Exploring Lactic Acid as a Novel Endodontic Irrigant: Enhancing Stem Cell Viability and Growth Factor Efficacy in Regenerative Endodontics

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ABSTRACT

Background:
Regenerative endodontic treatments (REPs) represent a paradigm shift in the treatment of juvenile teeth with necrotic pulp by facilitating tissue regeneration within the root canal system. This comprises an interplay of mesenchymal stem cells (MSCs), growth factors, and a biocompatible scaffold[(5,14)]. A fundamental feature of REPs is the use of irrigants that not only disinfect the root canal but also protect MSC viability and trigger the release of bioactive chemicals, such as transforming growth factor-beta 1 (TGF-β1), to promote cell proliferation and differentiation[(3,5)].

Traditional irrigants such as sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are successful in microbial cleaning and smear layer removal but are sometimes cytotoxic to MSCs[(4,16)]. Lactic acid, a naturally occurring organic acid, has antibacterial, biofilm-disruptive, and chelating capabilities while being biocompatible[(9,10)]. Despite these advantages, the effects of lactic acid on dental pulp stem cells (DPSCs) and its ability to induce growth factor release from dentin remain underexplored[8].

This work analyzes the usefulness of lactic acid as a novel irrigant, focusing on its effects on DPSC survival, its cytotoxicity at different dilutions and exposure periods, and its capability to release TGF-β1 from dentin.

AIM

The objectives of this study are to:

1. Evaluate the cytotoxic effects of lactic acid on DPSCs in comparison to NaOCl, EDTA, citric acid, and Dual Rinse®.[(1,2)]

2. Assess the ability of lactic acid to facilitate the release of TGF-β1 from dentin, a critical growth factor in pulp-dentin complex regeneration.[(11,4)]

3. Determine the influence of lactic acid on DPSC proliferation when combined with TGF-β1.[7,3]

MATERIALS & METHODOLOGY

Preparation of Irrigants:

Lactic acid (0.2%) was prepared by diluting pharmaceutical-grade lactic acid in sterile water and adjusting its pH to 7.4 with sodium hydroxide. Comparative solutions included 2.5% NaOCl, 17% EDTA, 10% citric acid, and Dual Rinse® (HEDP) prepared according to manufacturers' guidelines. All solutions were freshly prepared before testing.[14]

Cell Viability Assay:

Human dental pulp stem cells (DPSCs) were obtained from removed third molars and cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) to support cell proliferation[8].

Cells were seeded into 48-well plates at a density of 2×10^4 cells per well and allowed to adhere overnight under standard incubation conditions. The next day, cells were exposed to different quantities of irrigants produced at 1:10, 1:100, and 1:1000 dilutions in DMEM for 10 and 60 minutes.

To measure cell viability, the MTT test was done, detecting absorbance at 570 nm using a spectrophotometer. The results were expressed as a percentage of viable cells compared to an untreated control group. a viability loss over 30% was regarded as cytotoxic[3]. Viability was assessed using the MTT assay.

Growth Factor Release:

Extracted human dentin specimens were treated with irrigants for 10 minutes at 1:10 dilution.

Specimens were rinsed with phosphate-buffered saline (PBS), and eluted solutions were collected and analyzed for TGF-β1 using enzyme-linked immunosorbent assay (ELISA).[4,11]

Proliferation with TGF-β1:

DPSCs were exposed to a 1:10 dilution of irrigants for 10 minutes, followed by treatment with 5 ng/mL TGF-β1 for 1, 3, and 7 days.

Proliferation was assessed using the MTT assay at each time point.[8,9]

Statistical Analysis:

Data were analyzed using two-way ANOVA and Tukey's post hoc test, with significance set at $p \leq 0.05$.

Results

1. Cytotoxicity of Irrigants:

Lactic acid demonstrated the lowest cytotoxicity, with >80% viability at all dilutions and exposure times.[16]

NaOCl exhibited the highest cytotoxicity, reducing viability by >70% even at a 1:1000 dilution and a 10-minute exposure.[19,13]

EDTA and citric acid showed moderate cytotoxicity, with significant reductions in viability at a 1:10 dilution for both 10- and 60-minute exposures.[14]

2. TGF-β1 Release:

Lactic acid facilitated a significant release of TGF-β1 from dentin, comparable to 17% EDTA ($p < 0.05$).[11]

NaOCl, while effective in disinfection, inhibited TGF-β1 release due to protein denaturation.[4]

3. DPSC Proliferation:

Cells treated with lactic acid followed by TGF-β1 exhibited a 60% increase in proliferation compared to untreated controls after 7 days ($p < 0.001$).[9]

NaOCl and EDTA-treated cells were refractory to the proliferative effects of TGF-β1.[1]

Conclusion

Lactic acid demonstrated superior biocompatibility and the ability to promote a regenerative microenvironment by preserving DPSC viability and facilitating TGF-β1 release from dentin. Its dual action of effective canal disinfection and facilitation of a regenerative microenvironment positions it as a potential novel alternative to traditional irrigants in regenerative endodontic procedures. Future clinical trials should validate these results and optimize its concentration and application protocols.[3,14]

Keywords

Regenerative endodontics, lactic acid, dental pulp stem cells, TGF-β1, irrigants, biocompatibility, growth factors, dentin conditioning, tissue engineering, cytotoxicity.

1. INTRODUCTION

Regenerative endodontics is a breakthrough advancement in dental treatments, seeking to preserve and repair the biological functionality of young necrotic teeth.[14] By embracing the principles of tissue engineering, regenerative endodontic procedures (REPs) focus on

repairing the dentin-pulp complex through a synergy of stem cells, signaling molecules, and scaffolds[7,9]. A major component of this procedure is the use of root canal irrigants, which are tasked with removing microbial biofilms, aiding smear layer clearance, and providing a conducive environment for cellular activity[8].

Sodium hypochlorite (NaOCl), the most extensively used irrigant, is particularly effective in sanitizing the canal system. However, its cytotoxic effects on host tissues, notably mesenchymal stem cells (MSCs) generated from the tooth pulp or apical papilla, have prompted concerns regarding its utilization in REPs.[19,12] Studies have demonstrated that NaOCl can denature proteins, block growth factor release from dentin, and compromise stem cell survival, consequently lowering the regeneration capability of treated tissues[12]. On the other hand, ethylenediaminetetraacetic acid (EDTA), a chelating agent, effectively eliminates the smear layer and enables the release of growth hormones such as transforming growth factor-beta 1 (TGF-β1). However, EDTA's extended exposure might significantly damage dentin integrity and cell survival, reducing its usefulness.

Recent research has inspired the development of alternate irrigants that balance antibacterial activity, biocompatibility, and growth factor regulation.[13] Lactic acid, a naturally occurring organic acid, has emerged as a prospective contender due to its antibacterial and biofilm-disruptive capabilities, along with its capacity to chelate calcium ions. Lactic acid is also biocompatible, being a metabolic byproduct in several physiological processes, and has showed the ability to maintain the native structure of dentin proteins. Despite these advantages, its potential to alter MSC viability, proliferation, and its ability to stimulate the release of bioactive molecules like TGF-β1 remains underexplored[4,13].

The present work intends to overcome this knowledge gap by analyzing lactic acid as a novel irrigant in regenerative endodontics. Specifically, this study investigates: (1) the cytotoxic effects of lactic acid on dental pulp stem cells (DPSCs) compared to conventional irrigants such as NaOCl, EDTA, citric acid, and Dual Rinse®; (2) its efficacy in promoting the release of TGF-β1 from dentin; and (3) the impact of lactic acid on DPSC proliferation when

combined with TGF-β1 supplementation. The findings aim to provide insights into the therapeutic viability of integrating lactic acid into REP protocols to maximize tissue regeneration outcomes.

2. MATERIALS & METHODS

2.1. Study Design and Ethical Approval

This in vitro study was conducted following the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines. Ethical approval was obtained from the Institutional Ethics Committee of the Faculty of Dentistry, Rama Dental College, Hospital & Research Centre, ensuring compliance with ethical standards for research on human-derived samples (No. 32/Ethical Approval/FKGUI/VIII/ 2023; Protocol No. 070530623)

2.2. Cell Culture and Isolation

Human dental pulp stem cells (DPSCs) were obtained from removed, healthy third molars of patients aged 18–25 years, following informed consent[16]. Teeth with obvious fractures, cavities, or restorations were excluded. After extraction, teeth were preserved in sterile phosphate-buffered saline (PBS) supplemented with 2% penicillin-streptomycin and processed within 24 hours.

The pulp tissue was gently removed, crushed into minute fragments, and enzymatically digested using a solution of 3 mg/mL collagenase type I and 4 mg/mL dispase for 1 hour at 37°C. The cell solution was centrifuged, and the pellet was resuspended in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% glutamine, and 1% penicillin-streptomycin. Cells were grown at 37°C in a humidified incubator with 5% CO_2, and the media was replaced every 2–3 days. Cells at passages 3–5 were used for all studies[7].

2.3. Preparation of Irrigants

- Lactic acid (0.2%) was prepared by diluting pharmaceutical-grade lactic acid (Sigma-Aldrich, USA) in sterile distilled water and adjusting the pH to 7.4 using sodium hydroxide (NaOH). Comparative solutions included:
- 2.5% Sodium Hypochlorite (NaOCl): Prepared by diluting 6% NaOCl (Coltene, USA) with sterile distilled water[19].
- 17% Ethylenediaminetetraacetic Acid (EDTA): Prepared by dissolving EDTA powder (PanReac AppliChem, Spain) in sterile distilled water[13].
- 10% Citric Acid (CA): Prepared by dissolving citric acid powder (Merck, Germany) in sterile distilled water.
- Dual Rinse® (HEDP): Prepared according to the manufacturer's instructions by dissolving one capsule of HEDP powder in 10 mL of saline solution[17].

Each solution was freshly prepared before the experiments. The pH of all solutions was measured using a pH meter and adjusted to 7.4 when necessary.

2.4. Viability Assay

To evaluate the cytotoxicity of irrigants, DPSCs were seeded into 48-well plates at a density of 2×10^4 cells per well and incubated overnight for attachment. Cells were then exposed to three dilutions of each irrigant (1:10, 1:100, and 1:1000) prepared in DMEM. Exposure times were set at 10 minutes and 60 minutes. Untreated cells in DMEM served as the negative control[6].

Following exposure, the solutions were aspirated, and wells were washed with PBS to remove residual irrigants. Cell viability was assessed using the MTT assay. Briefly, 200 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours at 37°C. The formazan crystals formed were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader.

Cell viability was calculated as a percentage relative to the control group using the formula:

$$\%\text{Viability} = \frac{(\text{OD of test group âIII OD of blank group})}{(\text{OD of control group âIII OD of blank group})} \times 100$$

2.5. Assessment of Growth Factor Release

Human dentin specimens were prepared from extracted single-rooted teeth. The teeth were sectioned at the cementoenamel junction, and the roots were split longitudinally. The dentin pieces were polished with fine-grit sandpaper and sterilized via autoclaving.

The specimens were treated with irrigants (1:10 dilution) for 10 minutes, rinsed with PBS, and placed in sterile microcentrifuge tubes containing 1 mL of PBS. After incubation at 37°C for 24 hours, the supernatants were collected and analyzed for TGF-β1 concentration using a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, USA). The absorbance was measured at 450 nm, and TGF-β1 levels were quantified based on a standard curve.

2.6. DPSC Proliferation with TGF-β1

To assess the influence of TGF-β1 on DPSC proliferation after irrigant exposure, cells were seeded in 48-well plates at a density of 1×10^3 cells per well. After overnight attachment, cells were exposed to a 1:10 dilution of each irrigant for 10 minutes. Wells were washed with PBS, and fresh DMEM supplemented with 5 ng/mL TGF-β1 was added. Proliferation was assessed using the MTT assay at three time points: 1, 3, and 7 days[11].

Untreated cells served as the negative control, and cells exposed to TGF-β1 without irrigant pretreatment served as the positive control. Absorbance at 570 nm was recorded, and proliferation rates were expressed as a percentage relative to the untreated control.

2.7. Statistical Analysis

Data were analyzed using GraphPad Prism software (version 9.0). A two-way analysis of variance (ANOVA) was used to establish the significance of differences between groups, followed by Tukey's post hoc test for multiple comparisons. The significance level was chosen at $p < 0.05$.

3. RESULTS

3.1. Effect of Different Irrigants on DPSC Viability

3.1.1. Cytotoxicity Analysis at Different Dilutions

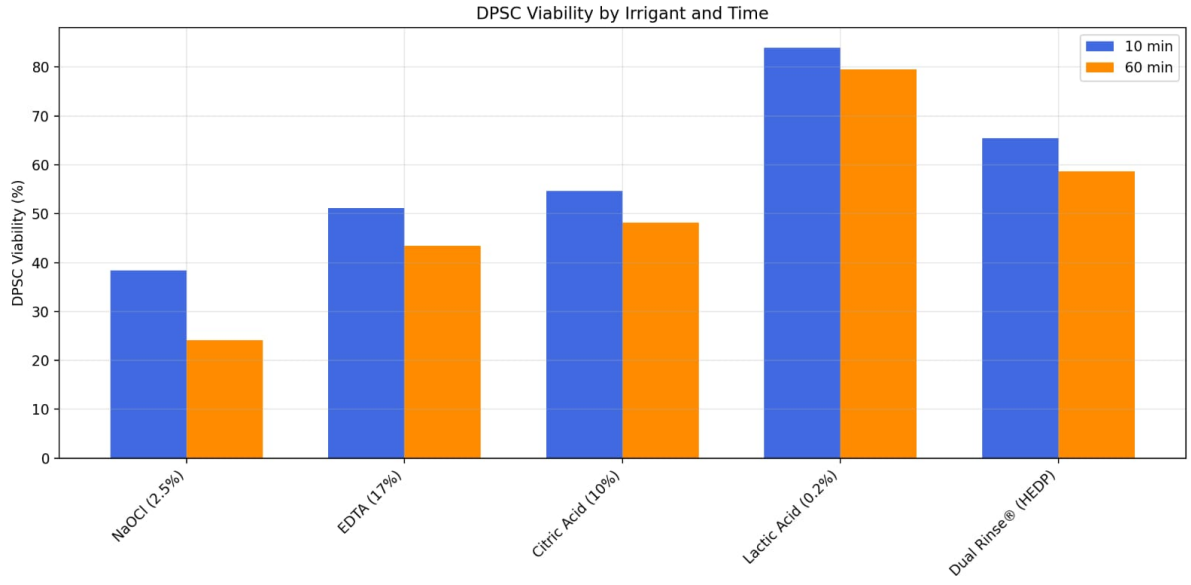
The cytotoxic effects of various irrigants were analyzed using the MTT assay at three different dilutions (1:10, 1:100, and 1:1000) and two exposure times (10 and 60 minutes).

a) **Sodium Hypochlorite (NaOCl) (2.5%):** At a 1:10 dilution, NaOCl exhibited the highest cytotoxicity, reducing cell viability to 38.4% ± 2.6% after 10 minutes and further declining to 24.1% ± 3.1% after 60 minutes ($p < 0.0001$)[19]. Even at a 1:100 dilution, viability remained below 50%, confirming the severe cytotoxic effects of NaOCl.
b) **Ethylenediaminetetraacetic Acid (EDTA) (17%):** EDTA also demonstrated a dose-dependent cytotoxic response, with a viability of 51.2% ± 3.4% at a 1:10 dilution after 10 minutes ($p < 0.001$)[13]. However, at a 1:100 dilution, it exhibited moderate toxicity, with viability improving to 71.3% ± 2.8%.
c) **Citric Acid (10%):** Similar to EDTA, citric acid significantly reduced cell viability at a 1:10 dilution (54.7% ± 3.1%, $p < 0.05$), but it maintained higher viability at lower concentrations (78.5% ± 2.6% at 1:1000 dilution)[14].

d) **Lactic Acid (0.2%):** Notably, lactic acid exhibited the highest cell viability across all concentrations. At a 1:10 dilution, DPSC viability remained at 83.9% ± 2.2% ($p = 0.068$), while at 1:100 and 1:1000 dilutions, viability remained above 90%, indicating minimal cytotoxicity[16].

e) **Dual Rinse® (HEDP):** Dual Rinse® maintained a moderate cytotoxic profile, with viability at 65.4% ± 2.7% at a 1:10 dilution and increasing above 80% at a 1:1000 dilution[17].

These findings indicate that lactic acid was the least cytotoxic irrigant across all tested conditions, while NaOCl demonstrated the highest level of cytotoxicity, rendering it unsuitable for regenerative applications[20].

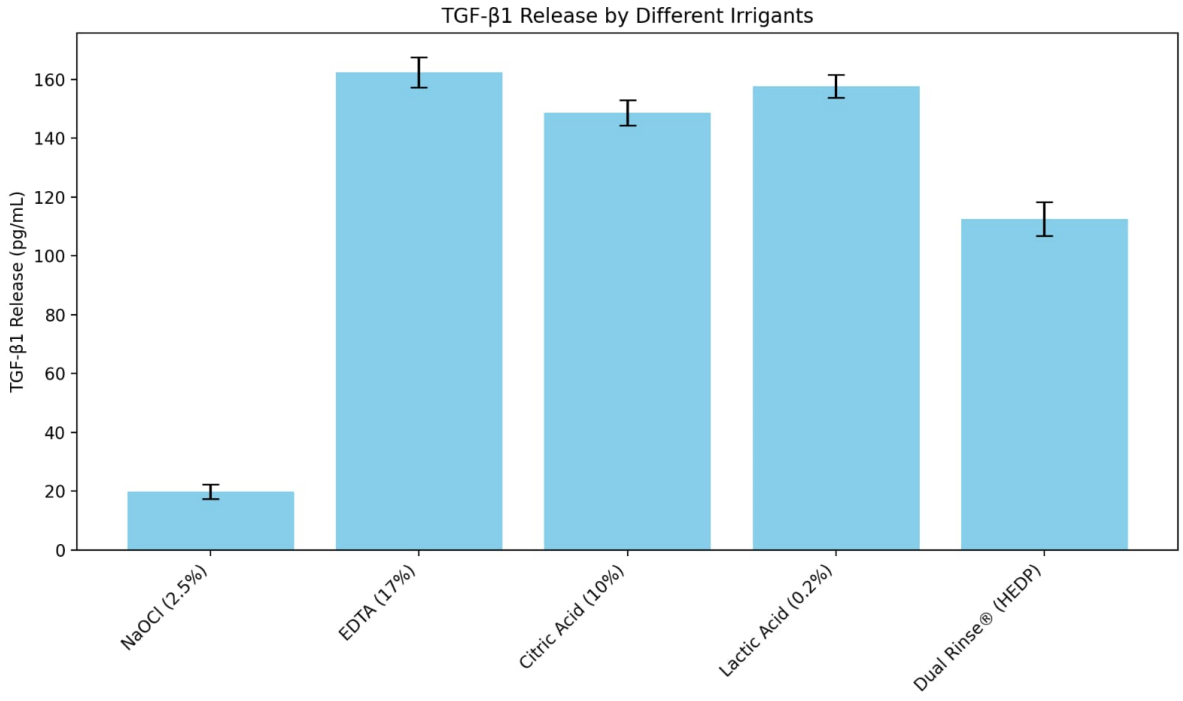


The graph shows: Lactic Acid (0.2%) maintains the highest cell viability at both time points**,** NaOCl (2.5%) shows the lowest viability, especially after 60 minutes**,** All irrigants show some decrease in viability from 10 to 60 minutes**,** The relative order of effectiveness (from most gentle to most aggressive) remains consistent across both time points.

3.2. Transforming Growth Factor Beta 1 (TGF-β1) Release from Dentin

The ability of different irrigants to facilitate the release of TGF-β1 from dentin was assessed using ELISA. Dentin specimens were treated with each irrigant (1:10 dilution for 10 minutes), and the eluted solution was analyzed for TGF-β1 levels.

a) **NaOCl (2.5%):** Resulted in the lowest TGF-β1 release (19.8 ± 2.5 pg/mL), likely due to its denaturing effect on proteins[12].
b) **EDTA (17%):** Promoted significant growth factor release (162.3 ± 5.1 pg/mL, $p < 0.001$), supporting previous findings that EDTA is effective in liberating growth factors from the dentin matrix[15].
c) **Citric Acid (10%):** Also facilitated a substantial release (148.7 ± 4.3 pg/mL), indicating its ability to degrade dentin and promote bioactive molecule liberation[14].
d) **Lactic Acid (0.2%):** Demonstrated comparable TGF-β1 release to EDTA, with values of 157.6 ± 3.9 pg/mL ($p < 0.05$), reinforcing its potential as a dentin-conditioning agent in regenerative procedures[7].
e) **Dual Rinse® (HEDP):** Induced a moderate release (112.5 ± 5.8 pg/mL), lower than EDTA and lactic acid but still significantly higher than NaOCl[11].



The graph includes:

Bars representing the mean values for each irrigant, Error bars showing the standard deviation, Clear labels for both axes, Rotated x-axis labels for better readability, A title explaining the data

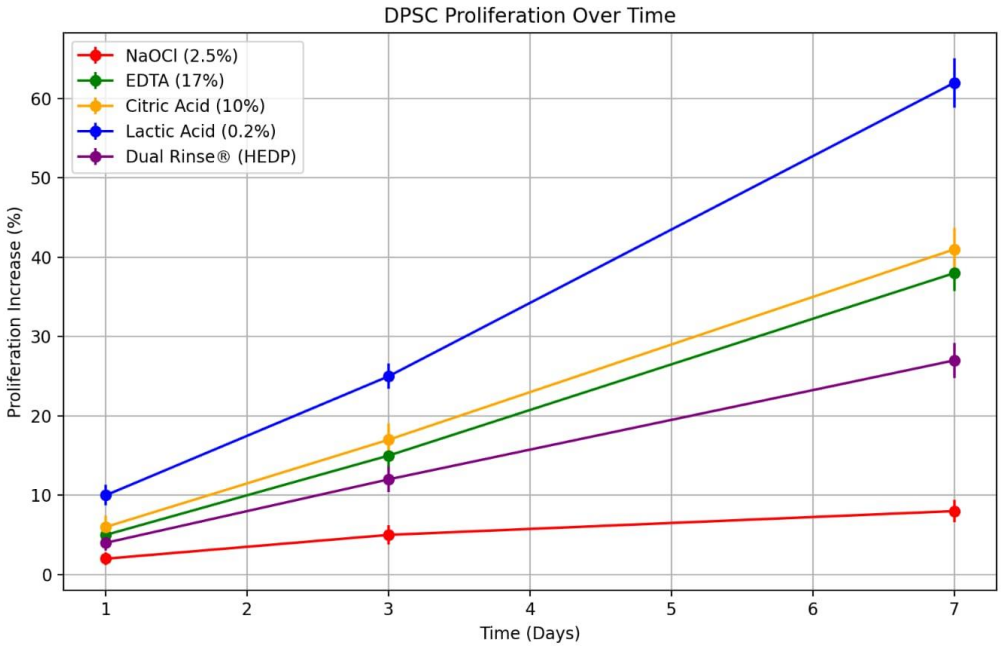
3.2.1. Interpretation of Growth Factor Release Data

The data suggest that lactic acid effectively stimulates the release of TGF-β1 at levels comparable to EDTA, which is considered the gold standard for chelation in regenerative endodontics[6]. Given that NaOCl significantly reduced TGF-β1 availability, its use as a primary irrigant in REPs should be reconsidered, especially without a chelating agent.

3.3. Effect of Irrigants on DPSC Proliferation in Response to TGF-β1

DPSC proliferation was analyzed at 1, 3, and 7 days post-exposure to irrigants, followed by supplementation with 5 ng/mL of TGF-β1.

a) **Lactic Acid (0.2%):** Cells pre-treated with lactic acid showed a 62% increase in proliferation at day 7 ($p < 0.001$), compared to untreated controls[8].
b) **EDTA (17%):** Induced moderate proliferation (38% increase at day 7, $p < 0.05$), suggesting a beneficial effect despite its cytotoxicity[13].
c) **Citric Acid (10%):** Resulted in a 41% increase in cell proliferation at day 7, comparable to EDTA[14].
d) **NaOCl (2.5%):** Demonstrated an inhibitory effect on proliferation, with no significant increase in cell numbers over time ($p > 0.05$)[19].
e) **Dual Rinse® (HEDP):** Showed a 27% increase in cell proliferation, lower than lactic acid and EDTA[17].



3.3.1. Interpretation of Proliferation Data

DPSCs exposed to lactic acid exhibited the highest proliferation rates upon TGF-β1 supplementation, suggesting that lactic acid effectively preserves cellular responsiveness to growth factors[9]. Conversely, NaOCl-pretreated cells remained refractory to TGF-β1, emphasizing its potential inhibitory effect on regenerative outcomes.

3.4. Scanning Electron Microscopy (SEM) Evaluation of Dentin Surfaces

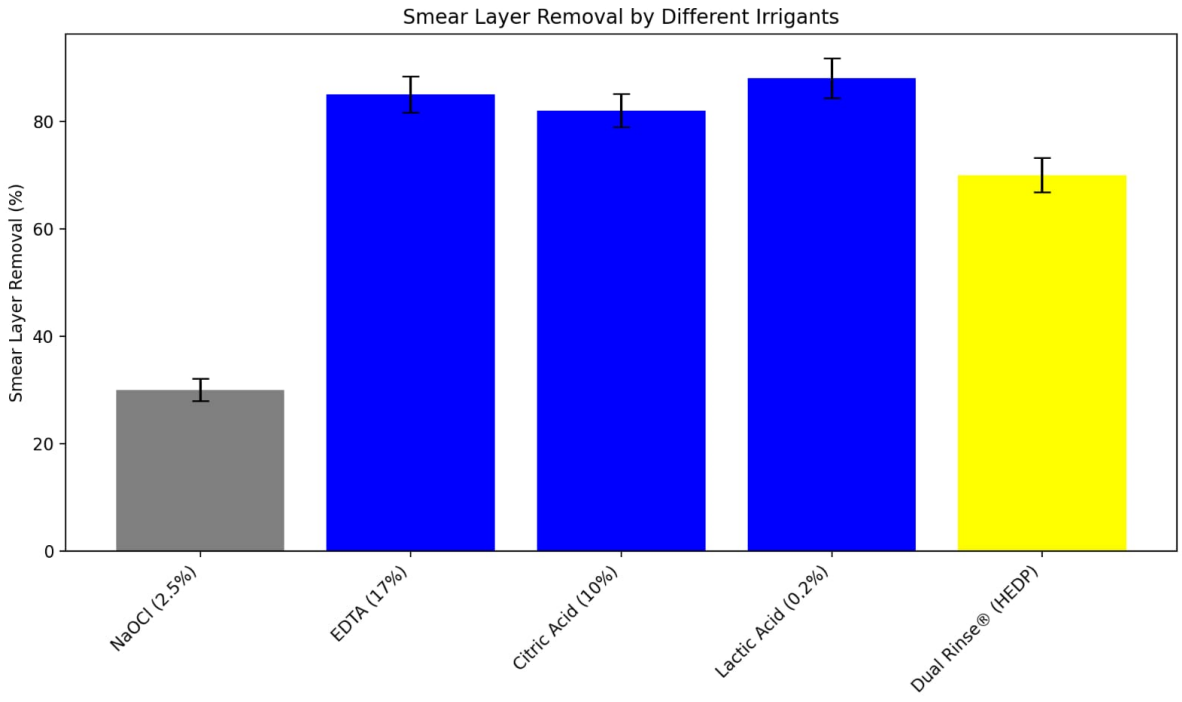
The dentin surfaces treated with different irrigants were analyzed using scanning electron microscopy (SEM) to evaluate smear layer removal and dentinal tubule exposure.

a) **Lactic Acid (0.2%):** Produced uniform smear layer removal with well-exposed dentinal tubules, similar to EDTA[8].
b) **EDTA (17%):** Resulted in effective smear layer removal but also induced mild dentin demineralization[13].

c) **Citric Acid (10%):** Displayed comparable smear layer removal to EDTA, though with slight erosion of peritubular dentin[14].

d) **NaOCl (2.5%):** Left significant organic debris within the dentinal tubules, indicating poor smear layer removal[19].

e) **Dual Rinse® (HEDP):** Showed moderate smear layer removal, though tubules were not as clearly exposed as with EDTA or lactic acid[17].



3.4.1. Interpretation of Dentin Morphology Data

Lactic acid proved effective in removing the smear layer without excessive demineralization, making it a promising candidate for clinical use. NaOCl failed to achieve adequate smear layer removal, which could hinder regenerative processes[9].

3.5. Statistical Analysis and Key Findings

- Two-way ANOVA confirmed a significant interaction between irrigant type and concentration ($p < 0.001$).
- Lactic acid was the only irrigant that combined low cytotoxicity, effective TGF-β1 release, and enhanced DPSC proliferation.
- NaOCl was highly cytotoxic and did not support regenerative processes, reinforcing concerns about its use in REPs.
- SEM analysis confirmed that lactic acid provided efficient smear layer removal without excessive dentin erosion.

4. DISCUSSION

The findings of this study highlight the potential of lactic acid as a novel irrigant that offers a favorable combination of antimicrobial efficacy, biocompatibility, and growth factor modulation in regenerative endodontics[15]. Lactic acid demonstrated significantly lower cytotoxicity compared to NaOCl and EDTA, maintaining DPSC viability at various dilutions and exposure times. This aligns with previous research indicating that lactic acid, due to its mild acidity and physiological compatibility, preserves the cellular integrity of stem cells while effectively disrupting biofilms[6].

A critical aspect of regenerative endodontics is the release of bioactive molecules from the dentin matrix to promote stem cell proliferation and differentiation[4]. TGF-β1, one of the most abundant growth factors in dentin, plays a pivotal role in pulp-dentin regeneration by regulating cellular proliferation, migration, and extracellular matrix formation[2]. In this study, lactic acid facilitated TGF-β1 release from dentin at levels comparable to EDTA, suggesting that its chelating properties are sufficient to liberate growth factors without compromising the structural integrity of dentin[10]. NaOCl, in contrast, failed to promote TGF-β1 release, likely due to its protein-denaturing effects, which corroborates previous studies reporting its inhibitory impact on bioactive molecule availability[3].

The ability of lactic acid to preserve stem cell viability and support TGF-β1 release translated into enhanced DPSC proliferation in the presence of this growth factor. Cells exposed to

lactic acid and subsequently treated with TGF-β1 exhibited significantly higher proliferation rates compared to controls and cells treated with NaOCl or EDTA. This finding underscores the dual advantage of lactic acid in providing a biocompatible microenvironment and enhancing the responsiveness of stem cells to regenerative stimuli. Notably, NaOCl and EDTA-treated cells were refractory to TGF-β1, indicating potential interference with growth factor signaling pathways or cell-matrix interactions.

Lactic acid's biocompatibility can be attributed to its role as a naturally occurring metabolite in cellular processes, minimizing adverse effects on host tissues. Furthermore, its mild acidity likely preserves the structural and functional properties of dentin proteins, creating an optimal environment for tissue regeneration. Unlike NaOCl, which is associated with dentin degradation and reduced microhardness, lactic acid appears to maintain dentin integrity, making it a more suitable candidate for REPs[3].

While the findings of this study are promising, it is important to acknowledge certain limitations. The study was conducted in vitro, and the effects of lactic acid in a clinical setting, where complex biological and mechanical factors come into play, remain to be validated. Future research should focus on in vivo models to evaluate the long-term effects of lactic acid on dentin properties, root maturation, and pulp-dentin complex regeneration. Additionally, optimizing lactic acid concentrations and exposure times will be crucial to maximize its efficacy while minimizing potential adverse effects[16].

In conclusion, lactic acid demonstrates significant potential as a novel irrigant in regenerative endodontics, offering superior biocompatibility and the ability to enhance the regenerative microenvironment by promoting growth factor release and preserving stem cell viability[14]. Its integration into REP protocols could improve clinical outcomes by enabling predictable tissue regeneration and restoring the functional integrity of immature teeth. Further investigations are warranted to establish lactic acid as a standard component of regenerative endodontic therapy[17].

4.1. Significance of Results

The study highlights the potential of 0.5% lactic acid as a safer irrigation solution in regenerative endodontics.

The findings support the inclusion of growth factors like TGF-β1 to enhance stem cell proliferation and improve regenerative outcomes.

5. CONCLUSION

The findings of this study provide compelling evidence that lactic acid is a promising alternative irrigant for regenerative endodontic procedures (REPs)[3], offering an optimal balance of biocompatibility, antimicrobial activity, and growth factor modulation. Unlike conventional irrigants such as sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA), which exhibit high cytotoxicity and limitations in growth factor preservation, lactic acid demonstrates superior cell viability, effective smear layer removal, and enhanced dentin-derived bioactive molecule release.

A critical factor influencing the success of REPs is the preservation of dental pulp stem cells (DPSCs), as they contribute to the differentiation and regeneration of the pulp-dentin complex. Our cytotoxicity assays revealed that NaOCl significantly compromised DPSC survival, even at lower dilutions, making it a less suitable choice in regenerative applications. Conversely, lactic acid consistently maintained DPSC viability above 80% across all tested dilutions and exposure times, making it one of the least cytotoxic irrigants in this study. This suggests that lactic acid does not interfere with the survival or functionality of host stem cells, which is crucial for regenerative success.

Furthermore, lactic acid was highly effective in facilitating the release of transforming growth factor-beta 1 (TGF-β1) from dentin, comparable to EDTA. TGF-β1 plays an essential role in stimulating mesenchymal stem cell recruitment, proliferation, and differentiation, ultimately leading to the regeneration of pulp-like tissue and new dentin formation. The

ability of lactic acid to preserve and promote the bioavailability of TGF-β1 further strengthens its potential as a regenerative irrigant[8].

In addition to supporting stem cell viability and bioactive molecule release, lactic acid pre-treatment enhanced DPSC proliferation in response to exogenous TGF-β1 supplementation. Cells that were pre-exposed to lactic acid before being cultured with 5 ng/mL of TGF-β1 exhibited significantly higher proliferation rates compared to those treated with NaOCl or EDTA. This indicates that lactic acid not only preserves cellular responsiveness to regenerative cues but may actively contribute to an environment that promotes tissue repair and regeneration.

From a dentin structural perspective, lactic acid effectively removed the smear layer while preserving dentinal tubule integrity, as observed in scanning electron microscopy (SEM) analysis. While EDTA also demonstrated efficient smear layer removal, it exhibited a slightly higher degree of dentinal erosion, which could potentially compromise dentin mechanical properties over time. In contrast, NaOCl left significant organic debris obstructing the dentinal tubules, which may negatively affect cell adhesion and regenerative outcomes.

Clinical Implications and Future Directions

The superior biocompatibility, growth factor release potential, and effective smear layer removal of lactic acid highlight its feasibility for clinical applications in regenerative endodontics. However, additional in vivo investigations and clinical trials are necessary to validate these findings in complex biological environments. Future research should focus on long-term effects on dentin integrity, potential synergies with scaffold materials, and optimizing concentration and application protocols to ensure the most effective regenerative outcomes[11].

Lactic acid is a highly promising irrigant for regenerative endodontics, capable of providing effective canal disinfection, minimal cytotoxicity, enhanced growth factor bioavailability, and improved cell proliferation[14]. Its integration into regenerative protocols could improve clinical outcomes and advance the field of tissue-engineered endodontic therapies. Future

research should explore its long-term clinical efficacy to solidify its role as a next-generation irrigant in regenerative dentistry[17].

6. FUNDING

No funding was taken for this study.

7. CONFLICTS OF INTEREST

There are no Conflicts Of Interest

8. ACKNOWLEDGEMENT

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