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IN VITRO BIOCOMPATIBILITY OF BIODENTINE AND MTA AS CALCIUM SILICATE-BASED ENDODONTIC MATERIALS ON MRC5 AND BHK-21 FIBROBLASTS

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ABSTRACT

Objectives: this study intended to evaluate the cytotoxicity of Biodentine and MTA materials to fibroblast cell lines. **Materials and Methods**: MRC-5 and BHK-21 fibroblast cell lines were incubated for 1 day in extracts from immersion of MTA and Biodentine materials in culture medium for either 1, 3, or 7 days as well as directly with fresh set materials immersed in culture medium. Fibroblasts cultured in DMEM were used as a control group. Cytotoxicity was evaluated using MTT assay. The data of cell cytotoxicity were analyzed statistically by two ways and one-way analysis of variance at a significance level of $P < 0.05$. **Results**: MTT viability assay revealed that MTA preserved cell viability better than Biodentine. It also revealed that 7th day extract significantly showed higher number of viable cells than 1stand 3rd days extracts ($P < .05$). **Conclusion**: Within the limitation of this study, Biodentine revealed more cytotoxicity than MTA, however, both materials exhibited biocompatibility and can be used in contact with living cells.

KEYWORDS

Biocompatibility, MTA, Endodontic, MRC5 and BHK-21.

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INTRODUCTION

The main purpose of root canal treatment is to prevent and eliminate microbial invasion. Unfortunately, non-adequately treated or pathologically invaded root canals may leak irritants leading to persistent pathological periapical lesions (Siqueira, 2001)[1]. In some cases, root canal treatment may be challenged with many factors that may prevent proper microbial elimination such as

complicated canal anatomy or presence of post supported restorations. In such case, periapical surgery may represent a valuable option for saving teeth.

During endodontic surgery, apical part of the root is resected and retrograde filling material is usually used to seal a prepared root-end cavity. Accordingly, the material comes in direct contact with periapical tissues; thus; it must provide an adequate an adequate environment that promotes healing and regeneration of the relevant tissues (Saidon, He, Zhu, Safavi, and Spångberg, 2003)[2].

Throughout the dental history, a wide variety of materials have been used for retrograde fillings. The physiochemical and biological properties of filling materials as well as their handling may prevent them from being considered ideal retrograde filling material. Calcium silicate based materials have gained popularity in recent years due to their good sealing ability, adaptation and excellent bioactivity (Han and Okiji, 2013)[3].

Mineral **t**rioxide **a**ggregate (MTA) was the first calcium-silicate material. It was developed as a retrograde filling material at Loma Linda University, California, USA. MTA has been described to show excellent bioactivity (Sarkar, Caicedo, Ritwik, Moiseyeva, and Kawashima, 2005[4], Reyes-Carmona, Felippe, and Felippe, 2009)[5] and biocompatibility (Ma, Shen, Stojicic, and Haapasalo, 2011[6], Samara *et al.,* 2011[7], Mozayeni, Milani, Marvasti, and Asgary, 2012)[8] toward living cells with good sealing ability (Galhotra *et al.,* 2013[9], Martell and Chandler, 2002)[10]. However, MTA suffers from inferior handling properties and long setting time (Torabinejad, Hong, Mc Donald, and Ford, 1995[11], Dammaschke, Gerth, Züchner, and Schäfer, 2005)[12]. Thus, development of new calcium silicate cements that act as replacement materials for MTA was of interest. Biodentine, calcium silicate based product, was specifically designed as a "dentine replacement" material. Biodentine is presented as powder and liquid components. The powder component consists oftri-calcium silicate, di-calcium silicate, calcium carbonate and zirconium oxide. The liquid one contains calcium chloride (as setting accelerator) and a hydro-soluble polymer (as a water reducing agent). It has been sated that Biodentine could represent a better adjunct for MTA as it has better biological acceptance and superior handling properties. The importance of using the bioceramic materials is the ability of these materials to release free calcium ions. This property is an essential for successful endodontic root end procedure because of the action of calcium on differentiation of osteoblasts and cement oblast cells, and hard tissue mineralization (Ebtehal, Perinpanayagam, and Mac Farland, 2006)[13]. This study was conducted to evaluate the cytotoxicity of Biodentine and MTA materials to fibroblast cell lines and to determine the concentration of free calcium ion in extracts delivered from MTA and Biodentine materials.

MATERIAL AND METHODS

Material

Normal human fetal lung fibroblast (MRC-5) and Baby hamster kidney fibroblasts, (BHK-21) cell lines. (The Holding Company for Biological Products and Vaccines, VACSERA, Agouza, Giza, Egypt). Dulbecco's Modified Eagle's medium, DMEM, and Trypsin EDTA (200mg/l versene (EDTA), 170.000 U Trypsin/L) (Lonza, Verviers, Belgium). Fetal Bovine Serum (FBS), (Euroclone, Milan, Italy). An antibiotic/anti-mycotic solution containing 1000 U/ml penicillin, 1000 µg/ml streptomycin and 25 µg/ml fungisone, Phosphate Buffer saline (PBS) and 3-(4,5-dimethyl-thiazoyl)-2,5-diphenyl-tetrazolium bromide (MTT) (Sigma-Aldrich, St Louis, MO, USA). Costar, 96-well and 24-well plates, Tissue culture treated polystyrene (#3512, Corning Inc., NY, USA), ELISA BioTek Lx800 microplate reader (BioTek, Bedfordshire, UK), high velocity vibrator (4000 rpm) (softly 8, De Gotzen S.R.L., Varese, Italy),incubator (Shellab incubator, Sheldon Manufacturing, Inc., Oregon, USA) and (Ultraviolet sterilization chamber, Aura 2000 MAC4, Euroclone S.p.A., v. Lombardia, Italy) were used.

METHODS

Preparation of filling material discs

MTA and Biodentine were mixed following the manufacturers' instructions under aseptic conditions. MTA was mixed in a 3:1 powder to liquid ratio on a sterile clean glass slab using flat stainless steel spatula for 1 minute. Biodentine was mixed by adding five drops of mixing solution (calcium chloride) into capsule containing Biodentine powder, then the capsule was inserted into a high velocity vibrator for 30 seconds.

Twenty samples of each material were prepared and shaped into discs using a sterile cylindrical polyethylene tube (8-mm diameter and 3-mm height). The materials were packed inside the polyethylene tubes in increments with light pressure against a sterile glass slab using a sterile metal spatula, and then the samples were covered with a sterile cover slab to remove excess material. To obtain the initial setting, all samples were kept for 3 hours in incubator at 37 ^{o}C and 95% relative humidity. Following initial setting, all samples were exposed to ultraviolet light for 20 minutes on each surface to ensure sterility of the samples.

Preparation of filling material extracts

Four discs of each material were transferred separately into 24-well culture plates and incubated at 37 ^{o}C in 1.5 mL serum free DMEM / 1% Pen-strep in a humidified atmosphere containing 5% CO_2 for 24 hours to allow elements, if it will, to leach from the filling material into the medium. The ratio of surface area of tested material to volume used for extract preparation was about 125 mm^2/mL in accordance with ISO standard 10993-5 (Hunter, Kirk, Robinson, and Kardos, 1998)[14]. The supernatant was then collected and referred to as ''material day-1 extract''.

Extracts of longer periods (3 and 7 days) were prepared by the same protocol used for preparing day-1 extract. After each time period of sample incubation, the conditioned medium (material extract) was then filtered using 0.20µm filter.

Ionized calcium concentration in each material extract was measured using Quantichrom calcium colorimetric assay kit (Bioassay Systems, Hayward, CA, USA) at optical density at 610 nm, using microplate reader.

To obtain different concentrations, each extract was divided into four aliquots and serially diluted with culture media (DMEM with 1% antibiotic) into 4 different concentrations (undiluted, 1:1, 1:2, 1:4 v/v). FBS was then added to different extracts concentrations to form 10% FBS extracts.

Preparation of cell lines

MRC5 and BHK-21 fibroblast cell lines were cultured in DMEM supplemented with 10% FBS, and 1% Pen-Strip at 37 ^{o}C and 95% humidity in a humidified incubator under an ambient air atmosphere containing 5% CO_2 for 24 hours to achieve 90 % confluence. Confluent cells were then detached from the culturing flask walls using Trypsin EDTA solution for 5 minutes, and aliquots were collected and sub-cultured.

Testing the effect of material extracts on fibroblast cell lines

BHK-21 and MRC5 cells were seeded separately into a 96-well plate; [(BHK-21= 1 ×105 cells/ml) and (MRC5= 0.5 × 105 cells/ml)]; in complete growth media (100 µL DMEM/10% FBS and 1% AB), and were incubated for 24 hours to achieve 90% confluence before adding the extracts. Then, the medium in each well was discarded and cells were incubated with new complete growth media containing different concentrations of MTA and Biodentine extracts (100µL) of each time point (day-1, day-3 and day-7 extracts). Cells in complete growth media was served as negative control group (cells of this group were not exposed to the test materials). After treatment, plates were incubated for another 24 hours prior to performing the MTT assay. Samples were measured in triplicates to ensure reproducibility.

Testing the effect of direct material placement (discs) on fibroblast cell lines

As described earlier, the BHK-21 and MRC-5 cells were cultured and plated at a density of 1×10^5 and 0.5×10^5 cells/ml for BHK-21 and MRC5 cells respectively in 24-well plate containing 2.8mL complete growth medium. Four discs of each test materials (MTA and Biodentine) were added separately to each cell line.

To prevent mechanical irritation of cells as a result of disc material weight, the tested discs were attached to inner surface of the plate cover, so that the material discs were actually hanging inside well plates. For control group, empty polyethylene tubes (8-mm diameter and 3-mm height) were incubated with cell lines. The well plates were then incubated for 24 hours before cell viability was examined using MTT assay.

MTT cell viability assay

The MTT assay was used to evaluate the viability of MRC5 and BHK-21 fibroblast after 24 hours incubation with tested materials. Freshly prepared MTT solution, 5 mg/mL, was added to each well and incubated in dark humidified atmosphere of 5% CO_2/ 37 ^oC for additional 4 hours. When the purple formazan crystals were formed around the cells, the supernatant was carefully removed, and dimethyl sulfoxide (DMSO) was added to each well and shake for 1 hour to dissolve the crystals. Optical densities were measured at wave length of 570nm (primary absorbance value) and a reference wavelength of 630nm (background absorbance value). The background absorbance was subtracted from the primary values. Cell viability was expressed as viability percentage (%) which was calculated using the following formula:

$$\% \text{ Viability} = \frac{OD_{\text{test well}}}{OD_{\text{negative control}}} \times 100$$

RESULTS

The effect of material extracts on fibroblast cell lines

MRC5 cell line

Results of cell viability of MRC5 fibroblasts under different concentrations of material extracts (derived from the MTA and Biodentine) are summarized in Table No.1 and presented in Figures No.1-2.

One way ANOVA indicated no significant difference on cell viability was observed between different concentrations of MTA extract [(p= 0.530) (p=0.761) (p= p=0.841)] or between different concentrations of Biodentine extract [(p=0.089) (p= 0.501) (p=0.073)] at day 1, day 3 or day 7 respectively.

For day 1 and day 3 extracts, one way ANOVA revealed significant differences regarding cell viability between the three tested groups (-ve control, MTA and Biodentine groups). Tukey's Post hoc pairwise comparison revealed that control group significantly had more viable cells than both MTA and Biodentine extracts at any concentration, while, there were no significant differences between MTA extracts and the corresponding Biodentine extracts concentrations. However, for day 7 extracts, Tukey's post hoc test revealed no significant difference between control group and Biodentine group at all concentrations (except with 1:4 diluted extract which revealed more viable cells in Biodentine group). It also revealed that all MTA concentrations significantly promoted cell proliferation more than control group and corresponding concentrations in Biodentine group.

It has been noted that day-7 extracts significantly promoted cell viability more than day-1 and day-3 extracts in both MTA and Biodentine groups.

BHK-21 cell line

Results of cell viability of BHK-21 fibroblasts under different concentrations of material extracts (derived from the MTA and Biodentine) are summarized in Tables No.2 and presented in Figures No.(3-4).

One way ANOVA indicated no significant difference on cell viability was observed between different concentrations of MTA extract [(p= 0.530) (p=0.761) (p= p=0.841)] or between different concentrations of Biodentine extract [(p=0.089) (p= 0.501) (p=0.073)] at day 1, day 3 or day 7 respectively. It revealed that day 7 extracts resulted in significant better cell viability than corresponding day 1 and day 3 extracts in both tested materials.

One way ANOVA showed that cell viability was significantly affected after exposure to materials extracts [table (2???)]. After cell line treatment with day 1 extracts, Tukey's post hoc test revealed that the control and MTA groups preserved cell viability significantly more than Biodentine group in all concentrations. While there were no significant differences between different MTA extract

concentrations and control group [table (2???)]. However, treatment with day-3 extracts resulted in significantly more viable cells in control group than MTA group which in turn had significantly more viable cells than Biodentine group. For day-7 extracts, one way ANOVA revealed no significant differences between tested groups except between MTA and Biodentine undiluted extract (Table No.3). Pairwise comparison revealed that undiluted MTA extract significantly maintained cell viability more than undiluted Biodentine extract.

Concentration of ionized calcium

Detailed values of free calcium ions concentrations in different extracts derived from MTA and Biodentine are summarized in Table No.3. T-test revealed that Biodentine significantly released more calcium ions than MTA at all-time points (1, 3 and 7 days extracts). Regarding time of extract, one-way ANOVA revealed that calcium concentration is significantly different among different extract preparation time within the Biodentine group while no significant difference was found between different MTA extracts. Post HOC test revealed that the 7th day Biodentine extract significantly contained less ionized calcium than that 1st and 3rd day extracts while there was no significant difference between 1st and 3rd day extracts.

The effect of material Discs on fibroblast cell lines (direct exposure)

Results of the cytotoxicity assay (Figure No.5) on the cell viability of BHK-21 and MRC5 fibroblasts after direct incubation with MTA and Biodentine discs are summarized in Table No.(4). One way ANOVA indicated that there were no significant differences between tested groups (Control, MTA and Biodentine) on cell line viability with either BHK-21 cell line (p=0.167) or with MRC5 cell line (p=0.303).

The toxic effects of materials used for endodontic therapy are of particular concern as they can cause degeneration of the periapical tissue and delay wound healing (De Deus, Ximenes, Gurgel-Filho, Plotkowski, and Coutinho-Filho, 2005)[15]. During endodontic surgery, apical part of the root must be sealed with an appropriate root end filling material, that's why an ideal root repair material should be biocompatible with human tissues. The purpose of this study was to assess whether Biodentine could be an appropriate alternative for golden standard MTA material when placed as a retrograde filling.

Fibroblasts are the major constituents of connective tissue, the predominant cell type of periodontal ligament and are the most important collagen producers in this tissue (Kumada and Zhang, 2010)[16]. Genuine cytotoxicity of the tested materials over the living tissues was performed using two different types of fibroblast cell lines (BHK-21 and MRC5).

This study demonstrated that all the materials tested released calcium ions and had the ability to form surface apatite crystals, both of which indicate their bioactivity. Biodentine released significantly more calcium ions and produced more surface crystals.

Results obtained from current research are in agreement with previous researches (Schröder, 1985[17], Keiser, Johnson, and Tipton, 2000[18], Ma *et al.,* 2011[6]; Han and Okiji, 2013)[3]. Bozeman *et al* (Bozeman, Lemon, and Eleazer, 2006)[19]. reported that both MTA materials released more Ca initially, followed by a decline and then rise in elution, also SIR Khan (Khan, Ramachandran, Deepalakshmi, and Kumar, 2012)[20] reported that MTA and Biodentine released significantly less calcium ions in 7th day than the initial 5 hours of contact of those materials with distilled water. Han and Okiji (Han and Okiji, 2013)[3] reported that Biodentine significantly released more calcium ions than MTA when materials were in contact with simulated body fluid, while Gandolfi *et al* (Maria Giovanna Gandolfi *et al.,* 2013)[21] reported a significant increase of calcium release by Biodentine compared with MTA when materials were immersed in water.

Setting reaction of calcium silicate includes the hydration of calcium silicates particles that results in the production of calcium hydroxide as byproduct of the reaction, the major source of calcium ions released from these materials (Y.-L. Lee *et al.,* 2004[22], Camilleri, 2007[23], 2008)[24]. In this regard, the higher calcium ion release of Biodentine material may be attributable to its higher calcium silicate content and thus, the amount of calcium hydroxide produced after the hydration reaction

may be higher compared with the MTA. Moreover, the increased calcium release in Biodentine may be related to increased content of calcium carbonate and calcium chloride. Contrary to our results, SIR Khan *et al* (Khan *et al.,* 2012)[20] reported more calcium released from MTA in comparison with Biodentine. A possible explanation of that may be related to different actions occurred with materials when subjected to different culturing fluids, the current study utilized phosphate containing culturing media while the former authors utilized distilled water.

Continuous release of calcium ions from silicate based material may play an important role in the repair process because they can pass through the cell membranes by depolarization or activation of membrane-bound calcium channels (Hunter *et al.,* 1998)[14], however, a high concentration of intracellular calcium ions can cause cytotoxicity and trigger cell death (Midy, Dard, and Hollande, 2001[25], Orrenius, Zhivotovsky, and Nicotera, 2003[26], Kang *et al.,* 2013)[27]. Results of the present study indicated high level of calcium ion in the extracts of both materials, and consequently, an increased pH of these extracts is expected. In previous study, authors reported that the gradual increase of alkalinity may decrease cell viability *ex vivo*; under *in vivo* conditions, this might be neutralized by the body tissue fluids (Mozayeni *et al.,* 2012)[8]. Samples used in the present study were freshly prepared and were totally immersed in culture media, this may have extended the time of setting reaction leading to continuous release of ionized calcium through the first days of samples immersion in the culture media, this may explain the increased ion concentration and low cell viability of cells related to day-1 and day-3 extracts. In present study, the increased pH was indicated by color change of the phenol red containing culture media once it was added to material samples.

The obtained data showed a significant reduction in cell viability related to day 1 and day 3 extracts than that of control group. High calcium ion concentration was observed in both 1st and 3rd days MTA and Biodentine extracts. Under cell culture conditions, the optimum calcium concentration in the culture media of osteogenic cells is known to be around 1.8–2.2 mmol/L (Schröder, 1985)[17]. However, the obtained results indicated that calcium concentrations in 1st and 3rd day extracts of MTA were 2.6 and 2.5 mmol/L respectively (after conversion of measuring units). Meanwhile calcium concentration of 1st and 3rd days Biodentine extracts were 5.9 and 5.05 mmol/L respectively. This high calcium level may justify the low cell viability upon treatment with tested materials.

The obtained results were in agreement with previous researches (Damas, Wheater, Bringas, and Hoen, 2011[28], Zhu, Yang, Zhang, and Peng, 2014)[29]. On the contrary, some studies (Saidon *et al.,* 2003[2], Damas *et al.,* 2011[28]; Mozayeni *et al.,* 2012[8], Nuñez, Bosomworth, Field, Whitworth, and Valentine, 2014)[30] reported more favorable tissue response belonged to MTA or Biodentine extracts in comparison to control groups. These findings might be related to the different stimulation modes and setting time applied for the tested materials, where they permitted the materials to set for more than 24 hours while it was only 4 hours in our study, which might overshadowed the materials' biological effects.

Both day-7 MTA and Biodentine extracts exhibited a remarkable proliferation of MRC5 and BHK cells. It has been observed that the level of calcium ion decreased in day 7 extract in contrast to 1st and 3rd days extracts. The present results revealed that the level of free calcium in 7th day extract was 2.05 and 3.95 mmol/L for MTA and Biodentine respectively. Obviously, the concentration of free calcium ion is not the only factor that may affect cell viability. In spite of high level of free calcium in Biodentine 7th day extract, cell proliferation was promoted in Biodentine group. A possible cause for that might be attributed to the presence of high level of hydroxyapatite after complete hydration of materials and calcium carbonate formation as a result of long incubation period. According to the authors believe, calcium hydroxide (which is a by-product of hydration of calcium silicate during setting) might convert into calcium carbonate upon contact with CO_2 present in air to produce a precipitate of calcium carbonate on the specimen.

Previous studies (Ono *et al.,* 2008[31], Nakamura, Nagai, Tanaka, Sekijima, and Yamashita, 2010[32], Kasaj, Willershausen, Junker, Stratul, and Schmidt, 2012)[33] revealed that hydroxyapatite could act as a stimulator of cellular activities in mesenchymal cells *in vitro*; furthermore, (Ma *et al.,* 2011)[6] reported that materials deposited calcium carbonate showed better cell viability. Formation of hydroxyapatite was speculated in the present study due to formation of a wide precipitation that was noticed in the well plate.

Biodentine-treated cells maintained a low cell viability in comparison with that expressed with MTA-treated cells and substantiated with other data by previous studies (Jang *et al.,* 2014[34], B.-N. Lee *et al.,* 2014)[35]. Probable explanation of that may be related to increased production of heavy metals that may decrease cell viability. In a study of (Jang *et al.,* 2014)[34], the authors reported that Biodentine released significantly higher levels of 5 heavy metals (arsenic, copper, iron, manganese and zinc) than MTA. These heavy metals may induce oxidative stress which may produce free radicals and lead to disruption of cell membrane and subsequently decrease cell viability.

Another possible cause is mainly related to calcium chloride which acts as a setting accelerator for Biodentine powder (Grech, Mallia, and Camilleri, 2013)[36]. Many previous studies (M G Gandolfi *et al.,* 2008[37], Kang *et al.,* 2013[27], B.-N. Lee *et al.,* 2014)[35] have reported that calcium chloride was mainly contributed to lower survival rate of cells when used as setting accelerator for MTA. Kang *et al* (Kang *et al.,* 2013)[27] and lee *et al* (B.-N. Lee *et al.,* 2014)[35] have reported that MTA mixed with calcium chloride showed lower biocompatibility and less cell viability than MTA mixed with distilled water. In another study, (M G Gandolfi *et al.,* 2008)[37] also reported that pure MTA showed better cell viability rate than other silicate based cement that contain calcium chloride as setting accelerator.

As mentioned earlier, increased free calcium concentrate ion might have a negative effect on cell viability. The level of calcium ions in Biodentine extracts was confirmed to be higher than the tolerable level for cell proliferation and might be responsible for the increased cell mortality in the current study.

Effect of material on MRC-5 and BHK-21 cell lines attachment and viability

On the contrary to the first section of this study, materials were tested for their ability to inhibit or stimulate cells attachments. Generally, most materials halt the attachment of cells to the stratum of the petri dishes or wells of tissue culture plates. Surprisingly, both cell lines were capable to form strata on the bottom of the well and were stimulated to proliferate in presence of tested materials.

Two possible explanations for these results were proposed; the materials were hanged to the well-plate's cover leaving considerable distance between the material itself and wells' bottoms, which allowed the calcium ions level to be gradually elevated in culture medium. So, cells may accommodate the raise in calcium ions and may induce proliferation. In respect to the first part of this study, the cells were directly exposed to full calcium concentration obtained from long period of incubation leaving no chance for cell to adapt to the new environmental changes and may proceed to cell death. Another possible reason is that the materials samples were immersed in increased volume of culture media to allow full coverage of discs inside wells (2.8 mL culture media) and this may decrease the concentration of calcium ions to a level that may be tolerated by cells and induced cell proliferation.

Table No.1: Mean ± SD *of optical density values percentages of MRC5 cell line after exposure to different concentrations of MTA and Biodentine extracts*

		No dilution				1:1				1:2				1:4				P-value
Day1 extract	Control	100.0	±	31.1	(a)	100.0	±	31.1	(a)	100.0	±	31.1	(a)	100.0	±	31.1	(a)	1
	MTA	68.0	±	7.5		61.9	±	6.2	(b)	63.2	±	12.0	(b)	62.5	±	11.5	(b)	0.530
	Biodentine	55.0	±	7.4	(b)	56.7	±	5.8	(b)	62.8	±	9.4	(b)	64.5	±	12.0	(b)	0.089
	p- value	0.000				0.000				0.001				0.001				
Day 3 extract	control	100.0	±	31.3	(a)	100.0	±	31.3	(a)	100.0	±	31.3	(a)	100.0	±	31.3	(a)	1
	MTA	62.5	±	14.7	(b)	65.1	±	18.6	(b)	70.2	±	19.2	(b)	71.0	±	25.2	(b)	0.761
	Biodentine	59.0	±	16.2	(b)	57.4	±	19.7	(b)	62.9	±	15.4	(b)	67.9	±	9.4	(b)	0.501
	p- value	0.001				0.003				0.006				0.019				
Day 7 extract	control	100.0	±	31.3	(a)	100.0	±	31.3	(a)	100.0	±	31.3	(a)	100.0	±	31.3	(a)	1
	MTA	209.1	±	54.7	(b)	238.0	±	74.4	(b)	256.3	±	42.1	(b)	261.8	±	26.5	(b)	0.841
	Biodentine	120.3	±	17.9	(a)	124.5	±	28.7	(a)	154.2	±	63.9	(a)	163.6	±	35.8	(c)	0.073
	p- value	0.000				0.000				0.000				0.000				

Values with different superscripts at the same column are significantly different at p value < 0.05

Table No.2: Mean ± SD *of optical density values percentages of BHK-21 cell line after exposure to different concentrations of MTA and Biodentine extracts*

		No dilution				1:1				1:2				1:4				P-value
Day1 extract	control	100.0	±	13.8	(a)	100.0	±	13.8	(a)	100.0	±	13.8	(a)	100.0	±	13.8	(a)	1
	MTA	89.3	±	14.6	(a)	89.1	±	8.8	(a)	86.0	±	11.1	(a)	81.5	±	8.1	(a)	0.406
	Biodentine	59.3	±	10.1	(b)	59.7	±	9.4	(b)	52.4	±	9.0	(b)	50.6	±	6.8	(b)	0.077
	p- value	0.000				0.000								0.000				
Day 3 extract	control	100.0	±	13.8	(a)	100.0	±	13.8	(a)	100.0	±	13.8	(a)	100.0	±	13.8	(a)	1
	MTA	78.8	±	9.5	(b)	71.7	±	17.9	(b)	68.9	±	22.2	(b)	67.6	±	18.0	(b)	0.539
	Biodentine	46.7	±	6.5	(c)	44.5	±	7.2	(c)	47.0	±	7.5	(c)	46.2	±	4.6	(c)	0.862
	p- value	0.000				0.000								0.000				
Day 7 extract	control	100.0	±	13.8	(ab)	100.0	±	13.8		100.0	±	13.8		100.0	±	13.8		1
	MTA	118.9	±	19.4	(a)	110.5	±	19.9		110.3	±	13.4		104.0	±	15.5		0.353
	Biodentine	96.4	±	20.6	(b)	96.5	±	20.1		89.9	±	17.9		90.1	±	13.6		0.770
	p- value	0.044				0.284				0.054				0.137				

Values with different superscripts at the same column are significantly different at p value < 0.05

Table No.3: Mean and standard deviation of free calcium ions concentration in the day 1, day 3 and day 7 extracts of tested materials (mg/dL)

	Free Calcium ion concentration (mg/dL)						
	Day 1 extract		Day 3 extract		Day 7 extract		p- value
MTA	10.6	$1.86^{(A)}$	10.17	$1.9^{(A)}$	8.2	$1.1^{(A)}$	0.148
Biodentine	23.4	$2.4^{(B, a)}$	20.7	$1.4^{(B, a)}$	15.4	$2.1^{(B, b)}$	0.001
p- value	0.000		0.000		0.002		

Values with different superscripts (upper cases) at the same column are significantly different at p value < 0.05

Values with different superscripts (lower cases) at the same raw are significantly different at p value < 0.05

Table No.4: Mean ± SD of optical absorbance values percentages of MRC-5 and BHK-21 cell line after direct exposure to MTA and Biodentine materials

S.No		MRC5		BHK-21	
1	Control	100	9.4	100	1.7
2	MTA	114.7	29.8	109.3	2.5
3	Biodentine	104.4	48.3	103.6	4.8
4	p- value	0.167		0.303	

Values with different superscripts at the same column are significantly different p-value < 0.05

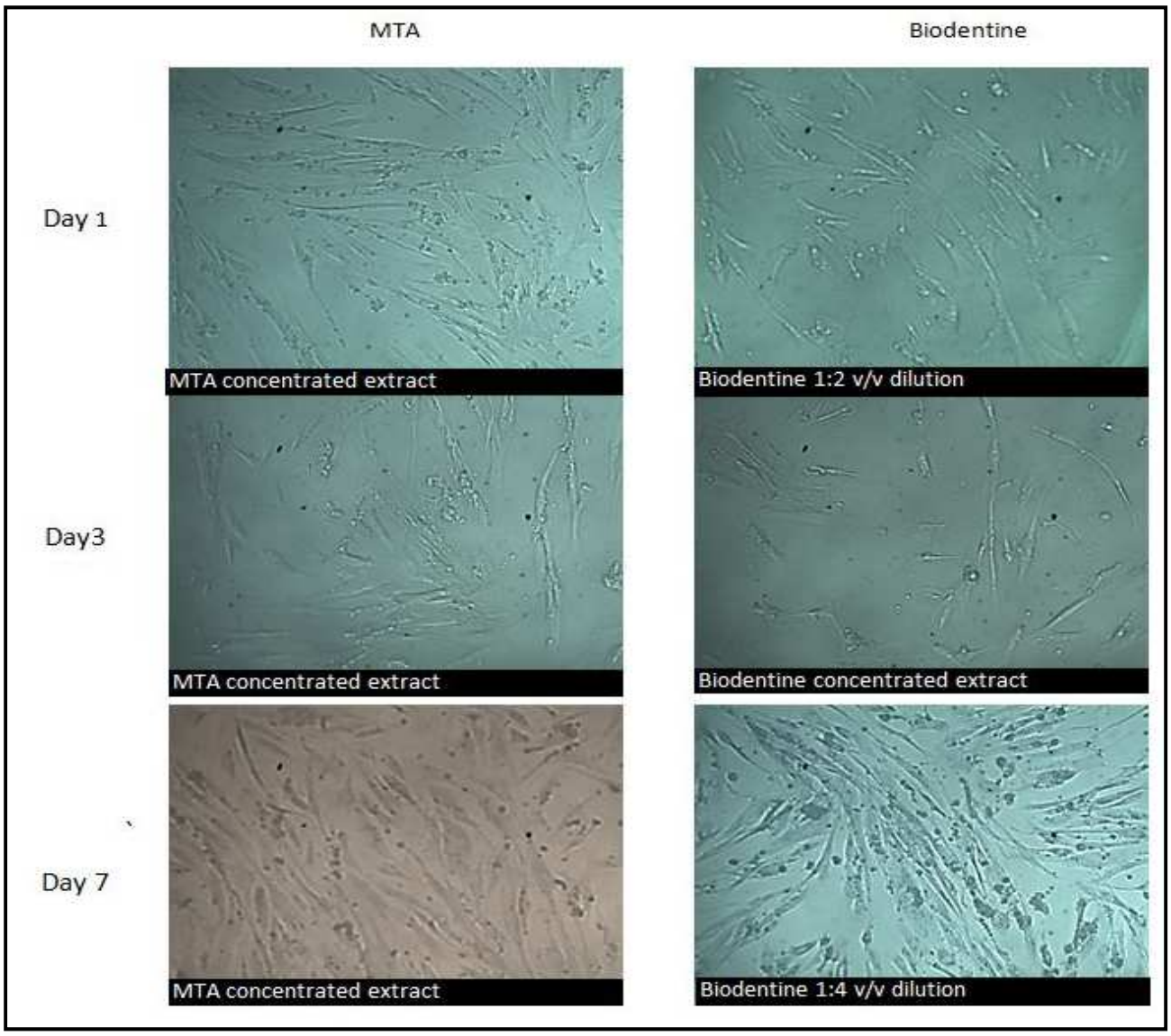

Figure No.1: Line chart demonstrating the MRC5 fibroblast cell line viability rate of the control group (DMEM) in comparison with extracts with various concentrations derived from MTA and Biodentine retrograde materials

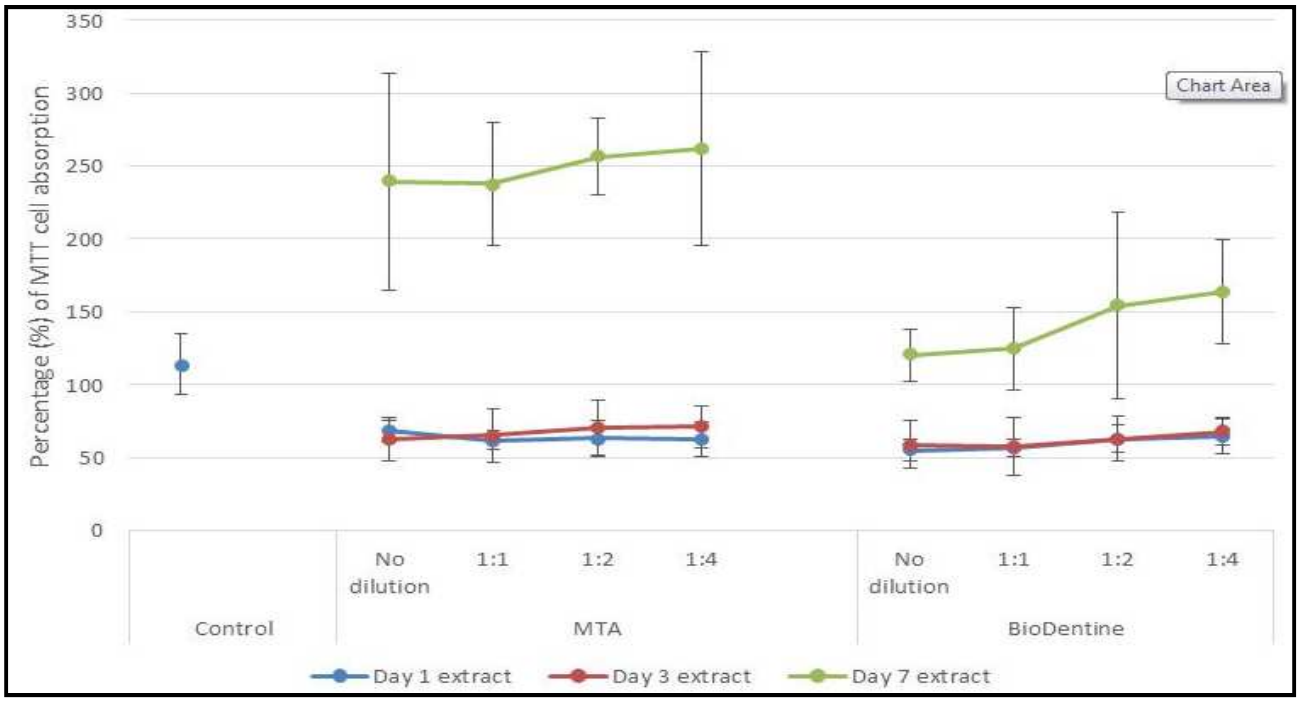

Figure No.2: Representative photo micrographs (x20 magnification) of MRC5 viable cells after incubation with different extracts derived from MTA and Biodentine materials Photomicrographs reveal increased viability of MRC5 cells treated with day 7 extracts

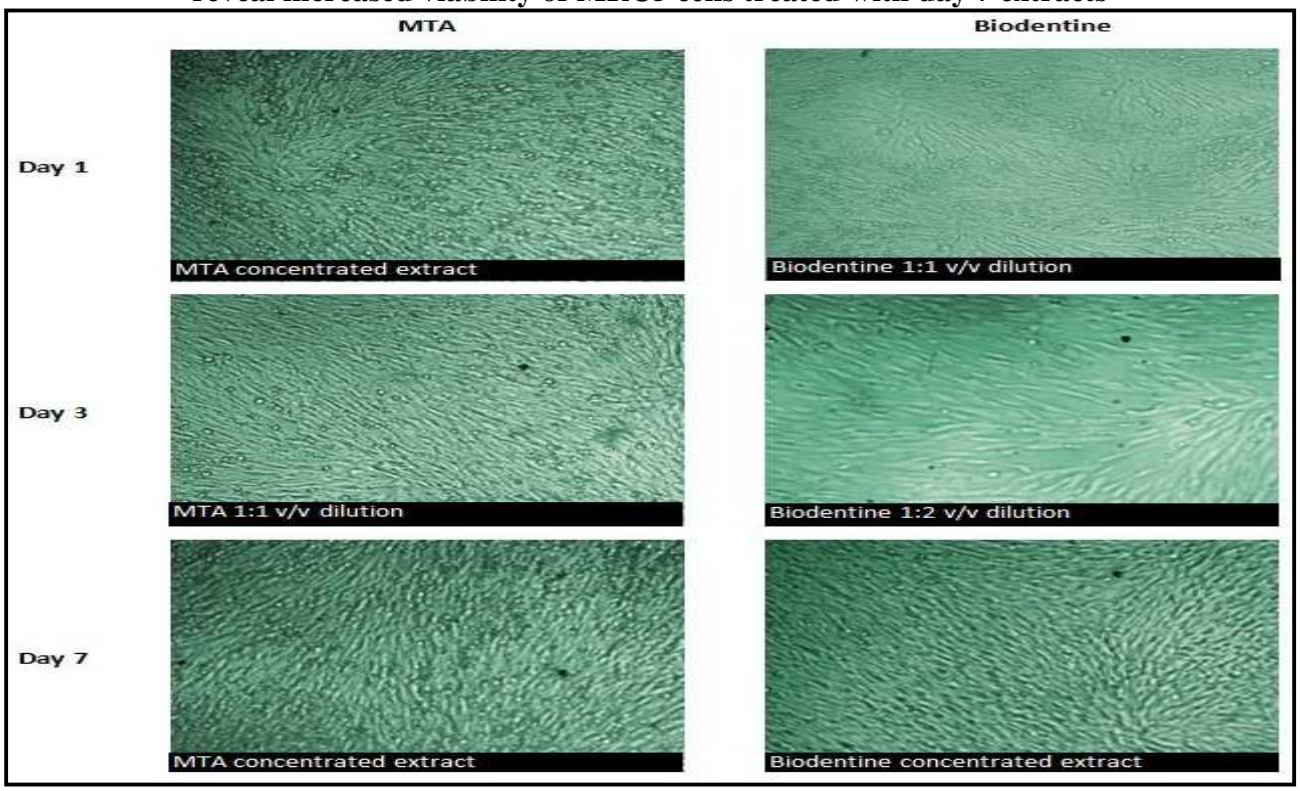

Figure No.3: Line chart demonstrating the BHK-21 fibroblast cell line viability rate of the control group (DMEM) in comparison with extracts with various concentrations derived from MTA and Biodentine retrograde materials

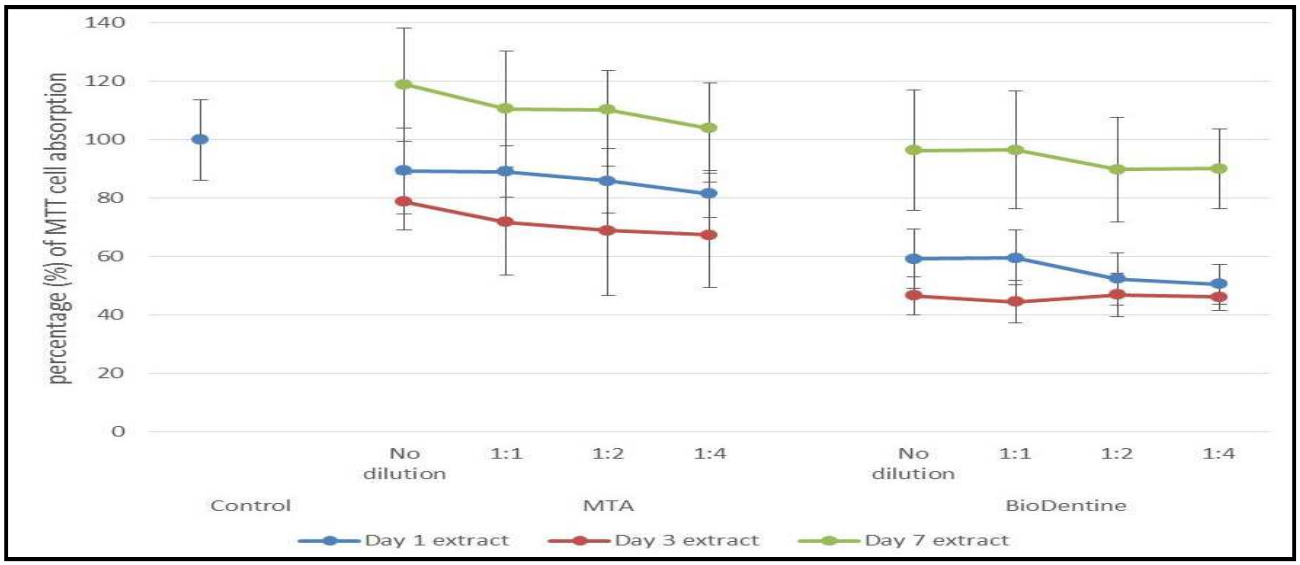

Figure No.4: Representative photo micrographs (x20 magnification) of BHK-21 viable cells after incubation with different extracts derived from MTA and Biodentine materials. Photomicrographs reveal increased cell viability of MTA extract in day1 and day 3 extracts.

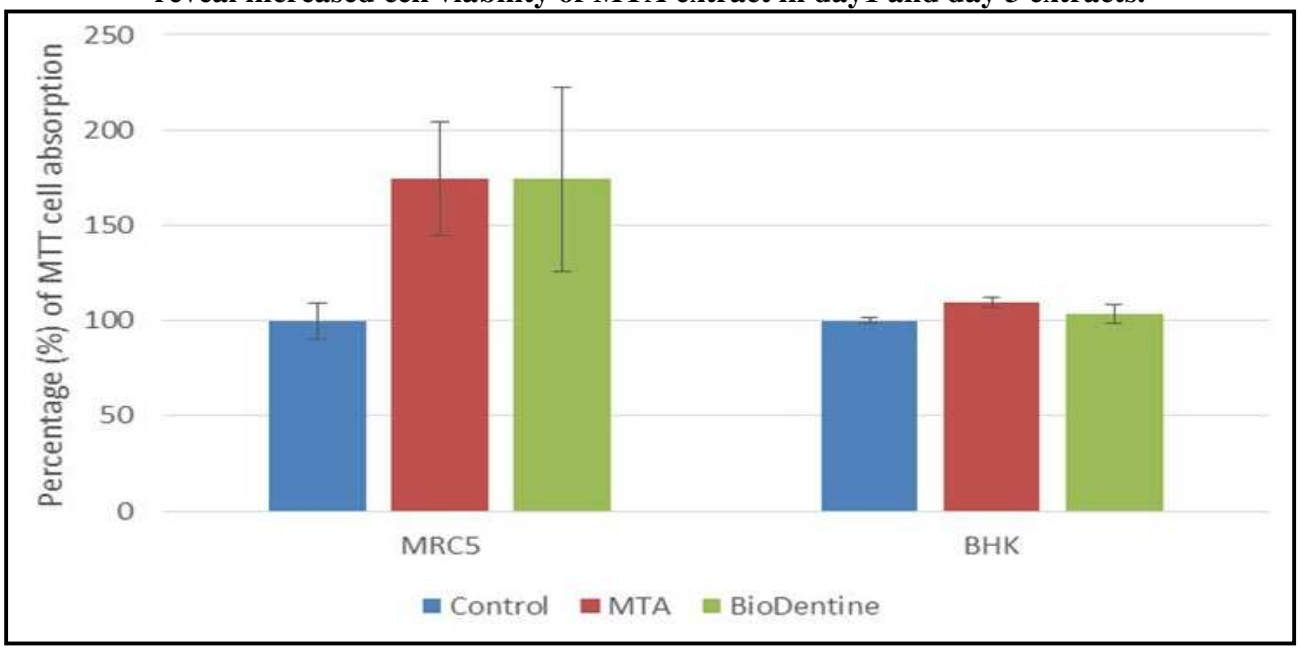

Figure No.5: Column graph comparing the BHK-21 and MRC5 cell viability after direct incubation with MTA and Biodentine retrograde materials Discussion

CONCLUSION

Within the limitation of this study, Biodentine revealed more cytotoxicity than MTA, however, both materials exhibited biocompatibility and can be used in contact with living cells.

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CONFLICT OF INTEREST

There is no conflict of interest to disclose.

BIBLIOGRAPHY

1. Siqueira J F. Aetiology of root canal treatment failure: Why well-treated teeth can fail, *International Endodontic Journal*, 34(1), 2001, 1-10.
2. Saidon J, He J, Zhu Q, Safavi K and Spångberg L S W. Cell and tissue reactions to mineral trioxide aggregate and Portland cement, *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, 95(4), 2003, 483-489.
3. Han L and Okiji T. Bioactivity evaluation of three calcium silicate-based endodontic materials, *International Endodontic Journal*, 46(9), 2013, 808-814.
4. Sarkar N K, Caicedo R, Ritwik P, Moiseyeva R and Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate, *Journal of Endodontics*, 31(2), 2005, 97–100.
5. Reyes-Carmona J F, Felippe M S and Felippe W T. Biomineralization ability and interaction of mineral trioxide aggregate and white portland cement with dentin in a phosphate-containing fluid, *Journal of Endodontics*, 35(5), 2009, 731-736.
6. Ma J, Shen Y, Stojicic S and Haapasalo M. Biocompatibility of two novel root repair materials, *Journal of Endodontics*, 37(6), 2011, 793-798.
7. Samara A, Sarri Y, Stravopodis D, Tzanetakis G N, Kontakiotis E G and Anastasiadou E. A comparative study of the effects of three root-end filling materials on proliferation and adherence of human periodontal ligament fibroblasts, *Journal of Endodontics*, 37(6), 2011, 865-870.
8. Mozayeni M A, Milani A S, Marvasti L A and Asgary S. Cytotoxicity of calcium enriched mixture cement compared with mineral trioxide aggregate and intermediate restorative material, *Australian Endodontic Journal*, 38(2), 2012, 70-75.
9. Galhotra V, Sofat A, Pandit I K, Gambhir R S, Srivastava N and Gugnani N. Comparative evaluation of microleakage of various retrograde filling materials: An in vitro study, *Journal of Natural Science, Biology, and Medicine*, 4(2), 2013, 403.
10. Martell B and Chandler N P. Electrical and dye leakage comparison of three root-end restorative materials, *Quintessence International*, 33(1), 2002, 30-4.
11. Torabinejad M, Hong C U, McDonald F and Ford T R P. Physical and chemical properties of a new root-end filling material, *Journal of Endodontics*, 21(7), 1995, 349-353.
12. Dammaschke T, Gerth H U V, Züchner H and Schäfer E. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements, *Dental Materials*, 21(8), 2005, 731–738.
13. Ebtehal A R, Perinpanayagam H and MacFarland D. Human alveolar bone cells interact with ProRoot and tooth-colored MTA, *Journal of Endodontics*, 32(9), 2006, 872-875.
14. Hunter A R, Kirk E E J, Robinson D H and Kardos T B. A slow release calcium delivery system for the study of reparative dentine formation, *Dental Traumatology*, 14(3), 1998, 112-118.
15. De Deus G, Ximenes R, Gurgel-Filho E D, Plotkowski M C and Coutinho-Filho T. Cytotoxicity of MTA and Portland cement on human ECV 304 endothelial cells, *International Endodontic Journal*, 38(9), 2005, 604-609.
16. Kumada Y and Zhang S. Significant type I and type III collagen production from human periodontal ligament fibroblasts in 3D peptide scaffolds without extra growth factors, *PLoS One*, 5(4), 2010, e10305.
17. Schröder U. Effects of calcium hydroxide-containing pulp-capping agents on pulp cell

migration, proliferation, and differentiation, *Journal of Dental Research*, 64(4), 1985, 541-548.

18. Keiser K, Johnson C C and Tipton D A. Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts, *Journal of Endodontics*, 26(5), 2000, 288-291.
19. Bozeman T B, Lemon R R and Eleazer P D. Elemental analysis of crystal precipitate from gray and white MTA, *Journal of Endodontics*, 32(5), 2006, 425-428.
20. Khan S I R, Ramachandran A, Deepalakshmi M and Kumar K S. Evaluation of pH and calcium ion release of mineral trioxide aggregate and a new root-end filling material, *EJ Dentistry*, 2, 2012, 166-169.
21. Gandolfi M G, Siboni F, Polimeni A, Bossù M, Riccitiello F, Rengo S and Prati C. *In vitro* screening of the apatite-forming ability, biointeractivity and physical properties of a tricalcium silicate material for endodontics and restorative dentistry. *Dentistry Journal*, 1(4), 2013, 41-60.
22. Lee Y L, Lee B S, Lin F H, Lin A Y, Lan W H and Lin C P. Effects of physiological environments on the hydration behavior of mineral trioxide aggregate, *Biomaterials*, 25(5), 2004, 787–793.
23. Camilleri J. Hydration mechanisms of mineral trioxide aggregate, *International Endodontic Journal*, 40(6), 2007, 462-470.
24. Camilleri J. Characterization of hydration products of mineral trioxide aggregate, *International Endodontic Journal*, 41(5), 2008, 408-417.
25. Midy V, Dard M and Hollande E. Evaluation of the effect of three calcium phosphate powders on osteoblast cells, *Journal of Materials Science: Materials in Medicine*, 12(3), 2001, 259-265.
26. Orrenius S, Zhivotovsky B and Nicotera P. Regulation of cell death: the calcium-apoptosis link, *Nature Reviews Molecular Cell Biology*, *4*(7), 2003, 552-565.
27. Kang J Y, Lee B N, Son H J, Koh J T, Kang S S, Son H H, Oh W M. Biocompatibility of mineral trioxide aggregate mixed with hydration accelerators, *Journal of Endodontics*, 39(4), 2013, 497-500.
28. Damas B A, Wheater M A, Bringas J S and Hoen M M. Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence bioceramic root repair materials, *Journal of Endodontics*, 37(3), 2011, 372-375.
29. Zhu L, Yang J, Zhang J and Peng B. A comparative study of BioAggregate and ProRoot MTA on adhesion, migration, and attachment of human dental pulp cells, *Journal of Endodontics*, 40(8), 2014, 1118-1123.
30. Nuñez C M C, Bosomworth H J, Field C, Whitworth J M and Valentine R A. Biodentine and mineral trioxide aggregate induce similar cellular responses in a fibroblast cell line, *Journal of Endodontics*, 40(3), 2014, 406-411.
31. Ono M, Kubota S, Fujisawa T, Sonoyama W, Kawaki H, Akiyama K, Yoshida Y. Promotion of hydroxyapatite-associated, stem cell-based bone regeneration by CCN2, *Cell Transplantation*, 17(1-2), 2008, 231-240.
32. Nakamura M, Nagai A, Tanaka Y, Sekijima Y and Yamashita K. Polarized hydroxyapatite promotes spread and motility of osteoblastic cells, *Journal of Biomedical Materials Research Part A*, 92(2), 2010, 783-790
33. Kasaj A, Willershausen B, Junker R, Stratul S I and Schmidt M. Human periodontal ligament fibroblasts stimulated by nanocrystalline hydroxyapatite paste or enamel matrix derivative. An in vitro assessment of PDL attachment, migration, and proliferation, *Clinical Oral Investigations*, 16(3), 2012, 745-754.
34. Jang Y E, Lee B N, Koh J T, Park Y J, Joo N E, Chang H S, Hwang Y C. Cytotoxicity and physical properties of tricalcium

silicate-based endodontic materials, *Restorative Dentistry & Endodontics*, 39(2), 2014, 89-94.

35. Lee B N, Lee K N, Koh J T, Min K S, Chang H S, Hwang I N, Oh W M. Effects of 3 endodontic bioactive cements on osteogenic differentiation in mesenchymal stem cells. *Journal of Endodontics*, 40(8), 2014, 1217-1222.
36. Grech L, Mallia B and Camilleri J. Characterization of set Intermediate Restorative Material, Biodentine, Bioaggregate and a prototype calcium silicate cement for use as root-end filling materials, *International Endodontic Journal*, 46(7), 2013, 632-641.
37. Gandolfi M G, Pagani S, Perut F, Ciapetti G, Baldini N, Mongiorgi R and Prati C. Innovative silicate-based cements for endodontics: A study of osteoblast-like cell response, *Journal of Biomedical Materials Research Part A*, 87(2), 2008, 477-486.

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