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Antimicrobial and Remineralizing Effects of L-Arginine as a Novel Intracanal Medicament in Enterococcus faecalis-Infected Root Canals: An In Vitro Study

Lu Qin
First Affiliated Hospital of Zhengzhou University
Yanmin Jia
First Affiliated Hospital of Zhengzhou University
Yiyi Wang
First Affiliated Hospital of Zhengzhou University
Xushuo Du
First Affiliated Hospital of Zhengzhou University
Lixin Fang
Wuhan University
Jiaqi Kang
First Affiliated Hospital of Zhengzhou University
Xiaoyan Sun
First Affiliated Hospital of Zhengzhou University
Qizhou Zhao
First Affiliated Hospital of Zhengzhou University
Yan Xiao
First Affiliated Hospital of Zhengzhou University
Huoxiang Zhou
Zhengzhou University
Suping Wang
wangsupingdent@163.com
First Affiliated Hospital of Zhengzhou University

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Abstract

Background Enterococcus faecalis (E. faecalis) is a predominant pathogen in refractory root canal infections. Calcium hydroxide [

$Ca(OH)_2$], the most widely used intracanal medicament, has limited efficacy against *E. faecalis* biofilms and may compromise mechanical properties of dentin. This study investigated the antimicrobial activity of L-arginine (L-Arg) against *E. faecalis* and its potential to promote remineralization of root canal wall dentin.

Methods

The minimum inhibitory concentration (MIC) and 24-hour growth inhibition of L-arginine against planktonic *E. faecalis* were determined. Biofilm inhibition was assessed by Crystal violet staining, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay, scanning electron microscopy (SEM), and colony-forming unit (CFU) counting. An ex vivo human root canal model using 36 single-rooted premolars was used to evaluate the antibacterial effect of L-Arg (0, 45, 90, 180 mg/mL) after 7days. For dentin remineralization assessment, 72 additional root specimens were randomly assigned to four groups (Control, $Ca(OH)_2$, L-Arg, L-Arg + $Ca(OH)_2$) for 7, 14, or 28 days. After incubation, dentin remineralization was evaluated using SEM, energy-dispersive X-ray spectroscopy (EDS), and Vickers microhardness testing.

Results

The MIC of L-Arg against *E. faecalis* was 90 mg/mL, and L-Arg significantly suppressed bacterial growth within 24 h ($p < 0.05$). Crystal violet staining, MTT assays, CFU counting, and SEM observations revealed a significant, concentration-dependent reduction in *E. faecalis* biofilm biomass in all L-Arg-treated groups ($p < 0.05$, $n = 6$). In the ex vivo root canal model, a 7-day sealing treatment with L-Arg significantly reduced viable *E. faecalis* counts ($p < 0.05$, $n = 6$). SEM examination showed distinct mineral deposition on the dentin surface in both the L-Arg and L-Arg + $Ca(OH)_2$ groups after 7, 14, and 28 days. On days 14 and 28, the mass percentages of Ca and P elements in the L-Arg and L-Arg + $Ca(OH)_2$ groups were significantly higher than those in the blank control and $Ca(OH)_2$ groups ($p < 0.05$, $n = 6$). On day 28, the microhardness values of the L-Arg and L-Arg + $Ca(OH)_2$ groups were significantly higher than those of the blank control and $Ca(OH)_2$ groups ($p < 0.05$, $n = 6$).

Conclusions

L-Arg effectively inhibits *E. faecalis* in both planktonic and biofilm states, reduces bacterial loads in infected root canals, and promotes calcium phosphate deposition and dentin microhardness, suggesting its potential as a novel intracanal medicament for dentin remineralization.

1 Introduction

Microbial root canal infections are a leading cause of endodontic failure and persistent apical periodontitis. *Enterococcus faecalis* (*E. faecalis*) is a Gram-positive bacterium frequently detected in endodontically treated teeth with periapical lesions, with a detection rate as high as 77% [1]. It possesses extraordinary resilience, allowing it to survive harsh conditions such as elevated pH, high salinity, and extreme temperatures [2]. Moreover, it preferentially colonizes areas that are notoriously difficult to clean, including the apical third of the root canal and the canal isthmus [3]. Within the root canal, *E. faecalis* exists mainly in planktonic and biofilm states. The biofilm configuration can increase bacterial tolerance to disinfectants by up to 1000-fold and simultaneously protect the pathogen from host immune surveillance [4].

Root canal treatment (RCT) combined with intracanal medications is routinely effective in controlling root canal infections. Among the various options, calcium hydroxide [$Ca(OH)_2$] is the preferred and most widely used intracanal medicament because it can inactivate endotoxins and maintain a highly alkaline environment that disrupts bacterial metabolism [5]. However, its inhibitory effect against *E. faecalis* is relatively limited, as this microorganism can survive in highly alkaline environments by leveraging a unique proton pump mechanism to actively transport protons into the bacterial cell and neutralize its internal pH [6]. More importantly, prolonged contact between $Ca(OH)_2$ and the root canal wall can disrupt the linkage between hydroxyapatite and collagen fibers [7], thereby weakening the dentin structure and increasing the susceptibility of the tooth to root fracture. Antibiotic agents are less effective against quiescent and dormant bacteria in the nutrient-poor root canal system and are not recommended for routine clinical use due to the risk of bacterial resistance. Chlorhexidine, although effective against a broad spectrum of oral microorganisms, requires higher concentrations to inhibit or disrupt *E. faecalis* biofilms, and such high concentrations exert cytotoxic effects on various host cells, including epithelial cells, fibroblasts, and stem cells [8]. Consequently, traditional intracanal medications face multiple challenges, including insufficient efficacy against *E. faecalis* biofilms, cytotoxicity, antimicrobial resistance, and the risk of root fracture.

L-arginine (L-Arg), a naturally occurring alkaline amino acid in the human body, has been reported to generate ammonia via L-Arg deiminase (ADI) pathway, thereby elevating local pH, suppressing acidogenic microbiota, and facilitating mineralization [9, 10]. Existing literature indicates that L-Arg can reduce the biomass of *E. faecalis* biofilms at the early (6 h) and mature (24 h) stages by approximately 50% and 60%–75%, respectively [11]. Due to the positively charged guanidino group on its side chain, L-Arg can electrostatically bind to the negatively charged dentin surface and dentinal tubule walls, locally modulating the microenvironmental pH toward alkalinity and creating favorable conditions for calcium and phosphorus ion deposition [12] Furthermore, L-Arg can participate in assembling an "L-Arg-calcium-

phosphate" complex that serves as a mineralization precursor, directionally guiding the nucleation and growth of hydroxyapatite crystals [13, 14]. Characterized by low cytotoxicity and minimal disruption to the oral microecology, L-Arg exerts a dual effect of suppressing bacterial biofilms and promoting the remineralization of dental hard tissues, which could effectively compensate for the compromised efficacy and detrimental effects of $Ca(OH)_2$. However, L-Arg has rarely been investigated in endodontic therapy, and its effects on infected root canals and root canal wall dentin remain to be systematically elucidated.

Therefore, this study aimed to introduce L-Arg into the intracanal inter-appointment dressing system and to evaluate its effects on *E. faecalis* and root canal wall dentin. We hypothesized that (1) L-Arg could exert effective antibacterial activity against *E. faecalis* in both planktonic and biofilm; (2) it would inhibit *E. faecalis* within infected root canals; (3) it would promote calcium and phosphorus deposition and enhance the microhardness of root canal wall dentin.

2 Materials and methods

2.1 Preparation of L-Arg and intracanal medicaments

L-Arg powder (Sigma-Aldrich, St. Louis, MO, USA) was prepared as stock solution and diluted with sterile distilled water or Brain Heart Infusion (BHI) broth (Haibo Biotechnology Co. Ltd., Qingdao, China) to desired concentrations according to experimental requirements. For the combined group, L-Arg powder and calcium hydroxide [$Ca(OH)_2$] powder were blended at a 1:1 mass ratio to form a homogeneous mixture. All medicaments were mixed with sterile distilled water to a paste consistency immediately before use.

2.2 Bacterial strain and culture condition

The standard strain *E. faecalis* (ATCC 29212) was obtained from the School of Stomatology, Zhengzhou University, and routinely cultivated in BHI broth at 37°C. For planktonic assays, overnight bacterial suspensions were adjusted to 5×10^5 CFU/mL and 1×10^6 CFU/mL, respectively. For biofilm formation, the bacterial suspension was adjusted to 1×10^6 CFU/mL in BHI broth, as described in our previous study [15].

2.3 Minimum inhibitory concentration (MIC) determination

To quantify the bacteriostatic effect of L-Arg against *E. faecalis*, the broth microdilution method was used to determine the MIC [16]. L-Arg was serially diluted two-fold in a 96-well plate to obtain concentrations ranging from 360 to 0 mg/mL. An equal volume of overnight bacterial suspension adjusted to 5×10^5 CFU/mL was then added into each well. After incubation at 37°C for 24 h, the MIC was determined by visual inspection. The assay was performed in triplicate.

2.4 Growth curves

To dynamically monitor the effects of L-Arg on *E. faecalis*, a growth curve asssy was performed as described previously [17]. Briefly, bacterial suspensions (1×10^6 CFU/mL) were mixed 1:1(v/v) with L-Arg at different concentrations (45, 90, and 180 mg/mL) in a 96-well plate and incubated at 37°C for 24 h. The optical density at 600 nm (OD_{600}) was detected at 1-h intervals using a microplate reader. All measurements were performed in six tuplicate (n = 6).

2.5 Biofilm formation and inhibition assay

For biofilm formation, *E. faecalis* suspension (1×10^6 CFU/mL) was cultured in 24-well plates containing sterile glass coverslips at the bottom and incubated at 37°C for 48 h to allow the formation of mature biofilms according to our previous study [15].

Biofilm biomass was quantified by crystal violet staining [17]. Briefly, planktonic bacteria were gently removed by rinsing with phosphate-buffered saline (PBS). The biofilms were fixed with 10% methanol for 15 min, washed with PBS, and air-dried. Then, 100 μL of 0.1% crystal violet solution was added to each well, and the plates were stained at room temperature for 30 min. After the staining solution was aspirated, the wells were washed again with PBS and air-dried. Subsequently, 200 μL of 33% glacial acetic acid was added into each well, followed by incubation for 30 min. Finally, a 200 μL aliquot from each well was transferred to a fresh 96-well plate, and the OD_{590} was measured to represent the total biofilm biomass.

The viability and proliferation of bacteria within the biofilms were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay [18]. *E. faecalis* (1×10^6 CFU/mL) were cultured for 24, 48, and 72 h, after which the biofilm-bearing glass coverslips were gently rinsed with PBS. One milliliter of MTT solution was added into each well, and the plates were incubated at 37°C under 5% CO_2 in the dark for 1 h. After incubation, the solution was replaced with 1 mL of dimethyl sulfoxide (DMSO), and the plates were gently agitated in the dark for 20 min. A 200 μL sample from each well was transferred to a new 96-well microplate, and the OD_{492} was recorded.

2.6 Scanning electron microscopy (SEM)

The structural morphology of the biofilms was characterized by SEM [19]. Biofilms grown on glass coverslips were flushed twice with PBS, fixed in 2.5% glutaraldehyde overnight, dehydrated through a graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%, and 100%), sputter-coated with gold, and examined under SEM (Hitachi SU8010, Tokyo, Japan) at 2000× -5000× magnification. Three images were randomly captured on each coverslip.

2.7 Ex vivo human root canal infection model

To assess the antimicrobial efficacy of L-Arg in infected root canals, 36 single-rooted human mandibular premolars extracted for orthodontic reasons were collected from the First Affiliated Hospital of Zhengzhou University. The procurement of samples and experimental protocols were approved by the Research and Clinical Trials Ethics Committee (Approval no. 2025-KY-2119-002).

The root canal infection model was established as described previously [20, 21]. Briefly, teeth were immersed in 1% thymol and examined under ×25 magnifying lens to exclude cracked, fractured, or previously endodontically treated teeth. The crowns were uniformly sectioned to obtain a root length of 12 mm. Each root canal was then prepared to a uniform diameter using nickel-titanium rotary endodontic files. During instrumentation, the canals were irrigated with 1 mL of 1% sodium hypochlorite (NaClO) and 17% ethylenediaminetetraacetic acid (EDTA), followed by a final rinse with 5 mL of sterile saline. After sterilization at 121°C for 30 min, three randomly root samples were incubated for 24 h to confirm sterility. The sterilized specimens that passed the sterility check were then sealed at the apical third using flowable composite resin. A suspension of *E. faecalis* (1×10^8 CFU/mL) was inoculated into the root canals and cultured at 37°C for 21 days. BHI broth was refreshed every 24 h, and the culture medium was completely replaced every 48 h. The supernatant was subcultured weekly to check for contamination by extraneous bacteria.

2.8 Assessment of antibacterial activity in infected root canals

The specimens were randomly assigned to four groups (n = 6) and treated with L-Arg at concentrations of 0, 45, 90, and 180 mg/mL. A 30 μL aliquot of the corresponding solution was introduced into each canal, and the canals were then temporarily sealed with utility wax. The specimens were immersed in sterile saline, and incubated at a constant temperature. After 7 days, the root canals were irrigated with 3 mL of distilled water. From each group, six specimens were randomly selected, and dentin chips were collected into 1 mL of PBS. After serial dilution, 30 μL of the suspension was plated onto BHI agar plates and incubated for 24 hours for colony-forming unit (CFU) counting. The remaining three specimens from each group were fixed, dehydrated, sputter-coated with gold, and observed by SEM.

2.9 Effects of L-Arg on root canal wall dentin

A total of 72 human single-rooted premolars extracted for orthodontic purposes were collected. The exclusion criteria and root canal preparation protocols were identical to those described above. The 72 prepared root specimens were randomly divided into four groups: Control, $Ca(OH)_2$, L-Arg, and L-Arg + $Ca(OH)_2$. The root canals in the control group were sealed with sterile saline. Each group was further divided into three subgroups (n = 6) according to the intracanal dressing duration: 7, 14, and 28 days. Except for the control group, the formulations for all groups were mixed with sterile distilled water to form a paste, which was then delivered into the root canals using a K-file. The coronal orifices and the apical third of the external root surfaces were sealed with flowable composite resin, and the specimens were stored in an incubator at 100% relative humidity. At the predetermined time points, the temporary sealants at the orifices were removed. The intracanal medicaments were removed by sterile saline irrigation combined with ultrasonic activation. Before evaluation, the middle third of each root specimen was sectioned and split longitudinally under running water. The specimens were polished with silicon carbide sandpaper under running water, and any specimens exhibiting cracks were discarded.

2.10 SEM evaluation of dentin ultrastructure

Three specimens from each subgroup were randomly selected for SEM characterization of the dentin wall in the middle third of the root canal [22]. The specimens were rinsed three times with 5 mL of PBS, dehydrated through a graded ethanol series, and dried. The dried root specimens were then vacuum-sputter-coated with gold and mounted onto the observation stage with conductive adhesive. Three random areas per specimen were selected to observe the surface morphological changes of the dentin wall at 2000× and 5000× magnification.

2.11 Energy-dispersive X-ray spectroscopy (EDS) analysis

Three additional specimens from each subgroup were randomly selected, rinsed with PBS, and subjected to elemental analysis at three randomly selected sites using an energy-dispersive X-ray spectrometer (EDS) (Oxford Instruments, Abingdon, UK). The relative mass percentages of calcium (Ca) and phosphorus (P) were determined and compared [23].

2.12 Microhardness evaluation

Three test specimens from each subgroup were randomly selected and embedded in self-curing acrylic resin with the longitudinal section facing upward. The surfaces were successively ground and polished with 800-, 1200-, and 1500-grit sandpaper. The specimens were then placed on a Vickers microhardness tester (Shanghai SCT, China)[24]. Three indentation sites were randomly and evenly distributed on each specimen. A load of 100 g (0.98 N) was applied for 15 s. The average value of the three indentations was recorded as the microhardness value for each specimen.

2.13 Statistical analysis

Statistical analysis and graphical presentation were performed using GraphPad Prism 10.6 software (San Diego, CA, USA) and all date were expressed as mean value ± standard deviation (SD). Two-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test was used for multiple comparisons to assess differences among groups regarding in colony counts, calcium and phosphorus contents, and microhardness values. $P < 0.05$ was considered statistically significant.

3 Results

3.1 L-Arg inhibits the growth of planktonic *E. faecalis*

The antimicrobial activity of L-Arg against planktonic *E. faecalis* was preliminarily evaluated by MIC assay. The culture medium became clear when the L-Arg concentration reached 90 mg/mL, indicating that the MIC of L-Arg against *E. faecalis* was 90 mg/mL (Fig. 1A). This result suggested that L-Arg exerted an inhibitory rather than a bactericidal effect. To further verify this inhibitory effect over time, a growth curve was generated (Fig. 1B). The growth of *E. faecalis* was inhibited in a concentration-dependent manner, being slowed at 45 mg/mL and completely suppressed at 90 mg/mL, which was consistent with the MIC result.

3.2 L-Arg suppresses *E. faecalis* biofilm biomass and bacterial viability in a concentration-dependent manner

To investigate the effect of L-Arg on *E. faecalis* biofilm biomass and bacterial viability, crystal violet staining and MTT assay were performed. Compared with the control group, all L-Arg-treated groups exhibited reduced biofilm biomass (Fig. 2A). Moreover, the total biofilm quantity decreased progressively with increasing L-Arg concentration, indicating that L-Arg effectively inhibited the development of *E. faecalis* biofilms ($p < 0.05$). The viability and proliferation of bacteria was assessed by MTT assay (Fig. 2B). At each incubation time point, the OD values in all L-Arg-treated groups were significantly lower than those of the control group ($p < 0.05$), further confirming that L-Arg suppressed *E. faecalis* biofilm formation. In the 45 mg/mL L-Arg group, the absorbance at 72 h was slightly higher than that at 24 and 48 h. However, at the same L-Arg concentration, the differences in absorbance among the 24-, 48-, and 72-h time points were minimal and not statistically significat ($p > 0.05$). Overall, L-Arg suppressed E. faecalis biofilms and viability in a concentration-dependent manner.

3.3 L-Arg disrupts the structural integrity of *E. faecalis* biofilms

To further verify the inhibitory of L-Arg on *E. faecalis* biofilms from a morphological perspective, SEM was performed to examine biofilm architecture after treatment with different L-Arg concentrations. In the control group, the biofilm appeared compact and heavily colonized, with densely packed bacterial cells (Fig. 3A). In contrast, the L-Arg-treated groups showed a marked reduction in biofilm abundance (Fig. 3B). Moreover, L-Arg treatment noticeably remodeled the biofilm architecture, and this disruption became more pronounced with increasing concentration, resulting in a progressively disorganized and dispersed structural network. These findings further support the concentration-dependent antibiofilm effect of L-Arg observed in the biomass and viability assays.

3.4 L-Arg reduces *E. faecalis* loads in infected root canals

To assess the antibacterial effect of L-Arg in infected root canals, colony counts and SEM were performed. The $\log_{10}$ reductions in colony counts after treatment with different L-Arg concentrations are showed (Fig. 4A). Compared with the control group, colony counts in all L-Arg-treated groups were significantly reduced ($p < 0.05$). In the L-Arg group, *E. faecalis* cells were tightly packed and had penetrated deeply into the dentinal tubules (Fig. 4B). At 45 mg/mL, a marked decrease in the bacterial population was observed (Fig. 4C). At 90 mg/mL, *E. faecalis* was sparsely distributed within the dentinal tubules (Fig. 4D). At 180 mg/mL, only sporadic bacterial cells were visible on the dentinal tubule surfaces (Fig. 4E). These findings indicate that L-Arg inhibited *E. faecalis* in a concentration-dependent manner within a certain range, but the effect reached a plateau at higher concentrations.

3.5 L-Arg promotes mineral deposition on root canal wall dentin

To evaluate the mineralizing effect of L-Arg on root canal wall dentin, SEM was performed. Dentin surfaces and dentinal tubules were examined at 2,000× and 5,000× magnification after 7, 14, and 28 days of intracanal dressing respectively. At day 7, the collagen fiber meshwork was faintly discernible on the dentin surface in the $Ca(OH)_2$ group (Fig. 5B), whereas a precipitation layer had formed on the dentin surface in the L-Arg group (Fig. 5C). Notably, the L-Arg + $Ca(OH)_2$ group exhibited a uniform and continuous deposition layer across the dentin surface (Fig. 5D). At 5,000× magnification, the dentinal tubule orifices in the $Ca(OH)_2$ group remained completely patent (Fig. 5F), In contrast, remineralized substances began to within some tubule orifices in the L-Arg group (Fig. 5G), and mineral precipitation with subsequent thickening were observable around the peritubular dentin in the L-Arg + $Ca(OH)_2$ group (Fig. 5H).

At day 14, the dentin surface in the L-Arg group was densely covered by a mineralized layer (Fig. 6C), while the L-Arg + $Ca(OH)_2$ group displayed a compact and substantial mineralized crust (Fig. 6D). At 5,000× magnification, block-like remineralized structures were formed within the dentinal tubules in the L-Arg group (Fig. 6G), leading to pronounced thickening of the tubule walls.

At day 28, the dentin surface in the L-Arg group appeared rough, with the intertubular collagen meshwork largely covered by a mineralized stratum (Fig. 7C). An even more compact mineralized layer was observed on the dentin surface in the L-Arg + $Ca(OH)_2$ group (Fig. 7D). At 5,000× magnification, most dentinal tubules in the L-Arg group were filled with massive remineralized structures, resulting in distinct wall thickening (Fig. 7G). Collectively, these results indicate that L-Arg promoted progressive mineral deposition on root canal wall dentin, and this effect was enhanced by combination with $Ca(OH)_2$.

3.6. L-Arg increases calcium and phosphorus contents in root canal wall dentin

To investigate the effect of L-Arg on the elemental composition of root canal wall dentin, EDS analysis was performed to determine the mass percentages of calcium (Ca) and phosphorus (P) on the radicular dentin surface (Fig. 8A). At day 7, although the mass fractions of Ca and P in all treatment groups were slightly higher than those in the control group, the differences among groups were minimal and not statistically significant ($p > 0.05$). At day 14, the mass percentages of Ca and P in both the L-Arg and L-Arg + $Ca(OH)_2$ groups were significantly higher than those in the control group ($p < 0.05$). By day 28, the mass fractions of both elements in the L-Arg and L-Arg + $Ca(OH)_2$ groups were significantly higher than those in the control and $Ca(OH)_2$ groups ($p < 0.05$). Over time, the mass percentages of Ca and P in the $Ca(OH)_2$ group showed a progressive decrease, whereas those in the L-Arg and L-Arg + $Ca(OH)_2$ groups

showed a steady increase. Collectively, L-Arg increased the Ca and P contents in root canal wall dentin, and this effect was enhanced by combination with $Ca(OH)_2$.

3.7 L-Arg enhances the microhardness of root canal wall dentin

To evaluate the effect of L-Arg on the microhardness of root canal wall dentin, Vickers microhardness testing was performed (Fig. 8B). At day 7, the differences in microhardness among all groups were not statistically significant ($p > 0.05$). At day 14, the microhardness in the L-Arg group was slightly higher than that in the control group, but the difference was not statistically significant ($p > 0.05$). In contrast, the L-Arg + $Ca(OH)_2$ showed a significant increase in microhardness ($p < 0.05$). At day 28, the microhardness values of both the L-Arg and L-Arg + $Ca(OH)_2$ groups were significantly higher than those of the control and $Ca(OH)_2$ groups ($p < 0.05$). With prolonged intracanal dressing time, microhardness values in the control and $Ca(OH)_2$ groups progressively decreased, whereas those in the L-Arg-containing groups showed a gradual and sustained increase. Altogether, L-Arg enhanced the microhardness of root canal wall dentin over time, particularly when combined with $Ca(OH)_2$.

4. Discussion

This study investigated, for the first time, the effects of L-Arg on *E. faecalis* and root canal wall dentin, and constructed an infected root canal model to explore its feasibility as an intracanal medication. Our results demonstrated that L-Arg exerted a strong inhibiting effect on both planktonic and biofilm *E. faecalis*. Furthermore, L-Arg promoted calcium and phosphorus deposition on the dentin surface and enhanced microhardness, indicating promising remineralization potential.

L-Arg is an endogenous alkaline amino acid predominantly present in human saliva [25, 26]. Although L-Arg has demonstrated cariostatic and remineralizing properties, its efficacy in root canal disinfection remains relatively underexplored [27]. *E. faecalis* is a principal etiological agent in post-treatment endodontic disease, driven by its robust biofilm formation, extensive antibiotic resistance, and capacity to invade dentinal tubules [28–32]. As a facultative anaerobe, *E. faecalis* survives the alkaline, nutrient-deprived intracanal environment via an active proton pump mechanism that maintains intracellular pH homeostasis beneath $Ca(OH)_2$ dressings [29, 30]. Our findings showed that L-Arg effectively suppresses *E. faecalis* biofilm formation, as evidenced by crystal violet staining, MTT assays, and SEM. L-Arg-treated groups exhibited a significant reduction colony counts, which was corroborated by the diminished bacterial load observed on SEM. Given the complex root canal anatomy, the net positive charge of L-Arg facilitates electrostatic adsorption to negatively charged dentin, potentially establishing a localized retention reservoir that extends its therapeutic window. Our findings indicate that L-Arg exerts a bacteriostatic rather than bactericidal effect. This distinction may be clinically relevant, as interappointment dressings are generally designed to persistently restrain residual bacterial proliferation

and disrupt biofilm re-establishment rather than fully replace irrigant efficacy. Thus, L-Arg holds considerable merit as a low-toxicity adjunctive intracanal component.

Multiple studies have shown that prolonged $Ca(OH)_2$ exposure significantly reduces the mineral-to-matrix ratio and impairs the mineral crystallinity of radicular dentin, thereby increasing fracture susceptibility [33–35]. The highly alkaline environment denatures matrix proteins, disrupts hydroxyapatite-collagen cross-links, and severs the inorganic infrastructure, ultimately reducing dentin microhardness and mechanical performance [36–39]. Previous study have reported that $Ca(OH)_2$ dressing compromises radicular dentin strength by 22% at 1 week, 26% at 2 weeks, 33% at 1 month, and up to 55% at 3 months [35]. In contrast to these adverse effects of $Ca(OH)_2$, L-Arg may counteract these deleterious effects through three principal mechanisms. First, the guanidino group of L-Arg electrostatically binds to carboxyl and phosphate groups on dentin surfaces, thereby establishing a baseline for remineralization [40, 41]. Second, the intrinsic alkalinity of L-Arg modulates the local microenvironmental by promoting the precipitation of calcium and phosphorus ions and the formation of amorphous calcium phosphate precursors which subsequently transition into apatite crystals [42]. Third, L-Arg binds with Ca^{2+} and PO_4^{3-} through its carboxyl and amino side chains to form stable ternary L-Arg-calcium-phosphate complexes that serve as mineral nucleation centers, directionally guiding crystal growth and enhancing mineralization efficiency [43]. Accordingly, combining L-Arg with $Ca(OH)_2$ may provide additional benefits for root canal wall dentin. L-Arg has been reported synergistic effects with fluoride or calcium carbonate by precipitating fluorapatite, reducing mineral solubility, and enhancing acid resistance [44–47]. As an alkaline amino acid, L-Arg elevates local pH, thereby favoring calcium phosphate synthesis and stabilization, and can also bind to negative sites on collagen and mineral interfaces [48–51]. when combined with $Ca(OH)_2$, which itself provides highly alkaline environment, L-Arg may further promote the formation of calcium-phosphate precursor and facilitate mineral deposition. Encouragingly, this hypothesis is supported by our findings that the L-Arg + $Ca(OH)_2$ group exhibited more pronounced mineral deposition and greater microhardness improvement than the $Ca(OH)_2$ group alone. Therefore, the combination of L-Arg and $Ca(OH)_2$ may not only mitigate the adverse effects of $Ca(OH)_2$ on dentin but also enhance its remineralization potential, suggesting a promising strategy for intracanal medication.

However, several limitations of this study should acknowledged. First, the precise molecular mechanisms underlying the antimicrobial effects of L-Arg were not fully elucidated. Second, the microbial evaluation was linited to *E. faecalis* and not include other prevalent endodontic pathogens. Third, no direct comparison was made between L-Arg and established gold-standard intracanal disinfectants. Fourth, the effects of L-Arg on radicular dentin were only preliminarily characterized, and the exact mechanism by which L-Arg promotes remineralization remains to be clarified. Finally, because all experiments were performed in vitro, they could not fully replicate the dynamic oral environment, and the long-term durability of L-Arg-enhanced dentin reinforcement warrants requires further investigation.

5. Conclusion

This study investigated the effects of L-Arg on *E. faecalis* and root canal wall dentin. L-Arg inhibited planktonic *E. faecalis* growth in a dose-dependent manner, with an MIC of 90 mg/mL, and suppressed biofilm formation. It also effectively reduced viable *E. faecalis* counts in infected root canals after 7 day of intracanal dressing. Furthermore, L-Arg promoted calcium and phosphorus deposition on root canal dentin surfaces and enhanced dentin microhardness. These findings suggest that L-Arg has promising potential as a dual-function intracanal medication combining antibacterial activity with dentin remineralization.

Declarations

Ethics approval and consent to participate

This in vitro study was approved by the Research and Clinical Trials Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Approval No. 2025-KY-2119-002). All experiments were performed following relevant guidelines and regulations regarding the experiments on the use of human tissue samples. Informed consent was obtained from each patient for using extracted teeth.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lu Qin, Yanmin Jia Yiyi Wang and Xushuo Du. The first draft of the

manuscript was written by Lu Qin and Yanmin Jia, and all authors revised and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Not applicable.

Data availability

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

References

1. Thammasitboon K, Teanpaisan R, Pahumunto N. Prevalence and virulence factors of haemolytic *Enterococcus faecalis* isolated from root filled teeth associated with periradicular lesions: a laboratory investigation in Thailand. Int Endod J. 2024;57:769–83.
2. Xu Z, Ma H, Jiang X, et al. Transcriptome analysis of calcium hydroxide tolerance in *Enterococcus faecalis*. Front Microbiol. 2025;16:1551824.
3. Siqueira Ye WH, Fan B, Purcell W, et al. Anti-biofilm efficacy of root canal irrigants against in-situ *Enterococcus faecalis* biofilms in root canals, isthmuses and dentinal tubules. J Dent. 2018;79:68–76.
4. Yang S, Meng X, Zhen Y, et al. Strategies and mechanisms targeting *Enterococcus faecalis* biofilms associated with endodontic infections: a comprehensive review. Front Cell Infect Microbiol. 2024;14:1433313.
5. Koparkar T, Kalyan S, Mandke L, et al. Efficacy of various proton-pump inhibitors and calcium hydroxide as intracanal medicaments against *Enterococcus faecalis*: an in vitro study. Endod. 2022;34(4):259–64.
6. Liu Y, Liu S, Zhi Q, et al. Arginine-induced metabolomic perturbation in *Streptococcus mutans*. J Oral Microbiol. 2022;14(1):2015166.
7. Al-Hiyasat AS, El-Farraj HS, Alebrahim MA. The effect of calcium hydroxide on dentine composition and root fracture resistance of human teeth: an in vitro study. Eur J Oral Sci. 2021;129(4):e12798.
8. Sebbane N, Abramovitz I, Kot-Limon N, et al. Mechanistic insight into the anti-bacterial/anti-biofilm effects of low chlorhexidine concentrations on *Enterococcus faecalis*: an in vitro study. Microorganisms. 2024;12(11):2297.
9. He J, Hwang G, Liu Y, et al. L-Arginine modifies the exopolysaccharide matrix and thwarts *Streptococcus mutans* outgrowth within mixed-species oral biofilms. J Bacteriol. 2016;198(19):2651–61.

10. Gao HY, Yang H, Wang HM, et al. Arginine inhibits cross-kingdom interactions and synergistic cariogenicity between Streptococcus mutans and *Candida albicans*. Front Cell Infect Microbiol. 2025;15:1633342.
11. Snell AP, Manias DA, Elbehery RR, Dunny GM, Willett JLE. Arginine impacts aggregation, biofilm formation, and antibiotic susceptibility in *Enterococcus faecalis*. FEMS Microbes. 2024;5:xtae030.
12. Fang P, Ye X, Tian L, et al. Modified casein-stabilized amorphous calcium phosphate nanoparticles prevent dental caries by inhibiting the growth of *Streptococcus mutans* and promoting the remineralization of tooth enamel. Colloids Surf B Biointerfaces. 2025;254:114815.
13. Bijle MN, Ekambaram M, Lo EC, et al. The enamel remineralization potential of fluoride varnishes containing arginine. J Dent. 2020;99:103411.
14. Qiu X, Liu J, Chen X. The combination of arginine and fluoride-containing bioactive glass acted synergistically in inhibiting enamel demineralization in permanent teeth. J Dent. 2024;149:105227.
15. Wang Y, Fang L, Wang P, et al. Antibacterial effects of silica nanoparticles loading nano-silver and chlorhexidine in root canals infected by *Enterococcus faecalis*. J Endod. 2025;51(1):54–63.
16. Lv S, Fan W, Fan B. Enhanced in vitro antibacterial effect against *Enterococcus faecalis* by using both low-dose cetylpyridinium chloride and silver ions. BMC Oral Health. 2023;23(1):299.
17. Fang L, Zhang Y, Cheng L, et al. Silica nanoparticles containing nano-silver and chlorhexidine to suppress *Porphyromonas gingivalis* biofilm and modulate multispecies biofilms toward healthy tendency. J Oral Microbiol. 2024;16(1):2361403.
18. Wang S, Fang L, Zhou H, et al. Silica nanoparticles containing nano-silver and chlorhexidine respond to pH to suppress biofilm acids and modulate biofilms toward a non-cariogenic composition. Dent Mater. 2024;40(2):179–89.
19. Parolia A, Kumar H, Ramamurthy S, et al. Effectiveness of chitosan-propolis nanoparticle against *Enterococcus faecalis* biofilms in the root canal. BMC Oral Health. 2020;20(1):339.
20. Mohamed AA, Fayyad DM, El-Telbany M, et al. Antibacterial biofilm efficacy of calcium hydroxide loaded on gum arabic nanocarrier: an in-vitro study. BMC Oral Health. 2024;24(1):215.
21. Sisodiya YS, Saha SG, Agarwal RS, et al. Time-dependent effect of nanoparticle-based intracanal medicament on root dentin microhardness and their antibacterial efficacy against *Enterococcus faecalis*. J Conserv Dent. 2026;29(8):895–901.
22. Wang Y, Zhang X, Zheng H, et al. Remineralization of dentin with cerium oxide and its potential use for root canal disinfection. Int J Nanomed. 2023;19:1–17.
23. Guo L, Tan S, Wen B, et al. Antibiofilm efficacy of ε-polylysine hydrochloride against *Enterococcus faecalis* biofilms and its biocompatibility: a promising root canal medicament. Dent Mater. 2026;S0109–5641(26):00452–5.
24. Carvalho NK, Barbosa AFA, Coelho BP, et al. Antibacterial, biological, and physicochemical properties of root canal sealers containing chlorhexidine-hexametaphosphate nanoparticles. Dent Mater. 2021;37(5):863–74.

25. Bijle MN, Naseer TK, Yiu C. Physical properties and enamel remineralization potential of arginine-fluoride varnishes. J Dent. 2024;148:104965.
26. Salomão KB, Campos LA, Nunes GP, et al. Association of arginine with a soluble source of calcium and fluoride: anticaries and antibiofilm effect on root dentin. J Dent. 2025;163:106194.
27. Kuriki N, Asahi Y, Okamoto M, et al. Synergistic effects of arginine and fluoride on human dental biofilm control. J Dent. 2024;149:105307.
28. Ruhal R, Sahu A, Koujalagi T, et al. Biofilm-specific determinants of enterococci pathogen. Arch Microbiol. 2024;206(10):397.
29. Wu B, Zhou Z, Hong X, et al. Novel approaches on root canal disinfection methods against *Enterococcus faecalis*. J Oral Microbiol. 2025;17(1):2475947.
30. Najafi K, Ganbarov K, Gholizadeh P, et al. Oral cavity infection by *Enterococcus faecalis*: virulence factors and pathogenesis. Rev Res Med Microbio. 2020;31(2):51–60.
31. Reyhani MF, Ghasemi N, Milani AS et al. Antimicrobial effect of nano-calcium hydroxide on the four- and six-week-old intra-canal *Enterococcus faecalis*biofilm J Dent (Shiraz). 2023;24(2):194–199.
32. Kim D, Kim E. Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part II. in vivo studies. Restor Dent Endod. 2015;40(2):97–103.
33. Yanyan S, Guangxin W, Guoqing S, et al. Effects of amino acids on conversion of calcium carbonate to hydroxyapatite. RSC Adv. 2020;10(61):37005–13.
34. Silva LR, de Lima KL, Santos AA, et al. Dentin thickness as a risk factor for vertical root fracture in endodontically treated teeth: a case-control study. Clin Oral Investig. 2021;25(3):1099–105.
35. Niranjan NT, Dastidar PG, Penukonda R, et al. Impact of calcium hydroxide and 2-hydroxyisocaproic acid on the microhardness of root dentine: an in vitro study. Odontology. 2023;112(3):711–17.
36. Nasr M, Abdou A, Bassiouny DM, et al. Antibiofilm effect of nano chitosan and calcium hydroxide intracanal medications and their effects on the microhardness and chemical structure of radicular dentine. BMC Oral Health. 2025;25(1):103.
37. Li ALB, Markvart M, Abbott PV. Effect of different concentrations of sodium hypochlorite on the compressive strength of endodontically treated roots. J Endod. 2022;48(3):370–4.
38. Hashem SN, Elhousiny MA. Microhardness and fracture resistance of radicular dentin treated with different concentrations of calcium hydroxide in endodontic regeneration procedures. Open Access Maced J Med Sci. 2021;9(D):324–9.
39. Said F, Moskovitz M. Effect of calcium hydroxide as a root canal dressing material on dentin fracture strength in primary teeth: in vitro study. J Clin Pediatr Dent. 2018;42(2):146–9.
40. Mohammadipour HS, Bagheri H, Babazadeh S, et al. Evaluation and comparison of the effects of a new paste containing 8% L-Arginine and $CaCO_3$ plus KNO_3 on dentinal tubules occlusion and dental sensitivity: a randomized, triple blinded clinical trial study. BMC Oral Health. 2024;24(1):507.
41. Zheng X, Cheng X, Wang L, et al. Combinatorial effects of arginine and fluoride on oral bacteria. J Dent Res. 2015;94(2):344–53.

42. Qi M, Long Z, Liu X, et al. Crystallization of smooth amorphous calcium phosphate microspheres to core-shell hydroxyapatite microspheres. RSC Adv. 2024;14(35):25369–77.
43. Ding J, Zhu X, Lin Q, et al. Effects of arginine-calcium carbonate pretreatment on the remineralizing and bonding performance of phosphorylated dentin. J Mech Behav Biomed. 2023;140:105718.
44. Méndez DAC, Cardenas Cuellar MR, Andrade FB, et al. Suppressive effect of arginine and sodium fluoride on enamel calcium loss and antibiofilm activity on mature oral microcosm biofilms. Biofouling. 2025;41(7):696–710.
45. Toledano M, Osorio E, Aguilera FS, et al. Dexamethasone-doped nanoparticles improve mineralization, crystallinity and collagen structure of human dentin. J Dent. 2023;130:104447.
46. Bhatia S, Vaidya M, Hegde V, et al. Comparative evaluation of the effect of EDTA, chitosan, etidronic acid, and silver citrate on the mineral content of root canal dentin using energy dispersive x-ray spectroscopy: an in-vitro study. Eur Endod J. 2025;10(2):173–80.
47. Moradian-Oldak J, George A. Biomineralization of enamel and dentin mediated by matrix proteins. J Dent Res. 2021;100(10):1020–9.
48. Zheng B, Zhao L, Chen L, et al. Phosphorylation of collagen fibrils enhances intrafibrillar mineralization and dentin remineralization. Nanoscale. 2024;16(24):11633–41.
49. Lopez-Fontal E, Gin S. Insights into calcium phosphate formation induced by the dissolution of 45S5 bioactive glass. ACS Biomater Sci Eng. 2025;11(2):875–90.
50. Toledano M, Osorio R, Vallecillo-Rivas M, et al. Zn-doping of silicate and hydroxyapatite-based cements: dentin mechanobiology and bioactivity. J Mech Behav Biomed Mater. 2021;114:104232.
51. Reyes-Carmona J. A new perspective on the biomineralization of radicular dentin. SVOA Dent. 2024;5:84–8.

Figures

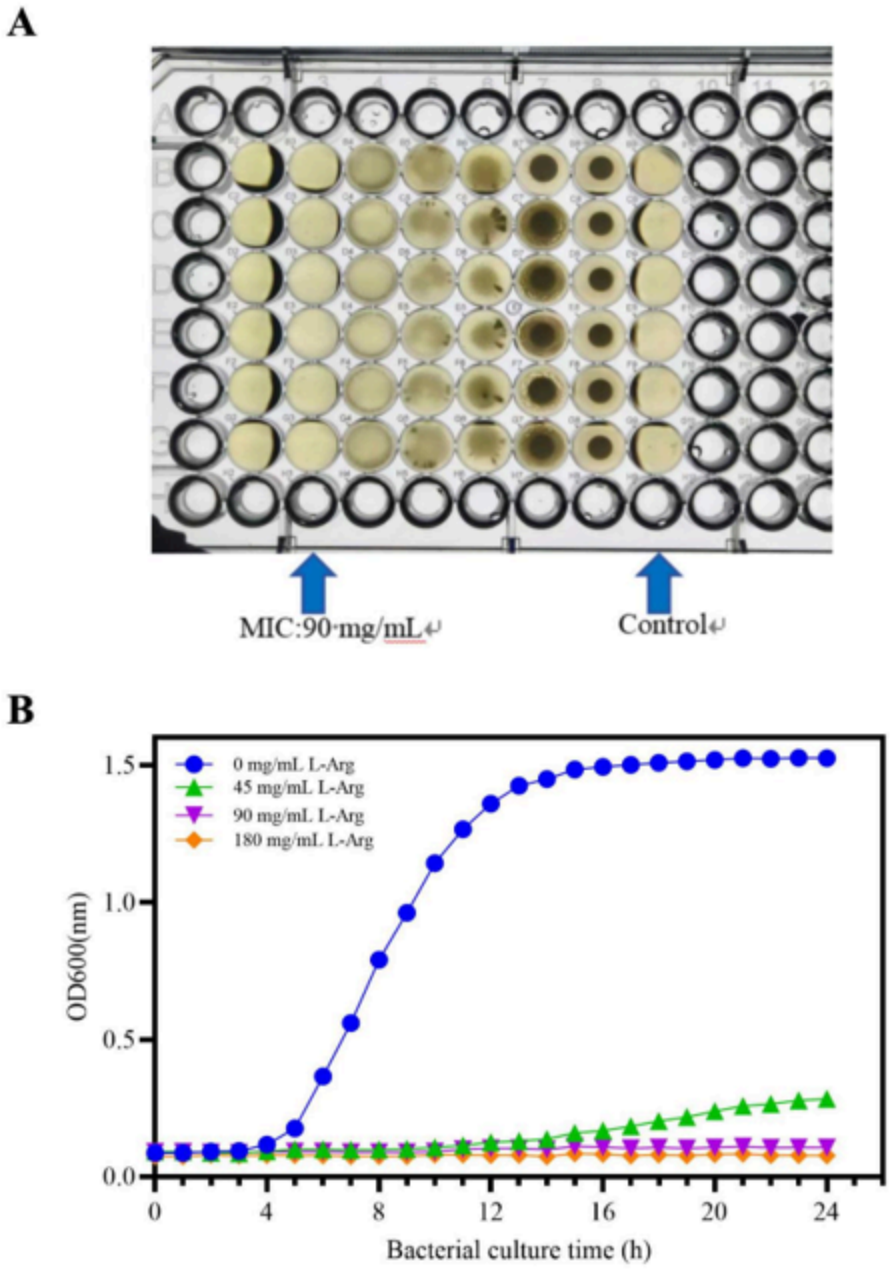

Figure 1

The effect of L-Arg on *E. faecalis* in the planktonic state. (A) The MIC value of L-Arg against *E. faecalis*. (B) Growth curves of *E. faecalis* after 24 hours cultivation in media containing different concentrations of L-Arg (mean ± SD; n = 6).

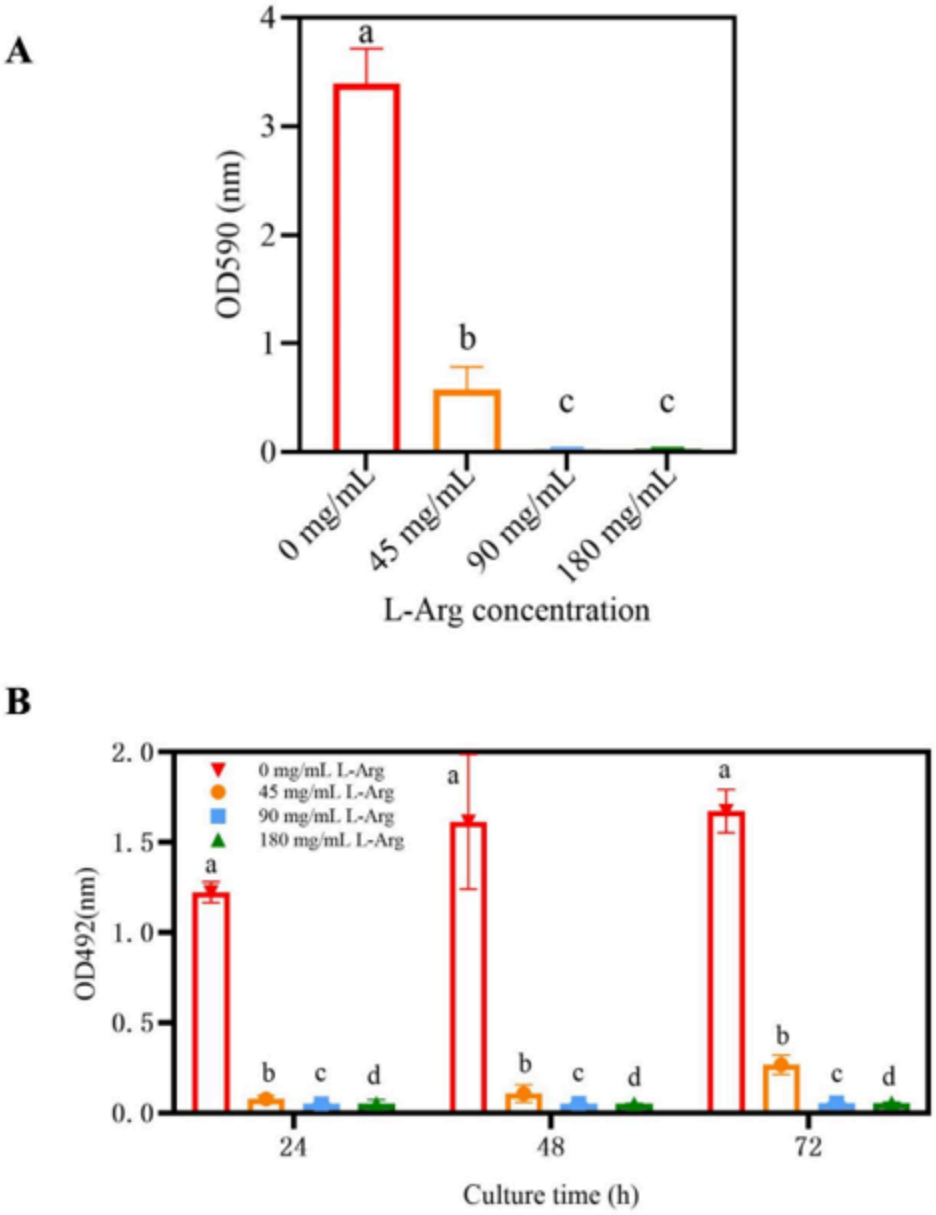

Figure 2

The effect of L-Arg on *E. faecalis* biofilms. (A) Crystal violet staining (mean ± SD; n = 6); Statistically significant at P < .05. (B) MTT assay (mean ± SD; n = 6); Statistically significant at P < .05.

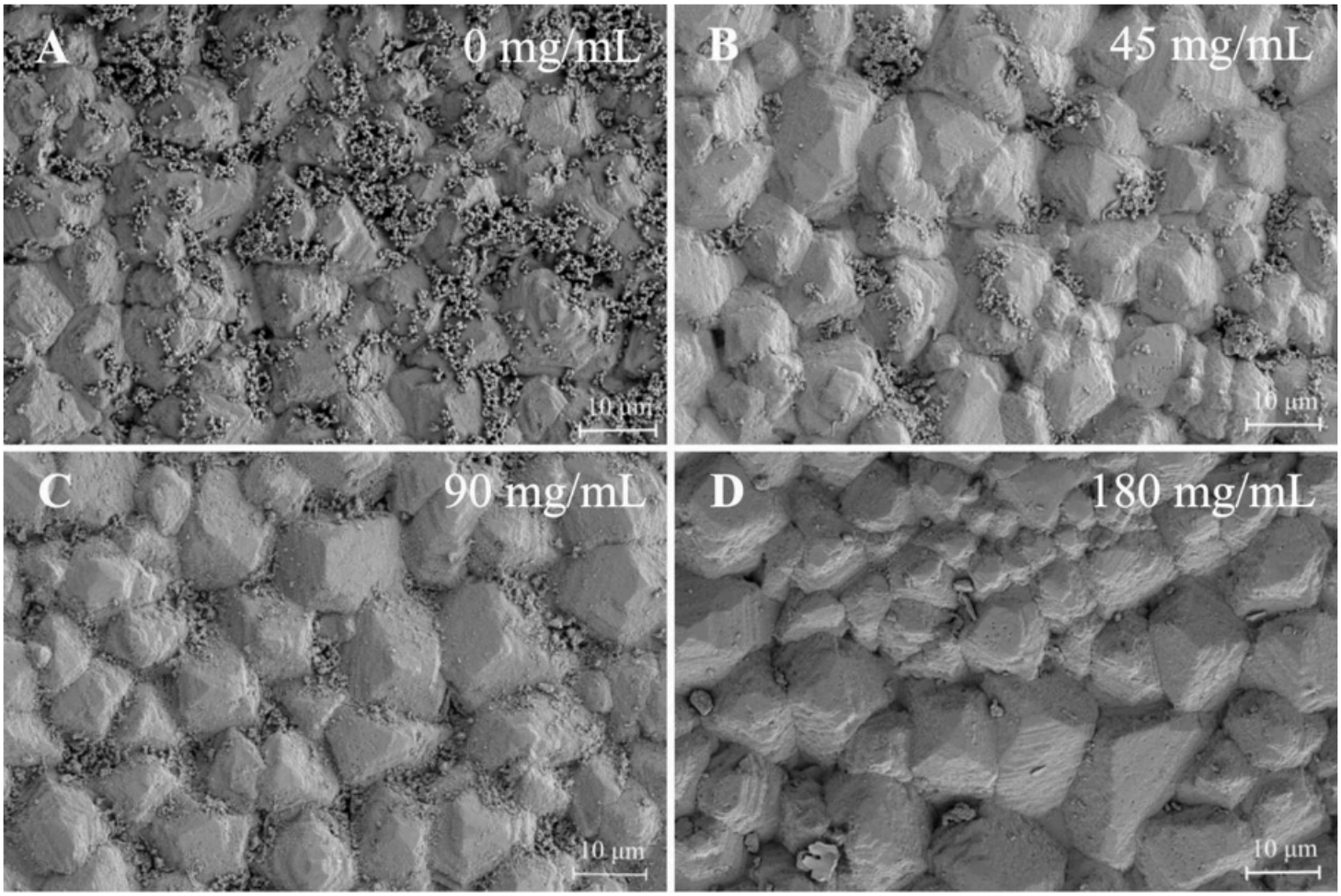

Figure 3

Representative SEM images of L-Arg against *E. faecalis* biofilms. (A) Control group; (B) 45 mg/mL L-Arg group; (C) 90 mg/mL L-Arg group; (D)180 mg/mL L-Arg group.

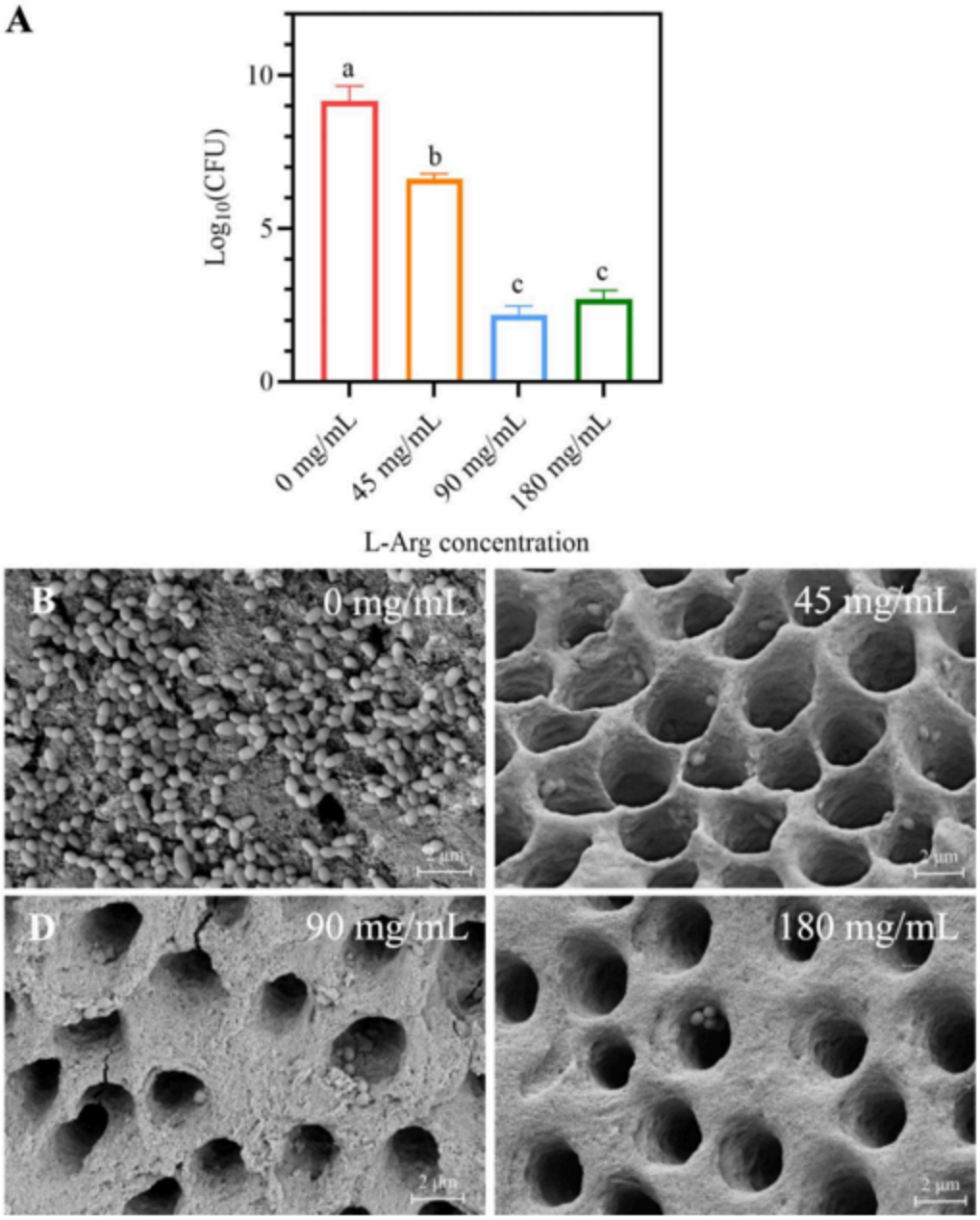

Figure 4

The effect of L-Arg on *E. faecalis* in the root canals in vitro. (A) Colony counts after 7 days of treatment with L-Arg at different concentrations (mean ± SD; n = 6); Statistically significant at P < .05; (B-E) SEM images of *E. faecalis* in the root canals after 7 days of treatment with L-Arg at different concentrations.

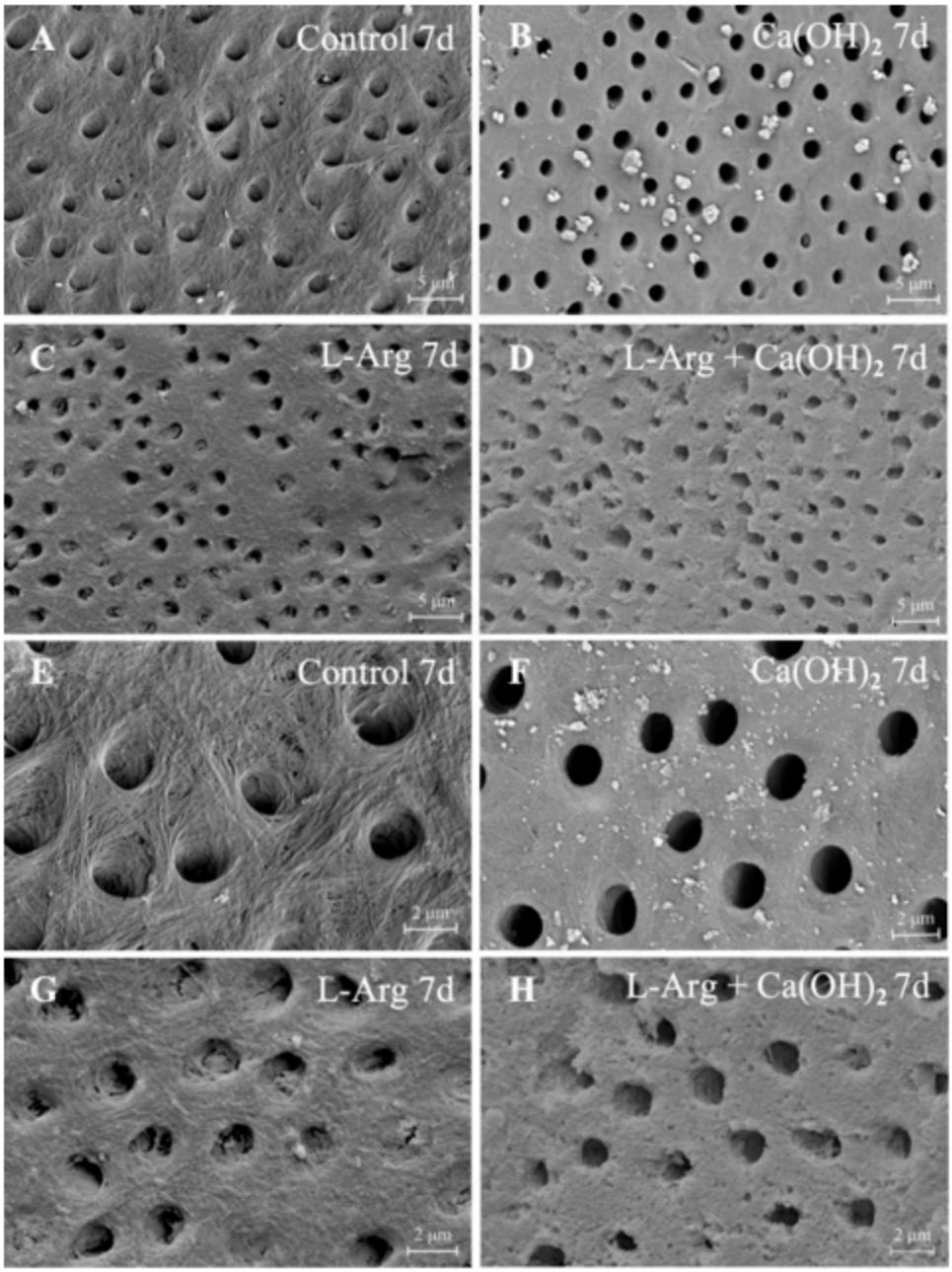

Figure 5

Morphological changes in the dentin of the root canal wall induced by L-Arg. (A-D) SEM images of the root canal wall dentin after 7 days of sealing treatment (×2000); (E-H) SEM images of the root canal wall dentin after 7 days of sealing treatment (×5000).

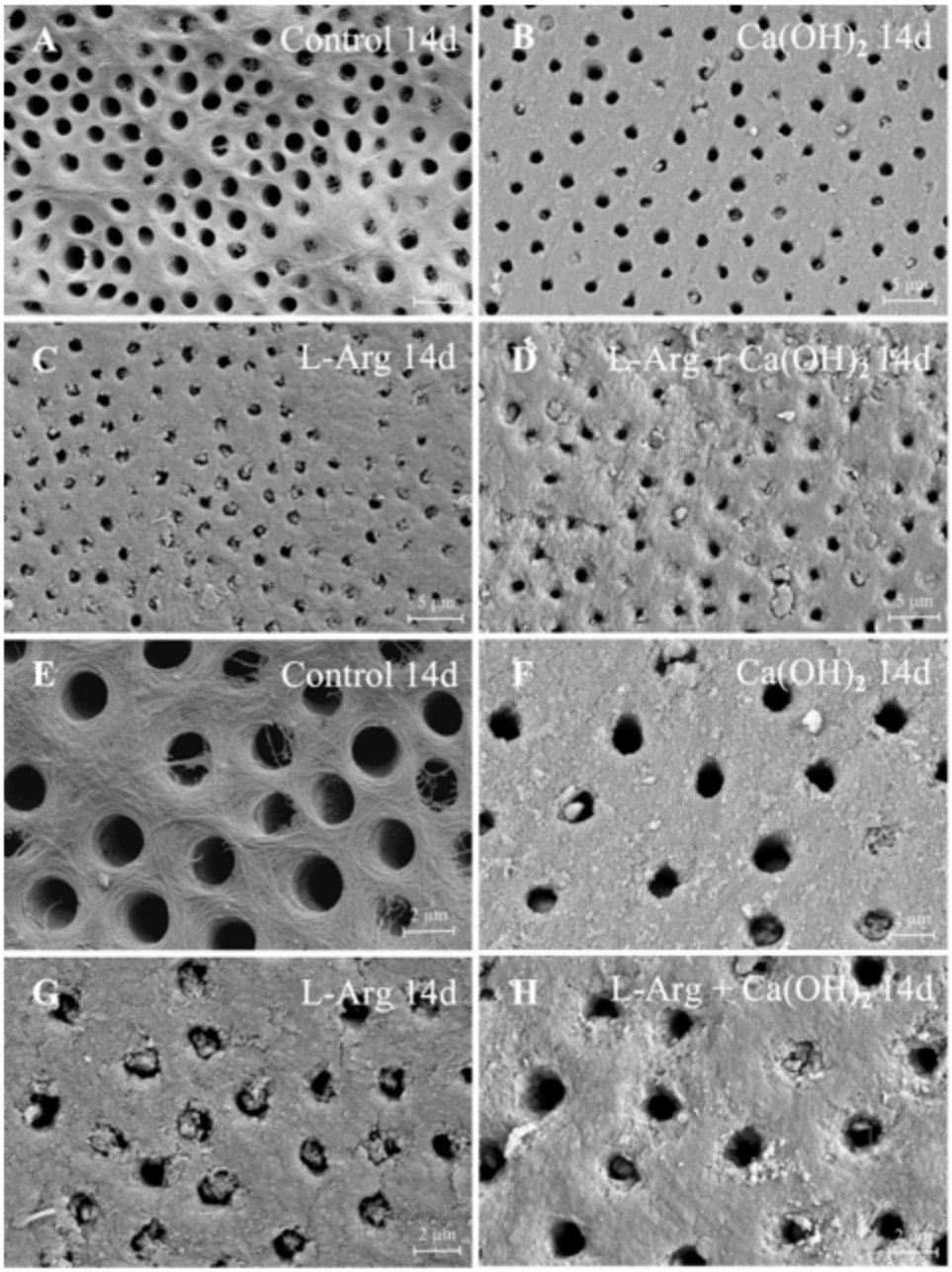

Figure 6

Morphological changes in the dentin of the root canal wall induced by L-Arg. (A-D) SEM images of the root canal wall dentin after 14 days of sealing treatment (×2000); (E-H) SEM images of the root canal wall dentin after 14 days of sealing treatment (×5000).

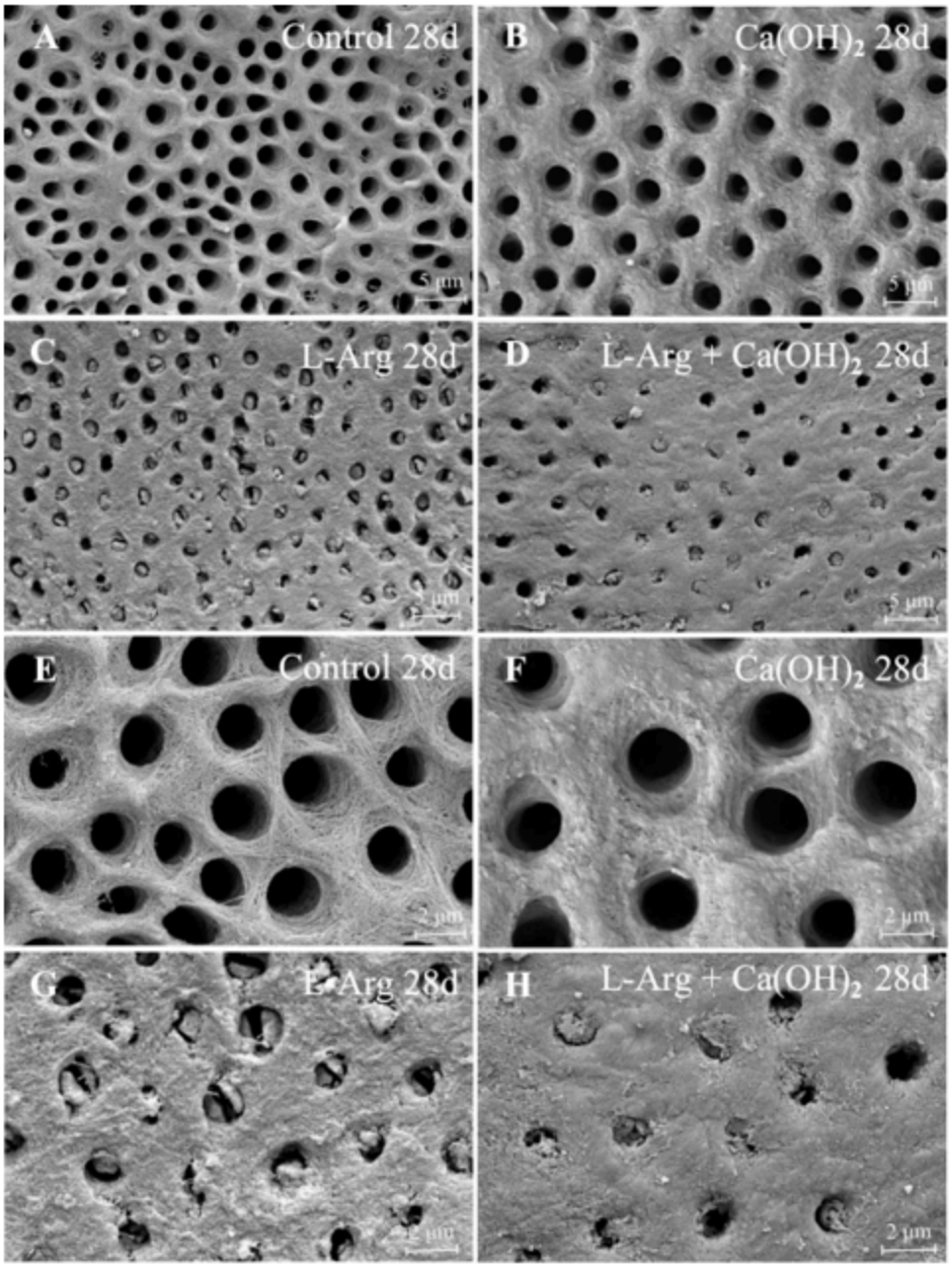

Figure 7

Morphological changes in the dentin of the root canal wall induced by L-Arg. (A-D) SEM images of the root canal wall dentin after 28 days of sealing treatment (×2000); (E-H) SEM images of the root canal wall dentin after 28 days of sealing treatment (×5000).

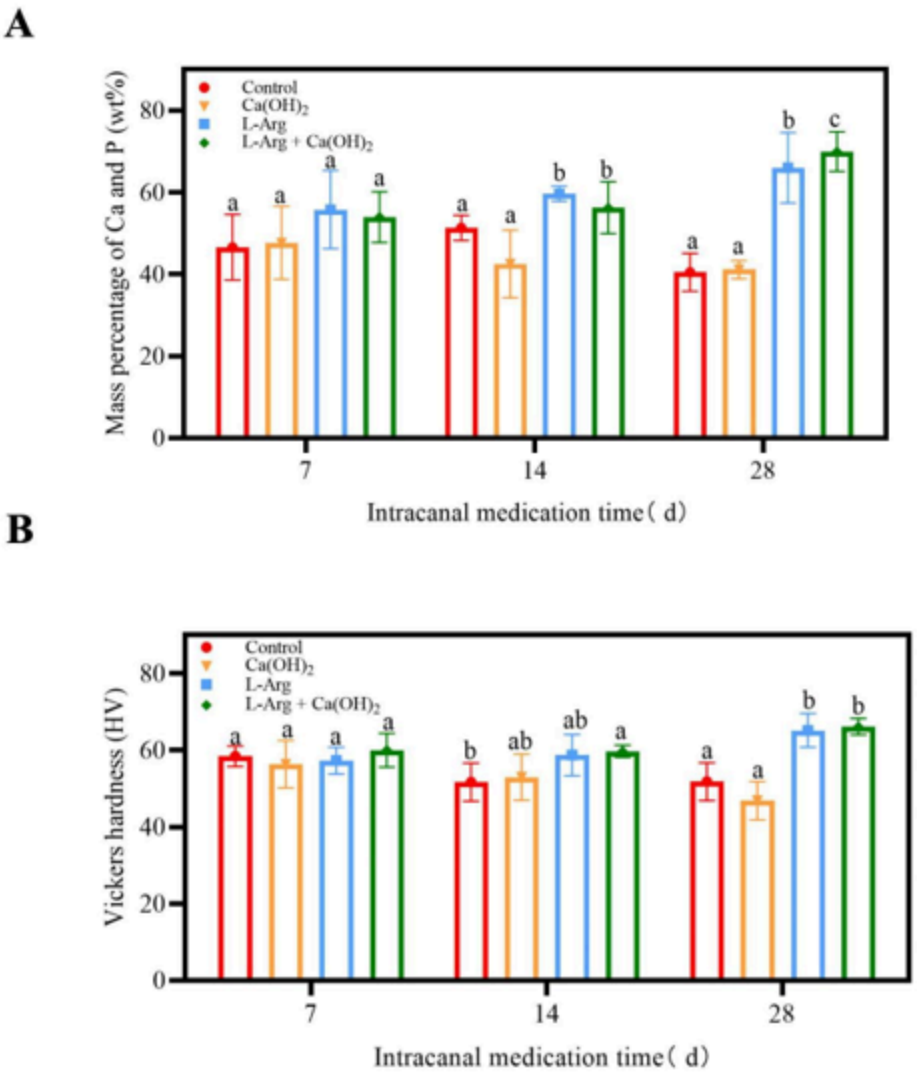

Figure 8

The effect of L-Arg on the dentin of the root canal wall. (A) Mass fractions of calcium and phosphorus in each group after medicamentation (mean ± SD; n = 6),Statistically significant at P < .05; (B) Microhardness values of the root canal wall dentin after medicamentation (mean ± SD; n = 6); Statistically significant at P < .05.