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# Evaluation of the effect of smear layer on the apical sealing ability of Mineral trioxide aggregate using a novel fluid filtration method: an in vitro study

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***ABSTRACT***
***Background and objective:***
*The influence of smear layer on the apical sealing ability of MTA is still controversial. The sealabilityof MTA with and without smear layer can be measured using fluid filtration method with more advantages than other leakage studies.*
***Aim:***
*To investigate the effect of the smear layer on apical microleakage in teeth obturated with Mineral Trioxide Aggregate (MTA) using fluid filtration method.*
***Methodology:***
*Fifty extracted human maxillary central incisor teeth without caries,cracks or open apices, decoronated at 14-mm from apex , pulp extirpated and apical patency was established with a size 10 K-file . In Group A,the smear (-) group (n=20), 10 mL of 17% Ethylene DiamineTetraacetic Acid (EDTA) followed by 10 ml of 5.25% Sodium hypochlorite ( NaOCl) was used and in Group B , the smear (+) group(n=20) , the root canals were irrigated with 10 mL of 5.25% NaOCl only. Apical 5mm parts of the teeth filled with MTA using a carrier gun and plugged. Leakage quantity was measured by fluid filtration and expressedas µL/cmH2O/ min$^{-1}$at 2nd and 30$^{th}$ days.*
***Results:***
*On the 2$^{nd}$ and 30$^{th}$ day the MTA obturated samples with smear layer (Group B) showed decreased leakage than the other group(Group A). Both the groups showed increase in leakage value during the study period. But there was no statistical significance when the increase in filtration was compared between.the groups.*
***Conclusion:***
*Presence of smear layer has a positive effect on the apical sealing ability on MTA on 2$^{nd}$ and 30$^{th}$ days. Irrespective of the presence or absence of smear layer, there was an increase in leakage when the samples were studied for 30 days.*
***Key words:*** *Mineral trioxide aggregate, Smear layer, obturation,dental leakage, filtration, EDTA*

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## I. Introduction

Microorganisms present inside the root canal may remain active in the dentinal tubules even after vigorous chemomechanical preparation .Thus perfect apical sealing is desirable to prevent the remaining bacteria and their endotoxins from reaching the root apex. Apical leakage is considered to be a common cause

for endodontic therapy failure and is influenced by many variables such as different filling techniques, the physical and chemical properties of sealers and the presence or absence of smear layer.

Conventionally ideal requirements of an obturating material requires that it should be bacteriostatic, seal apically and laterally, be nonirritating to periapical tissues, resist moisture, and provide radiopacity. Furthermore, the material should be sterile, nonshrinking, nonstaining, and easily placed and removed from the root canal system[1].Furthermore, it would be more desirable if an obturation material can offer additional properties that decrease bacterial survival and promote bioactive mechanisms necessary for regeneration and healing[2].

Apical leakage is influenced by many variables such as different obturation methods, the properties of the sealer used and the presence or absence of a smear layer. Root canal instrumentation produces a layer of organic and inorganic material called the smear layer that may also contain bacteria and their by-products. It can prevent the penetration of intracanal medicaments into dentinal tubules and influence the adaptation of filling materials to canal walls[3]. But the advantages and disadvantages of the presence of the smear layer in instrumented root canals are still controversial issues [4–6]because leakage studies in which the smear layer has been removed have yet to prove that removal is consistently beneficial.

Mineral Trioxide Aggregate (MTA) might have a profound advantage when used as canal obturation material because of its superior physiochemical and bioactive properties. MTA provides an effective seal against dentin and cementum and promotes biologic repair and regeneration of the periodontal ligament[7]. According to the present literature review several studies have shown that Ethylene Di Amine Tetraacetic Acid (EDTA) as a smear layer removing agent could possibly interfere with the setting characteristics as well as alter the surface and ultrastructural characteristics of MTA[8,9].But certain other studies have concluded that smear layer removal improved the marginal adaptation provided by MTA and subsequently improved the seal provided by the material[10,11] .

Thus, it seems that no clear consensus has been reached in the endodontic community as to whether the smear layer should be removed or left intact before sealing of the root canal space and whether the commonly used material for smear layer removal, EDTA could affect the apical sealing ability of MTA when used as an obturation material.

The sealing ability of MTA and other root filling material has been tested by using dye, fluid filtration[12–14], protein leakage[15], and bacterial leakage methods[13]. Among these methods fluid filtration possess several advantages like automatic recording, quantitative measurement, more precise , ability to record small volumes, no need to destroy samples, long term evaluation ,ability to assess both apical and coronal leakage and more sensitive. A computerized fluid filtration technique which has digital air pressure arrangement and makes use of infrared rays and photosensitive diodes has been used in many of the leakage studies[16]

But more recently a simpler and novel version of fluid filtration method has been studied that makes use of a digital camera to record the bubble position and uses Auto CAD 2006 softwareto locate the bubble position with a precision of 0.1mm and thereby assesses the amount of leakage quantitatively using a specific software equation .The use of digital camera makes the method more precise and permits rereading of the fluid displacement. The equipment itself is more inexpensive and user friendly[17].

This study aims to makeuse of this novel method of fluid filtration system to assess apical leakage of MTA when used as a root filling material with and without using EDTA to remove the smear layer inside the root canal.

## II. Methodology

This in vitro study was conducted in the Department of Conservative Dentistry &Endodontics, Government Dental College, Thiruvananthapuram. The study protocol was approved by the Scientific and Ethical Committee of Government Dental College, Thiruvananthapuram. Fifty extracted permanent human maxillary central incisor teeth that were free of caries, cracks or open apices were selected. The total sample size was divided into 2 groups of 20 patients each, which constituted the experimental group. Fiveteeth were taken as positive control and 5 teeth were selected as the negative control in order to check the efficiency of the fluid filtration system. The study groups are as follows:

**Group A – the smear (-) group (n=20)** **:** MTA obturation after smear layer removal

**Group B – the smear (+) group (n=20)** **:** MTA obturation without removal of Smear layer

**Positive control** **:** Canal instrumented and left unfilled.

**(n=5)**

**Negative control (n=5):**Instrumented and filled with cyanoacrylate cement

All the teeth were randomly assigned to each group.

**Sample preparation**

The external surfaces of the teeth were cleaned with curettes. The teeth were stored in a physiologic saline until use. The crowns of all teeth were removed to a specific level by using a diamond disc at low speed hand piece at 14-mm length. The pulp of each tooth was removed and apical patency established with a size 10 K-file (Kerr, Romulus, MI). Working length was established 1.0 mm short of the apical foramen, and the roots were instrumented to size 60 K file by using the step-back technique. A size 10 K-file was used during root canal preparation to maintain patency of the canal. The coronal part of the root canals was completed with Gates-Glidden drills (Maillefer, Ballaigues, Switzerland). (Figure 5).Between each file use, the canals were irrigated with 1 mL of 5.25% sodium hypochlorite solution ( NaOCl).

In the smear (+) group (n=20), the root canals were irrigated with 10 mL of 5.25% NaOCl only. In the smear (–) group (n=20), 10 mL of 17% EDTA followed by 10 mL of 5.25% NaOCl was used to remove the smear layer. All irrigating solutions were delivered via a 23-gauge needle inserted as far as possible into the canal without binding. The canals were finally irrigated with distilled water and dried with paper points (DiaDent, Almere, Netherlands).

**Obturation Technique**

Apical 5 mm of the teeth were filled with MTA. An MTA carrier gun was used to apply the MTA within the root canal. Then an endodontic plugger appropriate to master apical file was chosen, and the stopper was adjusted to the plugger 1 mm behind the working length. After all the prefilling preparations were completed, the MTA was mixed according to the manufacturer's recommended proportions and placed into a mortar. The MTA was taken from the mortar with the MTA gun and applied within the root canal where the tip of the gun reached. After that, the MTA within the canal was pushed to the apex of the root canal with the plugger to obtain a 1-mm apical filling, and the same application was repeated until a 5-mm filling was achieved.

The negative control samples (n=5) were instrumented and filled with cyanoacrylate cement. Positive control samples (n=5) were instrumented and left as unfilled. This was to check the efficiency of the assembled fluid filtration system.The samples were stored in isotonic saline solution in between the leakage assessment.

**Measurement OfSealability**

As multiple measurement of sealability of single sample is needed in this study, fluid filtration was preferred to bacterial and dye penetration studies. The apparatus for studying was set up according to method described by Javidi et al[17]. (Figure 7)

This system involves the evaluation of fluid transport in specimens calculated from bubble movement. It is necessary to apply pressure to fluid to move through the specimen and displace the bubbles. Therefore an oxygen tank equipped with a manometer to precise adjustment of pressure was utilized. A specific plastic tube was connected to the oxygen source and the end part was connected to an Erlen. Two holes were made on the Erlen's cap, one for the entrance of oxygen and the other for emersion of fluid.

The oxygen tube was fixed above the fluid in the Erlen, but the glass cylinder was entirely immersed in water (the end that removes the fluid) and the other side of this cylinder was connected to a micropipette (0.1 cc) by a plastic tube. This micropipette was fixed on a vertical plate and its other end was connected to a three-valve tube by a latex pipe (0.5 cm in diameter and approximately 2 cm in length).

The three-valve tube was equipped with a bilateral control faucet; when turned on, only two directions were connected. The upper side of the three-valve tube was connected to a syringe, which was used to create an air bubble through the micropipette. The diameter of the air bubble was not smaller than the internal diameter of the micropipette so that its movement will be a precise indicator for fluid movement in the micropipette. The lower side was used to connect specimens.

All of the connections of this system were smeared with cyanoacrylate glue and covered by multiple layers of Parafilm strips .This strip seals connection in experimental tubes to guarantee an impervious connection. Micropipette, connections and the three-valve tube were fixed on the vertical plate of the system made of compressed plastic. This vertical plate was mounted on a completely stable horizontal plate.The role of this horizontal plate was to provide stability for the vertical plate and to communicate between two parts of the system.

Part 1 consists of tubes, micropipette, pipes and a tooth sample that transfer pressure to the specimen and Part 2 contains a recorder of fluid transport. A digital camera (Nikon D3100 14.2MP) ( Figure 6) and a professional software (AutoCAD 2011,Autodesk, Inc.) was used in this system to record and measure the amount of bubble displacement. The camera was mounted on the horizontal plate using a small tripod with a distance of 32 cm from the vertical plate. It was placed on the middle of the micropipette to prevent minimal errors. Its distance from the micropipette was the closest possible to allow the camera to cover the entire range of the bubble's movement(Figure 8).

The outer surfaces of all teeth except the apical 2 mm were covered with Parafilm strips. The apical end of the root excluding apical foramen was covered by cyanoacrylate glue and inserted in latex with a 0.5 cm internal diameter and 5 cm length. The free end of the pipe was connected to the only free end of the three-valve tube (the lower end). This junction was sealed completely by Parafilm strip.

The control faucet was closed toward the tooth, so only the syringe and the micropipette was connected. The syringe was used to insert an appropriate air bubble to the micropipette. The camera was adjusted in the macrograph to take precise picture in a short distance. The control faucet was opened to the tooth and the syringe was removed from the pass. Now only the tooth and the fluid filtration system were connected. Then the major faucet of the oxygen tank was opened. The pressure was previously adjusted, since it should be constant during all steps of the experiment.

The first picture of the bubble position in the micropipette was taken after 30 sec to reach a balance in the system. Four subsequent pictures were taken at 2-minute intervals (2, 4, 6 and 8 min after the first one) ( Figure 9). At the end of the experiments, the faucet for the oxygen tank and then the control faucet to the samples were closed. The Parafilm strips were opened and the latex pipe was disconnected from the three-valve tube. The same steps were repeated for the next samples.

Five pictures of each sample were transferred to the computer. The bubble position in each picture was determined by professional software (Auto CAD 2011). The precision of this software was 0.1 mm, so with the device used in this study, the smallest recordable displacement was 0.031mm and the smallest recordable volume was about $10^{-8}$ litre.

Displacement measures were introduced to custom-made software designed for accelerating the calculations. This software calculates the mean displacement of the bubble per minute and then with a specific quotient converts the longitudinal displacement of the bubble into the volume of fluid passing from the samples showing it as µl/min/cm $H_2O$ unit. Thus one number for each sample represented the amount of leakage in the canal (µl/min/cm $H_2O$).

**Statistical Analysis**

Independent t test was used to analyze the values between Group A and Group B at each time period. Paired t test was used to compare fluid filtration at $2^{nd}$and $30^{th}$ day for each group.ANalysisof CO- VAriance (ANCOVA) test was used to compare the change in fluid filtration of groups during the storage period.

## III. Results

There was only negligible bubble displacement in the negative control group and complete fluid escape through the tooth apex in the positive control group that indicated the efficiency of the fluid filtration system.

When comparison of fluid filtration at day 2 was evaluated between the groupsit was significantly less ($p<0.01$) in group B, the smear (+) group (0.0297) as compared to GroupA , the smear (-) group (0.347). The independent t test ($t=5.03$, $p<0.01$) showed that the fluid filtration is significantly less among Group B (0.0312) as compared to Group A**.(Table 1)**

When comparison of fluid filtration at day 30 was evaluated between the groups it was significantly less ($p<0.01$) in Group B, the smear (+) group (.0312) as compared to Group A , the smear (-) group (0.0354). The independent t test ($t=4.19$, $p<0.01$) showed that the fluid filtration was still significantly less among Group B (0.0312) as compared to Group A.(**Table 1).**Thus at both 2 days and 30 days Group B showed significantly less leakage than Group A **.**

When comparison was made within Group A for the leakage at $2^{nd}$ day and $30^{th}$day it was observed that in Group A, there was 2.08 percent increase in fluid filtration. The paired t test ($p<0.01$) showed that the increase in fluid filtration in Group A was statistically significant at 0.01 level**.** Thus there was a statistically significant increase in leakage in theSmear (-) group, that is, Group A as shown in the figure **(Graph 1)**

Similarly when comparison of fluid leakage was done within Group B, the smear (+) group , it was observed that there was 5.07 percent increase in fluid filtration The paired t ($p<0.01$) showed that the increase in fluid filtration in group B was statistically significant at 0.01 level **( Table 2 ).**There was a significant increase in fluid leakage even in the smear (+) group, Group B, from day 2 to day 30.

In order to assess the total increase in leakage in each groups during the entire evaluation period ANCOVA test was used **( Table 3 ).**The value of the ANCOVA ($Fy.x = 3.52$, $p>0.05$) was not significant at

0.05 level. This means that there was comparable increase in fluid leakage in both Group A and Group B from day 2 to day 30.

## IV. Discussion

The smear layer in a cavity and that within the root canal is not comparable. There is difference in the tools used for cutting as well as greater variation in the number of dentinal tubules. There will be more of soft tissue remnants within the canal unlike in coronal cavities. The generation of a smear layer is almost inevitable during root canal instrumentation[4].

In our study in Group A the smear (-) group, we have used 10 ml of 17% EDTA followed by 10 ml of 5.25% sodium hypochlorite to remove the smear layer and final irrigation was done with distilled water since many studies have evaluated the efficacy of these irrigating solutions and have concluded that the alternate use of these two solutions was the most effective in smear layer removal from the canal walls[18,19]. Some studies have suggested that EDTA alters the surface characteristics and setting properties of MTA and hence can increase the apical leakage across MTA root fills[8,9]. They have suggested the possibility of chemical reaction between residual EDTA and MTA which accounts for the increased apical leakage. Since we wanted to assess this effect of EDTA on thesealability of MTA we used EDTA to remove the smear layer.

The canals were obturated with MTA only in the apical 5mm since this area is the most critical zone with maximum propensity for lateral and accessory canals. In addition to this , the high cost of this material makes it less cost effective to fill the entire canal especially in anterior teeth.The possibility of voids during orthograde apical plugging of MTA cannot be ruled out.A study that compared 3- to 5-mm MTA plugs against complete orthograde MTA obturation of root canals tested with a fluid filtration device revealed no significant difference in sealing ability after 4 weeks[20]

In our study the samples were subjected to fluid filtration 48 hrs after obturation with MTA and again after 30 days of obturation.This is in accordance with the study done by Martin et al,which compared the effect of microleakage in the root canal with MTA as an apical plug or as root canal filling material. They showed that after 48 hours, MTA root filling was better than MTA as an apical plug, but that there was no difference after 4 weeks. But in the study by Martin et al the effect of smear layer on the sealability was not evaluated[20]. Hence we wanted to evaluate the sealability of MTA after 48 hours as well as 30 days and to assess if there was any difference in the smear (+ )group and the smear (-) group.

Among the various methods to assess apical leakage , this study used a novel fluid filtration system reported by Javidi et al[17].Although other methods like dye penetration and bacterial studies have been used in other leakage studies, we chose fluid filtration system because the samples are not destroyed during the study and hence monitoring of the same samples could be done again after 30 days . Automatic recording allowed elimination of operator errors and precise recording of the values. Both quantitative and qualitative assessment was possible and the system is highly sensitive. This method ensured extreme precision and permitted rereading the fluid displacement since the bubble displacement was recorded using digital camera . $O_2$ pressure was used because it remains unchanged during the experiment and is adjustable for different clinical situations. It is also time saving. The system was claimed to have a high precision of $10^{-1}$Lfor the smallest recordable volume[17].

When the leakage at day 2 was evaluated and compared between the groups, it was found that Group A ,the smear (- ) group showed significantly higher leakage after 48 hrs during all the time periods when compared to Group B, the smear (+) group which was statistically significant This is contradictory to a similar study by Yildirim et al in which the leakage after 2 days was not significantly different between the smear( +) group and the smear( –) group[5]. However in this study a computerised fluid filtration system designed by Orcoglu et al[16]was used to assess the leakage .

When the amount of leakage was compared between Group A and Group B at 30 days the smear (+) group ; Group B showed significantly less leakage than Group A , the smear (-) group which was statistically significantand similar to the findings of Yildirim et al[5].

Comparison within Group A, the smear (-) group on the 2nd day and 30 th day revealed that there was a 2.08 % significant increase in leakage on day 30 when compared to the 2 nd day. This indicates that in Group A the sealability of MTA root fills was deteriorating over time which is clinically relevant.

Interestingly when comparison of leakage was made within Group B, the smear (+) group, during the 2nd day and the 30th day period it was observed that the percentage increase in leakage at 30 days was 5.07% when compared to that in 2 days. This is contradictory to another similar study in which no significance difference in leakage was observed 2 days and 30 days[5].

While assessing the overall difference in leakage between Group A and Group B during the total evaluation period, although there has been increased leakage in both the groups from day 2 to day 30, the increase in leakage in both the groups were comparable during the study period . This points out that thesealability of MTA deterioratedovertime and resulted in a comparable increase in leakage in the smear (+)

group and the smear (-) group. But the amount of leakage in Group B the smear (+) group was significantly less during all the time periods of observation when compared to Group A, the smear (-) group.

In a similar study authors have suggested that decreased leakage in smeared versus smear-free MTA-obturated canals might have been due to several factors like hydrophilic properties of MTA and particle size of MTA[5].As stated by Torabinejad et al the decrease in leakage in the smear (+) group ,Group B could be due to the fact that smear layer might act as a "coupling agent" enhancing the bond between the MTA and root canal dentin[21].

The leakage was increased overtime in both the smear (+) and the smear (-) groups although the amount of leakage was less in the smear (-) group during all the time periods.In a study by Timpawat et al that investigated the effect of removal of the smear layer on apical microleakage, using the fluid filtration method, it was concluded that the removal of smear layer caused significantly more apical microleakage than when the smear layer was left intact[22] .

The overtime increase in leakage in both the smear (-) group as well as the smear (+) group suggests that , in addition to the presence or absence of smear layer , there could be other variables like the properties and surface characteristics of MTA , the type of MTA used, the manipulation and setting characteristics of MTA , the effectiveness of orthograde placement technique for MTA and the solubility of the material , or the possibility of a chemical interaction with EDTA , which can influence the final seal provided by the material .In this study the increased leakage across MTA root fills irrespective of the presence or absence of smear layer might bedue to the in vitro experimental set up that could have compromised the bioactivity of MTA.

In another study it is observed that MTA does not contain endogenous phosphorous[23]. MTA need to foster phosphate groups from the surrounding tissue fluids in order to be bioactive .In our study isotonic saline was used for storage of the samples. This could have caused a decrease in the bioactivity of MTA which in turn plays an important role in improvement of its seal overtime.

## V. Conclusion

The following conclusions are drawn while interpreting the results of the present study. The retention of intracanal smear layer significantly decreases the apical microleakage of MTA as an obturationmaterial. The removal of smear layer significantly increases the apical leakage after MTA obturation .Irrespective of the presence or absence of smear layer , apical leakage of orthograde MTA apical plugs increases over time indicating a deterioration in its sealability.The amount of increase in apical leakage overtime is comparable irrespective of the presence or absence of smear layer indicating that the clinical significance of the influence of smear layer on the sealability of MTA obturation remains questionable .

**Limitations And Future Recommendations**

The experimental set up and environment might have an influence on the setting characteristics as well as bioactivity of MTA which in turn plays an important role in the sealability of MTA. Hence more studies in a clinical setting is recommended to assess the outcome even better. In this study the evaluation of leakage was done only for a period of 30 days. Hence longer follow up periods could be more informative of the long term sealability of MTA root fills.Further studies are recommended, which takes into consideration, other variables like the properties and surface characteristics of MTA, the type of MTA used, the manipulation and setting characteristics of MTA, the effectiveness of orthograde placement technique for MTA and the solubility of the material, the possible chemical reactions of MTA with other materials used for chemomechanical preparation of the root canals.

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**Conflicts of interest**

There are no conflicts of interest in this sudy.

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**Author Contributions**

The first two authors have equal contribution in developing concept, design, data collection, analysis, writing and editing of the article and should be considered as primary authors. All authors gave their final approval and agree to be accountable for all aspects of the work.

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**Table 1**

Comparison of fluid filtration at day 2 and day 30 between groups

| | | Mean | SD | N | t | P |
|---|---|---|---|---|---|---|
| Day2 | Group A | 0.0347 | 0.0033 | 20 | 5.03** | 0.000 |
| | Group B | 0.0297 | 0.0030 | 20 | | |
| Day 30 | Group A | 0.0354 | 0.0034 | 20 | 4.19** | 0.000 |
| | Group B | 0.0312 | 0.0030 | 20 | | |

** : Significant at 0.01 level

**Table 2**
Comparison of fluid filtration between 2nd and 30th day in Group B

| | Mean | SD | N | Mean percentage Difference | Paired t | p |
|---|---|---|---|---|---|---|
| Day2 | 0.0297 | 0.0030 | 20 | 5.07 | 14.43** | p<0.01 |
| Day 30 | 0.0312 | 0.0030 | 20 | | | |

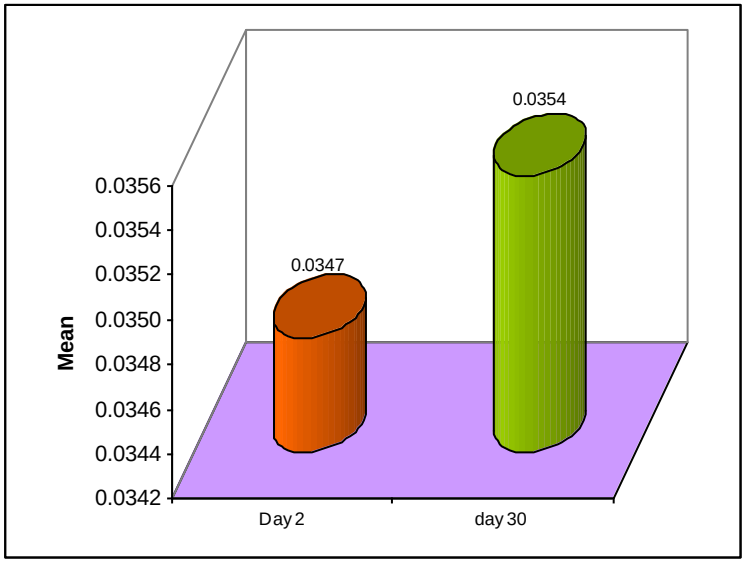
** : Significant at 0.01 level

**Table 3**
Comparison of effectiveness of intervention in Group A and Group B on fluid filtration (ANCOVA)

| Stage | | Mean ± SD | df | F | P |
|---|---|---|---|---|---|
| Day2 | Group A | 0.035 ± 0.003 | (1,38) | 25.28** | 0.000 |
| | Group B | 0.03 ± 0.003 | | | |
| Day 30 | Group A | 0.035 ± 0.003 | (1,38) | 17.54** | 0.000 |
| | Group B | 0.031 ± 0.003 | | | |
| Adjusted value at day 30 | Group A | 0.033 ± 0.0002 | (1,37) | 3.52 | 0.069 |
| | Group B | 0.034 ± 0.0002 | | | |

** : Significant at 0.01 level

**Graph 1**
Comparison of fluid filtration between 2nd and 30th day in group A




Dr Kiran V M, et. al. "Evaluation of the effect of smear layer on the apical sealingability of Mineral trioxide aggregate usinga novel fluid filtration method: an in vitro study." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 21(07), 2022, pp. 22-29.