Research Square

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Antibacterial and cellular responses to nano-α-tricalcium phosphate for pulp capping

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Abstract

This study evaluated the antibacterial effects, aqueous pH, and human dental pulp stem cell (hDPSC) responses to nano-α-tricalcium phosphate (nα-TCP) compared with mineral trioxide aggregate (MTA). Nano-α-TCP was tested at 0.02, 0.08, and 0.16 mg/mL. Antibacterial effects against *Lactobacillus acidophilus* were assessed by viable colony counts after 24-h contact. Aqueous pH was measured at 0, 3, and 24 h, scratch wound closure at 6 and 24 h, and alkaline phosphatase (ALP) on days 3, 7, 14, and 21. Data were analysed using analysis of variance and Tukey's post hoc comparisons ($\alpha = 0.05$). All nα-TCP concentrations reduced bacterial recovery relative to distilled water, with reductions of 81.2–92.8%; no colonies were recovered with MTA. Nano-α-TCP exhibited near-neutral to mildly alkaline pH, significantly below MTA. At 24 h, gap closure reached 23.93% and 22.04% with 0.16 and 0.08 mg/mL nα-TCP, respectively, exceeding MTA (12.32%) and medium-only controls (8.04%). ALP measurements were higher with 0.16 mg/mL nα-TCP than MTA on day 3, but no pairwise differences were detected on days 7–21. For ALP, the treatment–time interaction was significant, whereas the overall treatment effect was not. Nano-α-TCP reduced bacterial recovery and enhanced hDPSC gap closure at substantially lower aqueous pH than MTA. Its transient ALP response supports further biological investigation, without establishing odontogenic differentiation or clinical efficacy.

Introduction

Preserving a vital pulp requires control of the local microbial challenge while allowing the injured tissue to recover. Contemporary vital pulp therapy therefore depends on the condition of the remaining pulp, aseptic treatment and an effective restoration, as well as the properties of the material placed at the exposure site [1–3]. A candidate pulp capping material must be evaluated across these different requirements. Suppression of bacterial recovery alone gives little information about its effects on repair-associated cells.

Mineral trioxide aggregate is an appropriate comparator because it has a substantial history of endodontic use. Its hydration chemistry and interaction with environmental fluids influence its biological and antimicrobial behaviour [4]. The same material can behave differently under direct contact, extract exposure or clinically buffered conditions. Comparisons with experimental materials consequently need to describe the exposure system closely, rather than treat a nominal concentration as a complete description of the cellular environment.

Calcium phosphate materials offer an alternative chemical basis for investigating repair at the dentin–pulp interface. A systematic review of calcium phosphate and silicate materials identified favourable effects on several dental pulp stem cell functions, but also considerable heterogeneity in formulation and experimental methods [5]. These findings support material-specific investigation. They do not establish that every calcium phosphate formulation will produce the same cell response, or that a response observed in one cell population can be transferred to another.

Recent work from our research group has examined nα-TCP through complementary experimental approaches. Riany et al. assessed setting behaviour, antibacterial effects against *Streptococcus mutans* and fibroblast cytotoxicity [6]. Wulansari et al. investigated nα-TCP/silver nanoparticle resin composites using human dental pulp stem cells, odontogenic protein markers and mineral deposition [7]. These systems differ from the nα-TCP preparations examined here; the resin matrix and silver component prevent direct attribution of the composite findings to α-TCP alone. In a separate study, Widyastuti et al. evaluated solanesol and calcium hydroxide through TNF-α measurements, illustrating the need to examine inflammatory responses alongside other biological endpoints [8].

The present study evaluates bacterial colony recovery, pH, cell gap closure and alkaline phosphatase (ALP) responses. *Lactobacillus acidophilus* provides a defined bacterial challenge, although a single-species assay cannot represent the ecological complexity of carious dentin [9]. Gap closure offers a short-term cellular endpoint, whereas ALP measurements extend observation to 21 days. These outcomes address different aspects of material behaviour and require separate interpretation. An ALP response alone does not establish odontogenic differentiation.

We investigated whether the response to nα-TCP depended on dose and exposure time, and how that response compared with MTA. The primary questions were whether bacterial recovery decreased, whether the preparations generated a strongly alkaline environment, and whether the cellular endpoints changed over time. We did not assume that an improvement in one assay would predict improvement in the others.

Materials and methods

Ethics and donor information

Ethical committee approval was received from the Ethics Committee of the Universitas Trisakti (Approval no: 945/S1/KEPK/FKG/6/2025 Date: 18 June 2025). Informed consent was not required because this study was conducted entirely in vitro and did not involve human participants.

Material preparation

Alpha-tricalcium phosphate (α-TCP, HiMedia, Mumbai, India) powder was synthesized by dry high-energy ball milling (HEM) to produce nanoparticles. The process was carried out in a planetary ball mill with zirconia balls 2–5 mm in diameter as the grinding media inside a zirconia jar to avoid metal contamination. The ball-to-powder ratio (BPR) was set at 10:1, with a rotation speed of 400 rpm for 5 effective hours using a duty cycle of 15 min ON and 5 min OFF to prevent excessive temperature rise

(maximum < 60°C). The entire process was carried out under dry conditions at room temperature. α-Tricalcium phosphate (α-TCP) powder was assessed by particle size analysis before and after 30 h of high-energy milling. The resulting powder, designated nα-TCP, was prepared at 0.02, 0.08, and 0.16

mg/mL. ProRoot MTA (Dentsply Tulsa Dental Specialties, USA) served as the reference material in all assays. Distilled water was the antibacterial negative control; Dulbecco's modified Eagle medium (DMEM) alone was the scratch assay control.

Human dental pulp stem cell culture

Human dental pulp stem cells (hDPSCs) were independently isolated from the pulp tissue of human donor teeth and expanded in culture before use. Their stem cell phenotype was assessed by immunophenotyping at Prodia StemCell, Jakarta, Indonesia, on 3 November 2025. The cells expressed CD73 (99.4%), CD90 (96.5%), and CD105 (90.3%), with negligible expression of haematopoietic lineage markers (0.1%).

All cell culture procedures were conducted in a biosafety cabinet. Cells were maintained in Dulbecco's modified Eagle medium (DMEM; Gibco, Waltham, USA) supplemented with fetal bovine serum, 1% amphotericin B, and 1% penicillin–streptomycin (all from Gibco, Waltham, USA). Antimicrobial supplements remained in the medium throughout expansion until the cells reached the confluence required for experimentation. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 (Esco, Changi, Singapore). The medium was replaced regularly, and cells were passaged every 3–4 days until they reached 80% confluence.

Antibacterial assay

Bacterial suspension and colony counting

Lactobacillus acidophilus ATCC 4356 was inoculated into Mueller–Hinton broth (Merck, Darmstadt, Germany), incubated at 37°C for 24 h, and adjusted to a 0.5 McFarland standard. The initial bacterial population was determined by total plate count (TPC). The suspension was serially diluted to 10^{-6} in sterile 0.9% NaCl, and 1 mL of the final dilution was mixed with 20 mL of Mueller–Hinton agar in a sterile Petri dish. Colonies were counted after incubation at 37°C for 24 h, with dilution accounted for when calculating colony-forming units per millilitre (CFU/mL). Each sterilised test sample was placed in a sterile tube containing 1 mL of the standardised bacterial suspension and incubated at 37°C for 24 h. The remaining culturable bacterial population was then determined by TPC.

pH measurement

Eluate alkalinity was assessed by immersing disk specimens (6 mm × 2 mm) in 10 mL of deionized water at 37°C. The pH was measured at 0, 3, and 24 h using a calibrated digital pH meter (SevenCompact™, Mettler Toledo, Greifensee, Switzerland). Measurements were performed in triplicate, and mean values were reported.

Scratch wound closure assay

Scratch wound closure was assessed using a scratch wound assay. hDPSCs were seeded in 24-well plates and grown to confluence. A straight scratch was made through the centre of each monolayer using a a sterile 200-µL pipette tip, followed immediately by treatment with nα-TCP solutions at 0.16, 0.08, or 0.02 mg/mL. Control groups received DMEM alone or MTA solution. The scratched areas were photographed using an inverted microscope at 0, 6, and 24 h, and wound closure was quantified using ImageJ (version 1.54s).

Gap closure was calculated from the initial cell-free area (A_0) and the area remaining at the specified time (A) [10]:

$$Gap\ closure\ (\%) = [(A_0 - A) / A_0] \times 100$$

Alkaline phosphatase measurement

hDPSCs were seeded at 4 × 10³ cells/well in 96-well plates and cultured to 80% confluence before exposure to nα-TCP at 0.02, 0.08, or 0.16 mg/mL or to MTA. ALP was assessed on days 3, 7, 14, and 21 using a commercial colorimetric kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. After incubation, 50 µL of stop solution was added, and absorbance was read at 405 nm using a microplate reader (Stat Fax 2100, Palm City, FL, USA). The experiment was repeated three times.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The Shapiro–Wilk test was used to assess normality where applicable. Antibacterial outcomes were analysed using one-way analysis of variance (ANOVA), and pH outcomes using two-way ANOVA. Migration and ALP analyses included treatment and time as factors. Pairwise comparisons were performed using Tukey's test, with statistical significance set at $p < 0.05$. Statistical analyses and graph preparation were performed using GraphPad Prism version 10.4.2 (GraphPad Software, Boston, MA, USA).

Manuscript preparation

ChatGPT (OpenAI) assisted with drafting and revising the manuscript text and adapting its format to the journal requirements. No experimental data were generated by this tool. The authors are responsible for verifying the scientific content, citations and final wording

Results

Antibacterial effects

Shapiro–Wilk testing did not detect departures from normality in evaluable groups ($p > 0.05$). The means were 23.50, 17.75 and 9.00 CFU/mL at 0.02, 0.08 and 0.16 mg/mL, respectively. The distilled-water

group had a mean of approximately 125.3 CFU/mL, while no colonies were recovered in the MTA group. Relative to distilled water, the nα-TCP means corresponded to approximate reductions of 81.2%, 85.8% and 92.8%. Nano α-TCP significantly reduced viable *L. acidophilus* counts at all tested concentrations compared with the distilled-water control. Colony counts decreased with increasing nα-TCP concentration, with the lowest count observed at 0.16 mg/mL, followed by 0.08 and 0.02 mg/mL. None of the nα-TCP groups differed significantly from the MTA positive control. MTA also significantly reduced bacterial counts compared with distilled water (Fig. 1).

SD from three independent replicates per group (n = 3). Asterisks indicate significant

differences from aquadest ($p < 0.05$). No significant differences were

detected between nα-TCP and MTA

Aqueous pH

Shapiro–Wilk testing did not detect departures from normality ($p > 0.05$). At time zero, mean pH values were 6.887, 7.173 and 7.573 for nα-TCP at 0.16, 0.08 and 0.02 mg/mL, respectively, compared with 12.48 for MTA (Fig. 2). At 3 h, the corresponding means were 6.920, 7.320 and 7.677, versus 12.62 for MTA. The pH of nα-TCP preparations remained near neutral to mildly alkaline, whereas MTA exhibited markedly alkaline values throughout the observation period. At 0, 3, and 24 h, all nα-TCP groups had significantly lower pH values than MTA. Within the nα-TCP groups, the lowest concentration (0.02 mg/mL) consistently showed the highest pH, followed by 0.08 and 0.16 mg/mL. Descriptively, nα-TCP pH values changed little between 0 and 3 h and declined slightly at 24 h, while MTA maintained a high pH with a small upward trend (Fig. 2).

per group (n = 3). Asterisks indicate significant differences from MTA at the corresponding

time point ($p < 0.05$).

Scratch wound closure

At 6 h, mean closure was 7.733%, 6.803% and 5.797% for nα-TCP at 0.16, 0.08 and 0.02 mg/mL, respectively. MTA and DMEM yielded 3.693% and 4.973% (Fig. 3). The 0.16 mg/mL group exceeded DMEM and MTA. The 0.08 mg/mL group exceeded MTA, but not DMEM. No difference between nα-TCP doses was detected at this time. The higher-dose separation was more pronounced at 24 h. Closure reached 23.93% at 0.16 mg/mL and 22.04% at 0.08 mg/mL, compared with 11.05% at 0.02 mg/mL, 12.32% for MTA and 8.040% for DMEM. Both higher doses exceeded MTA and DMEM, while the two higher doses did not differ from each other. The 0.02 mg/mL group exceeded DMEM but did not differ from MTA.

At 24 h, the differences relative to MTA were 11.61 percentage points (95% confidence interval 9.212–14.01) for 0.16 mg/mL and 9.723 percentage points (95% confidence interval 7.322–12.12) for 0.08 mg/mL. Repeated-measures ANOVA identified a treatment–time interaction, $F(4, 10) = 58.36$, a

treatment effect, $F(4, 10) = 100.7$, and a time effect, $F(1, 10) = 792.6$ (all $p < 0.0001$). All material groups exceeded DMEM alone at 24 h (Fig. 3)

per group ($n = 3$). Asterisks indicate significant differences from negative control (DMEM)

at the corresponding time point ($p < 0.05$).

ALP response

Mean ALP concentrations at day 3 were 3.264, 2.804 and 2.141 ng/mL for nα-TCP at 0.16, 0.08 and 0.02 mg/mL, respectively, and 1.837 ng/mL for MTA (Fig. 4). The group of 0.16 mg/mL exceeded MTA by 1.427 ng/mL. It also exceeded 0.08 mg/mL by 0.4597 ng/mL and 0.02 mg/mL by 1.123 ng/mL. The 0.08 and 0.02 mg/mL groups showed intermediate values, without significant differences from MTA indicated in the figure.

ALP profiles diverged over time. At day 7, mean concentrations ranged from 2.820 to 3.167 ng/mL across the four groups. At day 14, the 0.08 mg/mL group had the lowest mean (1.428 ng/mL), while MTA had the highest (3.107 ng/mL); none of the pairwise comparisons was significant. At day 21, means were 2.504, 2.128 and 1.822 ng/mL for 0.16, 0.08 and 0.02 mg/mL nα-TCP, respectively, versus 2.536 ng/mL for MTA. No pairwise differences were detected at days 7, 14 or 21. The ANOVA identified a treatment–time interaction, $F(9,24) = 3.594$, $p = 0.0058$, and a corrected time effect, $F(2.317,18.54) = 4.470$, $p = 0.0220$ ($\varepsilon = 0.7725$). The overall treatment effect was not significant, $F(3,8) = 1.868$, $p = 0.2133$. These results indicate a day-specific early separation rather than sustained superiority over MTA.

Discussion

The present study identifies a specific combination of antibacterial and cellular responses to nano-α-tricalcium phosphate (nα-TCP): reduced recovery of *L. acidophilus*, enhanced scratch wound closure at the two higher concentrations, and an early ALP response that was not sustained relative to mineral trioxide aggregate (MTA). These responses occurred alongside a substantially lower aqueous pH than that of MTA. The findings support further investigation of nα-TCP as a component of experimental pulp-capping materials, but they do not establish dentin regeneration or clinical efficacy. This distinction matters because successful vital pulp therapy depends on the condition of the residual pulp, infection control and the integrity of the restoration, rather than on a single laboratory property of the capping material [1, 3].

The combined assays describe complementary material responses, but aqueous pH does not necessarily reflect conditions in buffered cell culture medium. Christie et al. identified substantial variation in formulations and experimental methods across calcium phosphate and silicate studies, limiting comparisons between material classes [5]. The present findings therefore apply to the preparations and exposure conditions tested.

Antibacterial effects and comparison with MTA

All nα-TCP concentrations reduced culturable *L. acidophilus* relative to distilled water. The reported reductions ranged from approximately 81.2% at 0.02 mg/mL to 92.8% at 0.16 mg/mL, with progressively lower mean colony counts as concentration increased. This numerical pattern supports a concentration-related antibacterial response within the tested range, although the comparison against the negative control should be distinguished from formal evidence of differences between nα-TCP concentrations. The effect is relevant to an initial evaluation of materials intended for use near carious dentin, where reducing the microbial challenge is a necessary part of preserving pulp vitality [1].

MTA yielded no recovered colonies, whereas the nα-TCP groups retained measurable colony counts. Although no significant differences between nα-TCP and MTA were reported, the absence of statistical significance should not be interpreted as equivalent antibacterial efficacy. An equivalence claim would require a prespecified acceptable difference and sufficient precision to exclude clinically important departures from that margin. Moreover, zero recovered colonies means that bacteria were not detected under the specified plating conditions; it does not prove sterilization or exclude organisms below the detection limit. Accordingly, the results support antibacterial effects of nα-TCP, while preserving the descriptive distinction between reduced recovery with nα-TCP and undetected recovery with MTA.

The comparison with our previous physicochemical study is informative but formulation-specific. Wulansari et al. evaluated nα-TCP–AgNPs resin biocomposites and reported antibacterial activity against both *S. mutans* and *L. acidophilus*, together with calcium and phosphate release and an alkaline pH profile [11]. The 1% AgNPs formulation gave the most favourable results in that system. The present study shows reduced *L. acidophilus* recovery without deliberately incorporating silver into the tested preparations. It therefore extends the experimental questions surrounding nα-TCP, but does not quantify the contribution of α-TCP to the earlier composite response. Silver loading, resin chemistry, specimen preparation and exposure conditions all differ, precluding a direct ranking of antibacterial potency across the two studies.

Aqueous pH and material mechanisms

The pH results provide an important qualification to the antibacterial findings. At baseline, nα-TCP values ranged from 6.887 to 7.573, compared with 12.48 for MTA; all nα-TCP groups remained significantly lower than MTA at the reported observation times. Thus, antibacterial effects occurred in a preparation whose separately measured aqueous pH was much closer to neutral. This combination does not support attributing the nα-TCP antibacterial response solely to strong alkalinization. However, pH was measured in deionized water rather than in the bacterial exposure suspension, so a direct relationship between pH and bacterial survival was not demonstrated.

Several mechanisms could be investigated to explain the response to nα-TCP. Changes in dissolved calcium and phosphate, particle contact with bacteria, and medium-dependent surface transformations are plausible candidates; none was measured here. In their study of Biodentine and ProRoot white MTA, Rathinam et al. linked extracellular calcium, intracellular calcium dynamics and subsequent hDPSC differentiation and mineralization, using calcium chelation to investigate the relationship [12]. Their

findings provide a mechanistic rationale for studying ion-mediated responses, but they do not demonstrate that the same pathway operates in nα-TCP. Ion-release measurements and experiments that independently vary pH and dissolved ions would be required before assigning the present findings to calcium signalling.

Scratch wound closure

The clearest cellular effect was enhanced scratch wound closure at 24 h. Closure reached 23.93% and 22.04% with nα-TCP at 0.16 and 0.08 mg/mL, respectively, compared with 12.32% for MTA and 8.040% for DMEM. The two higher concentrations exceeded both comparators, while the lowest concentration exceeded DMEM but did not differ from MTA. The reported differences relative to MTA were 11.61 and 9.723 percentage points for the two higher concentrations. These estimates describe a substantial separation within this assay without implying that a corresponding benefit would occur in an exposed pulp.

Peng et al. reported that calcium silicate extracts promoted human dental pulp cell migration, alongside odontogenic gene and protein responses [13]. The direction of the migration-associated response agrees with the present increase in scratch closure, supporting the relevance of examining pulp-cell behaviour after biomaterial exposure. Their extract chemistry included increased silicon, however, and their cells and exposure dilutions differed from ours. Their study cannot establish a calcium-specific mechanism for nα-TCP or provide a numerical benchmark for the closure percentages reported here. The useful comparison is that repair-associated cell behaviour can respond to the chemical environment generated by different bioceramics, while the active constituents require material-specific investigation.

Scratch closure must also be distinguished from migration alone. The method measures the disappearance of an initially cell-free area, which can reflect movement, proliferation and differences in survival or spreading. The manuscript does not describe suppression or independent measurement of proliferation during the assay. Balko et al. showed that scratch width, cell density and culture conditions can materially affect closure measurements and emphasized standardization [14]. Consequently, the present endpoint supports enhanced collective gap closure rather than isolated chemotaxis or increased migration velocity. Time-lapse tracking, proliferation measurements and an independently validated migration assay would help identify the process responsible for the observed effect.

Early ALP response

The ALP findings show an early separation followed by convergence, rather than sustained superiority over MTA. At day 3, 0.16 mg/mL produced the highest measured value and exceeded MTA and the two lower nα-TCP concentrations. No pairwise differences were detected at days 7, 14 or 21, and the overall treatment effect was not significant. The significant treatment–time interaction therefore carries more interpretive weight than a general ranking of materials: the effect of treatment depended on when it was assessed. It would be inappropriate to summarize these data as a persistent increase in ALP throughout the 21-day period.

Basheer et al. provide a relevant calcium phosphate comparison. Their strontium-substituted tetracalcium phosphate cement produced higher ALP values at days 7 and 14 and showed mineralization-related responses alongside MTA [15]. Our early day-3 separation differs from that later pattern. The discrepancy may reflect phosphate phase, strontium incorporation, cement formulation or exposure conditions, but the present experiment cannot distinguish among these possibilities. Importantly, their evaluation also included Alizarin Red and osteopontin, allowing ALP to be interpreted with additional endpoints. The comparison illustrates why an early ALP response in an unmodified α-TCP preparation should not be equated with the differentiation behaviour of a substituted calcium phosphate cement.

Gu et al. evaluated a biphasic calcium phosphate cement containing β-TCP and reported increased ALP activity, mineral deposition and DMP1 and DSPP gene and protein expression with 20% TCP incorporation compared with conventional calcium phosphate cement [16]. Their study also examined macrophage responses. This provides a closer biological framework for investigating calcium phosphate materials, while differing from the current powder preparations in composition and physical form. The multiple concordant endpoints in that study support a stronger interpretation of odontogenic potential than ALP alone. Here, the evidence remains an early ALP-associated response, with no direct measurement of matrix mineralization or a dentin-specific differentiation programme.

Our previous hDPSC study further reinforces this distinction. Wulansari et al. investigated nα-TCP/AgNPs resin composites using viability measurements, DSPP and DMP-1 proteins, and Alizarin Red staining; mineral deposits increased between days 14 and 21 [7]. These outcomes cannot be transferred directly to the present nα-TCP preparations because the silver component and resin matrix alter the exposure. Nevertheless, the earlier study provides an appropriate set of complementary endpoints for the next stage of investigation. The current study contributes a concentration- and time-resolved ALP profile and short-term gap closure data, while the earlier work supplies formulation-specific evidence of mineralization-related activity.

Study limitations

Several limitations should be considered when interpreting these findings. The in vitro design does not reproduce the vascular, immune, and inflammatory conditions of the dentin–pulp complex. The limited replication and potential donor variability also constrain generalisability. Antibacterial testing involved a single *L. acidophilus* strain under direct-contact conditions, which cannot represent the complexity of multispecies dentinal biofilms or establish long-term antimicrobial effectiveness. Furthermore, scratch wound closure may reflect both migration and proliferation, as these processes were not assessed independently. The pH measured in deionized water may differ from that in buffered culture medium or at the clinical material–tissue interface. Finally, sealing ability, mechanical properties, and long-term stability of a final pulp-capping formulation were not evaluated. Further studies using independent donors, dentin-associated biofilms, and tissue models are needed before clinical application can be considered.

Conclusion

Nano α-TCP reduced *L. acidophilus* recovery and enhanced hDPSC scratch wound closure, particularly at 0.08 and 0.16 mg/mL, while exhibiting a lower aqueous pH than MTA. The ALP response was limited to an early increase at 0.16 mg/mL, without sustained superiority over MTA. These findings support further investigation of nα-TCP for pulp-capping applications, although its odontogenic potential and clinical efficacy remain to be established.

Declarations

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Competing interests

The authors declare that they have no financial or non-financial competing interests relevant to the content of this manuscript.

Ethics approval

Ethical committee approval was received from the Ethics Committee of the Universitas Trisakti (Approval no: 894/S2-Sp/KEPK/FKG/5/2025 Date: 21 May 2025).

Consent to participate

Informed consent was not required because this study was conducted entirely in vitro and did not involve human participants.

Author contributions

Concept – SW., MOR.; Design – SW., MOR.; Supervision – AC., MOR.; Resources – S.W., JDT., NLR.; Materials – SW., JDT., NLR.; Data Collection and/or Processing – SW., JDT., NLR.; Analysis and/or Interpretation– MOR., SW., AC.; Literature Search – SW., JDT., NLR.; Writing – MOR., SW.; Critical Review – AC., MOR.

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Figures

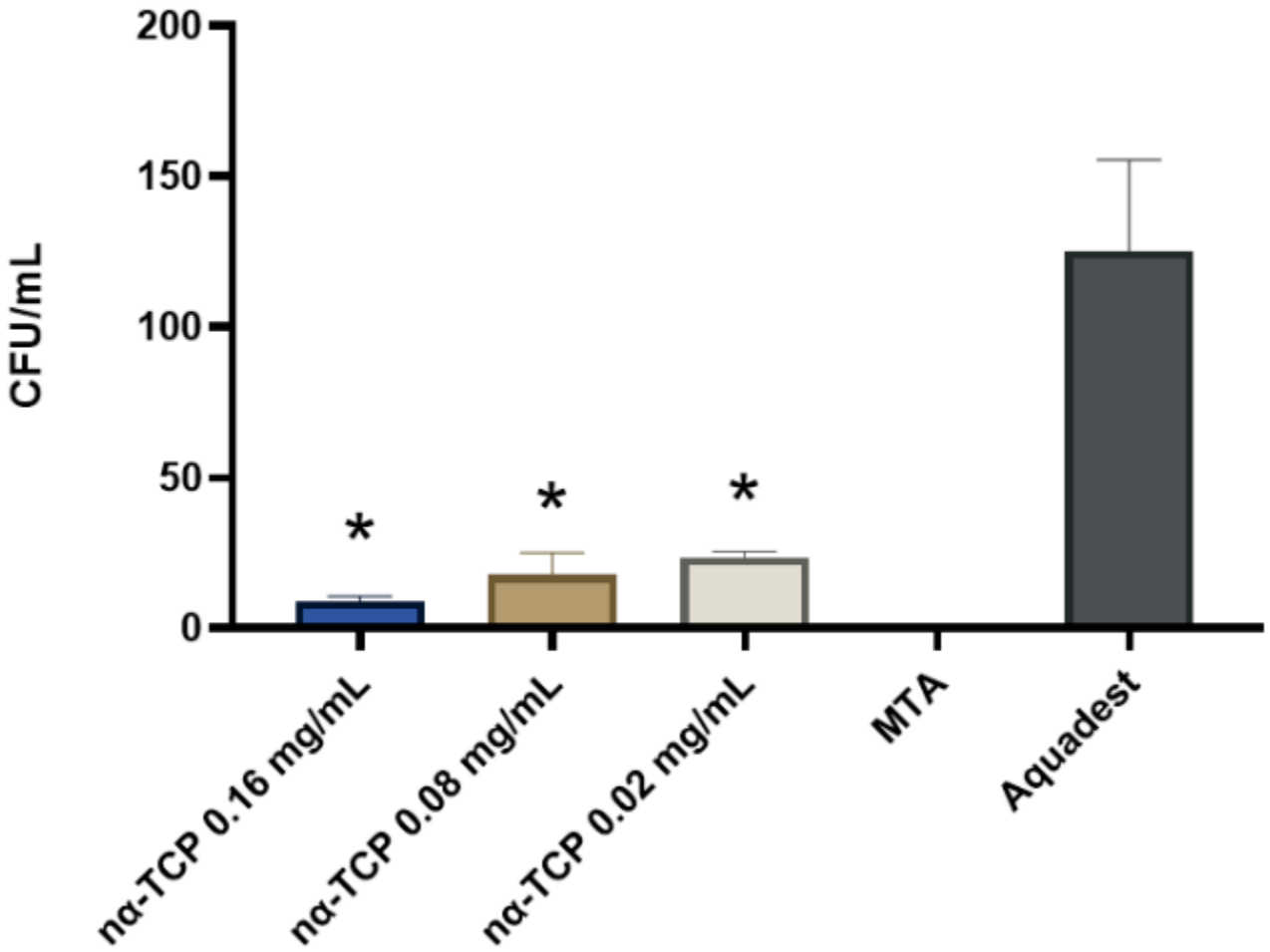


Figure 1

Viable Lactobacillus acidophilus counts after 24-h exposure to nα-TCP at 0.16, 0.08, or 0.02 mg/mL, mineral trioxide aggregate (MTA), or distilled water. Bars represent means and error bars represent SD from three independent replicates per group (n = 3). Asterisks indicate significant differences from aquadest ($p < 0.05$). No significant differences were detected between nα-TCP and MTA

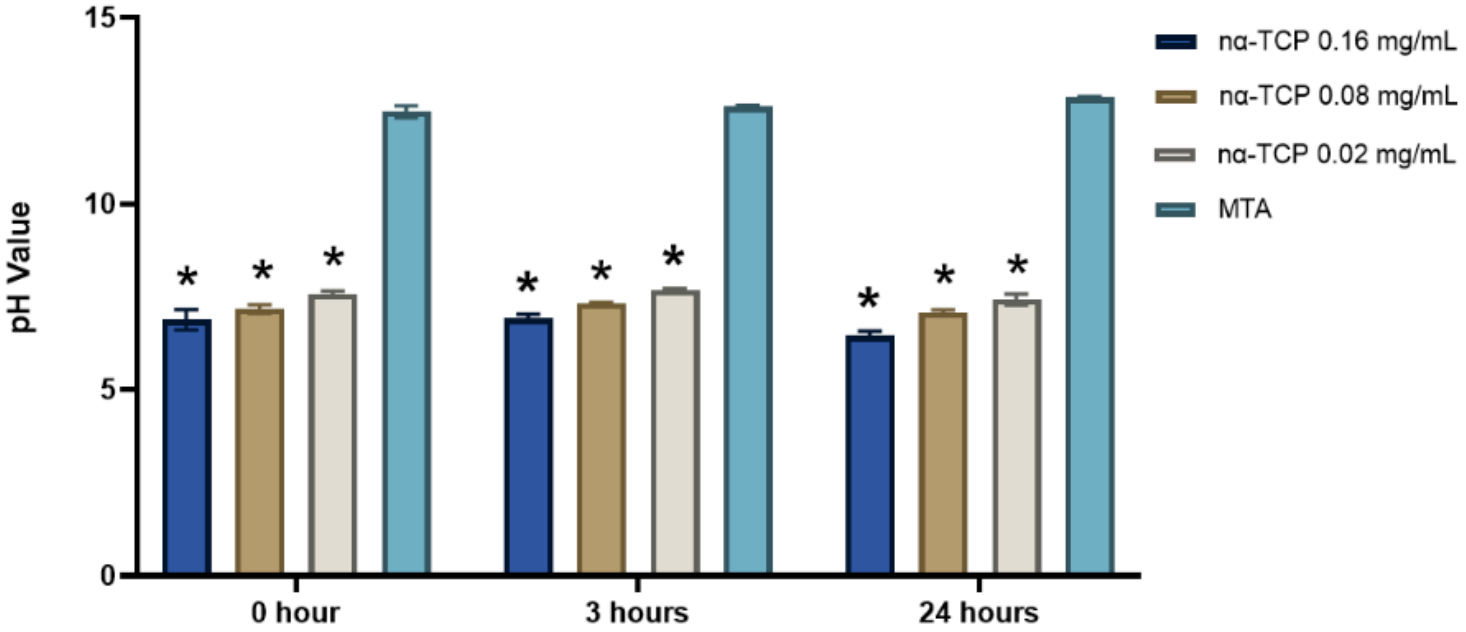


Figure 2

Aqueous pH of nα-TCP preparations and MTA at 0, 3, and 24 h. Nano-α-TCP concentrations were 0.16, 0.08, and 0.02 mg/mL. Bars represent means and error bars represent SD from three independent replicates per group (n = 3). Asterisks indicate significant differences from MTA at the corresponding time point ($p < 0.05$).

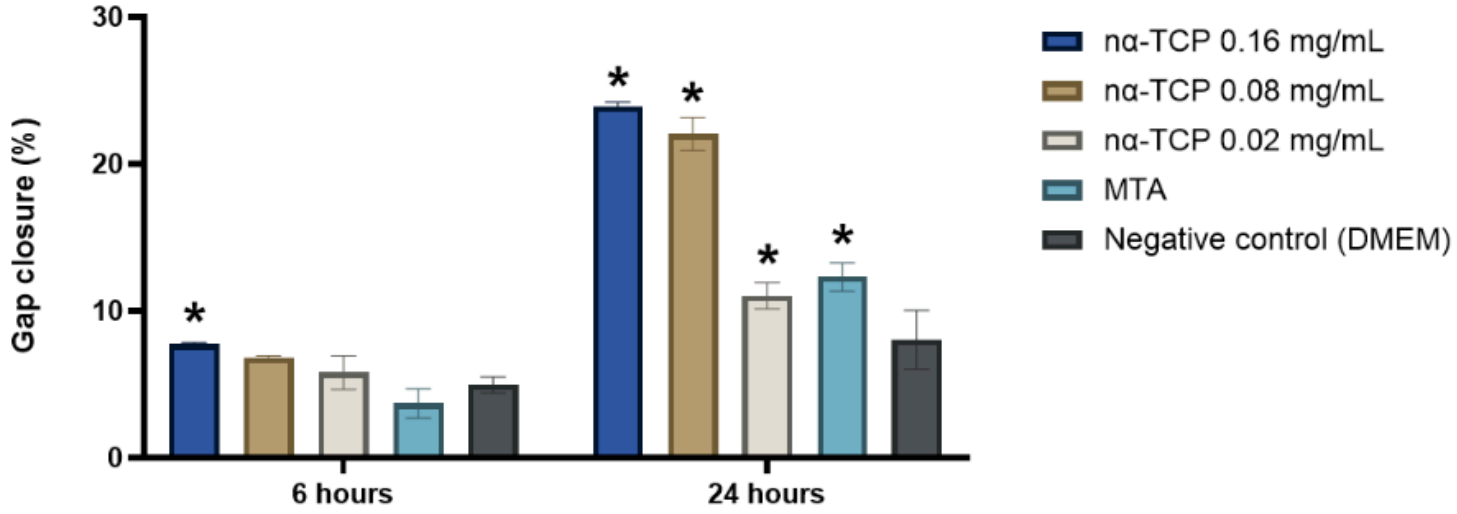


Figure 3

Scratch wound closure in hDPSCs exposed to nα-TCP at 0.16, 0.08, or 0.02 mg/mL, MTA, or DMEM alone at 6 and 24 h. Bars represent means and error bars represent SD from three independent replicates per group (n = 3). Asterisks indicate significant differences from negative control (DMEM) at the corresponding time point ($p < 0.05$).

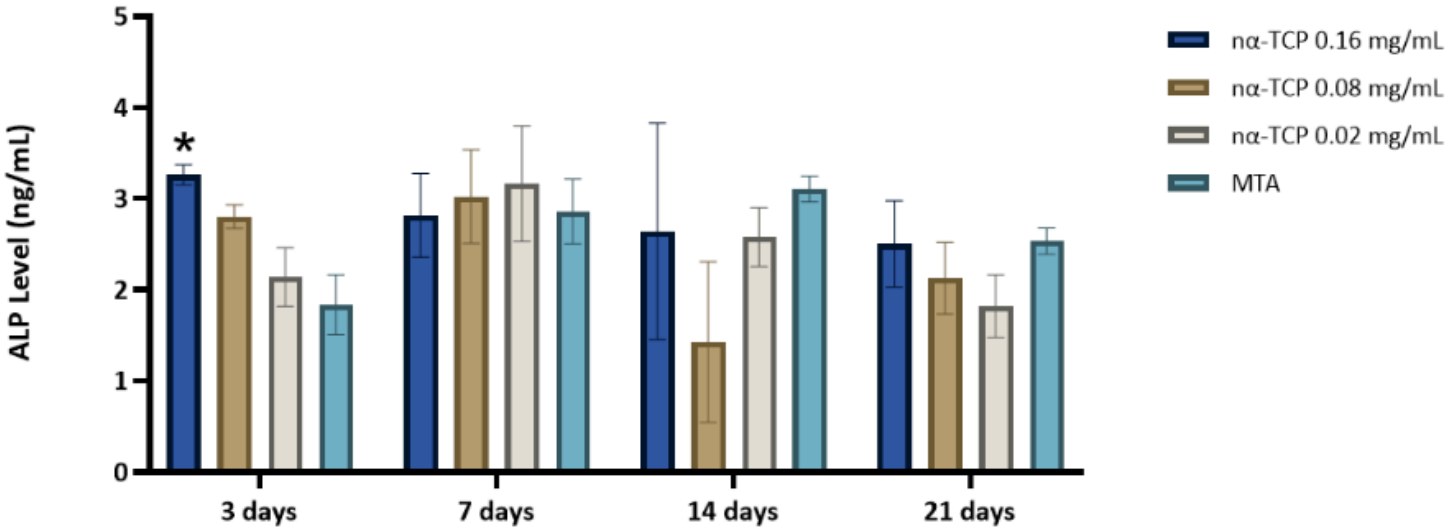


Figure 4

ALP measurements in hDPSCs exposed to nα-TCP at 0.16, 0.08, or 0.02 mg/mL or MTA on days 3, 7, 14, and 21. Bars represent means and error bars represent SD from three independent replicates per group (n = 3). The asterisk indicates a significant difference from MTA at the corresponding time point ($p < 0.05$).