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Micro-computed tomographic evaluation of the removal of a novel thermosensitive hydrogel containing $Ca(OH)_2$ -loaded PLGA nanoparticles from the root canal system

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Research Article

Keywords: Nanoparticles, Calcium hydroxide, Intracanal medicament, Thermosensitive hydrogel, Micro-computed tomography

Posted Date: October 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11024771/v1

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Additional Declarations: Competing interest reported. José Antonio González Sánchez, Fernando Duran-Sindreu, Elena Sánchez-López, and Firas Elmsmari are inventors on a patent related to the $Ca(OH)_2$-loaded PLGA nanoparticle technology evaluated in this study. The remaining authors declare no competing interests

Abstract

Objectives

To evaluate the removability of a novel thermosensitive hydrogel containing calcium hydroxide-loaded PLGA nanoparticles ($Ca(OH)_2$-NPs-gel) compared to a commercial $Ca(OH)_2$ paste from root canals using micro-computed tomography (micro-CT).

Materials and methods

Twelve extracted human maxillary molars were randomly assigned to two groups (n = 18 canals/group): $Ca(OH)_2$-NPs-gel and a commercial paste (Calcicur®; VOCO GmbH). Micro-CT evaluated volumetric changes after initial placement and after passive removal using sterile saline irrigation alone. A randomly selected subsample (n = 9 canals/group**)** underwent active removal using mechanical activation. Filling and removal efficiencies were statistically analysed.

Results

After passive removal, the $Ca(OH)_2$-NPs-gel demonstrated a significantly higher median removal efficiency (77.48%) than the commercial Ca(OH)2 paste (20.89%) (P = .001). No significant difference in initial filling efficiency was detected between groups (P = .366), indicating comparable initial filling values under experimental conditions. During active removal, performance was statistically similar (P = .537); mechanical activation did not significantly improve the washout of either medicament beyond passive irrigation levels.

Conclusions

The $Ca(OH)_2$-NPs-gel showed significantly higher removal efficiency with passive saline irrigation than the commercial Ca(OH)2 paste under the conditions of this study. This improved removability may help overcome traditional vehicle-related limitations and reduce residual intracanal medicament before obturation.

Clinical relevance:

Thermosensitive gels and nanotechnology represent promising strategies for improving intracanal drug delivery. However, the removal of thermosensitive intracanal gels has not yet been investigated. This micro-CT study demonstrates that a novel $Ca(OH)_2$-loaded nanoparticle thermogel can be efficiently removed by passive saline irrigation, supporting its potential clinical applicability as an intracanal medicament.

INTRODUCTION

Apical periodontitis is a biofilm-induced disease characterized by bacterial colonization of the apical portion of the root canal system [1]. Recent studies observed that it affects between 52% and 58.6% of the global adult population [2, 3]. Calcium hydroxide ($Ca(OH)_2$) is widely utilized as an inter-appointment medicament to eliminate surviving bacteria, prevent regrowth, and neutralize bacterial endotoxins [4–6]. However, complete removal of $Ca(OH)_2$ remains a persistent challenge, as conventional syringe irrigation alone has frequently been reported to be insufficient for complete $Ca(OH)_2$ removal [7–9]. Micro-CT studies have frequently detected greater amounts of residual $Ca(OH)_2$ in the apical third of the root canal across different irrigation and activation protocols [10–12]. This incomplete removal negatively impacts the final obturation by causing sealer dissolution over time, decreasing sealer penetration into the dentinal tubules, and forming void spaces [13–16]. Presently, micro-CT serves as an advanced, non-destructive technique to accurately evaluate the penetration and removal of these intracanal medicaments [17].

To overcome the limitations of traditional medications, advanced hydrogel-based systems integrated with nanotechnology have been developed for targeted drug delivery in endodontics [18–20]. Recently, a novel formulation was created by dispersing biodegradable $Ca(OH)_2$-loaded PLGA nanoparticles into a thermosensitive hydrogel [21, 22]. This material uniquely transitions from a fluid-like state at 10°C to an elastic-like solid gel at body temperature (37°C), enabling easy injection, precise adaptation to the root canal anatomy, and improved delivery to the apical third [19, 22, 23].

Notably, this $Ca(OH)_2$-NPs-gel achieves disinfection levels comparable to commercial pastes but requires an active ingredient concentration approximately 200 times lower (0.23% vs. 45%), which substantially improves its biosafety profile by reducing the risk of tissue damage upon extrusion [24]. Structurally, the hydrogel functions as a 3D scaffold with high water content, suggesting it may be more easily removed mechanically by irrigation than dense, viscous conventional pastes [19, 23]. Conversely, its thermosensitive properties also generate a significant increase in mucoadhesive strength, enhancing retention within infected areas but may complicate complete removal during the final stages of the procedure [22, 24]. To the authors' knowledge, no previous study has compared the removal of this novel $Ca(OH)_2$-loaded PLGA NPs gel with that of a commercial calcium hydroxide paste.

The aim of this in vitro study was to evaluate the removability of a novel thermosensitive hydrogel containing biodegradable calcium hydroxide-loaded PLGA nanoparticles ($Ca(OH)_2$-NPs-gel) compared to a commercially available calcium hydroxide paste (Calcicur®; VOCO GmbH, Cuxhaven, Germany) from the root canal system using micro-CT. The null hypothesis was that there is no significant difference in the volume of intracanal residues between the novel $Ca(OH)_2$-NPs-gel and the commercial $Ca(OH)_2$ paste when comparing both passive or active removal protocols.

MATERIALS AND METHODS

Sample Selection and Preparation

The research protocol was approved by the Ethics Committee of the Universitat Internacional de Catalunya (study number END-INVI-2022-02) and was conducted in accordance with the PRILE 2021 guidelines [25]. The sample size was calculated using Minitab v20 *(Minitab Inc., State College, PA, USA*) based on data from a previous publication by Araya-Cumsille et al. (2025) [26], considering an alpha error of 0.05 and a statistical power of 90%; while a minimum of 12 canals per group was required, the sample was increased to 18 canals per group to enhance statistical reliability. Twelve permanent human maxillary molars with mature apices, extracted for unrelated reasons, were selected. Initial radiographic examination via radiovisiography *(CS 5100; Carestream Health, Rochester, NY, USA)* and limited field of view cone-beam computed tomography (CBCT) *(CS 8200 3D; Carestream Health)* confirmed that each tooth presented three independent roots and excluded calcified canals, accessory canals, multiple foramina, and resorptive processes.

Standardized access cavities were prepared using a 016 round diamond bur *(Dentsply Maillefer, Ballaigues, Switzerland)* and an Endo-Z tungsten bur *(Dentsply Maillefer).* Working length (WL) was established with a size 15.02 K-file *(Dentsply Maillefer)*. Root canals were instrumented using Reciproc Blue® R25 files *(VDW, Munich, Germany)* and apically enlarged with ProFile® rotary instruments *(Dentsply Tulsa Dental Specialties, Tulsa, OK, USA)* to size 30.04 (mesial and distal) and 40.04 (palatal). Irrigation was performed using 4.5% NaOCl *(Heinkel Iberica, Barcelona, Spain)* and 10% citric acid *(Sigma-Aldrich, St. Louis, MO, USA)*. The irrigants were delivered via 10-mL Luer-lock syringes *(Medibase, Tremblay-en-France, France)* attached to 30G side-vented needles *(ProRinse®*; *Dentsply Maillefer)* placed 3 mm short of the WL.

The $Ca(OH)_2$-NPs-gel formulation (0.23% active concentration) was prepared according to the protocol described by Roig et al. (2024) [22] and stored at 4°C prior to application. The specimens were randomly allocated into two experimental groups (n = 12 teeth; 18 canals per group): the NPs-gel group and a commercial calcium hydroxide paste group *(Calcicur®; VOCO GmbH, Cuxhaven, Germany*). The medications were delivered using type 47 application cannulas *(VOCO GmbH)* inserted up to the WL, gradually withdrawing the syringe to ensure complete filling. Coronal accesses were sealed with Cavit® *(3M ESPE, Seefeld, Germany)*, and the specimens were stored at 37°C and 100% humidity for 72 hours.

After the 72-hour incubation, the temporary seals were removed, and the retrieval of the medicaments was performed in two consecutive phases:

In Phase 1 (passive removal), the medicament was removed from all specimens using passive irrigation with saline solution via a 30G side-vented needle *(ProRinse®*; *Dentsply Maillefer)* positioned 3 mm short of the WL. Sterile saline solution, stored at 6°C, was intentionally selected as the sole irrigant for the removal protocols to isolate and specifically evaluate the mechanical washout capabilities of the medicaments; this approach prevented any confounding chemical dissolution of the inorganic calcium hydroxide components that typically occurs when using chelating agents.

For Phase 2 (active removal), a randomly selected subsample (n = 9 per group) underwent supplementary saline irrigation activated with the XP-endo Finisher® (FKG Dentaire, La Chaux-de-Fonds, Switzerland). The instrument was operated at 800 rpm and 1 N·cm for 60 seconds using gentle 7- to 8-mm longitudinal in-and-out movements [27]. Because palatal canals exhibited greater initial volumes and wider buccolingual dimensions that could exceed the expansion range of the XP-endo Finisher, they additionally underwent passive ultrasonic irrigation with a Z-Silver tip (Zarc4Endo, Gijón, Spain) for three 20-second cycles, with irrigant replenishment between cycles [28].

Micro-CT scanning and analysis

Specimens were characterized using a high-resolution SkyScan 1272 micro-CT system *(Bruker microCT, Kontich, Belgium)* at three stages: (i) after medication placement, (ii) after passive removal, and (iii) after active removal. Up to three specimens were arranged vertically and inverted within the internal chamber per scan **(**Fig. 1A**)**. All scans were performed at 90 kV and 111 µA using a 0.25-mm Al filter, with a rotational step of 0.3° over 360°, frame averaging of 3, and an isotropic voxel size of 12 µm.

Raw data were reconstructed into 3D models using NRecon v.1.6.3 software *(Bruker microCT)*, and subsequent volumetric analysis and visualization were conducted using DataViewer v.1.5.2.4 *(Bruker microCT)* and CTAn v1.7.0.4 software *(Bruker microCT*). Region of interest (ROI) segmentation was executed to separate the three distinct roots of each specimen. For each canal, the ROI was anatomically bounded by a superior pulpal plane (SPP) positioned at the canal orifice and an inferior pulpal plane (IPP) positioned at the apical foramen.Two primary metrics were calculated: filling efficiency, which evaluated the volume of material successfully introduced relative to the total fillable canal space, and removal efficiency, which measured the percentage of removed material post-cleaning against the baseline post-filling volume **(**Fig. 1B**)**.

ROIs corresponding to the three individual roots of each tooth were manually defined in CTAn prior to volumetric analysis to ensure independent evaluation of each root canal. Following ROI definition, medicament segmentation was performed using specimen-specific greyscale thresholding. All image segmentation and volumetric measurements were carried out by a single calibrated examiner using standardized segmentation criteria. To minimize measurement bias, all datasets were anonymized before analysis, and the examiner remained blinded to the experimental allocation of the specimens throughout the segmentation and quantitative evaluation.

Because the tested medicaments exhibited different radiopacities, greyscale thresholds were individually calibrated for each specimen. Lower thresholds were used to segment the less radiopaque $Ca(OH)_2$-NPs-gel, whereas higher thresholds were applied to Calcicur® paste (VOCO GmbH), allowing consistent volumetric quantification of both materials.

Statistical Analysis

Statistical analyses were performed using SPSS v.23.0 (IBM Corp., Armonk, NY, USA) and Minitab v.20 (Minitab Inc., State College, PA, USA). Box plots were generated using Python Matplotlib v.3.10 (*Python Software Foundation, Wilmington, DE, USA*). Data normality was assessed using the Shapiro–Wilk and Anderson–Darling tests, while homogeneity of variances was evaluated using Levene's test. Parametric data were evaluated using independent-samples and paired Student's t-tests, whereas non-parametric data were analysed using the Mann-Whitney U test and the Wilcoxon signed-rank test. Statistical significance was established at $P < .05$ for all analyses.

RESULTS

Initial filling efficiency was analyzed showing that both intracanal medications demonstrated the capacity to achieve high volumetric coverage within the root canal system, with specific instances reaching filling rates exceeding 90%. Overall, the commercial paste (Calcicur®) group achieved a mean filling volume of 68.13% ± 15.47%, while the $Ca(OH)_2$-NPs-gel group reached a mean of 62.33% ± 21.86%. The data distribution for this phase was normal. An independent samples Student's t-test indicated no statistically significant differences in the initial volumetric filling percentages between the two experimental groups ($P = .366$) (Fig. 2).

When analyzing the passive removal phase with saline, the data did not meet the assumptions for normality (Shapiro-Wilk test: Calcicur®, $P = .024$). Utilizing the Mann-Whitney U test, a statistically significant difference was observed between the two experimental groups ($P = .001$). The novel $Ca(OH)_2$-NPs-gel group demonstrated a significantly higher median removal efficiency of 77.48% (IQR: 39.50% – 93.25%) compared to the commercial paste, which presented a median removal efficiency of 20.89% (IQR: 10.00% – 52.75%) (Fig. 3).

Regarding the impact of mechanical activation, the paired differences between passive and active removal fulfilled the assumption of normality in both experimental groups; therefore, paired Student's t-tests were used for the intra-group comparisons (n = 9 canals per group) to compare the baseline passive removal against the subsequent active removal. For the commercial paste, removal efficiency increased from a mean of 23.87% ± 18.44% after passive irrigation to 41.08% ± 26.30% after active irrigation with the XP-endo Finisher® and Z-Silver, though this difference was not statistically significant ($P = .130$) (**Figure S1A**).

For the $Ca(OH)_2$-NPs-gel sub-sample, the mean passive removal was 53.60% ± 24.83%, and after active removal, it was recorded at 50.02% ± 33.30% ($P = .800$). During the active removal phase, an independent Student's t-test indicated that the final performance of both materials was statistically similar ($P = .537$) (**Figure S1B**). These results indicate that the addition of this mechanical activation step did not produce a statistically significant improvement in removal efficiency for either the novel hydrogel or the commercial paste over the levels achieved with the passive protocol.

DISCUSSION

The removability of a novel thermosensitive $Ca(OH)_2$-NPs-gel compared to a commercially available $Ca(OH)_2$ paste from the root canal system was evaluated in the present study. The null hypothesis was partially rejected. A statistically significant difference was observed when passive removal was performed. The novel $Ca(OH)_2$-NPs-gel performed significantly better, removing 77.48% of the intracanal medicament from the root canals, compared to the 20.89% observed for the commercial $Ca(OH)_2$ paste (Calcicur®, VOCO GmbH, Cuxhaven, Germany). In contrast, when comparing active removal, no significant differences were observed between the two groups.

To validate the experimental model and ensure high reproducibility, this in vitro study was conducted in accordance with the PRILE 2021 guidelines [25]. In addition, to enhance the reliability and robustness, the sample size was increased to 18 canals per group, following the recommendations observed in previous studies [26]. In the present study, Micro-CT imaging was employed. As shown in previous studies [10–12], it allows for highly accurate volumetric assessments of the root canal system at multiple stages without altering the sample's anatomy. The validity of the standardized methodology was confirmed as no significant differences (P = .366**)** were observed in the initial volumetric filling percentages between the commercial Calcicur® (VOCO GmbH, Cuxhaven, Germany) paste (68.13% ± 15.47%) and the novel $Ca(OH)_2$-NPs-gel (62.33% ± 21.86%). Confirming this baseline volumetric homogeneity suggests that the significant differences observed during the removal phases may be attributable to the specific physicochemical properties of the evaluated medicaments, rather than variations in their initial placement.

Regarding the passive removal phase, the novel $Ca(OH)_2$-NPs-gel showed a significantly higher removal efficiency (77.48%) compared to the conventional $Ca(OH)_2$ (Calcicur®, VOCO GmbH, Cuxhaven, Germany) paste (20.88%) (P = .001). This difference highlights the critical role of the vehicle in the predictability of intracanal medicament removal. As observed by Nandini et al. [29] and Arslan et al. [30], the formulation's vehicle is a key factor in removal difficulty, with oil-based formulations proving more difficult to wash out than water-based solutions. In the context of this novel medicament, Roig et al. [22] developed the $Ca(OH)_2$-NPs-gel to specifically transition from a fluid-like state at 10°C to an elastic-like state at body temperature (37°C). Additionally, Roig-Soriano et al. [24] noted that this thermosensitive property provides a significant increase in mucoadhesive strength, which enhances the retention of the medicament within the infected zones. While it was initially hypothesized that this enhanced mucoadhesion might complicate complete removal during the final stages of the procedure, the present results alleviate this concern. The hydrogel-based formulation may have facilitated a highly efficient washout utilizing only passive saline irrigation, potentially overcoming the vehicle-related limitations in conventional pastes.

In addition to its superior removability, the formulation's low concentration may provide a significant biosafety advantage. Extrusion of conventional $Ca(OH)_2$ pastes risks severe complications, including tissue necrosis, nerve injury, and granuloma formation due to prolonged alkaline contact [31–34]. Conversely, the $Ca(OH)_2$-NPs-gel achieves comparable antibacterial efficacy with an active concentration approximately 200-fold lower [24]. It is this drastically reduced mineral load that may plausibly reduce

the risk of tissue necrosis upon accidental periapical extrusion [24], a biological benefit that is independent of its intracanal removability.

The greater removal efficiency achieved during passive irrigation may be related to the thermodynamic and structural characteristics of the hydrogel. At body temperature, Poloxamer 407 undergoes thermal gelation through dehydration of its polypropylene oxide units and subsequent micellar aggregation, forming a three-dimensional hydrophilic network with a high-water content [35–39]. This hydrophilic matrix could facilitate saline penetration and promote disruption of the gel network under fluid flow [40, 41]. These properties may have contributed to the greater removal efficiency observed for the $Ca(OH)_2$-NPs-gel compared with the conventional paste; however, the underlying mechanism was not directly evaluated in the present study.

On the other hand, no statistically significant differences were observed between the two groups (P = .537) when active removal was performed, showing a mean removal efficiency of 50.02% ± 33.30% for the $Ca(OH)_2$-NPs-gel and 41.08% ± 26.30% for the Calcicur® (VOCO GmbH, Cuxhaven, Germany) paste. Moreover, intra-group comparisons revealed no statistically significant volumetric reduction of the filling material between the passive and active removal phases for either group. This result contrasts with previous studies [7–9] in which activation procedures improved $Ca(OH)_2$ removal compared with conventional syringe irrigation.

To maximize cleaning efficacy during the active phase, the XP-endo Finisher® (FKG Dentaire, La Chaux-de-Fonds, Switzerland) and Z-Silver ultrasonic tip (Zarc4Endo, Gijón, Spain) were used. Recent studies by Wigler et al. [27] and Keskin et al. [42] have shown that the highly flexible, shape-memory alloy of the XP-endo Finisher® allows it to expand and adapt to root canal irregularities, significantly improving $Ca(OH)_2$ removal compared to conventional syringe irrigation. However, in the present study, mechanical activation with these instruments did not significantly enhance intracanal medicament removal. A plausible explanation is that the initial passive washout was highly effective in removing the bulk of the readily accessible material. In addition, because this study only used saline, rather than chelating agents, which chemically dissolve the inorganic components of $Ca(OH)_2$, the mechanical agitation provided by the instruments alone was insufficient to remove deeply embedded remnants. Furthermore, mechanical activation with instruments such as the XP-endo Finisher® may generate new radiopaque dentinal debris due to friction against the canal walls. Because highly sensitive, low-greyscale thresholds were required to segment the low-density $Ca(OH)_2$-NPs-gel in the Micro-CT analysis, it could be hypothesized that the software captured this newly generated dentin debris as residual material. This potential imaging artifact might explain why the recorded removal efficiency for the hydrogel sub-group did not improve, and even exhibited a slight, non-significant decrease following mechanical activation.

These findings may have implications for subsequent root canal obturation. Previous micro-CT studies have consistently detected greater amounts of residual $Ca(OH)_2$ in the apical third regardless of the irrigation or activation protocol used [10–12]. The presence of $Ca(OH)_2$ remnants leads to negative consequences, which include sealer dissolution over time and void formation at the gutta-percha-dentin

interface [13, 15]. Furthermore, Machado et al. [16] reported that residual $Ca(OH)_2$ significantly reduced both the penetration area and maximum penetration depth of an epoxy resin–based sealer within dentinal tubules, particularly in the apical third. In the present study, the $Ca(OH)_2$-NPs-gel achieved a median removal efficiency of 77.48% with passive saline irrigation. This improved removability may reduce the amount of medicament remaining before obturation; however, its effects on sealer penetration, interfacial adaptation, and apical sealing should be directly evaluated in future studies.

However, several limitations of the present study should be acknowledged. First, as an in vitro experimental model, it cannot replicate the complex thermodynamic conditions of the oral cavity in vivo, which is a significant factor when evaluating a thermosensitive hydrogel. Furthermore, while the primary sample size was robust, the active removal evaluation was conducted on a reduced subsample. This smaller sample size decreases the statistical power of this specific phase, potentially increasing the risk of a Type II error and limiting the ability to detect minor but clinically relevant differences in cleaning efficacy following mechanical activation. Moreover, to isolate and assess the mechanical washout capabilities of the medicaments, this study solely used sterile saline during the removal phases. Given that chelating agents such as 10% citric acid or 17% EDTA are superior for dissolving the inorganic components of $Ca(OH)_2$ [29], future studies should investigate the removability of this novel $Ca(OH)_2$-NPs-gel using chelators and thereafter evaluate its direct impact on the push-out bond strength of endodontic sealers.

CONCLUSION

Within the limitations of this in vitro study, the novel $Ca(OH)_2$-NPs-gel demostrated a significantly superior removal efficiency using solely passive saline irrigation compared to a conventional $Ca(OH)_2$ paste. This improved removability may help overcome traditional vehicle-related limitations of $Ca(OH)_2$, reinforcing the gel's potential as a promising intracanal medicament.

Declarations

Funding: This work was supported by the AGAUR European Regional Development Fund (ERDF; PROD00141), the UIC 2025 Valorization Project, and the Spanish Association of Endodontics (Asociación Española de Endodoncia [AEDE]) through the Rafael Miñana Laliga Research Grant and MICIU/AEI/10.13039/501100011033 (Grant PID2024-157231OB-C21).

Ethics approval: *This study was performed in accordance with the Declaration of Helsinki and its later amendments. The research protocol was approved by the Ethics Committee of the Universitat Internacional de Catalunya (study number END-INVI-2022-02).*

Consent to participate: Written informed consent was obtained from all patients for the donation and use of their extracted teeth for research purposes.

Competing interests: José Antonio González Sánchez, Fernando Duran-Sindreu, Elena Sánchez-López, and Firas Elmsmari are inventors on a patent related to the $Ca(OH)_2$-loaded PLGA nanoparticle technology evaluated in this study. The remaining authors declare no competing interests.

Data availability: The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation Process: During the preparation of this manuscript, OpenAI ChatGPT (GPT-5.6; OpenAI, San Francisco, CA, USA) and NotebookLM (Google, Mountain View, CA, USA) were used solely to assist with language editing, grammatical correction, and improvements in clarity, readability, organization, and wording across different sections of the manuscript. These tools were not used for study design, data collection, statistical analysis, generation of results, or scientific interpretation of the findings. All AI-assisted content was critically reviewed, edited, and verified by the authors, who take full responsibility for the accuracy, integrity, and final content of the manuscript. No patient-identifiable, sensitive, or confidential research data were provided to either AI tool

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Figures

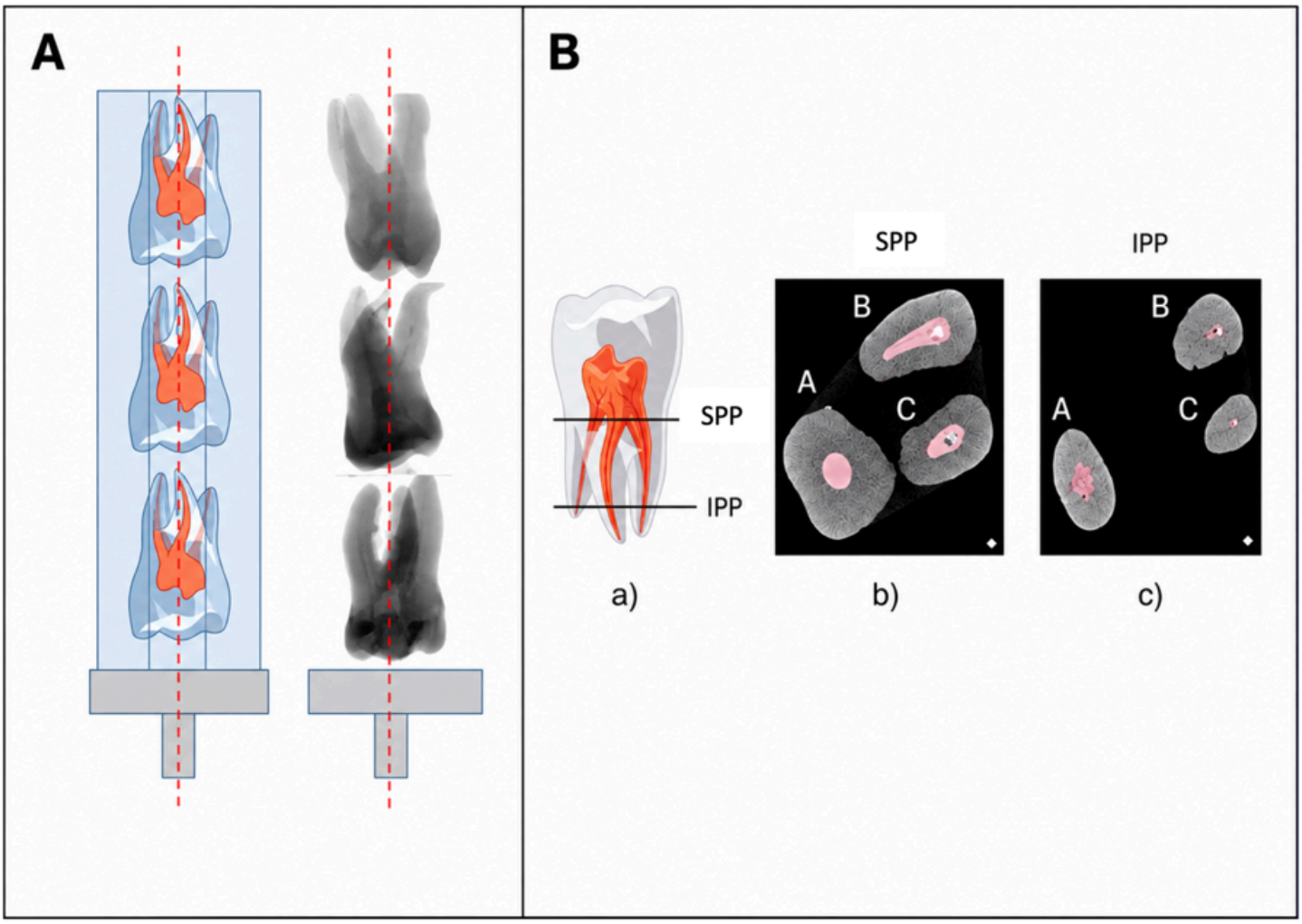

Figure 1

Schematic representation of dental specimen positioning and reference planes used for micro–computed tomography assessment. **(A)** Vertical arrangement of three dental specimens scanned simultaneously in an inverted orientation, with the roots directed upward: theoretical positioning scheme and representative specimen stack. **(B)**Definition of the superior pulpal plane (SPP) and inferior pulpal plane (IPP) used for root canal assessment: **(a)** location of the reference planes, **(b)** cross-sectional image at the SPP, and **(c)** cross-sectional image at the IPP.

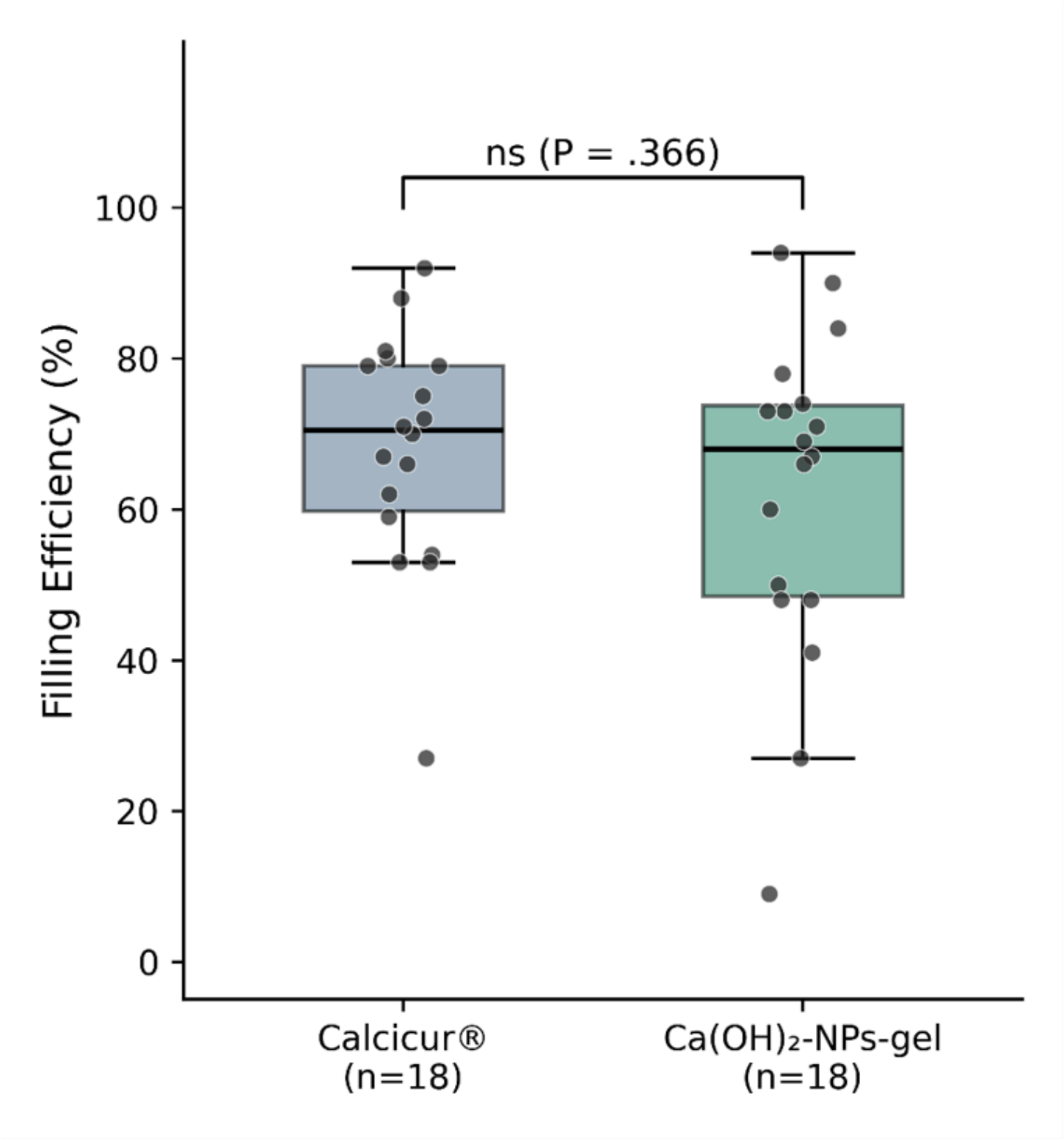

Figure 2

Box plot comparing initial volumetric filling between Calcicur® and $Ca(OH)_2$-NPs-gel.

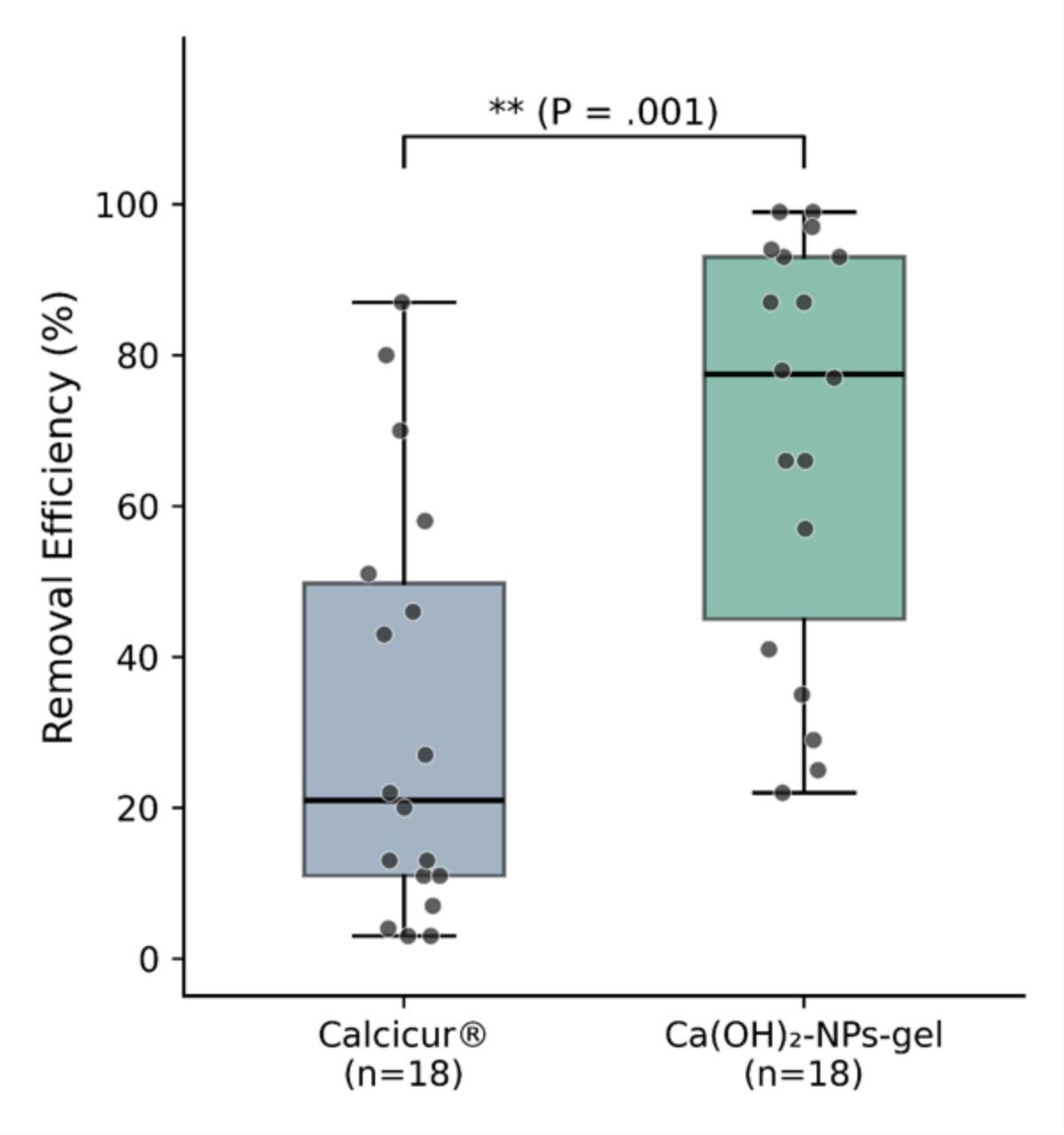

Figure 3

Box plot comparing passive removal efficiency between the two groups.

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- Suplementaryfigure.png