lack of adhesion property in guttapercha, sealer was introduced to overcome this limitation. Bonding of the sealer to dentine either by mechanical retention or chemical adhesion or both is important mainly to maintain a tight seal between them after treatment against bacterial leakage. Additionally, it is important to withstand stresses produced during function or due to successive treatment as post preparation or coronal restoration (8,9,10,11).

Table2:- Mean ± SD of gap width (μm) of EndoSequence BC, MTA Fillapex and AH Plus sealers groups.

| Sealer | EndoSequence BC | MTA Fillapex | AH Plus | P- value |
|--------|----------------|--------------|---------|----------|
|        | Mean SD        | Mean SD      | Mean SD |          |
| Coronal| 8.22 3.63      | 4.44 2.18    | 3.95 1.07 | 0.635 NS |
| Middle | 4.46 1.04      | 4.47 3.37    | 4.73 1.07 | 0.283 NS |
| Apical | 8.09 5.22      | 6.75 2.49    | 7.25 2.77 | 0.829 NS |
| Overall| 6.76 4.01      | 5.27 2.81    | 5.63 2.51 | 0.455 NS |

*= Significant, NS=Non-significant

In the present study, adaptation of the sealers to dentine wall have been tested in addition to the bond strength, as strong bond of the sealer to dentine wall does not assure that it covers and adapts to the entire surface of canal wall. It was proposed that, presence of interfacial gap areas due problems in the bond, manipulation or shrinkage of the sealer could allow leakage of bacteria and their byproducts (12,13).
The push-out bond strength test is one of widely used tests in endodontics research for evaluation of the bond strength of the material to dentine wall. It has been suggested that push-out test provides a good evaluation of bond strength because the load applied parallel to the dentine bonding interface simulates clinical stresses (10,14,15, 16). It has the advantages of testing different sections in the root canal besides the simplicity of sample preparation in comparison to other tests (17). However, its results and validity could be affected by some factors that should be taken into consideration during setting up the test. These factors are plunger sizes and root canal diameters and specimen orientation. Larger diameter of plunger size than canal space will cause the load applied to dentine instead of filling materials leading to much higher results. Smaller diameter of plunger size (50%-60%) than canal diameter will cause puncture of the filling material and decreases the bond strength result which will represent reading of strength of the filling material itself. (18,19) This effect disappears when the plunger diameter was 90% of the canal space, so it sets closer to the canal diameter and the stress will be concentrated near the sealer dentine interface. In the current study, three plunger sizes were used for coronal, middle and apical sections to decrease the effect of that variable as possible. In addition to plunger diameter, sample vertical alignment was done to be perpendicular to the applied load. The deviation from vertical alignment will result in frictional resistance during the push-out test which indicates higher results (9, 18). Pane et al (18) reported that deviation from vertical alignment up to 10° degrees did not significantly affect push-out strength.

Selection of straight root canal is an important aspect of the test to give sections that are vertically aligned to the applied load to yield purer forces (20) and produce less stress to the bonding interface during sample preparation (5). Additionally, deviations could happen during sectioning or aligning procedures. The root canal diameters and shapes of single rooted teeth are different between the specimens in the different groups and even in same group before and even after canal preparation. Therefore, the following efforts have been done to decrease this difference and yield uniform cross sections:
1. Crowns of the roots were decoronated at cemento enamel junction and root length was standardized to 16 mm which allow standardization of samples' mechanical preparation and obturation. Furthermore, the decoronation standardizes specimens' sectioning procedures in the experimental design.
2. Root canal preparation using protaper system up to size F5 have been done to yield uniform root canal preparation and cross sections at the three level of the root for all specimens. So the cross section of the samples at coronal middle and apical will be nearly the same, matching the three plungers selected for testing the push-out bond strength.
3. The specimens’ sections were carefully examined to select only root sections with a circular canal shape. Root dentine type and morphology are variable along the whole length of root canals and between specimens that may influence its ability to form bond with the sealers. Effort to standardize the dentine is directed to sample size selection and appropriate randomization (21, 22).

The results of the present study showed that over all AH Plus sealer had a significant higher bond strength than BC and MTA Fillapex. The higher bond strength of the AH Plus, could refer to its ability to form a covalent bond by an open epoxide ring to any exposed amino groups in collagen, long-term dimensional stability, and low polymerization stress (23). It has been reported that MTA Fillapex forms a hydroxyapatite hybrid layer which shows as tag-like structures. Low adhesion capacity of these structures maybe the cause of the low bond strength of MTA Fillapex. That also can be applied to EndoSequence BC which also forms a hydroxyapatite hybrid layer upon setting (23,24,25). This result came in accordance with the results of other studies (8, 15, 23, 25) and contradict to others. Assmann et al (20) reported no significant difference between the bond strength of AH Plus and the MTA Fillapex sealers, while Naser and Al-Zaka (24) and Shokouhinejad et al (26) reported no significant difference between AH Plus and BC sealers. This conflict may result from different methodological variations such as storing sectioned discs for 24 hours before testing, (20) using different core materials and obturation technique (24) or using only middle root sections during testing (26). It is also disagrees with Madhuri et al (27) who reported that BC sealer has higher bond strength than MTA Fillapex. This variation may be attributed to storage time before testing, obturation technique or one plunger size used during samples testing.

Regarding root sections between groups, the result showed that only the middle section showed a significant difference between groups. The middle section of AH Plus group have a statistically significant higher bond strength than both of BC and MTA Fillapex while there was no statistically significant difference between them.
Regarding root sections within the same sealer, the result showed that in AH Plus sealer group the middle section has statistically significantly higher mean bond strength value than the coronal and apical sections while there was no statistically significant difference between them. On the other hand, in both BC and MTA Fillapex groups there
was no statistically significant difference among the root sections. It has been suggested that bonding of root dentine is affected by anatomical and histological factors such as orientation of the dentine tubules, its number which decreases in an apical direction and its size which may be influenced by dentine treatment (28).

During this study, ESEM were selected for adaptation assessment. ESEM has been developed for the purpose of imaging hydrated and non-conducting samples in their natural condition without prior dehydration or conductive coating or preparation (28). ESEM can record specimens under moderate pressure, at low temperature, low vacuum and low voltage and in 100% humidity during the observation period producing high quality and less electrostatically distorted images (29,30,31,32).

In the present study, the result showed that there was no significant difference in gaps width between the three sealers. one of the possible causes for gap presence among the three sealers BC, MTA Fillapex and AH Plus is the obturation technique used. The current results come in accordance with Demiriz et al 2016 (33); while on the other hand, it come contradict to other studies (34,35,36). Polineni et al (34) reported that MTA Fillapex has poor adaptation than BC while the other studies reported that BC has better adaptation than AH Plus in coronal and middle sections (35) or apical section (36). This difference may be attributed to different obturation technique (34, 36), different core materials (34), sample preparation method (36), method of measurement (34) or to different canal preparation size (34, 35, 36).

In the current study, lateral compaction technique used during obturation which still the most used technique among practitioners (37). Demiriz et al 2016 (33); stated that incomplete polymerization, viscosity increases during the polymerization or polymerization shrinkage could be the cause of gap formation in AH Plus and MTA Fillapex who had a resin component. In the current study, samples were stored for one week to be sure that sealers were set completely.

Regarding gaps among root section of each sealer, BC group showed no significant difference between the three sections. In the other hand, apical section has statistically significant wider gaps than the coronal section in both MTA Fillapex and AH Plus group while there is no significant difference between middle and both apical and coronal sections. These results come in accordance with Polineni et al (34). On the other hand, this result is come contradict to Demiriz et al 2016 (33), who reported no significant difference between root sections of MTA Fillapex and AH Plus. The difference in the result might be related to the use of the different obturation technique. Furthermore, different method of measurement may lead to different results where they used only the highest value among the detected gap. Increased gap in apical sections could be referred to difficulties in smear layer removal from this part due to insufficient irrigation delivered to this area. Also, less compaction force applied to this section during obturation may be a cause.

Results of BC group come in contradict to Polineni et al (34) who reported significant wider gaps in apical section than coronal in previous study they used different core materials, different obturation technique, different sealer application method and method of measurement which may lead to this discrepancy. In the current results, although there was no significant difference between root sections, coronal section showed wider gap than both middle and apical sections which agreed with the result of previous study (35). This incidence and overall gaps of BC sealer might be a result of its hydrophilic nature. Al-Haddad et al (38) reported that EDTA irrigation for smear layer removal may alter the adhesion of bioceramic sealers by decreasing the wetting ability of dentine because Bioceramic-based sealers are hydrophilic by nature, in contrary AH Plus is hydrophobic which founds suitable environment in acidic medium of EDTA (38). In the current study, although saline was used for the final irrigation, it might have been insufficient to completely flush away EDTA increasing the wetting ability of dentine.

Conclusions:-
1. EndoSequence BC bioceramic sealer, MTA Fillapex MTA based sealer showed their inferiority regarding push-out bond strength when compared to AH Plus resin-based sealer.
2. Regarding interfacial adaptation, all of the three sealers EndoSequence BC, MTA Fillapex and AH Plus showed different grades of gap width along the whole length of root canal wall.
3. EndoSequence BC, MTA Fillapex and AH Plus sealers, all showed adhesive failure.
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