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ORIGINAL RESEARCH

Examination of surface porosity of current pulp capping materials by micro-computed tomography (micro-CT) method

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Abstract

When dental pulp is exposed, it must be covered with a biocompatible material to form reparative dentine. The material used, besides being biocompatible, should have an ideal surface structure for the attachment, proliferation and differentiation of dental pulp stem cells. This study aimed to evaluate the porosity of the microstructures of four pulp capping materials using micro-computed tomography (micro-CT). Biodentine, Bioaggregate, TheraCal and Dycal materials were prepared according to the manufacturer's instructions using 2×9 mm Teflon molds. A total of 60 samples, 15 in each group, were scanned using micro-CT. Open and closed pores and the total porosity of the microstructures of the materials were assessed. The findings obtained from the study were analyzed *via* the Kruskal-Wallis test followed by the Mann-Whitney U test. The porosity of Bioaggregate was significantly higher than that of Biodentine, Dycal and TheraCal in all porosity values. While Biodentine did not show a statistically significant difference in open and total porosity values from either TheraCal or Dycal, closed porosity values of Dycal were significantly higher than those of Biodentine and TheraCal. Because of the affinity of cells to porous surfaces, the pulp capping materials' microstructure may affect the pulp capping treatment's success. From this perspective, the use of Bioaggregate in direct pulp capping may increase the success of treatment.

Keywords

Calcium hydroxide cement; Calcium silicate cements; Micro-CT; Porosity

1. Introduction

Caries are one of the most common chronic diseases in the world. When deep caries is completely removed, pulp exposure may occur [1]. It is thought that pulp exposure that occurs as a result of dental trauma affects approximately 20% of children and adolescents in the permanent dentition period [2]. Direct pulp capping treatment is defined as sealing the exposed pulp area after mechanical or traumatic exposure by directly applying dental materials to protect the pulp and provide the formation of reparative dentine [3]. Direct pulp capping treatment is appropriate for children, teenagers and young adults with primary and permanent teeth diagnosed as having healthy pulp [1]. A previous study demonstrated the success rate of direct pulp capping in primary molars with 84% cariously exposed pulp and suggested direct pulp capping with bioceramics as an acceptable treatment option [4]. In a recent study, pedodontists in Turkey preferred partial pulpotomy with mineral trioxide aggregate (MTA) at a rate of 35% for pulp exposures of 1–2 mm caused by mechanical perforation in young permanent teeth, while 32.9% were treated with direct pulp capping [5]. Direct pulp capping is also recommended by the American Academy of Pediatric Dentistry (AAPD) for pulp exposures smaller than 1 mm in vital primary teeth [6, 7].

Regeneration of the dentine/pulp structure in teeth in which the pulp is exposed requires the use of pulp capping materials that imitate the architecture of the natural dental extracellular matrix and create a suitable environment for the adherence, proliferation, differentiation and biomineralization of dental pulp stem cells (DPSCs) [8, 9]. For this purpose, varied materials, including calcium hydroxide and calcium silicate cements, have been more commonly used in pulp capping treatment [10, 11].

Calcium hydroxide is a traditional pulp capping material and has long been accepted as the gold standard for pulp capping material in dentistry. However, calcium hydroxide can be resorbed over time [12], and when it is used, tunnel defects can cause bacteria and toxins to leak into the pulp, causing the pulp capping treatment to fail [13, 14]. There are several forms of calcium hydroxide, including Dycal, which was developed by L. D. Caulk Co. (Milford, DE, USA). Its main ingredient is calcium hydroxide. Since it is a low-cost material compared to newly emerging pulp capping materials, calcium hydroxide is still extensively used today. MTA is commonly used as an alternative to calcium hydroxide to form direct pulp capping. It has superior biocompatibility and effective sealing ability. However, MTA has major drawbacks, such as slow setting kinetics, dentine discoloration and complicated handling properties [15, 16]. Studies have shown that MTA causes grayish discoloration of the tooth structure [17, 18]. This outcome limits the use of MTA in anterior teeth [17, 18]. Due to these unfavorable effects, calcium silicate-based materials with different structures, such as Biodentine, Bioaggregate and TheraCal, have been introduced in dentistry to overcome some of these drawbacks.

Biodentine is a new calcium silicate-based restorative cement with mechanical properties similar to dentine. Like MTA, it can replace dentine in crowns and roots [19]. It has a beneficial effect on healthy pulp tissue when used directly in pulp capping therapy and stimulates reparative dentine formation [16]. Its main component is tricalcium silicate, and it also contains calcium carbonate and zirconium oxide. Its liquid is a calcium chloride solution containing a water-reduction agent [20].

Bioaggregate contains tricalcium silicate, dicalcium silicate, calcium phosphate monobasic, amorphous silicon dioxide and tantalum pentaoxide, which replaces the bismuth oxide used in traditional MTA for radiopacity [19]. Unlike conventional MTA, it contains no aluminum, which reduces its toxic effects on tissues [21]. Studies have shown comparable bioactivity, biocompatibility and sealing ability of Bioaggregate to those of MTA [19, 21, 22].

TheraCal (Bisco, Schaumburg, IL, USA) is a novel resin-containing light-cured calcium silicate-based material that acts as a pulp-capping material in vital pulp treatment. It has been stated that, much like other calcium silicate-based materials, TheraCal provides superior sealing and strong bioactivity in the pulp exposure area [23].

The extracellular matrix in the body is a three-dimensional (3D) network with nanostructures. Nanostructured architecture for tissue regeneration provides better microenvironments for cell adhesion, proliferation and differentiation [24]. Hence, biomaterials with micro- or nanostructured architecture have been developed and used in tissue engineering studies [25, 26]. This finding indicates that pores and porosities within the physical structure of the pulp capping materials may also be important in the success of pulp capping treatment, in addition to the importance of biocompatibility and the ability to stimulate the formation of reparative dentine. To the best of our knowledge, no study in the literature has evaluated the porosity of pulp capping materials for its importance in pulp capping treatment. Therefore, in this *in vitro* study, the aim was to evaluate and compare the porosity of four pulp capping materials using micro-computed tomography (micro-CT) and correlate their porosities with their effects as reported in previous pulp capping studies.

2. Materials and methods

2.1 Preparation of samples

The materials used in the study and their contents are given in Table 1.

In brief, all test materials were prepared following the manufacturer's instructions and then placed in Teflon molds (diameter: 9 mm, height: 2 mm) with the help of a carrier. Fifteen samples of each material were created (Fig. 1A). The materials were allowed to set: 4 hours for Bioaggregate, 12 minutes for Biodentine, 2 minutes for Dycal and immediately for TheraCal, because it polymerizes with the use of light from a II-generation light emitting diode (LED) light-curing device (Guilin Woodpecker Medical Instrument Co. Guilin, China; light intensity: 1200 mW/cm^2). Materials requiring time to set were kept at 37 °C in a 100% humidity environment.

2.2 Assessment of porosity

The characterization of the surface porosity of the materials was analyzed by micro-computed tomography (micro-CT) (Skyscan-Bruker 1172, Kontich, Belgium). After adjusting the camera's pixel size to 9 μm, the voltage to 100 kV, the milliampere to 100, the reconstructor rotation angle to 360°, and the rotation angle to 2°, all samples were scanned for approximately one hour. Images were reconstructed using NRecon software (version 1.6.10.6, Skyscan, Kontich, Belgium) (Fig. 1B). The volume of open and closed pores on the surface of the objects formed in three dimensions, as well as the percentage volume of the porosity, was examined using the CT Analyser program (version 1.16.4.1, Skyscan, Kontich, Belgium).

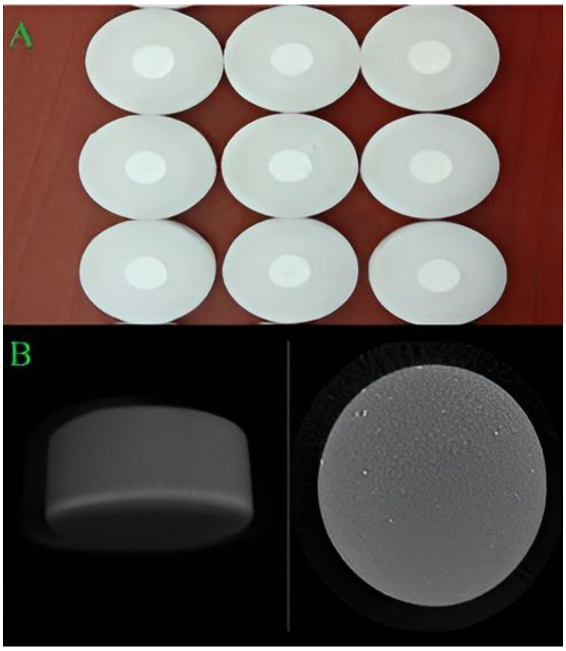

FIGURE 1. Preparation of samples and scanning for analysis. (A) Materials prepared in Teflon molds. (B) Micro-CT scanned image of a sample.

2.3 Volume of pores

The pores in the material, starting from a circular surface and forming a whole space on the other surface, are called closed pores. If the pores in the material begin from a surface and form half a gap, they are called open pores. The volume of the open

TABLE 1. Composition of pulp capping materials.

Material name	Contents	Manufacturer
Dycal		
	Base: 1,3-Butylene glycol disalicylate	
	Zinc oxide	
	Calcium phosphate	
	Calcium tungstate	
	Iron oxide pigments	
	Catalyst: Calcium hydroxide	Dentsply Caulk, Milford, DE, USA
	N-ethyl-o/p-toluene sulfonamide	
	Zinc oxide	
	Titanium dioxide	
	Zinc stearate	
	Iron oxide pigments (dentine color only)	
TheraCal LC		
	Type III Portland cement	
	Sr glass	
	Fumed silica	
	Barium sulfate	Bisco, Inc, Schaumburg IL, USA
	Bisphenol A glycidylmethacrylate	
	Poly dimethacrylate	
	Barium zirconate	
Biodentine		
	Powder: Tricalcium silicate	
	Dicalcium silicate	
	Calcium carbonate	
	Calcium oxide	
	Iron oxide	Septodont, Saint-Maur-des-fossés Cedex, France
	Zirconium oxide	
	Liquid: Calcium chloride	
	Hydrosoluble polymer	
	Distilled water	
Bioaggregate		
	Tricalcium silicate	
	Dicalcium silicate	
	Tantalum pentoxide	Innovative Bioceramics, Vancouver, Canada
	Monobasic calcium phosphate	
	Amorphous silicon dioxide	

and closed pores was calculated separately as a percentage. The sum of the open and closed pores gives the porosity of the materials.

2.4 Data analysis

IBM SPSS Statistics 22 for Windows software (IBM Corp. Released 2013. Version 22.0. Armonk, NY, USA: IBM Corp.) was used to carry out the statistical evaluation of the data obtained from the study. A non-parametric Kruskal-Wallis test was utilized since the quantitative data of the groups did not display normal distribution ($p < 0.05$). Significant differences among the groups were determined with the Kruskal-Wallis test ($p < 0.05$). The Bonferroni corrected Mann-Whitney U test was applied for pairwise comparisons of the groups. The significance levels were determined to be 0.008 for the Bonferroni corrected Mann-Whitney U test and 0.05 for other overall analyses.

3. Results

Significant differences among the groups were identified in open, closed and total porosity values ($p < 0.05$; Kruskal-Wallis H test). Results demonstrated that the porosity of Bioaggregate was significantly higher than Biodentine, Dycal and TheraCal materials in all porosity values ($p < 0.008$; Bonferroni corrected Mann-Whitney U test) (Table 2, Fig. 2).

While Biodentine did not show statistically significant differences in open and total porosity values as compared to TheraCal and Dycal materials ($p > 0.008$), Dycal was significantly higher in closed porosity values compared to Biodentine and TheraCal ($p < 0.008$) (Table 1).

The micro-CT images of the material interface are shown in Fig. 3. Bioaggregate was observed to be a much more porous structure than the others (Fig. 3A,B).

4. Discussion

The materials used in pulp capping treatment have to be biocompatible and provide a suitable microenvironment for dental pulp stem cells (DPSCs) to regenerate the dentine–pulp complex. In dentine–pulp regeneration, the success of the pulp capping treatment can be increased *via* the development of pulp capping materials with structures that allow DPSCs to adhere and create reparative tissues [27]. In one study, a three-dimensional nanofibrous gelatin scaffold with high porosities was created that mimics the nanostructure and chemical content of the dental extracellular matrix. The *in vitro* study showed that the nanofibrous gelatin scaffold offered a microenvironment superior to that of standard gelatin equivalents for cell adhesion, proliferation and differentiation [28]. In another study, Gupte and Ma [29] stated that porous scaffolds allow precursor cells to migrate into the material, providing a suitable environment for them to adhere, spread and proliferate. Bordini *et al*. [9] stated that the increase in cell proliferation in porous chitosan-calcium-aluminate scaffolds, when compared with scaffolds containing only chitosan, may be related not only to the scaffold composition but also to the scaffold architecture. Based on these studies, the porous structure of the pulp capping materials may increase the adhesion, proliferation and differentiation of the DPSCs for reparative dentine formation. Therefore, in this study, we compared the porosities of the four current pulp capping materials using micro-CT and correlated their porosities with their effects in previous pulp capping studies.

The presence of biologically active, semiporous material placed on the exposed pulp may increase the activation of DPSCs, cell colonization in areas adjacent to the material, and the formation of reparative dentine. The high rate of calcium release and apatite formation are the signals that appear at different times for DPSCs. This situation may explain the role and function of the calcium silicate biomaterials that stimulate the formation of new reparative tissues and clinical healing [30]. Therefore, in the present study, we preferred to use Biodentine, TheraCal and Bioaggregate as the new calcium silicate materials.

Calcium hydroxide is the traditional pulp capping material, and it has been accepted as the gold standard of pulp capping material for a very long time. The first outcome of calcium hydroxide placement on the exposed pulp area is the appearance of superficial necrosis due to its high potential of hydrogen (pH) (12.5) [31, 32]. When necrosis occurs, the pulp is stimulated to defend and mend itself by creating a reparative dentine bridge *via* the processes of cellular differentiation, extracellular matrix secretion and mineralization [32]. In the study by Modena *et al*. [31], calcium hydroxide was much more cytotoxic, reduced cell metabolism of cultured human dental pulp fibroblasts, and caused severe cell death. Canoğlu *et al*. [33] evaluated the success of direct pulp capping of primary molars with calcium hydroxide and mineral trioxide aggregate (MTA) after hemorrhage control with different solutions. Their study found the clinical success rate of MTA to be 98.3%, while the success of calcium hydroxide was 89.7%. Since it is a lower-cost material than newly emerging pulp capping materials, it continues to be used extensively today. Therefore, in this study, we used Dycal, a calcium hydroxide paste, to compare with calcium silicate-based materials.

Recent studies have shown that Bioaggregate is non-toxic to osteoblasts and human periodontal ligament cells, and it enhances mineralization and odontoblastic differentiation (concerning human DPSCs) [34–37]. In a study comparing the effects of ProRoot MTA, Biodentine and Bioaggregate on the formation of reparative dentine, the dental pulps of rats were covered with these materials, and the formation of the reparative tissue was examined histologically, immunohistochemically and with the use of micro-CT after four weeks [38]. Favorable effects on reparative processes were observed in all materials. The relative ratio of newly formed reparative dentine on micro-CT analysis was lower in Bioaggregate compared to MTA and Biodentine. However, Bioaggregate revealed a dense mineralized dentine on histological analysis [38]. The present study showed that the porosity of Bioaggregate was superior to other materials. This greater porosity of Bioaggregate may be a contributing factor in the dense mineralized dentine formation with Bioaggregate reported in the study by Kim *et al*. [38]. Kayad *et al*. [39] used a rabbit model to compare the effects of TheraCal and Biodentine on direct pulp capping. TheraCal created a dentine bridge

TABLE 2. Descriptive statistics of pulp capping materials.

Porosity (%)	Group	n	Mean Percentage	Std. Deviation	Minimum	Maximum
Open						
	Bioaggregate	15	5.47a	3.16	1.96	13.14
	Biodentine	15	0.04b	0.03	0.02	0.12
	Dycal	15	0.14b	0.14	0.01	0.41
	Theracal	15	0.07b	0.11	0.00	0.35
	Total	60	1.43	2.81	0.00	13.14
*p** value			0.0095			
Closed						
	Bioaggregate	15	3.29a	0.72	1.96	4.55
	Biodentine	15	0.13b	0.08	0.05	0.31
	Dycal	15	0.27c	0.16	0.08	0.64
	Theracal	15	0.09b	0.11	0.01	0.38
	Total	60	0.94	1.41	0.01	4.55
*p** value			0.0085			
Total						
	Bioaggregate	15	8.59a	2.56	6.09	15.52
	Biodentine	15	0.21b	0.09	0.09	0.36
	Dycal	15	0.35b	0.20	0.05	0.75
	Theracal	15	0.59b	1.69	0.01	6.67
	Total	60	2.43	3.89	0.01	15.52
*p** value			0.0096			

**Kruskal-Wallis test; a, b, c: Letters in the column show a significant difference ($p < 0.008$; Bonferroni corrected Mann-Whitney U test).*

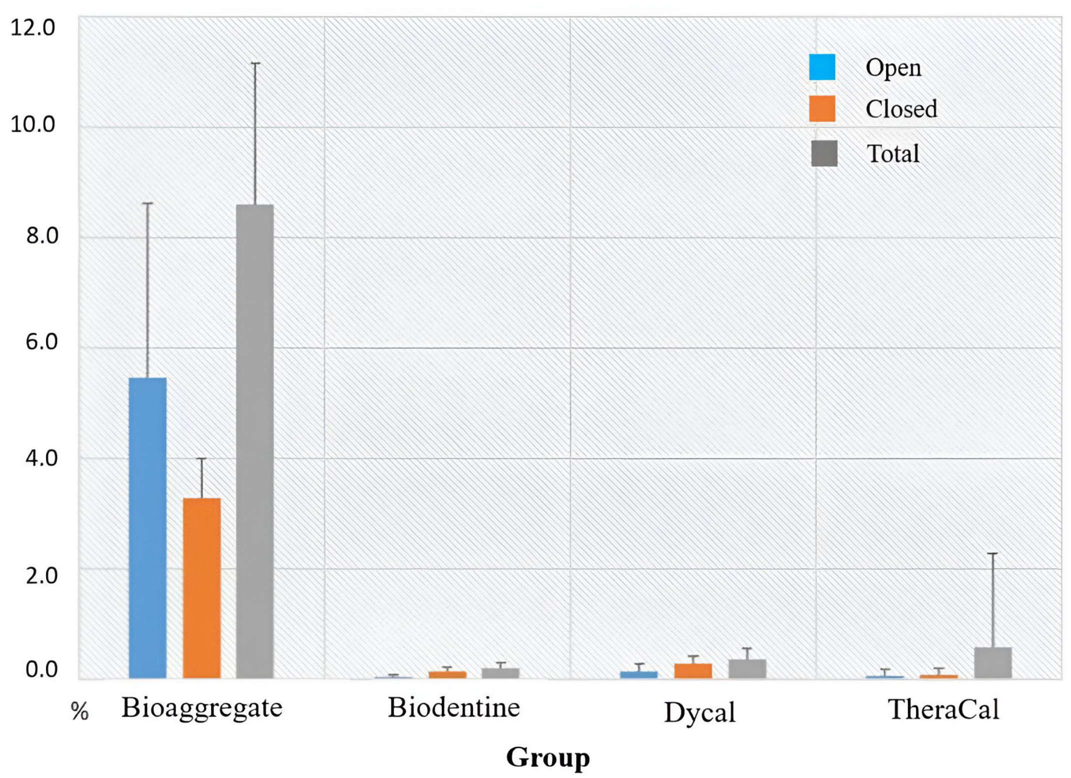

FIGURE 2. Graphical representation of the groups for each porosity values.

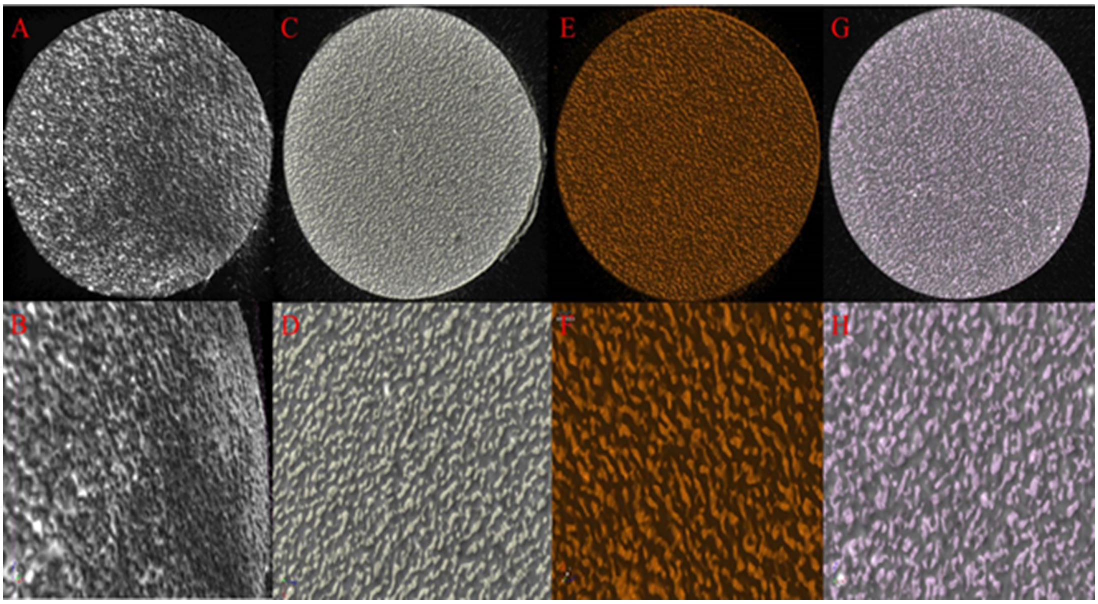

FIGURE 3. Micro-CT images of the materials' microstructure. (A,B) Bioaggregate, (C,D) Biodentine, (E,F) Dycal, (G,H) TheraCal (20× magnification).

in the second week, while Biodentine created a significantly thicker dentine bridge in the first and second weeks. The results of a randomized clinical trial showed that TheraCal was less successful than Biodentine but better than Dycal when evaluated clinically and radiographically [40]. Alazrag *et al.* [41] stated that the biocompatibility of TheraCal as a furcation perforation repair material is lower than that of MTA-Angelus and Biodentine. Kunert *et al.* [42] investigated the biocompatibility of commercially available bioactive materials. They found the cytotoxicity of TheraCal to be quite high compared to other bioactive materials. Collado-González *et al.* [43] stated that stem cells from human exfoliated primary teeth adhere and spread well on the surface of MTA-Angelus and Biodentine due to the good porosity of their microstructures. Another study comparing MTA with Biodentine revealed that Biodentine exhibited a greater capacity to increase the viability, adhesion and migration of human DPSCs [44]. The results of these studies demonstrate that calcium silicate-based Biodentine and Bioaggregate can be alternatives to MTA in the formation of reparative tissues for vital pulp therapies.

A study employing micro-CT to compare retrograde filling using Biodentine, Bioaggregate and MTA showed that the porosity of Biodentine was lower (in acidic pH) than that of the other materials, as in this study [45]. Similar to our study, Al-Sherbiny *et al.* [46] found Biodentine to be the material with the lowest open porosity. They also examined the porosity of capping materials, including Biodentine and MTA. Another study, in which the porosity of calcium silicate-based materials, including Bioaggregate and Biodentine, was examined by mercury porosimetry, found that the porosity of Bioaggregate was higher than that of Biodentine [47]. Similarly, we found that Bioaggregate had significantly higher porosity values than Biodentine and other materials tested. In a study investigating the porosity of calcium silicate-based materials, the porosity of TheraCal was found to be similar to Biodentine [30]. Notably, Biodentine did not show statistically significant differences from TheraCal in any porosity values in the current study.

The results of a study comparing the success of TheraCal and Dycal in direct pulp capping showed no significant difference between the two materials [48]. Grewal *et al.* [12] compared the direct contact of Biodentine and Dycal to dentine tissue in pulpotomy treatment and reported that Biodentine provided significantly thicker reparative dentine formation. Jalan *et al.* [49] also stated that Biodentine provides thicker and more continuous tertiary dentine formation than calcium hydroxide. In the present study, when we compared the porosities of calcium hydroxide, TheraCal and Biodentine, we found no significant difference between the materials in their open and total porosity values. However, there was a significant excess of Dycal from Biodentine and TheraCal in the closed-pore ratio. This reveals that the porosity of capping materials, as well as various other properties, affect the success of pulp capping.

The success of capping treatments also depends heavily on the physical and mechanical characteristics of the pulp capping materials. Majeed *et al.* [50] evaluated the push-out bond strength and surface microhardness of Biodentine, Bioaggregate and ProRoot MTA in coronal and apical root dentine. Of the three, Bioaggregate showed greater stable bond strength (0% adhesive-type bond failure) but inferior mechanical characteristics and bond strength [50]. Kucukyıldız *et al.* [51] found Dycal to be considerably lower than MTA-Angelus in terms of the microhardness value in their study. When TheraCal, Biodentine and MTA-Angelus were evaluated in terms of marginal adaptation, the highest frequency distribution of gap presence was observed in TheraCal, while the lowest ratio was seen in Biodentine [41]. The results of the same study showed that TheraCal had the lowest solubility, while Biodentine had the highest. The presence of resin in the structure of TheraCal may have reduced its biocompatibility and marginal adaptation while reducing its solubility [41]. Gupta *et al.* [52] stated that Biodentine as a root-end filling material caused less microleakage than Bioaggregate and MTA. However, no significant difference was observed between Bioaggregate and Biodentine.

The clinical usability of capping materials should also be considered. When evaluated in terms of clinical use, Biodentine takes 12–13 minutes to set, which is considerably less time than MTA (2.20–2.45 hours). Bioaggregate sets in 4 hours and requires permanent restoration after the final set. If the final restoration must be placed on the same treatment, a long setting time becomes a clinical disadvantage [53]. Dycal sets in approximately 2 minutes after application, whereas TheraCal is light-cured, which is advantageous for these materials. The bond strength of the capping material to the restorative material is also an important factor for clinical success. A recent review stated, despite the absence of data for some calcium silicate cements such as Bioaggregate, that Biodentine has a higher bond strength to the dentinal wall than other studied calcium silicate cements when blood is present [54]. Raina *et al*. [55] evaluated the bond strength of Dycal, MTA Plus, Biodentine and TheraCal in self-adhering and bulk-fill flowable composites and found that the lowest bond strength value occurred in Dycal, while the highest was with TheraCal in both groups. When comparing Biodentine and MTA, Biodentine showed a higher bond strength value than MTA. TheraCal's resin-based structure, which facilitates chemical adhesion and forms a robust interface between TheraCal and the bonding material, may be responsible for the high bonding strength of the product.

The search for new alternative solutions for pulp capping continues. Qiu *et al*. [56] stated that the calcium salt of 4-methacryloxyethyl trimellitate acid (4-MET), a novel bioactive adhesive monomer called CMET, exhibits advantageous biocompatibility and odontogenic induction potential. CMET may be a very promising adjuvant for pulp-capping materials. Franzin *et al*. [57] evaluated the mechanical, physicochemical and antimicrobial properties of a novel pulp capping material based on sodium trimetaphosphate. The experimental groups were two micro-(mTMP) and two nanoparticulate sodium trimetaphosphate (nTMP) groups, each containing zirconium oxide (ZrO_2), and a solution containing either chitosan or titanium oxide (TiO_2) nanoparticles (NPs). The compressive strength was higher and the setting time was shorter in the nTMP-containing groups, and those that contained TiO_2 nanoparticles also exhibited antimicrobial properties. Utilizing bioactive compounds in pulp capping agents has the potential to promote quick and more effective pulp capping [58]. Novel chitosan membranes with microspheres containing transforming growth factor beta 1 (TGF-β_1) for pulp capping provided 3–6 times more reparative dentine than Dycal or chitosan bilayer membranes without TGF [59]. Experiments on acute pulp repair in rats demonstrated the superior dentine regeneration characteristics of Zn-doped bioactive glass (BGz) micro-nano spheres when utilized as a pulp capping agent [60].

The limitations of this study were: 1. The positive effect of the materials directly on pulp capping was evaluated based only on their porosity. However, pulp capping material affects the success of pulp capping due to many other features. 2. The relationship between the porosity ratio, compressive strength and microleakage of the materials used in the study was not evaluated. Increasing porosity may lead to the failure of pulp capping by reducing the strength of the material under pressure and causing microleakage. For these reasons, comprehensive *in vivo* studies are needed to evaluate the degree of the effect of the porosity of pulp capping materials on the success of pulp capping treatment.

According to the results of this *in vitro* study, Bioaggregate was found to be superior to the other pulp capping materials in terms of porosity. In previous *in vivo* studies [27, 38] conducted on Bioaggregate, it was observed that this material yielded successful results in the formation of reparative dentine. Since the DPSCs, which play a role in forming reparative dentine, can be positioned in the porous structure of Bioaggragate, its porous structure may also play a role in the success of pulp capping treatment. However, in *in vivo* studies [12, 33, 39, 40, 48, 49] performed with other, less porous pulp capping materials, successful results were obtained. Thus, further studies should be conducted to determine which properties of the materials used in the pulp capping treatment primarily affect the success.

5. Conclusions

The high surface porosity of Bioaggregate may be a factor that increases the chance of successful pulp capping treatment by providing better adhesion and proliferation of human dental pulp cells.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

BD and MY—designed the research study. BD—performed the research; wrote the manuscript. MY—analyzed the data. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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