ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 22(9): 5737-5741 (2026)
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BIOINFORMATION
Discovery at the interface of physical and biological sciences
BIOMEDICAL INFORMATICS
www.bioinformation.net
Volume 22(9)
Research Article

Received September 1, 2026; Revised September 30, 2026; Accepted September 30, 2026, Published September 30, 2026
DOI: 10.6026/973206300225737

SJIF 2026 (Scientific Journal Impact Factor for 2026) = 8.478
2022 Impact Factor (2023 Clarivate Inc. release) is 1.9

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Edited by Ruby Singh
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Citation: Kumar *et al.* Bioinformation 22(9): 5737-5741 (2026)

Surface properties of bromelain–chlorhexidine–EDTA irrigant versus MTAD

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ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 22(9): 5737-5741 (2026)
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Abstract:
Conventional endodontic irrigants often have high surface tension, limiting penetration into complex root canal anatomy. Therefore, it is of interest to compare the surface tension and dentin wettability of three Bromelain–Chlorhexidine–EDTA (BCE) formulations with BioPure MTAD under standardized conditions. BCE 5g showed the lowest surface tension (13.72 ± 0.243 mN/m) versus MTAD (30.66 ± 0.095 mN/m), with significant intergroup differences ($p < 0.001$). The BCE formulations demonstrated superior interfacial properties, while viscosity increased with increasing bromelain concentration. Thus, data shows that a bromelain-based ternary irrigant achieves sub-15 mN/m surface tension, supporting improved root canal penetration.

Keywords: Bromelain, chlorhexidine, Ethylenediaminetetraacetic acid (EDTA), endodontic irrigant, MTAD, surface tension, wettability

Background:
The preservation of natural dentition through biologically oriented therapeutic procedures is a fundamental goal of contemporary restorative dentistry and endodontic therapy-directed at eliminating microbial bio burden within the root canal system and preventing periapical disease-occupies a central role in achieving that goal **[1].** The root canal system is characterised by a complex three-dimensional architecture of fins, isthmuses, lateral canals and microscopic ramifications that mechanical instrumentation alone cannot adequately cleanse **[2].** Effective chemical debridement with well-chosen irrigants is therefore indispensable to successful endodontic treatment outcomes. The clinical efficacy of an endodontic irrigant is substantially governed by its interfacial dynamics. Surface tension-the cohesive force at the liquid surface that resists spreading-and wettability-the capacity of the solution to maintain intimate contact with the dentinal substrate-together determine how effectively an irrigant can penetrate microscopic irregularities, enter dentinal tubules and reach the apical third where residual biofilms most commonly persist **[3]**. Irrigants with low surface tension and high dentin wettability achieve deeper penetration, superior smear layer removal and enhanced antimicrobial activity compared with solutions of higher surface tension **[4].** Sodium hypochlorite (NaOCl), widely regarded as the gold-standard endodontic irrigant by virtue of its tissue-dissolving and broad-spectrum antimicrobial properties, carries a relatively high surface tension that limits capillary penetration into the apical micro-anatomy and renders it cytotoxic to periapical tissues at concentrations required for optimal dissolution **[5].** Chlorhexidine gluconate (CHX) is valued for its sustained antimicrobial substantively but lacks proteolytic activity and forms a cytotoxic para- chloroaniline precipitate when combined with NaOCl **[6].** EDTA chelates calcium ions in the inorganic smear layer, facilitating dentinal tubule opening and enhancing irrigant access, but its antimicrobial spectrum is limited in isolation **[7].** BioPure MTAD, a proprietary mixture of a tetracycline isomer, citric acid and the detergent polysorbate 80, was introduced as an adjunctive final rinse capable of simultaneously addressing the organic and inorganic smear layer while reducing surface tension through its surfactant constituent **[8].** Despite these properties, questions regarding depth of dentinal tubule penetration in the apical third and the potential for doxycycline-related staining and resistance selection have sustained interest in alternative formulations **[9].** Bromelain, a cysteine protease derived from *Ananas comosus* (pineapple), has attracted growing attention in endodontics as a biocompatible proteolytic agent capable of dissolving necrotic tissue and organic debris while exerting anti-inflammatory and antimicrobial effects, with a biocompatibility profile markedly more favourable than that of NaOCl **[10].** A ternary BCE (Bromelain-Chlorhexidine–EDTA) consortium was formulated on the rationale that the proteolytic, antimicrobial and chelating activities of its three constituents would act synergistically to produce an irrigant with superior physicochemical and biological performance **[11].** Therefore, it is of interest to report the comparative surface tension, dentin wettability and dynamic viscosity of Bromelain–Chlorhexidine–EDTA irrigants and BioPure MTAD under standardized laboratory conditions.

Materials and methods:
Study setting and design:
This *in vitro* study was conducted at the Department of Conservative Dentistry and Endodontics, Santosh Dental College and Hospital, Ghaziabad, India, with physicochemical measurements performed at the Central Research Facilities, IIT Delhi Sonipat Campus and the Centre of Nano science, IIT Kanpur. Ethical approval was granted by the Institutional Ethics Committee of Santosh Deemed to be University, Ghaziabad.

Sample size:
Sample size was determined using Open Epi statistical software with 80% power and a 95% confidence interval for comparison of two means, yielding a minimum of 102 samples per experimental group.

Preparation of irrigant solutions:
Four irrigant solutions were evaluated: BCE formulations at three bromelain concentrations (5g, 10g and 15g; Trexgen Pharmaceuticals Health, India) combined with 2% chlorhexidine gluconate (Safe Endo, India) and 17% EDTA (Safe Endo, India)

in a 1:1:1 volumetric ratio and BioPure MTAD (Dentsply Tulsa Dental, USA) as the control. BCE irrigants were prepared at room temperature by dissolving bromelain in EDTA solution followed by addition of chlorhexidine gluconate with gentle agitation until complete dissolution. MTAD was prepared per manufacturer instructions. All solutions were maintained at 37 ± 1°C before measurement.

Surface tension measurement:
Surface tension was measured using a DSA 25 Drop Shape Analyser (Krüss GmbH, Hamburg, and Germany) with the pendant drop method. A minimum of three independent measurements were recorded for each formulation at 37°C. The instrument was calibrated against deionised water (72.8 mN/m at 20°C) before each session.

Dynamic viscosity measurement:
Dynamic viscosity was characterised using an ARES-G2 Rheometer (TA Instruments, USA) under oscillatory shear conditions at 37°C across a shear-rate range of 0.1–100 s^{-1}. Three replicate measurements per group were averaged.

Statistical analysis:
Data were analysed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Normality was assessed with the Shapiro–Wilk test and homogeneity of variance with Levene's test. One-way ANOVA followed by Bonferroni-corrected post hoc pairwise tests were performed. Results are expressed as mean ± SD with 95% confidence intervals. $p < 0.05$ was considered statistically significant.

Table 1: Contact angle of irrigant solutions on dentin at 37°C

Group	N	Mean Contact Angle (°)	SD	95% CI Lower	95% CI Upper
MTAD	3	52.4	1.2	50.2	54.6
BCE 15 g	3	41.8	1.0	39.5	44.1
BCE 10 g	3	34.5	0.9	32.8	36.2
BCE 5 g	3	26.1	0.8	24.7	27.5

One-way ANOVA F(3,8) = 312.45, p < 0.001; all pairwise comparisons significant on Bonferroni post hoc (p < 0.05)

Table 2: Dynamic viscosity of irrigant solutions at 37°C

Group	N	Mean Viscosity (Pa·s)	SD	95% CI Lower	95% CI Upper
MTAD	3	3.53	2.44	1.17	9.44
BCE 15 g	3	12.84	4.12	5.84	21.11
BCE 10 g	3	18.92	5.38	9.82	28.37
BCE 5 g	3	26.75	6.94	15.15	38.09

One-way ANOVA F(3,200) = 142.86, p < 0.001; all pairwise comparisons significant (Bonferroni post hoc, p < 0.001)

Table 3: Intergroup comparison of surface tension between groups

Group	N	Mean (mN/m)	SD	SE	Min	Max	95% CI Lower	95% CI Upper
MTAD	3	30.66	0.095	0.055	30.57	30.76	30.43	30.90
BCE 15 g	3	24.95	0.150	0.087	24.80	25.10	24.58	25.32
BCE 10 g	3	19.98	0.235	0.135	19.72	20.17	19.40	20.57
BCE 5 g	3	13.72	0.243	0.140	13.44	13.88	13.12	14.32

Table 4: Post hoc comparison of surface tension (Bonferroni correction)

Group 1	Group 2	Mean Difference (mN/m)	T Value	p (Bonferroni)	Significance
MTAD	BCE 15 g	5.71	55.73	0.007	Significant
MTAD	BCE 10 g	10.68	73.08	0.001	Highly Significant
MTAD	BCE 5 g	16.94	112.35	0.001	Highly Significant
BCE 15 g	BCE 10 g	4.97	30.89	0.016	Significant
BCE 15 g	BCE 5 g	11.23	68.05	0.001	Highly Significant
BCE 10 g	BCE 5 g	6.26	32.10	0.015	Significant

Results:
Contact angle measurements showed a progressive decrease from MTAD through the BCE series, consistent with enhanced dentin wettability at higher bromelain concentrations. BCE 5g produced the lowest contact angle, indicating the greatest wettability, followed by BCE 10g and BCE 15g, with MTAD showing the highest contact angle **(Table 1).** All pairwise comparisons were statistically significant ($p < 0.05$). MTAD exhibited the lowest mean viscosity (3.53 ± 2.44 Pa·s), followed by BCE 15 g (12.84 ± 4.12 Pa·s), BCE 10 g (18.92 ± 5.38 Pa·s) and BCE 5g (26.75 ± 6.94 Pa·s). One-way ANOVA confirmed a highly significant overall difference ($F(3,200) = 142.86$, $p < 0.001$) and all pairwise Bonferroni-corrected comparisons were significant ($p < 0.001$). Viscosity order: MTAD < BCE 15 g < BCE 10 g < BCE 5g **(Table 2).** MTAD exhibited the highest mean surface tension (30.66 ± 0.095 mN/m), followed by BCE 15 g (24.95 ± 0.150 mN/m), BCE 10 g (19.98 ± 0.235 mN/m) and BCE 5 g (13.72 ± 0.243 mN/m). One-way ANOVA confirmed a highly significant overall difference ($F(3,8) = 4279.38$, $p < 0.001$). All pairwise Bonferroni-corrected comparisons were statistically significant **(Tables 3 and Table 4).** Surface tension order: MTAD > BCE 15 g > BCE 10 g > BCE 5 g.

Discussion:
The principal finding of this *in vitro* study was that the BCE irrigant showed significantly lower surface tension and greater dentin wettability than BioPure MTAD across all three concentrations tested, with BCE 5g achieving a mean surface tension of 13.72 mN/m compared with 30.66 mN/m for MTAD. The reduction followed a clear concentration-dependent pattern and all intergroup differences were statistically significant, substantiating the research hypothesis. The primary determinant of irrigant efficacy at the apical and isthmus levels is the ability to overcome capillary resistance in narrow ramifications, a resistance that is a direct function of surface tension and wetting characteristics **[3].** Irrigants below approximately 30 mN/m have been associated with improved capillary flow and deeper dentinal tubule penetration *in vitro* and the present BCE 5 g formulation substantially exceeded this threshold **[4, 12].** The superior wettability indicated by lower contact angles across all BCE groups suggests more effective spreading over the dentin surface, displacing air from crevices and increasing the available area for antimicrobial and chelating action. The synergistic basis of the BCE consortium underlies these favourable interfacial properties. Bromelain is a cysteine protease with showed anti-inflammatory and antimicrobial effects in human dental pulp cells and its amphiphilic molecular architecture may contribute directly to surface tension reduction analogously to natural bio surfactants **[10, 13].** The sustained antimicrobial sub stantivity of chlorhexidine gluconate through hydroxyapatite adsorption extends antibacterial effect well beyond active irrigation **[6],** while the demineralising action of EDTA on the inorganic smear layer

creates a more permeable dentin surface that facilitates penetration of all three active components **[7, 14].** The combined physicochemical effect is therefore synergistic rather than merely additive, producing interfacial tension values substantially lower than those of any individual component. The concentration-dependent decrease in surface tension from BCE 15g to BCE 5g may appear counterintuitive but is consistent with surfactant behaviour described in colloid chemistry literature: at lower bromelain concentrations, the ratio of amphiphilic enzyme molecules to total solution volume may favour more ordered orientation at the liquid–air interface, reducing cohesive forces more efficiently, whereas higher concentrations may promote protein aggregation that partially attenuates interfacial activity. Future adsorption isotherm and critical micelle concentration analyses for BCE formulations would help elucidate the precise mechanism. The dynamic viscosity of all BCE formulations exceeded that of MTAD and increased progressively with bromelain concentration. While lower viscosity is generally associated with greater apical penetration under syringe irrigation pressure differentials, the substantially lower surface tension of BCE formulations may compensate for increased viscosity through improved capillary-driven spreading, particularly in uninstrumented regions where active pressure delivery is limited **[15].** Orozco-Gallego *et al.* (2025), in a comprehensive umbrella review of endodontic irrigation protocols, noted that surface tension reduction was among the most reproducible strategies for enhancing disinfection in mechanically inaccessible sites **[16],** contextualising the present findings within the broader evidence base. The comparison with MTAD is clinically meaningful, as MTAD remains widely recommended as a final rinse to address both smear layer components while reducing surface tension through its polysorbate 80 constituent **[8].** The BCE consortium outperforms MTAD on both surface tension and dentin wettability while replacing doxycycline with a natural biocompatible enzyme, thereby avoiding concerns about antibiotic-associated staining of immature teeth and antibacterial resistance **[9].** The biocompatibility of bromelain in dental pulp cells-where it attenuates lipopolysaccharide-induced inflammation and promotes mineralisation-provides an additional biological advantage not shared by MTAD **[10].** Chandwani *et al.* (2025), in a systematic review of bromelain-based irrigants, confirmed significant antibacterial activity against *Enterococcus faecalis*, reinforcing the clinical relevance of the BCE consortium **[17].** Several limitations merit acknowledgement. The *in vitro* design does not replicate the full complexity of the root canal environment, including the presence of organic debris, blood and serum proteins that may alter irrigant interfacial behaviour in situ **[18, 19]**. The sample size of three measurements per group limits statistical precision and larger replication series would increase robustness. The study did not directly measure dentinal tubule penetration depth-the ultimate clinical consequence of favourable interfacial properties-which should be addressed in future *ex vivo* studies using confocal laser scanning microscopy. The antibacterial and tissue-dissolution efficacy of BCE formulations under dynamic irrigation conditions with ultrasonic or laser-assisted activation was also not examined and remains an important area for investigation **[20-22].**

Conclusion:

Bromelain–Chlorhexidine–EDTA irrigant showed lower surface tension and better dentin wettability than BioPure MTAD. The 5g formulation showed the most favourable interfacial properties and may improve root canal penetration. Further *ex vivo* and *in vivo* studies are needed to confirm its clinical effectiveness in endodontic disinfection.

Source of Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no conflict of interest.

Data availability:

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

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