

# Comparative Evaluation of Cytotoxic and Genotoxic Effects of Three Resin-Based Sealers by 3,(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay and Comet Assay – An *In vitro* Study

## Abstract

**Background and Aim:** The complete sealing of the pulpal space contributes to the better healing potential after an root canal treatment, and root canal sealers are an integral part of this treatment which comes in contact with the periodontal tissue. Biocompatibility of an endodontic sealer plays an important role in the root canal treatment procedure. Hence, the aim of the study was to compare and evaluate the cytotoxicity and genotoxicity effect of three resin-based sealers on human periodontal fibroblast cells. **Settings and Design:** This *in vitro* study was conducted in Dr. Prabhakar Kore's Basic Science and Research Center and K. L. E V. K. Institute of Dental Sciences, Belagavi. **Materials and Methods:** Human periodontal fibroblasts were incubated with 15 specimens of Group 1 – AH Plus Group 2 – MTA Fillapex, and Group 3 - GuttaFlow 2, respectively. Cytotoxicity was assessed by 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and genotoxicity was assessed by Comet assay at time intervals at 24 h and 48 h. **Statistical Analysis:** Data were analyzed by the Kruskal–Wallis and Mann–Whitney U-test. **Results:** AH Plus and GuttaFlow 2 did not show any cytotoxicity or genotoxicity. MTA Fillapex was slightly cytotoxic throughout the time interval, and it also showed low genotoxicity throughout the time periods for which it was tested. **Conclusion:** AH Plus and GuttaFlow 2 did not show any cytotoxic and genotoxic effect; however, MTA Fillapex showed cytotoxic and genotoxic effect throughout the time interval.

**Keywords:** 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, comet assay, cytotoxicity, fibroblasts, genotoxicity, sealer

## Introduction

A complete sealing of the pulp canal space after cleaning and shaping is of prime importance for a successful endodontic treatment.<sup>[1]</sup> Endodontic sealer is in proximity with periapical tissues and its inadvertent extrusion may cause cellular injury, periapical inflammation which affects the outcome of treatment.<sup>[2]</sup> Hence, biocompatibility of a sealer plays a vital role for successful endodontic treatment.

AH Plus is a widely used endodontic sealer which consists of two paste systems: Paste A: Bisphenol-A epoxy resin, Bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, and iron oxide pigments and Paste B: Dibenzyl diamine, amino adamantane, tricyclodecane-diamine, calcium tungstate, zirconium oxide, silica, and silicone oil.<sup>[1]</sup>

MTA Fillapex (MTA-F; Angelus, Londrina, Brazil) is a material of excellent biological

property of white MTA which is composed of salicylate resin, resin diluent, natural resin, bismuth oxide, silica, mineral trioxide aggregate, and pigments.<sup>[1]</sup> Hence improve the properties of an endodontic sealer.

GuttaFlow 2 (coltene whaledent, GmBH + Co KG, Langenau, Switzerland) is a newly introduced sealer which is a silicone-based flowable filling system which combining gutta-percha powder with sealer in one single tube. It is comprised a flowable, nonheated gutta percha that slightly expands instead of shrinking. It contains micro silver which is different than guttaflow (coltenewhaledent, GmBH + Co KG) which contains nanosilver.<sup>[3]</sup> The genotoxicity of the guttaflow 2 has not been evaluated yet.

To assess the biocompatibility of a dental material, one of the initial screening tests is cytotoxicity which includes the various standardized

**Vaidehi Vikas  
Dhopavkar,  
Sunita S. Shivanand,  
Kishore Bhat,  
Anand C. Patil,  
Preeti K. Doddwad,  
Niraj Jayant  
Godbole**

*Department of Conservative  
Dentistry and Endodontics,  
KLE.V.K. Institute of Dental  
Sciences, Belgaum, Karnataka,  
India*

**Submitted :** 30-Apr-2020  
**Revised :** 22-Jul-2020  
**Accepted :** 05-Oct-2020  
**Published :** 21-Dec-2021

**Address for correspondence:**  
*Dr. Vaidehi Vikas Dhopavkar,  
807, Angol "C" Scheme,  
M. G. Road, Hindu Nagar,  
Belgaum - 590 006, Karnataka,  
India.  
E-mail: vaidehi1703@gamil.com*

Access this article online

**Website:**  
[www.contempclindent.org](http://www.contempclindent.org)

**DOI:** 10.4103/ccd.ccd\_213\_20

**Quick Response Code:**



**How to cite this article:** Dhopavkar VV, Shivanand SS, Bhat K, Patil AC, Doddwad PK, Godbole NJ. Comparative evaluation of cytotoxic and genotoxic effects of three resin-based sealers by 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and comet assay – An *in vitro* study. *Contemp Clin Dent* 2021;12:376-82.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, 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: WKHLRPMedknow\_reprints@wolterskluwer.com

cell culture methods and the most frequently used is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay which employs target cells.<sup>[4]</sup> The evaluation of genotoxicity testing is important for the detection of potential human toxicity of the sealers to adjacent periodontal tissues so that hazards can be prevented. Comet assay or single-cell gel electrophoresis is a test done for quantitative DNA damage assessment in mammalian cells.

The test for genotoxicity is highly sensitive and can detect the slightest level of DNA damage, and it even requires short time to complete with minimal cost and limited number of cells per sample. Resin-based sealers which are currently being used in endodontics exhibit a certain amount of toxicity.<sup>[5]</sup> However, there are fewer studies comparing both the cytotoxicity and genotoxicity for these resin-based sealers. Furthermore, the genotoxicity of guttaflow 2 has not been studied previously.

Hence, with this gap in the knowledge, this study aims to evaluate and compare cytotoxic and genotoxic effects of three different resin-based sealers: Group 1 – AH Plus (epoxy-based resin sealer), Group 2 – MTA Fillapex (MTA-based resin sealer), and Group 3 – guttaflow 2 (silicone-based sealer).

## Hypothesis

### Null hypothesis

The three resin-based sealers (epoxy-based, MTA-based, and silicone-based sealers) will not have any cytotoxic or genotoxic effect on human fibroblasts cells.

### Alternate hypothesis

The three resin-based sealers (epoxy-based, MTA-based, and silicone-based sealer) will have any cytotoxic or genotoxic effect on human fibroblasts cells.

## Materials and Methods

Forty freshly extracted intact human permanent teeth with healthy periodontal tissue, indicated for routine orthodontic extraction were collected and preserved in phosphate buffer saline (PBS) medium until its culture. Teeth with caries, external resorption, and inflamed periodontal tissue were excluded.

Test compounds were as follows: Group 1 – AH Plus sealer (AH-Plus; DENTSPLY DeTrey GmbH, Konstanz, Germany), Group 2 – MTA Fillapex sealer (MTA-F; Angelus, Londrina, Brazil), and Group 3 – GuttaFlow 2 sealer (coltene whaledent, GmbH + Co KG, Langenau, Switzerland).

## Methodology

### Procedure for the assessment of cytotoxicity of test compounds<sup>[6]</sup>

#### Cell isolation and culture

Human periodontal fibroblasts were isolated and cultured (S Ivanovski 2001) in 96 well plate. In brief, the

healthy periodontium tissue and the tooth extracted after orthodontic extractions were placed in PBS. The excised periodontal tissues were placed in tissue-culture dishes to allow the establishment of explant cultures. Subsequent sub-cultures were made in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), 1% antibiotic-antimycotic  $\times 100$ .<sup>[7]</sup>

### Sample preparation and elution

All the three sealers were mixed as per the manufacturer's instructions under sterile conditions. 15 discs for each sealer (2 mm in diameter and 2 mm in length) were fabricated in sterile Teflon molds. The test specimens were allowed to set in a humid chamber at 37°C for 24 h. All the discs were sterilized by keeping them in the laminar air flow for 15–20 min.

### Cytotoxicity test

Cytotoxicity was done by the MTT assay. The cell line was cultured in DMEM medium which was supplemented with 10% heat inactivated FCS. The cells were seeded in a 24 well-flat bottom microplate and maintained at 37°C in 95% humidity and 5% (Carbon Dioxide) CO<sub>2</sub> for overnight. The individual discs were treated in 24 well plate in DMEM using the ratio 1.25 cm<sup>2</sup>/ml between the surface of the sealer samples and the volume of medium in triplicates.

The cells were incubated for 24 and 48 h. After the incubation, the discs were removed and cells in the well were washed twice with phosphate buffer solution, and 20  $\mu$ L of the MTT staining solution (5 mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 37°C. After 4 h, 100  $\mu$ L of di-methyl sulfoxide was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using ELISA microplate reader.

The percentage of cell viability was calculated from the following formula:<sup>[6]</sup>

$$\% \text{ of cell viability} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Cytotoxicity was rated based on cell viability relative to control as given by Dahl *et al.* which was interpreted as:<sup>[8]</sup>

- Noncytotoxic (>90% cell viability)
- Slightly cytotoxic (60%–90% cell viability)
- Moderately cytotoxic (30%–59% cell viability)
- Strongly cytotoxic (<30% cell viability).

The percentage of cell viability for each sealer was recorded, and the results were tabulated and were subjected to the statistical analysis.

### Procedure for the assessment of genotoxicity of test compounds

The protocol for genotoxicity by alkaline method was carried as per given by Singh *et al.*<sup>[9]</sup>

Human periodontal fibroblasts were isolated, cultured (S Ivanovski 2001), and sample preparation was done same as that for cytotoxicity.<sup>[7]</sup>

#### Preparation of base slides

1% (500 mg per 50 ml PBS) was prepared and microwaved or heated until near boiling point till agarose mixture was dissolved. While normal melting agarose was hot, conventional slides were dipped up to one-third the frosted area and then gently removed. The underside of slide was wiped to remove agarose and slides were laid in a tray on a flat surface to dry. The slides were air dried or warmed at 5°C. A aliquot of 5 mL low melting agarose (LMA) was placed into vial in a 37°C water bath to cool and thereby stabilizing the temperature.

#### Cell treatment

The cell line were cultured in DMEM medium which was supplemented with 10% heat inactivated calf serum (FBS) and 1% antibiotic-antimycotic  $\times 100$  solution. The cells were seeded at a density of approximately  $5 \times 10^4$  cells/well in a 24-well plate flat bottom microplate and maintained at 37°C in 95% humidity and 5% CO<sub>2</sub> overnight. The individual discs were treated in 24 well plates in DMEM using the ratio 1.25 cm<sup>2</sup>/ml between the surface of the sealer samples and the volumes of medium in triplicates. The cells were incubated for another 24.48 h. After the incubation, the discs were removed.

Thus, a volume of 10  $\mu$ L of treated or control cells ( $1 \times 10^4$  cells) was added to 120  $\mu$ L of 0.5% low-melting point agarose at 37°C, layered onto a pre-coated slide with 1.5% regular agarose and covered with a coverslip. After brief agarose solidification in the refrigerator, the coverslip was removed and a third agarose layer (80  $\mu$ L LMA) was added to the slide. The coverslip was replaced until the agarose layer hardens (5–10 min). The slides were immersed into a lysis solution for about 1 h.

#### Electrophoresis of microgel slides

This procedure describes electrophoresis under pH >13 (alkaline conditions).

After at least 2 h at 4°C, the slides are gently removed from the lysis solution. The slides were allowed to set in the alkaline buffer for 20 min to allow for unwinding of the DNA and the expression of any alkali-labile damage. The slides were electrophoresed for 30 min.

The slides were then coated drop wise with neutralization buffer for at least 5 min. This was repeated for two more times. Slides were stained with 80  $\mu$ L 1X ethidium bromide and left for 5 min and then dipped in chilled distilled water to remove any excess stain. The coverslip was then placed over it, and the slides were dried before staining. The slides were drained, and then, it is kept for 20 min in cold 100% ethanol or cold 100% methanol for dehydration. The slides were air dried and are placed in an oven at 50°C for 30 min and

stored in a dry area. The slides were rehydrated with chilled distilled water for 30 min and stained with 10% ethidium bromide for 10 min and then covered with a fresh coverslip.

#### Evaluation of DNA damage

For the visualization of DNA damage, observations were made of EtBr-stained DNA using a 40x objective on a fluorescent microscope. To assess the quantitative and qualitative extent of DNA damage in the cells by measuring the length of DNA migration and the percentage of migrated DNA. Smaller fragments of DNA migrate further in the electric field compared with intact DNA and the cellular lysates thus resembled a “comet” with a bright fluorescent head and a tail region [Figure 1].

Each slide was analyzed using fluorescent microscope and 50 comets per sample were calculated at 24 h and 48 h. During the analysis, the edges and eventually damaged parts of the gel as well as debris, superimposed comets, comets of uniform intensity and comets without a distinct head (“clouds,” “hedgehogs,” or “ghost cells”) were excluded.

Two parameters were estimated:<sup>[10]</sup>

1. Tail length (the distance of DNA migration from the body of the nuclear core which was recorded as the distance from the perimeter of the comet head to the last visible point in the tail)
2. Tail intensity (percentage of DNA in the tail).

Image analysis was done by ImageJ and Open Comet analysis assay.

The comets were classified according to the extent of DNA damage into five categories as follows by Pereira *et al.*:<sup>[11]</sup>

1. Undamaged (damage <5%)
2. Low damage (5%–20%)
3. Medium level damage (20%–40%)
4. High level of damage (40%–85%)
5. Completely damaged (damage >85%).

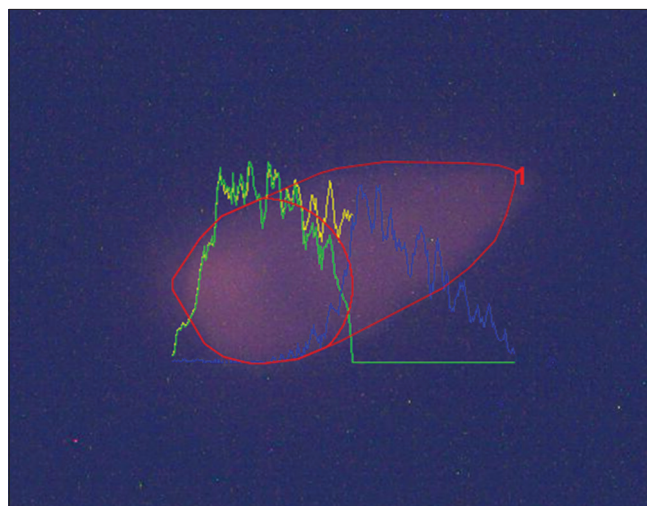


Figure 1: Comet seen in MTA Fillapex. Image analysis was done by ImageJ and Open Comet analysis assay



## Results

Three resin based sealers were evaluated and compared for cytotoxicity [Table 1] and genotoxicity [Tables 2 and 3] at 24 and 48 h, i.e., Group 1 – AH Plus; Group 2 – MTA-Fillapex; and Group 3 – guttaflow 2. Statistical analysis was done by Kruskal–Wallis test.

AH Plus and guttaflow 2 did not show any cytotoxicity or genotoxicity throughout the tested time interval, i.e., 24 h and 48 h. MTA Fillapex was more cytotoxic and genotoxic than AH Plus and guttaflow 2 [Graphs 1-3].

## Discussion

The null hypothesis of the study was rejected as there was a significant difference between the three groups. AH Plus and guttaflow 2 did not show any cytotoxicity or genotoxicity throughout the tested time interval, i.e., 24 h and 48 h. MTA Fillapex was more cytotoxic and genotoxic than AH Plus and guttaflow 2.

The preeminent considerations for a root canal procedures are biocompatibility and antimicrobial property of an endodontic sealer. Biologically unfavorable materials, which does not necessarily cause blatant clinical symptoms, but may have an impact on the healing processes in the periapical tissues and prolong or avert resolution of lesions.<sup>[12]</sup>

Hence, the main purpose of this study was to evaluate the toxicity of endodontic sealers suitable for its use in the clinical scenarios.

In this study, cytotoxicity and genotoxicity of three resin-based sealers which are Group 1 - AH Plus (Epoxy resin based), Group 2 – MTA Fillapex (MTA-based sealer, and Group 3 - GuttaFlow 2 (silicone-based sealer) were evaluated.

A myriad of techniques are accessible to evaluate the biocompatibility of materials where in the cell culture technique is an extensively employed method. Cytotoxicity is a complex phenomenon which can result in a wide spectrum of effects from a simple cell death to metabolic aberrancy with functional or route-specific changes,<sup>[13]</sup> whereas genotoxicity is one of the esteemed factors prevailing biocompatibility. Genotoxicity may cause damage to the cell genome that can significantly decrease the tissue's self-repairing potential and in the long term may cause neoplasia.

Although a number of tests are published in the literature for testing cytotoxicity like MTT assay, lactase dehydrogenase assay, Typhan blue staining, 2,3-bis-(2-methoxy-4-nitro-5-carboxyanilide), and genotoxicity tests such as chromosomal aberration, comet assay, and micronucleus assay test.<sup>[10]</sup>

In the present study, MTT assay was used to assess cytotoxicity and comet assay to evaluate genotoxicity.

MTT assay is a simple, fast, precise, sensitive, reproducible method for detecting *in vitro* cytotoxicity and cell proliferation.<sup>[13]</sup>

With respect to MTT assay, the MTT solution is a tetrazolium salt which is reduced to formazan by mitochondrial enzymes of viable cells proportionally to dehydrogenase activity. This perceives the signal which is produced by the activation of living cells. In this assay, the yellow MTT salt, which has a ring-shaped molecular structure is absorbed by the cells and then cleaved by an enzyme inside the mitochondria which gives rise to a product named Formazan which are purple-colored nonsoluble crystals.<sup>[13]</sup> To achieve this result, ELISA spectrophotometer was used for the identification of color intensity of the solution, which gives a highly accurate reading to check the cytotoxicity of material.

For genotoxicity, Comet assay or single-cell gel electrophoresis method was used which is a standard, noninvasive, and a powerful technique that directly measures DNA damage which occurs in individual cell types of nearly all kinds of cells. This assay is based on the principle that, the size of DNA fragments is reduced by its damage which is detected by applying an electrophoretic field to the lysed

**Table 1: Cytotoxicity of test compounds at 24 h**

Compound name	Mean of optical density	Cell viability (%)
Group 1 - AH Plus	0.2962	91
Group 2 - MTA Fillapex	0.2018	61.86
Group 3 - GuttaFlow 2	0.3052	93.56
Negative control	0.3262	100

**Table 2: Cytotoxicity of test compounds at 48 h**

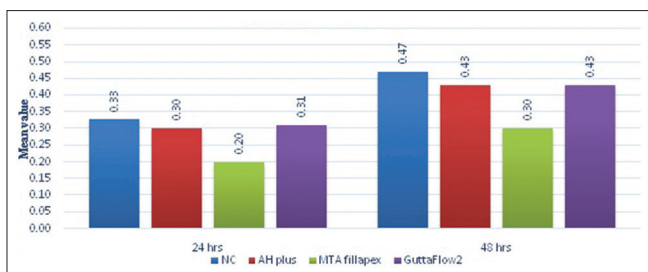
Compound name	Mean of optical density	Cell viability (%)
Group 1 - AH Plus	0.432	91.83
Group 2 - MTA Fillapex	0.2976	63.26
Group 3 - Guttaflow 2	0.4334	92.13
Negative control	0.4704	100

**Table 3: Genotoxicity of the test compounds (tail length assessment at 24 h)**

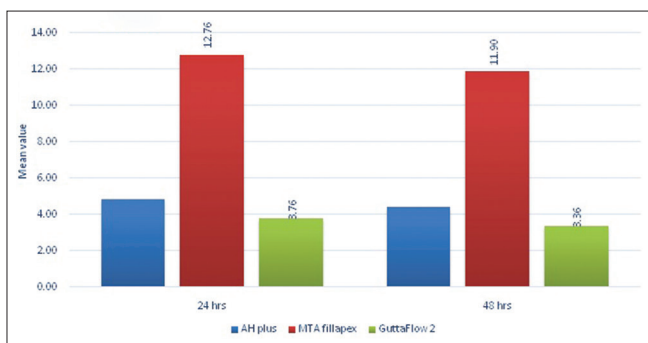
Compound name	Tail length assessment (average) (%)
Group 1 -AH Plus	4.82
Group 2 - MTA Fillapex	12.76
Group 3-Guttaflow 2	3.76

**Table 4: Genotoxicity of the test compounds (tail length assessment at 48 h)**

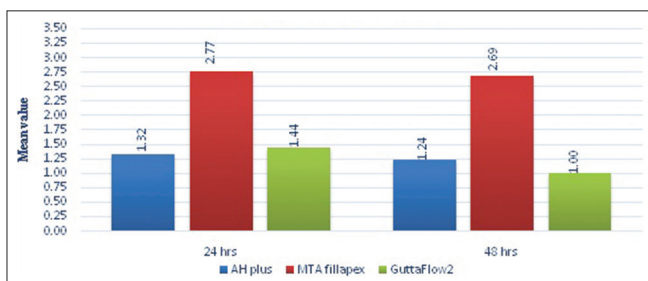
Compound name	Tail length assessment (average) (%)
Group 1 - AH Plus	4.42
Group 2 - MTA Fillapex	11.80
Group 3 - Guttaflow 2	3.36



Graph 1: Absorption Score checked for cytotoxicity at 24 and 48 h



Graph 2: Is of tail length which is checked for genotoxicity at 24 h and 48 h between three sealers



Graph 3: Is of tail intensity of genotoxicity which is checked at 24 h and 48 h between 3 sealers

cells where in the damaged cellular DNA fragments and intact DNA are separated, yielding to a classic “Comet tail” shape seen under a fluorescent microscope [Figure 1]. The extent of DNA damage is mostly evaluated by comet tail measurements by using the image analysis software which is ImageJ and Open Comet software.<sup>[14]</sup>

If a tested material is proven to be a cytotoxic to cultured cells *in vitro*, a similar behavior can be assumed *in vivo* in patients.<sup>[15]</sup> In addition, human cells can be cultured with a less number of transition, resulting in minimum cell changes due to cell culture manipulation.<sup>[16]</sup> The choice of cultured human periodontal ligament fibroblasts for the present study is justified by the close connection of endodontic sealers and cements to the periapical tissue.<sup>[17]</sup>

There is no standard time for cytotoxicity test, since each sealer is cytotoxic at different time intervals. Hence, after a final set of 24 h, analysis was done at 24 h and 48 h. Although cytotoxicity testing of freshly mixed sealer is

pertinent as it is placed into the root canal system in a freshly mixed and incompletely polymerized stage, it is important that after the setting the sealers are evaluated over extended periods because it is likely that, after or during the clinical application of the sealer, changes in cytotoxicity levels may be observed because of the diffusion of lethal components from the materials into the surrounding environment.

### Cytotoxicity and genotoxicity

Cytotoxicity of test materials and its cell viability were assessed by criteria given by Dahl *et al.*,<sup>[8]</sup> and genotoxicity was assessed by criteria given by Pereira *et al.*<sup>[11]</sup>

Genotoxicity of sealers was tested by ImageJ analysis software. Open Comet is a plugin for image processing program ImageJ. This can estimate the area and pixel value statistics of user-defined selections and intensity-threshold objects. It can also measure the distances and angles.

Considering the test materials used in this study, Group 1 - AH Plus has shown moderate cytotoxicity after initial mixing (Merdad *et al.*; Lodeine *et al.*) which decreases after setting (Merdad *et al.*). The authors have claimed that it might be due to the release of minute amounts of formaldehyde or release of amine added to accelerate the epoxy polymerization or epoxy resin components from the sealer.<sup>[16,18]</sup>

At 24 h, cytotoxicity was 91%, and at the end of 48 h, it was 91.83% [Table 1 and Graph 1]. In the present study, no cytotoxicity was observed with AH Plus. The results of the present study are in accordance with results shown by Karapinar-Kazandağ *et al.*, Camps *et al.*, and Azar *et al.*<sup>[2]</sup>

Previous studies done on genotoxicity of AH Plus by Leyhausen *et al.* and Van Landuyt *et al.* have shown that AH Plus is not genotoxic.<sup>[19,20]</sup> There have been contradicting results with epoxy resin-based sealers. AH Plus, AH 26, and topseal are shown to have genotoxic effect in the study done by Huang *et al.* in which the authors have stated that epoxy resin-based sealers have a dose-dependent increase in genotoxicity.<sup>[21]</sup>

Group 2 - MTA Fillapex which is a MTA-based sealer and has a low solubility, and easy handling,<sup>[22]</sup> however, the results related to its biological response are conflicting. Several researchers revealed that this material displayed high level of cytotoxicity and genotoxicity even after 90 days,<sup>[23]</sup> whereas some studies demonstrated that the cytotoxicity of MTA Fillapex decreased with time.<sup>[24]</sup>

However, the results of the present study proved that Group 2 - MTA Fillapex remained cytotoxic throughout the tested times. MTA Fillapex showed the cell viability decreased at the end of 24 h (61.86%) and at the end of 48 h (63.26%) after the final set [Tables 1,2 and 4]. The release of arsenic material from MTA Fillapex that reacts with the protein thiols and long-term exposure may be responsible for the increased cytotoxicity. Furthermore, the

presence of resin may play a secondary role in inducing cytotoxicity.<sup>[10]</sup>

The genotoxicity of MTA Fillapex was most probably associated to its composition where it contains only 30% MTA and resin components such as salicylate which has a higher potential concerned to cellular genotoxicity. MTA Fillapex also contains higher levels of arsenic, heavy metal, element as a contaminant, In a study done by Darrag and Fayyad in MTA, Fillapex was genotoxic.<sup>[10]</sup>

Furthermore, in a study done by Claudia *et al.*, in the author stated that MTA Fillapex showed cytotoxic and genotoxic effects. Salicylate has shown to initiate the process of apoptosis in human fibrosarcoma cells and has caused the fragmentation of cell genetic material, deciding its precipitation in the cytoplasm.<sup>[25]</sup>

A longer period may therefore be necessary for the toxicity to decrease over time to render it noncytotoxic. The results of this study are in accordance with the studies done by Loise *et al.* and Silva EJ *et al.*<sup>[1,26]</sup>

Group 3 which is GuttaFlow 2 is a silicone-based sealer. Genotoxicity of guttaflow 2 has not been documented previously. However, genotoxicity of guttaflow which is its previous version has been determined by (Brzovic *et al.*), and the author has stated that guttaflow sealer is not genotoxic and is biocompatible.<sup>[27]</sup>

Guttaflow 2 remained noncytotoxic throughout the present study. At the end of 24 h and 48 h, it showed a cell viability of 93.56% and 92.13%, respectively [Tables 1 and 2]. The higher biocompatibility of guttaflow 2 could be attributed to its microsilver particles in contrast to its previous version of guttaflow which had nanosilver. As microsilver has less toxicity than nanosealer, also the fewer atoms which are present on the surface of microsilver which causes less reactivity related to its volume which might be responsible for its low or no cytotoxicity. Furthermore, one of the components of guttaflow 2 is polydimethylsiloxane.<sup>[3]</sup>

Polydimethylsiloxane containing silicon-low temperature isotropic carbon alloy implants, which are usually placed on a metal graphite substrate in the form of either a subperiosteal implant or an endosteal blade are found to be very biocompatible.<sup>[3]</sup>

At 24 h and 48 h, it showed no or slight cytotoxicity which is statistically not significant. The result of the present study are in accordance with the results given by Preeti *et al.* and Silva *et al.*<sup>[3,28]</sup>

The correct choice of a sealer, however, should be considered not only according to the biological behavior but also on the joint evaluation of other parameters which are antimicrobial; physical, and chemical properties.<sup>[25]</sup>

### Limitations of the study

1. It is an *in-vitro* study
2. Different time intervals must be evaluated.

### Future recommendations

1. *In vivo* study with different time parameters
2. Different assays of cytotoxicity and genotoxicity.

### Conclusion

Considering the limitations of this study, as it is an *in vitro* study, an *in vivo* study needs to be carried out to know the cytotoxic and genotoxic potential of a sealer. The extended time intervals which would be for weeks or months needs to be evaluated. Different assays documented in the literature to evaluate cytotoxicity and genotoxicity should be carried out.

Under the parameters of the study, we can conclude that: guttaflow 2 whose genotoxicity is not evaluated yet is not cytotoxic or genotoxic at 24 and 48 h. MTA fillapex was more cytotoxic and genotoxic than AH plus and guttaflow 2 at both the intervals, i.e., 24 h and 48 h.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

### References

1. Silva EJ, Santos CC, Zaia AA. Long-term cytotoxic effects of contemporary root canal sealers. *J Appl Oral Sci* 2013;21:43-7.
2. Karapinar-Kazandağ M, Bayrak OF, Yalvac ME, Ersev H, Tanalp J, Sahin F, *et al.* Cytotoxicity of 5 endodontic sealers on L929 cell line and human dental pulp cells. *Int Endod J* 2011;44:626-34.
3. Mandal P, Zhao J, Sah SK, Huang Y, Liu J. *In vitro* cytotoxicity of guttaflow 2 on human gingival fibroblasts. *J Endod* 2014;40:1156-9.
4. Silva EJ, Neves AA, De-Deus G, Thais AM Moraes AP, Valentim RM, *et al.* Cytotoxicity and gelatinolytic activity of a new silicon-based endodontic sealer. *J Appl Biomater Funct Mater* 2015;13:e376-80.
5. Baraba A, Zelježić D, Kopjar N, Mladinić M, Anić I, Miletić I. Evaluation of cytotoxic and genotoxic effects of two resin-based root-canal sealers and their components on human leucocytes *in vitro*. *Int Endod J* 2011;44:652-61.
6. Rodrigues JC, Hegde MN, Kumari S. A comparative evaluation of root canal sealers on human gingival fibroblasts A cell culture study. *Endod* 2011;23:48-55.
7. Ivanovski S, Haase HR, Bartold PM. Isolation and characterization of fibroblasts derived from regenerating human periodontal defects. *Arch Oral Biol* 2001;46:679-88.
8. Dahl JE, Frangou-Polyzois MJ, Polyzois GL. *In vitro* biocompatibility of denture relining materials. *Gerodontology* 2006;23:17-22.
9. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, *et al.* Single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. *Environ Mol Mutagen* 2000;35:206-21.
10. Darrag AM, Fayyad DM. Genotoxicity of three endodontic sealers by single cell gel-electrophoresis/comet assay. *Tanta Dent J* 2014;11:85-92.
11. Pereira LL, Marcussi S, Sátiro LC, Pereira CA, Andrade LF,

- Davide LC, *et al.* Application of comet assay to assess the effects of white bean meal on DNA of human lymphocytes. *Brazilian J Pharm Sci* 2012;48:103-8.
12. Ørstavik D. Materials used for root canal obturation : Technical, biological and clinical testing. *Endod Top* 2005;12:25-38.
  13. Medeiros VJ, Ximenes R, Antônio R, Fidel S, Ávila TC. Cytotoxicity of root canal sealers on endothelial cell cultures. *Braz Dent J* 2013;24:15-20.
  14. Kayaalti Z, Söylemez E, Yalcin S, Söylemezoğlu T. Searching of standard comet assay parameters for detecting lymphocyte DNA damages using fourteen different test conditions. *J Adv Biol* 2010;3:234-41.
  15. Yoshino P, Nishiyama CK, Modena KC, Santos CF, Sipert CR. *In vitro* cytotoxicity of white MTA, MTA Fillapex® and Portland cement on human periodontal ligament fibroblasts. *Braz Dent J* 2013;24:111-6.
  16. Karimjee CK, Koka S, Rallis DM, Gound TG. Cellular toxicity of mineral trioxide aggregate mixed with an alternative delivery vehicle. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;102:e115-20.
  17. Economides N, Pantelidou O, Kokkas A, Tziafas D. Short-term periradicular tissue response to mineral trioxide aggregate (MTA) as root-end filling material. *Int Endod J* 2003;36:44-8.
  18. Merdad K, Pascon AE, Kulkarni G, Santerre P, Friedman S. Short-term cytotoxicity assessment of components of the epiphany resin-percha obturating system by indirect and direct contact millipore filter assays. *J Endod* 2007;33:24-7.
  19. Leyhausen G, Heil J, Reifferscheid G, Waldmann P, Geurtsen W. Genotoxicity and cytotoxicity of the epoxy resin-based root canal sealer AH plus. *J Endod* 1999;25:109-13.
  20. Van Landuyt KL, Geebelen B, Shehata M, Furche SL, Durner J, Van Meerbeek B, *et al.* No evidence for DNA double-strand breaks caused by endodontic sealers. *J Endod* 2012;38:636-41.
  21. Huang TH, Lee H, Kao CT. Evaluation of the genotoxicity of zinc oxide eugenol-based, calcium hydroxide-based, and epoxy resin-based root canal sealers by comet assay. *J Endod* 2001;27:744-8.
  22. Vitti RP, Prati C, Silva EJ, Sinhoreti MA, Zanchi CH, de Souza e Silva MG, *et al.* Physical properties of MTA Fillapex sealer. *J Endod* 2013;39:915-8.
  23. Zmener O, Martinez Lalis R, Pameijer CH, Chaves C, Kokubu G, Grana D. Reaction of rat subcutaneous connective tissue to a mineral trioxide aggregate-based and a zinc oxide and eugenol sealer. *J Endod* 2012;38:1233-8.
  24. Salles LP, Gomes-Cornélio AL, Guimarães FC, Herrera BS, Bao SN, Rossa-Junior C, *et al.* Mineral trioxide aggregate-based endodontic sealer stimulates hydroxyapatite nucleation in human osteoblast-like cell culture. *J Endod* 2012;38:971-6.
  25. Bin CV, Valera MC, Camargo SE, Rabelo SB, Silva GO, Balducci I, *et al.* Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. *J Endod* 2012;38:495-500.
  26. Konjhodzic-Prcic A, Jakupovic S, Hasic-Brankovic L, Vukovic A. Evaluation of biocompatibility of root canal sealers on L929 fibroblasts with multiscan EX spectrophotometer. *Acta Inform Medica AIM J Soc Med* 2015;23:135-7.
  27. Parirokh M, Forghani FR, Paseban H, Asgary S, Askarifard S, Mahani SE. Cytotoxicity of two resin-based sealers and a fluoride varnish on human gingival fibroblasts. *Iran Endod J* 2015;10:89-92.
  28. Camargo CH, Oliveira TR, Silva GO, Rabelo SB, Valera MC, Cavalcanti BN. Setting time affects *in vitro* biological properties of root canal sealers. *J Endod* 2014;40:530-3.