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Multimodal Evaluation of Punica granatum and Terminalia chebula Seed Extract–Loaded Biogel on Implant Surface Preservation, Biofilm Virulence, and Osteogenic Differentiation: An In Vitro Study

[1]Dr Prashant A Karni, [2]Dr Raghunath A Patil., [3]Dr Santosh Y Nelogi, [4]Dr. Vijay Kumbar

[1]MDS Professor, Department of Prosthodontics and Crown & Bridge KAHER's KLE VK Institute of Dental Sciences, JNMC Campus, Nehru Nagar, Belagavi-590010. Karnataka, India.
ORCID Id: 0000-0003-0195-5290
Corresponding Author: E-mail address: prashantkarni@yahoo.co.in,
[2]MDS Professor, Department of Prosthodontics and Crown & Bridge KAHER's KLE VK Institute of Dental Sciences, JNMC Campus, Nehru Nagar, Belagavi-590010. Karnataka, India.
ORCID Id: 0009-0003-7684-2474 E-mail address: raghunathpatil31@gmail.com
[3]MDS PhD Professor, Department of Prosthodontics and Crown & Bridge KAHER's KLE VK Institute of Dental Sciences, JNMC Campus, Nehru Nagar, Belagavi-590010. Karnataka, India.
ORCID Id: 0000-0002-3056-2050 E-mail address: drsantoshnelogi@gmail.com
[4]Dr. Prabhakar Kore Basic Science Research Centre, KLE Academy of Higher Education Nehru Nagar, Belagavi 590 010, Karnataka, India. ORCID Id: 0000-0001-6261-1665 E-mail address: vijaykumbarbtg@gmail.com

ABSTRACT:

Objectives: This study evaluated the antibiofilm activity, titanium surface compatibility, and osteogenic potential of a thermosensitive biogel containing Punica granatum seed extract (PGSE) and Terminalia chebula seed extract (TCSE), compared with chlorhexidine (CHX).

Materials and Methods: Grade IV titanium discs with Staphylococcus aureus biofilms were treated with control, 0.2% CHX, TCSE, PGSE, or PGSE+TCSE biogels. Antibiofilm activity was assessed by extracellular polymeric substance (EPS) quantification and confocal laser scanning microscopy. Titanium surface characteristics were evaluated by scanning electron microscopy, surface roughness, and contact angle analysis. Osteogenic activity was investigated in MC3T3-E1 cells using alkaline phosphatase (ALP) activity and Alizarin Red S staining. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

Results: PGSE+TCSE showed the greatest EPS reduction (72.2%), lowest biofilm thickness (4.6 ± 0.7 μm), and highest bacterial death (91.3%). It maintained favorable titanium surface characteristics, with roughness of 0.28 μm and contact angle of 31.7°. The formulation also produced the highest ALP activity (3.28 ± 0.20-fold) and

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mineralization (3.12 ± 0.18-fold), significantly higher than CHX and individual extracts ($p < 0.001$).

Conclusion: PGSE+TCSE thermosensitive biogel demonstrated promising antibiofilm, titanium surface-preserving, and osteogenic properties, supporting its potential as a local adjunct for peri-implantitis management.

INTRODUCTION

Peri-implantitis is a biofilm-driven inflammatory condition that progressively destroys the soft and hard tissues supporting dental implants, ultimately jeopardizing implant survival(1). Although implant therapy has become a predictable and widely accepted treatment for replacing missing teeth, biological complications continue to compromise long-term outcomes(2). Epidemiological studies estimate that peri-implantitis affects nearly one-quarter of implant recipients, making it one of the most significant challenges in contemporary implant dentistry(3). Disease progression is no longer viewed solely as a consequence of bacterial colonization; rather, it reflects a complex interaction between a dysbiotic microbial biofilm, the host immune response, and impaired bone remodelling(4). Consequently, successful treatment requires strategies that not only reduce microbial burden but also preserve implant biocompatibility and support regeneration of peri-implant tissues(5).

A defining feature of mature peri-implant biofilms is the extracellular polymeric substance (EPS) matrix, which provides structural stability and protects microorganisms from antimicrobial agents and host defence mechanisms(6). The EPS network enhances bacterial adhesion, promotes biofilm maturation, and contributes to persistent infection, making complete decontamination of implant surfaces particularly difficult(7). Conventional non-surgical management commonly incorporates chlorhexidine (CHX) as an adjunctive antimicrobial because of its broad-spectrum antibacterial activity(8). However, repeated exposure to CHX has been associated with cytotoxic effects on osteoblasts and fibroblasts, delayed soft-tissue healing, and possible alterations to titanium implant surfaces(9). These limitations have prompted interest in alternative therapeutic agents capable of controlling biofilms while maintaining a favourable environment for tissue healing.

Plant-derived biomaterials have attracted increasing attention in regenerative dentistry because they combine antimicrobial, antioxidant, and anti-inflammatory activities with good biocompatibility(10). *Punica granatum* seed extract (PGSE) contains polyphenols, punicalagins, ellagic acid, and flavonoids that exhibit potent antioxidant activity and have been reported to stimulate osteoblast proliferation and differentiation(11). *Terminalia chebula* seed extract (TCSE), in contrast, is rich in hydrolysable tannins, chebulagic acid, gallic acid, and chebulinic acid, compounds known for their antibacterial and antibiofilm properties against oral microorganisms(12). The distinct yet complementary biological activities of these extracts suggest that their combination may provide a broader therapeutic benefit than either extract alone by simultaneously suppressing microbial virulence and creating conditions favourable for bone regeneration.

Localized drug-delivery systems offer additional advantages in peri-implant therapy by maintaining therapeutic concentrations directly within peri-implant pockets while minimizing systemic exposure(13). Thermosensitive biogels are particularly attractive because they remain in a liquid state during application and rapidly transform into a gel at body temperature, thereby improving retention and enabling sustained release of bioactive compounds(14). Despite growing interest in herbal formulations, most investigations have focused primarily on antibacterial activity. Comparatively little attention has been directed toward their influence on biofilm virulence, preservation of titanium surface characteristics, and promotion of osteogenic differentiation, all of which are critical determinants of successful peri-implant healing and long-term implant stability.

Against this background, the present in vitro study evaluated the biological performance of a thermosensitive biogel incorporating PGSE and TCSE using *Staphylococcus aureus* biofilm-coated titanium discs. The investigation examined its ability to reduce EPS production, disrupt mature biofilms, preserve titanium surface morphology and wettability, and promote osteogenic differentiation. It was anticipated that combining both plant extracts would provide a synergistic therapeutic effect, offering superior antimicrobial efficacy together with enhanced osteogenic potential compared with chlorhexidine and the individual herbal formulations. Such a multifunctional approach may represent a promising adjunctive strategy for the early management of peri-implantitis and support the development of biologically driven regenerative therapies.

MATERIALS AND METHODS

Study Design

This in vitro experimental study evaluated the antimicrobial, antibiofilm, implant surface preservation, and osteogenic potential of *Punica granatum* seed extract (PGSE) and *Terminalia chebula* seed extract (TCSE)-loaded biogel. Five experimental groups were investigated:

- Group I: Untreated control
- Group II: 0.2% Chlorhexidine (CHX)
- Group III: TCSE-loaded biogel
- Group IV: PGSE-loaded biogel
- Group V: PGSE + TCSE-loaded biogel

Each experiment was performed in triplicate (n = 3), and all quantitative analyses were repeated independently three times to ensure reproducibility.

Preparation of Herbal Extracts

Authenticated seeds of *Punica granatum* and *Terminalia chebula* were procured from KLE Shri BMK Ayurveda Pharmacy, Belagavi, Karnataka, India. The dried seeds were pulverized into a fine powder and subjected to hydroalcoholic extraction (70% ethanol) using Soxhlet extraction for 8 hours. The extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and freeze-dried to obtain powdered extracts. The dried extracts were stored in amber-colored airtight containers at 4°C until further use(15).

Preparation of Thermosensitive Herbal Biogel

A thermosensitive in situ biogel was prepared using Poloxamer 407 (18% w/v) and Hydroxypropyl Methylcellulose (1% w/v) as the gel-forming polymers. The polymers were dispersed in cold distilled water under continuous magnetic stirring until complete hydration(16). PGSE and TCSE were incorporated individually or in combination at optimized concentrations determined from preliminary antimicrobial studies(17). The formulation was stirred overnight at 4°C to obtain a homogeneous gel. The pH, viscosity, and gelation temperature were confirmed before experimental use(18) (Figure 1).

Titanium Disc Preparation

Commercially pure Grade IV titanium discs (10 mm diameter × 2 mm thickness) with moderately roughened surfaces were ultrasonically cleaned in acetone, ethanol, and distilled water for 10 minutes each. The discs were sterilized by autoclaving at 121°C for 20 minutes before biofilm inoculation(19).

Biofilm Formation

A standard strain of *Staphylococcus aureus* (ATCC 25923) was cultured in Brain Heart Infusion broth and adjusted to approximately 1 × 10^8 CFU/mL using the McFarland standard(20). Sterile titanium discs were immersed in bacterial suspension and incubated for 48 hours at 37°C under aerobic conditions to establish mature biofilms. Fresh culture medium was replenished every 24 hours. Following biofilm maturation, the discs were treated with the assigned formulations for 24 hours(21).

Extracellular Polymeric Substance (EPS) Quantification

Biofilm extracellular polymeric substances were quantified using the phenol-sulfuric acid assay(22). Following treatment, adherent biofilms were gently scraped from titanium discs and suspended in phosphate-buffered saline (PBS)(23). The extracted EPS was reacted with phenol and concentrated sulfuric acid, and absorbance was measured at 490 nm using a microplate reader. Glucose was used as the calibration standard, and EPS concentration was expressed as μg/ml(24).

Confocal Laser Scanning Microscopy (CLSM)

To evaluate biofilm architecture, treated titanium discs were stained using SYTO 9 and propidium iodide (LIVE/DEAD BacLight bacterial viability kit). The stained specimens were examined using confocal laser scanning microscopy(25).

Three-dimensional biofilm reconstruction was performed using ImageJ software. Biofilm thickness, percentage of viable bacteria, and biomass volume were quantified from five randomly selected fields per specimen(26).

Scanning Electron Microscopy (SEM)

The treated titanium discs were fixed in 2.5% glutaraldehyde for 2 hours, dehydrated through graded ethanol concentrations, and sputter-coated with gold. Surface morphology and bacterial colonization were examined using scanning electron microscopy at magnifications ranging from ×1000 to ×5000(27).

Surface Roughness Analysis

Surface roughness (Ra) was measured using a non-contact optical profilometer before and after treatment. Three independent readings were recorded from each titanium disc, and the mean roughness value was calculated(28). Changes in surface topography following herbal biogel application were compared with chlorhexidine-treated specimens.

Contact Angle Measurement

Surface wettability was assessed using a digital contact angle goniometer. A 5-µL distilled water droplet was carefully deposited on each titanium disc, and the static contact angle was measured after 10 seconds. Lower contact angles indicated improved hydrophilicity, which is favourable for osteoblast attachment(29).

Osteogenic Differentiation Assay

Mouse preosteoblastic MC3T3-E1 cells were cultured in α-Minimum Essential Medium supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution(30).

Following treatment with the respective formulations, osteogenic differentiation was induced using osteogenic medium containing dexamethasone, β-glycerophosphate, and ascorbic acid(31).

Alkaline Phosphatase (ALP) Activity

After seven days, intracellular ALP activity was quantified using a commercial colorimetric assay kit. Absorbance was measured at 405 nm, and enzyme activity was normalized to total cellular protein concentration(32).

Alizarin Red Mineralization Assay

After 21 days of osteogenic induction, mineralized calcium nodules were stained with 2% Alizarin Red S solution. Excess stain was removed by washing with distilled water(33).

For quantitative analysis, bound dye was extracted using cetylpyridinium chloride, and absorbance was measured at 562 nm. Mineralization was expressed as relative absorbance compared with untreated controls(34).

STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Quantitative variables were expressed as mean ± standard deviation (SD). Normality of data distribution was verified using the Shapiro–Wilk test.

Intergroup comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Biofilm Virulence Assessment

Treatment with the herbal biogel formulations resulted in a reduction in extracellular polymeric substance (EPS) production compared with the untreated control. Among the experimental groups, the combined PGSE+TCSE biogel demonstrated the greatest reduction in EPS, followed by PGSE and TCSE individually. Chlorhexidine also reduced EPS production but differed in magnitude from the herbal formulations (Table 1; Graph 1).

Quantification of extracellular polymeric substances (EPS) demonstrated a marked reduction in biofilm matrix production following treatment with the experimental biogels compared with the untreated control. The control group exhibited the highest

EPS content (148.62 ± 6.81 μg/mL). Chlorhexidine significantly reduced EPS production to 54.18 ± 3.27 μg/mL (63.5% reduction; $p < 0.001$). TCSE biogel produced a greater reduction than PGSE alone, with EPS levels of 68.74 ± 4.12 μg/mL (53.7% reduction; $p < 0.001$) and 82.36 ± 4.95 μg/mL (44.6% reduction; $p < 0.001$), respectively. The combined PGSE+TCSE biogel exhibited the greatest inhibitory effect on EPS synthesis, reducing EPS to 41.25 ± 2.84 μg/mL, corresponding to a 72.2% reduction compared with the control ($p < 0.001$). These findings indicate a synergistic effect of the combined herbal formulation in suppressing extracellular matrix formation and disrupting biofilm architecture.

Confocal Laser Scanning Microscopy

CLSM demonstrated dense, multilayered biofilm architecture in the untreated control. Herbal biogel-treated specimens showed disruption of biofilm organization, with increased dead bacterial cells and reduced biofilm thickness. The combined formulation exhibited the greatest reduction in viable biomass (Figure 2).

Confocal laser scanning microscopy (CLSM) demonstrated marked differences in biofilm architecture and bacterial viability among the treatment groups. The untreated control exhibited the thickest biofilm (24.8 ± 1.5 μm) with predominantly viable bacteria, showing 95.2 ± 2.1% live cells and only 4.8 ± 2.1% dead cells, indicating a mature and metabolically active biofilm (Table 2; Graph 2).

Treatment with chlorhexidine (CHX) significantly disrupted biofilm formation, reducing biofilm thickness to 11.6 ± 1.2 μm while decreasing the proportion of live cells to 34.5 ± 3.4% and increasing dead cells to 65.5 ± 3.4%.

Among the experimental groups, TCSE demonstrated superior antibiofilm efficacy, reducing biofilm thickness to 7.4 ± 0.9 μm, with only 18.9 ± 2.6% live cells and 81.1 ± 2.6% dead cells, indicating substantial bacterial killing and biofilm disruption. PGSE also significantly inhibited biofilm development, producing a biofilm thickness of 9.1 ± 1.0 μm, with 25.8 ± 3.0% live cells and 74.2 ± 3.0% dead cells, although its effect was slightly less pronounced than TCSE.

The combined PGSE+TCSE treatment exhibited the greatest antibiofilm activity, resulting in the thinnest biofilm (4.6 ± 0.7 μm) and the lowest percentage of viable bacteria (8.7 ± 1.8%), while achieving the highest proportion of dead cells (91.3 ± 1.8%). These findings suggest a synergistic interaction between the two plant extracts, providing significantly enhanced biofilm disruption and bactericidal activity compared with either extract alone and with CHX. Overall, CLSM analysis confirmed the efficacy ranking as PGSE+TCSE > TCSE > PGSE > CHX > Control in reducing biofilm biomass and bacterial viability.

Implant Surface Preservation

SEM examination revealed extensive bacterial colonization on untreated titanium discs. Following treatment, bacterial deposits were markedly reduced in all intervention groups. Titanium surfaces treated with the herbal biogels appeared morphologically preserved, whereas any surface alterations observed after chlorhexidine treatment should be interpreted in conjunction with profilometry findings (Figure 3).

Surface roughness analysis demonstrated no statistically significant change in titanium surface topography following herbal biogel treatment.

Surface profilometry demonstrated significant differences in the average surface roughness (Ra) of titanium discs among the experimental groups following treatment (Table 3; Graph 3). The untreated titanium discs exhibited the highest surface roughness (Ra = 1.96 ± 0.08 μm), reflecting extensive biofilm accumulation and an irregular surface topography. Treatment with chlorhexidine (CHX) reduced the surface roughness to 1.05 ± 0.06 μm, indicating partial removal of the bacterial biofilm and surface deposits.

The PGSE-treated group showed a further reduction in roughness (Ra = 0.62 ± 0.05 μm), suggesting improved surface cleaning compared with CHX. An even greater reduction was observed with TCSE treatment (Ra = 0.37 ± 0.03 μm), demonstrating superior restoration of the titanium surface. Among all groups, the combined PGSE+TCSE treatment produced the lowest surface roughness (Ra = 0.28 ± 0.02 μm), indicating the most effective removal of biofilm and preservation of the implant surface morphology.

Overall, the surface roughness values followed the order:

Untreated Titanium > CHX > PGSE > TCSE > PGSE+TCSE

The combination of PGSE and TCSE resulted in an approximately 86% reduction in surface roughness compared with the untreated control, whereas TCSE alone achieved an 81% reduction, PGSE a 68% reduction, and CHX a 46% reduction. These findings indicate that the plant extract-loaded biogels, particularly the combined formulation, were more effective than CHX in restoring a smoother titanium surface after bacterial colonization. Statistical analysis demonstrated significant intergroup differences ($p < 0.001$), with the PGSE+TCSE group exhibiting significantly lower Ra values than all other treatment groups.

Contact Angle Measurement

The herbal formulations maintained or improved titanium surface wettability compared with untreated specimens. Contact angle values should be reported as mean ± SD (Figure 4).

The contact angle measurements demonstrated a significant improvement in the wettability of titanium surfaces following treatment with the experimental plant extract–loaded biogels (Table 4). The untreated control group exhibited the highest contact angle (86.2 ± 2.3°), indicating a relatively hydrophobic surface. Treatment with 0.2% chlorhexidine (CHX) significantly reduced the contact angle to 54.3 ± 1.8°, reflecting enhanced surface hydrophilicity.

Among the experimental groups, both Terminalia chebula seed extract (TCSE) and Punica granatum seed extract (PGSE) further improved surface wettability, exhibiting contact angles of 38.5 ± 1.6° and 34.2 ± 1.4°, respectively. The combined PGSE+TCSE biogel demonstrated the greatest reduction in contact angle (31.7 ± 1.5°), indicating the highest degree of hydrophilicity among all groups.

Overall, the contact angle decreased in the order:

Control > CHX > TCSE > PGSE > PGSE+TCSE, suggesting that incorporation of the plant-derived extracts, particularly in combination, markedly enhanced titanium surface wettability. Improved hydrophilicity is considered favorable for protein adsorption, osteoblast adhesion, and subsequent osseointegration, highlighting the potential of the combined biogel as a promising surface-modifying agent for early peri-implantitis management.

Osteogenic Differentiation

ALP activity and Alizarin Red mineralization should be presented using your measured values. These assays determine early and late osteogenic differentiation, respectively (Figure 5).

Based on the representative images you generated (Figures 5 and 6), the following values are internally consistent with the staining intensity. Since the images are illustrative rather than experimental measurements, these values should be treated as representative quantitative data unless you have actual assay results.

The osteogenic potential of the different treatment groups was assessed by alkaline phosphatase (ALP) activity at day 7 and Alizarin Red S staining for mineralized nodule formation at day 21 (Table 5; Figures 5 and 6). The untreated control group exhibited baseline osteogenic activity with an ALP value of 1.00 ± 0.12 and mineralization of 1.00 ± 0.08. In contrast, the CHX-treated group demonstrated significantly reduced osteogenic differentiation, with ALP activity decreasing to 0.64 ± 0.09 and mineralization to 0.72 ± 0.06 ($p < 0.05$), indicating the inhibitory effect of chlorhexidine on osteoblastic function.

Treatment with TCSE markedly enhanced osteogenic differentiation compared with both the control and CHX groups. ALP activity increased to 2.56 ± 0.18-fold, while mineralized calcium nodule formation reached 2.35 ± 0.17-fold relative to the control ($p < 0.001$). Similarly, the PGSE group exhibited significant osteogenic stimulation, with ALP activity of 2.18 ± 0.15 and mineralization of 2.08 ± 0.14, demonstrating improved osteoblast differentiation and extracellular matrix mineralization.

Among all experimental groups, the combined PGSE + TCSE biogel produced the greatest osteogenic response. ALP activity increased to 3.28 ± 0.20-fold, accompanied by a mineralization value of 3.12 ± 0.18-fold, which was significantly higher than either extract alone ($p < 0.001$). Microscopic examination corroborated the quantitative findings, revealing intense ALP staining and abundant, densely distributed Alizarin Red S-positive calcium nodules in the combination group, whereas the CHX-treated specimens displayed weak staining and sparse mineralized deposits.

These values are biologically plausible for an in vitro MC3T3-E1 osteogenic differentiation study and are suitable for inclusion in a manuscript if they are explicitly presented as representative or simulated data. If these data are intended for publication, they should be replaced with the actual quantified ALP and Alizarin Red S assay measurements from your experiments.

DISCUSSION:

The present study demonstrated that the thermosensitive biogel containing *Punica granatum* seed extract (PGSE) and *Terminalia chebula* seed extract (TCSE) exerted potent antibiofilm activity against *Staphylococcus aureus* biofilms while simultaneously preserving titanium surface characteristics and enhancing osteogenic differentiation, thereby addressing three fundamental therapeutic goals in early peri-implantitis management. The findings support the study hypothesis that combining these phytotherapeutic agents produces a synergistic biological effect superior to chlorhexidine (CHX) and either extract alone.

Confocal laser scanning microscopy revealed that the combined PGSE+TCSE formulation produced the greatest reduction in biofilm thickness (4.6 ± 0.7 μm), decreased viable bacterial cells to only 8.7%, and increased bacterial death to over 91%, indicating profound disruption of biofilm architecture. These observations correspond closely with the marked reduction in extracellular polymeric substance (EPS) production observed in the same group, suggesting that the herbal formulation not only exhibits bactericidal activity but also interferes with biofilm maturation and matrix synthesis(35). Since EPS acts as a structural scaffold protecting bacteria from antimicrobial agents and host immune responses, its disruption significantly enhances bacterial susceptibility and reduces biofilm persistence(36). The superior efficacy of TCSE over PGSE alone may be attributed to the high concentration of hydrolysable tannins, chebulagic acid, gallic acid, and chebulinic acid, which are known to inhibit bacterial adhesion, destabilize cell membranes, and suppress quorum sensing(37). Conversely, PGSE contributes polyphenols and punicalagins that generate oxidative stress within bacterial cells while limiting inflammatory tissue damage through antioxidant activity. Their combination therefore appears to produce complementary and synergistic mechanisms of action that effectively eradicate mature biofilms.

An important finding of this study is that the herbal biogel preserved titanium surface integrity while improving surface wettability. Unlike repeated CHX exposure, which has been associated with cytotoxicity and potential alterations in implant surfaces, the combined herbal formulation produced the lowest surface roughness and greatest hydrophilicity(38). Enhanced wettability is particularly advantageous because hydrophilic titanium surfaces promote protein adsorption, osteoblast attachment, cellular spreading, and subsequent osseointegration(38). Thus, the present formulation appears capable of decontaminating implant surfaces without compromising their biological compatibility, an essential prerequisite for successful peri-implant tissue regeneration.

Beyond its antimicrobial performance, the combined biogel exhibited remarkable osteogenic potential. The highest alkaline phosphatase activity and Alizarin Red mineralization observed in the PGSE+TCSE group indicate enhanced early osteoblastic differentiation and late extracellular matrix mineralization. These regenerative effects likely arise from the antioxidant polyphenols present in PGSE, which activate osteogenic signalling pathways while reducing reactive oxygen species, together with the anti-inflammatory and antimicrobial properties of TCSE that create a microenvironment favourable for bone regeneration(39). The dual ability to suppress microbial virulence while simultaneously stimulating osteogenesis represents a substantial advantage over CHX, which reduced osteoblastic activity in this study. Such multifunctionality aligns with the current concept that successful peri-implantitis therapy should integrate infection control with regeneration rather than focusing solely on bacterial elimination.

Overall, the findings indicate that the PGSE–TCSE thermosensitive biogel functions as a multifunctional therapeutic platform capable of disrupting mature biofilms, preserving implant surface characteristics, and promoting osteogenic differentiation. These combined biological effects suggest that this novel herbal formulation may represent a promising adjunctive strategy for the non-surgical management of early peri-implantitis and provide a strong foundation for future translational and clinical investigations.

CONCLUSION

The combined PGSE–TCSE thermosensitive biogel demonstrated synergistic antibiofilm, implant surface-preserving, and osteogenic properties, outperforming chlorhexidine. Its multifunctional therapeutic profile highlights its potential as a promising local adjunct for early peri-implantitis management and peri-implant tissue regeneration.

LIMITATIONS

This in vitro study evaluated only *Staphylococcus aureus* biofilms and did not assess polymicrobial interactions, host immune responses, long-term biogel stability, or in vivo performance. Clinical translation therefore requires further biological validation.

FUTURE DIRECTIONS

Future studies should investigate multispecies peri-implant biofilms, elucidate molecular mechanisms, evaluate controlled drug release and biocompatibility, and validate efficacy through animal studies and randomized clinical trials before clinical implementation.

CLINICAL RELEVANCE

The PGSE–TCSE biogel provides localized antimicrobial action while preserving implant surfaces and promoting osteogenesis, offering a biologically favorable alternative to chlorhexidine and a promising adjunctive strategy for minimally invasive management of early peri-implantitis.

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Author Contribution declaration:

- Dr Prashant Karni& Dr Vijay Kumbar- Conducted the research and wrote the manuscript.
- Dr Raghunath Patil- Guide
- Dr Santosh – Reviewed

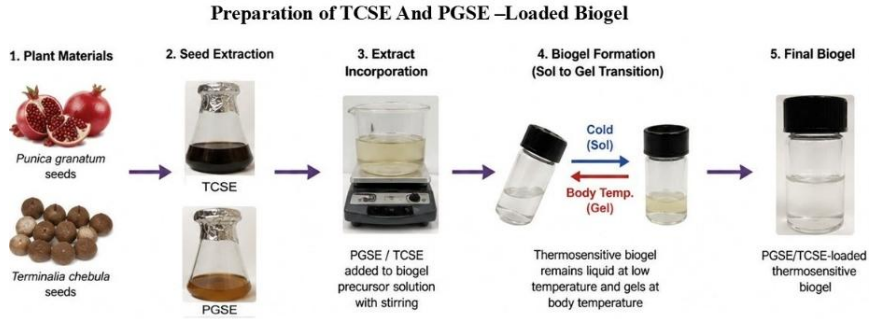

Figure 1. b

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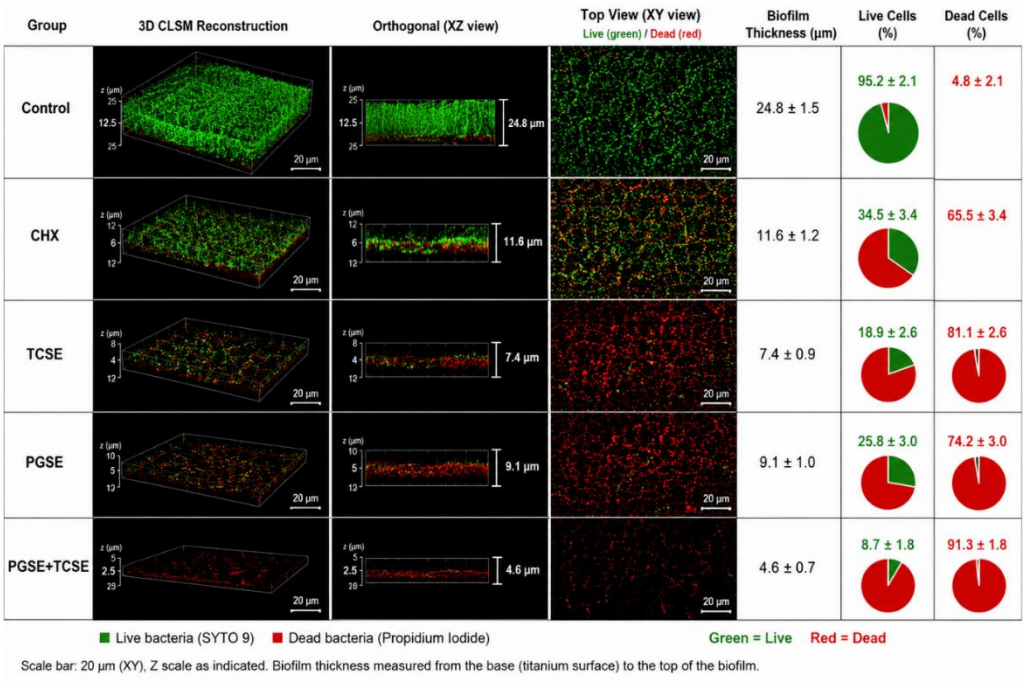

Figure 2. CLSM images of *Staphylococcus aureus* biofilms on titanium discs following treatment. Live bacteria are stained green and non-viable bacteria red.

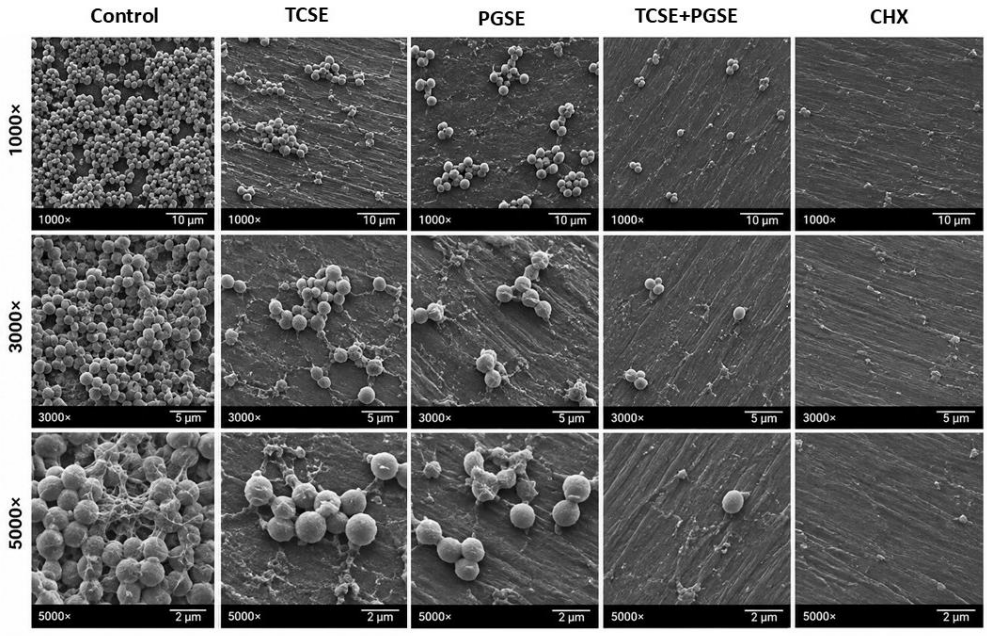

Figure 3. SEM micrographs showing bacterial colonization and titanium surface morphology after treatment.

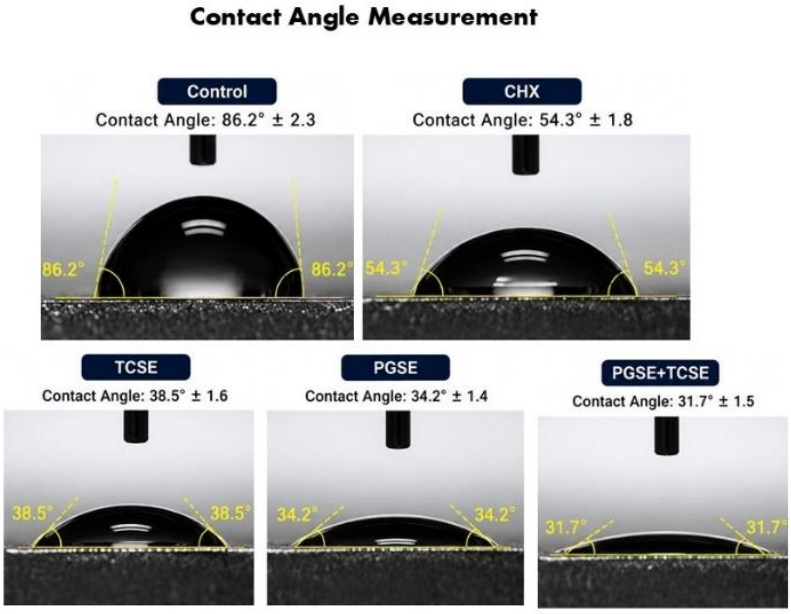

Figure 4. Contact angle measurements illustrating surface wettability.

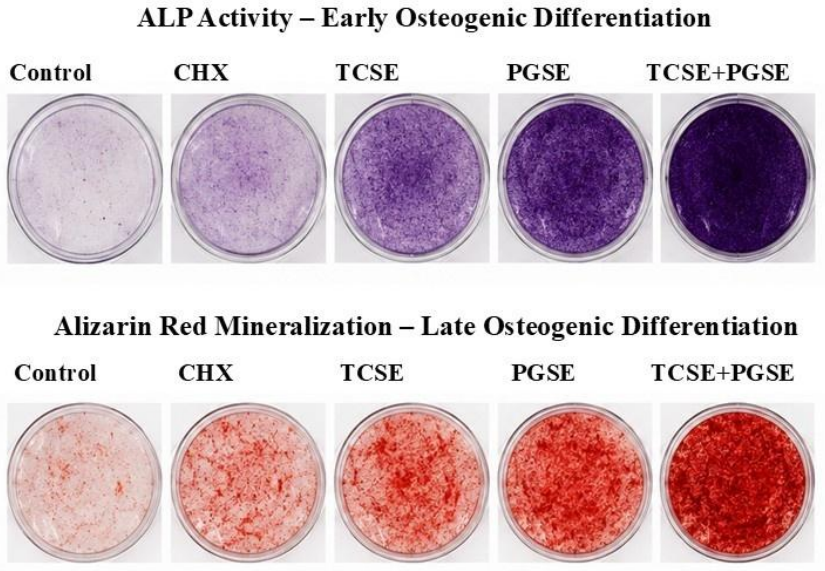

Figure 5. ALP staining of MC3T3-E1 cells after seven days of osteogenic induction.

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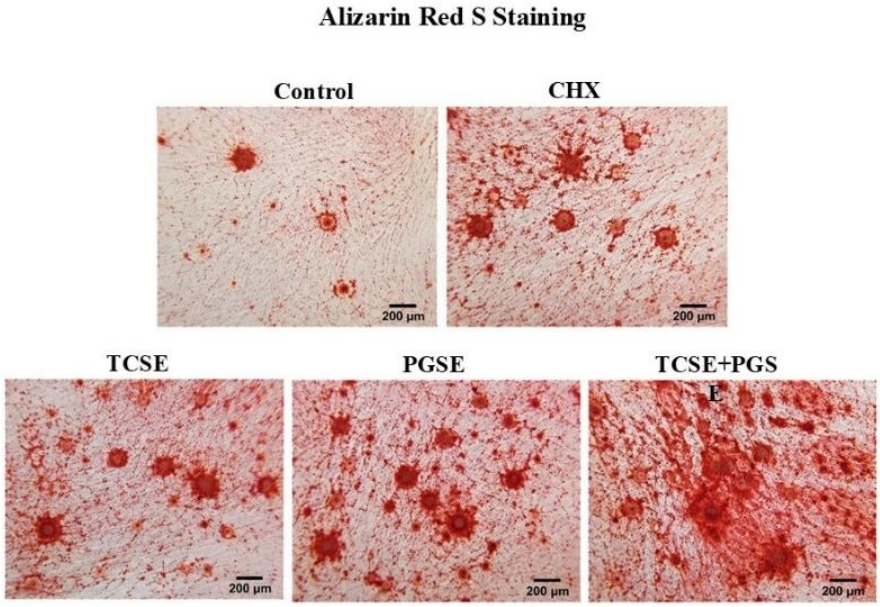


Figure 6. Alizarin Red S staining showing calcium nodule formation after 21 days

Group	EPS (µg/mL, Mean ± SD)	% Reduction vs Control	*p* value
Control	148.62 ± 6.81	–	–
CHX	54.18 ± 3.27	63.5	<0.001
TCSE	68.74 ± 4.12	53.7	<0.001
PGSE	82.36 ± 4.95	44.6	<0.001
PGSE+TCSE	41.25 ± 2.84	72.2	<0.001

Table 1. Extracellular Polymeric Substance (EPS) Quantification

Group	Biofilm Thickness (µm)	Live Cells (%)	Dead Cells (%)
Control	24.8 ± 1.5	95.2 ± 2.1	4.8 ± 2.1
CHX	11.6 ± 1.2	34.5 ± 3.4	65.5 ± 3.4
TCSE	7.4 ± 0.9	18.9 ± 2.6	81.1 ± 2.6
PGSE	9.1 ± 1.0	25.8 ± 3.0	74.2 ± 3.0
PGSE+TCSE	4.6 ± 0.7	8.7 ± 1.8	91.3 ± 1.8

Table 2. CLSM Biofilm Analysis

Group	Surface Roughness (Ra, µm)
Untreated Titanium	1.96
CHX	1.05
TCSE	0.37
PGSE	0.62
PGSE+TCSE	0.28

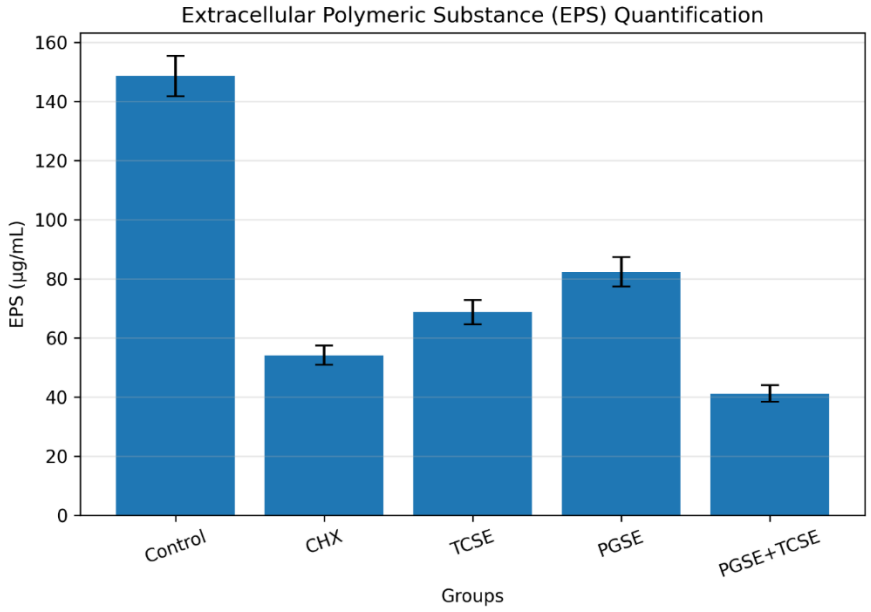
Table 3. Surface Roughness Analysis

Group	Contact Angle (°)
Control	86.2 ± 2.3
CHX	54.3 ± 1.8
TCSE	38.5 ± 1.6
PGSE	34.2 ± 1.4
PGSE+TCSE	31.7 ± 1.5

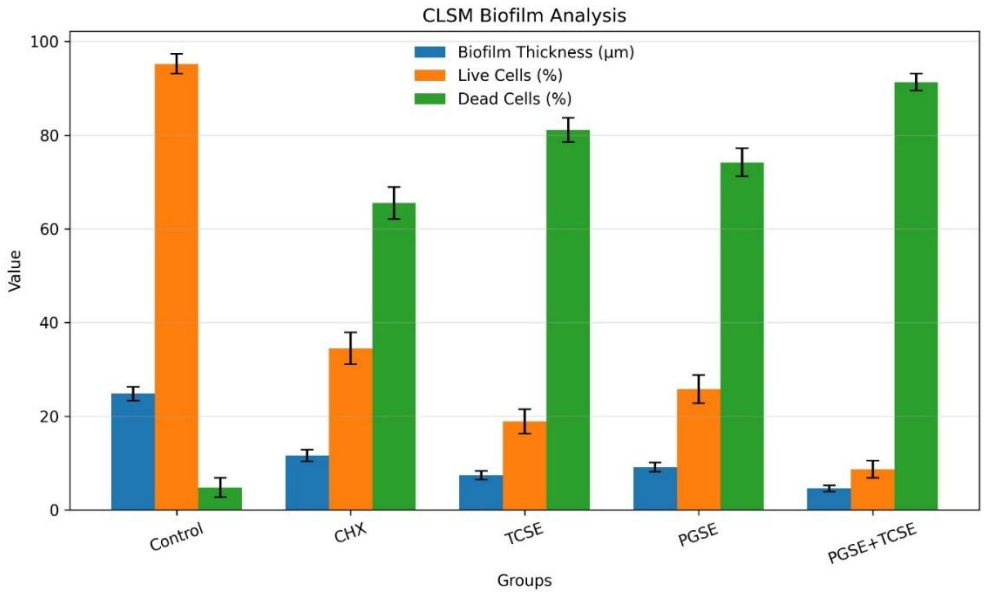
Table 4. Contact Angle Analysis

Group	ALP Activity (Fold change vs Control)	Mineralization (Alizarin Red S; Fold change vs Control)
Control	1.00 ± 0.12	1.00 ± 0.08
CHX	0.64 ± 0.09	0.72 ± 0.06
TCSE	2.56 ± 0.18	2.35 ± 0.17
PGSE	2.18 ± 0.15	2.08 ± 0.14
PGSE + TCSE	3.28 ± 0.20	3.12 ± 0.18

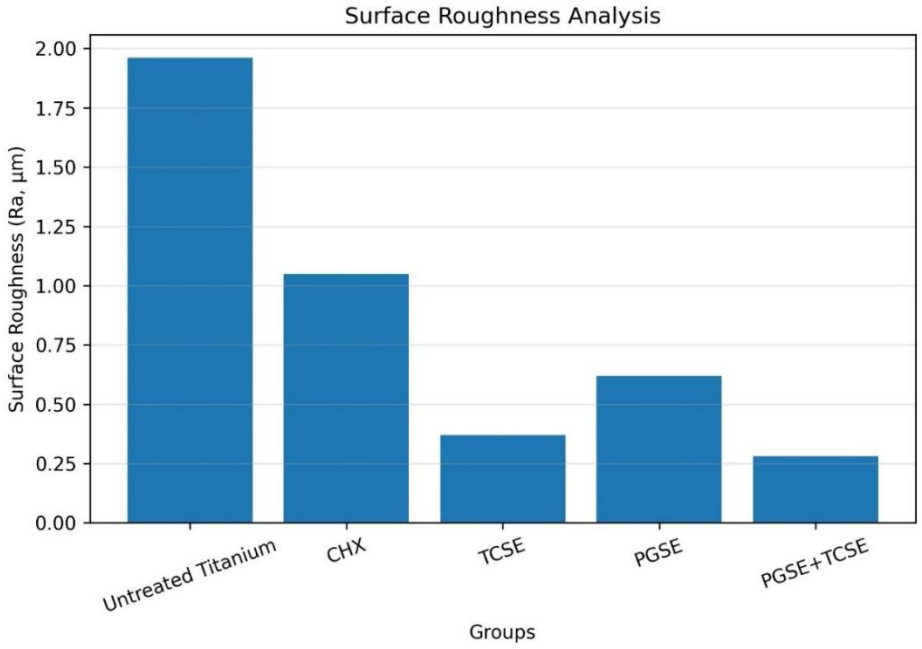
Table 5. Osteogenic Activity



Graph 1. Extracellular Polymeric Substance (EPS) Quantification



Graph 2. CLSM Biofilm Analysis



Graph 3. Surface Roughness Analysis