ORIGINAL RESEARCH

The Effects of Calcium Hydroxide–loaded Poly (Lactic-co-glycolic Acid) Biodegradable Nanoparticles in the *ex vivo* External Inflammatory Root Resorption Model

Patcharaporn Chaiyosang[1], Thanisorn Mahatnirunkul[2], Warat Leelapornpisid[3]

Abstract

Aim: To evaluate the calcium ions (Ca^{2+}) diffusion of calcium hydroxide-loaded poly(lactic-co-glycolic acid) biodegradable nanoparticles [$Ca(OH)_2$-loaded PLGA NPs] compared with conventional $Ca(OH)_2$ in a simulated external root resorption *ex vivo* model using inductively coupled plasma mass spectrometry (ICP-MS).

Materials and methods: Thirty human mandibular premolars were prepared by sectioning the root segments to create roots measuring 10 mm from the anatomical apex. The root canals were instrumented and irrigated. The external root surface cavities were created. The specimens were randomly divided into the following three groups: Poly(lactic-co-glycolic acid) (PLGA; control group, $n = 10$), conventional calcium hydroxide [$Ca(OH)_2$] (Metapaste, $n = 10$), and $Ca(OH)_2$-loaded PLGA NPs [15% $Ca(OH)_2$, $n = 10$]. The intracanal materials were placed in the root canals, and the teeth were stored in phosphate-buffered saline at 37°C. The release of Ca^{2+} was measured at 7, 30, and 60 days using ICP-MS.

Results: Both $Ca(OH)_2$-loaded PLGA NPs and Metapaste groups exhibited higher levels of Ca^{2+} release compared to the PLGA group at all time points. During the initial 7-day period, the $Ca(OH)_2$-loaded PLGA NPs exhibited a significantly greater release of Ca^{2+} compared to Metapaste. From day 7 to day 30, Metapaste displayed a significantly higher release of Ca^{2+} than the $Ca(OH)_2$-loaded PLGA NPs, but it experienced a subsequent decline in Ca^{2+} release after the 30-day period. After the 30-day mark, the $Ca(OH)_2$-loaded PLGA NPs once again exhibited a significantly higher release of Ca^{2+} compared to Metapaste.

Conclusion: The $Ca(OH)_2$-loaded PLGA NPs exhibited sustained release of Ca^{2+} that exceeded conventional $Ca(OH)_2$, particularly during the first week, demonstrating a greater amount of Ca^{2+} release.

Clinical significance: The utilization of $Ca(OH)_2$-loaded PLGA NPs as an intracanal medication for external inflammatory root resorption provided sustained release and had the potential to enhance the efficacy of inhibiting root resorption more effectively than conventional $Ca(OH)_2$.

Keywords: Calcium hydroxide, Calcium hydroxide-loaded poly(lactic-co-glycolic acid) biodegradable nanoparticles, External inflammatory root resorption.

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Introduction

External inflammatory root resorption refers to the permanent deterioration of the outer structure of a tooth. Once the external root resorption occurs, the tooth becomes unresponsive to pulp sensibility tests, and the presence of symptoms or clinical signs may vary. Radiographically, the tooth exhibits a loss of tooth structure accompanied by a radiolucency that affects the adjacent periodontal ligament (PDL) and bone.[1] The causes of external root resorption are diverse and encompass inflammatory conditions, traumatic injuries, pressure or mechanical stimulation, neoplastic conditions, systemic disorders, and idiopathic factors.[1,2] External inflammatory root resorption is one of the most critical pathologies which usually occur after dental trauma. The highest occurrence of external resorption was predominantly observed in cases of intrusive luxation (92.8%), followed by avulsion (89.0%), lateral luxation (80.2%), and extrusive luxation (77.4%).[3] The mechanism involves the destruction of the protective layer of root dentin and infection.[4]

Upon the detection of active external root resorption, the primary goal of treatment is to regulate the proliferation of pulpal bacteria, which serve as catalysts for the resorptive process. The preferred antibacterial protocol consisted of chemo–mechanical preparation, followed by long-term dressing with calcium hydroxide [$Ca(OH)_2$].[4,5] According to the International Association of Dental Traumatology (IADT) guidelines for the management of dental trauma, $Ca(OH)_2$ medication followed by 3 months replacement is recommended until there is evidence of hard tissue repair.[6] However, the prolonged administration of $Ca(OH)_2$ (exceeding 30 days) can induce a decrease in dentin fractural strength.[7] Moreover, the alkalinity and antibacterial properties can be reduced by buffering effects from dentin and hydroxyapatite.[8] Consequently, investigating

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$$n_1 = \frac{\left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2 \left[\sigma_1^2 + \frac{\sigma_2^2}{r}\right]}{\Delta^2}$$

$$r = \frac{n_2}{n_1}, \Delta = \mu_1 - \mu_2$$

Fig. 1: The formula for calculating sample size

novel intracanal medicaments for external inflammatory root resorption poses a significant challenge. Although there are several user-friendly modified products of $Ca(OH)_2$ available, such as Metapaste, Ultra-Cal, and Calcipex, the main component of these products is $Ca(OH)_2$ microparticles.

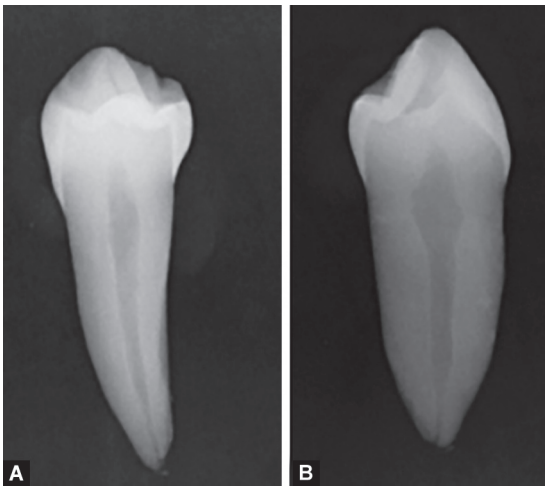
At present, nanotechnology has been introduced in the medical field. The utilization of nanotechnology for drug delivery protects drugs against rapid degradation or clearance, thereby improving drug performance.[9] In the field of dental science, Elmsmari et al. developed calcium hydroxide-loaded poly(lactic-co-glycolic acid) biodegradable nanoparticles [$Ca(OH)_2$-loaded PLGA NPs] which maintained high concentrations longer and penetrated deeper through dentinal tubules.[10] Moreover, Dianat et al. demonstrated that reducing the size of $Ca(OH)_2$-sized particles into nanoparticles had greater antimicrobial activity than conventional size without significantly changing the cytotoxicity to fibroblast cells.[11,12] Based on existing evidence, the utilization of nanotechnology for drug delivery may lead to the development of prolonged and steady $Ca(OH)_2$ release, which can help reduce the buffering effect caused by dentin and hydroxyapatite. Previous studies have indicated that calcium hydroxide-loaded poly(lactic-co-glycolic acid) biodegradable nanoparticles [$Ca(OH)_2$-loaded PLGA NPs] exhibit a superior depth of penetration within dentinal tubules in comparison to conventional $Ca(OH)_2$.[10] However, the diffusion of Ca^{2+}, which is a critical substance for inducing mineralization and indirectly reducing inflammation, from $Ca(OH)_2$-loaded PLGA NPs on the external root surface has not been evaluated.[13] This study aims to evaluate the Ca^{2+} diffusion of $Ca(OH)_2$-loaded PLGA NPs compared with conventional $Ca(OH)_2$ in a simulated external root resorption *ex vivo* model using inductively coupled plasma mass spectrometry (ICP-MS).

Materials and Methods

An *ex vivo* experimental study was carried out from January 2023 to June 2023, with ethical approval obtained from the research ethics committee of the Faculty of Dentistry, Chiang Mai University (56/2021).

Sample-size Calculation

Sample-size was determined from a similar study using the formula in Figure 1.[14,15] With α level type I error at 0.05 and β level type II error of 0.20 for the study, a sample size of 2 was obtained for each group; $p < 0.05$ is considered statistically significant.



Figs 2A and B: Preoperative radiographs of the root specimens in both (A) Buccolingual and (B) Mesiodistal directions

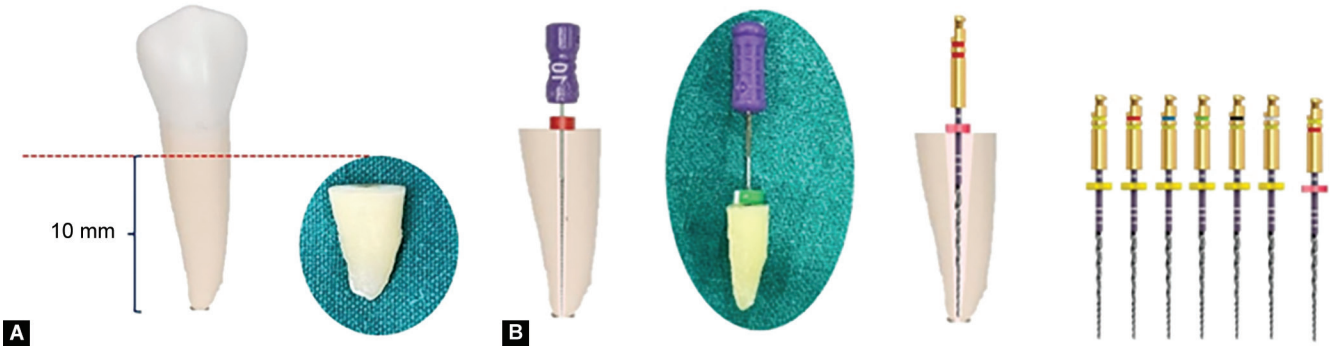
Tooth Selection

Thirty human single-root permanent mandibular premolars extracted for orthodontics reasons were collected. After extraction, the teeth were immediately stored in 0.1% thymol solution (M dent) until the experiment starts. The preoperative radiographs of the root specimens in both the buccolingual and mesiodistal direction were taken for observing root canal anatomy and abnormality by using the CS7600 (Fig. 2), a digital imaging plate (Carestream, Atlanta, Georgia, USA). The inclusion criteria comprised of teeth with a single straight root, a single-root canal, a minimum root length of 10 mm, and complete root formation. Teeth that displayed external root resorption, internal root resorption, canal obliteration, or a history of previous root canal treatment were excluded from the study.

The teeth were randomly assigned into 3 groups, each consisting of 10 teeth. The groups were differentiated based on the intracanal materials used such as PLGA (control group), conventional $Ca(OH)_2$ (Metapaste, META BIOMED, Cheongju, Korea), and $Ca(OH)_2$-loaded PLGA NPs (15%) prepared by the National Nanotechnology Center in Thailand.

Tooth Preparation

A root segment was prepared by sectioning below the cementoenamel junction at various lengths, by using a diamond disc (Meisinger, Neuss, Germany), to create roots of 10 mm equal length measured from the anatomical apex (Fig. 3A). The access to the canal was performed with a round diamond bur (Meisinger, Neuss, Germany) and a round steel bur (Meisinger, Neuss, Germany). Then, pulpal tissue was removed with a barbed broach (Dentsply Maillefer, Oklahoma, USA). The teeth were instrumented using a crown-down technique. After coronal flaring, the working length was determined by visualizing a 10-K file (Dentsply, Maillefer, Oklahoma, USA) at the apical foramen and deducting it by 0.5 mm. The canal was enlarged to a size of 20 K-file (Dentsply, Maillefer, Oklahoma, USA), creating a guide path for rotary instruments, and then instrumented with ZenFlex® (Kerr Corporation, Pomona, CA, USA) to a final apical preparation size of 55/06 (Fig. 3B). The canals were irrigated with 5 mL of 2.5% NaOCl over a 30-second period between each file size through a 25-gauge needle attached to the syringe. After the final instrumentation, the smear layer was removed with 2 mL of 17% ethylenediaminetetraacetic acid (EDTA)



Figs 3A and B: (A) Preparation of root segments by sectioning below the cementoenamel junction at various lengths to create roots of 10-mm equal length measured from the anatomical apex; (B) Determination of the working length and shaping of the canal to a final apical preparation size of 55/06 (ZenFlex®)

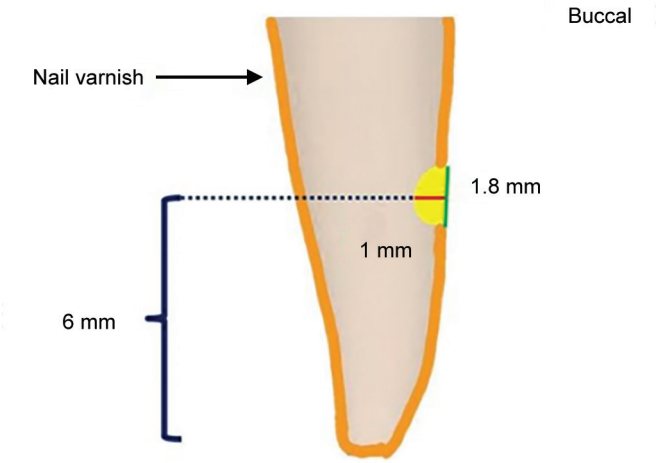


Fig. 4: Preparation of the external root surface cavities (1.0-mm deep and 1.8 mm in diameter)

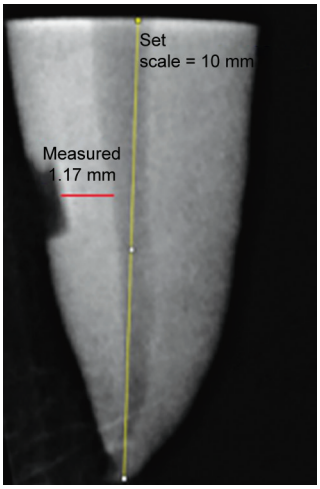


Fig. 5: Radiographs of the root specimens in the mesiodistal direction, and the quantification of remaining dentin thickness at the external root surface cavities by Image J software

followed by 2 mL of 2.5% NaOCl. The canals were dried with paper points.

External root surface cavities were prepared as described by Chamberlain et al. with modification by using flat-end cylindrical diamond bur (EDENTA, Hauptstrasse, Switzerland) with a 1.8 mm in diameter and a 4.0-mm head length, a cavity measuring 1.0-mm deep and 1.8 mm in diameter were made on the buccal external surface of each root, 6 mm coronally to the apex (Fig. 4).[16] Root surface cavities were rinsed with 3 mL 17% EDTA for 1 minute, followed by 3 mL distilled water. After preparing the tooth models, radiographs of the root specimens were taken in the mesiodistal direction, and the quantification of remaining dentin thickness at the external root surface cavities was performed utilizing Image J software (Fig. 5).

Placement of Intracanal Materials

The root specimens were then filled with different intracanal materials, namely, PLGA, Metapaste (pH 12–13), and $Ca(OH)_2$-loaded PLGA NPs (15%) (pH approximately 12.5), with 10 teeth assigned to each group (Flowchart 1). Access was cleaned with a cotton pellet leaving 2-mm space for Cavit G (3M ESPE, Seefeld, Germany). The teeth were immediately immersed in separate 20 mL vials filled with phosphate-buffered saline. During the testing period, the teeth were stored at 37°C.

Calcium Ion Measurement

The number of Ca^{2+} present in the solution was measured using ICP-MS. 10 mL of samples was measured. To verify the calibration, the following standard calcium solutions were prepared: 0.01, 0.025, 0.05, 0.075, 0.1, 0.125, 0.25, 0.5, and 1 ppm. The interpretation of blank, standard calcium and test solutions were performed by ICP-MS. The investigation of Ca^{2+} release was compared with a standard curve obtained from multiple dilutions of pure calcium in ultrapure water.

Data Analysis

The data were statistically analyzed using Statistical Package for the Social Sciences (SPSS), version 25.0, software (SPSS, Inc., Chicago, Illinois, USA). The remaining dentin at the external root surface cavities and the concentration of Ca^{2+} release were analyzed using a one-way factorial analysis of variance (ANOVA). Dunnett's T3 test was employed for multiple comparisons of the concentration of Ca^{2+} release at a significance level of 5%.

Results

After preparing the tooth models, the mean remaining dentin at the external root surface cavities was 1.203 ± 0.272, 1.204 ± 0.274,

Flowchart 1: Thirty root specimens were randomly divided into three groups based on the intracanal materials

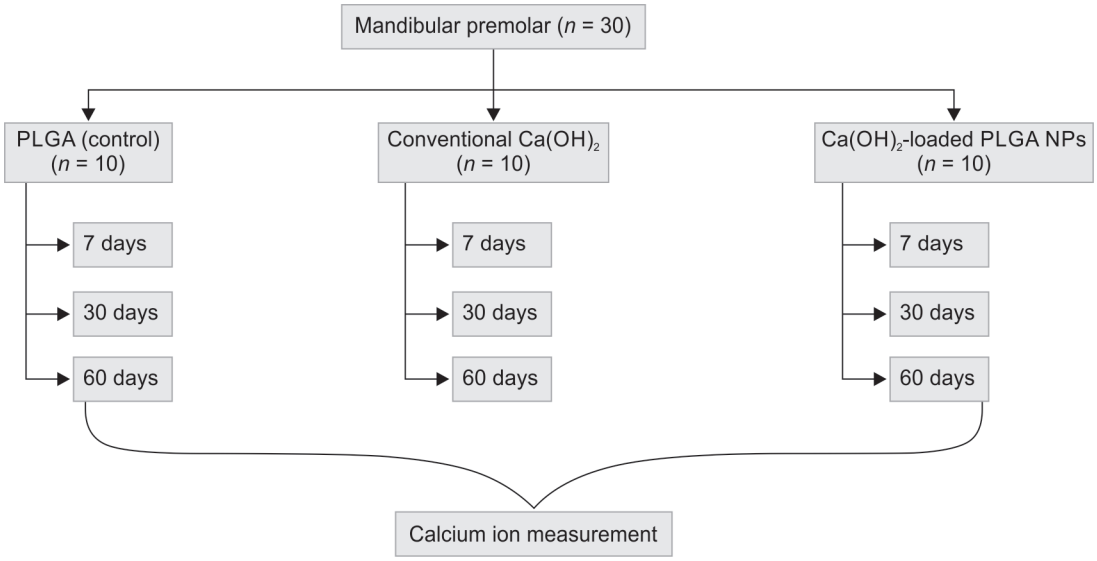


Table 1: Measurement of remaining dentine thickness at external surface cavities

	Dentine thickness		
Group	*Mean*	*SD*	*ANOVA*
PLGA	1.203	0.272	
Metapaste	1.204	0.274	0.799
$Ca(OH)_2$-loaded PLGA NPs	1.138	0.243	

SD, standard deviation

Table 2: Ca^{2+} release observed at different times

	Mean ± SD (ppb)		
Group	*7 days*	*30 days*	*60 days*
PLGA	187.80 ± 97.43	232.58 ± 108.91	665.13 ± 84.62
Metapaste	3,445.34 ± 438.02	5,879.55 ± 878.16	2,965.07 ± 269.86
$Ca(OH)_2$-loaded PLGA NPs	4,099 ± 294.38	3,865.21 ± 411.16	4,219.31 ± 815.20

SD, standard deviation

and 1.138 ± 0.243 mm in the PLGA, Metapaste, and $Ca(OH)_2$-loaded PLGA NPs groups, respectively. No statistical difference in remaining dentin thickness was found between all groups with $p > 0.05$ (Table 1). The comparison of initial measurements with subsequent periods revealed distinct patterns in the release of Ca^{2+} among the different groups. The PLGA group exhibited a negligible rise in Ca^{2+} levels throughout the entire study (day 7: 187.80 ± 97.43 ppb; day 30: 232.58 ± 108.91 ppb; day 60: 665.13 ± 84.62 ppb). The Metapaste groups demonstrated an initial increase in Ca^{2+} levels from 7 to 30 days, followed by a subsequent decline (day 7: 3,445.34 ± 438.02 ppb; day 30: 5,879.55 ± 878.16 ppb; day 60: 2,965.07 ± 269.86 ppb). In contrast, the $Ca(OH)_2$-loaded PLGA NPs consistently released Ca^{2+} over the entire duration of the study (day 7: 4,099 ± 294.38 ppb; day 30: 3,865.21 ± 411.16 ppb; day 60: 4,219.31 ± 815.20 ppb).

Analyzing the specific time points, at day 7, the detected Ca^{2+} levels followed the sequence of $Ca(OH)_2$-loaded PLGA NPs > Metapaste > PLGA. Between day 7 and day 30, the detected Ca^{2+} levels showed the order of Metapaste > $Ca(OH)_2$-loaded PLGA NPs > PLGA. From day 30 to day 60, the Ca^{2+} levels detected followed the order $Ca(OH)_2$-loaded PLGA NPs > Metapaste > PLGA. Statistical analysis revealed significant differences in the release of Ca^{2+} between the $Ca(OH)_2$-loaded PLGA NPs and Metapaste groups compared to the PLGA group at every time point. In the initial 7-day period, the $Ca(OH)_2$-loaded PLGA NPs exhibited a significantly higher release of Ca^{2+} compared to Metapaste ($p = 0.004$). From day 7 to day 30, Metapaste demonstrated a significantly greater release of Ca^{2+} than the $Ca(OH)_2$-loaded PLGA NPs ($p < 0.001$). However, after the 30-day mark, the $Ca(OH)_2$-loaded PLGA NPs displayed a significantly higher release of Ca^{2+} in comparison to Metapaste ($p = 0.002$) (Table 2; Fig. 6).

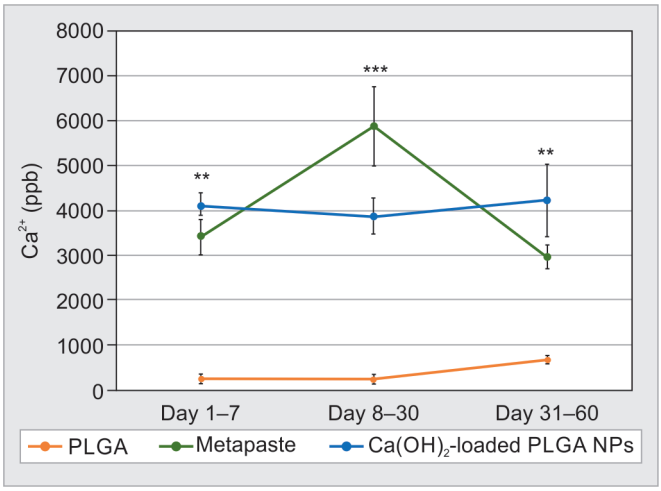


Fig. 6: The line graph demonstrated Ca^{2+} release observed at different time intervals

Discussion

Calcium hydroxide has been recommended by IADT as an intracanal medication for the purpose of inhibiting root resorption due to

its antimicrobial properties and ability to promote an alkaline environment making $Ca(OH)_2$ can stop inflammatory root resorption.[6,17] Moreover, the Ca^{2+} ions present in $Ca(OH)_2$ play a crucial role in the process of remineralization.[18,19] Furthermore, these ions indirectly contribute to reducing inflammation by promoting the activity of osteoblasts, which are indispensable for bone remodeling and repair.[13] However, $Ca(OH)_2$ has limitations. Prolonged usage may weaken dentin strength, and the buffering effects of dentin and hydroxyapatite can diminish its alkalinity and antibacterial properties.[7,8] Hence, this study was initiated due to the considerable challenge posed by investigating new intracanal medicaments for external inflammatory root resorption.

This study compares the effectiveness of $Ca(OH)_2$-loaded PLGA NPs with conventional $Ca(OH)_2$. By utilizing PLGA's drug delivery capabilities and incorporating $Ca(OH)_2$ nanoparticles, the $Ca(OH)_2$-loaded PLGA NPs are expected to enhance drug efficacy. The integration of nanotechnology in drug delivery systems, including the widespread use of PLGA, has been highlighted in previous research.[9] Furthermore, PLGA exhibits slow degradation as a matrix or encapsulating material, resulting in a gradual release of the drug over time and potentially reducing the loss of excess drugs into the environment.[20] Previous studies have also demonstrated that reducing drugs into nanoparticle size can improve their performance.[21]

Our findings have revealed the potential of PLGA in enhancing drug performance. The graph (Fig. 6) illustrates that Ca^{2+} from $Ca(OH)_2$-loaded PLGA NPs were continuously released throughout the entire duration of the study. Although Metapaste demonstrated a significantly higher Ca^{2+} release than the $Ca(OH)_2$-loaded PLGA NPs between days 7 and 30, it experienced a subsequent decline in Ca^{2+} release after the 30-day period. In contrast, the $Ca(OH)_2$-loaded PLGA NPs exhibited a sustained and considerably high release of Ca^{2+}. This indicates that PLGA possesses the ability to sustain drug release over an extended period, potentially leading to a reduction in the buffering effect.

Moreover, in the first week, the $Ca(OH)_2$-loaded PLGA NPs released a significantly higher amount of Ca^{2+} compared to Metapaste. This finding aligns with previous research conducted by Elmsmari et al., which indicated that the $Ca(OH)_2$-loaded PLGA NPs achieved a considerably greater depth of penetration within the dentinal tubules in comparison to the unencapsulated drug counterpart over a 7-day period.[10] Additionally, Farzaneh et al. found that the nanoparticle formulation of $Ca(OH)_2$ displayed superior penetration depths across all regions of the root dentinal tubules when compared to the conventional form.[22] As a result, the enhanced penetration capability of the $Ca(OH)_2$-loaded PLGA NPs may contribute to their ability to release a greater amount of Ca^{2+} at sites of external root resorption during the initial stage, surpassing the release achieved by Metapaste.

Based on these outcomes, $Ca(OH)_2$-loaded PLGA NPs show potential superiority over Metapaste in inhibiting root resorption. The sustained release of Ca^{2+} provided by $Ca(OH)_2$-loaded PLGA NPs significantly contributes to their enhanced efficacy, with implications for clinical applications in reducing inflammation and alleviating external root resorption. Additionally, Ca^{2+} plays a crucial role in promoting bone healing. In addition, the use of $Ca(OH)_2$-loaded PLGA NPs presents user-friendly benefits by eliminating the requirement for medication changes within a 2-month period. This has the potential to decrease the frequency of appointment visits.

However, further clinical research is necessary to validate and expand upon these findings. Despite careful inclusion criteria, this study has limitations. The variability in anatomy among the real teeth used hinders standardization. Furthermore, the *ex vivo* model employed falls short of fully replicating the clinical scenario. It fails to showcase the utilization of medication by infection and clastic cells, and certain drugs may be prone to washout from body fluids. Nevertheless, this study highlights the promising potential of $Ca(OH)_2$-loaded PLGA NPs as an alternative to Metapaste for effectively inhibiting root resorption.

Conclusion

The $Ca(OH)_2$-loaded PLGA NPs had higher Ca^{2+} release than Metapaste initially, but Metapaste had higher Ca^{2+} release from days 7 to 30. However, after 30 days, $Ca(OH)_2$-loaded PLGA NPs had higher Ca^{2+} release than Metapaste. This indicated $Ca(OH)_2$-loaded PLGA NPs exhibit a sustained release of Ca^{2+} that surpasses Conventional $Ca(OH)_2$.

Clinical Significance

The utilization of $Ca(OH)_2$-loaded PLGA NPs as an intracanal medication for external inflammatory root resorption provides sustained release and has the potential to enhance the efficacy of inhibiting root resorption more effectively than conventional $Ca(OH)_2$.

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