



EVALUATION OF THE ADHESION AND MORPHOLOGY OF STEM CELLS FROM APICAL PAPILLA IN DIRECT CONTACT WITH MTA AND BIODENTINE

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ABSTRACT:

Purpose: To investigate the use of tricalcium silicate cement Biodentine as an alternative to MTA in regenerative endodontic procedures by assessing the morphology and adhesion of stem cells from apical papilla in direct contact with these materials

Materials/Methods: Glass ionomer cement, MTA and Biodentine disks with identical size were created. Stem cells from apical papilla were incubated on these disks, and their size and morphology were assessed with a scanning electron microscope.

Results: No adhesion of SCAP was observed when incubating cells with glass ionomer disks, which can be explained by a decrease in pH and a delay in cell proliferation. Cell adhesion was observed in the groups incubated in the presence of MTA and Biodentine discs. Cells adhered to the calcium silicate cements have different morphology and can be round or flat, with several processes.

Conclusions: Stem cells from apical papilla adhere to MTA and Biodentine when in direct contact with discs made of these materials. The cells show different morphology and can be round or flat, with several processes. Calcium silicate cements have biocompatibility and bioactivity and they are suitable materials for the biological treatment of pulp and regenerative endodontics.

Keywords: cell adhesion, SCAP, MTA, Biodentine, regenerative endodontics,

INTRODUCTION

Cell adhesion plays a crucial role in cellular communication and it is fundamental to tissue development and regeneration. Cell adhesion is the ability of a single cell to attach to another cell or extracellular matrix. Most cells are firmly attached to a substrate *in vitro* [1]. Cell adhesion is associated with stimulatory signals that regulate cell differentiation, cell cycle, migration and renewal [2]. The affinity of cells to the substrate is extremely important in the creation and implementation of biomaterials. Adhesion is also essential for intercellular signaling and regulation [2].

There are different requirements for cell adhesion depending on the field of application and cell-specific characteristics [3]. Various techniques are used to analyze it in the study of biomaterials, the impact of environmental signals on cell cultures and to determine the adhesion properties of healthy or neoplastic cells [4, 5].

Many studies are trying to investigate the complex effects of bioactive materials on living tissues, especially on dental pulp. Calcium hydroxide has been used to treat pulp diseases for decades [6]. Due to its high pH, it causes superficial necrosis and a moderate inflammatory response in the pulp.

The healing process is initiated by resolving the inflammation, stem cell differentiation and formation of tertiary dentin [7]. In recent years, calcium silicate cements (CSCs) have increasingly replaced calcium hydroxide due to fewer adverse effects and a higher clinical success rate [8]. MTA is the gold standard in pulp therapy nowadays. It is also used in the treatment of perforations and for the creation of apical plugs in permanent teeth with incomplete root development [8].

MTA is a calcium silicate cement and it consists mainly of tricalcium silicate, dicalcium silicate and tricalcium aluminate, which form a matrix of calcium silicate hydrate during setting [9]. Its bioactivity is due to apatite precipitates, which are formed upon contact with phosphate-containing liquids and are deposited on the surface of the material [8, 9].

MTA is an expensive material with poor handling properties, a long setting time of several hours and a high chance of tooth discoloration, which makes its application challenging despite its proven clinical success [10]. New calcium silicate cements with optimized material properties, such as Biodentine, have been developed, in order to address these disadvantages. In addition to the typical components of tricalcium silicates, it contains fillers of calcium carbonate to improve mechanical stability, as well as zirconium dioxide [11]. The liquid is supplemented with plasticizing polycarboxylate and calcium chloride to improve its handling properties and decrease the setting time [12]. The material is biocompatible and it induces den-

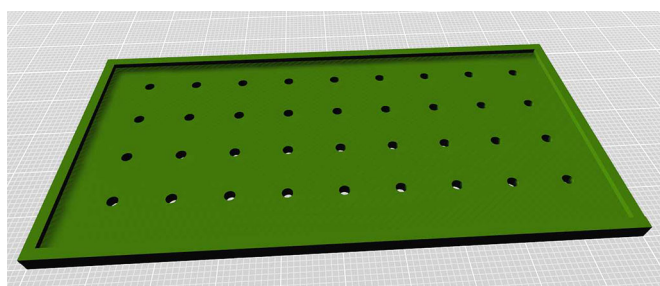
tin bridge formation with properties comparable to that of MTA [13]. This makes the new CSCs a convenient and affordable alternative to the MTA.

The aim of the present study was to investigate the use of tricalcium silicate cement Biodentine as an alternative to MTA in regenerative endodontic procedures by assessing the morphology and adhesion of stem cells from apical papilla in direct contact with these materials.

MATERIALS AND METHODS

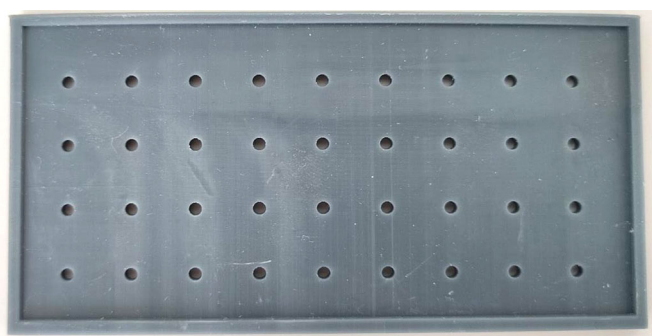
Using the online 3D computer modeling program Tinkercad (Autodesk, San Rafael, California, United States), a plate with 36 holes with a diameter and height of 2 mm was created. The boundaries of the plate were modelled with a height of 4 mm in order to prevent its deformation and bending during the printing process (Figure 1).

Fig. 1. Plate with holes, modelled in CAD-program



The prepared model was exported to a stl-file and three samples were printed with a 3D - printer Form 3 (Formlab, Somerville, Massachusetts, United States). The printed plate is shown in Figure 2.

Fig. 2. Printed plated with calibrated openings.



In each of the plates were prepared 36 disks of the tested materials according to the manufacturers' instructions:

- First group - Biodentine (Septodont, Saint-Maur-des-Fossés, France), further condensed with a plugger and ultrasonic tip;
- Second group - MTA (MTA Angelus, Angelus,

Lindoiia, Brazil), condensed with a plugger and ultrasonic tip;

- Third group (control) - glass ionomer cement (G-Cem, GC Corporation, Tokyo, Japan).

From each group, 20 disks with preserved shape and size were selected, which were sterilized with ultraviolet light in a laminar flow cabin for 30 minutes (Figure 3).

Fig. 3. Disk, prepared for sterilization and incubation of stem cells



Stem cells from apical papilla (SCAP) were isolated from third molars with incomplete root development, extracted for orthodontic purposes. Informed consent was signed by the patients or their parents. The apical papillae were carefully removed from the root and cut into small pieces with a scalpel blade. Then they were placed in 1ml solution of 3 mg/ml collagenase type I and 4 mg/ml dispase for 1hat 37 ° C, 5% CO2 and 50% humidity in an incubator. The suspension was centrifuged for 4 min at 3000 rpm and the precipitated cells were resuspended in 1 ml of culture medium. The cells were seeded in 2 cm diameter plastic plates (Greiner Bio-One, Frickenhausen, Germany) with culture medium and 20% fetal bovine serum and cultured at 37 ° C, 5% CO2 and 50% humidity.

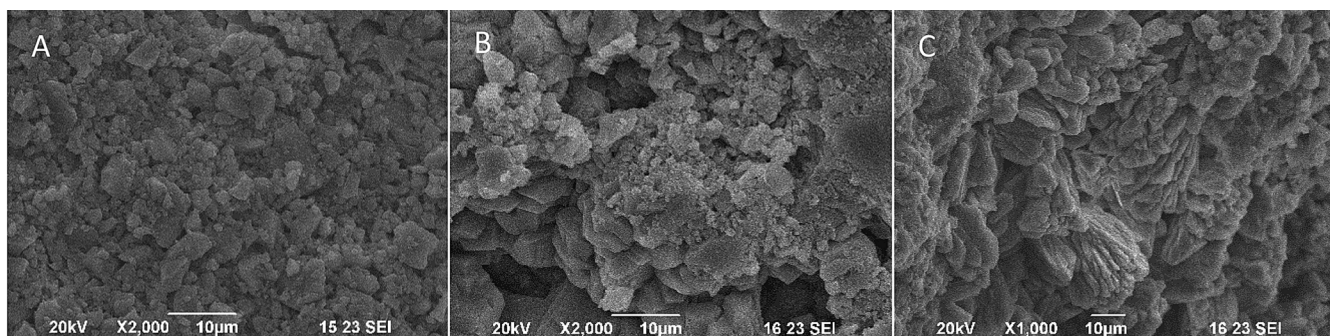
The cells from the third passage were incubated on pre-prepared disks of test materials in 96-well plates for 24 hours at 37 ° C, 5% CO2 and 50% humidity. Additionally, several segments of each group were placed in a control medium without cell incubation.

After the incubation period, the samples were fixed with 3% glutaraldehyde in 0.1 M phosphate buffer and observed by scanning electron microscope (SEM). The morphology of the cells and the presence or absence of cell processes were determined.

RESULTS

Figure 4 shows representative SEM images of the studied materials without cell incubation.

Fig. 4. SEM images of GIC (A), Biodentine (B) and MTA (C) without cell incubation.



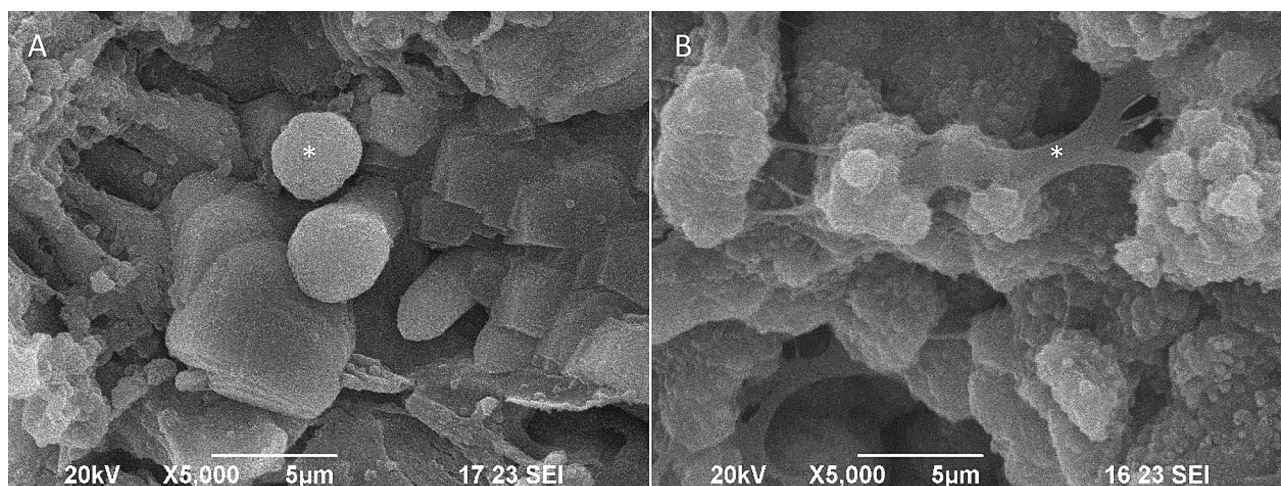
The obtained images show a typical characteristic of cements after immersion in a buffer solution with the presence of irregularly shaped crystals in GIC (Figure 4A) or fibrillar, rhomboid and hexagonal shaped crystals in MTA and Biodentine (Figure 4B, C).

No adhesion of SCAP was observed when incubating cells with glass ionomer disks, which can be explained

by a decrease in pH and a delay in cell proliferation, as evident by the paler color of the culture medium. Cell adhesion was observed in the groups incubated in the presence of MTA and Biodentine discs (Figure 5, asterisk).

Cells adhered to the CSCs have different morphology and can be round (Figure 5A) or flat, with several processes (Figure 5B).

Fig. 5. SEM images of MTA (A) and Biodentine (B), showing the morphology and adhesion of SCAP (asterisk).



DISCUSSION

The experimental setup in the presented study mimics the clinical protocol in a standard regenerative endodontic procedure, which allowed us to draw our own conclusions. No cell adhesion was observed in the GIC samples, which may be due to impaired SCAP proliferation as a result of acidification of the culture medium or a change in the physicochemical characteristics of the material upon contact with it. Studies have shown that the viscoelastic properties of materials alter the ability of cells to attach, migrate and differentiate significantly [14]. At the same time, some of these materials could stimulate cell adhesion by creating surface roughness. Despite the fact that GIC have pronounced surface roughness, no cell adhesion was observed, which probably confirms the observation that the low pH inhibits cell proliferation.

Ultraviolet light was used in the present study to disinfect the samples, because it does not have a negative effect on the surface properties of dentin and endodontic ma-

terials, unlike autoclaving, and this would not interfere with cell proliferation. Dental pulp stem cells proliferate on the surface of MTA, dentin filings and Bio-Oss, with the slowest proliferation on Bio-Oss, showing a much higher ability of MTA and dentin to attract cells. This defines them as good materials for pulp regeneration [15].

The use of new products in the biological treatment of pulp or in regenerative endodontics requires the creation of a barrier for microorganisms and blood clots and necessitates adequate biocompatibility and bioactive properties to induce tissue repair or stimulate cell differentiation and regeneration [16, 17]. A high concentration of Biodentine in the culture medium significantly reduces the proliferation of stem cells, which is probably due to the fact that this CSC increases their differentiation [18]. This property may be useful during regenerative endodontic procedures.

Cell adhesion is a good indicator for quality assessment of the used materials, as biocompatible cements main-

tain cell adherence [19]. The use of SEM provides valuable information about the behavior of cells in direct contact with biomaterials. Sample preparation involves fixation and dehydration, which may lead to changes in the surface or composition of the materials and related problems for quality and complete observation of the samples [20]. In the present study SCAP adhere to MTA and Biodentine disks, with cells having different morphology. Spindle-shaped or flat cells with processes observed in contact with biomaterials are a good indicator of their low cytotoxicity [19]. Cells of this shape were also observed in this study (Figure 5).

MTA and Biodentine are known to stimulate the adhesion of dental pulp stem cells and gingival fibroblasts [21]. The materials have very good sealing properties, as Biodentine allows better isolation of the root canal and furcation defects [22]. In some cases, with MTA-based materials, cell adhesion is limited by the presence of several round cells on the surface of the material [17]. However, another study showed that cells in contact with MTA have the same morphology as those that grow only in a culture medium [23]. In contrast, cells in contact with Biodentine are covered with calcium ions and exhibit the morphological characteristics of apoptotic cells. One possible explanation is that the high concentration of calcium

ions in this case leads to changes in the cytoskeleton and destruction of microvilli resulting in the onset of a signaling cascade, which can lead to the activation of apoptosis. It is possible that the differences in morphology are due to different types of adhesion that depends on the type of substrate on which the cells grow [23]. The composition of both types of cement should not be neglected. MTA uses bismuth oxide for an opacifier, and Biodentine uses zirconium oxide, which is not toxic to somatic human cells or murine fibroblasts [24].

CONCLUSION

The present study proves that SCAP adhere to MTA and Biodentine when in direct contact with discs made of these materials. The cells show different morphology and can be round or flat, with several processes. The results support the reported in the literature properties of biocompatibility and bioactivity of CSCc and their indisputable indications as suitable materials for biological treatment of pulp and regenerative endodontics.

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REFERENCES:

1. Sagvolden G, Giaever I, Pettersen EO, Feder J. Cell adhesion force microscopy. *Proc Natl Acad Sci USA*. 1999 Jan 19;96(2):471-6. [[Crossref](#)]
2. Huang S, Ingber DE. The structural and mechanical complexity of cell-growth control. *Nat Cell Biol*. 1999 Sep;1(5):E131-8. [[Crossref](#)]
3. Huang W, Anvari B, Torres JH, LeBaron RG, Athanasiou KA. Temporal effects of cell adhesion on mechanical characteristics of the single chondrocyte. *J Orthop Res*. 2003 Jan; 21(1):88-95. [[Crossref](#)]
4. Palmer CP, Mycielska ME, Burcu H, Osman K, Collins T, Beckerman R, et al. Single cell adhesion measuring apparatus (SCAMA): application to cancer cell lines of different metastatic potential and voltage-gated Na⁺ channel expression. *Eur Biophys J*. 2008 Apr;37(4):359-68. [[Crossref](#)]
5. Kwon KW, Choi SS, Lee SH, Kim B, Lee SN, Park MC, et al. Label-free, microfluidic separation and enrichment of human breast cancer cells by adhesion difference. *Lab Chip*. 2007 Nov;7(11):1461-8. [[Crossref](#)]
6. Schroder U. Effects of calcium hydroxide-containing pulp-capping agents on pulp cell migration, proliferation, and differentiation. *J Dent Res*. 1985 Apr;64 Spec No:541-8. [[Crossref](#)]
7. Briso AL, Rahal V, Mestreneur SR, Dezan Junior E. Biological response of pulps submitted to different capping materials. *Braz Oral Res*. 2006 Jul-Sep;20(3):219-25. [[Crossref](#)]
8. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review—part II: leakage and biocompatibility investigations. *J Endod*. 2010 Feb;36(2):190-202. [[Crossref](#)]
9. Darvell BW, Wu RC. “MTA”-an Hydraulic Silicate Cement: review update and setting reaction. *Dent Mater*. 2011 May;27(5):407-22. [[Crossref](#)]
10. Oliveira LV, da Silva GR, Souza GL, Magalhaes TEA, Barbosa GLR, Turrioni AP, et al. A laboratory evaluation of cell viability, radiopacity and tooth discoloration induced by regenerative endodontic materials. *Int Endod J*. 2020 Aug;53(8):1140-52. [[Crossref](#)]
11. Nowicka A, Lipski M, Parafiniuk M, Sporniak-Tutak K, Lichota D, Kosierkiewicz A, et al. Response of human dental pulp capped with biodentine and mineral trioxide aggregate. *J Endod*. 2013 Jun; 39(6):743-7. [[Crossref](#)]
12. Luo Z, Kohli MR, Yu Q, Kim S, Qu T, He WX. Biodentine induces human dental pulp stem cell differentiation through mitogen-activated protein kinase and calcium-/calmodulin-dependent protein kinase II pathways. *J Endod*. 2014 Jul;40(7):937-42. [[Crossref](#)]
13. Tziafa C, Koliniotou-Koumpia E, Papadimitriou S, Tziafas D. Dentinogenic responses after direct pulp capping of miniature swine teeth with Biodentine. *J Endod*. 2014 Dec;40(12):1967-71. [[Crossref](#)]
14. Alghilan MA, Windsor LJ, Palasuk J, Yassen GH. Attachment and proliferation of dental pulp stem cells on dentine treated with different regenerative endodontic protocols. *Int Endod J*. 2017 Jul;50(7):667-75. [[Crossref](#)]
15. Lymperi S, Taraslia V, Tsatsoulis IN, Samara A, Velentzas AD, Agrafioti A, et al. Dental Stem Cell Migration on Pulp Ceiling Cavities

Filled with MTA, Dentin Chips, or Bio-Oss. *Biomed Res Int.* 2015; 2015:189872. [[Crossref](#)]

16. Gandolfi MG, Spagnuolo G, Siboni F, Procino A, Riviuccio V, Pelliccioni GA, et al. Calcium silicate/calcium phosphate biphasic cements for vital pulp therapy: chemical-physical properties and human pulp cells response. *Clin Oral Investig.* 2015 Nov;19(8):2075-89. [[Crossref](#)]

17. Rodríguez-Lozano FJ, García-Bernal D, Oñate-Sánchez RE, Ortolani-Seltenerich PS, Forner L, Moraleda JM. Evaluation of cytocompatibility of calcium silicate-based endodontic sealers and their effects on the biological responses of mesenchymal dental stem cells. *Int Endod J.* 2017 Jan;50(1):67-76. [[Crossref](#)]

18. Küçükkaya S, Görduysus MÖ, Zeybek ND, Müftüoğlu SF. In Vitro Cytotoxicity of Calcium Silicate-Based

Endodontic Cement as Root-End Filling Materials. *Scientifica (Cairo).* 2016; 2016:9203932. [[Crossref](#)]

19. Akbulut MB, Uyar Arpacı P, Unverdi Eldeniz A. 'Effects of novel root repair materials on attachment and morphological behaviour of periodontal ligament fibroblasts: Scanning electron microscopy observation'. *Microsc Res Tech.* 2016 Dec; 79(12): 1214-21. [[Crossref](#)]

20. Sequeira DB, Seabra CM, Palma PJ, Cardoso AL, Peca J, Santos JM. Effects of a New Bioceramic Material on Human Apical Papilla Cells. *J Funct Biomater.* 2018 Dec 16;9(4). [[Crossref](#)]

21. Luo Z, Li D, Kohli MR, Yu Q, Kim S, He WX. Effect of Biodentine™ on the proliferation, migration and adhesion of human dental pulp stem cells. *J Dent.* 2014 Apr;42(4):490-7. [[Crossref](#)]

22. Makhlof M, Zogheib C, Makhlof AC, Kaloustian MK, El Hachem C, Habib M. Sealing Ability of Calcium Silicate-based Materials in the Repair of Furcal Perforations: A Laboratory Comparative Study. *J Contemp Dent Pract.* 2020 Oct 1; 21(10):1091-97. [[PubMed](#)]

23. Bossu M, Mancini P, Bruni E, Uccelletti D, Preziosi A, Rulli M, et al. Biocompatibility and Antibiofilm Properties of Calcium Silicate-Based Cements: An In Vitro Evaluation and Report of Two Clinical Cases. *Biology (Basel).* 2021 May 26;10(6). [[Crossref](#)]

24. Köseoglu S, Pekbagr Yan KT, Kucukyilmaz E, Saglam M, Enhos S, Akgün A. Biological response of commercially available different tricalcium silicate-based cements and pozzolan cement. *Microsc Res Tech.* 2017 Sep;80(9):994-99. [[PubMed](#)]

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