RESEARCH PAPER

Comparative Assessment of Biodentine and Mineral Trioxide Aggregate on the Viability, Migration, and Odontogenic Differentiation of Human Dental Pulp Stem Cells: An In Vitro Study

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ABSTRACT

Introduction: Bioactive calcium silicate-based materials have been widely used in vital pulp therapy due to their reparative dentin-producing and cellular survival-promoting properties. The biological effects of Biodentine and mineral trioxide aggregate (MTA) on dental pulp stem cells might differ.

Objective: To assess the effect of Biodentine versus MTA on the viability, migration and odontogenic differentiation of human dental pulp stem cells (hDPSCs).

Methods: An experimental in vitro comparative study was conducted at the Fatima Jinnah Dental College, Karachi, from 1st November 2025 to 30th April 2026. Human dental pulp stem cells (hDPSCs) were incubated with Biodentine and MTA extracts, and untreated cells were used as controls. The MTT assay, scratch wound-healing assay, and quantitative real-time PCR for the detection of alkaline phosphatase activity and Alizarin Red S staining were used to determine cell viability, migration, and odontogenic differentiation, respectively.

Results: At 72 hours, cell viability was higher with Biodentine than MTA (95.6 ± 4.2% vs. 91.7 ± 5.4%). At 48 hours, wound closure was 91.2 ± 6.8% and 83.5 ± 8.0%, respectively. Alkaline phosphatase activity at day 14 was 4.16 ± 0.68 U/mg protein versus 3.62 ± 0.61 U/mg protein. At day 21, mineralization absorbance was 1.12 ± 0.18 versus 0.96 ± 0.16. DSPP expression was 4.82 ± 1.14 versus 3.91 ± 0.98-fold, respectively.

Conclusion: Both Biodentine and mineral trioxide aggregate demonstrated favorable effects on human dental pulp stem cell viability, migration, and odontogenic differentiation. Biodentine, however, presented relatively greater cellular viability, migration, mineralization and expression of odontogenic markers, indicating a more desirable regenerative response in the current experimental conditions.

Keywords: Biodentine; Mineral Trioxide Aggregate; Human Dental Pulp Stem Cells; Cell Viability; Cell Migration; Odontogenic Differentiation; Vital Pulp Therapy

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INTRODUCTION

The importance of vital pulp therapy has been growing in modern conservative dentistry due to its ability to preserve pulp vitality and prevent or delay root canal treatment.[1] These procedures are biologically successful largely due to the relationship between the pulp-capping material and the resident cells that make up the dental pulp and mediate tissue repair and regeneration.[2] Human dental pulp stem cells (hDPSCs) are a source of mesenchymal stem cells that exhibit a high degree of self-renewal and the capability to differentiate into odontoblast-like cells, and thus have a high degree of regenerative potential.[3] The cells participate in the processes of cellular proliferation, migration, production of extracellular matrix, and

mineralization, which are responsible for dentin–pulp complex regeneration.[4] Therefore, the biological characteristics of the pulp-capping materials, namely the viability, migration, and odontogenic differentiation of stem cells, play an important role in the success of the pulp healing process.[5]

Mineral trioxide aggregate (MTA) has been extensively used as a sealing material, pulp cap, reparative material, and for hard-tissue formation due to its biocompatibility, sealing ability, bioactivity, and hard-tissue formation properties.[6] The calcium silicate formulation promotes the release of calcium ions and the creation of an alkaline environment, potentially enhancing cellular activity and mineralized tissue deposition.[7] MTA does have some drawbacks, however, such as long setting time, poor handling characteristics, risk of discoloration, and a relatively high cost.[8] Several of these disadvantages were overcome in the development of a calcium silicate-based bioactive dentin substitute known as Biodentine.[9] It possesses better handling characteristics, relatively low setting time, and good physicochemical properties.[10] While both materials have the potential of stimulating reparative dentin formation, the biological response of dental pulp-derived stem cells to either material could differ based on chemical composition, ion release, surface properties, and interaction with the cell microenvironment.

Recent advances in regenerative endodontics have highlighted the importance of evaluating dental materials beyond conventional physical and mechanical properties.[11] Cell viability is a critical factor for biocompatibility evaluation because the viability of the pulp stem cells can be affected by cytotoxic reactions, which can impact the survival and regenerative ability of the pulp stem cells.[12] In a similar way, cellular migration is necessary for wound healing, as it allows progenitor cells to migrate to sites of pulp injury and take part in wound healing.[13] Another important biological response is odontogenic differentiation, as hDPSCs can assume an odontoblast-like morphology and express dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP-1), and alkaline phosphatase (ALP).[14] These parameters can be assessed in vitro, offering a controlled experimental method to understand the regenerative potential of bioactive dental materials and detect differences not detected by conventional biocompatibility testing methods.

The biological activity of pulp-capping materials, therefore, should be evaluated not only by cytotoxicity but by a comprehensive evaluation of cellular survival, reparative migration, and odontogenic differentiation. Differences in physicochemical composition and cellular interactions between Biodentine and MTA should be explored under standardized in vitro conditions, while both materials have been shown to have the ability to regenerate. Evaluating the three complementary biological parameters simultaneously in human dental pulp stem cells can give a better understanding of the relative regenerative abilities of the cells. The present study was conducted to compare the viability, migration, and odontogenic differentiation of human dental pulp stem cells when cultured with Biodentine and mineral trioxide aggregate in vitro. The results can be used to understand the biological benefits of these two widely used bioactive materials, thereby offering experimental data for material selection in vital pulp therapy and regenerative endodontics.

METHODS

A comparative study was conducted in an experimental in vitro setting at Fatima Jinnah Dental College, Karachi, Pakistan. The study was conducted over six months, from 1st November 2025 to 30th April 2026.

The required sample size was determined using the OpenEpi online calculator for comparing two independent means based on the results of Birant et al. (2021). The mean cell viability of Biodentine was 62.93 ± 31.45%, while that of ProRoot MTA was 53.69 ± 30.22%; the expected mean difference was 9.24%, and the pooled standard deviation was 30.84%.[15] A 95% confidence level, 5% level of significance, 80% statistical power, and 20% beta error were considered. Thus, the sample size of each group was calculated as 175 observations (175 for Biodentine and 175 for MTA).

The human dental pulp stem cell cultures that met the predetermined laboratory eligibility criteria were selected by the non-probability purposive sampling method. The experimental groups were established, in which the pulp-capping materials used were the characterized hDPSC cultures. Biodentine extract was given to group A and MTA extract was given to group B. An untreated cell culture group was also maintained as a negative control to obtain baseline cellular responses.

This included human dental pulp stem cell cultures from healthy dental pulp tissue isolated from extracted permanent teeth (18–30 years old donors). Possible candidates were cultured with confirmed mesenchymal stem cell properties, reasonable cell viability, appropriate fibroblast-like morphology, and growth properties. To reduce variations related to long-term cell culture, cells were selected from passages 3–5. Cultures with microbial or fungal contamination, abnormal morphology, poor cellular growth, low baseline viability, or loss of characteristic mesenchymal stem cell markers were excluded. The previously exposed or experimented cell cultures were also excluded.

Healthy permanent teeth that were to be extracted were collected under aseptic conditions after receiving institutional ethical approval and informed consent from the respective donors. The dental pulp tissue was carefully collected, put into a suitable sterile transport medium, and processed in a sterile environment in the laboratory. Human dental pulp stem cells were extracted and cultivated in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and antibiotics, and grown in a standard incubator at 37°C and 5% CO_2.[16] The isolated cells were identified using their morphological characteristics and flow cytometry for expression of surface markers of mesenchymal stem cells.

The cells with the right characteristics were expanded and used in the next experiments.

Biodentine and MTA were molded using the manufacturers' instructions. The materials were activated and kept in culture medium to generate the material-conditioned extracts under controlled conditions. The extracts were filtered if necessary and used for the cultured hDPSCs. Cells in the experimental group were treated with Biodentine, MTA or left untreated (control). The percentage of viable cells was determined by MTT assay at predetermined time intervals and compared with the untreated control. A standardized scratch was made in confluent cell monolayers, and wound closure was quantified by microscopic observation of the monolayers at the time of the scratch and at the time of observation. To assess cellular migration, the percentage of wound closure was calculated.

Odontogenic differentiation was evaluated by culturing the hDPSCs in odontogenic induction medium in the presence of the material extracts. Alkaline phosphatase activity was used as an early marker of differentiation, and mineralization was determined by staining with Alizarin Red S. The odontogenic differentiation markers dentin sialophosphoprotein (DSPP) and dentin matrix protein-1 (DMP-1) were evaluated by quantitative real-time polymerase chain reaction (qRT-PCR). Experiments were carried out under standard laboratory conditions, technical replicates were taken, and independent experimental repetitions were performed to minimize the variation of measurements. The observations were recorded on a structured lab data sheet.

All data were analyzed with IBM SPSS Statistics (version 26). The mean and standard deviation of the quantitative variables (cell viability percentage, wound closure percentage, alkaline phosphatase activity, mineralization levels, and relative gene expression) were calculated. The Shapiro–Wilk test was used to determine the normality of continuous data. Data from Biodentine, MTA and control groups were compared using one-way analysis of variance (ANOVA), and using Tukey's test for normally distributed data. Two-way ANOVA was conducted to determine the effects of treatment group, time, and the interaction between treatment group and time for comparisons among different observation periods. Non-normally distributed data were analyzed using the Kruskal-Wallis test and then compared with the post hoc tests. Experimental replicates were done independently, and technical replicates were averaged prior to statistical testing. A p-value < 005 was regarded as being statistically significant.

RESULTS

The baseline characteristics of hDPSC cultures were comparable across the experimental groups. Baseline cell viability was high and all cultures had a fibroblast-like morphology with appropriate expression of mesenchymal stem cell markers (Table 1).

At 24, 48 and 72 hours, the cells treated with Biodentine had higher viability compared to cells treated with MTA, while both treatment groups had lower viability than the untreated control ($p<0.001$) (Table 2).

Cell migration increased progressively over 48 hours in all groups. Biodentine had the highest wound closure at all observation points, while the untreated control had the highest migration ($p<0.001$) (Table 3).

Alkaline phosphatase activity was highest in Biodentine-treated cells, followed by MTA-treated cells and controls ($p<0.001$) on days 7 to 14 (Table 4).

All groups had higher mineralization between days 14 and 21. The mineral deposition was highest in the cells treated with Biodentine, followed by MTA and the untreated cells ($p<0.001$) (Table 5).

Both material-treated groups were found to be higher than controls when it comes to the expression of all three odontogenic differentiation markers, with Biodentine having the highest relative expression of all three ($p<0.001$) (Table 6).

Table 1

Allocation and baseline characteristics of hDPSC cultures

Characteristic	Biodentine (n=175) n (%)/Median (IQR)/Mean ± SD	MTA (n=175) n (%)/Median (IQR)/Mean ± SD	Untreated control (n=30) n (%)/Median (IQR)/Mean ± SD
Cell passage,	4 (3–5)	4 (3–5)	4 (3–5)
Baseline viability, %	96.2 ± 2.1	96.0 ± 2.2	96.4 ± 1.9
Fibroblast-like morphology	175 (100.0)	175 (100.0)	30 (100.0)
CD90-positive cells, %	96.1 ± 2.4	95.8 ± 2.6	96.3 ± 2.2
CD105-positive cells, %	94.8 ± 3.1	94.5 ± 3.0	95.0 ± 2.8
CD34/CD45-negative cells, %	97.2 ± 1.8	97.0 ± 1.9	97.4 ± 1.7

Table 2

Cell viability assessed by MTT assay

Time point	Biodentine (%)	MTA (%)	Control (%)	Overall p-value
24 hours	91.8 ± 5.6	88.6 ± 6.2	98.1 ± 2.0	<0.001
48 hours	94.2 ± 4.8	90.1 ± 5.9	98.4 ± 1.8	<0.001
72 hours	95.6 ± 4.2	91.7 ± 5.4	98.6 ± 1.6	<0.001

Table 3

Cell migration assessed by scratch wound-healing assay

Time point	Biodentine wound closure (%)	MTA wound closure (%)	Control wound closure (%)	Overall p-value
0 hours	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	—
12 hours	38.6 ± 8.4	32.1 ± 8.7	42.8 ± 7.9	<0.001

24 hours	71.4 ± 9.2	62.8 ± 9.6	78.2 ± 8.1	<0.001
48 hours	91.2 ± 6.8	83.5 ± 8.0	96.1 ± 3.9	<0.001

Table 4
Alkaline phosphatase activity during odontogenic induction

Observation day	Biodentine (U/mg protein)	MTA (U/mg protein)	Control (U/mg protein)	Overall p-value
7 days	2.84 ± 0.51	2.46 ± 0.48	1.72 ± 0.39	<0.001
14 days	4.16 ± 0.68	3.62 ± 0.61	2.35 ± 0.47	<0.001

Table 5
Mineralization assessed by Alizarin Red S staining

Observation day	Biodentine (absorbance, 405 nm)	MTA (absorbance, 405 nm)	Control (absorbance, 405 nm)	Overall p-value
14 days	0.68 ± 0.12	0.59 ± 0.11	0.31 ± 0.08	<0.001
21 days	1.12 ± 0.18	0.96 ± 0.16	0.52 ± 0.12	<0.001

Table 6
Relative expression of odontogenic differentiation markers by qRT-PCR

Marker	Biodentine (fold change)	MTA (fold change)	Control (fold change)	Overall p-value
DSPP	4.82 ± 1.14	3.91 ± 0.98	1.00 ± 0.24	<0.001
DMP-1	3.96 ± 0.91	3.28 ± 0.84	1.00 ± 0.22	<0.001
ALP	3.41 ± 0.82	2.89 ± 0.76	1.00 ± 0.20	<0.001

DISCUSSION

The present in vitro study comparatively evaluated the effects of Biodentine and mineral trioxide aggregate (MTA) on the viability, migration, and odontogenic differentiation of human dental pulp stem cells (hDPSCs). The results revealed good cellular response on both materials, but Biodentine exhibited higher cell viability, migration, ALP activity, mineralization, and expression of odontogenic markers. These findings suggest differences in the biological responses of hDPSCs to the two calcium silicate-based materials.

The increase in viability of Biodentine was in line with that of Birant et al. (2021), who showed good cytocompatibility in both Biodentine and ProRoot MTA groups, with a numerical increase in viability for Biodentine that did not reach statistical significance.[17] Likewise, Manaspon et al. (2021) showed that both materials were effective for the proliferation of hDPSCs and had moderate cytotoxicity.[18] Their results corroborate the positive cellular responses that were found in the present study; however, the difference in cellular response among materials could be different depending on their exposure conditions and assays.

In 2021, a study on the biological characteristics and odontogenic differentiation effects of calcium silicate-based pulp-capping materials and found that Biodentine and MTA were associated with good biological responses.[19] Both materials promoted cell proliferation and mineralization, while other pulp-capping materials had lesser responses. The results of the present study corroborate with the increased mineralization and odontogenic activity found in both treatment groups. In the context of cellular differentiation, Assadian et al. (2022) noted that Biodentine, OrthoMTA, and calcium-enriched mixture cement were able to promote the proliferation of dental pulp stem cells while simultaneously expressing odontogenic markers.[20]

The results of the migration were also corroborated by Manaspon et al. (2021) who found that hDPSCs maintained good migration properties in the presence of Biodentine and ProRoot MTA extracts than the other pulp-capping materials.[18] A study by Assadian et al. (2022) showed good results for the proliferation and expression of odontogenic genes when using Biodentine, OrthoMTA and calcium-enriched mixture cement. They found that both Biodentine and OrthoMTA stimulated dentinogenic markers, such as DSPP and DMP-1.[20] In the same way, Yune et al (2023) showed that enamel matrix derivative (EMD) and hydraulic calcium silicate cement (HCC) stimulated odontogenic and osteogenic differentiation of hDPSCs.[21] The results concur with those of the present study in which enamel matrix derivative was not added to the increased expression of odontogenic markers seen in the present study.

In the 2024 journal volume, Phang et al. looked at how the shape of the Biodentine specimens and the length of elution time affected responses in dental pulp stem cells. They also found that mineralization and gene expression related to differentiation were enhanced by the extracts derived from the discs but not by the powdered extracts.[22] This is a relevant finding for the present study because it has stressed the need for a controlled preparation of the materials and the conditions of the extracts when interpreting the differences between cellular viability and mineralization.

In addition, Esen et al. (2024) tested the cytotoxicity and bioactivity of calcium silicate-based cements, using hDPSCs. They stated that Biodentine had comparatively higher ALP activity and favorable growth-factor responses which correlated with the increased ALP activity in the present study.[22] Likewise, Koutrouli et al. 2024 reported that MTA and Biodentine promoted an increased cell viability and proliferation in a 3D tissue-engineered deciduous pulp model, with a higher and earlier cellular response for Biodentine.[23]

The relatively good results obtained with Biodentine in the parameters studied should be understood in the context of an in vitro experimental model. There was no response to the material that was as good as the untreated cellular conditions, as seen in the untreated control group with the highest viability and migration. In addition,

scratch wound closure which measures cell migration could be affected by cell migration and proliferation. Likewise, the genes and the activity of ALP and mineralization present complementary but different markers of odontogenic differentiation. The results do not conclude the biological response to both materials to be superior, but indicate a favorable response in this experimental data, with Biodentine demonstrating higher values.

Strengths and limitations

An important strength of the present study was the assessment of cell viability, migration, and multiple odontogenic differentiation parameters, providing a broader evaluation of material–cell interactions. But there were some drawbacks to the study. The results obtained in vitro had to be extrapolated for in vivo conditions. In addition, differences in the concentrations of materials extracted and exposure time as well as differences in individual responses to stem cells may affect the results. The scratch assay could not completely distinguish cellular migration from proliferation. These findings should be confirmed by further studies on 3D pulp models and in vivo studies.

CONCLUSION

Both Biodentine and mineral trioxide aggregate showed good biological reactions in human dental pulp stem cells, which can be used in vital pulp therapy. In the current experimental model, Biodentine, however, showed a comparatively higher cell viability, migration, alkaline phosphatase activity, mineral deposition, and expression of odontogenic differentiation markers when compared to MTA. These findings suggest a potentially more favorable in vitro regenerative response with Biodentine. However, more standardized in vitro and in vivo studies are needed to determine if these differences are clinically significant.

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