

Comparative Evaluation of Cytotoxic and Genotoxic Effects of Three Root End Repair Materials on Human Periodontal Fibroblast Cells

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Abstract

Aim: The purpose of the study was comparative evaluation of cytotoxic and genotoxic effects of three root end repair materials on human periodontal fibroblast cells.

Materials and Methodology: Human periodontal fibroblasts were incubated with fifteen specimens of group 1 - MTA Angelus, group 2 - Neo MTA Plus and group 3 - MTA Repair HP respectively. Cytotoxicity was assessed by MTT Assay and Genotoxicity was assessed by Comet assay at time intervals at 24 hours; 48 hours and 72 hours. Data was analysed by Kruskal Wallis and Mann-Whitney U test.

Results: Group 1 MTA Angelus and Group 2 Neo MTA Plus did not show any cytotoxicity or genotoxicity. Group 3 MTA Repair HP was slightly cytotoxic after 48hours of time interval and it also showed low genotoxicity which was statistically not significant.

Conclusion: MTA Angelus and Neo MTA Plus did not show cytotoxic and genotoxic effect however MTA Repair HP showed slight cytotoxic after 48 hours and genotoxic effect throughout the time interval, however it was statistically not significant.

Keywords: MTT Assay; Comet Assay; MTA Angelus; MTA Repair HP; Neo MTA Plus

Introduction

The intricacy of root canal system, persistent periapical pathology, inadequate instrumentation and presence of physical barriers may necessitate surgical endodontic therapy. Root end surgery is the last resort to maintain endodontically treated teeth with persistent periapical pathology [1].

Unlike orthograde filling materials, root-end filling materials communicate directly with vital periapical tissues. The tissue response to these materials becomes important and may further influence the outcome of surgical endodontic treatment [4].

Mineral trioxide aggregate (MTA) appears to be the most promising material to date, as it comes closest to being the ideal material for root-end filling. Nevertheless, MTA has some drawbacks such as a long setting time, difficult handling characteristics, presence of toxic elements in the material composition [6].

New advances of MTA have been introduced such as MTA Repair HP and NeoMTA Plus, based on tricalcium silicates focusing on improving its physiochemical properties, without affecting its biocompatibility.

MTA Repair HP, a calcium silicate based cement which shows high repair plasticity, improving its handling characteristics [7] and NeoMTA Plus which is a new finer powder, tricalcium silicate-based bioactive cement [8]. Contains tantalum oxide as radiopacifier instead of bismuth oxide, avoiding the discolouration and producing calcium hydroxide, which is necessary to induce mineralized tissue formation [10].

In-vitro cytotoxicity and genotoxicity tests are used to detect toxic effects caused by a material or its extract in cell culture, facilitating the reproducibility of test results and providing highly reliable data from standardized protocols in a rapid and inexpensive way [9].

The laboratory assays of cytotoxicity are the initial screening test in assessing the biocompatibility of root end repair materials [1]. MTT-Assay is a comparatively fast, accurate and simple technique for cytotoxicity evaluation and is currently the most commonly used method to test cell growth rate and toxicity of the material. Cytotoxicity is determined by using the MTT Assay.

The evaluation of genotoxicity testing is important for the detection of potential human toxicity of the material to adjacent periodontal tissues so that hazards can be prevented [10].

The single-cell, gel (comet) assay has been recognised as a rapid, simple, and consistent method of evaluating the genotoxicity of materials used in dental practice [9]. Genotoxicity is determined by using comet assay.

However, there are fewer studies comparing both the cytotoxicity and genotoxicity for these new formulation root end filling materials.

Purpose of the Study

The purpose of this study was to evaluate and compare cytotoxicity and genotoxicity of three different root end filling materials: MTA Angelus, NeoMTA Plus and MTA Repair HP.

Materials and Methodology

Cytotoxicity analysis

Test group includes, group 1-MTA Angelus, group 2-NeoMTAPlus and group 3-MTARepair HP each consisting of 15 samples. 2 x 2 mm disc of these materials were made into Teflon moulds and then allowing the material to set for 24hrs in humidifier into which 2.5 ml of Dulbecco modified eagle medium was added and then incubated for 24 hours at 37°C. Cultivation of human periodontal fibroblasts from freshly extracted teeth and their seeding and cultivation went simultaneously.

Then the inoculation of test compounds was done into 96 well plate seeded with periodontal fibres, after which it was incubated for 24 hours at 37°C, and the materials were tested at 24 hours, 48 hours and 72 hours.

MTT protocol for cytotoxicity started which include adding of 20 µl of 5 mg/ml of MTT reagent and then incubating for 24 hours.

After which the MTT reagent and the media were removed, 200 µl of dimethyl sulfoxide was added, and optical density checked at 472 nm under spectrophotometer.

Genotoxicity analysis

Test groups include group 1-MTA Angelus, group 2-NeoMTA Plus and group 3-MTA Repair HP. 5 slides per treatment was taken and incubated for 24 hours at 37°C. After which Cells were suspended in ethyl methyl sulphonate at 5 mmol/L and 10 µL of the above suspension

was added to 120 µL of low melting point agarose at 37°C and then Layering was done with precoated 1.5% regular agarose and covered with a coverslip. Agarose was gelled and cover slip was removed.

The slides were immersed in lysis solution at 4°C and then immersion in alkaline buffer after which electrophoresing of slides for another 20 minutes at 25V was done followed by neutralizing in 0.4 mol/L tris-HCL for 15 minutes.

Later Staining done by Ethidium Bromide and checked by standard protocol by Gel Viewing System and Integrated Software. 50 cells per slide were checked for genotoxicity.

Statistical analysis

Data was entered in Microsoft excel and analyzed using SPSS for windows, Version 21; SPSS Inc. (Chicago, IL, USA). Descriptive statistics were used to calculate percentages and mean values. Following Statistical tests were used.

Test applied were: Kruskal wallis ANOVA test and Mann Whitney U test.

Viability will be measured by formulae [9]:

$$\text{Percentage of viable cells} = \frac{\text{No. of viable cells} \times 100}{\text{Total no. of cells}}$$

Results

Cytotoxicity results of test groups using human periodontal fibroblasts

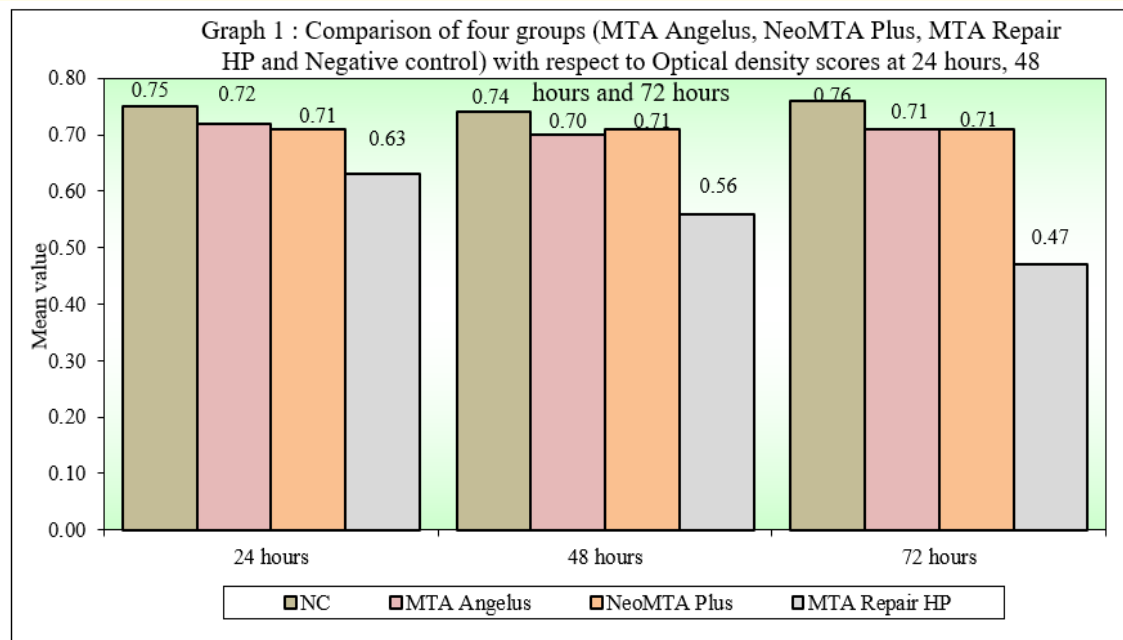


Figure 1: Comparison of four groups with respect to absorption scores at 24 hours, 48 hours and 72 hours by Kruskal Wallis ANOVA test.

Cytotoxicity of test compounds at 24 hours, 48 hours and 72 hours' time interval				
Serial number	Compound name	Time interval	Mean of optical density	Cell viability
1.	Group 1-MTA Angelus	24 hours	0.72	92%
		48 hours	0.70	91%
		72 hours	0.71	90%
2	Group 2-NeoMTA Plus	24 hours	0.71	96%
		48 hours	0.71	90%
		72 hours	0.71	90%
3	Group 3-MTA Repair HP	24 hours	0.63	91%
		48 hours	0.56	75%
		72 hours	0.47	66%
4	Negative Control	24 hours	0.75	100%
		48 hours	0.74	100%
		72 hours	0.76	100%

Table 1: Master chart showing optical density values; mean of optical density and percentage of cell viability at 24 hours; 48 hours and 72 hours.

Observation from figure 1

The above graph depicts mean; standard deviation with respect to different absorption scores of each group at control; 24 hours; 48 hours; 72 hours using Kruskal Wallis ANOVA. Pair wise comparison was done using Mann-Whitney U test.

At 24 hours there was no significant difference overall between all three test groups but in pair wise comparison using Mann-Whitney U test there was significant difference between the test compounds group 3 and Negative Control.

At 48 hours there was an overall significant difference between all three test groups but there was no significant difference in pair wise comparison between test groups.

At 72 hours there was an overall significant difference between all three test groups. Also, there was a significant difference in pair wise comparison between test groups along with negative control.

Observation from figure 2

This table depicts graph and values at different time intervals at 24 hours; 48 hours, 72 hours for tail length parameter by Kruskal Wallis ANOVA test and pair wise comparison by Mann-Whitney U test.

At 24 hours there was no significant difference between all three groups and all the test compounds were non-genotoxic.

At 48 hours there was no significant difference between all three groups but according to descriptive statistics group 1 and group 2 was non genotoxic and group 3 was slightly genotoxic.

At 72 hours there was no significant difference between all three groups but according to descriptive statistics group 1 and group 2 was non genotoxic and group 3 was slightly genotoxic.

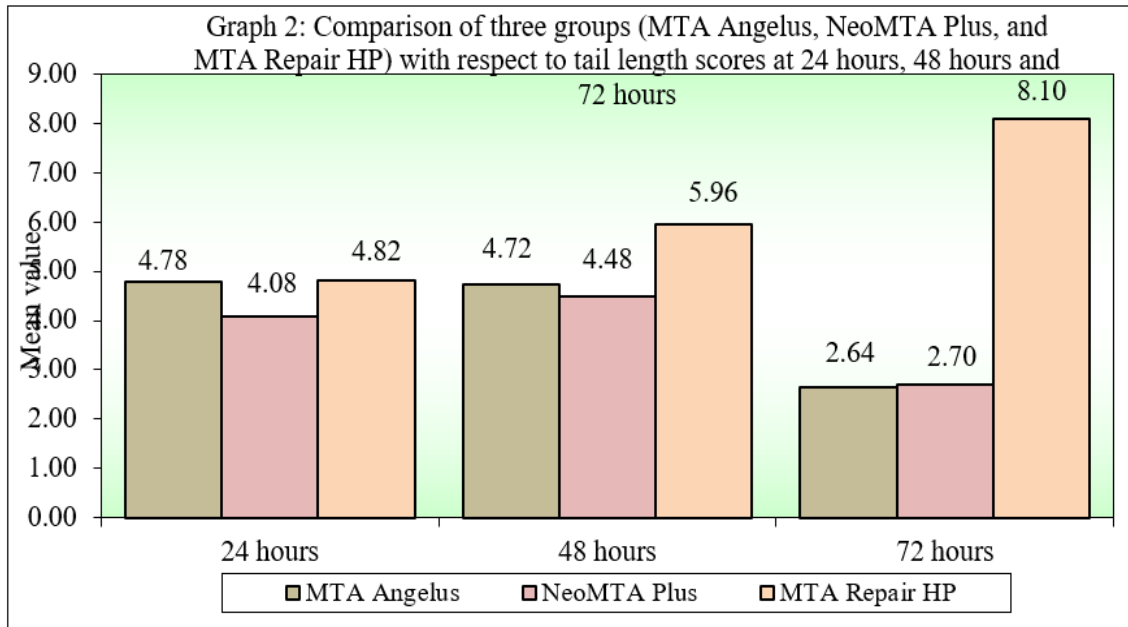


Figure 2: Comparison of four groups with respect to tail length scores at 24 hours, 48 hours and 72 hours by Kruskal Wallis ANOVA test.

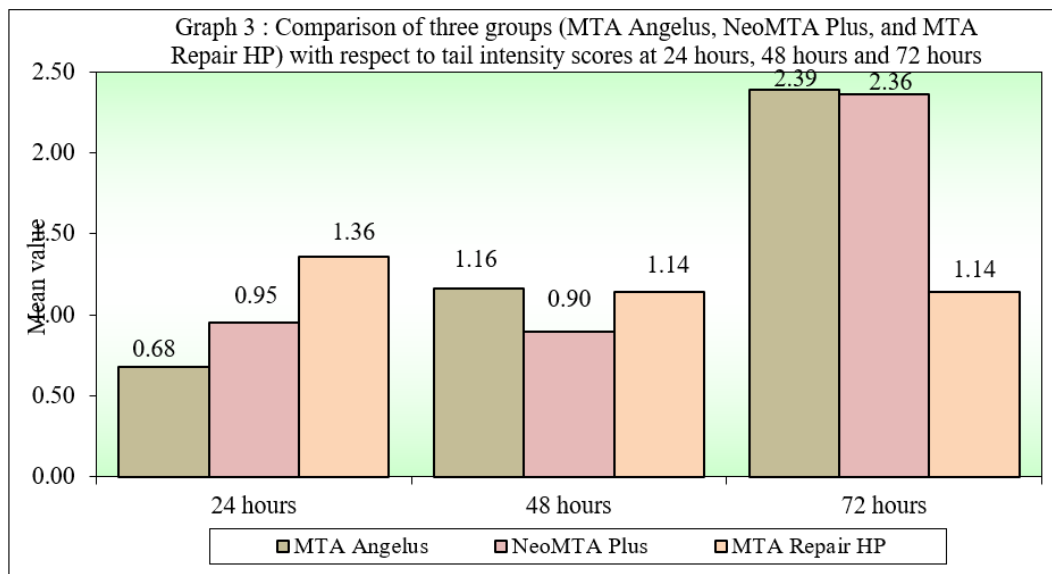


Figure 3: Comparison of four groups with respect to tail intensity scores at 24 hours, 48 hours.

Observation from figure 3

This table depicts graph and values at different time intervals at 24 hours; 48 hours, 72 hours for tail intensity parameter. Pair wise comparison was done by Kruskal Wallis and Mann-Whitney U test.

There was no significant difference between all three test groups.

Discussion

The term biocompatibility states capability of materials in dealing well with the host response. The cytotoxic and genotoxic potential of materials is commonly evaluated to determine their biocompatibility before the conveyance of clinical studies [17].

It has been reported in previous studies that mineral trioxide aggregate has no cytotoxic [3,18,19] and genotoxic [20-23] effects *in vitro*. For these reasons, the aim of this study was to evaluate the biocompatibility of new formulations MTA Repair HP and NeoMTA Plus compared to MTA Angelus.

Cytotoxicity and genotoxicity tests are important to evaluate cellular damage and biological effect of new root end repair material [25].

Cytotoxicity

Various test systems are accessible to determine the cytotoxicity of a biomaterial in cultured mammalian cell populations [26]. Colorimetric assays which measure the activity of enzymes that reduce MTT or similar dyes (XTT, MTS, WSTs) to formazan dyes, giving a purple color. These assays allow assessment of cell viability and proliferation in cell culture assays, which provide information on whether a material is cytotoxic or not. Cell viability can be compared against a negative control [27].

The assay used in the present study used the tetrazolium salt MTT to measure mitochondrial dehydrogenase activity [13]. It is a pale yellow substrate that produces a dark blue formazan product when cleaved by active mitochondria, and so the reaction only occurs in living, metabolically active cells [3]. It is based on succinate dehydrogenase mitochondrial enzyme activity, resulting in the conversion of tetrazolium salt into insoluble violet formazan crystals, whose absorbance is proportional to the amount of living cells [28]. Mineral trioxide aggregate is a hydrophilic substance likely to release ionic components; it would be more apt to interfere with intracellular enzyme activities than influence membrane permeabilities. Therefore, the MTT assay was chosen for the present study [26].

Because the metabolic activity of the cells in culture was measured along a period of time, the increase in the value of absorbance along the time can be interpreted as an indirect measurement of proliferation of cells [20]. Notably high values of absorbance in MTT assay and cell viability were observed in the presence of MTA Angelus extracts and moderate absorbance values in the presence of NeoMTA Plus and MTA Repair HP. Using hPDL fibroblasts, results revealed no significant difference in all three root end repair materials (MTA Angelus, NeoMTA Plus and MTA Repair HP) upto 48 hours. However, there was a decrease in absorbance value with MTA Angelus, NeoMTA Plus and MTA Repair HP from 48hrs to 72hrs compared to control group. According to the present results, MTA Angelus and NeoMTA Plus was non cytotoxic at 24 hours (92% and 96% cell viability respectively), 48 hours (91% and 90% cell viability respectively) and 72 hours (90% and 90% cell viability respectively), whereas MTA Repair HP was slightly cytotoxic both at 48 hours (75% cell viability) and 72 hours (66% cell viability). This could be related to the concentration of Silicon and strontium released from the materials. According to Camilleri, *et al.* 2015 and Christopher, *et al.* 2018 the concentration of silicon and strontium in MTA Repair HP was lower than the concentration of the same elements in other materials. Previous studies indicated that abundant silicon ions could be released from calcium silicate-based bioceramics upon exposure to an aqueous environment, thus significantly improving the biological performance of osteoblasts and periodontal ligament cells [29,30]. It has also been reported that strontium preferentially stimulates cell proliferation of mesenchymal and osteoblastic cells [31,32] and it is also known to be crucial for the proliferation and mineralization of PDL cells and may play multiple important roles in the biological function of periodontal regeneration [33].

Genotoxicity

Genotoxicity is one of the valued 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 be the cause of development of neoplasia [14,15].

In the present study for genotoxicity, Comet Assay or Single cell gel electrophoresis method was used which is a standard, non-invasive, 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 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. The extent of DNA damage is mostly evaluated by comet tail measurements by using image analysis software which is ImageJ and open comet software [14].

In the present study more DNA damage was associated with MTA Repair HP (Table 1) compared to MTA Angelus and NeoMTA Plus, however there was no statistical significant difference seen between all three groups. The reason for genotoxic behavior could be related to the composition of MTA Repair HP which consists of minimum content of strontium and silicon ions compared to MTA Angelus and NeoMTA Plus, as well as the lower Ca:P atomic ratio of MTA Repair HP could be correlated to the presence of the plasticizer. A direct comparison of the present results was not possible due to unavailability of literature regarding evaluation of the genotoxicity of MTA Repair HP.

The present study confirmed that tricalcium silicate-based materials associated with tantalum oxide showed cytocompatibility. Although 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 root end filling material.

Conclusion

Within the limitations of this *in vitro* study, MTA Angelus and NeoMTA Plus showed non cytotoxicity and non-genotoxicity, whereas MTA Repair HP showed slight cytotoxicity after 48 hours and slight genotoxicity however which was statistically not significant.

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