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ORIGINAL ARTICLE

Response of stem cells from human exfoliated deciduous teeth (SHED) to three bioinductive materials – An in vitro experimental study

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KEYWORDS

Bioinductive materials;
Cytotoxicity;
Functional differentiation;
SHEDs;
Vital pulp therapy

Abstract *Introduction:* Stem cells have unmatched capacity and potential for regeneration and when used alone or in combination with scaffolds to replace or repair damaged cells, can differentiate into any mature cell.

Aim: To evaluate the functional differentiation potential of EMD (Enamel Matrix Derivative), MTA (Mineral Trioxide Aggregate) and Biodentine on Stem Cells from Human Exfoliated Deciduous teeth (SHED).

Objective: To determine functional differentiation potential (osteogenic/odontogenic) of various biomaterials on SHED.

Material and method: SHED derived from 5th linear passage after sub-culturing were treated with EMD, MTA and Biodentine individually and their effect on cell viability was compared and evaluated by MTT (3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide) assay for 7 days. Alizarin red S staining was used to assess mineralization potential of these materials by the staining calcium deposits for 14 days. The results were analyzed using One-way ANOVA, Post hoc Tukey's test for multiple comparisons.

Results: It was observed that EMD imparted the highest cell viability at the end of 7 days ($p < 0.001$) followed by Biodentine and MTA. Likewise EMD showed highest potential to enhanced mineralization and expression of dentine sialoprotein ($p < 0.001$) followed by Biodentine and MTA at the end of 14 days ($p < 0.001$).

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Conclusion: It can be concluded that all the tested materials are bioinductive to SHED. EMD can be used for various vital pulp therapies as that of Biodentine and MTA with predictable as well as enhanced success rate.

1. Introduction

Stem cells are primitive cells are found in all multi-cellular organisms that are characterized the capacity to differentiate into any mature cell type (Narang and Sehgal, 2012). These cells have an unmatched potential for regeneration and are used frequently or in combination with scaffolds to replace or repair damaged cells (Becker et al., 1963). Dental progenitor cells found in pulp tissues allows possible therapeutic modality for dentin and pulp (Hargreaves et al., 2008). Studies in the field of regenerative endodontics have demonstrated that few dental materials have ability to help deposit hard tissue and maintain vitality of exposed pulp termed as bioinductive materials (Kuratate et al., 2008; Nair et al., 2008).

Bioinductive materials when placed in direct contact with wounded dentin or pulp, exhibit adequate biocompatibility and bioactivity to heal it (Al-Hezaimi et al., 2013; Nowicka et al., 2013). These materials have ability to induce hard tissue formation. They are biocompatible and nontoxic, non-resorbable, and unaffected by blood/saliva contamination. From last few years, more effective pulp capping materials have been successfully developed (Nowicka et al., 2013). Of these, MTA (Mineral Trioxide Aggregate) and Biodentine, exhibit reliable effects than previously used one (Wongwatanasanti et al., 2018; El Meligy et al., 2016). MTA has shown to induce proliferation of murine undifferentiated pulp cells to odontoblast-like cells and support adhesion, proliferation as well as migration of human mesenchymal stem cell or human dental pulp stem cells (Wongwatanasanti et al., 2018). It also possesses high potential for mineralization with relatively few inflammatory reactions in clinical use thus making it material of choice for endodontic therapies (Kuratate et al., 2008; Nair et al., 2008). Biodentine is a calcium silicate- based bioactive restorative material with dentine like mechanical properties and can be used as a dentine substitute. It stimulates tertiary dentine formation and possesses potential to differentiate dental pulp and mesenchymal stem cells to enhance mineralization (El Meligy et al., 2016). EMD (Enamel Matrix Derivative) is a protein extract from unerupted porcine tooth buds containing approximately 90% amelogenin and smaller amounts of tufelin, ameloblastin, enamelin and other non-amelogenin proteins (Wang et al., 2018). When used for therapeutic purpose, it has proved to form dentine like islands bridging the width of coronal pulp at the pulpotomy site. It increases the expression of odontoblast/osteoblast cell markers in stem cells from human exfoliated deciduous teeth (SHED) and this property helps improve pulp tissue repair and regeneration (Ishizaki et al., 2003; Wang et al., 2018).

Evidences from experimental studies have demonstrated effects of EMD, MTA and Biodentine on human dental pulp tissues either separately or in combination with other materials through various treatment modalities at variable success rates (Nowicka et al., 2013; Tsai et al., 2018). There are numerous research papers and reports available in the literature regarding biocompatibility of EMD, MTA and Biodentine, either compared alone or with each other in one or the other way (Araújo et al., 2018; Min et al., 2009). However, when data about effect of direct contact of these materials on stem cells from human exfoliated deciduous teeth (SHED) was searched, very limited data was found.

Keeping these factors in consideration, the present in-vitro study was conducted to evaluate the functional differentiation potential EMD, MTA and Biodentine when placed in direct contact with SHED through MTT assay and Alizarin Red S staining method.

2. Objective of the study

The objective of study was to evaluate functional differentiation (osteogenic/ odontogenic) potential of stem cells from human exfoliated deciduous teeth (SHED) under the influence of EMD, MTA and Biodentine.

3. Hypothesis of the study

SHED under direct influence of biomaterials like EMD, MTA and Biodentine demonstrate functional differentiation (osteogenic/odontogenic) potential.

4. Material and method

This in –vitro experimental study was carried out at Department of Pediatric and Preventive Dentistry and Department of molecular Biology and Immunology after gaining clearance from institutional ethical committee.

4.1. Isolation and culture of SHED

All the procedures performed in this study were conducted following protocols given by Goorha and Reiter (2017). Intact caries free primary teeth, close to exfoliation, were extracted from children aging between 8 and 12 years under strict aseptic conditions. The access cavity was made into tooth pulp chamber using high speed air rotor and sterile coolant, to gain access to pulp issues. The pulp tissue was carefully extirpated from the root and pulp chamber with sterile barbed broach and sharp spoon excavator. The tissue tags were suspended in screw capped test tubes containing transport media (50:50 ratio of Dulbecco's Modified Eagle's Medium: F12 with HEPES buffer, and 1% antibiotic actinomycotic solution, Himedia laboratories, Mumbai, India). The samples were sent to the laboratory for isolation and culture of stem cells (Goorha & Reiter, 2017; Tsai et al., 2018). At laboratory, pulp tissues were minced in a petri-dish using sterile blade into small pieces and placed in prewarmed 37 °C washing medium

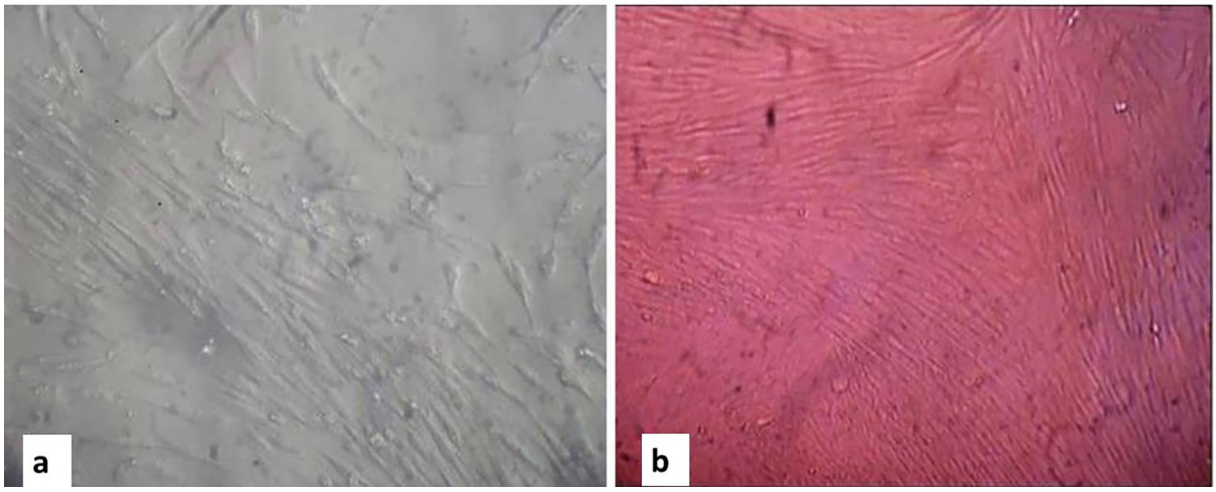

Fig. 1 Isolation of SHED from dental pulp after 4th passage, (a) unstained (b) H&E stained.

Table 1 Various bioinductive materials used in the study.

Groups	Materials used
Group 1 (Negative control)	Cells maintained in regular culture medium
Group 2 (Positive control)	Cells maintained in osteoinduction medium
Group 3	Cells cultured with MTA
Group 4	Cells cultured with Biodentine
Group 5	Cells cultured with enamel matrix derivative (Emdogain)

(Delbecco's phosphate buffered saline without calcium or magnesium and 1% antibiotic actinomycotic solution) to clean all the debris from the tissues. The pulp with washing media was transferred into centrifuge tube and centrifuged at 800 rpm for 5 min at room temperature to form a tissue pellet. The washing medium was aspirated and the pellet was resuspended in 4 ml culture medium supplemented with 3 mg/ml collagenase and 4 mg/ml dispase II for and incubated for 1 h at 37 °C, till the tissue underwent enzymatic dissociation. The cell cultures were further subjected for 4 steps enzymatic digestion and re-suspended in 5–10 ml phosphate buffered saline to obtain pellets of cells. These pellets were re-suspended in 5 ml of DMEM to obtain single-cell suspensions, incubated at 37 °C in 5% CO2 in 24 well micro titer plates for 7 days (Goorha and Reiter, 2017). The culture medium was changed every three days until the cell confluence was achieved (Guven et al., 2013). The outgrown cells at confluence were subcultured in growth medium, DMEM supplemented with 10% fetal bovine serum (FSB), 2 mmol L^{-1} of L-glutamine (Himedia, Mumbai, India) as well as 1% of PSA antibiotic solution (penicillin, streptomycin and amphotericin, Himedia, Mumbai, India) and incubated at 37 °C in a humidified atmosphere of 5% CO2 and 15% O_2. The cells derived at the end of this procedure were subcultured at a ratio of 1:4 and termed as SHED (Stem Cells from Human Exfoliated Deciduous teeth) (Fig. 1). These SHED were further identified and characterized following guidelines of the ISCT (International Society of Cellular Therapy) to confirm the primary stem cells mesenchymal phenotype with specific surface markers. Surface antigens of SHED were determined using antibodies conjugated to fluorophores using flow cytometry (Tomás-Catalá et al., 2018; Yu et al., 2016). The antibody markers used were CD90, CD73 and CD105. The estimation of the sample size used for the present study was based on the method of "resource equation". In the present study, cells thus derived were further divided in five groups based on treatment with test materials and control groups. The total number of cells per groups used was 5000–10,000 per wells (Charan & Kantharia, 2013).

4.2. Sample preparation

In this study, SHED derived from 5th linear passage after subculturing and maintained in DMEM (HIMEDIA laboratories, Mumbai, India) were used as negative control however cells cultured and maintained in osteoinduction medium was used as positive control (Vishnubhalaji et al., 2012). The test materials used in this experimental study were MTA (MTA+, Angelus, Londrina, PR, Brazil), Biodentine (Septodont, St-Maur-des-Fosses, Cedex, France) and EMD gel (enamel matrix derivative- Strauman company, Basel, Switzerland) (Table 1). These materials were mixed as per manufacturer's instruction. One scoop of MTA powder and a drop of distilled water were mixed for 30 s till homogenous consistency was achieved. Biodentine was mixed by adding five drops of the liquid into the capsule containing 1-gram powder on amalgamator at a speed of 4000 rpm for 30 s. Premixed Biodentine and MTA were further added to culture medium to achieve final concentration of 1 mg/ml (Slompo et al., 2015; Woo et al., 2013). 0.7 ml of Emdogain gel containing 30 mg/mL enamel matrix derivative was diluted with sterile distilled water to achieve final concentration of 100 µg/ml solution (Kwon et al., 2014).

4.3. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay

To analyze effects of bioinductive materials on SHED in terms of viability and proliferation rates, the MTT assay was carried out. In this procedure, SHED were seeded at a

density equal to 5000 cells/cm^2 on a 96 well plate and cocultured with 200 µml media conditioned by the different test materials for 7 days through a colorimetric 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide metabolic activity assay (MTT assay kit, Himedia laboratories, Mumbai, India). By using this coculture system, mixing and direct contact of materials to SHED were avoided. On 7th day cell viability was measured by MTT assay. MTT solution (1 mg/mL) was added to each well for 2 h in the culture incubator. The solution was then discarded, and 1 ml isopropanol was added to each well. The plate was shaken for 30 min at room temperature to solubilize the formazan crystals (Maeda et al., 2015; Vishnubhalaji et al., 2012). Two hundred microliter of isopropanol was transferred to a 96-well tissue culture plate. A spectrophotometer (RI Technologies, Bangkok, Thailand) was used to measure the absorbance values at wave lengths of 570 nm (Fig. 2).

Cell viability was calculated using the formula: (OD- optical density)

$$\text{Cell survival} = \frac{\text{OD values of experimental wells}}{\text{OD values of control wells}} \times 100$$

4.4. Alizarin red S staining

For conducting this procedure, similar coculture system was used for determining differentiation potential of the test materials on SHED. The cell culture media conditioned with all the test materials and control groups were mixed with SHED (10,000 cells per well) in 6 well plates for 14 days in 400 µml growth medium, DMEM supplemented with 10% fetal bovine serum (FSB), 2 mmol L^{-1} of L-glutamine. The culture medium was changed every three days to maintain vitality of the cells (Guven et al., 2013). At the end of 14th day, the treated stem cells were fixed for 10 min with cold 100% methanol (Himedia laboratories, Mumbai, India) and washed with sterile water twice and then stained with 2% alizarin red (Alizarin Red S staining kit - Himedia laboratories, Mumbai, India) for 10 min at room temperature. The alizarin red was removed, and specimens were washed with sterile water to remove excess color. The specimens were visualized under a light microscope for the presence of calcified nodules to determine positive or negative staining (Seo et al., 2013) (Fig. 3).

4.5. Statistical analysis

Results of the all test material were analyzed using SPSS Inc software (Windows, version 16.0 Chicago, USA) by using tools of descriptive statistics such as Mean, and SD for representing quantitative data. One-way ANOVA test was applied to compare measurements of mean of five groups. Post hoc data analysis which follows one-way ANOVA was done by using Tukey's multiple comparison test was also used. Post hoc test analyses multiple pair-wise individual comparison among group.

5. Results

5.1. Isolation and characterization of SHED

The presence of stem cells derived from the dental pulp of exfoliating primary teeth was confirmed and characterized using specific antibodies for CD73, CD90 and CD105, as SHED, towards which positive expression was recorded (Slompo et al., 2015; Tsai et al., 2018).

5.2. MTT assay

The incubation of SHED with various dilutions of bioinductive materials resulted in a significant cell proliferation as compared to control groups. It was evident from the analysis that, out of three biomaterials tested, EMD gel showed highest cell viability and proliferation followed by biodentine. However, MTA showed least cell viability and proliferation. Table 2 and Fig. 4 reveals that at the end of 7 days, highest amount of cell viability was seen with EMD group (absorbance: 1.864 ± 0.272) followed by Biodentine group (absorbance: 1.684 ± 0.186). MTA and Negative control group shows equal cell viability (absorbance: 1.485 ± 0.04) ($p < 0.001$).

5.3. Alizarin red S staining assay

Incubation of SHED with various dilutions of biomaterials resulted in significant mineralized nodule formation. It was evident from the analysis that, out of three biomaterials tested, EMD gel showed highest osteoinduction/odontoinduction capacity followed by biodentine and MTA respectively. Table 3

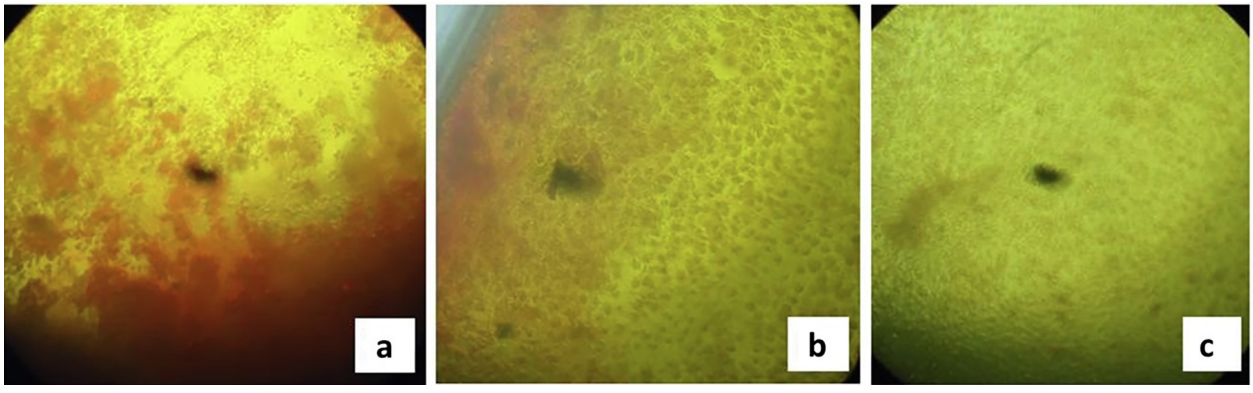

Fig. 2 MTT assay of SHED treated with (a) MTA, (b) Biodentin, (c) Emdogain.

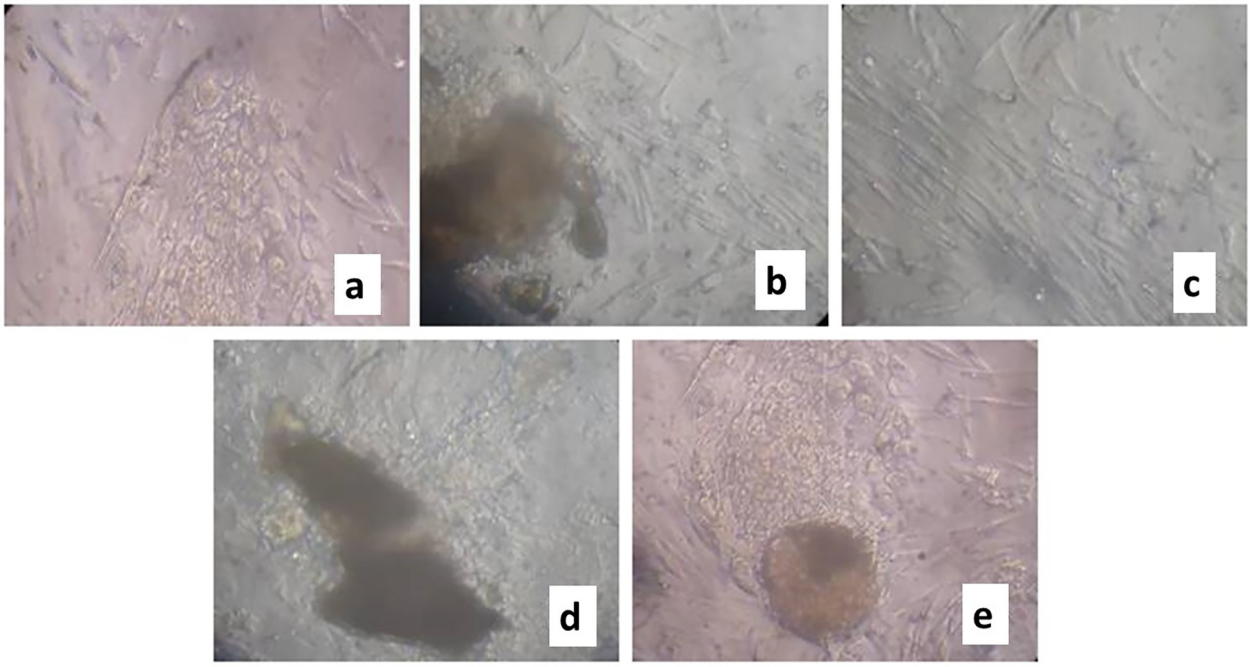

Fig. 3 Alizarin Red S staining of SHED grown with, (a) growth media, (b) osteoinduction media, (c) MTA, (d) Biodentin, (e) Emdogain.

Table 2 Results of Bioinductive material with MTT assay on SHEDs.

Groups	Mean	Std Dev (S.D)	Anova F Test	p value/significance
Group I (Negative control)	1.485	0.04	F = 15.909	$p < 0.001^{**}$
group III (MTA)	1.485	0.064		
Group IV (Biodentine)	1.684	0.186		
Group V (Emdogain)	1.864	0.272		

$p > 0.05$ – not significant, *$p < 0.05$ – significant.
** $p < 0.001$ –highly significant.

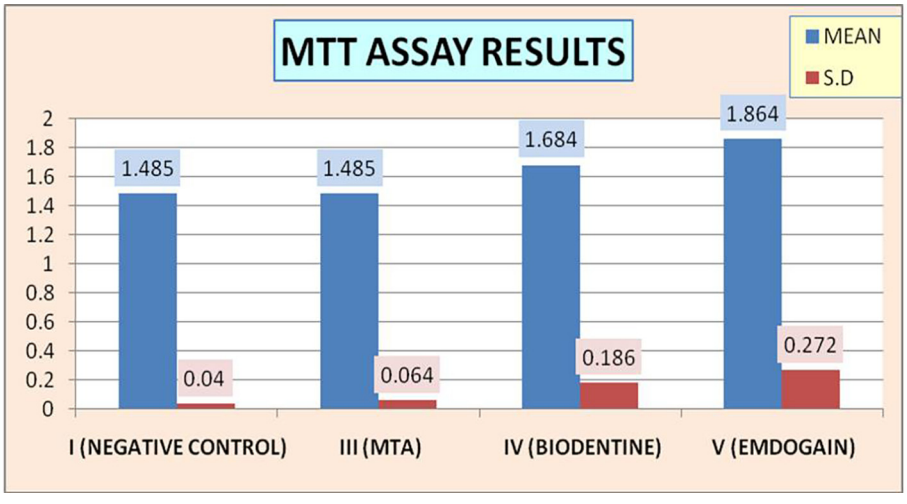

Fig. 4 Graph showing MTT assay results of SHED with different bioinductive materials.

Table 3 Results of Alizarin red staining on SHEDs with different bioinductive materials.

Groups	Mean	Std Dev (S.D)	Anova F Test	p value/significance
Group I (Negative control)	0.937	0.033	F = 96.952	$p < 0.001^{**}$
Group II (Positive Control)	1.814	0.042		
Group III (MTA)	1.492	0.005		
Group IV (Biodentine)	1.562	0.054		
Group V (Emdogain)	1.728	0.110		

$p > 0.05$ – not significant, $*p < 0.05$ – significant.
** $p < 0.001$ – highly significant.

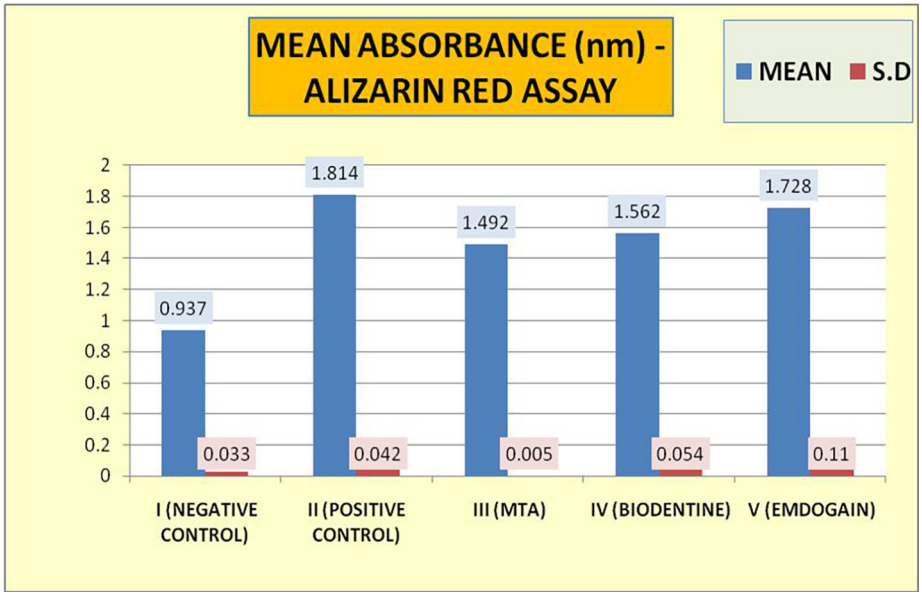

Fig. 5 Graph showing Alizarin Red S staining results with different bioinductive materials.

and Fig. 5 reveal that at the end of 14 days; the highest amount of osteoinductive/odontogenic activity was seen in EMD group (absorbance: 1.728 ± 0.110) ($p < 0.001$); followed by Biodentine (absorbance: 1.562 ± 0.054) and MTA (absorbance: 1.492 ± 0.005) as compared to positive control (cells maintained in odontogenic media- 1.814 ± 0.042) and negative control (cells maintained in regular media- 0.937 ± 0.033).

6. Discussion

The traditional pulp capping/regenerative endodontic procedures advocate pulp- dentine wound to be treated with calcium hydroxide in order to protect injured tissue, induce the formation of reparative dentine and maintain pulp vitality (Al-Hezaimi et al., 2013). With advances in material science, latest bioinductive materials showed promising results in pulp therapy of primary as well as permanent teeth. Regenerative endodontic procedures along with principles of tissue engineering aim to regenerate damaged dental structures, including dentin and root as well as the pulp dentin complex helping restore tissue functions. To achieve this, it becomes necessary for clinicians to understand the nature and behavior of stem cells and their regeneration potential. The therapies utilizing principles of regenerative endodontics connotes application of bioinductive materials in direct contact with wounded pulp-dentin comlex. This exerts adequate bioactivity promoting undifferentiated mesenchymal stem cells differentiating to either odontoblastoid, osteoblastoid or cementoblastoid cells to heal it (Al-Hezaimi et al., 2013; Nowicka et al., 2013).

In vitro cytotoxicity and functional differentiation studies with human dental pulp cells represent a useful apparatus for analyzing the reaction, behavior and fate of cells when placed in direct contact with test materials promoting animal free methods for experiments (Tomás-Catalá et al., 2018; Vinken and Blaauboer, 2017). In the present study, it was decided to use SHED as these cells are directly originated from neural crest cells and exhibit less maturity. They have higher proliferation rate than any other source of stem cells. The potential of SHED to differentiate into other type of cells are more than dental pulp stem cells (DPSC) and bone marrow mesenchymal stem cells (BMMSC). These cells can differentiate into multiple types of cells such as odontoblast, osteoblast, chondroblast, adipocytes and neurons too. The population double (PD) value of SHED is more than 140, which is relatively much better than DPS (60–120 PD) and BMMSC (30–50 PD) (Zainuri et al., 2018). SHED were successfully used as model cell line in this study unlike other studies (de Menezes et al., 2009; Collado-González et al., 2017), to test

functional differentiation properties of bioinductive materials like MTA, Biodentine and Emdogain, through direct contact method. Such cells are multipotent in nature and capable of self-renewal through multi-lineage differentiation. The tissues derived from these cells include mesenchymal components of teeth including odontoblasts, pulp, apical vasculature and periodontal ligament (Abbas and Sharpe, 2008). Though these cells belong to heterogeneous population, they share common molecular characteristics with neural crest cells and stem cells in vitro (Shi et al., 2005). These stem cells show positive indicators for antibodies against human MSC (mesenchymal stem cells) with specific surface markers like CD73, CD90, CD 105 (Buttke et al., 1993).

In the present study, stem cells suspended in regular growth medium that contained DMEM supplemented with 10% fetal bovine serum (FSB), 2 mmol L^{-1} of L-glutamine, was used as negative control group. Stem cells suspended in "osteoinduction medium" a specifically conditioned medium was used as positive control group. The osteoinduction medium used in this study comprised of DMEM (Dulbecco's Modified Eagle's Medium), 20% FBS, 50 μg/ml ascorbic acid, 50 ηmol/L β glycerol phosphate and 10–8 mol/L dexamethasone (Guven et al., 2013). The cells were suspended in media containing test materials rather than incubating them directly with test materials. It was done to reduce cytotoxic effects of test materials associated with direct contact with the stem cells. This experimental set up simulates clinical situations, where the dental pulp cells reside in close vicinity to blood vessels and nerve fibers receiving the solubalized factors released from the capping materials (Vishnubhalaji et al., 2012). When the SHED were cultured in regular culture medium, only viable cells eluted, to indentify the functional differentiation potential of SHED than in osteoindution medium.

MTT assay was carried out to analyze effects of bioinductive materials on viability and proliferation rate of SHED. MTT is a tetrazolium salt based colorimetric assay that works on a principle of measuring metabolic activity of mitochondrial enzymes from viable cells. In this assay, there is reduction of yellow 3-(4, 5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide by mitochondrial succinate dehydrogenase. When MTT enters the cells, it passes into the mitochondria where it is reduced to an insoluble, dark purple formazan product (Maeda et al., 2015). During the processing, cells are solubalized with an organic solvent (DMSO, Iso proanol, 3-(4,5-dime thylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) releasing formazan. This solubilized formazan product with reagent is measured using spectrophotometer. In this way, the cytotoxicity of the material was confirmed through MTT assay, which is suggestive of the metabolic activity of stem cells in culture media, measured along the stipulated span of time. As the value of absorbance increases with time, it can be interpreted as increased level of cellular activity and viability, thus an indirect measurement of cell proliferation. Amongst the material tested in the present study, highest amount of cell viability was observed with Emdogain followed by Biodentine and least with MTA. However as per the results obtained, all the test materials exhibited significant biocompatibility at given concentrations and maintained viability of majority of the stem cells. Thus, our results are in agreement with previous studies that reported the similar findings (Collado-González et al., 2017; Tran et al., 2012).

Alizarin red S (ARS) staining is an anthroquinone dye based calorimetric and functionality assay, used widely to evaluate calcium deposits in cell culture (Guven et al., 2013). The ARS staining is quite versatile because the dye can be extracted from stained monolayer of cells and readily assayed. This assay provides a sensitive tool for recovery and semi-quantification of ARS in a stained monolayer cell (Paranjpe et al., 2011). Degree of mineralization is assessed by extracting calcified mineral at low pH, neutralizing it with ammonium hydroxide and detecting through colorimetric analysis at 405 nM in 96-well format (Wang et al., 2011). This assay is more sensitive than other assays like cetylpyridinium chloride (CPC) extraction method, as it can detect small traces of calcific deposits in the cells. The destained ARS dye ranging from 30uM- 4 mM shows a linear relationship with the absorbance at 405 nM, which makes sample dilution prior to measurement unnecessary. In this study, this quantification assay was applied to the osteogenic induction medium of SHED. Cells in each group were cultured in different osteogenic differentiation media for 18 days, fixed for ARS staining and quantified for mineral deposit using the kit. It was observed that Emdogain promoted highest calcified nodule formation, followed by Biodentine and least by MTA. From Emdogain group, 84% of cells showed functional differentiation into calcified tissue forming cells followed by Biodentine treated cell (showing 66% proliferation) and least with MTA treated cells (59% proliferation). Positive control group showed 93% cell differentiation. The highest amount of calcified nodule formation amongst the tested material was seen with Emdogain revealing its higher bioactivity than others. Min et al. (2009) measured calcific nodule formation in odontoblast like cells through alizarin red s staining. It was observed that odontoblast like cells inducing dentinogenesis exhibit DMP-1, DSPP, OCN and MEPE gene expression. Similar type of gene expression is shared by osteogenic cells differentiating in bone marrow, which also forms calcific nodules. These results confirmed positive induction potential and immunophenotyping for genetic expression of DMP-1, DSPP, OCN, and MEPE odontogenic markers as compared to MTA (Ishizaki et al., 2003; Kaida et al., 2008). Seo et al. (2013) demonstrated positive role of MTA as bioinductive material for odontogenic differentiation on hDPSCs. However, in the present study, SHED were used and results of the study are in similar inclination to the above one. Chang et al. (2014) reported that biodentine can be used as potential pulp capping agent with favorable compatibility, triggering similar levels of inflammatory response but later odontoblastic differentiation in comparison to MTA in hDPSC. The results obtained in present study are in agreement to their results. Nowicka et al. (2013) reported that both MTA and Biodentine are biocompatible and exhibit promising bioinductive (odontogenic) differentiation potential. In another study, when SHEDs were treated with MTA, calcium hydroxide and Biodentine, the highest progressive increase in DMP-1 gene expression was seen with MTA, followed by calcium hydroxide and least with Biodentine (Araújo et al., 2018), and results of present study are in partial contradiction to results of previous one. Min et al. (2009) and Wang et al. (2018) reported the synergistic effect of Emdogain and MTA to differentiate SHED to odontoblast like cells and enhance mineralization. Kémoun et al. (2007) observed that Emdogain enhances calcium deposition as well as elevates several

mineralization markers. Duan et al. (2011) reported that Emdogain enhances osteogenic differentiation of induced pluripotent stem cell and results of the present study are also in similar agreement to the above study reports.

Stem cells derived from dental pulp and periodontal ligament demonstrates expression of growth factors like BMP, FGF, and TGF that regulates dentin, cementum and bone formation (Shi et al., 2005). When a suitable and biocompatible pulp capping agent is applied onto a wounded pulp in a favorable environment, a series of processes is initiated that stimulate dental pulp stem cells differentiate into odontoblastoid cells accelerating hard tissue deposition on vital pulps. This process was termed as 'reparative dentinogenesis' (Guven et al., 2013). Thus, it is obviously evident that, though cells possess integral growth factors, materials harboring bioinductive molecules are equally important to induce and enhance stem cell differentiation into odontoblast like cells (Lin & Rosenberg, 2011). These results support our proposition that EMD has highest contribution for expression of odontoblastic differentiation markers. Thus, in accordance with the results of previous studies our results prove that EMD can be used through direct contact method as direct pulp capping or pulpotomy agent to enhance healing of pulp from primary teeth. Apart from biocompatibility, this study also tested the cell differentiation ability to provide data on safe use of bioinductive material for pulp capping procedures as well as regenerative therapy.

7. Conclusion

Based on the results of present study, it can be concluded, that all the tested materials exhibited satisfactory biocompatibility to maintain cell viability and encouraged proliferation and functional differentiation of SHED. Out of three biomaterials tested, Emdogain showed highest cell viability, proliferation and osteoinduction/ odontoinduction capacity followed by biodentine and MTA respectively. However, further in-vivo experimental studies should be carried out in future to investigate their efficacies.

8. Finding source and Sponsorship

Nil.

Declaration of Competing Interest

None.

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