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A Thermosensitive Gel Containing Biodegradable Nanoparticles Carrying Calcium Hydroxide as Antibacterial Intracanal Therapy

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Keywords: calcium hydroxide | endodontics | intracanal medication | nanoparticles | PLGA | thermosensitive gel

ABSTRACT

Aim: To characterise and evaluate mucoadhesive strength and antibacterial properties of a nanotechnological formulation for endodontic disinfection based on biodegradable nanoparticles dispersed in a thermosensitive gel containing calcium hydroxide (Ca(OH)₂-NPs-gel).

Methodology: Morphology of Ca(OH)₂-NPs-gel was studied using transmission electron microscopy. Moreover, Ca(OH)₂-NPs-gel was sterilised using gamma irradiation (25 kGy), and the stability after the sterilisation process was studied by measuring Ca(OH)₂-NPs-gel average size, polydispersity index, zeta potential and encapsulation efficiency. To assess the ex vivo mucoadhesive strength, extracted single-rooted human teeth were used to measure the force necessary to separate the formulation from the teeth. In addition, the short-time stability of Ca(OH)₂-NPs-gel was evaluated monthly, analysing entrapment efficacy, backscattering and transmittance of Ca(OH)₂-NPs-gel stored at different temperatures (4°C, 25°C and 37°C). Furthermore, the antibacterial analysis of Ca(OH)₂-NPs-gel was performed against *Enterococcus faecalis* inoculated in extracted human single-root teeth and evaluated by confocal and scanning electron microscopy. Finally, the metabolic activity of bacteria was studied through a resazurin assay to evaluate bacterial survival after treatment.

Results: Ca(OH)₂-NPs-gel owned a round shape and a smooth surface without particle aggregation. Sterilisation did not induce an alteration in Ca(OH)₂-NPs-gel physicochemical properties and Ca(OH)₂-NPs-gel presented a high adhesion strength. In addition, 4°C was the best temperature to store Ca(OH)₂-NPs-gel. Regarding the antibacterial therapeutic efficacy, Ca(OH)₂-NPs-gel possesses suitable antibacterial properties, indicating that it efficiently reduces bacterial biofilms.

Conclusion: Calcium hydroxide-loaded PLGA nanoparticles dispersed in a thermosensitive gel have been developed, optimised and characterised, obtaining excellent antibacterial properties and achieving bacterial disinfection levels similar to those of commercial formulations.

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1 | Introduction

Calcium hydroxide ($\text{Ca}(\text{OH})_2$) is a strong base that dissociates into calcium and hydroxyl ions upon contact with aqueous fluids. It is a white, odourless powder (Mohammadi and Dummer 2011). $\text{Ca}(\text{OH})_2$ possesses remarkable antibacterial properties and the ability to repair tissues (Freeman et al. 1994; Mohammadi and Dummer 2011; Panzarini et al. 2007). Due to its ability to block bacterial enzymes and facilitate mineralisation in dentine, $\text{Ca}(\text{OH})_2$ is one of the most employed intracanal medicaments in endodontics and dental traumatology (Mohammadi and Dummer 2011). However, various studies that evaluated bacterial reduction through microbiological culture demonstrated a decrease in bacterial count, yet a limited therapeutic efficacy was found after administering $\text{Ca}(\text{OH})_2$ as intracanal medication since 30% of the canals still contained a viable bacterial population (Ørstavik et al. 1991; Sathorn et al. 2007).

In this sense, bacteria may persist following intracanal treatment for several reasons, such as their confinement within anatomical variations of the canal space that are impervious to debridement attempts. Bacteria may penetrate up to 200 μm into the dentinal tubules (Love 1996); they may form biofilms or they may be inherently resistant to the medication (Siqueira and Lopes 1999). The properties of intraradicular bacteria and their propensity to form biofilms imply that the compound mechanism of action and its capacity to reach the target are crucial. It is of utmost importance to retain intracanal medications in the root canal for the necessary time to eliminate bacteria and their metabolic by-products (Pedrinha et al. 2022). In this area, the most popular $\text{Ca}(\text{OH})_2$ is marketed in paste form, supplemented with other compounds to enhance its properties, including stronger antibacterial and dissociation capabilities (Valverde et al. 2017). The primary effect relies on the high pH of calcium hydroxide (12.5), which can alter enzymatic activity, thereby disrupting cellular metabolism. However, the pH of $\text{Ca}(\text{OH})_2$ can be buffered by dentine, organic debris and tissue fluids, impacting its efficacy.

Moreover, in addition to ionic dissociation, the ability of $\text{Ca}(\text{OH})_2$ to effectively penetrate into the interior of the dentinal tubules before dissociating is crucial for its antibacterial efficacy since direct contact between the bacteria and $\text{Ca}(\text{OH})_2$ is required. Therefore, one of $\text{Ca}(\text{OH})_2$ main drawbacks could be the lack of direct interaction due to dental tubule anatomical complexities (Elmsmari et al. 2021). Moreover, it has been demonstrated that bacteria can penetrate up to 400 μm into the dentinal tubules while the average penetration of $\text{Ca}(\text{OH})_2$ is between 28 and 126 μm in the coronal section (Zand et al. 2017). Furthermore, some studies have demonstrated that the buffering effect that dentine exerts on $\text{Ca}(\text{OH})_2$ pastes must be considered. This effect can counteract the antibacterial properties of $\text{Ca}(\text{OH})_2$ and neutralise the pH increase. In this area, several authors investigated the vehicle impact on several $\text{Ca}(\text{OH})_2$ pastes and found that the vehicle is essential for the diffusion and dissociation of $\text{Ca}(\text{OH})_2$ (Gomes Camões et al. 2004; Pacios et al. 2003; Pedrinha et al. 2022). Some studies suggest that ionic dissociation is higher in low viscosity media causing a rapid effect, whereas the high molecular weight of currently marketed vehicles reduces $\text{Ca}(\text{OH})_2$

dispersion into the tissues and maintains the paste for extended periods of time (Athanasiadis et al. 2007). In order to address all these problems, a medication where the active ingredient is released gradually and continuously preventing pH saturation may constitute a suitable solution.

For this purpose, new advancements in intracanal medications have been attempted following different approaches. Some strategies involve integrating antimicrobial medicines along with delivery systems, such as antimicrobial peptides and nanoparticles, as they could substantially reduce the incidence of bacterial resistance (Ordinola-Zapata et al. 2022). The limitation in $\text{Ca}(\text{OH})_2$ capacity to achieve contact with pathogens has been overcome by encapsulating it inside nanoparticles (NPs) (Elmsmari et al. 2021). By using NPs, we have increased the ability of $\text{Ca}(\text{OH})_2$ to penetrate into dentinal tubules and achieve a prolonged and more constant release of $\text{Ca}(\text{OH})_2$, reducing the required therapeutic dose and side effects (Khan et al. 2015; Yetisgin et al. 2020). Furthermore, $\text{Ca}(\text{OH})_2$ encapsulation ($\text{Ca}(\text{OH})_2$ -NPs) also contributes to maintaining a high alkaline pH, enhancing its antibacterial capacity (Moodley and Ibrahim 2021; Sirén et al. 2014). Therefore, by using NPs made of poly(lactic-co-glycolic acid) (PLGA) and encapsulating $\text{Ca}(\text{OH})_2$, the limitations have been reduced. According to the literature, the efficacy of triclosan-loaded PLGA and PLA nanoparticles for the treatment of periodontal disease has been assessed. Triclosan was used because it is an antimicrobial agent with high efficacy against plaque-forming bacteria (Weatherly and Gosse 2017). Significant penetration into the dentinal tubules was achieved with both formulations, but PLGA nanoparticles provided better results. In this application, a reduction in gingival inflammation was achieved due to the rapid release of triclosan (Piñón-Segundo et al. 2005). Moreover, Leelapornpisid et al. (2024) also investigated the efficacy of biodegradable PLGA nanoparticles loaded with calcium hydroxide as an intracanal medication against endodontopathogenic microorganisms in a multi-species biofilm model, demonstrating a significantly lower viable cell density than $\text{Ca}(\text{OH})_2$.

Moreover, NPs average size below 200 nm allows NPs to enter the dentinal tubules (Lenzi et al. 2013), achieving direct contact between bacteria and active compounds, eliminating pathogens and their by-products. Furthermore, NPs dispersed in gelling forms may increase their adhesion to infected zones, allowing an increased contact between the $\text{Ca}(\text{OH})_2$ -NPs and bacteria (Folle et al. 2024).

Despite their main advantages, $\text{Ca}(\text{OH})_2$ -NPs are produced in a liquid form, which is suitable for their clinical application but lacks mucoadhesive properties that may enhance tissue retention. Therefore, the production of thermosensitive hydrogels constitutes a suitable solution (Li and Guan 2011). These gels present a fluid constitution until they reach 37°C; at this temperature, the interaction between their hydrophobic and hydrophilic segments alters, transitioning towards a gel-like structure (Dumortier et al. 2006). In this area, a polymer widely used for the production of thermosensitive gels is Poloxamer 407 (PA 407), which would enable $\text{Ca}(\text{OH})_2$ -NPs to be administered in a fluid form, facilitating its entry into the root canal system and subsequent gel formation, avoiding

subsequent infections. Calcium hydroxide nanoparticle gel ($\text{Ca}(\text{OH})_2$ -NPs-gel) represents an innovative advancement over previous nanoparticle-based formulations developed for intracanal disinfection by offering a synergistic combination of enhanced antimicrobial activity, improved drug delivery and prolonged therapeutic effects, all within a biocompatible and easy-to-use gel matrix (Song and Ge 2019; Forier et al. 2014; Gholami et al. 2018).

On a previous study, we have examined the rheological properties of $\text{Ca}(\text{OH})_2$ -NPs dispersed in a smart gel ($\text{Ca}(\text{OH})_2$ -NPs-gel) as well as demonstrate that it provides a prolonged release and a high penetration into the dentinal tubules (Roig et al. 2024). Therefore, the main hypothesis of this study was to observe the morphological features of the $\text{Ca}(\text{OH})_2$ -NPs-gel, confirm that physicochemical parameters were maintained after sterilisation and test whether $\text{Ca}(\text{OH})_2$ -NPs-gel will improve mucoadhesive strength and antibacterial activity against *Enterococcus faecalis* biofilms.

2 | Materials and Methods

For the mucoadhesive and antibacterial assessments, ethical approval was obtained from the Universitat Internacional de Catalunya with ethical code (END-INVI-2022-02). Additionally, the manuscript of this laboratory study has been written according to Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu et al. 2021).

2.1 | Materials

Poly-D,L-lactic-co-glycolic acid (PLGA) Resomer RG 503 H was purchased from Evonik Industries (Essen, Germany); $\text{Ca}(\text{OH})_2$ 98% extra pure was from ACROS Organics (Fisher Scientific, Waltham, Massachusetts, USA). Poloxamer 188 (P188), Poloxamer 407 (PA407) and dimethyl sulfoxide (DMSO) were supplied by Sigma-Aldrich (St. Louis, Missouri, USA). Acetone was from Fisher Scientific. All formulations were produced using Milli-Q water (Millipore Sigma, Burlington, Massachusetts, USA). All other reagents were of analytical grade.

2.2 | Preparation of $\text{Ca}(\text{OH})_2$ -NPs-Gel

Preparation of the $\text{Ca}(\text{OH})_2$ -NPs was performed using the solvent displacement method (Elmsmari et al. 2021; Sánchez-López, Gomes, et al. 2020). In this method, the drug, $\text{Ca}(\text{OH})_2$, was dissolved in the organic phase, constituted by DMSO and PLGA. The aqueous phase was formed by a surfactant solution (P188) in Milli-Q water. Once both phases were obtained, the organic phase was added drop by drop over the aqueous phase, under magnetic stirring. Finally, the organic solvents were eliminated using a Rotavapor R-210/215 (Buchi, Flawil, Switzerland) (Elmsmari et al. 2021).

Thermosensitive gels containing $\text{Ca}(\text{OH})_2$ -NPs were prepared using PA 407. In order to prepare them, 1.8 g PA 407 (18%) was

dissolved in $\text{Ca}(\text{OH})_2$ -NPs. The mixture was stirred at 1290 rpm for 5 min and left to stand overnight at 4°C (Arafa et al. 2018; Gonzalez-Pizarro et al. 2019).

2.3 | $\text{Ca}(\text{OH})_2$ -NPs-Gel Morphology

The study of the $\text{Ca}(\text{OH})_2$ -NPs-gel morphology was carried out by preparing a pool of three samples and using negative staining, for which uranyl acetate (2%) was used. The staining was performed by placing $\text{Ca}(\text{OH})_2$ -NPs-gel on copper grids that were activated by UV light. Transmission electron microscopy (TEM) was used to observe the morphology of $\text{Ca}(\text{OH})_2$ -NPs-gel by means of a JEOL 1010 microscope (Akishima, Japan), and several images from different grid sections were undertaken (Llorente et al. 2023; Thiruchenthoooran et al. 2022). In addition, using ImageJ processing software (GNU General Public License, USA), the average size (Z_{av}) of $\text{Ca}(\text{OH})_2$ -NPs-gel was also calculated (Galindo et al. 2022).

2.4 | Sterilisation Assays

$\text{Ca}(\text{OH})_2$ -NPs-gel sterilisation was performed using a 25 kGy irradiation source (Aragogamma, Spain). According to the European Pharmacopoeia, in the case of the sterilisation of pharmaceutical products, 25 kGy is the suitable dose to achieve, as it allows maintaining around 10^{-6} sterility assurance level (SAL) (Esteruelas et al. 2023; Ramos Yacasi et al. 2016; Thiruchenthoooran et al. 2022). To evaluate the stability of $\text{Ca}(\text{OH})_2$ -NPs-gel during sterilisation, their physicochemical parameters were studied. Therefore, physicochemical variables (Z_{av} , polydispersity index [PI] and zeta potential [ZP]) were analysed before and after irradiation using Zetasizer Nano ZS (Malvern Instruments, UK) (Elmsmari et al. 2021). Encapsulation efficiency (EE) was also indirectly analysed before and after sterilisation using an inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 8300; PerkinElmer, USA) to quantify $\text{Ca}(\text{OH})_2$ (Elmsmari et al. 2021).

2.5 | Ex Vivo Mucoadhesive Strength

The mucoadhesion force is defined as the force necessary to separate the formulation from a mucosal layer (Esteruelas et al. 2022). For this study, a modified balance was used, in which a falcon with a Teflon cylinder was placed on one of the arms, and on the other, an empty falcon was added to evaluate the mucoadhesion force required to separate the sample from the Teflon cylinder (Koffie et al. 2011). As a model for the bioadhesion tests, extracted single-rooted human teeth were used. After developing the test using Teflon in contact with one tooth (area of 11.58 mm²), mucoadhesion was also measured using a second tooth (area of 8.62 mm²). For this, both tissues and samples were placed in the thermostatic glass at 32°C and 10 µL of samples were added. Phosphate-buffered saline (PBS) was used as a blank, since it does not possess mucoadhesive properties (Takeuchi et al. 2005). Both samples, $\text{Ca}(\text{OH})_2$ -NPs and $\text{Ca}(\text{OH})_2$ -NPs-gel, were left in contact for 5 min so adhesion forces could be established. Subsequently, water was added

drop by drop in the falcon hawk of the opposite arm of the scale. Finally, when the limit force was separated from the mucosal formulation, the weight of the water added was quantified (Salunke and Patil 2016).

Mucoadhesive strength (dyne/cm²) was obtained by applying the following equation, where m is the mass required for detachment in grams, g is the acceleration due to gravity (980 cm/s²), and A is the surface area of mucosa exposed to the formulation (cm²):

$$\text{Mucoadhesive strength} = \frac{m \hat{A} \cdot g}{A}$$

2.6 | Short-Term Stability Test of Ca(OH)₂-NPs-Gel

Stability of Ca(OH)₂-NPs-gel was evaluated by analysing the properties of three individual samples of Ca(OH)₂-NPs-gel, and several aliquots were stored at three different temperatures (4°C, 25°C and 37°C). Physicochemical parameters (Z_{av} , PI, ZP and EE) were monthly analysed by triplicate, as specified elsewhere (Elmsmari et al. 2021). Additionally, backscattering (BS) and transmittance (T) profiles were measured each month using the Turbiscan Lab instrument (Formulation, France), which enables the identification of destabilisation processes such as creaming, sedimentation, flocculation or coalescence (Celia et al. 2009). This equipment owns a light source capable of emitting near-infrared light and T and BS detectors at angles of 45° and 180° from the incident beam, respectively. In this case, a total volume of 10 mL of sample was placed in a glass measurement cell. BS and T measurements were performed once a month for 24 h at intervals of 1 h (Du et al. 2020; Galindo et al. 2022).

2.7 | Antibacterial Assay

For the antibacterial analysis, an ex vivo model was performed using a method previously described (Teulé-Trull et al. 2024). Teeth extracted for reasons unrelated to this study were used after all patients accepted and signed the informed consent for tooth donation. For the preparation of the teeth, they were cut using a milling machine (Buehler, USA) and a teeth-specific cutting guide, allowing high precision and flat cuts without defects and 5 mm height blocks from the teeth apex. Then, teeth were instrumented with a single file reciprocating system (Reciproc R25; VDW, Munich) according to the manufacturer's instructions up to the working length, and irrigated with 4 mL of NaOCl 4.2% (Heinkel Iberica, Spain). Subsequently, the teeth were splinted by their axial axis ($n = 24$) and the blocks were reassembled and coated with nail polish to seal the block unions. Finally, these teeth were sterilised using gas plasma (Sterrads Plasma, Johnson and Johnson, USA). In order to guarantee sterility of samples, all teeth were rehydrated on Brain Heart Infusion (BHI) medium (Fisher Scientific, Waltham, Massachusetts, USA) and incubated at 37°C for 2 days, also validating the sterility of all samples by medium turbidity. Moreover, internal sterility controls were included to ensure sterility throughout the entire experiment: an untreated tooth was incubated in BHI and three falcon tubes containing only with BHI media alone were included.

The media in these controls was replaced in the same manner and frequency as the experimental groups.

Strains of *E. faecalis* (CECT 795) were thawed and placed in BHI for 24 h at 37°C, in an anaerobic atmosphere. For bacterial inoculation, dentine blocks were placed in wells and allowed to rehydrate in BHI for 30 min. Subsequently, the contents of the wells were aspirated, and BHI previously mixed with *E. faecalis* at an optical density of 0.1 at 600 nm was inoculated into each well. The plate was placed in the incubator at 37°C for 21 days (to have a mature *E. faecalis* biofilm), with BHI replacement every 3 days. After this time, the dentine blocks were moved to other clean wells and treatments were applied.

A total of 23 samples were prepared for antibacterial assays. Of these, 20 samples were randomly assigned to two experimental groups, while the remaining three served as untreated controls. Each experimental group received 0.5 mL of a specific medication placed in the canal: Group 1 ($n = 10$) was treated with Ca(OH)₂-NPs-gel (0.23% Ca(OH)₂), and Group 2 ($n = 10$) with CalciCur, a commercially available calcium hydroxide product with a stated concentration of 45% Ca(OH)₂. Following a 24-h incubation period, the samples were rinsed with 1× PBS using the gravity mode of a pipette controller to avoid turbulent flows. The samples from each group were then distributed for specific analyses, with half of the samples from each group allocated to each analysis type: six for confocal laser scanning microscopy (CLSM), six for bacterial metabolic activity and eight for scanning electron microscopy (SEM).

For CLMS, six samples (Group 1, $n = 3$; Group 2, $n = 3$) were analysed by observing three sections of each tooth (coronal, middle and apical sections) at 3, 6 and 9 mm from the apex. Live/dead staining (Sigma-Aldrich, St. Louis, Missouri, USA) was used to visualise the samples, which were then studied with a confocal microscope (Leica Microsystems, USA) at 10× and 63× magnifications (Teulé-Trull et al. 2024). Images were processed using LAS X Life Sciences software (Leica Microsystems, USA) (Tawakoli et al. 2013).

The metabolic activity of bacteria was assessed in six samples (Group 1, $n = 3$; Group 2, $n = 3$) using a 7 µg/mL resazurin solution (Sigma-Aldrich, St. Louis, Missouri, USA). Bacteria convert resazurin into resorufin in the presence of metabolic activity, inducing colour and fluorescence modification that can be detected by fluorometric and/or UV-Vis absorbance. Then, tooth samples were rinsed externally with sterile PBS; tooth blocks were disassembled, covered with 1 mL resazurin solution and incubated for 10 min under orbital agitation at 150 rpm to enhance the solubility of resorufin salt within the dentinal tubules. Subsequently, the absorbance at 570 and 600 nm was measured using a plate reader (Infinite M Nano+, Tecan). Metabolic activity was normalised against the untreated bacteria group, which was considered 100% (Dinh et al. 2023).

Subsequently, eight samples (Group 1, $n = 4$; Group 2, $n = 4$) were studied using a scanning electron microscope (SEM, JEOL JSM 5410, Tokyo, Japan) at a voltage acceleration of 10 kV. To carry out this study, the specimens were previously coated with a layer of gold by means of sputtering coating equipment (AGB7340, Essex, UK).

2.8 | Statistical Analysis

Statistical analysis of the results was performed using either Student's *t*-test or one-way ANOVA, previously confirming normal distribution (Anderson-Darling test). In the sterilisation assays, statistics were performed comparing each parameter before and after sterilisation using Student's *t*-test (overall significance level was set at 0.05, unless otherwise stated). In the same way, in the EE quantification, expressed in % of Ca(OH)_2 -NPs-gel evaluated monthly during storage at 4°C, 25°C and 37°C, statistics of the results were performed comparing each value against the initial timepoint by means of Student's *t*-test. In the ex vivo mucoadhesive strength, differences between groups were analysed by one-way ANOVA. Sterilisation assays, EE quantifications and ex vivo mucoadhesive strength were performed by triplicate. Finally, antibacterial assay differences between groups were analysed by one-way ANOVA. Subsequently, to assess whether there were significant differences between the different groups in the ex vivo mucoadhesive strength and antibacterial assay, a statistical Tukey post-test was carried out (GraphPad Prism 8.0, Boston, USA). Sample size was calculated using Minitab v17 (State College, Pennsylvania, USA), data from a previous publication (Elmsmari et al. 2021) and considering a margin of error of 10%.

3 | Results

3.1 | Ca(OH)_2 -NPs-Gel Morphology

The developed Ca(OH)_2 -NPs-gel was observed by TEM after negative staining. In that sense, the visualisation of the NPs (Figure 1) revealed that Ca(OH)_2 -NPs-gel presented a uniform

spherical shape dispersed in the amorphous thermosensitive gel without any signs of aggregation or compaction. Furthermore, NPs Z_{av} was below 200 nm, as previously reported using dynamic light scattering (DLS). In addition, Z_{av} of Ca(OH)_2 -NPs-gel obtained from the images was also analysed, obtaining a Z_{av} of 137.3 ± 4.26 nm.

3.2 | Sterilisation Assays

To confirm that the physicochemical properties of Ca(OH)_2 -NPs-gel were preserved, Z_{av} , PI, ZP and EE were assessed before and after γ -radiation. As it can be observed in Table 1, no significant differences were obtained before and after sterilisation. Therefore, sterilisation did not have a detrimental effect on the characteristics of the Ca(OH)_2 -NPs-gel, remaining within the accepted limits.

3.3 | Ex Vivo Mucoadhesive Strength

Mucoadhesive strength of Ca(OH)_2 -NPs and Ca(OH)_2 -NPs-gel was also studied and compared. Mucoadhesivity was evaluated either with the formulation in contact with dentine and Teflon or between two dentine samples (Figure 2). For each experiment, Ca(OH)_2 -NPs presented values of 691.33 and 920.48 dyne/cm², tooth-teflon and tooth-tooth respectively. These mucoadhesion strength results were similar to those obtained in the blank since no mucoadhesion was found. In the case of Ca(OH)_2 -NPs-gel a mucoadhesive force of 13 028.06 tooth-teflon and 19 860.31 dyne/cm² tooth-tooth was observed. These results indicate that Ca(OH)_2 -NPs-gel produces a significant increase in mucoadhesive strength ($p < 0.0001$) against Ca(OH)_2 -NPs.

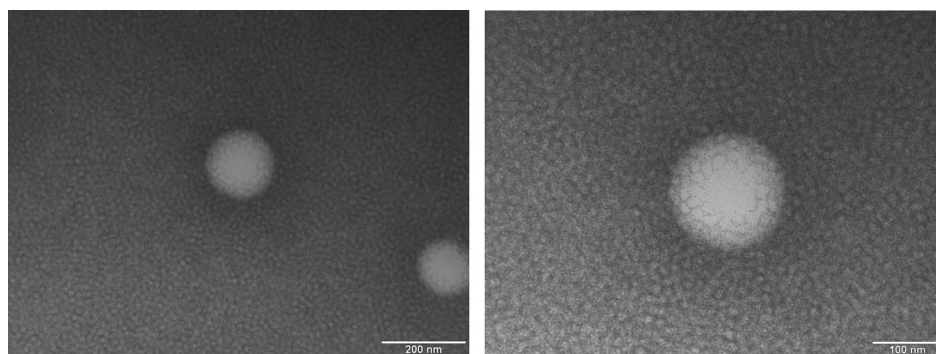


FIGURE 1 | TEM images of Ca(OH)_2 -NPs-gel. Scale bar of 200 and 100 nm respectively.

TABLE 1 | Comparison between physicochemical parameters after and before γ -radiation.

	$Z_{av} \pm SD$ (nm)	PI $\pm SD$	ZP $\pm SD$ (mV)	EE $\pm SD$ (%)
Before sterilisation	157.5 ± 2.7	0.067 ± 0.015	-31.1 ± 0.4	91.96 ± 3.1
After sterilisation	156.3 ± 1.2	0.087 ± 0.004	-29.2 ± 0.3	83.79 ± 4.2
Statistics	ns	ns	ns	ns

Note: Z_{av} , PI, ZP and EE are shown. Results correspond to mean $\pm$ standard deviation (SD). Statistics were performed comparing each parameter before and after sterilisation using Student's *t*-test ($p < 0.05$). The differences are presented as statistically non-significant (ns) or statistically significant (s).

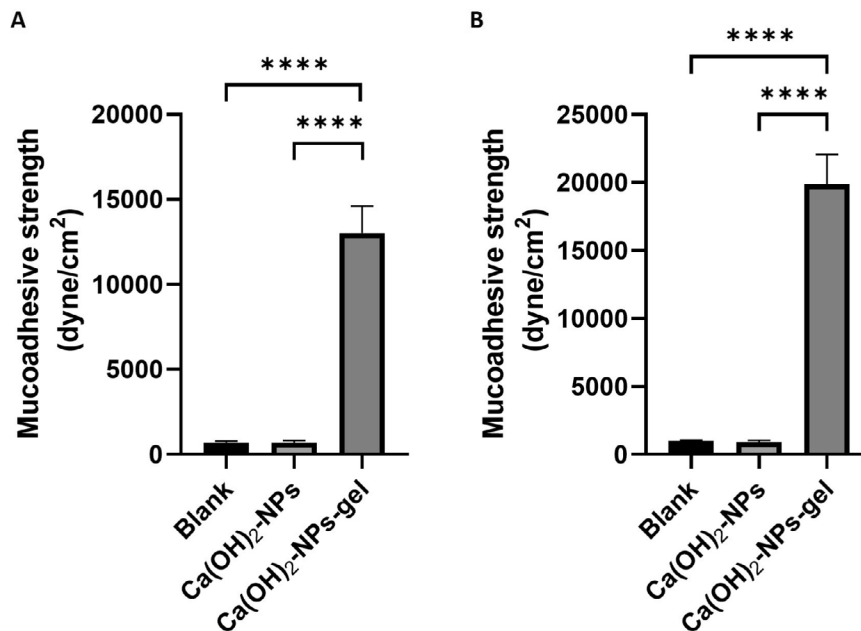


FIGURE 2 | Comparison of mucoadhesive strength of Ca(OH)₂-NPs-gel, Ca(OH)₂-NPs and Blank using (A) Tooth-teflon contact or (B) Tooth-to-tooth contact. Differences between groups were analysed by one-way ANOVA (*****p* < 0.0001) followed by Tukey post hoc test.

3.4 | Short-Term Stability Test of Ca(OH)₂-NPs-Gel

The short-term stability of Ca(OH)₂-NPs-gel was studied at three storage temperatures, and BS profile, T profile and EE were monthly measured (Galindo et al. 2022; Thiruchenthooran et al. 2022). As can be observed in Figure 3A, Ca(OH)₂-NPs-gel stored at 4°C did not show differences above 10% in the BS nor T profile even after 8 months of storage (Figure S1). This indicates that at 4°C, Ca(OH)₂-NPs-gel was stable for at least 8 months.

After being stored at 25°C, it was also observed that neither T nor the BS show an increase of more than 10% during the first 6 months. However, after 6 months, a destabilisation phenomenon was observed. Therefore, Ca(OH)₂-NPs-gel may be stored at this temperature during a limited period of time, being preferable 4°C as storage temperature. Finally, Ca(OH)₂-NPs-gel stored at 37°C showed to be unstable after the first month.

Moreover, as shown in Table 2, in accordance with BS and T results, EE values at 4°C did not show differences after 8 months of storage. In the case of 25°C, a relevant decrease in the EE occurs at 5 months. Finally, after being stored at 37°C, an important decrease in the EE was observed at the second month.

3.5 | Antibacterial Assay

To evaluate the antibacterial effect of Ca(OH)₂-NPs-gel, a live/dead staining assay was performed. It was analysed with the combination of FITC (fluorescein isothiocyanate)/Texas Red interference filter and then was studied with a confocal microscope. FITC was used to distinguish between live and dead cells in the dental tissue samples observed under a confocal microscope. This technique provides valuable information about cell viability and the health of the tissue under study. The FITC study

shows strong fluorescence (green), indicating a large number of bacterial aggregates. As can be observed in Figure 4, the merge of live/dead staining in the control group is predominantly green (live) compared to red (damaged cells). Conversely, in the case of both Ca(OH)₂-NPs-gel and Calciur, red staining prevails over green, indicating an increased concentration of damaged bacteria membranes.

These results can also be observed in Figure 5A, where this live/dead bacterial staining is reported at higher magnification and separated by tooth sections. The three tooth sections of the control groups show a predominance of live bacteria, while in both Calciur and Ca(OH)₂-NPs-Gel there is a predominance of bacterial death. Comparing these results with those obtained through the metabolic activity analysis, which would quantitatively indicate bacterial inactivation, the results are consistent. Metabolic activity (Figure 5B) shown by the marketed Ca(OH)₂ and Ca(OH)₂-NPs-gel was about 50%, being statistically significant compared to the control (*p* < 0.001), but there was no significant difference between both Ca(OH)₂ groups. However, it is worth noting that the marketed Ca(OH)₂ concentration was 5% whereas the optimised Ca(OH)₂ NPs-gel contained 0.23% of Ca(OH)₂.

Finally, an analysis of bacterial presence was carried out by SEM 72h after treatment application. According to the data obtained (Figure 6), all groups were covered by a dense *E. faecalis* biofilm. The cracks observed in all biofilms are attributed to the dehydration process required for SEM analysis.

4 | Discussion

Ca(OH)₂-NPs-gel stands out from other nanoparticle-based formulations due to its unique combination of enhanced antimicrobial activity, improved drug delivery, prolonged therapeutic effects and biocompatibility, all within an easy-to-use

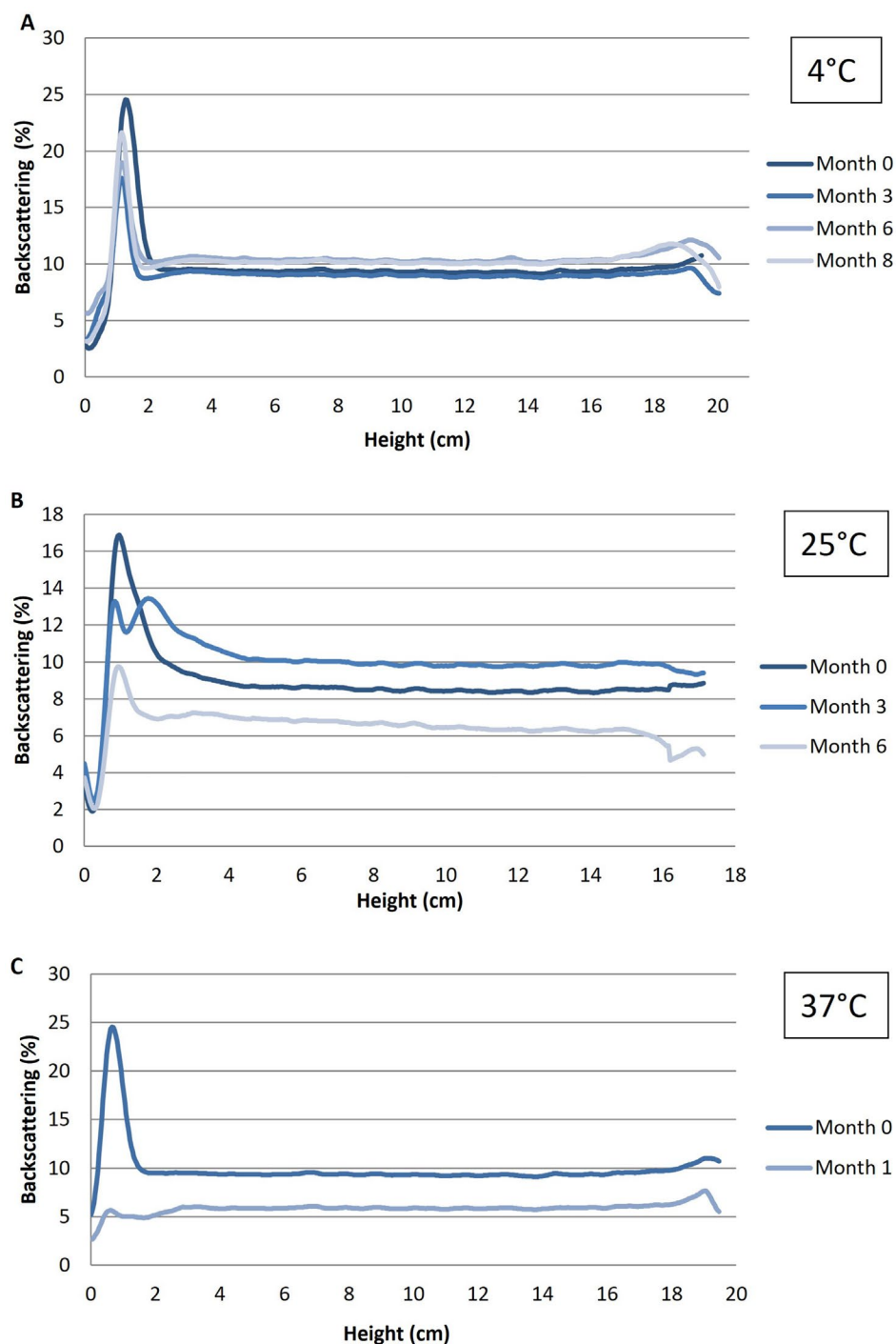


FIGURE 3 | Backscattering profiles measured monthly of Ca(OH)_2 -NPs-gel at (A) 4°C, (B) 25°C and (C) 37°C.

gel matrix (Carrêlo et al. 2022; Hamidi et al. 2008). NPs were formed by PLGA polymer since it is a biocompatible and biodegradable polymer accepted by regulatory agencies, and the gel matrix helps to prolong the release of the calcium hydroxide nanoparticles, providing a sustained antibacterial effect (Haseeb et al. 2016). In addition, the viscosity and density of the preparation, as well as the type of vehicle used, are determining factors. Gel-type formulations, due to their increased viscosity, enable denser and more controlled canal fillings, thereby reducing the risk of accidental extrusion. Furthermore, the vehicle employed in commercial calcium hydroxide pastes may influence their biological safety profile. Recently, Alnæs

et al. (2024) reported that polyethylene glycol, used as a vehicle in commercial calcium hydroxide formulations, may induce anaphylactic shock. To characterise Ca(OH)_2 -NPs-gel, it was measured by TEM, confirming an NPs average size below 200 nm. Ca(OH)_2 -NPs-gel showed a smooth surface and an average size similar to that acquired by DLS. In fact, the size obtained by DLS was slightly higher than the size measured by TEM (157.5 ± 2.7 nm and 137.3 ± 4.26 nm, respectively). This may be due to the fact that DLS measures the hydrodynamic radius of the NPs, whereas TEM measures the observed NPs size (Karow et al. 2015). This is of special importance since NPs possess a large surface area-to-volume ratio, potentially leading

TABLE 2 | Encapsulation efficiency (EE) expressed in % of $\text{Ca}(\text{OH})_2$ -NPs-gel evaluated monthly during storage at 4°C, 25°C and 37°C.

Time (months)	EE (%)		
	4°C	25°C	37°C
0	86.72 ± 0.27	80.93 ± 0.11	84.23 ± 0.41
1	80.39 ± 0.28	80.70 ± 0.17	81.09 ± 0.45
2	89.19 ± 0.40	78.30 ± 0.56	75.05 ± 0.45*
3	84.45 ± 0.12	79.78 ± 0.10	77.50 ± 0.14*
4	89.48 ± 0.38	81.14 ± 0.21	73.84 ± 0.28*
5	87.97 ± 0.5	71.79 ± 0.12*	58.38 ± 0.11***
6	87.32 ± 0.16	69.46 ± 0.50*	—
7	91.61 ± 0.38	74.82 ± 0.10*	—
8	87.78 ± 0.11	45.36 ± 0.22***	—

Note: Statistics of the results were performed comparing each value against the initial timepoint by means of Student's *t*-test (**p* < 0.05, ****p* < 0.001).

to a more rapid and extensive release of calcium and hydroxyl ions, which accelerates the disinfection process (Lungkapintha and Louwakul 2019). The higher charge density and surface areas of nanoparticles can also enable greater antibacterial efficacy (Lungkapintha and Louwakul 2019).

Furthermore, sterilisation is a safe and cost-effective method frequently used in the medical industry to eliminate microorganisms and is also a requirement for endodontic medications (Hasanain et al. 2014; Nasim and Hemmanur 2021; Ramos Yacasi et al. 2016). However, PLGA is a thermosensitive polymer and cannot be sterilised using autoclave or ethylene oxide; $\text{Ca}(\text{OH})_2$ -NPs-gel was sterilised using γ -radiation. To confirm that physicochemical properties are not affected by radiation, $\text{Ca}(\text{OH})_2$ -NPs-gel was evaluated before and after this process. No significant differences were obtained in the main physicochemical parameters before and after the sterilisation, confirming that $\text{Ca}(\text{OH})_2$ -NPs-gel was suitable for γ -irradiation, preserving their physicochemical properties (Hasanain et al. 2014).

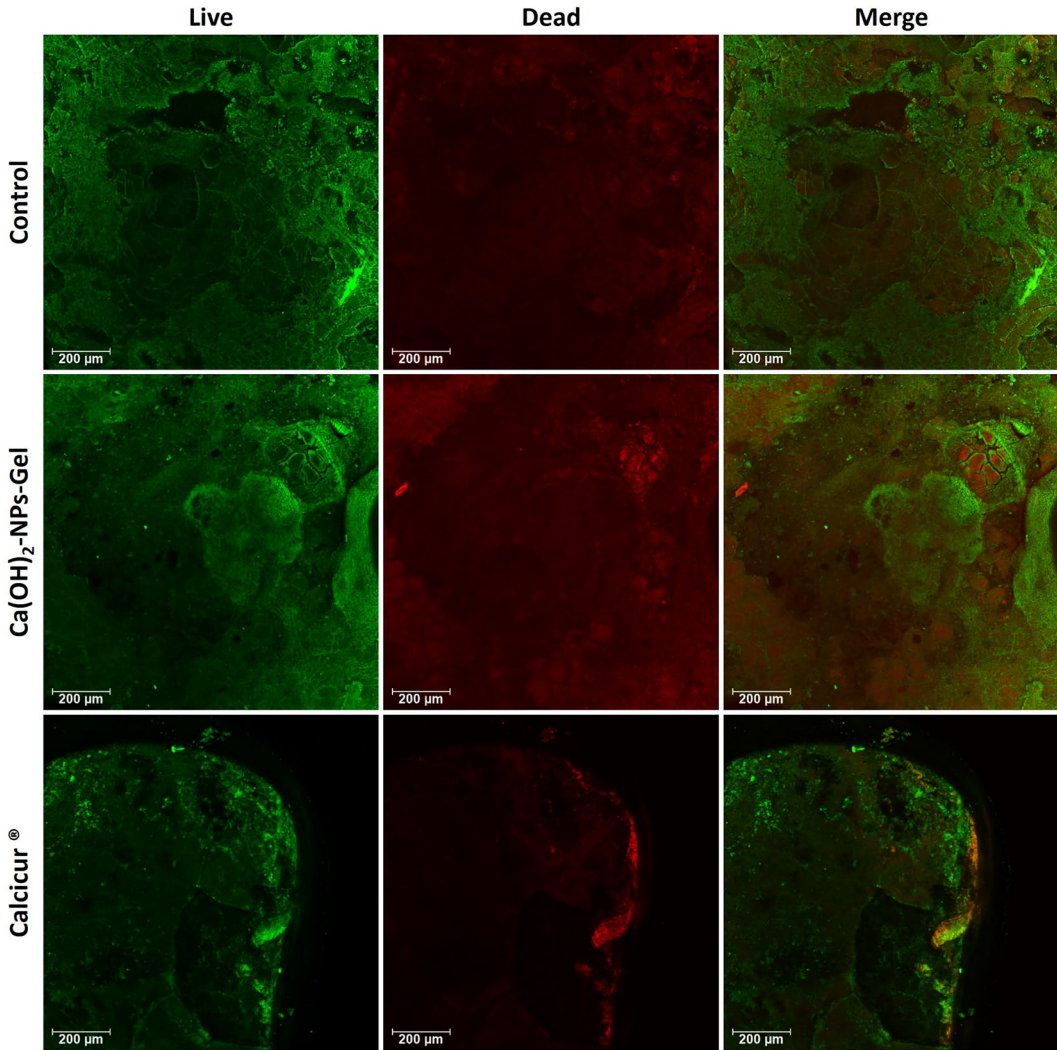


FIGURE 4 | Antibacterial capacity. Confocal microscopy images taken at 10× of live/dead stained dentine blocks 24 h after placing $\text{Ca}(\text{OH})_2$ -NPs-gel, CalciCur and control, split between live, dead and the merge of the both stains.

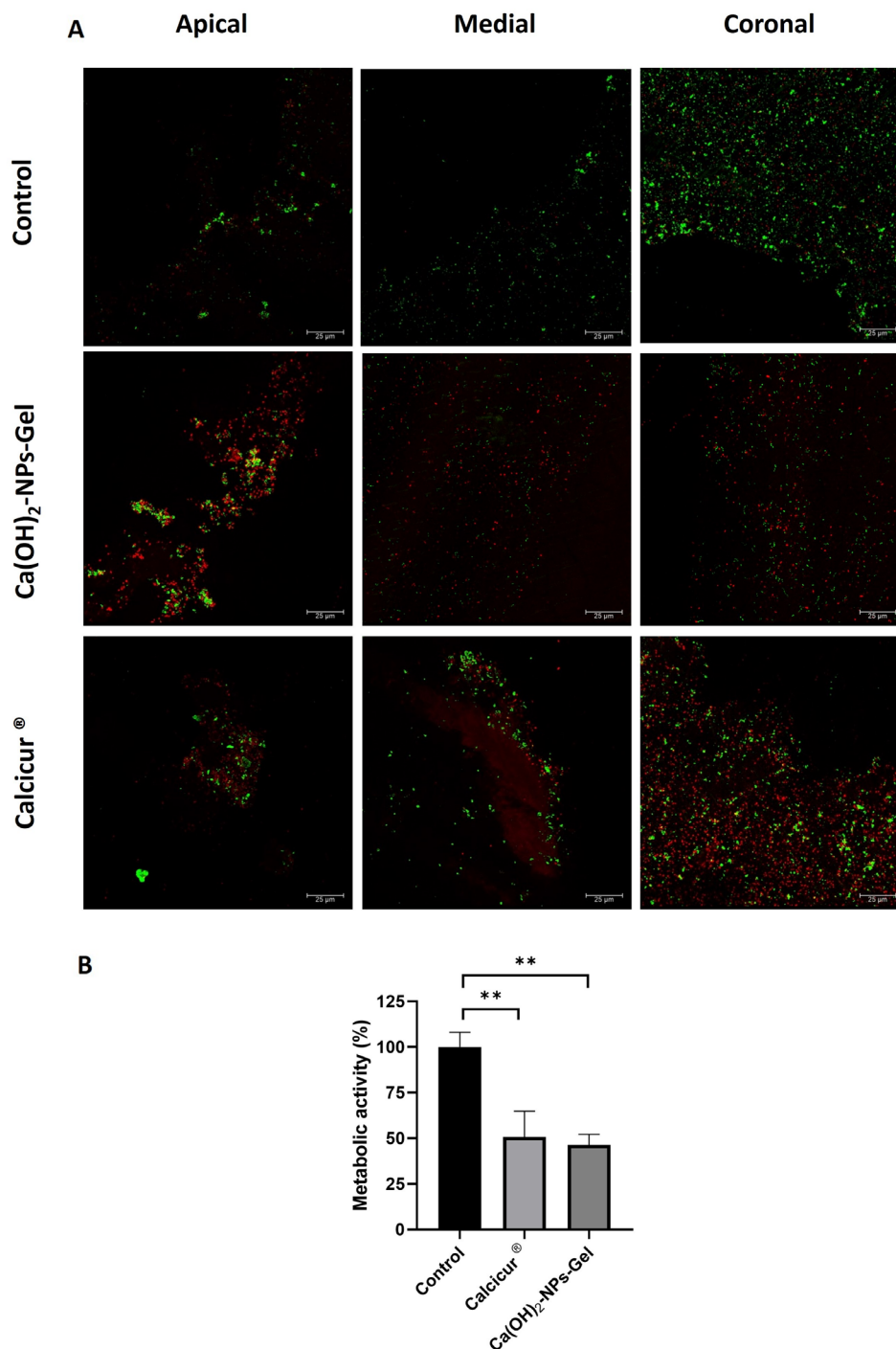


FIGURE 5 | Antibacterial capacity. (A) Confocal microscopy images at 63× magnifications of live/dead stained dentine blocks 24 h after placing Ca(OH)₂-NPs-gel, CalciCur or control at 63× magnification. (B) Metabolic activity on dentine blocks 24 h after placing Ca(OH)₂-NPs-gel, CalciCur or control. Differences between groups were analysed by one-way ANOVA (** $p < 0.001$) followed by Tukey post hoc test.

Mucoadhesive strength of sterile Ca(OH)₂-NPs-gel was also evaluated. In this regard, there were no significant differences between Ca(OH)₂-NPs and the blank control. This was expected since Ca(OH)₂-NPs in liquid form do not possess mucoadhesive properties (Dumortier et al. 2006; Rahman et al. 2014). In fact, the mucoadhesion strength obtained by the blank may be a result of the force produced by the surface tension of the liquid (Kleinheins et al. 2023). Upon contact inside the tooth at body temperature (37°C), the Ca(OH)₂-NPs-gel undergoes thermal gelation due to the aggregation

of PA 407 copolymer chains into micellar structures (Folle et al. 2021; Giuliano et al. 2020)—initiated by the dehydration of its polypropylene oxide units—thereby significantly enhancing mucoadhesive strength and improving retention (Fakhari et al. 2017). Moreover, a slight increase in mucoadhesion strength is observed in the case of the assay carried out with the tooth-tooth contact surface. This could be due to the fact that the tooth is a more porous surface than the teflon, so that the Ca(OH)₂-NPs-gel would increase its adhesion when introduced into these pores. Ca(OH)₂-NPs-gel showed a high

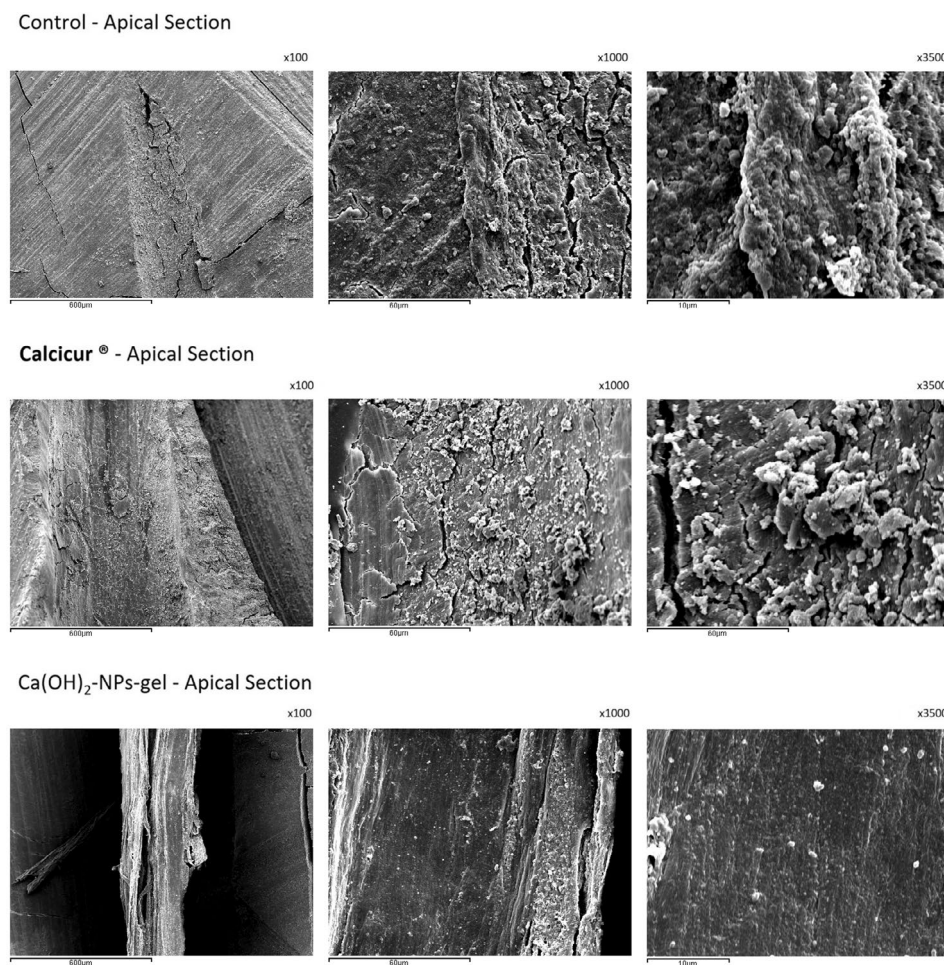


FIGURE 6 | Scanning electron microscopy (SEM) analysis of bacteria on the root canal surface, 72 h after treatment.

mucoadhesion compared with Ca(OH)_2 -NPs, but at the same time, not as high as other gels (Morsi et al. 2017), allowing the Ca(OH)_2 -NPs-gel to flow and distribute into the tooth and reach deeper into the canals (Giuliano et al. 2018). In addition, the formulation's thermosensitive properties allow the irrigation in liquid form inside the root canal, only increasing its viscosity in contact with body tissues (Husain 2018).

Short-term stability was also evaluated at three different temperatures, thus demonstrating that at 4°C, Ca(OH)_2 -NPs-gel was stable for 8 months after its production. This temperature is commonly used to preserve NPs for a long period of time (Abdelwahed et al. 2006). These results agree with other studies carried out with PLGA NPs, where 4°C is the preferable storage temperature (Dong et al. 2006; Esteruelas et al. 2023; Galindo et al. 2022; Sánchez-López et al. 2016). Moreover, at 25°C the results show that Ca(OH)_2 -NPs-gel remains stable for 5 months. This is especially relevant since Ca(OH)_2 -NPs-gel may be transported at 25°C but afterwards stored for long periods at 4°C. Finally, it is worth noting that at 37°C, Ca(OH)_2 -NPs-gel was not stable. This may be due to PLGA's thermosensitive profile, since, as demonstrated in other studies, this storage temperature may cause its degradation (Kumar et al. 2012).

Since the present formulation is aimed to act as antibacterial, its therapeutic efficacy was assessed *ex vivo*. It has been previously

elucidated that the antibacterial effect of Ca(OH)_2 occurs through the release of oxidising hydroxyl ions (Wang et al. 2012). These free radicals act on bacteria where they denature proteins, damage the cytoplasmic membrane and damage DNA (Dizdaroglu and Jaruga 2012). However, a possible complication in the action of hydroxyl ions is the buffering effect of dentine and hydroxyapatite against alkaline substances such as Ca(OH)_2 , leading to a substantial decrease in the antibacterial capacity of Ca(OH)_2 (Mohammadi and Dummer 2011; Sathorn et al. 2007; Teoh et al. 2018). The studies performed showed that Ca(OH)_2 -NPs-gel preserves the effect of CalciCur without showing significant differences between both formulations. These results are in line with the results obtained using the live/dead staining, thus demonstrating that Ca(OH)_2 -NPs-gel showed similar antibacterial properties to CalciCur but, as previously demonstrated, Ca(OH)_2 -NPs-gel was able to achieve a prolonged release and increased penetration (Roig et al. 2024). In previous studies developed by Leelapornpisid et al. (2024), Ca(OH)_2 -NPs showed superior bacterial reduction against free Ca(OH)_2 . However, the formulation assessed contained 6.5 times higher concentration of Ca(OH)_2 , and different bacterial strains (including *Candida albicans* and *Streptococcus gordonii*) were tested, which hinders the direct comparison with our results. These differences highlight the need for future studies to include a broader spectrum of endodontic pathogens to fully evaluate the antibacterial potential of Ca(OH)_2 -NPs-gel.

Moreover, the activity against biofilms was also assessed by SEM. After 72 h of the treatment application (either Calciur and Ca(OH)_2 -NPs-gel), qualitative observation through SEM showed a decrease in the number of colonies and aggregates compared to the control, but some stable and mature biofilms were still observed. The dentine buffer may be preventing its effect from being complete, seeing this remaining biofilm in places such as the apical section (Mohammadi and Dummer 2011; Sathorn et al. 2007; Teoh et al. 2018). In addition, in a previous publication, it was demonstrated that Ca(OH)_2 -NPs-gel was able to penetrate further than Ca(OH)_2 -NPs (Roig et al. 2024) and this fact may increase the potential antibacterial effect. However, future studies should focus on validating these findings in more clinically relevant models, such as in vivo settings.

Ca(OH)_2 -NPs-gel formulation represents a nanotechnological advancement in intracanal medication. The main limitation of conventional Ca(OH)_2 is its limited penetration into dentine tubules and bacterial niches (Wang et al. 2012). In this sense, our previous study demonstrated that nanoparticles exhibited significantly greater penetration into the tubules (Roig et al. 2024). Direct contact with bacteria inside their habitats is crucial. Additionally, the thermosensitive gel dispersion allows for prolonged and controlled release, thereby maintaining a therapeutic concentration for a longer period. The gel formulation contained only 0.23% Ca(OH)_2 , whereas the commercial formulation used contained 45% of the active compound. Achieving comparable antibacterial efficacy with approximately 200 times less active ingredient represents a significant benefit, as it could reduce systemic exposure and the likelihood of adverse effects. Notably, Ca(OH)_2 -NPs-gel achieved similar antibacterial effects to the commercial formulation despite using approximately 200 times lower Ca(OH)_2 concentration (0.23% vs. 45%). This sustained and controlled release (Roig et al. 2024) may reduce the need for retreatment and minimise potential side effects, offering practical advantages for both clinicians and patients (Heng 2018; Jain 2020; Roig et al. 2024). Besides, the formulation exhibits a greater penetration capacity than the free drug in the innermost areas of the tooth, where root canals are smaller. The fact that the Ca(OH)_2 -NPs gel reaches the deeper areas is crucial, as this is one of the main drawbacks of current Ca(OH)_2 drugs, as they are unable to eliminate bacteria in this area due to their inability to access to the most apical sections of root canals (Song and Ge 2019; Yohan and Chithrani 2014).

According to literature (Hauman and Love 2003; De Moor and De Witte 2002; Nelson Filho et al. 1999), the periapical extrusion of conventional calcium hydroxide carries serious and unpredictable clinical risks. Reported consequences may include tissue necrosis (Kim et al. 2009), significant neurological injury (Ahlgren et al. 2003), foreign body granuloma or cyst formation and antrolith development (Bramante et al. 2008). The severity of these lesions is attributed to the direct contact of the highly alkaline material with vital tissues, which in turn affects the local concentration and activity of the compound.

In the evaluation of a new intracanal medication, it would be important to investigate its removability due to its unique properties. The novel thermosensitive gel formulation differs from traditional pastes, and the vehicle may affect Ca(OH)_2 removal. The

greater penetration of the gel into the dentinal tubules, reaching deeper areas, is beneficial for disinfection, but can also make it difficult to completely remove the material. While enhanced mucoadhesion may favour intracanal retention, it may also hinder removal. Therefore, future studies should thoroughly investigate the removal of new intracanal medicaments, and a deep analysis of removability is therefore essential before clinical use.

Therefore, in this article, the development and characterisation of a novel formulation based on gel-dispersed biodegradable nanoparticles able to encapsulate Ca(OH)_2 was performed, and its antimicrobial performance was assessed ex vivo. Ca(OH)_2 -NPs-gel constitutes not only a novel paradigm in endodontic disinfection but also provides a smart approach to obtain a prolonged Ca(OH)_2 release with increased mucoadhesivity, which enhances nanoparticles' penetration into dentinal tubules, providing suitable antimicrobial performance with a formulation containing around 200-fold less amount of active compound than the marketed available pastes. Additionally, it is worth noting that although Ca(OH)_2 -NPs-gel requires a multi-step production as well as extensive physicochemical characterisation, their cost is relatively low compared with other types of nanoparticles. Despite the results obtained, the present research is limited to the study of the physicochemical parameters of the formulation and its ex vivo antimicrobial capacity. In this area, further experiments using additional timepoints, removal of intracanal medication, biocompatibility and cytotoxicity studies, and in vivo models of endodontic infection compared with the current marketed available products will be required in order to corroborate the disinfection capacity performance of Ca(OH)_2 -NPs-gel.

5 | Conclusion

A nanotechnological formulation for endodontic disinfection based on biodegradable nanoparticles dispersed in a thermosensitive gel containing calcium hydroxide (Ca(OH)_2 -NPs-gel) was developed and characterised. Morphological studies showed that Ca(OH)_2 -NPs-gel owned a nanometric particle size below 200 nm with a smooth surface and no aggregation signs. Moreover, Ca(OH)_2 -NPs-gel presented enhanced mucoadhesive strength against Ca(OH)_2 -NPs. Furthermore, even though Ca(OH)_2 -NPs-gel contains less Ca(OH)_2 amount than the marketed formulation, it exhibits an ex vivo antibacterial effect similar to the commercial Ca(OH)_2 .

Author Contributions

Xavier Roig-Soriano: investigation, methodology, writing – original draft. **Luis María Delgado:** investigation, supervision, writing – review and editing. **Firas Elmsmari:** investigation, methodology. **Fernando Duran-Sindreu:** funding acquisition, investigation, supervision, writing – review and editing. **Miriam Teulé:** investigation, methodology. **Marta Espina:** investigation, methodology, formal analysis. **Gerard Esteruelas:** investigation, methodology. **Maria Luisa García:** funding acquisition, investigation, writing – review and editing. **José Antonio González Sánchez:** funding acquisition, investigation, supervision, writing – original draft. **Elena Sánchez-López:** funding acquisition, investigation, supervision, writing – original draft.

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Conflicts of Interest

Dr. Firas Elmsmari, Dr. Elena Sánchez-López, Dr. Fernando Duran-Sindreu, Dr. José Antonio González Sánchez and Dr. Maria Luisa Garcia report a licensed patent with the Universitat Internacional De Catalunya and University of Barcelona—Composition comprising nanoparticles, method for the preparation of a composition comprising nanoparticles and uses of the composition for dental treatment. European patent with the file number: EP20382504. The other authors have stated explicitly that there are no conflicts of interest in connection with this article.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- Abdelwahed, W., G. Degobert, S. Stainmesse, and H. Fessi. 2006. "Freeze-Drying of Nanoparticles: Formulation, Process and Storage Considerations." *Advanced Drug Delivery Reviews* 58, no. 15: 1688–1713. <https://doi.org/10.1016/j.addr.2006.09.017>.
- Ahlgren, F. K. E. K., A. C. Johannessen, and S. Hellem. 2003. "Displaced Calcium Hydroxide Paste Causing Inferior Alveolar Nerve Paraesthesia: Report of a Case." *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* 96, no. 6: 734–737. <https://doi.org/10.1016/j.tripleo.2003.08.018>.
- Alnæs, M., T. Storaas, H. K. Vindenes, A. B. Guttormsen, and L. Björkman. 2024. "Anaphylaxis After Treatment With an Endodontic Material Containing Polyethylene Glycol." *Journal of Endodontics* 50, no. 11: 1649–1654. <https://doi.org/10.1016/j.joen.2024.09.002>.
- Arafa, M. G., R. F. El-Kased, and M. M. Elmazar. 2018. "Thermoresponsive Gels Containing Gold Nanoparticles as Smart Antibacterial and Wound Healing Agents." *Scientific Reports* 8, no. 1: 1–16. <https://doi.org/10.1038/s41598-018-31895-4>.
- Athanassiadis, B., P. V. Abbott, and L. J. Walsh. 2007. "The Use of Calcium Hydroxide, Antibiotics and Biocides as Antimicrobial Medicaments in Endodontics." *Australian Dental Journal* 52, no. 1S: S64–S82. <https://doi.org/10.1111/j.1834-7819.2007.tb00527.x>.
- Bramante, C. M., S. M. Luna-Cruz, C. R. Sipert, et al. 2008. "Alveolar Mucosa Necrosis Induced by Utilisation of Calcium Hydroxide as Root Canal Dressing." *International Dental Journal* 58, no. 2: 81–85. <https://doi.org/10.1111/j.1875-595x.2008.tb00180.x>.
- Carrêlo, H., A. R. Escoval, P. I. P. Soares, J. P. Borges, and M. T. Cidade. 2022. "Injectable Composite Systems of Gellan Gum:Alginate Microparticles in Pluronic Hydrogels for Bioactive Cargo Controlled Delivery: Optimization of Hydrogel Composition Based on Rheological Behavior." *Fluids* 7, no. 12: 375. <https://doi.org/10.3390/fluids7120375>.
- Celia, C., E. Trapasso, D. Cosco, D. Paolino, and M. Fresta. 2009. "Turbiscan Lab® Expert Analysis of the Stability of Ethosomes® and Ultradeformable Liposomes Containing a Bilayer Fluidizing Agent." *Colloids and Surfaces B: Biointerfaces* 72, no. 1: 155–160. <https://doi.org/10.1016/j.colsurfb.2009.03.007>.
- De Moor, R. J. G., and A. M. J. C. De Witte. 2002. "Periapicallesions Accidentally Filled With Calcium Hydroxide." *International Endodontic*

Journal 35, no. 11: 946–958. <https://doi.org/10.1046/j.1365-2591.2002.00597.x>.

- Dinh, M. N., M. Hitomi, Z. A. Al-Turaihi, and J. G. Scott. 2023. "Alamar Blue Assay Optimization to Minimize Drug Interference and Inter-Assay Viability." Preprint, bioRxiv. <https://doi.org/10.1101/2023.03.16.532999>.
- Dizdaroğlu, M., and P. Jaruga. 2012. "Mechanisms of Free Radical-Induced Damage to DNA." *Free Radical Research* 46, no. 4: 382–419. <https://doi.org/10.3109/10715762.2011.653969>.
- Dong, W. Y., M. Körber, V. López Esguerra, and R. Bodmeier. 2006. "Stability of Poly(d,l-Lactide-Co-Glycolide) and Leuprolide Acetate in In-Situ Forming Drug Delivery Systems." *Journal of Controlled Release* 115, no. 2: 158–167. <https://doi.org/10.1016/j.jconrel.2006.07.013>.
- Du, S., H. Wang, F. Jiang, and Y. Wang. 2020. "Diabetic Retinopathy Analysis—Effects of Nanoparticle-Based Triamcinolone." *Journal of Nanoscience and Nanotechnology* 20, no. 10: 6111–6115. <https://doi.org/10.1166/jnn.2020.18569>.
- Dumortier, G., J. L. Grossiord, F. Agnely, and J. C. Chaumeil. 2006. "A Review of Poloxamer 407 Pharmaceutical and Pharmacological Characteristics." *Pharmaceutical Research* 23, no. 12: 2709–2728. <https://doi.org/10.1007/s11095-006-9104-4>.
- Elmsmari, F., J. A. González Sánchez, F. Duran-Sindreu, et al. 2021. "Calcium Hydroxide-Loaded PLGA Biodegradable Nanoparticles as an Intracanal Medicament." *International Endodontic Journal* 54, no. 11: 2086–2098. <https://doi.org/10.1111/iej.13603>.
- Esteruelas, G., L. Halbaut, V. García-Torra, et al. 2022. "Development and Optimization of Riluzole-Loaded Biodegradable Nanoparticles Incorporated in a Mucoadhesive in Situ Gel for the Posterior Eye Segment." *International Journal of Pharmaceutics* 612: 121379. <https://doi.org/10.1016/j.ijpharm.2021.121379>.
- Esteruelas, G., E. B. Souto, M. L. Espina, and E. Sánchez-López. 2023. "Diclofenac Loaded Biodegradable Nanoparticles as Antitumoral and Antiangiogenic Therapy." *Pharmaceutics* 15, no. 1: 102. <https://doi.org/10.3390/pharmaceutics15010102>.
- Fakhari, A., M. Corcoran, and A. Schwarz. 2017. "Thermogelling Properties of Purified Poloxamer 407." *Heliyon* 3, no. 8: e00390. <https://doi.org/10.1016/j.heliyon.2017.e00390>.
- Folle, C., N. Díaz-Garrido, E. Sánchez-López, et al. 2021. "Surface-Modified Multifunctional Thymol-Loaded Biodegradable Nanoparticles for Topical Acne Treatment." *Pharmaceutics* 13, no. 9: 1–23. <https://doi.org/10.3390/pharmaceutics13091501>.
- Folle, C., A. M. Marqués, N. Díaz-garrido, et al. 2024. "Gel-Dispersed Nanostructured Lipid Carriers Loading Thymol Designed for Dermal Pathologies." *International Journal of Nanomedicine* 19: 1225–1248. <https://doi.org/10.2147/IJN.S433686>.
- Forier, K., K. Raemdonck, S. C. De Smedt, J. Demeester, T. Coenye, and K. Braeckmans. 2014. "Lipid and Polymer Nanoparticles for Drug Delivery to Bacterial Biofilms." *Journal of Controlled Release* 190: 607–623. <https://doi.org/10.1016/j.jconrel.2014.03.055>.
- Freeman, K., J. R. Ludington, A. Timothy, G. J. Pinero Svec, and J. Hoover. 1994. "Continuously Infused Calcium Hydroxide: Its Influence on Hard Tissue Repair." *Journal of Endodontics* 20, no. 6: 272–275. [https://doi.org/10.1016/S0099-2399\(06\)80814-9](https://doi.org/10.1016/S0099-2399(06)80814-9).
- Galindo, R., S. Elena, M. Espina, et al. 2022. "Development of Peptide Targeted PLGA-PEGylated Nanoparticles Loading Licochalcone-A for Ocular Inflammation." *Pharmaceutics* 14: 285.
- Gholami, A., A. Ebrahiminezhad, N. Abootalebi, and Y. Ghasemi. 2018. "Synergistic Evaluation of Functionalized Magnetic Nanoparticles and Antibiotics Against *Staphylococcus aureus* and *Escherichia coli*." *Pharmaceutical Nanotechnology* 6, no. 4: 276–286. <https://doi.org/10.2174/2211738506666181031143048>.

- Giuliano, E., D. Paolino, M. C. Cristiano, M. Fresta, and D. Cosco. 2020. "Rutin-Loaded Poloxamer 407-Based Hydrogels for in Situ Administration: Stability Profiles and Rheological Properties." *Nanomaterials* 10, no. 6: 3–5. <https://doi.org/10.3390/nano10061069>.
- Giuliano, E., D. Paolino, M. Fresta, and D. Cosco. 2018. "Mucosal Applications of Poloxamer 407-Based Hydrogels: An Overview." *Pharmaceutics* 10, no. 3: 1–26. <https://doi.org/10.3390/pharmaceutics10030159>.
- Gomes Camões, I. C., M. R. Salles, O. Chevitarese, and L. N. L. F. Gomes. 2004. "Diffusion of $\text{Ca}(\text{OH})_2$ Associated With Different Vehicles: Chromatographic Study (High-Performance Liquid Chromatography)." *Journal of Endodontics* 30, no. 1: 30–34. <https://doi.org/10.1097/00004770-200401000-00006>.
- Gonzalez-Pizarro, R., P. Carvajal-Vidal, L. Halbault Bellowa, A. C. Calpena, M. Espina, and M. L. Garcia. 2019. "In-Situ Forming Gels Containing Fluorometholone-Loaded Polymeric Nanoparticles for Ocular Inflammatory Conditions." *Colloids and Surfaces B: Biointerfaces* 175: 365–374. <https://doi.org/10.1016/j.colsurfb.2018.11.065>.
- Hamidi, M., A. Azadi, and P. Rafiei. 2008. "Hydrogel Nanoparticles in Drug Delivery." *Advanced Drug Delivery Reviews* 60, no. 15: 1638–1649. <https://doi.org/10.1016/j.addr.2008.08.002>.
- Hasanain, F., K. Guenther, W. M. Mullett, and E. Craven. 2014. "Gamma Sterilization of Pharmaceuticals—A Review of the Irradiation of Excipients, Active Pharmaceutical Ingredients, and Final Drug Product Formulations." *PDA Journal of Pharmaceutical Science and Technology* 68, no. 2: 113–137. <https://doi.org/10.5731/pdajpst.2014.00955>.
- Haseeb, R., M. Lau, M. Sheah, et al. 2016. "Synthesis and Characterization of New Chlorhexidine-Containing Nanoparticles for Root Canal Disinfection." *Materials* 9, no. 6: 452. <https://doi.org/10.3390/ma9060452>.
- Hauman, C. H. J., and R. M. Love. 2003. "Biocompatibility of Dental Materials Used in Contemporary Endodontic Therapy: A Review. Part 1. Intracanal Drugs and Substances." *International Endodontic Journal* 36, no. 2: 75–85. <https://doi.org/10.1046/j.1365-2591.2003.00631.x>.
- Heng, P. W. S. 2018. "Controlled Release Drug Delivery Systems." *Pharmaceutical Development and Technology* 23, no. 9: 833. <https://doi.org/10.1080/10837450.2018.1534376>.
- Husain, M. A. 2018. "Dental Anatomy and Nomenclature for the Radiologist." *Radiologic Clinics of North America* 56, no. 1: 1–11. <https://doi.org/10.1016/j.rcl.2017.08.001>.
- Jain, K. K. 2020. *Drug Delivery Systems. Methods in Molecular Biology*. Vol. 2059. 3rd ed. Springer.
- Karow, A. R., J. Götzl, and P. Garidel. 2015. "Resolving Power of Dynamic Light Scattering for Protein and Polystyrene Nanoparticles." *Pharmaceutical Development and Technology* 20, no. 1: 84–89. <https://doi.org/10.3109/10837450.2014.910808>.
- Khan, I., M. Khan, M. N. Umar, and D. H. Oh. 2015. "Nanobiotechnology and Its Applications in Drug Delivery System: A Review." *IET Nanobiotechnology* 9, no. 6: 396–400. <https://doi.org/10.1049/iet-nbt.2014.0062>.
- Kim, J. W., K. M. Cho, S. H. Park, et al. 2009. "Overfilling of Calcium Hydroxide-Based Paste Calcipect II Produced a Foreign Body Granuloma Without Acute Inflammatory Reaction." *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 107, no. 3: e73–e76. <https://doi.org/10.1016/j.tripleo.2008.10.019>.
- Kleinheins, J., N. Shardt, M. El Haber, et al. 2023. "Surface Tension Models for Binary Aqueous Solutions: A Review and Intercomparison." *Physical Chemistry Chemical Physics* 25, no. 16: 11055–11074. <https://doi.org/10.1039/d3cp00322a>.
- Koffie, R. M., C. T. Farrar, L. J. Saidi, C. M. William, B. T. Hyman, and T. L. Spires-Jones. 2011. "Nanoparticles Enhance Brain Delivery of Blood-Brain Barrier-Impermeable Probes for in Vivo Optical and Magnetic Resonance Imaging." *Proceedings of the National Academy of Sciences of the United States of America* 108, no. 46: 18837–18842. <https://doi.org/10.1073/pnas.1111405108>.
- Kumar, G., N. Shafiq, and S. Malhotra. 2012. "Drug-Loaded PLGA Nanoparticles for Oral Administration: Fundamental Issues and Challenges Ahead." *Critical Reviews in Therapeutic Drug Carrier Systems* 29, no. 1: 149–182. <https://doi.org/10.1615/critrevtherdrugcarriersyst.v29.i2.20>.
- Leelapornpisid, W., P. Wanwatanakul, and T. Mahatnirunkul. 2024. "Efficacy of Calcium Hydroxide-Loaded Poly(Lactic-Co-Glycolic Acid) Biodegradable Nanoparticles as an Intracanal Medicament Against Endodontopathogenic Microorganisms in a Multi-Species Biofilm Model." *Australian Endodontic Journal* 50, no. 1: 89–96. <https://doi.org/10.1111/aej.12812>.
- Lenzi, T. L., C. D. A. B. Guglielmi, V. E. Arana-Chavez, and D. P. Raggio. 2013. "Tubule Density and Diameter in Coronal Dentin From Primary and Permanent Human Teeth." *Microscopy and Microanalysis* 19, no. 6: 1445–1449. <https://doi.org/10.1017/S1431927613012725>.
- Li, Z., and J. Guan. 2011. "Thermosensitive Hydrogels for Drug Delivery." *Expert Opinion on Drug Delivery* 8, no. 8: 991–1007. <https://doi.org/10.1517/17425247.2011.581656>.
- Llorente, X., G. Esteruelas, L. Bonilla, et al. 2023. "Riluzole-Loaded Nanostructured Lipid Carriers for Hyperproliferative Skin Diseases." *International Journal of Molecular Sciences* 24, no. 9: 8053. <https://doi.org/10.3390/ijms24098053>.
- Love, R. M. 1996. "Regional Variation in Root Dentinal Tubule Infection by *Streptococcus gordonii*." *Journal of Endodontics* 22, no. 6: 290–293. [https://doi.org/10.1016/S0099-2399\(96\)80261-5](https://doi.org/10.1016/S0099-2399(96)80261-5).
- Lungkapin, P., and P. Louwakul. 2019. "Reduction of Endotoxin From Human Root Canals by Calcium Hydroxide Nanoparticles." *IOP Conference Series: Materials Science and Engineering* 526, no. 1: 5–9. <https://doi.org/10.1088/1757-899X/526/1/012020>.
- Mohammadi, Z., and P. M. H. Dummer. 2011. "Properties and Applications of Calcium Hydroxide in Endodontics and Dental Traumatology." *International Endodontic Journal* 44, no. 8: 697–730. <https://doi.org/10.1111/j.1365-2591.2011.01886.x>.
- Moodley, D., and A. I. O. Ibrahim. 2021. "Nanotechnology in Endodontics." In *Nanotechnology for Dentistry Applications*. IOP Publishing. <https://doi.org/10.1088/978-0-7503-3671-0ch8>.
- Morsi, N., M. Ibrahim, H. Refai, and H. El Sorogy. 2017. "Nanoemulsion-Based Electrolyte Triggered in Situ Gel for Ocular Delivery of Acetazolamide." *European Journal of Pharmaceutical Sciences* 104: 302–314. <https://doi.org/10.1016/j.ejps.2017.04.013>.
- Nagendrababu, V., P. E. Murray, R. Ordinola-Zapata, et al. 2021. "PRILE 2021 Guidelines for Reporting Laboratory Studies in Endodontology: A Consensus-Based Development." *International Endodontic Journal* 54, no. 9: 1482–1490. <https://doi.org/10.1111/iej.13542>.
- Nasim, I., and S. Hemmanur. 2021. "Intracanal Medicaments—A Review of Literature." *International Journal of Dentistry and Oral Science* 8, no. 5: 2643–2648.
- Nelson Filho, P., L. A. Bezerra Silva, M. R. Leonardo, L. Sabbag Utrilla, and F. Figueiredo. 1999. "Connective Tissue Responses to Calcium Hydroxide-Based Root Canal Medicaments." *International Endodontic Journal* 32, no. 4: 303–311. <https://doi.org/10.1046/j.1365-2591.1999.00225.x>.
- Ordinola-Zapata, R., W. C. Noblett, A. Perez-Ron, Z. Ye, and J. Vera. 2022. "Present Status and Future Directions of Intracanal Medicaments." *International Endodontic Journal* 55, no. S3: 613–636. <https://doi.org/10.1111/iej.13731>.
- Ørstavik, D., D. K. Kerekes, and O. Molven. 1991. "Effects of Extensive Apical Reaming and Calcium Hydroxide Dressing on Bacterial Infection

- During Treatment of Apical Periodontitis: A Pilot Study." *International Endodontic Journal* 24, no. 1: 1–7. <https://doi.org/10.1111/j.1365-2591.1991.tb00863.x>.
- Pacios, M. G., M. L. De La Casa, M. D. L. A. Bulacio, and M. E. López. 2003. "Calcium Hydroxide's Association With Different Vehicles: In Vitro Action on Some Dentinal Components." *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* 96, no. 1: 96–101. [https://doi.org/10.1016/S1079-2104\(02\)91705-1](https://doi.org/10.1016/S1079-2104(02)91705-1).
- Panzarini, S. R., R. Holland, V. De Souza, W. R. Poi, C. K. Sonoda, and D. Pedrini. 2007. "Mineral Trioxide Aggregate as a Root Canal Filling Material in Reimplanted Teeth. Microscopic Analysis in Monkeys." *Dental Traumatology* 23, no. 5: 265–272. <https://doi.org/10.1111/j.1600-9657.2006.00456.x>.
- Pedrinha, V. F., M. R. Cardenas Cuellar, M. C. de Barros, et al. 2022. "The Vehicles of Calcium Hydroxide Pastes Interfere With Antimicrobial Effect, Biofilm Polysaccharidic Matrix, and Pastes' Physicochemical Properties." *Biomedicine* 10, no. 12: 3123. <https://doi.org/10.3390/biomedicines10123123>.
- Piñón-Segundo, E., A. Ganem-Quintanar, V. Alonso-Pérez, and D. Quintanar-Guerrero. 2005. "Preparation and Characterization of Triclosan Nanoparticles for Periodontal Treatment." *International Journal of Pharmaceutics* 294, no. 1–2: 217–232. <https://doi.org/10.1016/j.ijpharm.2004.11.010>.
- Rahman, H. S., A. Rasedee, S. K. Yeap, et al. 2014. "Biomedical Properties of a Natural Dietary Plant Metabolite, Zerumbone, in Cancer Therapy and Chemoprevention Trials." *BioMed Research International* 2014: 1–20. <https://doi.org/10.1155/2014/920742>.
- Ramos Yacasi, G. R., M. L. García López, M. Espina García, A. Parra Coca, and A. C. Calpena Campmany. 2016. "Influence of Freeze-Drying and γ -Irradiation in Preclinical Studies of Flurbiprofen Polymeric Nanoparticles for Ocular Delivery Using d-(+)-Trehalose and Polyethylene Glycol." *International Journal of Nanomedicine* 11: 4093–4106. <https://doi.org/10.2147/IJN.S105606>.
- Roig, X., L. Halbaut, F. Elmsmari, et al. 2024. "Calcium Hydroxide-Loaded Nanoparticles Dispersed in Thermosensitive Gel as a Novel Intracanal Medicament." *International Endodontic Journal* 57: 1–921. <https://doi.org/10.1111/iej.14041>.
- Salunke, S. R., and S. B. Patil. 2016. "Ion Activated in Situ Gel of Gellan Gum Containing Salbutamol Sulphate for Nasal Administration." *International Journal of Biological Macromolecules* 87: 41–47. <https://doi.org/10.1016/j.ijbiomac.2016.02.044>.
- Sánchez-López, E., M. A. Egea, A. Cano, et al. 2016. "PEGylated PLGA Nanospheres Optimized by Design of Experiments for Ocular Administration of Dexibuprofen-In Vitro, Ex Vivo and In Vivo Characterization." *Colloids and Surfaces B: Biointerfaces* 145: 241–250. <https://doi.org/10.1016/j.colsurfb.2016.04.054>.
- Sánchez-López, E., D. Gomes, G. Esteruelas, and L. Bonilla. 2020. "Metal-Based Nanoparticles as Antimicrobial Agents: An Overview." *Nanomaterials* 10, no. 2: 1–39. <https://doi.org/10.3390/nano10020292>.
- Sathorn, C., P. Parashos, and H. Messer. 2007. "Antibacterial Efficacy of Calcium Hydroxide Intracanal Dressing: A Systematic Review and Meta-Analysis." *International Endodontic Journal* 40, no. 1: 2–10. <https://doi.org/10.1111/j.1365-2591.2006.01197.x>.
- Siqueira, J. F., and H. P. Lopes. 1999. "Mechanisms of Antimicrobial Activity of Calcium Hydroxide: A Critical Review." *International Endodontic Journal* 32, no. 5: 361–369. <https://doi.org/10.1046/j.1365-2591.1999.00275.x>.
- Sirén, E. K., E. Kerosuo, E. Lavonius, J. H. Meurman, and M. Haapasalo. 2014. "Ca(OH)₂ Application Modes: In Vitro Alkalinity and Clinical Effect on Bacteria." *International Endodontic Journal* 47, no. 7: 628–638. <https://doi.org/10.1111/iej.12199>.
- Song, W., and S. Ge. 2019. "Application of Antimicrobial Nanoparticles in Dentistry." *Molecules* 24, no. 6: 1–15. <https://doi.org/10.3390/molecules24061033>.
- Takeuchi, H., J. Thongborisute, Y. Matsui, H. Sugihara, H. Yamamoto, and Y. Kawashima. 2005. "Novel Mucoadhesion Tests for Polymers and Polymer-Coated Particles to Design Optimal Mucoadhesive Drug Delivery Systems." *Advanced Drug Delivery Reviews* 57, no. 11: 1583–1594. <https://doi.org/10.1016/j.addr.2005.07.008>.
- Tawakoli, P. N., A. Al-Ahmad, W. Hoth-Hannig, M. Hannig, and C. Hannig. 2013. "Comparison of Different Live/Dead Stainings for Detection and Quantification of Adherent Microorganisms in the Initial Oral Biofilm." *Clinical Oral Investigations* 17, no. 3: 841–850. <https://doi.org/10.1007/s00784-012-0792-3>.
- Teoh, Y. Y., B. Athanassiadis, and L. J. Walsh. 2018. "Comparison of Commercial Calcium Hydroxide Pastes for Prolonged Antibacterial Effect Using a Colourimetric Assessment." *Materials* 11, no. 3: 1–9. <https://doi.org/10.3390/ma11030348>.
- Teulé-Trull, M., E. Demiquels-Punzano, R. A. Pérez, et al. 2024. "Revision of Ex Vivo Endodontic Biofilm Model Using Computer Aided Design." *Journal of Dentistry* 149: 105270. <https://doi.org/10.1016/j.jdent.2024.105270>.
- Thiruchenthoooran, V., M. Świtalska, L. Bonilla, et al. 2022. "Novel Strategies Against Cancer: Dexibuprofen-Loaded Nanostructured Lipid Carriers." *International Journal of Molecular Sciences* 23, no. 19: 11310. <https://doi.org/10.3390/ijms231911310>.
- Valverde, M. E., P. Baca, L. Ceballos, M. V. Fuentes, M. Ruiz-Linares, and C. M. Ferrer-Luque. 2017. "Antibacterial Efficacy of Several Intracanal Medicaments for Endodontic Therapy." *Dental Materials Journal* 36, no. 3: 319–324. <https://doi.org/10.4012/dmj.2016-102>.
- Wang, Z., Y. Shen, and M. Haapasalo. 2012. "Effectiveness of Endodontic Disinfecting Solutions Against Young and Old *Enterococcus faecalis* Biofilms in Dentin Canals." *Journal of Endodontics* 38, no. 10: 1376–1379. <https://doi.org/10.1016/j.joen.2012.06.035>.
- Weatherly, L. M., and J. A. Gosse. 2017. "Triclosan Exposure, Transformation, and Human Health Effects." *Journal of Toxicology and Environmental Health—Part B: Critical Reviews* 20, no. 8: 447–469. <https://doi.org/10.1080/10937404.2017.1399306>.
- Yetisgin, A. A., S. Cetinel, M. Zuvun, A. Kosar, and O. Kutlu. 2020. "Therapeutic Nanoparticles and Their Targeted Delivery Applications." *Molecules* 25: 2193. <https://doi.org/10.3390/molecules25092193>.
- Yohan, D., and B. D. Chithrani. 2014. "Applications of Nanoparticles in Nanomedicine." *Journal of Biomedical Nanotechnology* 10, no. 9: 2371–2392. <https://doi.org/10.1166/jbn.2014.2015>.
- Zand, V., H. Mokhtari, A. Hasani, and G. Jabbari. 2017. "Comparison of the Penetration Depth of Conventional and Nano-Particle Calcium Hydroxide Into Dentinal Tubules." *Iranian Endodontic Journal* 12, no. 3: 366–370. <https://doi.org/10.22037/iej.v12i3.16421>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** [iej70032-sup-0001-AppendixS1.zip](#). **Figure S1:** [iej70032-sup-0002-FigureS1.docx](#).