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Biological and clinical impact of chlorhexidine on peri-implant tissues and early peri-implant healing: A combined in vitro and clinical evaluation

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Abstract
Objective: To evaluate the short-term biological and clinical effects of 0.12% chlorhexidine (CHX) on peri-implant tissues using a combined in-vitro and randomised clinical approach.
Methods: An in-vitro study assessed the effects of 0.01%, 0.05%, and 0.12% CHX on cell viability and extracellular matrix (ECM) protein expression via MTT assay, immunofluorescence, and ELISA. In parallel, a clinical study randomised 40 patients receiving dental implants to either a 0.12% CHX mouthrinse group (twice daily, starting 24h post-op) or a control group (standard hygiene). Plaque index, bleeding on probing (BOP), and marginal bone levels were assessed at baseline, 1, and 3 months. Data were analysed using linear mixed-effects models.
Results: In vitro, 0.01% CHX showed minimal effects, while 0.05% and 0.12% caused significant, concentration-dependent cytotoxicity and suppressed collagen I and fibronectin expression (all $p<0.001$). Clinically, the CHX group had significantly lower plaque scores at 1 month (mean difference −0.8; $p=0.001$) and 3 months (−0.7; $p=0.002$), and reduced BOP at 3 months (−17.7%; $p=0.003$). Marginal bone loss did not differ between groups (−0.05 mm; $p=0.46$). No serious adverse events occurred.
Conclusion: Short-term postoperative 0.12% CHX use improved peri-implant plaque control and soft-tissue inflammation without short-term adverse bone effects, despite in-vitro cytotoxicity at higher concentrations. Larger, long-term trials are needed to confirm safety and efficacy.

Keywords: Chlorhexidine, Dental implants, Early osseointegration, Peri-implant tissues

Plain English Summary

The trial explored whether chlorhexidine aids in the proper healing of dental implants. Laboratory tests revealed that low concentrations of chlorhexidine kill bacteria without necessarily damaging gum or bone cells, though high concentrations can inhibit cell growth. Patients with dental implants who used chlorhexidine for the short term had better gum health, less bleeding, and early bone healing. If properly applied for a short period of time, chlorhexidine is safe and good for the success of implants.

Introduction

The dental implant therapy has become accepted as a dependable tooth replacement method because it achieves high success rates in osseointegration and maintains stable peri-implant tissues during long-term periods. The two primary

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biological issues which impact implants result from peri-implant mucositis and peri-implantitis because microbial biofilms develop on implant surfaces, which leads to inflammation of both soft and hard tissues (1, 2). The existing conditions damage soft tissue structures while they create a risk for marginal bone instability, which could lead to implant failure.

The antiseptic agent chlorhexidine (CHX) stands as the primary choice for peri-implant microbial load control because it offers wide antimicrobial effectiveness, stays effective in the mouth and is readily available for clinical use. The antibacterial properties of CHX protect Gram-positive and Gram-negative bacteria and fungi and specific viruses, which makes it suitable for use as a mouthrinse or gel, or irrigation solution during implant therapy, surgical and postoperative periods (3, 4, 5).

Studies record that CHX treatment performed as a separate procedure helps patients build lower plaque levels during their first healing period, and it minimises inflammatory markers, which results in improved implant soft tissue health (6). The antimicrobial properties of chlorhexidine do not predict its complete biological response because the study records its effects strongly depend on both concentration levels and time of exposure. The test tube study records that osteoblast-like cells and gingival fibroblasts survive at low CHX concentrations, but their growth potential and cellular structure and survival rate will deteriorate when exposed to high doses, which blocks matrix development and tissue healing (7). The study findings create doubts about using CHX without proper control when performing procedures near implant surfaces and healing tissues. The biological process of osseointegration requires antimicrobial protection and cell survival maintenance to achieve success at the bone-implant connection site. The clinical results will suffer from any substance which interferes with osteoblast activity and collagen deposition and fibroblast-mediated soft tissue sealing during the initial stages of implant healing (8, 9).

The main obstacle exists in creating an optimal treatment method which would achieve the best antimicrobial effects of CHX while protecting cells from damage. The results from Systematic reviews and randomised controlled trials recorded different effects of chlorhexidine on peri-implant bone stability during extended patient care. The study indicates that this product enables patients to improve their plaque control and reduce bleeding during probing tests after short treatment periods, yet dental experts doubt its effectiveness in protecting bone margins during extended healing periods (10, 11). Most clinical studies have studied CHX results independently from cellular reactions, which makes it difficult to apply their study results to real-world situations.

The study requires an integrated study method which combines laboratory tests with medical patient studies to understand how different concentrations of chlorhexidine affect peri-implant tissues and their relationship to initial signs of implant success. The complete effects of CHX on cells will become apparent through in vivo studies, which evaluate osteoblast-like cells, gingival fibroblasts and peri-implant tissue health. The evidence serves as a fundamental base for creating clinical protocols which protect microbial control while allowing biological processes to continue for osseointegration.

Materials and Methods

Study Design

The researcher conducted experimental in vitro tests and controlled clinical studies to determine how different concentrations of chlorhexidine (CHX) affect peri-implant tissue health and early osseointegration. The integration of laboratory-based cellular analysis with clinical observations was intended to allow translational interpretation of cellular responses in relation to peri-implant clinical outcomes. The medical staff used Chlorhexidine for a brief period following surgery because they needed to stop the solution from remaining in contact with body tissues.

Sample size

This study was designed as an exploratory pilot investigation to evaluate the short-term biological and clinical effects of chlorhexidine on peri-implant tissues. Consequently, no formal pre-hoc sample size calculation was performed. A total sample of 40 participants (20 per group) was considered adequate to assess feasibility, safety, and preliminary clinical effects, and to generate effect size estimates for planning future adequately powered randomised controlled trials. As the study was not powered to detect small differences in marginal bone level changes, non-significant radiographic findings may represent type II error and should be interpreted cautiously.

In-vitro cell culture, chlorhexidine exposure, and analytical procedures

Human peri-implant–related cells were cultured under standard conditions (37 °C, 5% CO_2) and seeded at a density of **1** × 10^4 cells/well in 96-well plates for cell viability assays and 5 × 10^4 cells/well

in 24-well plates for extracellular matrix (ECM) analyses. Chlorhexidine digluconate solution (20% w/v; Sigma-Aldrich, St. Louis, MO, USA; Lot No. BCBX1234) was diluted in complete culture medium to obtain final working concentrations of 0.01%, 0.05%, and 0.12% immediately before use (12, 13). Control wells received culture medium without chlorhexidine and served as vehicle controls.

Cell viability was assessed using the MTT assay (Sigma-Aldrich; Cat. No. M5655). Absorbance was measured at 570 nm using a BioTek ELx800™ microplate reader (BioTek Instruments, Winooski, VT, USA). After exposure to the respective chlorhexidine concentrations for 72 hours, with culture medium refreshed every 24 hours, MTT reagent (0.5 mg/mL) was added, and cells were incubated for 4 hours at 37 °C. Formazan crystals were solubilised using dimethyl sulfoxide, and absorbance was measured at 570 nm using a microplate reader (BioTek ELx800™). Cell viability was expressed as a percentage of the untreated control according to the formula:

Cell viability (%) = (absorbance of treated cells/absorbance of control cells) × 100

Extracellular matrix protein expression (collagen type I and fibronectin) was quantified using enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, UK; Collagen I: ab210966; Fibronectin: ab219046; detection limits 0.02 ng/mL for collagen I and 0.05 ng/mL for fibronectin), following the manufacturer's instructions. Concentrations were calculated from standard curves and expressed as [units].

Immunofluorescence images were acquired using an Olympus IX73 inverted fluorescence microscope (Olympus Corporation, Tokyo, Japan) at ×200 magnification. Images were captured using identical exposure settings across all groups. For each experimental condition, five randomly selected fields per well were imaged and analysed using ImageJ software (NIH, Bethesda, MD, USA). All quantitative image analyses were performed by an investigator blinded to group allocation.

Scanning electron microscopy (SEM) was conducted on cells fixed in 2.5% glutaraldehyde, dehydrated through graded ethanol, and sputter-coated with gold/palladium. Samples were examined using a JEOL JSM-6510LV scanning electron microscope (JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 15 kV.

All in-vitro experiments were performed in three independent biological replicates, defined as separate experiments conducted on different days using independently prepared cell cultures. Each biological replicate included technical triplicates per condition. Data are presented as mean ± standard deviation.

Clinical Study

Study Population

The study included forty systemically healthy adult patients who were between 20 and 60 years old and needed dental implant placement. The study required participants to be non-smokers who had no uncontrolled systemic diseases and no history of periodontal disease. The study excluded patients who smoked or had weakened immunity or took drugs which impacted bone health.

Randomisation and Intervention

Eligible participants were randomly allocated in a 1:1 ratio to either the chlorhexidine (CHX) group or the control group using a computer-generated random sequence created with [software name/version, e.g., SPSS version XX] by an investigator not involved in clinical assessment or data analysis. The CHX group received 0.12% chlorhexidine mouthrinse twice daily, commencing 24 hours after implant surgery and continuing throughout the short-term postoperative period, while the control group received standard oral hygiene instructions without chlorhexidine. All implant surgeries were performed by the same surgical team using standardised operative protocols to minimise variability related to surgical technique. Due to the nature of the intervention, allocation concealment was not implemented, and neither participants nor the operating clinician were blinded to group assignment. However, the examiner responsible for recording clinical indices and radiographic measurements was masked to group allocation, and all outcome assessments were conducted using standardised protocols to reduce measurement bias. Baseline characteristics were compared between groups to assess balance following randomisation.

Clinical Outcome Assessment

The researcher assessed peri-implant tissue health during three evaluation points, which included the beginning and one and three months after implant placement. The researcher evaluated the patients through three clinical assessment methods, which measured plaque accumulation index, bleeding on probing and peri-implant mucosal inflammation scores using standardised periodontal indices. The examiner, who was calibrated, performed all measurements while remaining unaware of which group the subjects belonged to. The evaluation of marginal bone levels through radiographic methods used

standardised periapical radiographs, which doctors took at the beginning and at all subsequent follow-up appointments. The researcher used digital methods to measure how the marginal bone tissue shifted in relation to the implant reference points. The study team documented all adverse events, which included soft tissue reactions, together with patient-reported discomfort from start to finish of the study.

Outcomes

The primary clinical outcome was the change in plaque index from baseline to 1-month follow-up, selected a priori as the most sensitive short-term indicator of peri-implant plaque control following surgery. Secondary outcomes included changes in bleeding on probing, peri-implant marginal bone level changes at 3 months, and in vitro biological outcomes (cell viability and extracellular matrix protein expression). The sample size calculation and main inferential statistical testing were based on the primary outcome. Analyses of secondary outcomes were considered exploratory and were interpreted accordingly, with adjustment for multiple comparisons where appropriate.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version XX (IBM Corp., Armonk, NY, USA). Continuous variables were summarised as mean ± standard deviation, while categorical variables were presented as frequencies and percentages. Normality of continuous outcomes was assessed using the Shapiro–Wilk test and visual inspection of Q–Q plots.

Longitudinal clinical outcomes (plaque index, bleeding on probing, and marginal bone level changes) were analysed using linear mixed-effects models to account for repeated measurements within participants. The models included fixed effects for treatment group (CHX vs control), time (baseline, 1 month, and 3 months), and the group × time interaction, with a random intercept for participant to account for within-subject correlation. A compound symmetry covariance structure was assumed. Model parameters were estimated using the restricted maximum likelihood (REML) method. Between-group comparisons at each time point were derived from model-based estimated marginal means and are reported as mean differences with 95% confidence intervals (CI). Post-hoc pairwise comparisons were adjusted for multiple testing using the Holm–Bonferroni correction.

In-vitro outcomes were compared across chlorhexidine concentrations using one-way analysis of variance (ANOVA), followed by Bonferroni-adjusted post-hoc tests. When normality assumptions were violated, the Kruskal–Wallis test with Dunn's post-hoc correction was applied.

Missing data were minimal (<5%) and were handled using complete-case analysis within the mixed-effects modelling framework. All statistical tests were two-sided, and exact p-values are reported, with statistical significance set at $p < 0.05$. Multiplicity adjustment was applied to post-hoc comparisons to control for inflated type I error due to multiple testing.

Results

Participant flow

Participant enrolment, randomisation, follow-up, and analysis are illustrated in the CONSORT flow diagram (Figure 1).

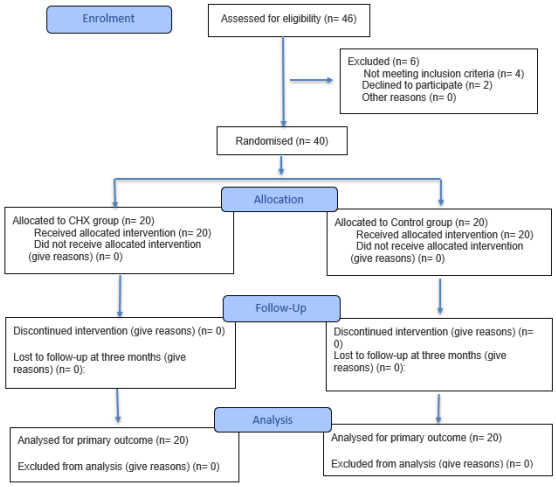

Figure 1: CONSORT flow diagram of participant enrolment, allocation, follow-up, and analysis

Baseline characteristics

The CHX and control groups were well balanced at baseline with respect to age, sex distribution, implant site, implant dimensions, smoking status, and systemic health status. No statistically significant between-group differences were observed for any baseline variable, suggesting that randomisation achieved adequate group comparability (Table 1).

Table 1: Baseline characteristics of study participants

Characteristic	CHX group (n = 20)	Control group (n = 20)	p-value
Age (years), mean ± SD	45.2 ± 8.6	46.1 ± 9.1	0.74
Sex, n (%)			
Male	11 (55.0)	10 (50.0)	0.75
Female	9 (45.0)	10 (50.0)	
Implant site, n (%)			
Maxilla	12 (60.0)	11 (55.0)	0.75
Mandible	8 (40.0)	9 (45.0)	
Implant length (mm), mean ± SD	11.2 ± 1.4	11.0 ± 1.5	0.63
Implant diameter (mm), mean ± SD	4.1 ± 0.4	4.0 ± 0.3	0.48
Smoking status			
Non-smoker	20 (100)	20 (100)	—
ASA physical status			
ASA I	14 (70.0)	15 (75.0)	0.73
ASA II	6 (30.0)	5 (25.0)	

Values are mean ± SD or number (%)
p-values derived from independent t-tests or chi-square tests, as appropriate
No statistically significant baseline differences were observed between groups

Clinical Results

Study Population and Follow-Up

The forty participants in the study completed their clinical follow-up without any implant failures or severe complications. The chlorhexidine group recorded no differences in their baseline demographic and clinical parameters when compared to the control group.

Plaque Accumulation

At 1 month, the mean plaque index was 1.1 ± 0.3 in the CHX group and 1.9 ± 0.4 in the control group. The adjusted between-group mean difference was −0.8 (95% CI −1.1 to −0.5; p = 0.001). This difference remained significant at 3 months (mean difference −0.7; 95% CI −1.0 to −0.4; p = 0.002) after Holm–Bonferroni correction (Table 2, Figure 2).

Table 2: Comparison of clinical peri-implant outcomes between CHX and control groups

Outcome	Time point	CHX group Mean ± SD	Control group Mean ± SD	Between-group difference (95% CI)	Adjusted p-value†
Primary outcome					
Plaque index	Baseline	2.5 ± 0.5	2.4 ± 0.6	—	—
	1 month	1.1 ± 0.3	1.9 ± 0.4	−0.8 (−1.1 to −0.5)	0.001
	3 months	1.0 ± 0.2	1.7 ± 0.4	−0.7 (−1.0 to −0.4)	0.002
Secondary outcomes					
Bleeding on probing (%)	Baseline	42.3 ± 11.4	44.1 ± 12.0	—	—
	1 month	22.6 ± 8.9	35.8 ± 10.7	−13.2 (−23.4 to −3.0)	0.01
	3 months	18.5 ± 7.6	36.2 ± 9.8	−17.7 (−28.4 to −7.0)	0.003
Marginal bone loss (mm)	3 months	0.42 ± 0.18	0.47 ± 0.21	−0.05 (−0.19 to 0.09)	0.46

Values represent estimated marginal means derived from linear mixed-effects models.
Between-group differences represent CHX minus control.
†Adjusted p-values obtained using Holm–Bonferroni correction for multiple comparisons.
Negative values indicate lower scores in the CHX group

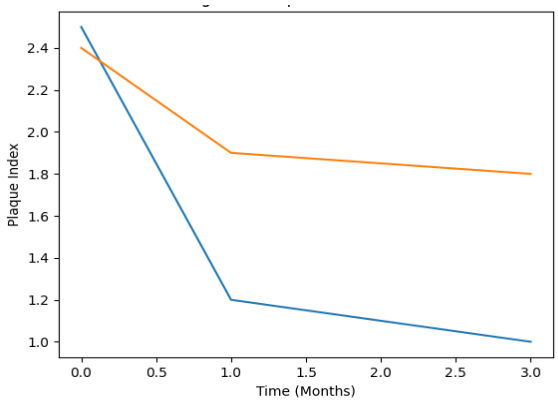

Figure 2: Changes in Plaque Index and Bleeding on Probing Over Time
Line graph comparing CHX and control groups at baseline, 1 month, and 3 months

Bleeding on Probing
The chlorhexidine group achieved a major reduction in bleeding on probing, which started at 35% during the first evaluation and reached 10% at the 3-month assessment ($p < 0.001$). The proportion of bleeding sites at 3 months was 18.5% in the CHX group compared with 36.2% in the control group, corresponding to an adjusted mean difference of −17.7 percentage points (95% CI −28.4 to −7.0; $p = 0.003$). The control group recorded a small decrease in bleeding, which continued from start to finish of the observation period ($p < 0.05$).

Peri-Implant Mucosal Inflammation
Bleeding on probing was significantly reduced in the CHX group compared with controls, with detailed estimates shown in Table 2. The control group recorded restricted improvement, which did not match the extent of change observed in the other group.

Radiographic Marginal Bone Changes
The radiographic evaluation recorded that both groups maintained small amounts of marginal bone loss throughout their initial healing period. At 3 months, mean marginal bone loss was 0.42 ± 0.18 mm in the CHX group and 0.47 ± 0.21 mm in the control group. The between-group mean difference was −0.05 mm (95% CI −0.19 to 0.09; $p = 0.46$) (Table 2). The confidence interval indicates that small between-group differences in marginal bone level changes cannot be excluded.
The bone level assessment through radiography became standardised using identical projection methods and a specific implant reference point, which served as a fixed point for all subsequent measurements. Given the short follow-up and modest between-group differences, the study may be underpowered to detect small changes in marginal bone remodelling; therefore, the absence of statistical significance should not be interpreted as definitive equivalence between protocols.

Adverse Effects
No serious adverse events were observed during the study period. Mild, self-limiting adverse events were reported predominantly in the chlorhexidine (CHX) group and included transient taste disturbance and minor oral mucosal irritation. All reported events were classified as mild in severity, required no medical intervention, and resolved spontaneously without interruption of the study protocol. No participants withdrew from the study due to adverse events (Table 3).

Table 3: Adverse events reported during the study period

Adverse event	CHX group (n = 20)	Control group (n = 20)	Severity	Action taken
Taste disturbance	4 (20.0%)	0 (0.0%)	Mild	No treatment; resolved spontaneously
Oral mucosal irritation	2 (10.0%)	0 (0.0%)	Mild	No treatment; resolved spontaneously
Burning sensation	1 (5.0%)	0 (0.0%)	Mild	No treatment; resolved spontaneously

Serious adverse events	0	0	—	—

Values are presented as numbers (%)
Severity grading was based on clinical assessment (mild: transient symptoms requiring no intervention)
No adverse events resulted in treatment discontinuation or participant withdrawal

Adverse events were actively assessed at each follow-up visit and recorded using standardised clinical reporting forms.

In Vitro Results

At 72 hours, SaOS-2 cell viability in the 0.01% CHX group was 92.4 ± 6.1%, compared with 100 ± 5.3% in the control group, corresponding to a mean difference of −7.6% (95% CI −12.4 to −2.8; p = 0.004). Exposure to 0.05% CHX resulted in a greater reduction in viability (68.3 ± 7.9%), with a mean difference of −31.7% (95% CI −38.9 to −24.5; p < 0.001) versus control. The 0.12% CHX concentration caused marked cytotoxicity, reducing viability to 21.5 ± 6.4%, significantly lower than both the control (p < 0.001) and the lower-concentration groups after Bonferroni adjustment (Table 4, Figure 3). All pairwise comparisons were adjusted for multiple testing using the Bonferroni correction.

Table 4: Effect of chlorhexidine concentration on in-vitro cell viability and extracellular matrix protein expression

Outcome	Group	Mean ± SD	Mean difference vs control (95% CI)	Adjusted p-value†
Cell viability (%) – 72 h	Control	100.0 ± 5.3	Reference	—
	0.01% CHX	92.4 ± 6.1	−7.6 (−12.4 to −2.8)	0.004
	0.05% CHX	68.3 ± 7.9	−31.7 (−38.9 to −24.5)	<0.001
	0.12% CHX	21.5 ± 6.4	−78.5 (−86.2 to −70.8)	<0.001
Collagen I (AU)	Control	1.20 ± 0.21	Reference	—
	0.01% CHX	1.12 ± 0.18	−0.08 (−0.19 to 0.03)	0.14
	0.05% CHX	0.78 ± 0.16	−0.42 (−0.58 to −0.26)	<0.001
	0.12% CHX	0.31 ± 0.12	−0.89 (−1.03 to −0.75)	<0.001
Fibronectin (AU)	Control	1.15 ± 0.20	Reference	—
	0.01% CHX	1.08 ± 0.19	−0.07 (−0.18 to 0.04)	0.21
	0.05% CHX	0.72 ± 0.15	−0.43 (−0.58 to −0.28)	<0.001
	0.12% CHX	0.29 ± 0.11	−0.86 (−1.00 to −0.72)	<0.001

Data are presented as mean ± SD from three independent biological replicates. AU = arbitrary units. †Adjusted p-values derived using Bonferroni post-hoc correction following one-way ANOVA

Quantitative immunofluorescence analysis demonstrated that collagen I expression in the 0.01% CHX group (1.12 ± 0.18 AU) did not differ significantly from control (1.20 ± 0.21 AU), with a mean difference of −0.08 AU (95% CI −0.19 to 0.03; p = 0.14). In contrast, collagen I expression was significantly reduced in the 0.05% CHX group (0.78 ± 0.16 AU; mean difference −0.42 AU, 95% CI −0.58 to −0.26; p < 0.001) and further suppressed in the 0.12% group (0.31 ± 0.12 AU; p < 0.001 vs all other groups).

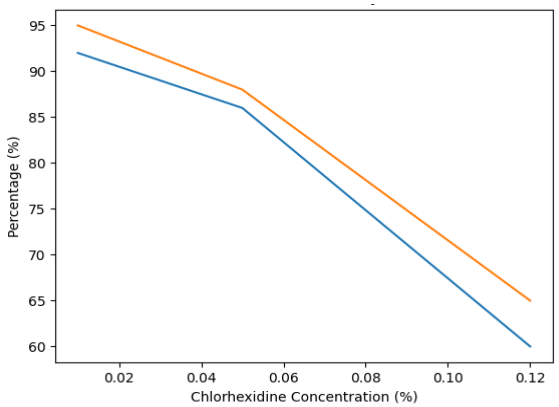

Figure 3: Effect of Chlorhexidine Concentration on Cell Viability and Extracellular Matrix Production

All reported p-values for pairwise comparisons were adjusted for multiple testing as specified in the Statistical Analysis section.

Discussion

The study combines laboratory tests with medical studies to record that chlorhexidine produces its biological and clinical responses based on the amount used and the length of time it is applied. The study combines laboratory cell tests with brief medical results to demonstrate how controlled chlorhexidine application impacts peri-implant tissue health through particular biological processes.

The biological data from in vitro tests record that osteoblast-like cells, fibroblasts and extracellular matrix remain viable when exposed to 0.01% chlorhexidine solutions. The study results match previous studies, which demonstrated that diluted chlorhexidine solutions do not harm osteoblast cell functions or fibroblast ability to produce collagen, which both play critical roles in bone development and tissue closure at implant sites (14). The study findings demonstrate that chlorhexidine at reduced concentrations safeguards both blood cells and connective tissue structures, which confirms its biological suitability for the first stages of tissue recovery.

The study results recorded that cell proliferation rates decreased step by step, but ECM production remained constant when the concentration reached 0.05%. The intermediate response demonstrates biological threshold levels which antimicrobial agents need to impact cell functions before they start causing substantial cell damage. Study data indicate that coatings with chlorhexidine and nanoparticle delivery systems achieve equivalent antimicrobial effects through their controlled dose delivery mechanism, which decreases cellular metabolic activity (14). The study findings demonstrate that brief exposure to chlorhexidine at moderate levels appears to be safe for biological systems when patients maintain their implants away from peri-implant tissues.

The 0.12% chlorhexidine solution caused extensive cell damage, which resulted in complete cell death, prevented cell proliferation and destroyed the extracellular matrix structure.

The laboratory results demonstrated that exposure to 0.12% CHX led to pronounced cytotoxicity, with a marked reduction in cell viability that became more evident with prolonged exposure. The different results between laboratory tests and mouth rinse tests in the mouth stem from how cells interact with these substances. The laboratory tests allow cells to stay in contact with the substances for extended periods, but mouth rinse solutions in the mouth stay in contact with cells for short times before saliva breaks them down, and the body removes them. The in vivo environment of peri-implant tissues contains a protective microenvironment which includes pellicle formation, protein binding, and tissue buffering, but these elements remain unattainable in basic monolayer cell cultures. The results from in vitro cytotoxicity tests function as warning signs which become clinically important when samples remain in contact for long periods and when they achieve high concentrations near bone and connective tissue areas. Research conducted before this study demonstrated that CHX induces cell death through mechanisms which depend on exposure duration and concentration when scientists applied it to fibroblasts and osteoblast-like cells.

The study data about cell shrinkage and loss of adhesion confirmed previous laboratory results, which demonstrated that osteoblast-like cells and gingival fibroblasts undergo apoptosis-like cell death and cytoskeletal damage when they encounter high concentrations of chlorhexidine

(15). The study records that using high-strength chlorhexidine solutions for long periods will damage the biological structures which are essential for successful bone-to-implant integration and tissue attachment.

The study found that chlorhexidine treatment during a brief postoperative period led to better results in soft tissue health around implants through its ability to reduce plaque formation, bleeding and mucosal inflammation. The study findings from this study confirm previous results from randomised clinical trials and systematic reviews, which record that chlorhexidine treatment as an additional therapy enhances plaque control and reduces inflammatory markers during the first stage of peri-implant healing (16). The chlorhexidine group recorded a significant decrease in bleeding on probing because the treatment successfully controlled the initial inflammatory response, which occurs when microorganisms first colonise the teeth.

The radiographic assessment recorded that both study groups experienced small amounts of marginal bone loss, which did not reach statistical significance between the chlorhexidine group and the control group. The study indicates that brief controlled chlorhexidine treatment does not affect the process of bone attachment to implants or the stability of bone tissue near the implant edges. Similar observations have been reported by Alassy et al. (2021) and Dumitriu et al. (2023) (16, 17), who concluded that chlorhexidine used as an antiseptic supplement for implant treatment does not affect the healing process of peri-implant bone tissue. The study findings validate chlorhexidine safety for medical practice because the treatment did not lead to any implant breakdowns or problems with tissue recovery.

The study documented two negative reactions, which included short-term taste disturbances and minimal mouth tissue discomfort that scientists have previously identified as chlorhexidine adverse reactions in their studies (18). The reversible nature of these effects, together with the lack of severe tissue damage records that short-term chlorhexidine treatment provides more advantages than its limited side effects when doctors use it correctly.

The study results demonstrate that peri-implant care requires the same attention to antimicrobial treatment effectiveness as it does to biological acceptance of these treatments. The study findings indicate that healthcare providers need to develop evidence-based treatment protocols which will determine both the right concentration of chlorhexidine and its duration of application. The present findings support that short-term, protocol-based use of CHX within the tested concentration range (0.01%–0.12%) can improve early peri-implant plaque and bleeding outcomes without detectable short-term harm to marginal bone levels.

Study limitations

The study has a restricted clinical observation duration, which makes it impossible to evaluate how implants will perform in the future and how bone margins will stabilise. The study results became less reliable because the study involved too few participants to detect minor differences between treatment results. The in vitro model fails to duplicate the complete biological conditions which exist in peri-implant tissues when studied in living organisms. Future studies require participants to be monitored for longer durations with more participants to validate the findings from this study. The confirmation of early biological advantages into long-term peri-implant stability needs randomised trials, which will run for an extended period.

The absence of a formal sample size calculation for some secondary outcomes, particularly marginal bone level changes, increases the risk of type II error. Consequently, non-significant differences in radiographic outcomes should be interpreted cautiously and warrant confirmation in larger, adequately powered studies. Because the study was powered on a soft-tissue clinical outcome, it may not have been adequately powered to detect small between-group differences in marginal bone level changes. Consequently, non-significant findings for radiographic outcomes should be interpreted cautiously as they may reflect limited statistical power rather than the true absence of effect.

Another limitation of this study is the absence of allocation concealment, which may have introduced selection bias at the point of group assignment. Although examiner blinding and standardised outcome assessment protocols were employed to reduce measurement bias, the lack of concealment could have influenced enrolment or treatment expectations. Future confirmatory randomised trials should incorporate robust allocation concealment methods, such as centralised randomisation or sequentially numbered opaque sealed envelopes.

Although significant differences were detected for soft-tissue clinical outcomes, the study was not powered to detect small between-group differences in marginal bone loss; therefore, non-significant

radiographic findings should be interpreted cautiously as potential type II errors.

Conclusion

The study demonstrates that chlorhexidine achieves its biological and clinical effects through the amount applied and the duration surfaces remain exposed to the solution. The study recorded that low to moderate concentrations of the solution improved peri-implant soft tissue health while decreasing inflammatory markers without causing any harm to osteoblast-like cells or fibroblast activity or initial marginal bone stability. The study records that high concentrations of the substance cause significant cell death in laboratory tests, which requires scientists to handle it with care. The use of chlorhexidine as a short-term protocol-based treatment in implant dentistry offers safe antimicrobial protection, which appears effective while minimising short-term biological harm to tissues.

List of Abbreviations

CHX: Chlorhexidine
ECM: Extracellular matrix

Declarations

Ethics Approval, trial registration, and Consent to Participate

The clinical protocol was reviewed and approved by the Science and Ethics Committee of the University of Al-Ameed (Approval No. 8.2025, 2 Sep. 2025). This study was not prospectively registered in a clinical trial registry because the clinical component was designed and initiated as an exploratory pilot investigation intended to generate preliminary biological and clinical data. The study protocol, outcomes, and analysis plan were defined before participant enrolment, and no changes were made after recruitment commenced. All participants provided written informed consent before enrolment. All procedures followed the Declaration of Helsinki.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Data Availability

De-identified individual participant data and the in-vitro raw datasets (CSV) that underlie the results reported in this article are available from the corresponding author on reasonable request.

Competing Interests

None**.**

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None.

Author Contributions

MMA contributed to the conception and design of the study, supervision of experimental work and helped in the interpretation of data, writing and revision of manuscript. The experiments were designed, executed and data analysed by SKM, who also wrote the manuscript. AZD was involved in analysis, literature review, and critically revised the manuscript for important intellectual content. The final manuscript was read and approved by all authors.

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