Biocompatibility, Bioactivity and Gene Expression Analysis of SHEDS Cultured in Various Calcium Silicate Based Cements: A Systematic Review and Meta-Analysis of *in Vitro* Studies

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***Aim**: To assess the biocompatibility, bioactivity and gene expression analysis of SHEDs incubated in various Calcium Silicate Based Cements. **Study design**: Following PRISMA statement, a search was carried out in the electronic databases–PubMed, Scopus, Embase, Google Scholar, JSTOR, and DOAJ from January 2000 to 31 May 2021. In vitro trials examining the response of SHEDs to the treatment with CSCs were eligible. **Results:** 10 trials were included after the selection process. These trials involved the assessment of cell viability, cell migration, cell adhesion, mineralization potential and gene expression analysis of SHEDs cultured in MTA, Biodentine, EndoCem Zr, RetroMTA, TheraCal & iRoot BP plus. **Conclusion**: MTA, Biodentine, EndoCem Zr, RetroMTA and iRoot BP showed adequate biocompatibility, bioactivity and genetic expression towards SHEDs, to support their clinical use in vital pulp treatment of primary teeth.*

***Keywords:** SHED, CSCs, biocompatibility, bioactivity, gene expression analysis*

INTRODUCTION

In primary teeth, the main goals of Vital Pulp Therapy (VPT) are to heal the reversible pulpal lesions and preserve pulp vitality/function. The efficacy of VPT is influenced by several factors, including intensity of inflammation and infection, adequate vascularization, achieving hemostasis, antibacterial property, sterilization of the exposed site, adequate coronal seal and cytocompatibility of pulp protecting agents. The presence of appropriate blood supply is mandatory for active formation/function of the odontoblasts, which plays the most important role in the success of VPT.[1-4] Calcium hydroxide was preferred earlier, in vital pulp procedures because it had antimicrobial qualities and it encouraged dentin bridge development. Mineral Trioxide Aggregate (MTA), on the other hand, was the foremost Calcium Silicate based Cement (CSC) to be widely preferred in such procedures, and has proven to be more successful than calcium hydroxide.[5,6]

CSCs have been extensively researched for vital pulp procedures throughout the previous two decades. However, due to long setting time, tooth discoloration, difficult handling and high cost of MTA, the search for the ideal CSCs are ongoing. At present, new varieties of MTA such as EndoCem Zr and RetroMTA, BioAggregate, Biodentine, iRoot BP Plus, EndoSequence Root Repair Material (ERRM), Calcium Enriched Matrix (CEM), Theracal LC and others are commercially available CSCs.

If the material can induce a positive response from the host, it is regarded as being bioactive, provided the bioactive material also elicits a biological response near the interface and encourages bond formation between the material and the tissue.[7] The bioactivity

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concept can be regarded to be closely linked to biointeractivity, i.e. information exchanged inside a biological system. This also suggests that a bioactive material should be able to induce a chemical reaction within the body fluids while being compatible with the tissue's repair processes.[8]

On the other hand, Stem cells derived from Human Exfoliated Deciduous teeth (SHED) possess immunosuppressive properties., acquired via dental pulp explants or through isolation of pulp tissue from exfoliated deciduous teeth.[9] Even though isolation of SHED is based on the pulp tissue, their differentiation ability is not only restricted to odontoblasts but also extends to various cell forms, including adipocytes, osteoblasts, neurons and endothelial cells.[10]

Since the introduction of a variety of MTA-like and CSCs in the market, a substantial amount of trials have been performed, concentrating on the response of these cements to SHEDs.[11,12] Literature search confirms the CSCs biocompatibility, odontogenic features and bioactivity when cultured in dental pulp stem cells isolated from permanent dentition.[13,14] Based on SHEDs proven mesenchymal origin, multipotentiality, and odontogenic differential potential, there is a need for an updated critical evaluation of their effects when incubated in various CSCs.

Hence the aim of this review was to investigate the biocompatibility, bioactivity and gene expression analysis of SHEDs incubated in various CSCs.

MATERIALS AND METHOD

Protocol registration

The main items used to describe systematic review and meta-analyses (the Preferred Reporting Items for Systematic Reviews and Meta-Analyses)[15] were referred to guide the study protocol. Furthermore, this study was registered in an international database of prospectively registered systematic reviews in health and social care, known as PROSPERO (CRD42020220599).

Review question

The research question was developed using the population (P), intervention (I), comparison (C), outcome (0), and study design framework. What is the response of SHEDs (P), incubated in culture media conditioned with various CSCs (I), when compared to unconditioned culture media (C), for cell viability & proliferation, mineralization, wound healing and gene expression analysis (O) in, *in vitro* studies (S)?

Search strategy

Specific search strategies were developed and performed in the electronic *databases*–PubMed, Scopus, Embase, Google Scholar, JSTOR, and DOAJ. In addition, data reported in the present review were primarily intervened based on the timeline selected from January 2000 to 31 May 2021. The whole framework was designed substantially based on key terms selected by their relevance to our investigations (Table 1). Besides, key terms were assembled by the utility of "AND" and "OR" Boolean operators. The search strategies used in this analysis along with the findings obtained from diverse databases are tabulated in Table 1.

Inclusion criteria

In vitro assays, trials assessing the effects of SHEDs on various CSCs for biocompatibility and bioactivity, studies assessing the gene expression analysis of SHEDs cultured in various CSCs and studies published in English.

Exclusion criteria

Studies assessing clinical evaluation; stem cell extracted from the animal; sealers preparation and characterization of cement; unavailable full articles (In press) and articles on the side-line topic were excluded. In addition, we also excluded thesis, books, systemic reviews, and reviews. Manuscripts with study designs other than in vitro assays, trials investigating stem cells other than SHEDs and/or trials studying the biological response of cements other than CSCs, were also excluded.

Study selection and data extraction process

Two independent researchers (MV, DU) assessed the title and abstract of the selected papers based on the inclusion and exclusion criteria. The reviewers examined each publication individually and retrieved data using a data extraction form designed expressly for this study. The following information was included on this form–author's name, year of publication, type of CSC used, type of culture media, type of assay, incubation time and results. Any discrepancies between the two reviewers were addressed by consulting a third reviewer (SA).

Quality assessment of included studies

The selected trials were individually reviewed for underlying methodological risk of bias using the 'Modified CONSORT checklist of items for reporting *in vitro* research of dental materials[16], recording the compliance with each of the criteria or items covered in the checklist.

RESULTS

Study selection process

From the electronic databases, a total of 344 studies were found. Following the removal of research based on title and abstract screening, 25 papers were left for full-text evaluation. Following a thorough review, further 17 studies were found to be ineligible for the following reasons: isolation of other types of stem cells (n=10), *in vivo* studies (n=3) and studies involving dental cement other than CSCs (n=4). Finally, the systematic review involved ten studies, two of which were selected from the references. Figure1 explains the search procedure used to find the selected studies.

Characteristics of included studies

The methodology used by the selected trials to examine cell proliferation, apoptosis and necrosis, cell migration, analysis of matrix calcium deposition, and gene expression analysis of SHEDs cultured in or without various CSCs are summarized in Table II.

The present review included trials wherein SHEDs were derived from healthy children ranging in age from 3-12 yrs old for the in vitro biological tests. SHEDs were favored for trials in these studies at the 3rd -6th passages, with the highest SHEDs at the 10th passage[18] and the lowest at the 2nd passage.[22] The various CSCs analyzed were Mineral Trioxide Aggregate (MTA) used in six trials[17,20,21,23,25,26], Biodentine used in seven trials[17,18,21,22,24-26], Theracal in two trials[17,26] and RetroMTA[19], EndoCem Zr[19] and iRoot BP Plus[24] used in one trial.

Figure 1: PRISMA flow diagram.

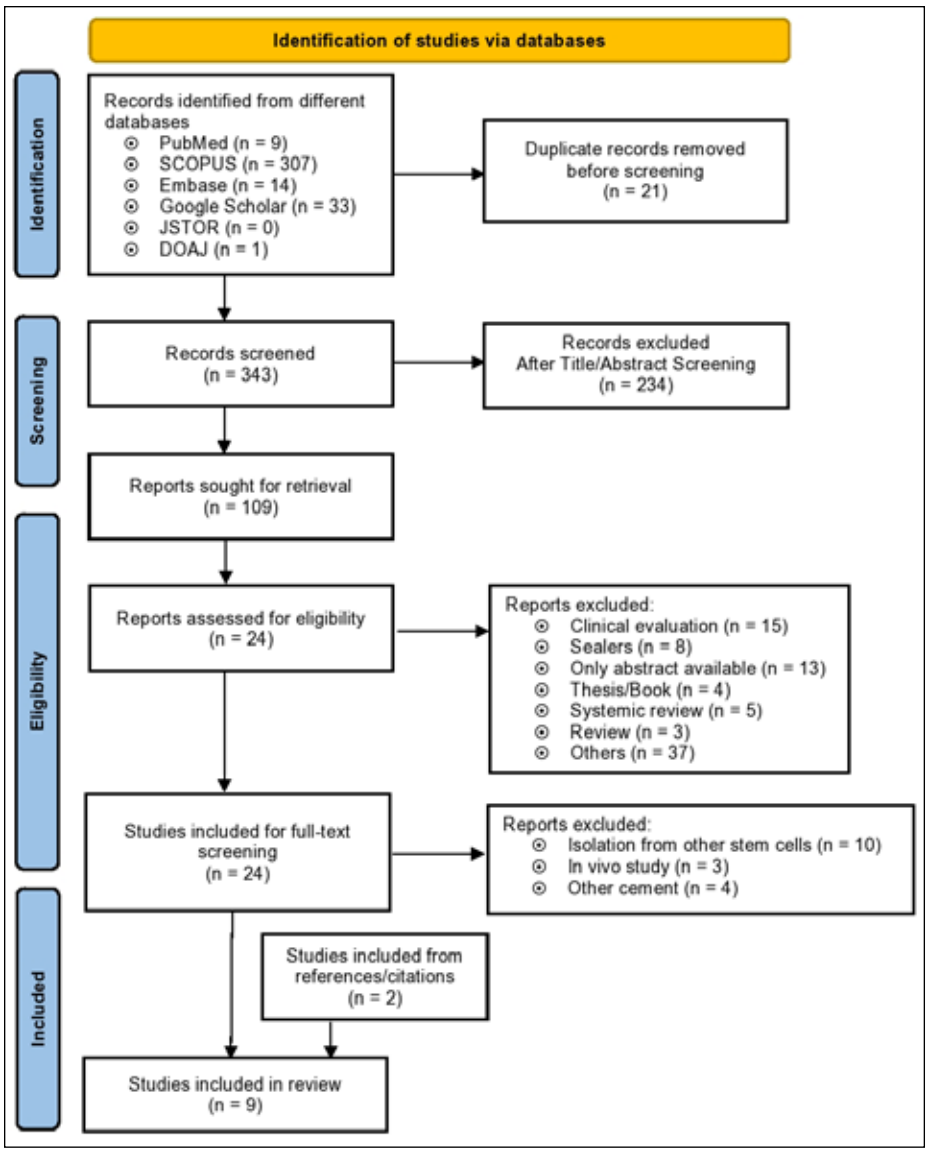


Table 1: Search strategy

S. No.	Search Strategy	PubMed	Scopus	Embase	Google Scholar	JSTOR	DOAJ
#1	((((calcium silicate) OR (calcium orthosilicate)) OR (calcium silicate hydrate)) OR (Mineral trioxide aggregate)	4412	555,344	58,498	10,700	3257	93
#2	(stem cells from human exfoliated deciduous teeth) OR (SHED)	11,633	5745	425,474	12,200	353,258	124
#3	((((((cytocompatibility) OR (bioactivity)) OR (odontogenic differentiation)) OR (biomineralization)) OR (cell differentiation)) OR (gene expression)	1,605,890	11,260,000	1,000,000	18,000	311,496	302,293
#4	#1 and #2 and #3	9	307	14	33	0	1

Biocompatibility

Following assay were performed to analyze SHEDs viability-3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay[17,21,22,24-26], Cell Counting Kit -A (CCK8) assay[18,20],EZ-Cytox Cell assay[19] and WST-1 cell proliferation assay.[23] SHEDs cultured in Biodentine observed greater cell viability than MTA in three trials[17,21,26], whereas Araujo LB *et al* [25] reported MTA with more cell viability than Biodentine. A trial conducted by Yun J *et al* [19] concluded that SHEDs cultured in EndoCem Zr showed more cell viability than RetroMTA. SHEDs incubated in iRoot BP, exhibited much greater cell migration and proliferation rates than MTA, although both were much greater than the negative control.[20]

In five trials, wound healing, transwell migration, and other comparable tests were used to measure cell migration.[20,23-26]. Tsai C *et al* [23] and Collado G *et al* [26] preferred Annexin V/7-AAD dual staining to examine necrosis and apoptosis. SHEDs cultured in MTA[20,21,25,26] and Biodentine[21,23,25,26] reported a greater cell migration when compared to negative control. Araujo LB *et al* [25] reported SHEDs incubated in Biodentine with greater wound healing ability through a sulforhodamine -B assay. However, the same trial also observed that MTA and Biodentine with lower cell migration and cell proliferation when compared to positive control.

In the present review, four trials also confirmed that SHEDs promoted cell adhesion and/or cell growth and spreading through immunofluorescence or scanning electron microscope.[20,22,24,26]

Bioactivity

Five trials preferred the Alizarin Red staining method to analyze the degree of mineralization of SHEDs cultured in various CSCs[18,20,21,22,26]. These trials revealed high potential for mineralization in SHEDs cultured in MTA[20,21], Biodentine[18,22,26] and iRoot[20]. Wang J *et al* [20] reported a high potential for mineralization in SHEDs cultured in Biodentine and iRoot BP when compared to MTA. The trial also concluded that iRoot BP had a high cellular activity when compared to MTA and control through alkaline phosphatase assay.

3.2.3 Gene expression analysis (Table III)

Nam OK *et al* [17] conducted a trial to analyze the effects of CSCs on gene expression changes in SHEDs. The SHEDs cultured in MTA reported a decrease in CCL-5 and an increase in IL-18 expression. Whereas, SHEDs cultured in Biodentine and Theracal increased the expression of CXCL6, suggesting inflamed gingival tissue or odontoblastic layer of carious teeth. Interestingly, enhanced Wnt/β-catenin and IFN-γ signaling on treatment with Biodentine and Theracal indicated the probable cause of reparative dentin formation.

In another study, researchers scrutinized the osteo/odontogenic gene expression in non-inductive and inductive SHED culture on treatment with various concentrations of Biodentine. Genes, namely ALP, BGLAP, DSPP, MSX2 upregulated and MSX2 downregulated in the non-inductive environment. Whereas RUNX2 upregulated in an inductive environment. Hence, suggesting chances of fluctuation in ion release and biomineralization.[22]

Table II: Descriptive data from selected studies.

SL. No.	Author Details	Type of CSC used	Cell variant	Type of culture media	Type of Assay	Duration	*/Result
1	Nam OK et al. 2020 [17]	Mineral Trioxide Aggregate, Biodentine & TheraCal LC.	SHEDs.	α- minimum essential medium + 10% FBS + 100 U/mL penicillin + 100 mg/mL streptomycin.	**Cell Viability (MTT assay)**	24-hrs & 72-hrs.	TheraCal LC > Biodentine > MTA. p = > 0.05.
					Gene expression analysis (qRT-PCR).	72-hrs.	The scatter plot analysis revealed overlapping of gene expressions between MTA, Biodentine & TheraCal treated cells.
2	Jung Y et al. 2020 [18]	Biodentine.	1.SHEDs (5- Yrs old, Male). 2.< 10th passage.	15% FBS + 2 mM GlutaMAX + 1 mM L-ascorbic acid + 1% penicillin/ streptomycin.	**Cell Viability (CCK-8 Assay).**	3-min,6-min,12-min & 24-hrs.	50% > 25% > 12.5% > control. p = < 0.05.
					Odontogenic differentiation (ARS).	9-days.	12 min, 6 min, 3 min > odontogenic medium > growth medium (12.5%). p = < 0.05.
						15-days.	12 min > 6 min > 3 min > odontogenic medium > growth. p = <0.05.
3	Yun J et al. 2019 [19]	RetroMTA, EZ-Seal, and EndoCem Zr.	SHEDs.	CEFOgro™ DPSC medium (CEFO).	**SHEDs characterization.**		SHEDs expressed high levels of CD 29, CD105, CD 146, STRO-1 and low levels of CD 34.
					EZ-Cytox Cell Assay (MTS Assay).	3-days.	EndoCem Zr > EZ > RetroMTA > Control. p = 0.043.

Table II: Descriptive data from selected studies (continued)

SL. No.	Author Details	Type of CSC used	Cell variant	Type of culture media	Type of Assay	Duration	*/Result
4	Wang J et al. 2019 [20]	MTA & iRoot BP Plus .	1SHEDs (6-10yrs old).	α – MEM + 10% FBS + 100 U/mL of penicillin + 100 mg/mL of streptomycin (for negative control).	**Cell viability assay (CCK8).**	1-day, 3-days, 5-days 7-days.	7-days. iRoot BP, MTA > negative control ($p<0.05$) .3,5-days iRoot BP >MTA> negative control ($p<0.05$).
			2. 4th 6th passage.		**Transwell migration assay.**	24-hrs.	iRoot BP plus and MTA promoted wound healing.
					Wound healing assay	24-hrs.	iRoot BP >MTA> negative control ($p<0.05$).
					Immunofluorescence staining	1-day, 3-days &5-days.	iRoot BP plus and MTA promote adhesion.
					ALP activity assay.	7-days & 14- days.	iRoot BP >MTA> negative control ($p<0.05$).
					Alizarin Rd staining.	21-days.	iRoot BP >MTA> negative control ($p<0.05$).
5	Dahake PT et al. 2019 [21]	MTA & Biodentine (1mg/mL).	1.SHEDs (8 - 12 yrs old). 2. 5th passage.	DMEM + 10% FBS, 2 mmol/L of L-glutamine + 1% of penicillin, amphotericin -B & streptomycin (for negative control).	**SHEDs characterization.**		SHEDs expressed high levels of CD 73, CD 90 & CD 105.
					Cell Viability assay (MTT Assay).	7-days.	Biodentine > MTA > control. $p = < 0.001$.
				DMEM + 20% FBS + 50 µg/ml ascorbic acid + 50 ηgmol/L β-glycerol phosphate + 10–8 mol/L dexamethasone (for positive control).	Odontogenic differentiation (Alizarin red S staining).	14-days.	Biodentine > MTA > control. $p = < 0.001$.
6	Athanasiadou E et al. 2018 [22]	Biodentine. (1:1, 1:2,1:4,1:8,1:16, 1:32, 1:64 & 1:128 diluted eluates).	1.SHEDs (3–10 yrs old). 2. 2nd – 6th passage.	α- Minimal essential medium (MEM) + 15% FBS + 100 µM L-ascorbic acid phosphate + 100 units/ml penicillin + 100 mg/mL streptomycin + 0.25 mg/ml Amphotericin B (for negative control). 0.01 mM dexamethasone sodium phosphate + 1.8 mM KH_2PO_4 + 5 mM β-glycerol phosphate (for positive control).	**SHEDs characterization.**		SHEDs expressed high levels for CD 90/Thy-1, CD 146,CD 49f/a6-integrin, CD 81), endothelial (CD 105) and neural (Nestin) markers. Lower expression was observed for STRO-1, CD 24, CD 31, CD 34 & the embryonic markers Nanog and Oct3/4.
					Cell Viability assay (MTT Assay).	24-hrs, 72-hrs &120-hrs.	1:16, 1:32, 1:64 Biodentine > control $p = < 0.05$.
					Cell viability test. (Live/dead fluorescence staining).		>90% remained viable.
					Odontogenic differentiation (Alizarin Red S staining).	14-days.	1:4, 1:8, 1:16, 1:32 Biodentine > control. Control > 1:1, 1:2, 1:4, 1:8, 1:16 Biodentine. $p = < 0.05$.
					Genetic expression analysis (qRT-PCR).	7-days & 14-days.	Significant up-regulation of DSPP and Runx2 at higher dilutions and a peak in expression of BMP-2, BGLAP and MSX-2 at 1:8 dilution on day-7.

Table II: Descriptive data from selected studies (continued)

SL. No.	Author Details	Type of CSC used	Cell variant	Type of culture media	Type of Assay	Duration	*/Result
7	Tsai AI et al. 2018 [23]	ProRoot MTA.	SHED (5-7 yrs old). 3rd – 4th passage.	α- MEM +15% FBS + 100 μM L-ascorbic acid phosphate + 2 mM L-glutamine + 100 units of Anti-biotic-Antimycotic (for negative control).	**SHEDs characterization.**		SHEDs were positive for the expressions of CD 105, CD 90, CD 73, CD 44 and CD 29.
					Cell proliferation assay (WST-1 assay).	1-day, 2-days & 3-days.	Control (direct contact & indirect contact))> MTA. p < 0.0001(1-day) ; p < 0.01 (2-days) ; p < 0.05 (3-days).
					Detection of apoptosis (fluorescent TUNEL assay).	2-days.	MTA (direct contact) > MTA (indirect contact).
					Apoptosis (Annexin – V/7-AAD stain).	2-days.	Positive staining.
8	Hasweh N et al 2018 [24]	Biodentine (0.02, 0.2, 2, 20 mg/mL).	SHED (5-6 yrs old). 3rd passage.	α Minimal essential medium + 2 mM Lglutamine + 100 units/ml penicillin + 100 mg/ml strepto-mycin + 0.25 mg/ml amphotericin B + 4000 unit/ ml heparin + 5% Plasma Lysate.	**SHEDs characterization.**		SHEDs were positive for CD 90 (99%), CD105 (92%), CD 73 (92%), and CD 44 (87%) and negative for CD 34, CD 45, HLA-DR & CD-11b (4%).
					Cell viability assay (MTT Assay).	2-days, 4-days, 6-days.	0.02, 0.2, 2 mg/mL > 20mg/mL. p < 0.0001.
					Wound healing Assay	24-hrs	0.2 and 0.02 mg/mL > 2 mg/mL.
					Transwell migration assay	24-hrs	0.02,0.2,2mg/mL > control. p <0.0037.
					Cell Adhesion assay	1-hr	No significant change.
9	Araujo LB et al 2018 [25]	MTA, and Biodentine. (1mg/mL) .	SHEDs (7-8 yrs old) 4th -8th passage.	α - MEM medium + 10% FBS + 1% penicillin and streptomycin (for negative control) α - MEM medium + 20% FBS + 1% penicillin and streptomycin (for positive control).	**MTT Assay**	1-day, 3-days, 5-days,7-days.	3,5-days: Positive control > MTA > BD > negative control. p < 0.05. 7-days: MTA > BD > negative control. p < 0.05.
					Determination of cell density (SRB Assay)	1-day, 3-days, 5-days,7-days.	1,3,5,7-days: Postive control > MTA, Biodentine. p < 0.05. 3,5-days: Biodentine> MTA p < 0.05.
					Cell Migration assay.	Overnight	Biodentine, MTA > negative control. p < 0.005.
					Gene expression analysis (qRT-PCR).	1-day, 7-days, 14-days, 21-days.	A greater levels of DMP-1 gene was expressed in MTA from 7th to 21st day.

Table II: Descriptive data from selected studies (continued)

SL. No.	Author Details	Type of CSC used	Cell variant	Type of culture media	Type of Assay	Duration	*/Result
10	Collado G et al 2018 [26]	Biodentine, MTA, Theracal LC and IRM (1:1,1:2, 1:4)	SHED (6-9 year)	DMEM medium serum-free + penicillin/ streptomycin. (for negative control).	**SHEDs characterization.**		SHEDs reported positive expression for CD 73, CD 105 & CD 90.(>95%).
					MTT Assay	24-hrs, 48-hrs & 72-hrs.	1.MTA> negative control : 48,72-hrs ($p<0.01$). 2.Biodentine > negative control : 48,72-hrs ($p<0.001$). 3.Biodentine > MTA :48,72-hrs ($p<0.01$).
					Apoptosis (Annexin – V/7-AAD stain).	72-hrs	> 87 % viable cell in Biodentine and MTA Angelus.
					Cell Migration assay.	24-hrs & 48- hrs	Biodentine > negative control; MTA > negative control. $p < 0.001$.
					Scanning electron microscopy.	3-days.	A high-quality cell growth and spreading observed with Biodentine & MTA.
					Alizarin Red Staining	7-days,14-days & 21-days.	Biodentine > negative control 7-days ($p < 0.01$) 14-days ($p<0.05$) 21-days ($p<0.001$).

Table III: Comprehensive details of gene expression analysis from the selected studies.

Studies	Calcium silicate based cement	Gene expression analysis		
		Gene	**Description**	**Result**
Nam OK et al. 2020 [17]	MTA, Biodentine, TheraCal.	CCL5	C-C motif chemokine ligand 5	BD, TC ↑
		IL-18	Interleukin 18	MTA ↑
		ICOSLG	Inducible T-cell co-stimulator ligand	MTA ↑
		MAP4K1	Mitogen-activated protein kinase kinase kinase kinase 1	BD, TC ↑
		LTB	Lymphotoxin beta	BD, TC ↑
		KLRD1	Killer cell lectin-like receptor D1	BD ↑
		CXCL6	C-X-C motif chemokine ligand 6	BD, TC ↑
		C3AR1	Complement component 3a receptor 1	BD, TC ↑
		SELL	Selectin L	BD, TC ↑

Table III: Comprehensive details of gene expression analysis from the selected studies (continued)

Studies	Calcium silicate based cement	Gene expression analysis		
		Gene	Description	Result
				Non-inductive: 1:1 < 1:2 > 1:4 < 1:8 > 1:16 < 1:32; 1:1 > 1:2 < 1:4 > 1:8 < 1:16 > 1:32 (< 0.01)
		ALP	Alkaline phosphatase	Inductive: 1:1 < 1:2 > 1:4 < 1:8 > 1:16 < 1:32; 1:1-1:32 (< 0.01) ↑
				Non-inductive: 1:1 - 1:32 (< 0.01) ↑
		BMP-2	Bone Morphogenetic Protein 2	Inductive: 1:1, 1:2 and 1:8; 1:2 (< 0.01) ↑
				Non-inductive: 1:2, 1:8 (p < 0.05); 1:1, 1:2 (p < 0.01) and 1:4 (p < 0.05)
		BGLAP	Bone Gamma-Carboxyglutamate Protein	Inductive: 1:1 and 1:8 (p < 0.01); ½ and 1:4 (p < 0.05) ↓
				Non-inductive: 1:4, 1:8 and 1:16 (p < 0.01) ↑
		DSPP	Dentin sialophosphoprotein	Inductive: 1:32 (p < 0.05); 1:16 (p < 0.01) ↑
				Non-inductive: 1:4 to 1:32 (p < 0.01)
		MSX2	Msh Homeobox 2	Inductive: 1:2 -1:32 ↓
				Non-inductive: 1:16 (p < 0.05) and 1:32 (p < 0.01) ↓
Athanasiadou et al 2018[22]	Biodentine	RUNX2	Runt-related transcription factor 2	Inductive: 1:16 (p < 0.01) and 1:32 (p < 0.01) ↑
Araujo LB et al 2018 [25]	MTA, calcium hydroxide and Biodentine.	DMP-1	Dentin Matrix Protein-1	MTA > CH > BD > Control ↑
		GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	MTA, CH, BD, Control ↑

Table IV : Quality assessment of included studies.[16]

Sl No.	Studies	1	2	2a	3	4	5	6	7	8	9	10	11	12	13	14
1	Nam OK et al. 2020 [17]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
2	Jung Y et al. 2020 [18]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	N
3	Yun J et al. 2019 [19]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	N	Y
4	Wang J et al. 2019 [20]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	N
5	Dahake PT et al. 2019 [21]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	N	N	N
6	Athanasiadou et al 2018[22]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	Y
7	Tsai AI et al 2018[23]	Y	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y
8	Hasweh N et al 2018 [24]	Y	Y	Y	Y	Y	Y	N	N	N	N	Y	Y	N	Y	Y
9	Araujo LB et al 2018 [25]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	N
10	Collado-Gonzalez et al 2017 [26]	Y	Y	Y	Y	Y	N	N	N	N	N	Y	Y	Y	Y	N

Y: reported on the article; N: not reported on the article

Araujo L *et al* [25] reported a progressive increase in expression of gene GAPDH in SHEDs cultured in MTA, Biodentine and calcium hydroxide. While upregulation of DMP-1 odontogenic marker was significantly reported in SHEDs cultured in MTA, whereas with the passage of time SHEDs incubated in Biodentine and calcium hydroxide treated also expressed the same.

Quality assessment of included studies

Table IV shows the findings of the quality assessment using a modified CONSORT checklist based on standards for reporting preclinical in vitro studies on dental materials[16].The abstract, introduction, intervention, hypothesis, and outcomes were all present in 100% of the selected studies. There was no provision for randomization, an allocation concealment strategy, implementation, or blinding in any of the experiments. While statistical tools were used in 90% of the investigations. Around 20% of the trials, cited sample size, whereas around 80% worked on mentioning work constraints. 50% of the trials were completed following protocol, with 70% of studies benefiting from financing.

Assessment of meta-analysis

A comprehensive treatment of weighted random studies over data extracted from selected studies were observed (Figures 2, 3). These analyses reported statistically significant positive effects on SHEDs cultured in Biodentine and MTA. Furthermore, a significant and nil heterogeneity, I^2 value was reported in Biodentine and MTA respectively. Thus, making the studies feasible for meta-analysis.[27] In this analysis, we observed values of Z as 1.31 (Biodentine) and 1.67 (MTA) at $\alpha = 0.05$, whereas the reported critical value for the same was 1.645. Thus, test statistic accepted the null hypothesis, stating that Biodentine and MTA showed an insignificant cytotoxic effect on SHEDs.

DISCUSSION

Several *in vitro* trials have been conducted to assess the biological properties of CSCs toward hDPSCs. These trials confirmed the biocompatibility and bioactive properties of CSCs towards hDPSCs.[28-31] However, the literature search offers limited knowledge regarding the effect of biological properties of CSCs towards SHEDs. Thus, the purpose of this systematic review was to perform a systematic appraisal of the information, regarding the existing research on SHEDs biocompatibility, bioactivity and gene expression analysis when cultured in various CSCs.

Ten trials met the previously defined inclusion criteria and the same were assessed for the present review after a thorough search and selection method. Despite its limitations, the study sample included a wide variety of cell viability assays, wound healing assays, cell adhesion, gene expression analysis and other bioactivity experiments on the biological effect of SHEDs to six different commercially existing CSCs (MTA, Pro RootMTA, EndoCem Zr, RetroMTA, Biodentine, iRoot BP and Theracal.

In general, the *in vitro* biological experiments included in this review were carried out by incubating SHEDs in standardized conditions with various CSC dilutions for defined time periods and reporting a range of outcome variables with a positive and/or negative control specimen as a reference. All of the included studies provided the characteristics of the test specimens employed as controls, as shown in Table II. The findings of the various biocompatibility and bioactivity testing were provided in the majority of research using just a negative control specimen as a reference,[17,18,19,20,23,24,26] whereas the remaining trials employed a positive as well as negative control specimen as a reference.[21,22,25]

The methodological heterogeneity of selected trials leads to a wide range of results. However, as indicated in Table III and IV, substantial results from the SHEDs proliferation and viable ability, adhesion, mineralization and wound healing assays tended to favor the incubation in Biodentine, MTA, EndoCem Zr, RetroMTA and iRoot BP over unconditioned medium culture. However, the small number of comparisons, combined with similar outcomes, resulted in inadequate evidence to recommend the usage of a single CSC. These findings are comparable with those described in the existing literature on the use of CSCs in vital pulp procedures.[32-35]

Theracal, on the other hand, demonstrated significant unfavorable outcomes in biocompatibility and bioactivity testing when incubated in SHEDs. Remarkably, our findings are congruent with those of a previous randomized controlled trial, in which both MTA and Biodentine outperformed Theracal as partial pulpotomy agents.[36,37] As a result, the information supporting Theracal's biological qualities towards SHEDs could be classified as conflicting, necessitating additional trials on its use in vital pulp procedures.

Figure 2: Forest plot for evaluation of cell viability of SHEDs cultured in MTA.

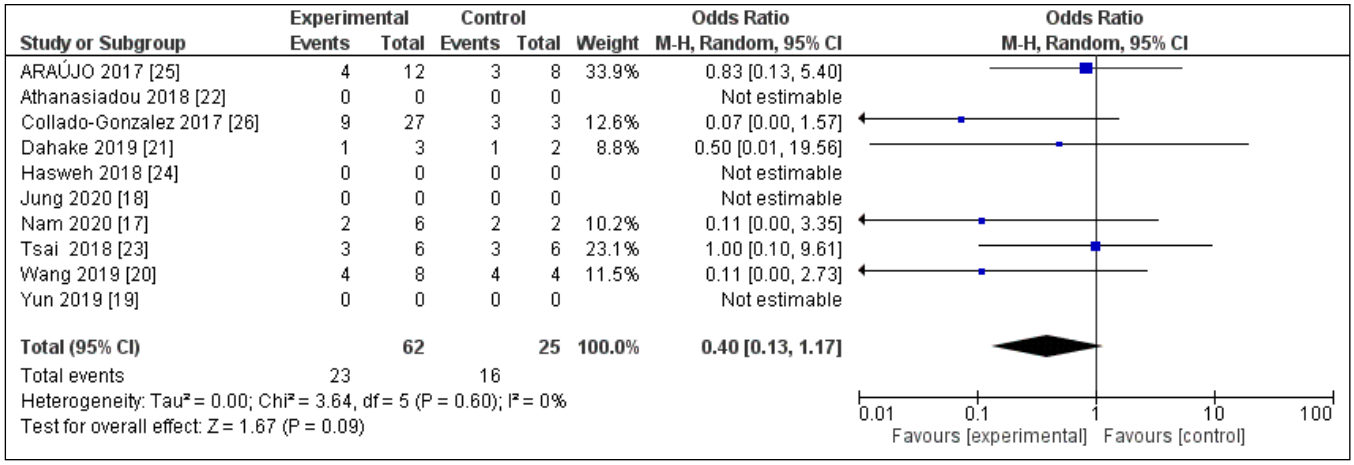
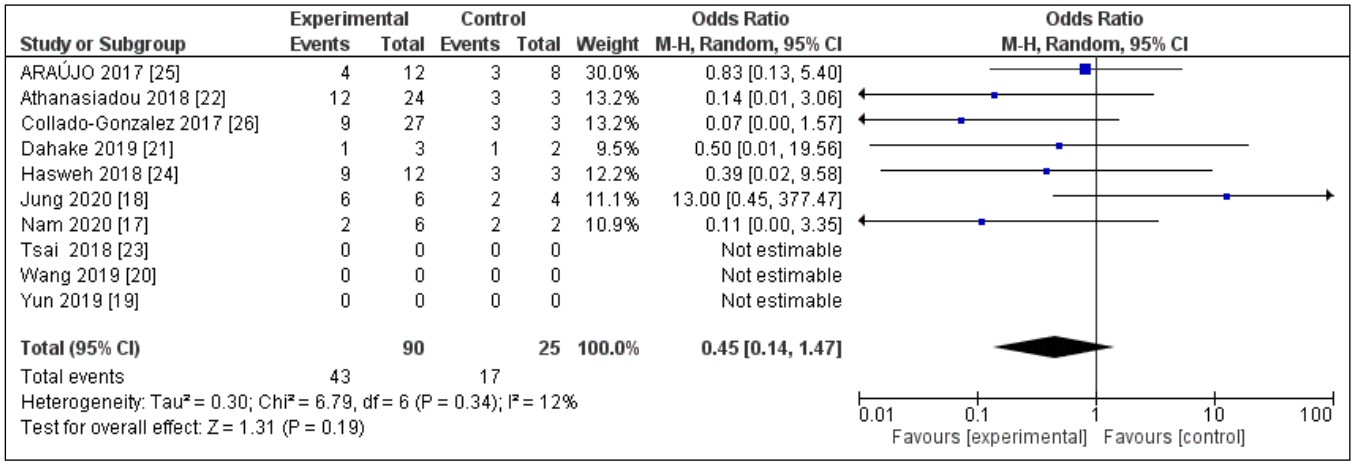


Figure 3: Forest plot for evaluation of cell viability of SHEDs cultured in Biodentine.



Different CSC concentrations were measured among the selected trials, as shown in the methodological summary (Table II). Material preparation was carried out in all cases following the instructions provided by the respective manufacturers. However, when it came to concentration, studies showed lot of variations. Material dosage was chosen in some cases[21,24,25] based on earlier work[38-42], while others[22,26] referred to the corresponding ISO requirements for specimen preparation and examined a series of cement dilutions.

The expression of osteo/odontogenic markers was only examined using RT-qPCR.[17,22,,25] In the first study, the expression of dentin Matrix Protein-1 (DMP-1) by SHEDs was examined after incubating with 1mg/mL MTA or Biodentine, and a rise in DMP-1 was detected. Such a marker outperformed a negative control test specimen after a 21-days of incubation period.[25]In the second study, the expression of numerous osteo/odontogenic markers was investigated in a series of Biodentine dilutions, and the results revealed that the biological effects of this CSC are concentration dependent.[22] Whereas, Araujo L *et al* [25] reported a progressive increase in expression of gene GAPDH in SHEDs cultured in MTA, Biodentine and calcium hydroxide.[25]

To the best of our understanding, this is the first comprehensive review and meta-analysis of SHEDs *in vitro* biological response to CSCs. Sanz JL *et al* [43] examined the bioactive properties and cytocompatibility of hydraulic CSCs cultured on SHEDs. However, they did not assess the gene expression analysis and could not perform the meta-analysis. Previous evaluations assessed CSCs biological *in vitro* capacities toward human teeth pulp cells[44] and specific types of Dental Stem Cells, specifically the cells isolated from permanent teeth (hDPSCs)[45] and apical papilla (hSCAPs). [46]

CONCLUSION

The results of *in vitro* tests evaluating the cell proliferation, differentiation, mineralization and migration ability of SHEDs cultured in various CSCs–MTA, EndoCem Zr, RetroMTA Biodentine, and iRoot BP Plus showed that they have adequate biocompatibility, bioactive properties and genetic expressions indicating that they can be used safely in primary dentition for VPT procedures.

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