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## Original Research Article

## An in vitro comparison of fracture resistance of immature teeth subjected to apexification using three different bioactive materials

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## ABSTRACT

**Aim:** To compare the fracture resistance of immature teeth subjected to apexification with three different bioactive materials.**Materials and Methods:** Sixty non-carious, single-rooted premolar teeth with straight canals were decoronated at cemento-enamel junction, standardizing 13 mm length. After access preparation, the canals were accessed with #10 K-file followed by biomechanical preparation using Ni-Ti ProTaper Gold rotary files upto F3 and irrigation was done using 2 mL 3% NaOCl, 17% EDTA and 5 ml of saline. To simulate immature roots with open apices, #1–5 Peeso reamers were passed through the apex enlarging to 1.5 mm diameter. Later, samples were randomly divided into 4 different groups with 5mm apical plug in each group, Group I: GuttaFlow BioSeal, Group II: Biodentine, Group III: EndoSeal MTA and Group IV: Control group. Samples were filled with an apical plug of 5mm. Later, remaining part of the root canals was obturated with gutta-percha cones and AH Plus sealer by lateral condensation. To stimulate periodontal ligament lining of the root surfaces, C-Silicone light body impression material was used. The samples were stored for 1 week in 100% humidity at 37°C to ensure that the sealer set in an environment that simulate the clinical situation. Later, fracture resistance was evaluated using the universal testing machine.**Results:** Group II (Biodentine) showed highest fracture resistance followed by Group I (GuttaFlow BioSeal) and Group III (EndoSeal MTA)**Conclusion:** Biodentine apexification groups showed highest fracture resistance values followed by GuttaFlow BioSeal and the least values were shown by EndoSeal MTA.This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.For reprints contact: [reprint@ipinnovative.com](mailto:reprint@ipinnovative.com)

## 1. Introduction

Traumatic dental injuries most often occur in young and adolescent age groups where the root apex is still in the process of maturation. These injuries often lead to pulpal necrosis, leading to cessation of root formation in the developing roots, producing immature root apex, characterized by wide, open apices and thin dentin walls. The endodontic treatment of such teeth has been a

challenge as a lack of apical stop poses difficulty in working length determination and obturation.<sup>1</sup> The performance of bioactive materials is largely attributable to their capacity of producing spontaneously an apatite layer when in contact with phosphate-containing physiological fluids, which can be used as apical stop and root strengthening.<sup>2</sup>

The aim of this in vitro study was to compare the fracture resistance of immature teeth subjected to apexification by three different bioactive materials like Biodentine, GuttaFlow BioSeal and EndoSeal MTA. Since there have not been enough comparative studies between

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these materials, we have chosen them to compare here. The null hypothesis was that there will be no difference between the tested materials in providing the fracture resistance of immature teeth.

## 2. Materials and Methods

Sixty non-carious, single-rooted premolar teeth with straight canals were collected for the study. The samples were decoronated at cemento-enamel junction, standardizing 13 mm length. Access preparation was performed and the canals were accessed with #10 K-file. Biomechanical preparation was done using Ni-Ti ProTaper Gold rotary files upto F3. The canals were irrigated using 2 mL 3% NaOCl, 17% EDTA and 5 ml of saline. To simulate immature roots with open apices, #1–5 Peeso reamers were passed through the apex enlarging to 1.5 mm diameter (#5 Peeso reamer) (Figure 1). To simulate periodontal ligament lining of the root surfaces, C-Silicone light body impression material was used. Later, samples were embedded in acrylic blocks (Figure 2) and randomly divided into 4 different groups.

### 2.1. Grouping method

The sixty experimental root samples were randomly divided into four groups:

- Group 1: GuttaFlow BioSeal apical plug
- Group 2: Biodentine apical plug
- Group 3: EndoSeal MTA apical plug
- Group 4: Control group (non-instrumented canals, intact teeth)

Group 1 and 3: Endo Seal MTA sealer (Maruchi, Korea) and GuttaFlow BioSeal sealer (roeko Coltene, Switzerland) come as premixed syringes which were inserted into the apical portions and backfilled to create a 5 mm apical plug, which was then radiographically verified.

Group 2: Biodentine (Septodont) is available as powder and liquid formulation. Powder is premixed in capsule to which liquid was added and triturated in amalgamator as per manufacturer's instructions. The formed mass was then carried with an amalgam carrier into the canal to create a plug which was then condensed into the apical portion with hand pluggers (GDC) – no. RCP 1/3 and RCP 9/11. The 5mm plug created was radiographically verified.

Group 4: Control group teeth were directly simulated for PDL lining and embedded in acrylic blocks.

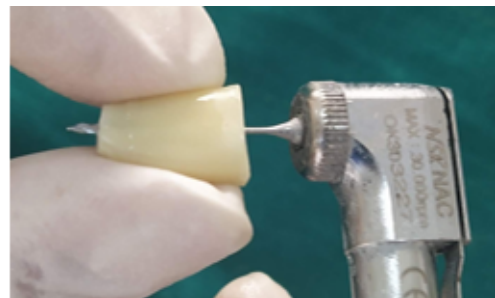
Later, remaining part of the root canals was obturated with gutta-percha cones and AH Plus sealer by lateral condensation. The samples were stored for 1 week in 100% humidity at 37°C to ensure that the sealer set in an environment that simulate the clinical situation. Later, fracture resistance was evaluated using the universal testing machine with the speed of 0.5mm/minute.

### 2.2. Statistical analysis

All the collected data of forces at which fracture of teeth occurred in Newton were subjected to statistical analysis using SPSS/PC version 20 software (IBM). A one-way analysis of variance was used to compare the forces at which the fracture of roots obturated with different materials occurred. Pair-wise comparison of four groups was performed by Scheffe's post hoc procedure. Statistical analysis was performed at 95% level of confidence.

## 3. Results

In experimental groups, the mean fracture resistance value was higher in Group 2 (root canals obturated with Biodentine) when compared to Group 1 and 3 (root canals obturated with GuttaFlow Bioseal and Endoseal MTA respectively), with no statistically significant difference between group 1 and 3 ( $p > 0.05$ ). The mean fracture resistance value was higher in Group 4 (control group, without instrumentation) when compared to experimental groups. ( $p > 0.05$ ).



**Fig. 1:** Simulation of immature apex by passing peeso reamer



**Fig. 2:** Sample embedment in acrylic block

**Table 1:** Mean, standard deviation and standard error of fracture resistance values for the experimental and control groups.

Groups	n	Mean (N)	SD	SE
Group 1	15	280.96	24.36	6.29
Group 2	15	354.17	82.66	21.34
Group 3	15	261.57	19.19	4.95
Group 4	15	378.10	137.01	35.37

SD: Standard deviation, SE: Standard error, n: number of samples

**Table 2:** Pair wise comparisons of four groups (1, 2, 3, 4) with respect to fracture resistance (Newton) by Scheffe's Posthoc procedure.

	Group 1 (p)	Group 2 (p)	Group 3 (p)	Group 4 (p)
Group 1		0.122	0.935	0.020*
Group 2	0.122		0.029*	0.885
Group 3	0.935	0.029*		0.003*
Group 4	0.020*	0.885	0.003*	

\*P<0.05 statistically significant.

#### 4. Discussion

The completion of root development and closure of the apex occurs up to 3 years after eruption of the tooth and unfortunately, traumatic injuries may occur during this period and may result in pulpal inflammation or necrosis.<sup>3</sup> Unfortunately, approximately 50% of the traumatized teeth are diagnosed with pulpal necrosis and incomplete root formation. Immature teeth have thin dentin walls and thus, are more fragile than the mature teeth and remain very sensitive to fracture, especially in the cervical area.<sup>4</sup>

Treatment option for such teeth revolves around two approaches, regeneration or apexification. Traumatic injury might result in complete destruction of HERS, which would reduce the success rates of regeneration and hence, an alternative treatment strategy like apexification could be utilized.<sup>3</sup> Main aims of the treatment for such immature teeth should be to produce an apical barrier, against which an obturating material is to be placed thereby preventing the extrusion of material into the surrounding tissues and providing a restoration that can reinforce or toughen immature dentin.<sup>5</sup>

Apexification by Calcium hydroxide (CH) treatment has shown adequate apical healing by means of the induction of an apical barrier and the agent's antibacterial capability, caused by a high pH. However, these teeth showed a 50% reduction in strength over 1 year, and were compromised by cervical root fractures because of changes in the organic matrix of the dentin.<sup>1</sup> To counteract the drawbacks of Calcium Hydroxide apexification treatment, materials based on bioactive technology like Mineral Trioxide Aggregate (MTA), Biodentine, Calcium Enriched Mixture Cement (CEM) and Bioaggregate were utilized.<sup>6</sup>

Bioactive materials present good adhesion and sealing properties by forming apatite crystals on the material surface, a material/dentine interface and dentinal tubules when in contact with biological fluids such as phosphate solutions. The formation of an interfacial layer and tag-like

structures in the dentine may minimise the microleakage and increase the push-out strength by improving the marginal adaptation of the bioactive cements.<sup>7</sup>

Mineral trioxide aggregate (MTA), which has a good root sealing ability and a high degree of biocompatibility, has been demonstrated to have good potential as an aid in the formation of apical hard tissue. Robert Lawley et al. demonstrated that the MTA apical barrier induced apical hard tissue formation.<sup>8</sup>

Biodentine is also referred to as "bioactive dentine substitute," which has improved physical, mechanical, and handling properties as compared to MTA. A specific feature of Biodentine is its capacity to continue improving the compressive strength with time over several days. It reaches up to 300 MPa after 1 month, which is almost equal to that of the compressive strength of natural dentin (297 MPa).<sup>1</sup>

GuttaFlow BioSeal is a newly introduced sealer with bioactive properties which provided tissue repair along with obturation. It is composed of Gutta-percha powder particles, polydimethylsiloxane, platinum catalyst, zirconium dioxide, calcium silicate, nano-silver particles and bioactive glass ceramic. Calcium silicate, which, upon contact with biological tissues, releases natural repair constituents and aids in the regeneration of periapical tissues.<sup>1</sup> Hence, this novel bioactive sealer was used in this study. Calcium ions are essential for the process of differentiation and mineralization of mineralizing cells. Gandolfi, et al.<sup>9</sup> demonstrated that the formation of calcium phosphate in biological-like environment reduces the interface open porosity with the time.<sup>10</sup>

Thus, in the clinical condition sealers that are capable of forming calcium phosphate might be predicted to enhance sealing with time thereby increasing the fracture resistance of endodontically treated teeth. Additionally, they are capable of creating a bonelike apatite, also creating an osteoinductive environment promoting cell bioadhesivity.<sup>10</sup> Since, there are very few comparative studies in literature between GuttaFlow BioSeal, Biodentine and MTA used

as apexification material, we have selected the mentioned materials.

Mandibular premolars with circular cross-section were selected which simulated the clinical situation better, where chewing forces are maximum.<sup>1</sup> To remove the smear layer and open the dentinal tubules for sealer penetration, irrigation using 2 ml of 17% EDTA and 2 ml 3% sodium hypochlorite was done alternatively with final rinse of 2 ml of saline.<sup>11</sup>

To simulate immature teeth, the canals were instrumented with Peeso reamers (#1–#5) until a size 5 Peeso was passed 1 mm beyond the apex to simulate open apex. Here we have performed apex enlargement upto Peeso reamer 5 (1.5mm)<sup>12,13</sup> Stuart et al.,<sup>14</sup> Tanalp et al.,<sup>15</sup> and Seto et al.<sup>16</sup> used a similar methodology for preparation of root canals.<sup>1</sup> The samples were then embedded in acrylic resin blocks for homogenous stress distribution and PDL simulation was done with Condensation silicone impression material to approximate the clinical scenario.<sup>17</sup> After their embedment in acrylic blocks, apical plugs of previously mentioned bioactive materials were made, which facilitated adequate condensation using hand pluggers against the acrylic surface.<sup>18</sup>

For apexification, a study by Hachmeister et al.<sup>19</sup> suggested a 3-5 mm thickness of the material as apical barrier. We have selected the thickness of apical plug to be 5 mm as previous studies have shown maximum success rates at 4-5mm thickness.<sup>19</sup> All the samples were then stored in an incubator for 24 hours for the apical plug to set followed by obturation of rest of the canal with Gutta percha and AH Plus sealer (Dentsply, Maillefer, Ballaigeus, Switzerland) which is an epoxy resin-based sealer with good flow capacity. A study by Rahimi et al.<sup>20</sup> showed no statistical significance between the lateral condensation and Obtura II techniques.<sup>20</sup>

Universal Testing Machine (Instron) has been used for measurement of fracture resistance of teeth in many studies. In this study, continuous load was vertically applied along the longitudinal axis of the teeth as here the load entirely transfers to the root which would result in decreased bending moments and maximum stresses located much more cervical, leading to smaller stresses.<sup>21</sup>

The results of our study demonstrate a statistically significant difference of ( $p < 0.05$ ) between Group 2 [Biodentine (354.17N)] and Group 3 [EndoSeal MTA (261.57)], Group 1 [GuttaFlow BioSeal (280.96)] and Group 4 [Control group (378.1 N)], Group 3 [EndoSeal MTA (261.57)] and Group 4 [control group (378.1 N)] (Table 1).

There was no statistically significant difference of ( $p > 0.05$ ) found between Group 2 [Biodentine (354.17N)] and Group 1 [GuttaFlow BioSeal (280.96)], Group 1 [GuttaFlow BioSeal (280.96)] and Group 3 [EndoSeal MTA (261.57)], Group 2 [Biodentine (354.17N)] and Group

4 [Control group (378.1 N)] (Table 2). Hence, the null hypothesis of our study was rejected as there was significant difference found amongst the various groups.

The lower mean for EndoSeal MTA could be attributed to the fact that it does not actually bond to dentin, rather it deposits hydroxyapatite interfacially, which only increases the frictional resistance of the filling material.<sup>21</sup> Moreover, EndoSeal MTA is premixed with nonaqueous but water-miscible carriers and, contrary to powder forms of MTA, uses only the environmental moisture to initiate and complete the setting reaction. These differences might predispose EndoSeal MTA to lower dislodgement resistance.<sup>22</sup>

Another chosen bioactive material was biodentine based on the previously mentioned appetite forming properties and excellent rise in compressive strength during prolonged periods. Also, the ease of availability and ease of manipulation lead us to its selection as one of the materials tested. Biodentine yielded maximum fracture resistance to compressive forces in our study which was statistically significant as compared to EndoSeal MTA. This was in accordance to a study done by R. Yasin et al.<sup>23</sup> and Grech L et al.<sup>24</sup> which demonstrated lowest degree of solubility for Biodentine and confirmed the deposition of hydroxyapatite crystals on material surface in presence of synthetic tissue fluid, thereby increasing its retention in the root apices aiding to improved fracture resistance.<sup>25</sup>

The rationale for choosing GuttaFlow BioSeal sealer for apical plug was based on the novelty of this material and its ease of manipulation (pre-mixed syringes), faster setting time and ease of availability. Additionally, sealers with a paste formulation showed to enhance the fracture strength due to the superior flow and greater penetration into dentinal tubules. Moreover, zirconium oxide found in the formulation of this sealer has a high fracture and tensile strength along with low Young's modulus as cleared out by Omran et al.<sup>10</sup>

Additionally, Gandolfi, et al.<sup>9</sup> demonstrated that the formation of calcium phosphate in biological-like environment reduces the interface open porosity with the time. Thus, in the clinical condition sealers that are capable of forming calcium phosphate might be predicted to enhance sealing with time there by increasing the fracture resistance of endodontically treated teeth. Additionally, they are capable of creating a bonelike apatite, also creating an osteoinductive one promoting cell bioadhesivity.<sup>10</sup> Hence, since the fracture resistance values were higher than MTA and closer to Biodentine, this novel material can be taken into consideration in apexification procedures.

Limitation of this study may be the lack of simulation of various masticatory forces as different stomatognathic occlusion and masticatory patterns along with the surrounding alveolar bone density might influence the occlusal loads on endodontically treated teeth. Thus, by

simulating oral cavity conditions as much as possible, valuable results can be obtained.<sup>26</sup>

## 5. Conclusion

Biodentine apexification groups showed highest fracture resistance values followed by GuttaFlow Bioseal and the least values were shown by EndoSeal MTA. Hence, within the limitations of this in-vitro study, it can be concluded that newly introduced bioactive sealer GuttaFlow BioSeal can be utilized in apexification procedures as an artificial barrier as an alternative to Biodentine.

## 6. Conflict of Interest

None.

## 7. Source of Funding

None.

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