



Effectiveness of Sectioning Method and Filling Materials on Roughness and Cell Attachments in Root Resection Procedure

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Abstract

Objectives The purpose of the present study was to investigate the created roughness and cell attachment of intact teeth (C), obturated teeth with bioceramic (BR), or epoxy resin (AH) after root resection using piezoelectric ultrasonic and carbide bur.

Materials and Methods Three groups of first mandibular premolars were used in the present study: control group (without any preparation or obturation) (C); second group was obturated with an epoxy resin sealer (AH, AH Plus Jet); and finally, the third one was obturated with a bioceramic sealer (BR, BioRoot RCS). All teeth were incubated for 4 months at 37°C. After that, the samples were sectioned using tungsten carbide bur or piezoelectric ultrasonic. Roughness and then cell attachment of periodontal ligament cells on the sectioned surfaces were investigated by profilometer and confocal microscope, respectively.

Statistical Analysis Data were statistically analyzed using one-way analysis of variance.

Results After root resection, no significant difference was found between the roughness among the three groups sectioned using the piezoelectric technique ($p > 0.05$). In contrast, concerning the sectioned samples by burs, C demonstrated a rougher surface compared with BR ($p < 0.05$). There was a significant higher cell attachment in BR compared with AH in the piezoelectric groups ($p = 0.047$), while no statistically significant difference was found between the groups sectioned with bur ($p > 0.05$).

Conclusion Dentists are now focused on the use of calcium silicate-based sealers due to their bioactivity. The present study advises dentists to use bioceramic sealer which could improve the dentin characteristics which ameliorate the cell attachment.

Keywords

- ▶ apicoectomy
- ▶ bioceramic
- ▶ cell attachment
- ▶ roughness
- ▶ tooth resection

* These authors contribute equally to this work.

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Introduction

Surgical endodontic therapy becomes the treatment of choice when teeth exhibit inadequate responses to nonsurgical treatments, retreatment, or when nonsurgical methods prove ineffective.¹ The goal of endodontic surgery, also known as apicoectomy, is to maintain the tooth and eliminate periradicular pathosis, thereby restoring the health and functionality of the tooth's periodontium.²

Root-end resection, retrograde cavity preparation, and root-end filling are essential parts of endodontic microsurgery procedure. Apical anatomy studies suggest that during surgical procedures, it is recommended to resect the root apex by 2 to 3 mm. This ensures the removal of the majority of unprepared and unfilled accessory canals, thereby effectively eliminating potential reservoirs of pathogens.^{3,4} Following these steps, an ideal material for root-end filling should be applied. This material should possess biocompatibility, strong adhesion to tooth structure, dimensional stability, resistance to dissolution, antibacterial properties, and ease of use.⁵⁻⁷ The objective is to create a hermetic seal at the apex, preventing the entry of microorganisms into the root canal.^{8,9}

Endodontic microsurgery provides precise and predictable outcomes as well as eliminates the assumptions associated with specific instruments.¹⁰ When performing tooth resection, it is crucial to minimize surface roughness and the occurrence of microfractures, as they can significantly influence the success or failure of retrograde treatment. Therefore, the size, shape, and material of the drill are important factors that affect the results of surface quality.¹¹

Ultrasonic devices have become increasingly gained popularity for preparing root-end cavities, surpassing the use of carbide or diamond burs.¹² Various studies have indicated that instruments such as diamond-coated tips and ultrasound devices are commonly used for cavity preparation.¹³⁻¹⁵ Other studies comparing different instruments and techniques for root-end resection, including steel fissure bur, tungsten carbide bur, Zekrya bur, diamond-tip piezoelectric, ultrasonic device, and Er:YAG laser, have consistently concluded that the tungsten carbide bur can be considered the most suitable method for apical resection.^{16,17}

To achieve optimal root resection, it is crucial to employ a suitable method and instruments such as carbide bur¹⁷ or piezoelectric technique¹⁸ that results in a regular and smooth apical surface with minimal cracks to ensure a successful treatment outcome.¹⁹ Achieving a smooth surface is important in reducing the exposure of dentinal tubules on the resected root surface and minimizing apical leakage. Rough and irregular surfaces can act as irritants, accumulate debris, and potentially stimulate resorption during the healing process. Furthermore, creating an appropriate surface condition is crucial to facilitate favorable cellular attachment and promote optimal healing outcomes by synthesizing new matrix components.^{20,21} In ideal healing responses, the attachment of periodontal ligament (PDL) fibroblasts to the resected tooth structure is critical. The ability of these cells to attach to the root surface plays a significant role in promoting proper healing and favorable treatment outcomes.²²

Previous studies shown the importance of bioactive endodontic sealers in root canal treatment, particularly in remineralization and mineral infiltration as well as deposition into the dentinal tubules, compared with traditional epoxy resin sealer.²³⁻²⁵ Moreover, these bioceramic sealers could infiltrate into dentinal tubules and fill them. In addition, their chemical composition includes calcium silicate elements that have biological and physicochemical properties that affect the dentin structure by the release of Ca^{2+} and the alkaline pH.²³ Yoo et al demonstrated that the use of calcium silicate-based materials could lead to the deposition of minerals within the dental tubules after 4 months of age.²⁶ However, the impact of this mineral deposition on the quality of resected surface, including surface roughness and cell attachment, has not been investigated in the literature.

The purpose of this *in vitro* study is to evaluate the change in roughness and cell attachment in intact, and obturated teeth with bioceramic or epoxy resin sealers following sectioning procedure using piezoelectric or bur techniques. The null hypothesis is that the obturation material or the sectioning technique has no effect on cell attachment and roughness values.

Materials and Methods

Teeth Preparation

Ninety-six single-rooted, single-canal first mandibular premolars with complete development, extracted for periodontal or orthodontic reasons, were included in the present *in vitro* study. The ethical protocol number: CE-2024-50 was obtained from the "Comité d'Ethique des Facultés de Médecine, d'Odontologie, de Pharmacie, des Ecoles d'Infirmières, de Kinésithérapie, de Sages-Femmes et des Hôpitaux Universitaires de Strasbourg" in order to use the extracted teeth in research studies.

The samples were randomly divided into three equal groups ($n = 32$) as follows:

Group 1 "control group C": Intact teeth with no endodontic/restorative preparation.

Group 2: Teeth prepared and obturated with AH Plus Jet "AH" (Dentsply DeTrey GmbH, Konstanz, Germany).

Group 3: Teeth prepared and obturated with BioRoot RCS "BR" (Septodont, Louisville, Colorado, United States).

All the cusps were flattened by polishing with a silicon carbide paper (120-grit) using a polishing machine (Escil, Chassieu, France) to standardize the length of root canal at 20 ± 1 mm. For Groups 2 and 3, access cavities were prepared by using diamond burs and ultrasonic tips under loops (Eighteenth, Changzhou City, Jiangsu Province, China). A 10-K-file was used to the manual scouting and the working length was determined to be 0.5 mm from the apical foramen. The root canals were prepared by using Ni-Ti MG3 Bleu sequence up to 25/06 (Dental Perfect, Shenzhen City, Guangdong Province, China) powered by an endodontic motor (Dental Perfect). A single operator, an expert endodontist, prepared all the teeth. Each canal was irrigated with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) (Septodont) and

5 mL of 3% of sodium hypochlorite (Septodont) an activated with the “Ultra Dancer” device (Dental Perfect). After drying with paper points, the canals were obturated using single cone technique with AH in Group 2 and BR and Group 3.

All teeth (Groups 1–3) were stored in phosphate-buffered saline (PBS $\times 10$, Dominique Dutscher, Bernolsheim, France) for 4 months at 37°C in an incubator.

Apicoectomy Procedure

After aging for 4 months, each tooth sample was surrounded by melted wax to simulate 2- to 3-mm-thick PDL except for the last apical 3 mm. The teeth were then embedded in acrylic self-curing resin (OrthocrylEQ, Dentaaurum, Ispringen, Germany), leaving the apical part (3 mm) of the root exposed. Subsequently, the wax was replaced with a silicon-based impression material, following the method outlined in a previous study.²⁷ To ensure blinded evaluation, all teeth were assigned codes ranging from 1 to 96. The sectioning of the teeth was performed perpendicular to their longitudinal axis, 3 mm from the apex, under cooling water. This procedure was carried out by same operator using two distinct techniques for each group, creating six subgroups ($n = 16$) as follows:

Groups C_P, AH_P, and BR_P: piezoelectric ultrasonic bone surgery system (NSK, VarioSurg3, Tokyo, Japan) in endo mode without burst coupled using a piezoelectric ultrasonic insert (Model: US3, Woodpecker Medical Instrument Co, Guangxi, China) (\blacktriangleright Fig. 1A).

Groups C_B, AH_B, and BR_B: tungsten carbide bone cutter bur (H254LE.314.012, Komet, Paris, France) at a speed of 150,000 RPM (\blacktriangleright Fig. 1B).

Each bur or insert was used for five procedures before being replaced with a new one.

Surface Roughness

All samples were examined using an optical profilometer (InfiniteFocus SL, Bruker Alicona, Graz, Austria) at a magnification of $\times 10$, with a lateral resolution of 5 μm and a vertical resolution of 1 μm . The arithmetic mean roughness (Ra) of each sample was determined across five distinct areas, following to ISO 4287 guidelines. Each Ra measurement was conducted along a profile extending at least 12.5 mm in length, following a sawtooth pattern perpendicular to the surface relief, and utilized a cutoff filter (Lc) of 2,500 μm .

Cell Attachment

Eighteen resected samples ($n = 3$ for each subgroup) were utilized. The apices underwent sterilization using PSM-UV (ADS Laminaire, Aulnay-sous-bois, France) for 60 minutes. Human PDL cells were extracted from alveolar ligament of an extracted tooth with the donor's consent. These cells were cultured in Dulbecco's modified Eagle medium (Dominique Dutscher) high glucose supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin, maintained at 37°C in a 5% CO₂ atmosphere. Routine passaging was done using trypsin EDTA, and the cells were used between passages 3 and 5. The

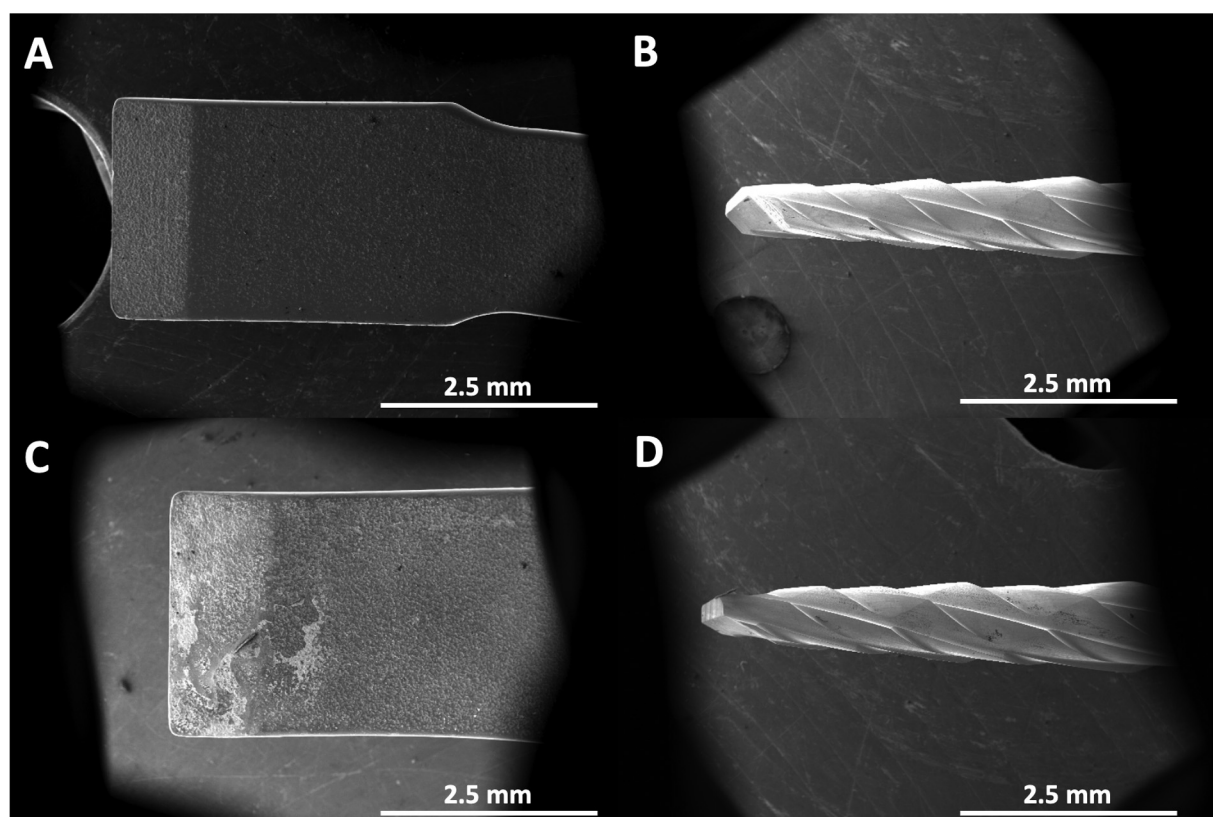


Fig. 1 Scanning electron microscope images demonstrated: (A) new piezoelectric ultrasonic bone surgery; (B) new tungsten carbide bone cutter bur; (C) used piezoelectric ultrasonic bone surgery for five times; and (D) used tungsten carbide bone cutter bur for five times.

apices were placed in a 48-well plate, held upright by the apical part using silicone grease (Beckman Coulter, Inc., Brea, California, United States) to expose the sectioned surface (►Fig. 2). For seeding, 40,000 cells in 400 μ L of medium were added to each well and incubated for 48 hours at 37°C.

Confocal Observation

After 48 hours of incubation at 37°C, the culture medium was removed, and cells were fixed with 4% paraformaldehyde (Euro-medex, Souffelweyersheim, France) in PBS for 30 minutes. Subsequently, the fixed cells were incubated with phalloidin 1/500 and DAPI 1/1,000 in PBS for 10 minutes, followed by a rinse in PBS. Observations were conducted using Zeiss LSM 710 confocal microscope (Zeiss, Heidelberg, Germany) at a $\times 20$ magnification, employing two wavelengths of 405 and 561 nm.

Scanning Electron Microscopy

Initially, a new and a used sectioning instrument from each group were examined using a scanning electron microscope (SEM) (FEI Company, Eindhoven, The Netherlands, 10 kV) at a magnification of $\times 30$ with a working distance of 10 mm. Additionally, to observe the structural changes and dentinal tubules, three randomly samples of BR were examined after aging for 4 months. For this evaluation, the samples were fractured longitudinally in the middle and horizontally (3 mm from the sectioned surface), mounted on SEM stubs, and sputter-coated with a gold-palladium alloy (20/80 weight %) using a sputtering device (Technics, California, United States), and subjected to SEM observation.

Statistical Analysis

Data were statistically analyzed with SigmaPlot release 11.0 (Systat Software, Inc., San Jose, California, United States). One-way analysis of variance was used to determine whether significant differences existed between the groups for roughness and cell attachment tests. The statistical significance level was set at $\alpha = 0.05$.

Results

Roughness

No statistically significant difference in surface roughness between BR samples and those prepared using the piezoelectric techniques ($p > 0.05$) within each group. Moreover,

no significant difference was found between the three groups sectioned by using piezoelectric technique ($p > 0.05$). In contrast, samples sectioned with burs showed that group C_B had a rougher surface compared with BR_B ($p < 0.05$). However, there were no statistically significant differences between C_B and AH_B or between AH_B and BR_B ($p > 0.05$) (►Fig. 3).

Cell Attachment

No significant differences were found between piezoelectric and bur in each group ($p > 0.05$). In contrast, comparing the cell attachment between the three groups (C, AH, and BR), there was a significantly higher cell attachment in BR compared with AH and C in piezoelectric groups ($p = 0.047$), while no statistically significant difference was found between the groups sectioned with bur ($p > 0.05$) (►Fig. 4).

Discussion

The aim of endodontic microsurgery is to preserve teeth that have not responded to the traditional orthograde treatment or retreatment. Various factors can affect the healing of the resected tooth, including the formation of cracks during apicoectomy and root-end preparation,¹⁶ retrograde cavity design,²⁸ retrograde filling materials,²⁹ and the homogeneity and roughness of the sectioned surfaces.¹⁹

In addition, certain materials used in root canal obturation may exert biological,³⁰ mechanical,^{28,31} and physico-chemical³² effects on the tooth's canal system, including dentinal tubules and overall structure. BioRoot RCS was used in the present study due to its previously observed bioactivity such as Ca^{2+} release, pH changes, and the remineralization process.²³ Conversely, epoxy resin materials such as AH Plus Jet[®] have not shown calcium ion release,³³ or the lower pH levels associated with bioceramic materials²⁵ which could not promote the remineralization process.

In the present study, two root-resection instruments were compared among three teeth groups: intact teeth, teeth obturated with bioceramic, and teeth obturated with epoxy resin materials. The utilization of piezoelectric and tungsten carbide burs was grounded in their recognition as the foremost techniques for ensuring a safe and effective endodontic microsurgical treatment.^{17,18} The selection of the intact teeth as a control group was strategic given that one

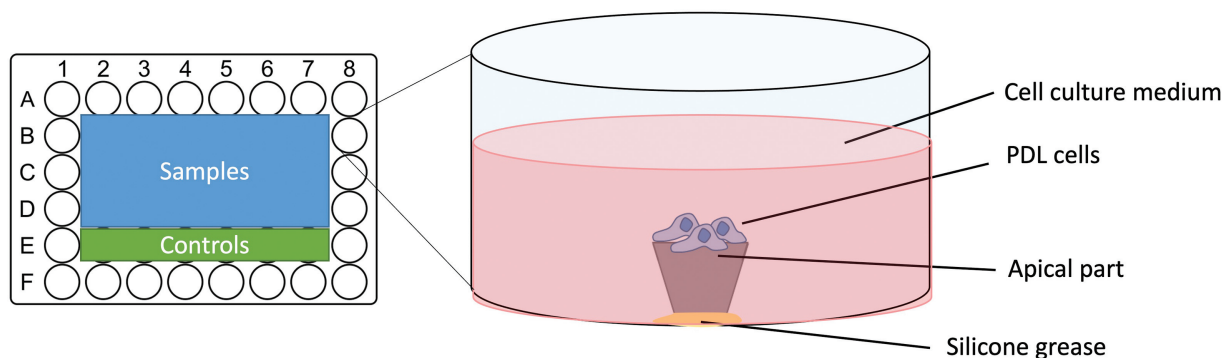


Fig. 2 Schematic graph demonstrating human periodontal ligament (PDL) cells seeding procedure onto the sectioned surface.

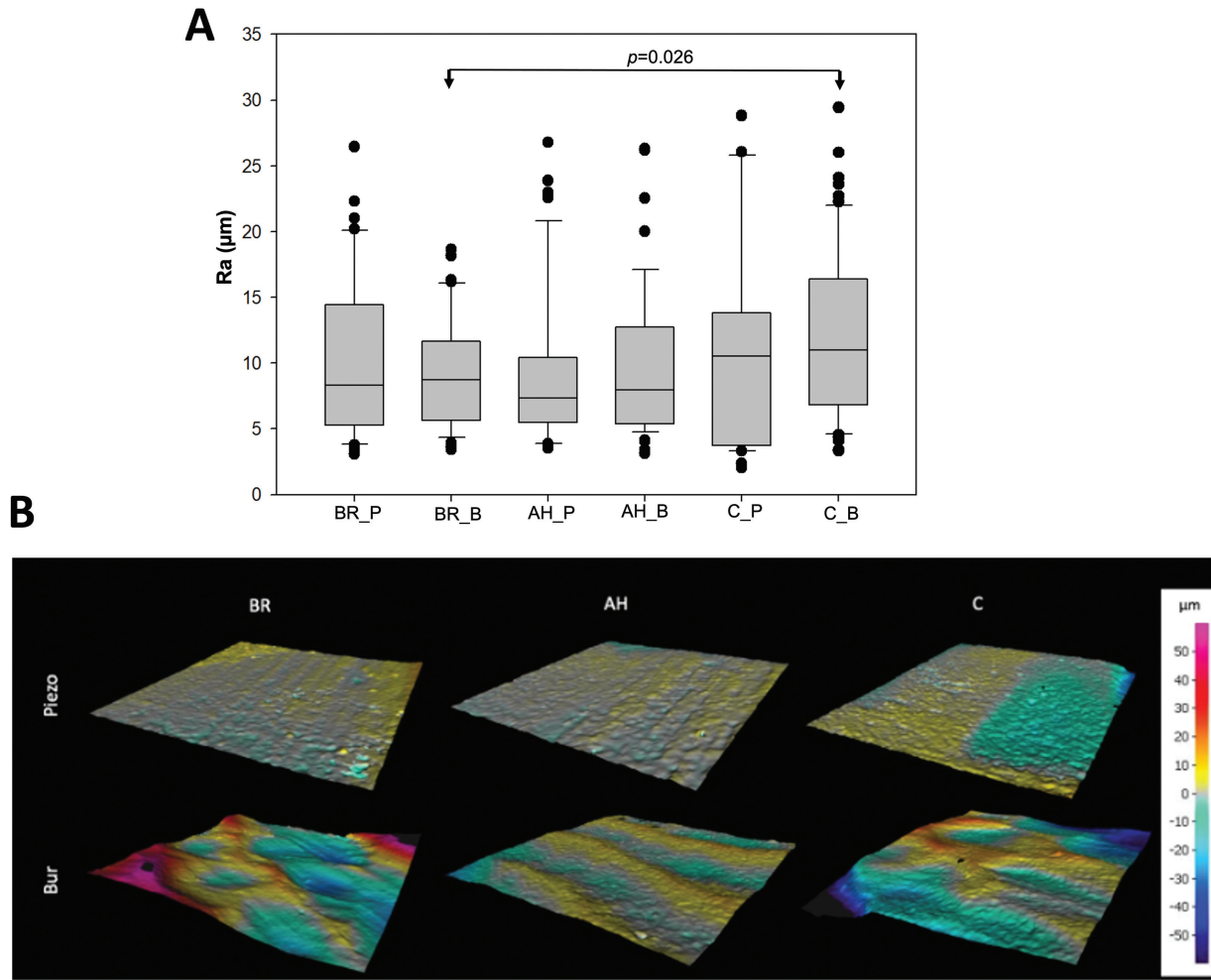


Fig. 3 (A) Schematic graph demonstrated the means and standard deviations (Ra “µm”) of all analyzed surfaces using a profilometer. C: Intact teeth with no endodontic/restorative preparation; AH: teeth prepared and obturated with AH Plus Jet; BR: teeth prepared and obturated with BioRoot RCS; P: piezoelectric; and B: bur (* $p < 0.05$). (B) Micrographs taken by a profilometer demonstrated the roughness of sample surfaces. C: Intact teeth with no endodontic/restorative preparation; AH: teeth prepared and obturated with AH Plus Jet; and BR: teeth prepared and obturated with BioRoot RCS.

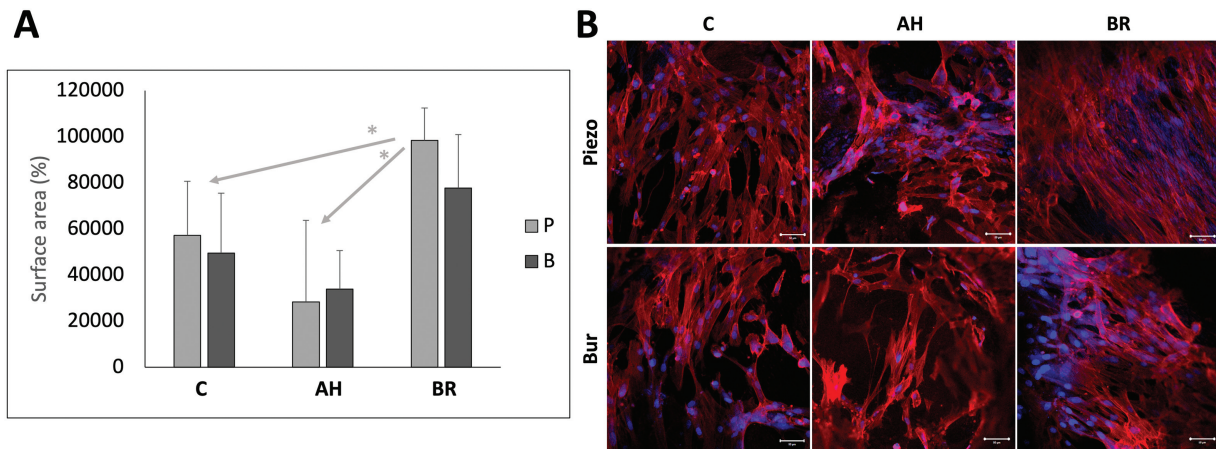


Fig. 4 (A) Percentages of covered area by cells using confocal microscope images. C: Intact teeth with no endodontic/restorative preparation; AH: teeth prepared and obturated with AH Plus Jet; BR: teeth prepared and obturated with BioRoot RCS; P: piezoelectric; and B: bur. (B) Confocal microscopy images demonstrating cell attachment onto the different tested surfaces after 48 hours of incubation at 37°C. C: Intact teeth with no endodontic/restorative preparation; AH: teeth prepared and obturated with AH Plus Jet; and BR: teeth prepared and obturated with BioRoot RCS.

indication of the apicoectomy process is the inaccessibility of orthograde treatment,³⁴ leaving the apical portion undisturbed. Additionally, using intact teeth without any chemical treatment or obturation material effects on their dentin serves as baseline for comparison with the treated groups (AH and BR).

Profilometry was used to analyze surface roughness post-sectioning. No significant differences were detected between the piezoelectric and bur preparations within each group ($p > 0.05$). In accordance to our study, Ekici et al¹⁸ demonstrated no significant difference between the roughness of surfaces resected by piezoelectric and carbide bur techniques. However, they noted that the carbide bur method achieved similar roughness levels with an advantageous less time than the piezoelectric technique. Notably, the C_B group exhibited rougher surface compared with BR_B ($p < 0.05$), necessitating a partial rejection of the null hypothesis. All teeth were sectioned at a 90-degree angle to minimize exposed dentinal tubules and surface roughness, thereby reducing contamination risk.³⁵ A uniform and smoother surface is preferable for promoting healing, minimizing contamination and facilitating the application of root-end filling materials.^{18,36}

Cell attachment was evaluated using a confocal microscope after 48 hours of incubation at 37°C, then percentage of the area covered by the cells was quantified. No significant differences were found between piezoelectric and bur techniques within each group ($p > 0.05$) which is in accordance with the roughness results. In contrast, when the cell attachment was compared between the three groups (C, AH, and BR), there was a significantly higher cell attachment in BR compared with C and AH in the piezoelectric groups ($p = 0.047$), while no significant difference was found between the groups sectioned with bur ($p > 0.05$). Regardless of the sectioning technique, when the results of piezoelectric and bur in each group were cumulated, BR demonstrated higher cell attachment compared with AH and C ($p = 0.002$). This could be attributed to the cytotoxicity reported in epoxy resin materials and the better biocompatibility of bioceramic materials with dental cells.³⁷ These findings could also be

related to the fact that bioceramic materials have bioactive properties as described previously^{24,25} and could create an optimal environment for intratubular mineralization after 4 months of obturation.²⁶ Therefore, it could be hypothesized that the realization of Ca^{2+} ions from bioceramic materials could increase the amount of calcium ions in root dentin as well as the mineral deposition in dentinal tubules (filled dentinal tubules) (→ Fig. 5). The mineral deposition was also observed in a previous study where the authors used mineral trioxide aggregate and the samples were incubated in PBS during only 1 month.³⁸ Moreover, it is plausible that the released calcium ions could play an important role to promote and enhance the cell attachment.³⁹ The primary objective postapicoectomy is the periapical regeneration through the deposition of bone, PDL, and cementum onto the resected surface.⁴⁰

This *in vitro* study has several limitations. The methodology used to simulate the PDL and the application of PBS during all the storage periods could be useful to avoid teeth desiccation. However, the study remains an *in vitro* study, and as such, it may not accurately represent the attachment of only PDL cells to dentinal surfaces *in vivo* conditions. In addition, more samples could provide a more comprehensive understanding of the cell attachment rates. Further research should be performed to investigate the mechanical changes in dentin structure due to the use of bioceramic as an initial filling material. The releasing of calcium ions could modify the morphology and the mechanical properties of dentin structure; thus, the mechanical properties of this structure should be studied in further research by using microhardness, flexural, and compression strength tests. It would also be beneficial to examine the progression of mineral deposition within the dentinal tubules at various aging periods following bioceramic obturation.

Conclusion

Within the limitations of the present *in vitro* study, teeth obturated with bioceramic material could play a positive role on the cell attachment rate compared with epoxy resin sealer

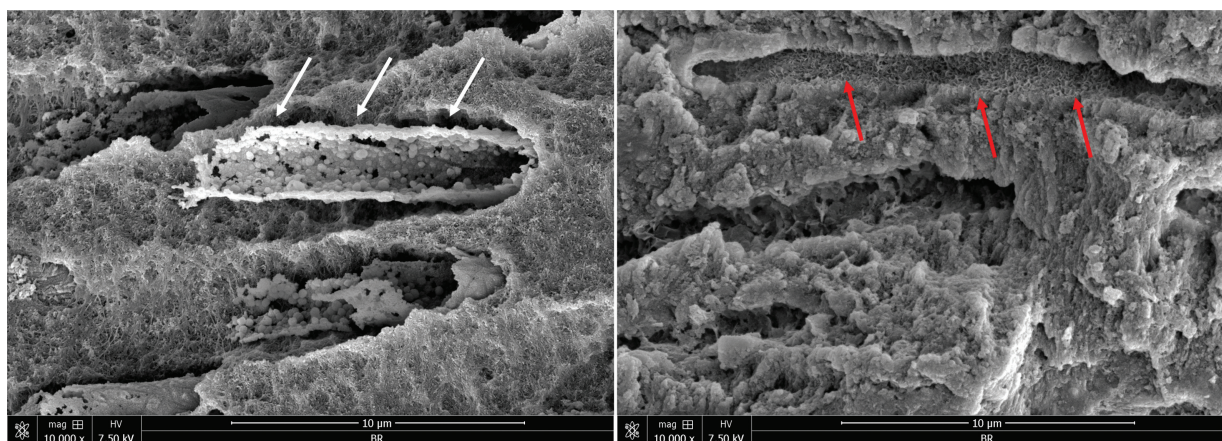


Fig. 5 Scanning electron microscope images demonstrate the mineral deposition (red arrows) and calcium silicate infiltration (white arrows) of BioRoot RCS into dentinal tubules after 4 months of aging period in phosphate-buffered saline at 37°C.

after an apical resection using carbide bur. No remarkable differences were found concerning the roughness of the different techniques or obturations materials. Further *in vivo* and *in vitro* studies should be performed to confirm these findings.

Conflict of Interest

None declared.

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