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Original Article

Efficacy of Autologous Mesenchymal Adipose Stem Cells for Dental Hard Tissue Formation in DPC: An In Vivo Animal Study

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ABSTRACT

Background: Direct pulp capping (DPC) is a procedure in which the exposed pulp of the tooth is protected with a suitable material. Currently, fat tissue (adipose stem cells) is known as a source of mesenchymal/stromal stem cells (MSCs).

Objectives: The purpose of the present study was to determine the effect of cell therapy in DPC using adipose-derived stem cells (ASCs), in addition to a collagen/hydroxyapatite hybrid scaffold, on dental hard tissue formation.

Methods: Mesenchymal stem cells were extracted from dogs. Then, DPC was performed in class V cavities prepared on the premolars of the same dogs in three groups using mineral trioxide aggregate (MTA), ASCs plus hydroxyapatite/collagen hybrid scaffold, and hybrid scaffold alone. After 12 weeks, the dogs were sacrificed and the teeth were fixed in formalin. Fixed decalcified specimens were stained and evaluated histopathologically to determine the presence of calcified bridge formation, characterize its structure, quantify its thickness, and assess pulp vitality.

Results: According to the results of the Kruskal-Wallis non-parametric test, significant differences were noted among the three groups in terms of vitality, dentinal bridge thickness, continuity of the bridge, type of bridge, and periapical lesion. The ASCs plus scaffold group showed dentinal bridge thickness similar to that observed with scaffold alone. The performance of MTA in this regard was superior to the other two groups.

Conclusion: In the present study, despite the formation of a calcified barrier following the pulp cap with ASCs, which indicates the potential of forming hard tissue by this cell line, this amount is not clinically sufficient.

Keywords: Adipose stem cell, Direct pulp capping (DPC), Scaffold, Dog

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Introduction

Dental caries remains a major challenge in terms of oral and dental health (Duangthip & Chu, 2020). Upon initiation, tooth decay causes slight inflammation of the pulp and periapical tissue and is not often associated with pain. However, its progression results in irreversible pulpitis, which is often painful and leads to pulp necrosis and periapical disease. Tooth decay is a multifactorial lesion that results from the accumulation of carious bacteria on the tooth surface (Sharma et al., 2015). Currently, preservation of pulp vitality of permanent teeth with mechanical or carious exposure of dental pulp is a challenging topic, since sufficient evidence does not exist to help clinicians make a correct clinical decision in such cases (Duncan, 2022).

Preservation of pulp vitality and restoring the function of dentin-pulp complex are desirable outcomes of vital pulp therapy (Komabayashi & Zhu, 2010; Zeng et al., 2024). Different methods have been introduced to preserve pulp vitality in teeth with extensive caries (Komabayashi & Zhu, 2010). Excavation of carious lesion, pulp capping, and pulpotomy are among the treatment modalities performed for deeply carious teeth with reversible pulpitis (Duncan, 2022). In direct pulp capping (DPC), exposed pulp is protected from further traumatization by applying a capping agent to cover the exposure site (Morotomi et al., 2019). This allows reparative dentinogenesis and formation of a dentinal bridge. This modality is well accepted by patients because it is lower in cost and less time-consuming compared with root canal therapy (Komabayashi & Zhu, 2010).

Vital pulp therapy is a well-known therapeutic approach aimed at preserving the vitality of dental pulp and inducing the pulp tissue to regenerate and form a dentin-pulp complex (Hanna et al., 2020). Injured dental pulp has limited potential to heal. In the presence of mild and slowly progressive stimuli, odontoblasts may form a dentinal barrier at the exposure site. However, in the presence of strong and rapidly progressive stimuli, post-mitotic odontoblasts cannot proliferate to replace the injured odontoblasts. Under these circumstances, newly differentiated odontoblast-like cells commence the process of repair (Baldión et al., 2018). Variable outcomes have been reported so far for DPC, and the reported success rates vary from 37 to 50% for carious pulp exposures and up to 92% for mechanical pulp exposures (Komabayashi & Zhu, 2010; Islam et al., 2023).

Infection control and biocompatibility of pulp capping materials are important factors that determine treatment outcomes. Calcium hydroxide [$Ca(OH)_2$] has been widely used for DPC and has long been regarded as the “gold standard” (Kim et al., 2020). However, the unfavorable quality of the created dentinal bridge and the lack of sealing of the dentinal walls were among the reasons for the collapse of this material (Islam et al., 2023). Also, the alkaline pH of $Ca(OH)_2$ leads to necrosis around the damaged tissue (Ba-Hattab et al., 2016). Mineral trioxide aggregate (MTA) is a well-known introduced cement that has been proven to be suitable for DPC (Kim et al., 2020; Torabinejad et al., 1993; Torabinejad et al., 1995; Petta et al., 2020). It is well capable of inducing reparative dentinogenesis (Parirokh et al., 2018).

Compared to $Ca(OH)_2$, MTA leads to dentin formation at a faster rate and with a higher structural quality (Torabinejad et al., 1993). However, important limitations, such as long setting time and tough application are among the disadvantages of MTA (Kim et al., 2020; Torabinejad et al., 1995). Dentin adhesives, (Kato et al., 2011) hydroxyapatite (Briso et al., 2006), bioactive glasses (Gong et al., 2014), calcium silicate-based cements (Tran et al., 2012), biodentine (Petta et al., 2020), resin-modified calcium silicates (Kim et al., 2020), and bioceramics (Gala-Garcia et al., 2010), have also been suggested for use as DPC agents to induce dentinal bridge formation. Although studies reveal that calcium silicate-based cements have more effective results in dentinal bridge formation than calcium hydroxide cements (Peskersoy et al., 2021; Abdel Sameia et al., 2020). It should be noted that the teeth included in this study did not have caries or inflammation; consequently, there was no need to reduce inflammation, although minimal inflammation can in turn play a significant role in supporting the differentiation of dentin and deposition of mineralized dentin.

Cell therapy as a conservative approach has been considered for pulp therapy and management of traumatized teeth in recent years (Obeid et al., 2013).

Tissue engineering using stem cells, scaffold, and growth factors is a new modality for the management of defects (Ashri et al., 2015). Adult stem cells have self-renewal potential and multilineage differentiation, which are important properties for postnatal development. These cells can serve as a rich source for tissue engineering purposes (Wofford et al., 2020). Considering the capability of mesenchymal stem cells to differentiate and form tooth-like structures, researchers have been in search of methods to successfully use these cells to regenerate the lost dental hard tissue (Hernández-Monjaraz et al., 2018).

Currently, adipose tissue is recognized as a source of mesenchymal/stromal stem cells (MSCs), which are abundant and easily and minimally invasively obtainable. Adipose-derived stem cells (ASCs) are stem cells derived from the adipose tissue. They have high proliferation ability and can differentiate into cardiomyocytes, chondrocytes, and osteocytes under suitable conditions (Krawczenko & Klimczak, 2022). Adipose stem cells are increasingly used in tissue engineering and cell therapy studies in vitro and in vivo. The osteogenic ability of ASCs is comparable to that of bone marrow-derived stem cells (BMSCs) (Dai et al., 2016).

Scaffolds are a main component of tissue engineering (Wofford et al., 2020). It enables the formation of tissues and interaction of cells. Scaffolds are used to transfer stem cells and/or growth factors to the defective site (Sharma et al., 2014). They are available in natural and synthetic, rigid and non-rigid, and degradable and non-degradable forms (Sharma et al., 2014). Collagen scaffolds have chemical and structural properties similar to those of protein structures in dental hard tissue and have high tensile strength. They enable optimal adhesion, proliferation, and migration of cells (Sharma et al., 2014; Dong & Lv, 2016). Hybrid scaffolds made of collagen, tricalcium phosphate, and hydroxyapatite have improved mechanical and osteoinductive properties (Ielo et al., 2022). Thus, collagen/hydroxyapatite hybrid scaffold was used in this study.

The purpose of this study was to evaluate the efficacy of DPC with ASCs and a collagen/hydroxyapatite hybrid scaffold for the formation of dental hard tissue. The null hypothesis was that the efficacy of DPC with ASCs and hybrid scaffold for the formation of dental hard tissue would be similar to that of MTA and scaffold alone.

Materials and Methods

This animal study was performed according to the guide for the care and use of laboratory animals under the supervision of a veterinarian and ARRIVE guidelines (Smith et al., 2007). The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences and the Local Ethics Committee for Animal Experiments at the Faculty of Veterinary Medicine, University of Tehran.

Isolation of ASCs

This study was conducted on four healthy mixed-breed dogs aged 18 to 24 months (Figure 1).

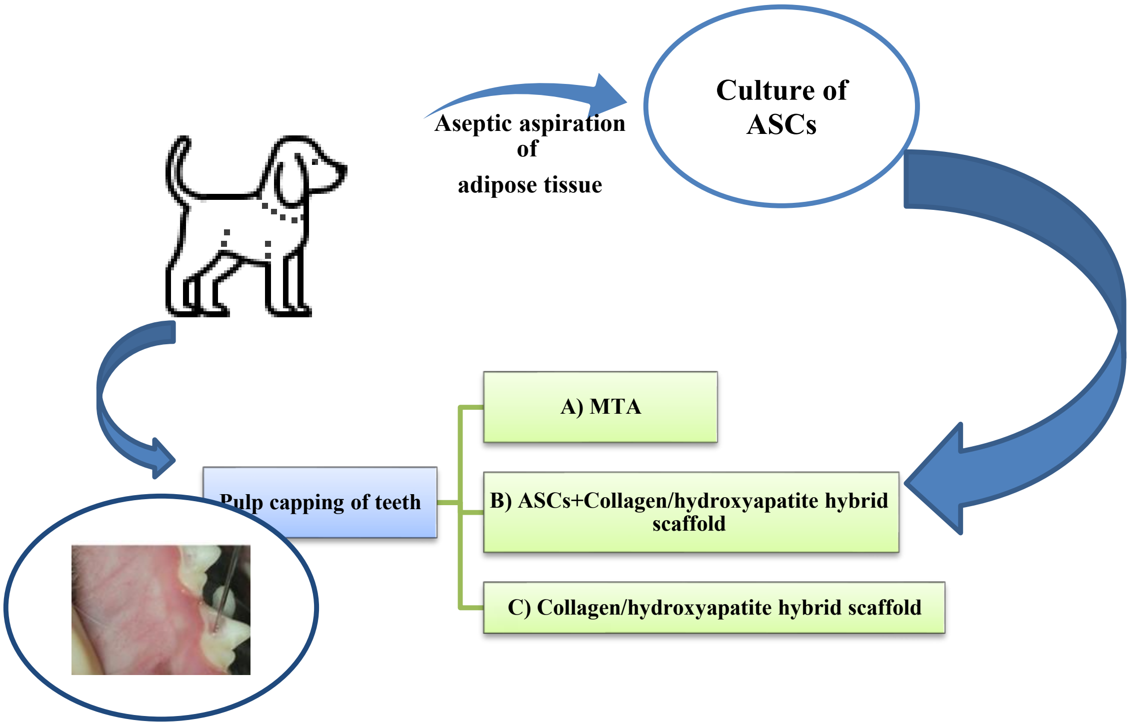

Figure 1. Schematic summary of the methodology

MTA: Mineral trioxide aggregate; ASCs: Adipose-derived stem cells.

General anesthesia was induced intravenously with 10 mg/kg of 10% ketamine (Alfasan, Woerden, Holland) and 0.27 mg/kg diazepam (Zepadic, Caspian Tamin Baxter healthcare, Puerto Rico). Interscapular and inguinal areas in dogs were prepped and draped for aseptic aspiration of adipose tissue. A small incision of 2 cm in length was made to aspirate 10 mL of subcutaneous adipose tissue (Figure 2). Aspirated adipose tissue was immersed in phosphate-buffered saline (PBS) containing 1% antibiotic (penicillin-streptomycin, solution 100X, BioWest, France) and transferred to a cell culture laboratory for the isolation of ASCs.

After the surgical procedure, dogs' teeth were subjected to scaling and polishing (Figure 3). For pain control, 2 mg/kg tramadol (DarouPakhsh Pharmaceutical, Iran) was administered intramuscularly before the dogs gained recovery.

Culture of ASCs

ASCs were isolated by enzymatic digestion. Briefly, adipose tissue was rinsed with PBS for several times and minced into small particles (0.5 cm^3) using scissors and surgical scalpel under sterile conditions. Efforts were made to separate blood vessels and connective tissue from yellow adipose tissue. After mincing, type II collagenase enzyme (0.075%) was added to adipose tissue and the mixture was transferred to a 50 mL sterile centrifuge tube and placed in an incubator shaker at 37 °C for one hour to allow enzymatic digestion by collagenase. The mixture was then filtered to separate large undigested fat particles. The obtained solution was centrifuged at 1410 rpm for five minutes. After two rounds of centrifugation under sterile conditions, the supernatant (containing cell plaque) was removed and added to a T25 cell culture flask along with Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin in a 1:100 ratio. The medium was not refreshed for 36 hours in order to allow the cells to adhere to the bottom of the flask. After the first 36 hours, the medium was refreshed every two weeks. The flask was incubated at 37 °C under 5% CO_2 and 95% humidity until reaching 80-90% confluence. The cells were passaged three times. Third-passage cells were removed from the bottom of the flask by applying trypsin and purified at 400 rpm centrifugation. The cells were suspended in 0.2 mL of culture medium and transferred to a syringe for injection. Isolated colonies were differentiated into adipose cells under specific inductive conditions to confirm their stemness (Solali et al., 2015) (Figure 4). To confirm differentiation of stem cells into adipocytes, flow cytometry was performed to assess the expression of CD34, CD44, and CD105 markers (Solali et al., 2015).

Formation of collagen/hydroxyapatite hybrid scaffold

The collagen/hydroxyapatite hybrid scaffold was synthesized as described by Bahrami et al. in 2017. To prepare hydroxyapatite, solutions containing calcium and phosphorus were used. In addition, calcium nitrate tetrahydrate [$Ca(NO_3)_2 \cdot 4H_2O$] and diammonium phosphate [$(NH_4)_2HPO_4$] were used. Distilled water was used to prepare aqueous mixtures of collagen and hydroxyapatite. To fabricate the scaffolds, a freeze-drying technique was used. Because the aim was to create a 3D scaffold, the prepared layers were divided into smaller pieces and joined together using a 10% collagen solution.

Pulp capping of teeth

After induction of anesthesia as explained earlier, general anesthesia was maintained by isoflurane inhalation (AERRANE, Baxter healthcare, Puerto Rico). Thirty-nine maxillary and mandibular teeth from four healthy 18–24-month-old male dogs were selected for DPC through visual examination. The procedure was performed under general anesthesia. The teeth were

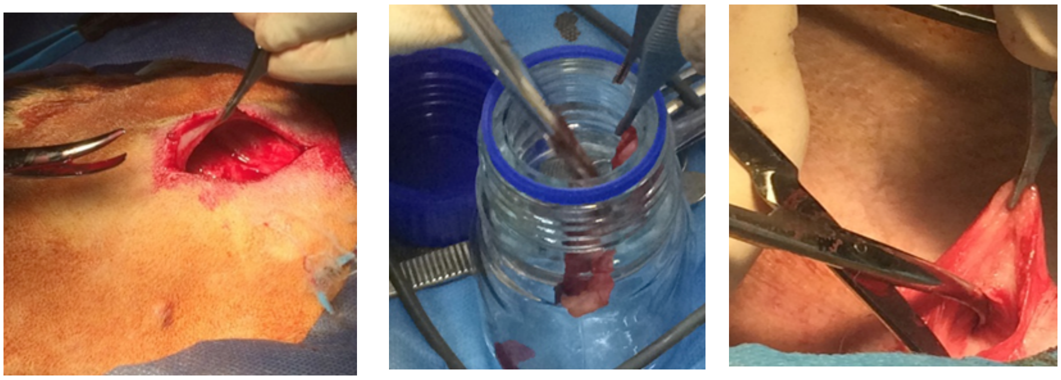

Figure 2. The surgical process of taking fat from a dog

Figure 3. Scaling and polishing of dogs' dentition

randomly separated into three study groups (n=13 per group). The DPCs of teeth were performed by: A) MTA, B) ASCs and collagen/hydroxyapatite hybrid scaffold, and C) collagen/hydroxyapatite hybrid scaffold alone.

Maxillary and mandibular premolars of the same dogs from which, ASCs were isolated were rinsed with chlorhexidine, and Class V cavities were prepared on their buccal surfaces 1 mm above the gingival margin. The cavities were about 4 mm height, 4 mm wide, and 3 mm deep (4×4×3). Cavities were prepared using an 008 round diamond bur (Jota 842 FG 014) on a low-speed handpiece under copious irrigation with sterile water. The cavities were prepared deeper at the center to observe the shadow of the pulp chamber through dentin. Dental pulp was then mechanically exposed using the sharp tip of a dental explorer (sharp tip of a c-shaped dental explorer). Sterile cotton rolls dipped in saline were used to control bleeding. Using block randomization, the teeth were assigned to the following three groups (n=13) for DPC:

Group A: MTA (ProRoot MTA, Arizona, USA) was mixed with sterile saline and was used to cap the exposure site. The carrier was used to dispense the material into the defect site, and the ProRoot MTA root repair material was condensed into the cavity with a small amalgam plugger, cotton pellets, or paper points. The placement of ProRoot MTA root repair material was confirmed with a radiograph. If an adequate barrier had not been created, the ProRoot MTA root repair material was rinsed out of the defect and the procedure was repeated. A wet cotton pellet was used to remove excess moisture from the site, and the access preparation was sealed with a temporary restoration for a minimum of four hours. After four hours, or at another appointment, a rubber dam was used and the ProRoot MTA root repair material was examined, expecting this material to be hard. In the absence of hardening, the MTA would be rinsed and the application repeated. It should be noted that the ProRoot MTA root repair material remained as a permanent part of the filling.

Group B: ASCs were injected into the defective site using a Hamilton syringe (Hamilton Company, Reno, Nevada, USA) and the exposure site was covered with collagen/hydroxyapatite hybrid scaffold. To seed the cells onto the collagen/hydroxyapatite scaffold, the scaffold was cut to 8 mm in diameter. They were subsequently washed with PBS and placed in 24-well chambers. The scaffolds were sterilized by exposing the 24-well plates

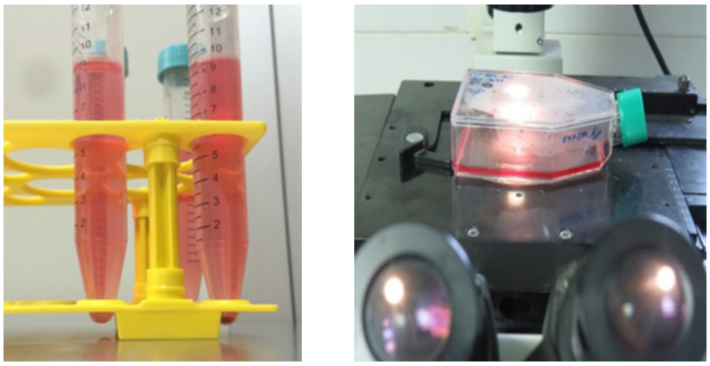

Figure 4. Cell culture and observation of cells under a microscope

Figure 5. Clinical sequence of DPC in dog's tooth

A) Class V cavity was prepared on the buccal surface of NO# 407 tooth with low-speed handpiece and round bur under saline irrigation; B) Pinpoint exposure of dental pulp using the sharp tip of an explorer; C) Bleeding control by cotton rolls; E) ASCs were injected to the defect site using a Hamilton syringe; E) Placement of collagen/hydroxyapatite scaffold; F) Glass ionomer cement restoration of the cavity

to an ultraviolet hood for one hour. They were then inverted and placed under the UV hood for one hour to sterilize all surfaces. At the seeding stage, 2.2×10^6 hEnSCs derived from the third passage were diluted in the 500 µL of DMEM + FBS 10% culture solution and injected onto the collagen/HA scaffold.

Group C: Collagen/hydroxyapatite hybrid scaffold was used alone to cover the exposure site.

Light-cure resin-modified glass ionomer cement (Voco GmbH, Cuxhaven, Germany) was then applied to restore the cavity (Figure 5).

Due to ethical considerations, the teeth could not be evaluated radiographically; thus, teeth with caries lesions probably associated with a periapical lesion were not considered in this study.

Histological slide preparation and histomorphometric evaluation

Twelve weeks after the procedure, the dogs were euthanized by intravenous injection of thiopental sodium (Rotexmedica, Trittau, Germany). Tooth specimens were fixed in 10% formalin for one week. Decalcification was performed by the immersion of specimens in 10% nitric acid. In next step, the teeth were sectioned in a buccolingual direction longitudinally at the exposure site. Specimens were paraffin-embedded and serially sectioned at the exposure site; 5 µm-thick slices were prepared for histological analysis (Figure 6). After hematoxylin and eosin (H&E) staining, the slides were inspected under a light microscope (E400, Nikon, Japan) at x40, x100, x200 and x400 magnifications by a pathologist blinded to the grouping of specimens.

Photographs were also taken at 40× magnification with a digital camera (E8400, Nikon, Japan). Table 1 summarizes the factors evaluated in histological analysis.

The creation of dentinal bridge (in microns) was measured histologically by calculating the mean value using at least three cross-sections using IHMM version 1.0 software (SBMU, Iran) (Eskandarizadeh et al., 2008). The Kruskal-Wallis test was used to compare the parameters among the three groups. Dunn's test was applied for pairwise comparisons at $P<0.05$ level of significance.

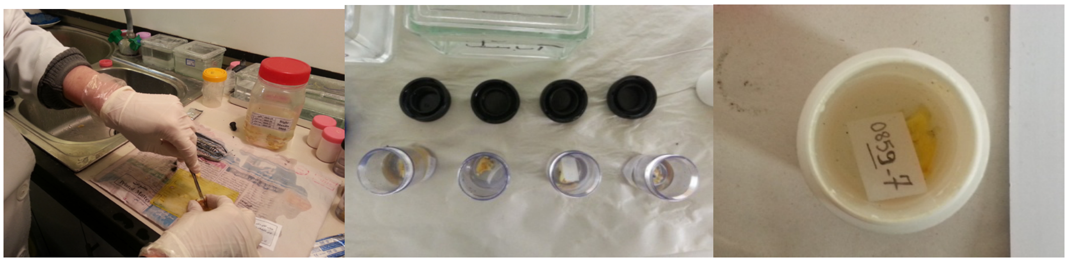

Figure 6. Steps of buccolingual cutting of decalcified samples

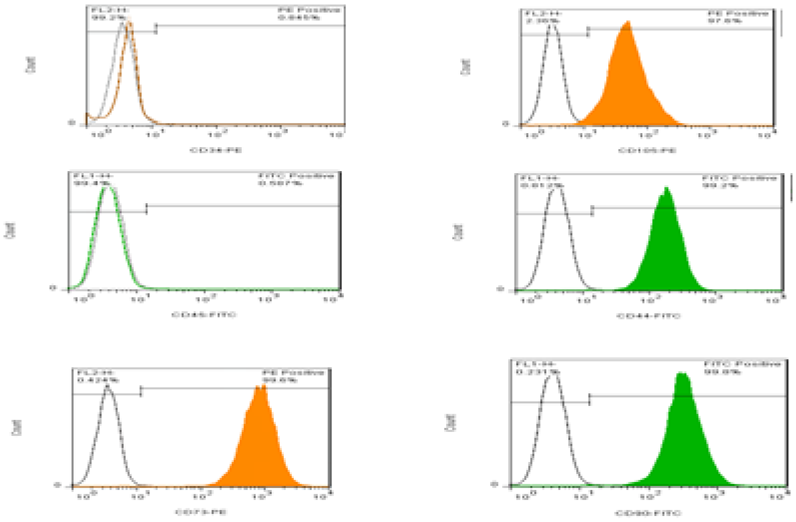

Figure 7. Flow cytometric analysis of mesenchymal cells by FlowJo software

Note: phycoerythrin was used for the detection of CD34, CD73, and CD105 markers, and fluorescein isothiocyanate-conjugating antibodies were used for detection of CD44, CD45, and CD90.

The score 0, attributed to no bridge formation, and the score 1, illustrating the formation of a calcified bridge SPSS version 24.0 was used for statistical analysis of the data. The frequency and percentage of degree of pulpal vitality (Eskandarizadeh et al., 2008), thickness and uniformity of bridge formation (Faraco Junior & Holland, 2004), type of bridge formation (Dominguez et al., 2003), inflammation (Bidar et al., 2014), and odontoblastic differentiation (Eskandarizadeh et al., 2008) were evaluated and noted in the three groups.

The comparison of variables among the three groups was analyzed statistically using the Kruskal-Wallis test. Pairwise comparisons were done using Dunn's test. The $P<0.05$ was considered statistically significant.

Results

Analysis of stem cells

Flow cytometric analysis

In this study, the expression of CD34, CD44, and CD105 markers was evaluated, and it was found that cells were positive for CD44 and CD105 markers, which confirmed that stem cells were of mesenchymal origin. Cells were negative for CD34, which confirmed the absence of hematopoietic stem cells (Figure 7).

Differentiation into osteoblasts and adipocytes

After 21 days, ASCs differentiated into adipocytes as shown by oil red O staining (Figure 8).

Histomorphometric evaluation

The mean dentinal bridge thickness was 0.12 µm in the MTA (positive control) group, 0 µm in the scaffold group (negative control), and 0.02 µm in the ASC group (test group) (Table 2; Figures 9 and 10).

Statistical analysis

The non-parametric Kruskal-Wallis test showed significant differences among the three groups in terms

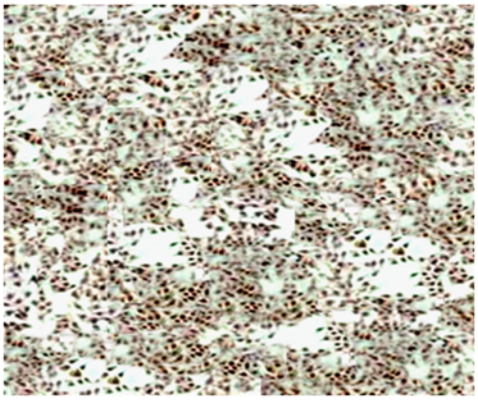

Figure 8. Histological view of the differentiation of mesenchymal stem cells into adipocytes

Table 1. Parameters evaluated in histological analysis

Factors	Scores	Characterization
Formation of calcified bridge (Torabinejad et al., 1993)	0	No bridge formed
	1	Formation of calcified bridge
Structure of calcified bridge formed (Tran et al., 2012)	0	Absence of bridge; no formation of calcified bridge or presence of a hard tissue wall around the defect site
	1	Interrupted bridge; formation of hard tissue only around the exposed site
	2	Continuous bridge; complete formation of dental hard tissue bridge
Type of dentinal bridge formed (Tsuji et al., 2014)	0	No formation of dentinal bridge
	1	Formation of osteodentin bridge
	2	Formation of dentinal bridge with an abnormal tubular pattern
	3	Formation of dentinal bridge with a normal tubular pattern
Vitality of pulp tissue (Torabinejad et al., 1993)	0	No sign of necrosis
	1	Signs of pulp necrosis
Inflammation (Wang et al., 2014)	0	Normal tissue and no inflammation; presence of 0-1 inflammatory cells in the microscopic field
	1	Mild; presence of 2-5 inflammatory cells in the microscopic field
	2	Moderate; presence of 6-15 inflammatory cells in the microscopic field
	3	Severe; presence of more than 15 inflammatory cells in the microscopic field
Odontoblastic differentiation (Torabinejad et al., 1993)	0	Presence of the odontoblastic layer
	1	No differentiation and absence of the odontoblastic layer

of vitality of dental pulp tissue (P<0.0001), dentinal bridge thickness (P<0.0001), continuity of the bridge (P<0.0001), type of bridge (P<0.0001), odontoblastic differentiation (P<0.0001) and periapical lesion (P<0.0001).

Vitality

Pairwise comparisons of the groups by the Dunn's test revealed a significant difference in terms of vitality between the MTA and scaffold groups (P<0.001), but the difference between the MTA and ASC groups was not significant (P=0.074). In other words, the ASC group had pulp vitality similar to the MTA group.

Thickness of the dentinal bridge

Pairwise comparison of the groups using the Dunn's test in terms of thickness of the dentinal bridge showed significant differences between the MTA and scaffold groups (P<0.0001) and between the MTA and ASC groups (P<0.001), but the difference between the scaffold and ASC groups was not significant in this regard (P=0.560). In other words, the ASC group had the same performance as the scaffold group in terms of dentinal bridge thickness, and the performance of MTA group was superior to that of the other two groups.

The Mean±SD dentinal bridge thickness was 0.12±0.07 µm in the MTA (positive control), 0 µm in the scaffold (Kruskal-Wallis test, P<0.05), and 0.04±0.02 µm in the ASC group. The mean thickness of the formed dentinal bridge in the MTA group was significantly more than that in the scaffold and ASC groups.

Continuity of bridge formation

Pairwise comparison of the groups using the Dunn's test in terms of continuity of bridge revealed significant differences between the MTA and scaffold (P<0.0001) and between the MTA and ASC (P<0.0001) groups. However, the difference between the scaffold and ASC groups was not significant (P=0.648). In other words, the performance of scaffold was similar to that of ASCs in terms of conti-

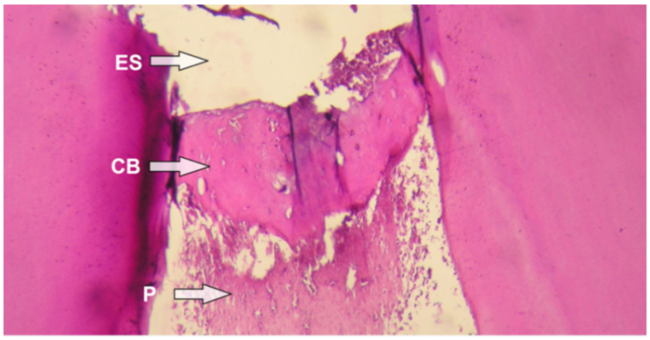

Figure 9. Histomorphometric view of the dental pulp of a premolar tooth from a dog, 12 weeks after direct pulp capping (hefmatoxylin and eosin, ×40 magnification)

Note: An interrupted osteodentin bridge was formed in the ASC group. ES indicates the exposure site capped with the pulp capping material. CB indicates the formation of an interrupted osteodentin bridge. P indicates the dental pulp.

nuity of bridge forming, and the performance of MTA was superior to that of the other two groups.

Type of bridge

Pairwise comparison of the groups using the Dunn's test in terms of type of bridge revealed significant differences between the MTA and scaffold ($P<0.0001$) and the MTA and ASC ($P<0.001$) groups. However, the difference between the scaffold and ASC groups was not significant ($P=0.628$). As a result, scaffold exhibited the same behavior as ASCs in terms of the type of bridge formed.

Odontoblastic differentiation:

Pairwise comparison of the groups using the Dunn's test in terms of odontoblastic differentiation showed significant differences between the MTA and scaffold groups ($P<0.0001$) and between the MTA and ASC ($P<0.001$) groups. However, the difference between the scaffold and ASC groups was not significantly different in this regard. In other words, scaffold and ASCs performed similarly, while MTA differed from them (Table 2).

Discussion

Histological analysis revealed that MTA resulted in the formation of a calcified barrier at the end of the three-month course of the study, which is in line with the findings of previous studies (Torabinejad et al., 1995). The success of MTA in this respect is attributed to its optimal physical and bioactive properties (Dong & Xu, 2023). In the current study, MTA was significantly more success-

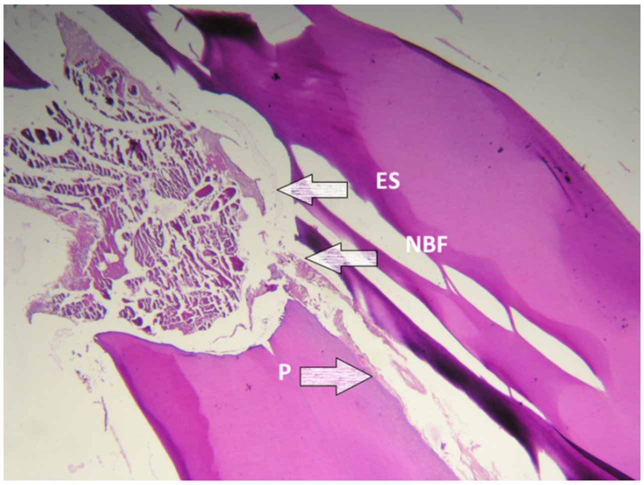

Figure 10. Histomorphometric view of the dental pulp of a premolar tooth in a dog, 12 weeks after direct pulp capping (H&E, ×100 magnification)

Note: NBF indicates no bridge formation in the ASC group. ES indicates the exposure site capped with pulp capping material. P indicates the necrotic pulp tissue.

Table 2. Histomorphometric results

Group	Total	No. (%)								
		Vitality		Continuity of Bridge			Type of Bridge			
		0	1	0	1	2	0	1	2	3
	10	9	1	1	9	1	3	6	1	6
MTA	10(100)	9(90)	1(10)	1(10)	9(90)	1(10)	3(30)	6(60)	1(10)	6(60)
Scaffold	12(100)	2(16.7)	10(83.3)	11(91.7)	1(8.3)	11(91.7)	1(8.3)	0(0)	11(91.7)	1(8.3)
ASCs	10(10)	5(50)	5(50)	8(80)	2(20)	8(80)	2(20)	0(0)	8(80)	2(20)
Total	32(100)	16(50)	16(50)	20(62.5)	12(37.5)	20(62.5)	6(18.75)	6(18.75)	20(62.5)	9(28.1)

Group	No. (%)							
	Degree of Inflammation				Calcified Bridge Formation		Odontoblastic Differentiation	
	0	1	2	3	0	1	0	1
	0	3	8	2	0	0	5	5
MTA	0(0)	3(30)	8(80)	2(20)	0(0)	0(0)	5(50)	5(50)
Scaffold	0(0)	0(0)	1(8.3)	4(33.3)	4(33.3)	3(25)	0(0)	12(100)
ASCs	0(0)	0(0)	3(30)	1(10)	5(50)	1(10)	0(0)	10(100)
Total	0(0)	3(9.3)	12(37.5)	7(22)	9(28)	4(12.5)	5(15.6)	27(84.3)

MTA: Mineral trioxide aggregate; ASCs: Adipose-derived stem cells.

ful in the formation of a calcified barrier following DPC compared to ASCs and the collagen/hydroxyapatite hybrid scaffold. The mean thickness of the calcified barrier was 0.12 µm, 0.02 µm, and 0 µm in the MTA, ASC, and collagen/hydroxyapatite scaffold groups, respectively. These values were significantly different. The mean thickness of the formed calcified barrier was 0.02 µm in the ASC group, which indicates that this cell line is also capable of forming hard tissue. However, the thickness of the formed barrier was not clinically sufficient. It should be noted that if class I cavities had been prepared instead of class V, the pulpal floor would provide better blood supply to the exposure site (Obeid et al., 2013), and more favorable results may be obtained. However, pulp horns extend to areas close to the occlusal surface of teeth in dogs; thus, class V cavities were used in the current study to prevent restoration loss due to masticatory forces. However, blood supply to the exposure site in class V cavities is not as efficient as that in class I cavities because the buccolingual width of the pulp chamber of premolar teeth in dogs is small, and there is insufficient space behind the exposure site for adequate blood supply to stem cells. In the current study, calcified bridge formation occurred in 20% of cases treated with DPC using ASCs and collagen/hydroxyapatite scaffold, which was lower than the rate in the MTA group. This highlights the lower potential of ASCs for osteogenesis and their higher sensitivity to hypoxia.

Better blood supply could have been obtained in the area if molars or canine teeth of dogs had been used. However, premolar teeth of dogs (four per each quadrant) are often used in pulp capping studies due to limitations, such as difficult access to molars, the challenging decalcification process required for preparing histological slides, and the small number of canine teeth (ethical considerations). It should be noted that using canines would require euthanizing too many animals because dogs have only four canines, which would be insufficient for our study; our sample size would require many more teeth to be meaningful.

Alves et al. compared the osteogenic potential of BMSCs and ASCs in an animal study on young dogs and showed that methyl thiazol tetrazolium conversion, alkaline phosphatase activity, collagen synthesis, percentage of mineralized area, Osterix expression, bone sialoprotein expression, and osteocalcin expression were all greater with the use of BMSCs compared to ASCs; these results highlighted the lower osteogenic potential of ASCs compared to BMSCs in dogs (Alves et al., 2014), which is in line with our findings. Insignificant formation of the dentinal bridge by ASCs may be due to hypoxia as stated by Tsuji et al., (2014). It is probable that the susceptibility of cells to hypoxia is mainly due to cell density and their insufficient osteogenic capability. In addition, it should be noted that if we had utilized molar teeth, the blood supply would have been much better. However, access to molars was limited. Moreover, radiography could be helpful before the procedure, provided that we are allowed to do this due to ethical considerations, in order to evaluate inflammation, and this requires further research to see the real potential of ASCs as compared with MTA.

The purpose of this study was to evaluate the potential of ASCs to induce dental hard tissue formation following DPC. For this purpose, ASCs were used in this study since they are abundant and easily available (Tsuji et al., 2014; Wang et al., 2014; Salvatore et al., 2021). They are capable of seeding in tissues and responding to signals (Wang et al., 2014). Moreover, their gene expression and differentiation capabilities are similar to those of dental pulp and periodontal ligament stem cells (Peng et al., 2021). They are also capable of differentiation into special cells, such as muscle cells, hepatic cells, brain cells, pancreatic cells, and epithelial cells (Wang et al., 2014). ASCs are mesenchymal stem cells derived from the adipose tissue. They are capable of adhering to cell culture flasks and expanding in vitro. Moreover, they can differentiate into multiple cell lineages (Irioda et al., 2016). In contrast to BMSCs, ASCs can be isolated from the adipose tissue in abundance via a minimally invasive procedure (Ciuffi et al., 2017). Their capability to differentiate into osteoblast-like cells has been previously confirmed as well (Mirkhani et al., 2022).

In this study, enzymatic digestion was used to isolate ASCs. Histological analysis was performed to evaluate the formation of a dentinal bridge after DPC, which is the gold standard for this purpose. Formation of a continuous dentinal bridge at the pulp-dentin border provides an ideal prognosis since it protects dental pulp against external stimuli. However, the formation of any type of hard tissue bridge can also be considered a success in DPC (Farzad-Mohajeri et al., 2022). In the current study, IHMM version 1.0 software (SBMU, Iran) was used to quantitatively assess the formation of dentinal bridge for reliable comparison of the results.

Conclusion

It seems that DPC with ASCs under the current conditions cannot yield a clinically desirable outcome. However, considering the formation of hard tissue in a limited number of samples in this study and the importance of preserving pulp vitality, future studies are needed to focus on other aspects related to this topic, such as the use of different growth factors, scaffolds, and cell implantation conditions.

Ethical Considerations

Compliance with ethical guidelines

This animal study was performed according to the Guide for the Care and Use of Laboratory Animals under the supervision of a veterinarian and ARRIVE guidelines (Smith et al., 2007). The study protocol was approved by the Research Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran (Code: IR.TUMS.REC.1394.928) and the Local Ethics Committee for Animal Experiments at the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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Authors' contributions

Conceptualization and study design: Sedighe Sadat Hashemikamangar, Mir Sepehr Pedram, Mohammad Mehdi Dehghan, and Saeed Farzad-Mohajeri; Data collection, analysis, and interpretation: Parisa Doroudgar and Sedighe Sadat Hashemikamangar; Initial draft preparation: Parisa Doroudgar, Naghmeh Bahrami, and Behnaz Behniafar; Review, editing and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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References

Abdel Sameia, M. M., Darrag, A. M., & Ghoneim, W. M. (2020). Two calcium silicate-based materials used in direct pulp capping (in-vivo study). *Tanta Dental Journal, 17*(2), 78-83. [DOI:10.4103/tdj.tdj_5_20]

Alves, E. G., Serakides, R., Boeloni, J. N., Rosado, I. R., Ocarino, N. M., & Oliveira, H. P., et al. (2014). Comparison of the osteogenic potential of mesenchymal stem cells from the bone marrow and adipose tissue of young dogs. *BMC Veterinary Research, 10,* 190. [DOI:10.1186/s12917-014-0190-y] [PMID]

Ashri, N. Y., Ajlan, S. A., & Aldahmash, A. M. (2015). Dental pulp stem cells. Biology and use for periodontal tissue engineering. *Saudi Medical Journal, 36*(12), 1391–1399. [DOI:10.15537/smj.2015.12.12750] [PMID]

Ba-Hattab, R., Al-Jamie, M., Aldreib, H., Alessa, L. & Alonazi, M. (2016) Calcium hydroxide in endodontics: An overview. *Open Journal of Stomatology, 6*(12), 274-289. [DOI:10.4236/ojst.2016.612033]

Bahrami, N., Malekolkottab, F., Ebrahimi-Barough, S., Alizadeh Tabari, Z., Hamisi, J., & Kamyab, A., et al. (2017). The effect of purmorphamine on differentiation of endometrial stem cells into osteoblast-like cells on collagen/hydroxyapatite scaffolds. *Artificial Cells, Nanomedicine, and Biotechnology, 45*(7), 1343-1349. [DOI:10.1080/21691401.2016.1236804] [PMID]

Baldión, P. A., Velandia-Romero, M. L., & Castellanos, J. E. (2018). Odontoblast-like cells differentiated from dental pulp stem cells retain their phenotype after subcultivation. *International Journal of Cell Biology, 2018,* 6853189. [DOI:10.1155/2018/6853189] [PMID]

Bidar, M., Naghavi, N., Mohtasham, N., Sheik-Nezami, M., Fallahrastegar, A., & Afkhami, F., et al. (2014). Mineral trioxide aggregate and portland cement for direct pulp capping in dog: a histopathological evaluation. *Journal of Dental Research, Dental Clinics, Dental Prospects, 8*(3), 134–140. [DOI:10.5681/joddd.2014.025] [PMID]

Briso, A. L., Rahal, V., Mestrener, S. R., & Dezan Junior, E. (2006). Biological response of pulps submitted to different capping materials. *Brazilian Oral Research, 20*(3), 219–225. [DOI:10.1590/s1806-83242006000300007] [PMID]

Ciuffi, S., Zonefrati, R., & Brandi, M. L. (2017). Adipose stem cells for bone tissue repair. *Clinical Cases in Mineral and Bone Metabolism: The Official Journal of the Italian Society of Osteoporosis, Mineral Metabolism, and Skeletal Diseases, 14*(2), 217–226. [DOI:10.11138/ccmbm/2017.14.1.217] [PMID]

Dai, R., Wang, Z., Samanipour, R., Koo, K. I., & Kim, K. (2016). Adipose-derived stem cells for tissue engineering and regenerative medicine applications. *Stem Cells International, 2016,* 6737345. [DOI:10.1155/2016/6737345] [PMID]

Dominguez, M. S., Witherspoon, D. E., Gutmann, J. L., & Opperman, L. A. (2003). Histological and scanning electron microscopy assessment of various vital pulp-therapy materials. *Journal of Endodontics, 29*(5), 324-333. [DOI:10.1097/00004770-200305000-00003] [PMID]

Dong, C., & Lv, Y. (2016). Application of collagen scaffold in tissue engineering: Recent advances and new perspectives. *Polymers, 8*(2), 42. [DOI:10.3390/polym8020042] [PMID]

Dong, X., & Xu, X. (2023). Bioceramics in endodontics: Updates and future perspectives. *Bioengineering (Basel, Switzerland), 10*(3), 354. [DOI:10.3390/bioengineering10030354] [PMID]

Duangthip, D., & Chu, C. H. (2020). Challenges in oral hygiene and oral health policy. *Frontiers in Oral Health, 1,* 575428. [DOI:10.3389/froh.2020.575428] [PMID]

Duncan H. F. (2022). Present status and future directions-Vital pulp treatment and pulp preservation strategies. *International Endodontic Journal, 55*(Suppl 3), 497-511. [DOI:10.1111/iej.13688] [PMID]

Eskandarizadeh, A., Parirokh, M., Eslami, B., Asgary, S., & Eghbal, M. (2008). A comparative study between mineral trioxide aggregate and calcium hydroxide as pulp capping agents in dog's teeth. *Dental Research Journal, 2*(2), 1-10. [Link]

Faraco Junior, I. M., & Holland, R. (2004). Histomorphological response of dogs' dental pulp capped with white mineral trioxide aggregate. *Brazilian Dental Journal, 15*(2), 104–108. [DOI:10.1590/s0103-64402004000200004] [PMID]

Farzad-Mohajeri, S., Pedram, M. S., Saeedifar, N., Mashhadi-Abbas, F., Dehghan, M. M., & Bahrami, N., et al. (2022). Direct pulp capping with autologous bone marrow derived stem cells in dogs. *Veterinary Research Forum: An International Quarterly Journal, 13*(2), 193–200. [DOI:10.30466/vrf.2020.122172.2874] [PMID]

Gala-Garcia, A., Teixeira, K. I., Wykrota, F. H., Sinisterra, R. D., & Cortés, M. E. (2010). Bioceramic/poly (glycolic)-poly (lactic acid) composite induces mineralized barrier after direct capping of rat tooth pulp tissue. *Brazilian Oral Research, 24*(1), 8–14. [DOI:10.1590/s1806-83242010000100002] [PMID]

Gong, W., Huang, Z., Dong, Y., Gan, Y., Li, S., & Gao, X., et al. (2014). Ionic extraction of a novel nano-sized bioactive glass enhances differentiation and mineralization of human dental pulp cells. *Journal of Endodontics, 40*(1), 83–88. [DOI:10.1016/j.joen.2013.08.018] [PMID]

Hanna, S. N., Perez Alfayate, R., & Prichard, J. (2020). Vital Pulp Therapy an Insight Over the Available Literature and Future Expectations. *European Endodontic Journal, 5*(1), 46–53. [DOI:10.14744/eej.2019.44154] [PMID]

Hernández-Monjaraz, B., Santiago-Osorio, E., Monroy-García, A., Ledesma-Martínez, E., & Mendoza-Núñez, V. M. (2018). Mesenchymal Stem Cells of Dental Origin for Inducing Tissue Regeneration in Periodontitis: A Mini-Review. *International Journal of Molecular Sciences, 19*(4), 944. [DOI:10.3390/ijms19040944] [PMID]

Ielo, I., Calabrese, G., De Luca, G., & Conoci, S. (2022). Recent Advances in Hydroxyapatite-Based Biocomposites for Bone Tissue Regeneration in Orthopedics. *International Journal of Molecular Sciences, 23*(17), 9721. [DOI:10.3390/ijms23179721] [PMID]

Irioda, A. C., Cassilha, R., Zocche, L., Francisco, j. c., cunha, r. c., & ferreira, p. e., et al. (2016). human adipose-derived mesenchymal stem cells cryopreservation and thawing decrease α4-integrin expression. *Stem Cells International, 2016,* 2562718. [DOI:10.1155/2016/2562718] [PMID]

Islam, R., Islam, M. R. R., Tanaka, T., Alam, M. K., Ahmed, H. M. A., & Sano, H. (2023). Direct pulp capping procedures - Evidence and practice. *The Japanese Dental Science Review, 59,* 48–61. [DOI:10.1016/j.jdsr.2023.02.002] [PMID]

Kato, C., Suzuki, M., Shinkai, K., & Katoh, Y. (2011). Histopathological and immunohistochemical study on the effects of a direct pulp capping experimentally developed adhesive resin system containing reparative dentin-promoting agents. *Dental Materials Journal, 30*(5), 583–597. [DOI:10.4012/dmj.2011-022] [PMID]

Kim, Y., Lee, D., Song, D., Kim, H. M., & Kim, S. Y. (2020). Biocompatibility and Bioactivity of Set Direct Pulp Capping Materials on Human Dental Pulp Stem Cells. *Materials (Basel, Switzerland), 13*(18), 3925. [DOI:10.3390/ma13183925] [PMID]

Komabayashi, T., & Zhu, Q. (2010). Innovative endodontic therapy for anti-inflammatory direct pulp capping of permanent teeth with a mature apex. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics, 109*(5), e75–e81. [DOI:10.1016/j.tripleo.2009.12.034] [PMID]

Krawczenko, A., & Klimczak, A. (2022). Adipose tissue-derived mesenchymal stem/stromal cells and their contribution to angiogenic processes in tissue regeneration. *International Journal of Molecular Sciences, 23*(5), 2425. [DOI:10.3390/ijms23052425] [PMID]

Mirkhani, S. M. H., Amini Sedeh, S., & Esfahanian, V. (2022). Comparison of osteoblastic differentiation of human periodontal ligament stem cells through application of two β-tricalcium phosphate products: An in vitro study. *Journal of Dentistry (Shiraz, Iran), 23*(1 Suppl), 183–189. [DOI:10.30476/dentjods.2021.86700.1350] [PMID]

Morotomi, T., Washio, A., & Kitamura, C. (2019). Current and future options for dental pulp therapy. *The Japanese Dental Science Review, 55*(1), 5–11. [DOI:10.1016/j.jdsr.2018.09.001] [PMID]

Obeid, M., Saber, S.elD., Ismael, A.elD., & Hassanien, E. (2013). Mesenchymal stem cells promote hard-tissue repair after direct pulp capping. *Journal of Endodontics, 39*(5), 626-631. [DOI:10.1016/j.joen.2012.12.012] [PMID]

Parirokh, M., Torabinejad, M., & Dummer, P. M. H. (2018). Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part I: vital pulp therapy. *International Endodontic Journal, 51*(2), 177–205. [DOI:10.1111/iej.12841] [PMID]

Peng, Z., Liu, L., Zhang, W., & Wei, X. (2021). Pluripotency of dental pulp cells and periodontal ligament cells was enhanced through cell-cell communication via STAT3/Oct-4/Sox2 signaling. *Stem Cells International, 2021*, 8898506. [DOI:10.1155/2021/8898506] [PMID]

Peskersoy, W., Lukarcanin,J., Turkun, M. (2021). Efficacy of different calcium silicate materials as pulp-capping agents: Randomized clinical trial. *Journal of Dental Sciences, 16*(2), 723–731. [DOI:10.1016/j.jds.2020.08.016] [PMID]

Petta, T. M., Pedroni, A. C. F., Saavedra, D. F., Faial, K. D. C. F., Marques, M. M., & Couto, R. S. D. (2020). The effect of three different pulp capping cements on mineralization of dental pulp stem cells. *Dental Materials Journal, 39*(2), 222–228. [DOI:10.4012/dmj.2018-349] [PMID]

Salvatore, G., De Felici, M., Dolci, S., Tudisco, C., Cicconi, R., & Campagnolo, L., et al. (2021). Human adipose-derived stromal cells transplantation prolongs reproductive lifespan on mouse models of mild and severe premature ovarian insufficiency. *Stem Cell Research & Therapy, 12*(1), 537. [DOI:10.1186/s13287-021-02590-5] [PMID]

Sharma, G., Puranik, M. P., & K R, S. (2015). Approaches to arresting dental caries: An update. *Journal of Clinical and Diagnostic Research: JCDR, 9*(5), ZE08–ZE11. [PMID]

Sharma, S., Srivastava, D., Grover, S., & Sharma, V. (2014). Biomaterials in tooth tissue engineering: A review. *Journal of Clinical and Diagnostic Research: JCDR, 8*(1), 309–315. [DOI:10.7860/jcdr/2014/7609.3937] [PMID]

Smith, J. A., van den Broek, F. A., Martorell, J. C., Hackbarth, H., Ruksenas, O., & Zeller, W., et al. (2007). Principles and practice in ethical review of animal experiments across Europe: Summary of the report of a FELASA working group on ethical evaluation of animal experiments. *Laboratory Animals, 41*(2), 143–160. [DOI:10.1258/002367707780378212] [PMID]

Solali, S., Kaviani, S., Soleimani, M., & Zonoubi Z. (2015). Isolation and characterization of mesenchymal stem cells derived from adipose tissue. *Koomesh, 16*(4), 505-511. [Link]

Torabinejad, M., Hong, C. U., McDonald, F., & Pitt Ford, T. R. (1995). Physical and chemical properties of a new root-end filling material. *Journal of Endodontics, 21*(7), 349-353. [DOI:10.1016/s0099-2399(06)80967-2] [PMID]

Torabinejad, M., Watson, T. F., & Pitt Ford, T. R. (1993). Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *Journal of Endodontics, 19*(12), 591–595. [DOI:10.1016/s0099-2399(06)80271-2] [PMID]

Tran, X. V., Gorin, C., Willig, C., Baroukh, B., Pellat, B., Decup, F., Opsahl Vital, S., Chaussain, C., & Boukpessi, T. (2012). Effect of a calcium-silicate-based restorative cement on pulp repair. *Journal of Dental Research, 91*(12), 1166–1171. [DOI:10.1177/0022034512460833] [PMID]

Tsuji, W., Rubin, J. P., & Marra, K. G. (2014). Adipose-derived stem cells: Implications in tissue regeneration. *World Journal of Stem Cells, 6*(3), 312-321. [DOI:10.4252/wjsc.v6.i3.312] [PMID]

Wang, X., Zhang, H., Nie, L., Xu, L., Chen, M., & Ding, Z. (2014). Myogenic differentiation and reparative activity of stromal cells derived from pericardial adipose in comparison to subcutaneous origin. *Stem Cell Research & Therapy, 5*(4), 92. [DOI:10.1186/scrt481] [PMID]

Wofford, A., Bow, A., Newby, S., Brooks, S., Rodriguez, R., & Masi, T., et al. (2020). Human fat-derived mesenchymal stem cells xenogenically implanted in a rat model show enhanced new bone formation in maxillary alveolar tooth defects. *Stem Cells International, 2020*, 8142938. [DOI:10.1155/2020/8142938] [PMID]

Zeng, Q., Chen, M., Zheng, S., Wei, X., & Liu, H. (2024). Efficacy of vital pulp therapy for carious pulp injury in permanent teeth: a study protocol for an open-label randomized controlled noninferiority trial. *Trials, 25*(1), 700. [DOI:10.1186/s13063-024-08559-y] [PMID]