

INVESTIGATION OF CYTO-TOXICITY OF AN INNOVATIVE BIOFACTOR MTA PRODUCT IN DIFFERENT HUMAN CELLS: A PILOT STUDY

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ABSTRACT

INTRODUCTION: Mineral trioxide aggregate (MTA) is one of the most popular and convenient material among current endodontic materials. In addition to MTA advantages, there are also features that need to be improved. Color stability and consistency are notorious concerns in MTA, and materials need improvement regarding these aspects. There are companies, which work on these contents.

OBJECTIVES: The aim of this study was to investigate the cyto-toxicity of Biodentine, Angelus MTA, BIOfactor MTA on 3T3L-1 fibroblast, and Saos-2 osteosarcoma cell lines with different dilution of all products.

MATERIAL AND METHODS: 3T3-1 fibroblast was applied to stimulate pulp cells in cell viability test. In order to generate an osteoblastic cell-like model, only Saos-2 cells were used. MTT test was utilized to measure cell cyto-toxicity in different dilution of all MTA products. SPSS package program was applied to perform Kruskal-Wallis, one-way ANOVA and two-way ANOVA tests.

RESULTS: According to results, while Biodentine, Angelus MTA and BIOfactor MTA showed cyto-toxic effects in 1 : 1 and 1 : 2 dilutions, they were not cyto-toxic in 1 : 4 and 1 : 8 dilutions.

CONCLUSIONS: MTA products have similar cyto-toxicity. BIOfactor MTA has a cyto-toxic effect as other similar products.

KEY WORDS: MTT assay, Biodentine, cyto-toxicity, BIOfactor MTA, Angelus MTA.

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INTRODUCTION

Repair techniques are quite important in conservative dentistry approach in order to keep the tooth healthy in the mouth [1, 2]. Mineral trioxide aggregate (MTA) is a bio-compatible material that is frequently used for

this purpose [3, 4]. MTA is utilized for conservative root fracture repair [2], perforation repair [5], pulp capping [6], apical plug formation for apexification [7], retrograde fillings in apical surgery [8], and coronal barrier formation [1] in re-vascularization treatment. MTA interacts with live tissues and body fluids in all these

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applications, and these chemical/biological interactions produce changes in physical properties of this biological milieu [9, 10].

Since the early 1990s, MTA's physical, chemical, and biological properties have been studied [11, 12]. Certainly, new components were introduced into the MTA structure to find the optimal combination. An ideal endodontic material should have properties, such as covering [13], dimensional stability [14, 15], color stability [16, 17], radiopacity, non-dissolution in interaction with body fluids [18], and fluency and easy placement [19] as well as alkaline pH, calcium ion release [20], bio-activity [21], and cell attachment and bio-compatibility [22].

While many of these features are available in MTA, some are missing. Color stability and consistency are continuous concerns for MTA, and materials need to be improved in these areas [15]. Therefore, different materials are introduced to the market.

A brand-new root repair material called BIOfactor MTA was released on the market. It is advised to use the same powder and liquid mixture as with other brands; however, it is asserted that BIOfactor MTA's powder is now better and finer. Setup duration, hydration time, and color stability have all been improved as a result of this change. Additionally, the company asserted that depending on clinician's desired preference, the cement may be made in either a flowable or thick consistency [23].

OBJECTIVES

The present study aimed to compare the cyto-toxicity of Biodentine (Septodont, Saint-Maur-des-Fosses, France) and White Angelus MTA (Angelus, Brazil) brand products, which are available on the market, with BIOfactor MTA (Imicryl Dental, Konya, Turkey) that has been currently released in Turkey on 3T3L-1 fibroblast and Saos-2 osteosarcoma cell lines, and to find the most appropriate dilution that can be used in future studies.

MATERIAL AND METHODS

In the study, three different calcium silicate-based products were examined to evaluate their cytotoxicity, including Biodentine (Septodont, France) (BD), White Angelus MTA (Angelus, Brazil) (WAM), and BIOfactor MTA (Imicryl Dental, Turkey) (BM).

PREPARATION OF SPECIMENS

Extraction liquids were collected in three wells of a 24-well plate. BD, WAM, and BM materials were prepared according to manufacturers' instructions. The thickness of each sample was 2 mm, and they were sterilized by exposing to ultraviolet (UV) light for 30 minutes.

OBTAINING EXTRACTION LIQUID

The prepared and sterilized samples were filled with DMEM (Dulbecco's modified eagle medium, Biochrom Ag; Germany) and cultured for 24 hours in a 37°C incubator containing 5% CO₂. After 24 hours, DMEM solutions were collected again. These solutions were considered as super-solutions.

INVESTIGATION OF CYTO-TOXIC PROPERTIES OF BD, WAM, AND BM

MTT test was performed to determine materials' cyto-toxicity potential, and it was carried out in the cell culture laboratory. 3T3L-1 fibroblast and Saos-2 osteosarcoma cell lines were used in cyto-toxicity assay. The cells were cultured at 37°C in a humidified 5% CO₂ atmosphere, and were maintained in the completed medium of Dulbecco's modified eagle medium (DMEM) with high glucose (Capricorn, CP18-2472) supplemented with 10% fetal bovine serum (FBS) (Biological Industries-04-001), 1% L-glutamine (Capricorn, GLN-B), and Penicilin (100 U/ml)-streptomycin (100 µg/ml) (Capricorn, CP15-1396). Active cells in logarithmic growth phase, which covered 80-85 percent of the surface of the flask were plated in each well in a 96-well plate. For each well, 10,000 cells were plated and incubated for adaptation for 24 hours. The prepared super-solution was diluted at 1 : 1, 1 : 2, 1 : 4, and 1 : 8 ratios, and were used for MTT test. After incubation, DMEM medium was removed. New extraction liquid medium that was previously prepared at 1 : 1, 1 : 2, 1 : 4, and 1 : 8 dilutions were added to each well and incubated for 24 hours. DMEM medium containing 10% FBS was used as the control group. Following incubation, MTT test was performed. MTT dye was homogenized by combining it with DMEM medium that did not contain phenol red, and a stock MTT solution was prepared at a final concentration of 5 mg/ml. The medium of each well was discarded, and MTT solution in 10% of completed medium was added to each well and incubated for 3 hours at 37°C. Afterwards, lysis solution (1% triton-X, 10% 0.1 mol/l HCl, 89% isopropanol) was added to each well. Optical density of the produced formazan was calculated using spectrophotometer readings at 570 and 630 nanometers as a reference. All experimental procedures and MTT test were repeated four times.

STATISTICAL ANALYSIS

SPSS package program was applied for statistical analysis of the data. Kruskal-Wallis method was used to compare groups and dilutions for analyzing cell viability data, because the number of observations within the groups was limited and it did not provide the assump-

tion of normal distribution. Lettering was done in cases where there was a statistically significant difference by using corrected p -values from paired comparisons.

RESULTS

There was a statistically significant difference in cell viability in the BD group in the 3T3L-1 fibroblast cell line ($p < 0.05$). The statistical significance of 1 : 4 and 1 : 8 dilutions was the same as that of the negative control group; however, 1 : 2 and 1 : 1 dilutions had a statistically significant negative effect on cell viability. The positive control group had a statistically significant negative impact on cell viability when compared with the other groups (Table 1).

In the 3T3L-1 fibroblast cell line, a statistically significant change in cell viability was identified in the WAM group ($p < 0.05$). The statistical significance of 1 : 4 and 1 : 8 dilutions was the same as that of the negative control group; however, 1 : 2 and 1 : 1 dilutions had a statistically significant negative effect on cell viability. The positive control group presented a statistically significant negative impact on cell viability when compared with the other groups (Figure 1).

Statistically significant difference was found in 3T3L-1 fibroblast cell line in terms of cell viability in the BM group ($p < 0.05$). The statistical significance of 1 : 4 and 1 : 8 dilutions was the same as that of the negative control group; however, 1 : 2 and 1 : 1 dilutions showed a statistically significant negative effect on cell viability. The positive control group had a statistically significant negative impact on cell viability when compared with the other groups.

In the BD group, there was a statistically significant difference in cell viability in Saos-2 osteosarcoma cell line ($p < 0.05$). The statistical significance of 1 : 4 and 1 : 8 dilutions was the same as that of the negative control group; however, 1 : 2 and 1 : 1 dilutions showed a statistically significant negative effect on cell viability. The positive control group had a statistically significant negative impact on cell viability when compared with the other groups.

In the Saos-2 osteosarcoma cell line, a statistically significant difference in cell viability was reported in the WAM group ($p < 0.05$). The statistical significance of 1 : 4 and 1 : 8 dilutions was the same as that of the negative control group; however, 1 : 2 and 1 : 1 dilutions demonstrated a statistically significant negative effect on

TABLE 1. Results of all materials and all dilutions

Group	Material	1 : 1	1 : 2	1 : 4	1 : 8	NK	PK	p -value
3T3L1	BD	0.4497 ± 0.037	0.6633 ± 0.0462	0.949 ± 0.0372	1.07 ± 0.0691	1.0603 ± 0.0751	0.2833 ± 0.0395	0.007
	WAM	0.4807 ± 0.0656	0.7233 ± 0.091	0.9137 ± 0.072	1.005 ± 0.0682	1.0603 ± 0.0753	0.2833 ± 0.0397	0.009
	BM	0.463 ± 0.057	0.6863 ± 0.1009	0.9747 ± 0.0657	1.0167 ± 0.0683	1.0603 ± 0.0752	0.2833 ± 0.0396	0.009
Kruskal-Wallis test	p -value	0.733	0.491	0.495	0.393	–	–	–
Saos-2	BD	0.6257 ± 0.0315	0.7797 ± 0.0652	0.88 ± 0.0311	1.1053 ± 0.147	1.037 ± 0.0174	0.3333 ± 0.0055	0.006
	WAM	0.6157 ± 0.0393	0.7807 ± 0.0748	0.9137 ± 0.04	1.0397 ± 0.0646	1.037 ± 0.0176	0.3333 ± 0.0057	0.007
	BM	0.548 ± 0.0872	0.7357 ± 0.1238	0.8923 ± 0.058	1.0063 ± 0.1027	1.037 ± 0.0175	0.3333 ± 0.0056	0.008
Kruskal-Wallis test	p -value	0.391	0.731	0.578	0.576	–	–	–

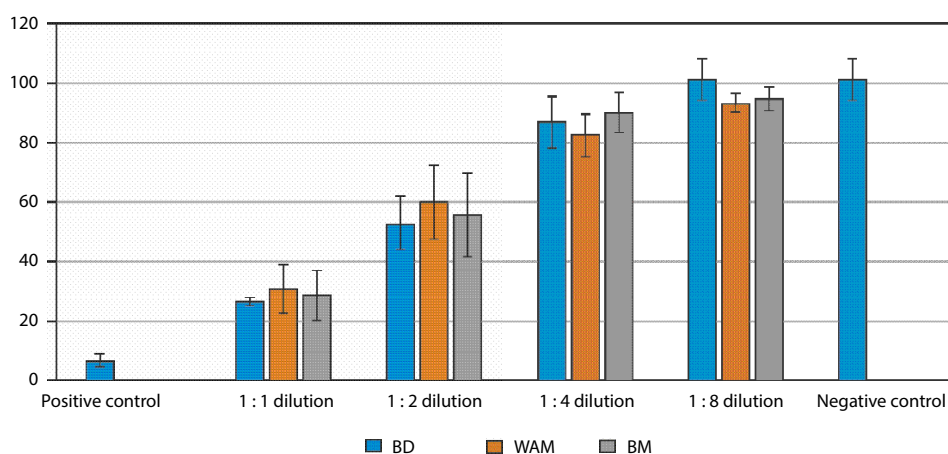


FIGURE 1. % cell viability (3T3L1)

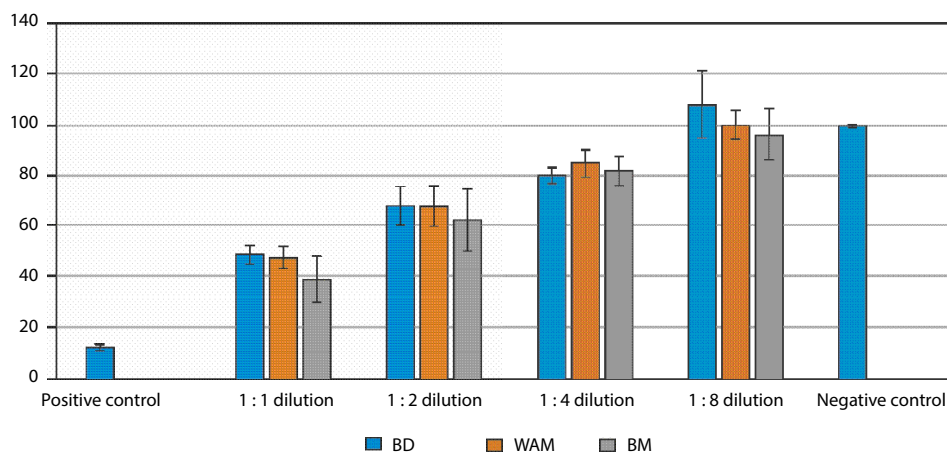


FIGURE 2. % cell viability (Saos-2)

cell viability. The positive control group had a statistically significant negative impact on cell viability when compared with the other groups (Table 1).

In the BM group, there was a statistically significant difference in cell viability in the Saos-2 osteosarcoma cell line ($p < 0.05$). The statistical significance of 1 : 4 and 1 : 8 dilutions was the same as that of the negative control group; however, 1 : 2 and 1 : 1 dilutions showed a statistically significant negative effect on cell viability. The positive control group had a statistically significant negative impact on cell viability when compared with the other groups (Figure 2). When each dilution was examined separately, no statistical difference between the groups was discovered ($p > 0.05$).

DISCUSSION

In dentistry, vital pulp therapy has been applied more reliably, and the success of perforation repairs has increased in endodontics with the introduction of bio-ceramic materials to the market. Certainly, these materials have expanded by gaining many different properties. This variety is possible with items' various constituents. This differentiates the cyto-toxicity of these products on live fibroblast cells. These compounds have been shown to induce dentin development in the wounded pulp as well as enhance dental pulp vitality in vital pulp therapies [24]. Capping materials must be safe and support pulp health in living pulp [25]. MTA is a bio-active cement introduced to the market by Torabinejad in the early 1990s as a viable physical endodontic repair and root-end filling material [26]. It has been proven that MTA induces mineralization of the exposed pulp, and has the potential to preserve pulp vitality. As a result, MTA's indications for the use have grown dramatically since its' initial application, and recently has been recognized as a superior material over $\text{Ca}(\text{OH})_2$ in many clinical applications [27, 28]. To offer radiopacity, MTA powder is a refined combination of Portland cement and

bismuth oxide. This cement's main component phases include tricalcium and dicalcium silicates as well as tricalcium aluminate [29, 30]. That is why white MTA to the materials tested in our study was added.

In the response to limitations of MTA, BD introduced a novel tricalcium silicate-based cement to the market. This relatively new bio-material is claimed to have similar qualities as MTA, and is now being researched for crucial pulp therapy techniques. BD is designed as a permanent, bio-compatible dentin substitute that can be applied to the full volume of the cavity in a single-session, or in an observation period before composite restoration using the sandwich approach [31]. Second tested material in the current study was BD.

BM is a new type of calcium silicate-based cement that was recently released for pulp capping, pulpotomies, apexification, root perforation repairs, root-end filling, and apical plug treatments. The components of BM powder include tricalcium and dicalcium silicate, tricalcium aluminate, and ytterbium oxide. The liquid is composed of water-soluble carboxylated polymer and demineralized water in amounts ranging from 0.5 to 3%. Depending on the type of treatment, this cement can be made in a fluid or dense form. BM, according to the manufacturer, has a finer powder for faster hydration, easier handling features, stronger sealing, and a shorter curing time, and it does not cause tooth discoloration. Additionally, BM seems to be a more cost-efficient product [32].

In our investigation, we intended to compare the cytotoxicity of this new product to that of BD that the authors described as gold standard for the application in endodontics. According to our readings, there is no literature on cyto-toxicity related to BM.

The effects of various bio-materials on cell viability and osteogenic gene expression are crucial for tissue engineering and regenerative medicine. Many studies have been conducted to investigate the survival of cells and the expression of various osteogenic markers in pulp capping materials [33].

Because it grows quickly and is resistant, the fibroblast cell line was chosen for this investigation for its ease of usage. Due to their reproducibility, established cell lines, such as these, have been suggested by ISO for testing biological responses of dental materials [34].

In general, cell viability assays assess the effects of the tested substance on cell growth and cyto-toxicity. Tetrazolium reduction, resazurin reduction, and protease activity tests are the most often used types of assays, which determine the metabolism or enzymatic activity of living cells. Another test that can be applied in this regard is luminogenic ATP experiment. The rate of viable cells is assessed directly in this experiment by measuring the amount of ATP. Although the ATP test is the fastest and most sensitive, inexpensive tests with adequate performance are more commonly used. Among them, the most used are MTS, XTT, WST-1, and MTT [35].

We chose the MTT assay to investigate the cyto-toxic effect of BD, WAM, and BM bio-materials on fibroblasts and osteosarcomas in our investigation. We accomplished this by testing the impact of all dilutions on cytotoxicity using the extract, which was generated directly and in varying dilutions. While 1 : 1 and 1 : 2 diluted solutions were cyto-toxic to fibroblasts and osteosarcoma cells, 1 : 4 and 1 : 8 diluted solutions were not.

Youssef *et al.* [24] obtained similar results to those of the present study. Proroot MTA, BD, Ca (OH)₂, and Emdogain were employed in this research, and it was discovered that all these compounds presented various cyto-toxic effects on dental pulp cells.

Rather than our findings, in a study by Zhou *et al.* [36], 1 : 1 and 1 : 2 dilutions of BD and AM did not show cytotoxic effects on fibroblasts.

CONCLUSIONS

In terms of cyto-toxicity, the findings of the current study indicate that the recently released BM should not be treated differently than WAM and BD. Since it is a less expensive and domestically produced substance, it appears to be safe in use regarding cyto-toxicity. According to the findings, it seems appropriate to use 1 : 4 and 1 : 8 diluted solutions in the biological compatibility assessment tests to be performed with these substances.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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