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Chitosan-based biodegradable dental chips impregnated with chlorhexidine gluconate for the treatment of periodontitis

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Chlorhexidine (CHX) is a synthetic cationic biguanide commonly used in dentistry to control infections. In the present study, cost-effective biodegradable dental chips of CHX (0.2%) with varying concentrations of high-molecular-weight chitosan (2%, 3%, and 4%) were prepared using the solvent casting method. The identification, purity, and interaction between the active drug and excipients have been confirmed using FTIR spectrometry. The formulated chips exhibited a pH of around 5, with excellent folding endurance of ≥1000, a thickness of 0.34–0.42 mm, and a moisture loss of about 10–14%. Organoleptically, the chips were consistent for at least three months at room temperature. The assay of CHX was performed using a validated HPLC method. The content uniformity of the chips was found to be greater than 90%, indicating a uniform distribution of the active drug. The release of CHX from the chips slowed down from 31 to 72 h with an increase in polymer concentration from 2 to 4%. The release followed the Higuchi model for 2% and 3% chips and the Korsmeyer–Peppas model for 4% chips. All the chips demonstrated stability for only one month under accelerated temperature and humidity conditions (*i.e.*, 40 °C/75% RH). Significant antimicrobial activity has been observed for both placebo and CHX-loaded chips against various standard and clinical isolates, with good activity on cementum. The formulated CHX dental chips offer an economical and effective drug delivery system for treating periodontal infections, due to their potent antimicrobial effect and sustained drug release, which facilitates the desired therapeutic effects.

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1. Introduction

Oral health is a fundamental component of overall health, and it is known to be affected by various oral diseases. One of those diseases is periodontitis, which causes bone loss due to the presence of harmful bacteria in the gingival pockets. If periodontitis is not treated promptly, it can lead to tooth loss, resulting in significant functional, aesthetic, and psychological impacts on affected individuals. Additionally, this condition may negatively affect speech, nutrition, quality of life, and self-esteem. It may also trigger systemic inflammation, potentially leading to severe conditions such as atherosclerosis,[1,2] myocardial infarction,[3,4] and diabetes mellitus.[5–9] Identifying a relationship between periodontal disease and certain systemic conditions or events can enhance care and attention to overall health as a preventive or therapeutic strategy.

Scaling and root planing (SRP) is the non-surgical treatment for early periodontitis. At the same time, antimicrobials such as chlorhexidine (CHX) are often used as adjunctive therapy and are known to yield effective therapeutic results. CHX is a synthetic drug usually used to treat pathologies in the oral cavity. CHX possesses unique qualities that have made it the standard criterion substance for many years. These qualities include its bacteriostatic, bactericidal, and antimicrobial effects, high substantivity, lack of systemic toxicity, and minimal susceptibility to bacterial resistance. CHX prevents biofilm development and plaque formation on the tooth's surface by inhibiting the adherence of microorganisms, thereby maintaining a pathogen-free environment that regenerates the periodontium and restores normal physiology.[10–12] It is used both as a prophylactic and a therapeutic agent. Prophylactically, it is used for pre- and post-operative disinfection, while as a therapeutic agent, it is used in periodontal therapy.

CHX chips are one of the local drug delivery devices; when used as an adjunct to SRP, the chances of keeping the patient disease-free increase many folds.[13] These chips are thin, flat, implantable therapeutic devices that maintain contact with the target tissue and sustain controlled drug release. These chips possess appropriate physical properties that enable them to affix to the desired location and remain unobtrusive to the

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target site. CHX, when released from the chips, reaches inaccessible periodontal pockets, delivers accurate doses of drugs with minimal side effects, and improves the patient's overall health.[14,15]

Considering all the advantages of a chip-based local drug delivery system, this study has been designed to develop a chitosan-based intra-pocket local drug delivery system with a reservoir of CHX that prolongs its release and maintains therapeutic levels for the required period. It will help to inhibit the inaccessible subgingival bacteria without harming tissues. Chitosan-based CHX dental chips will be developed and evaluated for polymer concentration effect, dissolution, drug release, antimicrobial cementum activity on isolates and cementum, physical and mechanical characterization. The formulated CHX dental chips, due to their improved antimicrobial activity and enhanced drug dose retention, could facilitate the desired therapeutic effects for an extended period. The study of polymer concentration, substantivity of CHX from the chips on the cementum, and the simplicity and economy of the formulation will add value to the existing literature. The data generated from this study will help formulation scientists in developing an effective CHX-based delivery system for periodontal infections.

2. Materials and methods

2.1. Materials

Chitosan (high molecular weight, 200–600 mPa s) was obtained from TCI, Japan. Glycerol (99.0–101.0%) and acetic acid (99.5–100.5%) were obtained from Sigma Aldrich, Germany, whereas CHX gluconate (19.0–21.0%) was sourced from Hangzhou Starshine, China. Freshly prepared, double reverse osmosis deionized water with a zero TDS (total dissolved solids) level was used throughout the study (FineTech Water Treatment, Pakistan). Simulated saliva (sorbic acid-free) was procured from Baqai Compounding Pharmacy, Karachi. All other chemicals and reagents were of analytical grade; therefore, they were used without further purification.

2.2. Methods

2.2.1. FTIR spectroscopy. The Nicolet iS5 FTIR spectrometer (Thermo Fisher Scientific, USA) was used to record the IR spectra. The sample was placed on an ATR diamond crystal (iD7 ATR, Thermo-Fischer Scientific, UK), and the spectra were collected by performing 64 scans in the range of 4000–700 cm^{-1} at a resolution of 4 cm^{-1}. The spectra were processed by OMNIC software (version 9.0, Thermo Fisher Scientific, USA).

The interaction between the drug and excipients was investigated using Fourier Transform Infrared (FTIR) spectrometry. The drug with each excipient was mixed separately in a 1 : 1 ratio, and the changes were observed using an FTIR spectrometer (Nicolet iS5, Thermo Fisher Scientific, USA), similarly as described above, for up to seven days. The spectra of the chips were also analyzed similarly.

2.2.2. pH measurements. The pH of the gels was recorded using a digital pH meter (Elmetron CP-501, Poland; sensitivity ±0.01 pH units). Commercially available buffer tablets (Merck) with pH levels of 4.0 and 7.0 were used to prepare buffer solutions for calibrating the pH meter at room temperature (25 ± 2 °C). All measurements were made with a combination glass electrode immersed directly into the gel, and the readings were recorded in triplicate. The temperature of the gels was measured using a probe attached to the pH meter.

The pH of the chips was measured by placing 200 mg of the chips in a beaker and adding 25 ml of water to it. The chip was manually stirred in the water for a few minutes, and pH measurements were made as described above.

2.2.3. Formulation of CHX chips

2.2.3.1. Preparation of chitosan gels. A total of three batches of gel were prepared (Table 1) using high-molecular-weight chitosan at concentrations of 2%, 3%, and 4%. The concentration of CHX (0.2%) remained the same in all batches, while glycerol (5%) was added as a plasticizer. All the chemicals were dissolved in an acetic acid solution (2%), which served as the solvent. The gel was then transferred to glass jars and kept at room temperature. Placebo gels were also prepared similarly.

2.2.3.2. Preparation of CHX chips. About 4 g of the gel was weighed and poured uniformly into the silicon cups. The gel took approximately three days (~72 h) to completely dry and form thin chips at ambient room temperature (25 ± 2 °C). The dried chips were then removed from the silicon cups, packed in aluminum foil, and stored in a desiccator for further studies.

2.2.4. Thickness measurements. A calibrated digital vernier caliper was used to measure the thickness of each chip at five different positions, including the middle and four corners. The mean of these readings at various points was taken, and the standard deviation was calculated. Each experiment was performed in triplicate.

2.2.5. Folding endurance. To calculate the folding endurance, the placebo and CHX-loaded chips (size 4 × 4 cm) were individually folded at an angle of 180° repeatedly at the exact location until a crack or breakage was observed,[16] or when the reading reached 1000 folds. The number of times the chip folded was recorded, and each reading was taken in triplicate.

2.2.6. Determination of moisture content. The chips of each formulation were cut into 2 × 2 cm pieces (100 mg) from each batch in triplicate, and the initial weights were noted on

Table 1 Composition of CHX gel

	Composition (%, w/v)		
Ingredients	F1	F2	F3
CHX	0.2	0.2	0.2
Chitosan	2	3	4
Glycerin	5	5	5
Acetic acid solution (2%, v/v)	Q.S.[a]	Q.S.[a]	Q.S.[a]

[a] Q.S. = quantity sufficient to produce 100%.

a digital weighing balance (AY220, Shimadzu, Japan). After recording the initial weight, the chips were stored in a desiccator with silica beads for 72 h. The chips were reweighed, and the difference in weight was calculated using the following formula.[17]

$$\%\,\text{Moisture loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

2.2.7. Swelling index ratio. The 2 × 2 cm pieces of chips (100 mg) from all the formulations were uniformly cut in triplicate. The initial weight was recorded by a digital weighing balance (AY220 Shimadzu, Japan). The sieve of foil was made by puncturing minute holes in it. The simulated saliva (pH 6.8) was maintained in a water bath at 37 °C.[18] The sieve containing the chip was immersed in 15 ml of simulated saliva in a Petri dish. The weight was recorded at pre-decided intervals of 30, 60, 90, 120, 150, 180, and 210 s. A change in the weight of the chip was recorded at each time interval until a constant weight was achieved. Every time, the chips were slightly wiped off using tissue paper.[19] The swelling index (S.I.) ratio of the chips was calculated using the following formula.[20]

$$\text{S.I.} = \frac{W_t - W_0}{W_0}$$

where W_t = weight of chip at time t, and W_t = weight of chip at zero time.

2.2.8. Organoleptic studies. The organoleptic properties of the chips were thoroughly evaluated over six months by healthy volunteers (n = 5; male = 2, female = 3). This evaluation included a physical examination of color, appearance (smooth and glossy), odor, taste, and visual inspection for microbial growth. Each parameter was monitored to detect any physical changes or variations in the chips during the study period.

2.2.9. Scanning electron microscopy (SEM). An analytical scanning electron microscope (JSM-6380A, JEOL, Japan) equipped with an EDS detector (EX-54175jMU, JEOL, Japan) was used for a detailed topographic study of the chips (with and without CHX). The samples were cut to size, placed on the stub, and attached with double-sided adhesive tape. All the samples were coated with gold particles for electron beam conduction using a Quick Auto Coater (JFC-1500, JEOL, Japan), an ion sputtering device. The surface and cross-sectional views of the chips were obtained at an accelerated voltage of 15 kV, and images were taken at various magnifications.

2.2.10. Assay of CHX chips. A simple, rapid, and sensitive reversed-phase (RP) high-performance liquid chromatography (HPLC) method, as reported by Scholz *et al.*,[21] was used for the assay of CHX in the prepared chips. The assay of CHX was carried out on an HPLC system (LC-10AD, Shimadzu, Japan) equipped with a UV detector (SPD-10AV, Shimadzu, Japan). Each time, 10 μl of the respective dilution was injected into the sampler (CTO-10A, Shimadzu, Japan), and an ultrasonic cleaner unit (FSF-010S, Shimadzu, Japan) was used to remove bubbles from the solvent system. The assay was performed at room temperature (25 ± 2 °C). Detection was achieved in under 10 min using a C18 column with specifications of 5 μm, 175 Å, and 4.6 × 250 mm (Thermo Fisher Scientific, USA) at a wavelength of 251 nm with a flow rate of 1.0 ml min^{-1}. The mobile phase consisted of acetonitrile–phosphate buffer (35 : 65, v/v, pH 3.0), which contained sodium dihydrogen phosphate (0.08 M) and triethylamine (0.5%).

2.2.10.1. Sample preparation. The stock solution for pure CHX (2.5 mg per 100 ml) was prepared in water and stirred for 5 min on a magnetic stirrer. Appropriate dilutions were prepared from this stock solution. The assay of the CHX-loaded chips (containing 8 mg CHX per chip) was performed by cutting the chips into small pieces, immersing them in 100 ml of water, and stirring them on a magnetic stirrer at a slow speed for 24 h. Approximately 2 ml of the sample was taken from this stock and diluted with water to 10 ml. The prepared chip solution (16 μg ml^{-1}) was then injected into the HPLC for analysis, and the area under the curve was noted.

2.2.11. Validation of HPLC assay. The HPLC method for determining CHX was validated according to the guidelines of the International Council for Harmonization (ICH).[22] The validation parameters included system suitability, linearity, range, accuracy, precision, and sensitivity. The details of these parameters are provided in the SI.

2.2.12. Drug content uniformity test. Each formulation chip was cut into four equal parts, weighing approximately 1 g. Each cut part was separately dissolved in 25 ml of water and stirred slowly on a magnetic stirrer for 24 h. A 2 ml sample was taken from this solution and further diluted to 10 ml (16 μg ml^{-1}), and the assay was performed as described earlier. Each experiment was repeated in triplicate, and the standard deviations were calculated.

2.2.13. *In vitro* drug release testing. The dissolution test was performed using the basket method (Type-I dissolution apparatus, GDT-6L, Galvano Scientific, Pakistan). The dissolution medium consisted of 900 ml of simulated saliva (pH 6.8), maintained at 37 °C, and the chip (4 g) was placed in a basket immersed in the medium, which was then rotated at 50 rpm. The samples (5 ml) of the solution were withdrawn at definite time intervals up to 4320 min. To maintain a constant volume (sink condition), 5 ml of the dissolution medium was added each time after removing the sample aliquot for analysis. The solution was passed through a 0.22 μm membrane filter (Durapore, Millipore, Ireland) and subjected to HPLC analysis according to the method mentioned above. The test for each formulation was carried out in triplicate, and the release was expressed as a percent cumulative drug release. Various kinetic models were applied to study the release profiles, including zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models, and the R^2 values were calculated.

Zero-order:

$$C_0 - C_t = k_0 - t$$

First-order:

$$\ln\,(C_0/C_t) = kt$$

Higuchi:

$$C_t = k_H t^{1/2}$$

Korsmeyer–Peppas:

$$C_t/C_0 = kt^n$$

Hixson–Crowell:

$$C_0^{1/3} - C_t^{1/3} = kt$$

where, C_0 = initial concentration; C_t = concentration at time t, k_H = Higuchi dissolution constant; k = release rate constant; and n = slope.

2.2.14. Stability studies. The formulated chips were stored in a stability chamber (Binder, Germany) for three months at a temperature of 40 °C and a relative humidity of 70%. The chips were removed each month for analysis by HPLC. The organoleptic properties of placebo and CHX-loaded chips were compared to those stored at room temperature.

2.2.15. Antimicrobial analysis. The antimicrobial activity of blank and sample chips was tested against standard ATCC (American Type Culture Collection) cultures of *Staphylococcus aureus* (ATCC 6538), *Streptococcus mutans* (ATCC 35668), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) using the disc diffusion method. Similarly, clinical isolates of *S. aureus*, *S. mutans*, *E. coli*, *P. aeruginosa*, and *Candida albicans* were also used. The chips were punched using a punching machine to obtain uniform discs with a diameter of 6 mm. The Petri plates (8.5 × 8.5 cm) of trypticase soy agar (Condalab, Spain) were prepared by pouring a constant amount of the medium (~19 ml) to obtain a thickness of 4 mm. The culture of each microorganism was standardized using a 0.5 McFarland standard and spread evenly over the plates using a sterile cotton swab. The discs of blank and sample chips for each formulation were placed at equal distances on the Petri plates and incubated at 37 °C for 24 h for bacteria and at 30 °C for 36 h for *C. albicans*. After the completion of the incubation period, the zones of inhibition were measured by recording the diameter of the zone around the discs at three different locations using a vernier caliper, and the mean diameter was calculated. Each experiment was repeated in triplicate.

2.2.16. Substantivity of CHX on cementum. An *in vitro* study was designed to assess the efficacy of the CHX chip and CHX solution on root cementum against various isolates, as discussed in the above section. The study was performed after taking approval from the Institutional Review and Ethics Board (IREB) of Baqai Medical University, Karachi (Ref: BMU-EC/03-2022). The extracted non-carious teeth were collected and sliced into discs of 6 mm using diamond disc burs (C01/190, 0.30 mm, and C06/220, 0.15 mm). The samples were divided into three groups and medicated for seven days to check the antimicrobial effect of CHX substantivity on the cementum. A total of three test tubes containing the sliced teeth were filled with 5 ml of simulated saliva. Out of these three tubes, one test tube containing saliva served as the control; in the second tube, a 0.2% CHX solution (0.09 ml) was added, while in the third tube, a CHX chip (2 × 2 cm) was placed (Fig. S1). After seven days, the antimicrobial activity was determined using a procedure similar to the one described above.

3. Results and discussion

3.1. Identification and purity determination of CHX and excipients

In the present study, before commencing the formulation work, the purity of each ingredient and the detection of any potential impurities or degradation products were confirmed by performing FTIR spectrometry. CHX consists of a central hexamethylene chain connected with a biguanide group on both sides and two symmetric 4-chlorophenyl rings at the end (Fig. S2a). The FTIR spectrum for the CHX solution (Fig. S2a) exhibits a broad band corresponding to an intense N–H stretch between 3700 and 2800 cm^{-1}. The absorption area in the range of 1800–1400 cm^{-1} relates to the fingerprint region of CHX. The absorption peaks at around 1640 and 1540 cm^{-1} are due to the stretching and bending vibrations of C=N and N–H, respectively. The region in 1500–1400 showed the peaks between C=C stretching, C–H deformation and bending, and C–N–H bending vibrations.[23,24] The peak observed at around 1093 cm^{-1} is related to the stretching of the chloride bonded to the aromatic ring of the CHX.[23] The chlorine atoms attached to the phenolic group are responsible for the biological activity of the drug.[25] The FTIR spectrum peaks of CHX (Fig. S2a) agree with those reported in the literature,[23,24,26] thus confirming that the compound used is in its pure form.

Chitosan is a linear polysaccharide composed of randomly distributed β-(1,4)-linked D-glucosamine and *N*-acetyl-D-glucosamine units (Fig. S2b). The FTIR spectrum of the chitosan polymer (Fig. S2b) exhibits a broad peak corresponding to the stretching vibrations of the O–H and N–H groups, located between 3600 and 3000 cm^{-1}. A small peak around 2900 cm^{-1} corresponds to the aromatic C–H stretching.[24] The region between 1700–1300 cm^{-1} belongs to the C=O stretching of the acetylated carbon amide group, $-NH_2$ bending, C–O stretching of the primary hydroxyl groups, and C–H bending vibrations. The peak at 1200–1000 cm^{-1} corresponds to the C–O–C and C–O–H stretching vibrations, while the peak around 900 cm^{-1} represents C–O and C–C stretching vibrations.[24,27,28]

Glycerin is a simple triol compound (Fig. S2c). The FTIR spectrum of glycerin (Fig. S2c) exhibits a broad peak in the region of 3500–3000 cm^{-1}, corresponding to the –OH stretching vibrations of the glycerin molecule. A twin peak in the 3000–2800 cm^{-1} region could be attributed to the C–H stretching. The region between 1600–800 cm^{-1} corresponds to the C–O stretching of the primary hydroxyl groups, as well as C–H and C–C deformation, bending, and stretching vibrations.[24]

3.2. Interaction studies

Interaction studies are crucial for ensuring the stability, safety, and efficacy of pharmaceutical dosage forms, which can be

influenced by the multichemical combinations.[29] CHX was mixed with chitosan and glycerin in a 1 : 1 ratio to identify any interaction between them. The spectrum obtained from the mixture of CHX with chitosan is shown in Fig. S3a, and with glycerin is shown in Fig. S3b. It is interesting to note that the pattern in the combined spectra (Fig. S3a and b) did not change much when compared with the spectra of pure CHX (Fig. S2a), chitosan (Fig. S2b), and glycerin (Fig. S2c) samples. No new peaks have been identified, while the peaks of both compounds in a similar region are found to be merged, resulting in a slight shifting of the peaks (Fig. S3).

Likewise, no change has been observed in the FTIR spectra of the formulated chips (formulation discussed in the later section) at different ratios (Fig. S4), which showed the same characteristic peaks as observed in the parent molecule (Fig. S2). The spectral analysis revealed minimal changes, indicating a negligible level of interaction. Consequently, all the compounds are compatible and suitable for incorporation into the formulation process.

3.3. Formulation of CHX chips

A thorough literature review was conducted prior to the formulation of CHX chips.[18,30–35] The CHX chips were prepared using a simple solvent casting method, in which all the ingredients were mixed and dissolved in a 2% acetic acid solution to form a gel. The viscosity measurement details of the gels are reported in the SI. After drying at room temperature for a specific time (~72 h), the CHX chips were obtained. The concentration of CHX has been selected based on its optimal bactericidal activity, as recommended in the Handbook of Pharmaceutical Excipients.[34]

Chitosan has been used as a gelling and viscosity-enhancing agent in this formulation due to its multiple benefits in drug delivery systems. The most significant advantage is its biodegradability and non-toxicity,[34] which is valuable in medical applications. Chitosan degrades into its constituent monomers, one of which is glucosamine, a compound used as a dietary supplement for joint health due to its anti-inflammatory properties.[36,37] It exhibits excellent mucoadhesive properties due to reactive hydroxyl and amino groups, which provide excessive hydrogen bonding and a positive charge.[38] This property also enables it to interact with the negatively charged mucous surface, playing a role in the sustained release of drugs through prolonged adhesion.[39] Additionally, it has low production costs and is easy to process.[40] Chitosan also exhibits antimicrobial activity against various microbes, including bacteria and fungi.[41]

Glycerin is a biocompatible and non-toxic material widely used in pharmaceutical and cosmetic preparations due to several properties.[34] In this study, it has been used as a plasticizer to maintain the moisture content of the formulation, thereby preventing brittleness and cracking of the chips, which allows for their easy insertion into the desired location. It is known to improve the mechanical properties, enhance drug delivery, and maintain the formulation's integrity over time.[42–44]

Acetic acid is used as the solvent in the manufacture of CHX chips. Its ability to dissolve chitosan helps form a homogenized solution, which, after casting and drying, produces a solid, smooth chip. Chitosan solutions or gels prepared with acetic acid have continuous, compact, and flexible surfaces, compared to those prepared with tartaric and citric acids, which can make the texture brittle and prone to breakage during handling.[45–47]

3.4. Physical characterization of CHX chips

3.4.1. pH measurements. The determination of pH is vital as highly acidic or basic components may irritate tissues of the body. In the present study, the pH of the gels before drying was observed to be acidic, with a value of around 4–5 for all three formulations (Table S1). It suggests that the formulations exhibit weak acidic characteristics, likely due to the addition of 2% acetic acid, which is ideal for enhancing the antimicrobial activity of chitosan and CHX. No pH adjustment was made in the gels before drying. In contrast, the pH values of the placebo and drug-loaded chips were almost identical (Table 2). The paired two-tailed *t*-test conducted on the pH values between the placebo and drug-loaded chips revealed no significant difference ($p = 0.822$).

It is interesting to note that the activity of CHX and chitosan is pronounced in acidic environments.[34] CHX exhibits its activity at a slightly acidic pH, whereas many microbes cause oral infections in pH conditions that are neutral to slightly basic. Therefore, the antimicrobial activity of CHX is enhanced in an acidic environment.[34,48] Similarly, chitosan behaves as a cationic polyelectrolyte in acidic conditions due to the protonation of amino groups. The cationic nature of chitosan enables it to play an antimicrobial role by interacting with negatively charged species or surfaces of microbial cell membranes. It disrupts membrane integrity and leads to the leakage of intracellular components, thereby rendering a wide range of microorganisms, including bacteria, fungi, and certain viruses, ineffective.[49,50]

3.4.2. Thickness of chips. Measuring the thickness of the prepared dosage form is an essential factor. Dose accuracy is directly related to thickness, which further ensures the aesthetics and uniformity of the product.[51] Generally, an ideal chip should exhibit a thickness between 0.05 and 1 mm;[52] however, a less variable thickness indicates good quality and consistency of the product.[53] The thickness of all the prepared chip formulations (both placebo and drug-loaded) ranged from 0.34 to 0.42 mm and increased with increasing polymer concentration (Table 2). The nominal standard deviation

Table 2 Physical parameters of the chips

Formulation	pH ± SD	Thickness (mm) ± SD	Folding endurance (folds)	Moisture loss (%) ± SD
1	4.6 ± 0.1	0.34 ± 0.01	1000	10.3 ± 0.41
2	5.0 ± 0.1	0.38 ± 0.02	>1000	13.0 ± 0.58
3	5.0 ± 0.1	0.42 ± 0.02	>1000	13.7 ± 0.49

values suggest that the formulation procedure and casting technique were adequate to produce chips with uniformity and consistent thickness (Table 2). Statistically, the paired sample two-tailed *t*-test revealed no significant difference in the thickness of the placebo and drug-loaded chips ($p = 0.650$).

3.4.3. Folding endurance of the chips. The elasticity and flexibility of the prepared chips are considered important physical characteristics, as they facilitate easy handling and application at the administration site.[54] The folding count of 300 is considered excellent for the films/chips. The higher the number, the better the mechanical strength and toughness of the product.[16,55]

The folding endurance results showed excellent flexibility for all three formulations (Table 2), with no significant difference. Due to the same readings, statistical calculations could not be made since no effort was made to exceed 1000 folds. The formulation prepared with 2% chitosan (F1) showed a folding endurance of 1000 folds, while the other two showed an endurance of over 1000 folds (Table 2). Thus, all the prepared chips exhibited excellent folding endurance values, indicating their good flexibility, possibly due to the incorporation of the plasticizer. These results, therefore, suggest that chips with such high folding endurance would be practically beneficial in clinical applications by dentists.

3.4.4. Moisture content of the chips. The percent moisture loss is a crucial parameter in assessing the reliability and physical stability of the chips, particularly under dry storage conditions. This test also indicates higher moisture absorption capacity, which is associated with improved mucoadhesion.[56] The percentage of moisture loss for all prepared chips is found to be approximately 10% or more (Table 2). The paired sample two-tailed *t*-test revealed no significant difference in moisture content between the placebo and drug-loaded chips ($p = 0.536$). These results thus support the claim that prepared chips will have good mucoadhesion, due to the presence of chitosan, which is known to have excellent mucoadhesion properties.[38]

Moreover, it has also been observed that the percentage moisture loss increases with an increase in polymer concentration (Table 2), due to the hygroscopic nature of the polymer. Chitosan is known to absorb more moisture after drying.[34] Thus, the more chitosan, the higher the moisture absorption will be. No difference in moisture loss has been observed between CHX-loaded and placebo chips.

3.4.5. Swelling index ratio of the chips. The swelling index describes a polymer's ability to absorb water. When the polymer is exposed to the solvent, there is an increase in volume due to the free energy of mixing, whereas the elastic retractile force opposes the deformation; hence, equilibrium is achieved, which dictates the extent of swelling. Thus, swelling is the state of balance between these two forces.[28,57]

The high swelling index values (Fig. 1) indicate that water molecules can easily penetrate the polymer.[16] The results show that the chip prepared with 2% chitosan (F1) has the highest swelling value compared to those containing higher percentages of chitosan (Fig. 1). These results align with the moisture

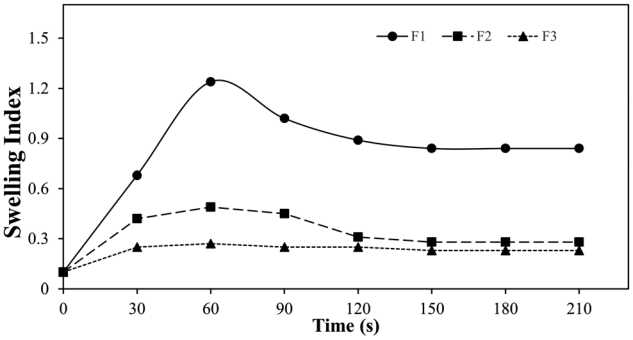

Fig. 1 A plot of swelling index *versus* time of the CHX-loaded chips. (F1) CHX-loaded chip with 2% chitosan, (F2) CHX-loaded chip with 3% chitosan, and (F3) CHX-loaded chip with 4% chitosan.

content data (Table 2), which indicates that chips with 2% chitosan exhibited lower moisture content; consequently, they absorbed more moisture for swelling than other formulations (Fig. 1). The swelling index ratio difference between chips containing 3% (F2) and 4% (F3) chitosan is found to be in a similar pattern but with a lesser difference (Fig. 1). The results also suggest that all chips quickly achieved a steady state of swelling. The pattern remained the same for both placebo and drug-loaded chips. Based on these findings, it can be stated that the chips with 2% chitosan (F1) can absorb more saliva and swell in the periodontal pocket compared to formulations F2 and F3.

3.4.6. Organoleptic properties of the chips. The organoleptic evaluation of all formulations was performed for both CHX-loaded and placebo chips at room temperature (25 ± 2 °C) and elevated temperature (40 ± 2 °C), and the results are reported in Table 3. Both placebo and drug-loaded chips showed identical results. The color and appearance of all the chips have been observed visually. It has been noted that the chips with lower polymer concentrations appeared almost colorless. On the contrary, the chips with higher polymer concentrations became slightly yellow since chitosan exerts a yellowish shade.[34,58,59] The storage temperature and duration also affected the color of the chips, as they darkened over time, particularly at higher temperatures, resulting in a color change from yellow to golden (dark yellow) to brown (Table 3). The color change has also been observed in placebo chips, thus making it evident that the change is due to chitosan at elevated temperature. Other workers have also observed a similar color change, particularly at higher temperatures, which may be attributed to the Maillard browning reaction or the interaction between chitosan and acetic acid.[58,60–66]

All formulations appeared smooth with a glossy texture, which remained unchanged at any temperature for six months (Table 3). The odor of all the chips was found to be pungent, like vinegar, which is attributed to the incorporation of an acetic acid solution as a solvent. The chips are also found to be tasteless despite having a vinegar-like odor. No visible growth has been observed in any formulations for six months, due to the antimicrobial properties of CHX, chitosan, and

Table 3 Organoleptic evaluation of the formulated chips

		Color		Appearance		Odor		Taste		Visual growth	
Time (month)	Formulation	RT	ET	RT	ET	RT	ET	RT	ET	RT	ET
0	F1	C	C	SG	SG	P	P	TL	TL	−ve	−ve
	F2	C	C	SG	SG	P	P	TL	TL	−ve	−ve
	F3	Y	Y	SG	SG	P	P	TL	TL	−ve	−ve
1	F1	C	Y	SG	SG	P	P	TL	TL	−ve	−ve
	F2	C	Y	SG	SG	P	P	TL	TL	−ve	−ve
	F3	Y	G	SG	SG	P	P	TL	TL	−ve	−ve
2	F1	C	G	SG	SG	P	P	TL	TL	−ve	−ve
	F2	C	G	SG	SG	P	P	TL	TL	−ve	−ve
	F3	Y	B	SG	SG	P	P	TL	TL	−ve	−ve
3	F1	C	G	SG	SG	P	P	TL	TL	−ve	−ve
	F2	Y	B	SG	SG	P	P	TL	TL	−ve	−ve
	F3	Y	B	SG	SG	P	P	TL	TL	−ve	−ve
4	F1	C	G	SG	SG	P	P	TL	TL	−ve	−ve
	F2	Y	B	SG	SG	P	P	TL	TL	−ve	−ve
	F3	Y	B	SG	SG	P	P	TL	TL	−ve	−ve
5	F1	Y	G	SG	SG	P	P	TL	TL	−ve	−ve
	F2	G	B	SG	SG	P	P	TL	TL	−ve	−ve
	F3	B	B	SG	SG	P	P	TL	TL	−ve	−ve
6	F1	Y	G	SG	SG	P	P	TL	TL	−ve	−ve
	F2	G	B	SG	SG	P	P	TL	TL	−ve	−ve
	F3	B	B	SG	SG	P	P	TL	TL	−ve	−ve

RT = room temperature (25 ± 2 °C), ET = elevated temperature (40 ± 2 °C), C = colorless, Y = yellow, G = golden (dark yellow), B = brown, S = smooth, G = glossy, P = pungent, TL = tasteless, −ve = no growth.

acetic acid. The organoleptic observations for the placebo chips are similar to those of the CHX-loaded chips. Based on the organoleptic findings, it is predicted that the chips could remain physically stable if stored at temperatures below 25 °C.

3.4.7. Microscopic analysis of the chips. SEM was performed to study the surface morphology of chips prepared with and without CHX. The surface images of placebo and CHX-loaded chips are shown in Fig. 2. A comparison is made between placebo (Fig. 2a–c) and CHX-loaded chips (Fig. 2d–f), as well as the impact of polymer concentration on the microscopic surface morphology. No significant differences were observed between the placebo and CHX-loaded chips. Both sample types appeared homogeneous with rough surfaces. One possible reason could be the casting methodology of the chips after complete gelatinization of the matrix, which is why a similar surface morphology is observed. Previously, Queiroz *et al.*[23] also observed rough surfaces through SEM for the films of CHX and starch.

In terms of the concentration effect, the primary difference noted is that the surface of the chips becomes more compact and denser with an increase in the polymer concentration (Fig. 2). This compactness was also observed during the preparation of gels, due to the rise in the viscosity of the solutions (Table S1). All chips appeared rough or uneven under microscopic examination (Fig. 2). The microscopic roughness of the chip surface could be attributed to the drying of the high-molecular-weight chitosan used in all chip formulations. The roughness of the chips and their compactness with respect to chitosan concentration can also be observed in the cross-sectional images (Fig. 3). This roughness or unevenness of the chips was not detectable through the naked eye, likely due to the smoothness of the chips surface occurring by the presence of glycerin, as observed organoleptically (Table 3).

3.5. Chemical analysis of the chips

3.5.1. Assay of CHX-loaded chips. The analysis of pure CHX and the formulated CHX-loaded chips was performed using HPLC, following the method described by Scholz *et al.*[21] Since the reported method was performed at 20 °C and used artificial saliva as the calibration standard and methanol for sample dilution, it was revalidated. The validation was performed in terms of system suitability (Table S2), linearity and range (Table S3), accuracy and precision (Tables S4 and S5), and sensitivity (Table S2) in our lab conditions for the reassurance of the assay results using the water and mobile phase as the diluting solvents. The technique demonstrated reliable system suitability in accordance with the recommended values,[67,68] and the validated method was found to be suitable for the assay of pure CHX and its chips (Table S6). The validation data is provided in the SI, and the typical chromatogram and calibration curve are shown in Fig. S5a and S5b, respectively.

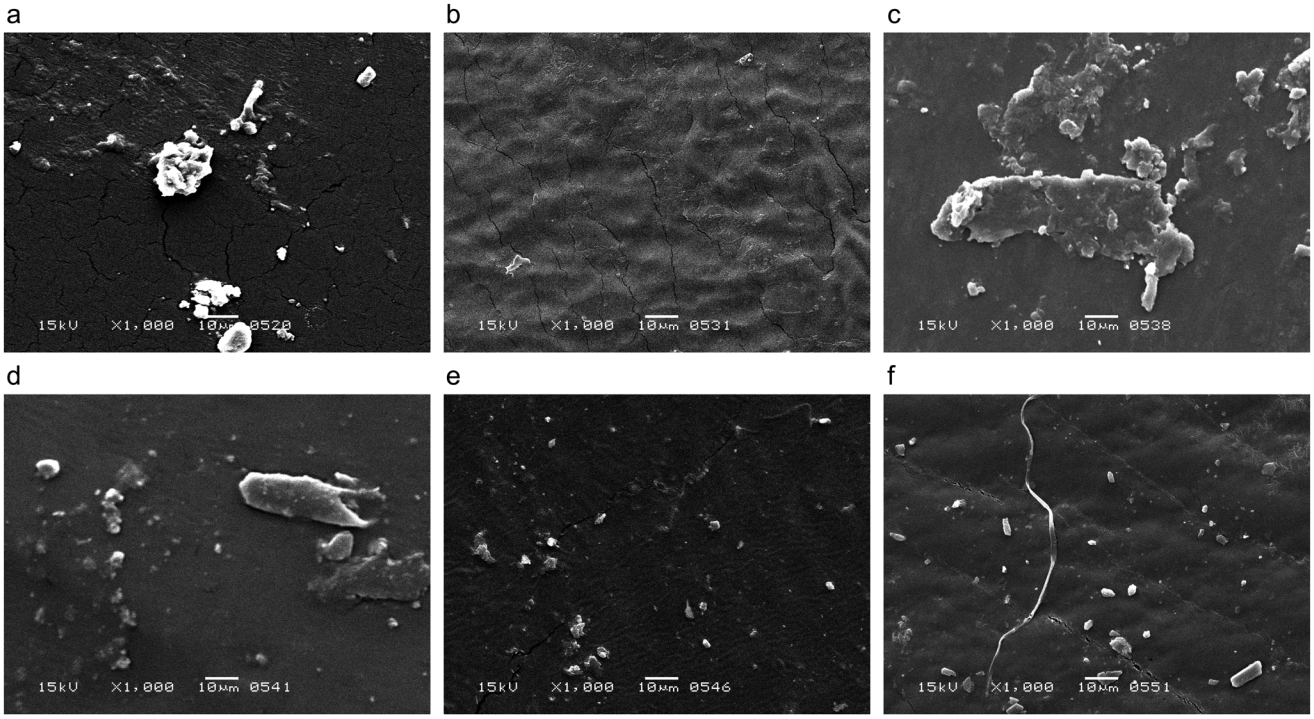

Fig. 2 The surface morphology of the chips by SEM. (a) Placebo chip with 2% chitosan, (b) placebo chip with 3% chitosan, (c) placebo chip with 4% chitosan, (d) CHX-loaded chip with 2% chitosan, (e) CHX-loaded chip with 3% chitosan, and (f) CHX-loaded chip with 4% chitosan.

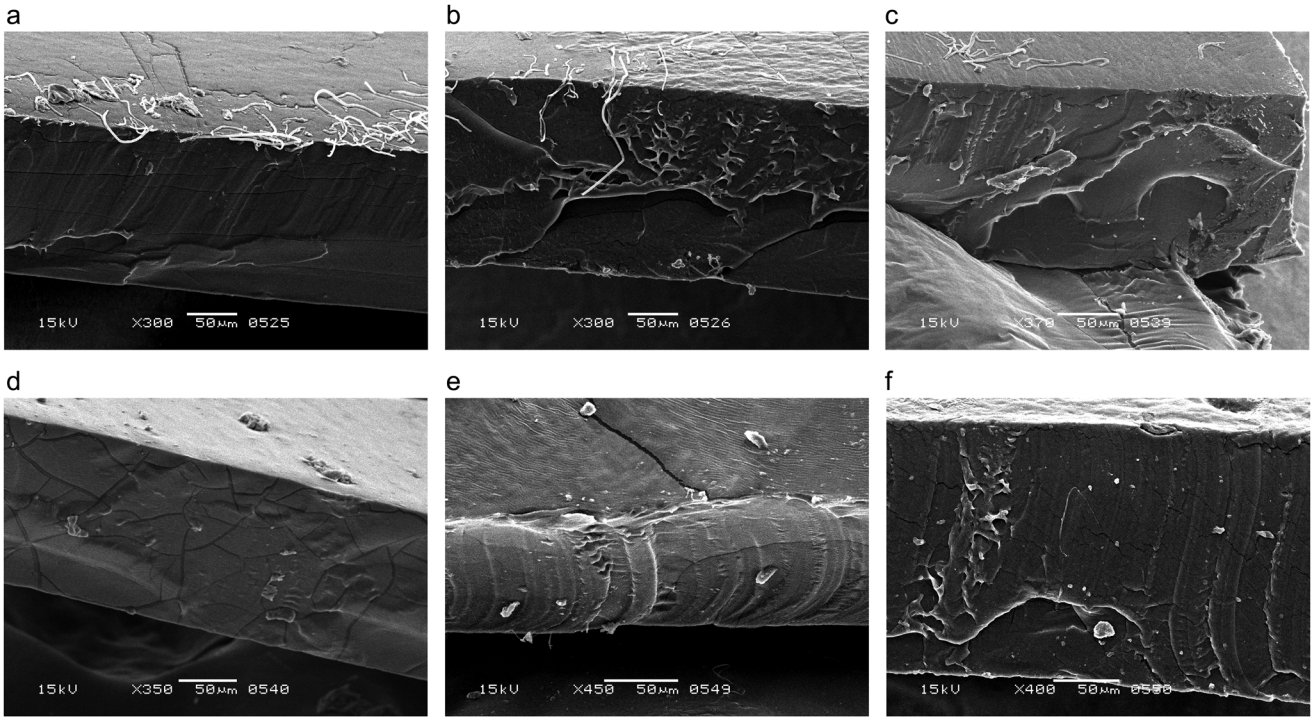

Fig. 3 The cross-sectional view of the chips by SEM. (a) Placebo chip with 2% chitosan, (b) placebo chip with 3% chitosan, (c) placebo chip with 4% chitosan, (d) CHX-loaded chip with 2% chitosan, (e) CHX-loaded chip with 3% chitosan, and (f) CHX-loaded chip with 4% chitosan.

3.5.2. Drug content uniformity in the chips. Each formulation chip was cut into four parts, and each piece was subjected to an HPLC assay to determine the uniformity of CHX content across the chip. The results demonstrated that CHX is uniformly distributed across the chip of each formulation; hence, the formulation procedure is suitable for producing uniform CHX-

Table 4 CHX content uniformity in the formulated chips

	Recovery (%)		
Part	F1	F2	F3
A	97.50	92.81	89.38
B	94.69	95.94	90.63
C	94.38	96.88	91.25
D	96.25	92.19	94.69
Total recovery (%) ± SD	95.70 ± 1.45	94.45 ± 2.30	91.48 ± 2.27

loaded chips (Table 4). The minor variations in the results, as evident from the standard deviation values, may be due to errors in cutting, weighing, or analysis (Table 4). The slight decrease in recovery values in chips with higher polymer concentrations could be due to the comparatively slow release of CHX (Table 4).

3.6. Study of the *in vitro* drug release from the chips

The release of the drug from its polymeric matrix is essential in determining the formulation's efficacy. In this study, the release rate of CHX from different chips in simulated saliva has been determined using a Type I dissolution apparatus. The cumulative release of CHX with respect to time is shown in Fig. 4. A decrease in the release of CHX has been noted with an increase in the polymer concentration (Fig. 4). The chips containing 2% chitosan have shown a comparatively higher release of CHX (~31 h) as compared to those containing 3% (~46 h) and 4% chitosan (~72 h). The increase in the polymer concentration was also noted to increase the viscosity of the gels at the time of preparation (Table S1), which resulted in the formation of thickened or dense chips, as confirmed in the thickness measurements (Table 2) and SEM study (Fig. 2). Thus, the higher the concentration of the chitosan, the more densely packed polymeric matrix encapsulating the drug will be formed. Such a dense matrix reduced the available pathways for the drug to diffuse out, eventually slowing the release of CHX. Such an effect is often desirable when controlled or sustained release of a drug is needed.

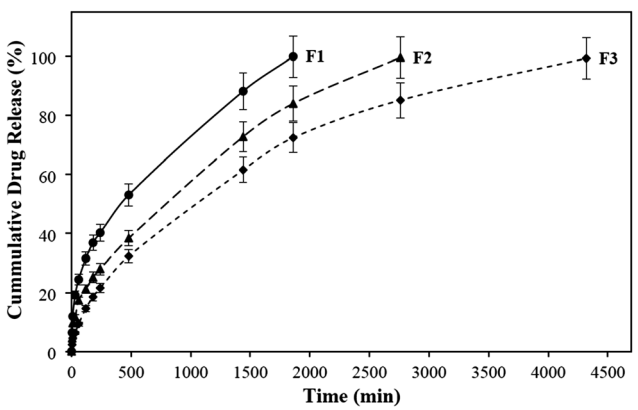

Fig. 4 Release pattern of CHX from the formulated chips at different time intervals. (F1) CHX-loaded chip with 2% chitosan, (F2) CHX-loaded chip with 3% chitosan, and (F3) CHX-loaded chip with 4% chitosan.

Mathematical models are often applied to understand the factors affecting drug release, as it is considered an integral part of product development. Various kinetic models have been used to determine the release pattern of CHX from the chitosan chips. The results indicated that the release of CHX from the chips follows the Higuchi model, where the cumulative drug release is linear to the square root of time (Fig. S6–S8). This linearity suggests that the release of the drug from the swollen polymer matrix is primarily based on diffusion. Interestingly, at higher concentrations, the release of CHX from the chips is also found to become linear to log cumulative drug release *versus* log time, *i.e.*, it begins to follow the Korsmeyer–Peppas model, along with the Higuchi model (Fig. S8). This change in release pattern indicates that at higher concentrations, polymer swelling leads to erosion, which also plays a role alongside diffusion in the drug release (non-Fickian transport). Thus, it is predicted that a further change in the concentration of the polymer or further sustained release will change the release kinetics of the drug from the chips. It is assumed that the longer the sustained release of the drug, the higher the patient's compliance, as the application frequency of the chip will be longer compared to faster release models, like tablet or capsule dosage forms that require frequent dosing.

3.7. Stability of CHX in polymeric chips

Stability studies refer to the duration during which a pharmaceutical product maintains its various properties (physical, chemical, microbiological, and pharmacokinetic) throughout its shelf life from the date of manufacture. According to ICH,[22] determining a product's shelf life is based on the point at which the active compound degrades by 10% over time, resulting in a concentration decrease to 90% of its original level. Since the increase in polymer concentration affects the assay of CHX in the formulated chips (Table 5), as also observed in the content uniformity test (Table 4), the 10% degradation has been calculated from the initial zero-time reading, rather than 100%. In this study, accelerated stability testing was performed on the formulated chips for up to three months, as more than 10% degradation was observed from the initial concentration after one month in all chips (Table 5). The decreased stability of CHX is attributed to its thermosensitive nature and is known to decompose at temperatures of 40 °C or higher,[69–71] as also observed during the organoleptic studies (Table 3). Therefore, this limitation of the formulated chips should be

Table 5 Stability data of CHX-loaded polymeric chips at accelerated conditions

	Recovery ± SD (%)		
Month	F1	F2	F3
0	96.63 ± 3.58	94.98 ± 3.50	87.13 ± 4.15
1	92.51 ± 3.15	86.82 ± 3.95	82.57 ± 3.40
2	76.76 ± 2.69	76.03 ± 2.65	68.72 ± 3.85
3	63.80 ± 1.50	66.33 ± 2.70	59.75 ± 1.15

addressed in future research studies under refrigerated and real temperatures, along with a change of the packaging material (*e.g.*, aluminum strips). Such studies could help improve the stability of the formulated chips since better physical stability (up to three months) was observed during the organoleptic studies (Table 3). Meanwhile, from the storage and patient perspective, it is suggested to counter the stability issue by compounding them extemporaneously and utilizing them within a month.

3.8. Antimicrobial activity of the chips

3.8.1. Determination of antimicrobial activity against standard and clinical cultures. The oral cavity, through the formation of dental plaque, can serve as a reservoir for potentially pathogenic bacteria, including *S. aureus*, *S. mutans*, *P. aeruginosa*, and *Enterobacteriaceae.* The main etiological factor for dental caries is known to be *S. mutans*. In contrast, the presence of *P. aeruginosa* in the oral cavity is particularly concerning in individuals with cystic fibrosis, as it has the potential to cause respiratory infections.[72,73] *E. coli* is more commonly associated with the gastrointestinal tract. If present in the oral cavity, it may have systemic implications if there are oral health imbalances or compromised mucosal barriers.[74,75]

One of the causative organisms of periodontitis is *S. aureus*, which is also implicated in the etiology of various systemic diseases and hematological malignancies.[76] *S. aureus* can also produce a wide range of exotoxins, noted in oral isolates, such as exfoliative toxin and enterotoxin.[77] Similarly, among many species of oral microbiota, fungi constitute a small but significant part, due to their presence in both healthy and medically compromised individuals. The most common fungal microbiota is *C. albicans*.[78] It has been reported that the inhibition of *C. albicans* by CHX lasts longer than that of common antifungals, such as amphotericin B and nystatin.[18,79]

The antibacterial activity of the formulated chips, both placebo and CHX-loaded, has been determined against different microbes, and the results are reported in Table 6. A significant antimicrobial activity against all cultures, including ATCC and clinical isolates, has been noted for both placebo and CHX-loaded chips (Table 6). It has been observed that the activity in placebo chips increases with an increase in the concentration of chitosan (Table 6). The activity in placebo chips is due to the presence of chitosan and acetic acid in the formulation (Table 6), as both possess antimicrobial properties.[18,34,80,81]

On the other hand, the activity in CHX-loaded chips decreases slightly with an increase in chitosan concentration, and the maximum zones are noted for the chips containing 2% chitosan (Table 6). This could be because an increase in polymer concentration results in a higher chip thickness (Table 2) and slower drug release (Fig. 4), thereby slowing down the diffusion of the drug from the chips across the medium. Comparatively, the zones of inhibition are found to be higher for the CHX-loaded chips than for the placebo chips (Table 6). Similarly, the zones are relatively bigger for standard ATCC cultures than for the clinical isolates (Table 6). Statistically, a combined paired sample two-tailed *t*-test indicated significant differences between the antimicrobial activity of placebo and drug-loaded chips against *S. aureus* (ATCC: $p = 0.000013$; C.I.: $p = 7.8 \times 10^{-12}$), *S. mutans* (ATCC: $p = 0.000077$; C.I.: $p = 1.32 \times 10^{-8}$), *P. aeruginosa* (ATCC: $p = 0.000005$; C.I.: $p = 4.64 \times 10^{-7}$), *E. coli* (ATCC: $p = 0.00011$), and *C. albicans* (C.I.: $p = 1.56 \times 10^{-15}$). On the contrary, a non-significant difference has been noted against the clinical isolate of *E. coli* (C.I.: $p = 0.327$). These results thus suggest that using CHX in the formulated chitosan chips would be an effective drug delivery system for combating periodontal infections over a longer duration in the majority of cases.

3.8.2. Substantivity of CHX on the cementum. This parameter has been designed and studied to assess the substantivity of CHX on the cementum. As the chips are designed to fit in the periodontal pocket, the part of the tooth that comes into direct contact would be the cementum; therefore, it is essential to check the substantivity of CHX on this part of the tooth. The results demonstrated significant antimicrobial activity in all formulations against the root cementum (Table 6). Among all the tested samples, the CHX-loaded chips

Table 6 Zones of inhibition of the placebo, formulated chips, and cementum discs against different microorganisms

	Zones of inhibition (mm) ± SD									
	Formulated chips on petri dishes						Cementum discs			
	F1		F2		F3					
Microorganism	Placebo	Chip	Placebo	Chip	Placebo	Chip	F1	F2	F3	CHX
S. aureus (ATCC 6538)	17.3 ± 0.6	31.5 ± 0.5	18.7 ± 0.6	29.5 ± 0.5	22.0 ± 1.0	28.5 ± 0.5	31.5 ± 0.5	29.0 ± 0.6	28.6 ± 0.3	28.3 ± 0.8
S. aureus (C.I.)[a]	8.0 ± 0.4	21.6 ± 0.6	8.0 ± 0.6	21.0 ± 0.5	8.3 ± 0.5	21.0 ± 0.6	19.3 ± 0.2	18.7 ± 0.4	18.1 ± 0.7	16.5 ± 0.5
S. mutans (ATCC 35668)	21.7 ± 0.5	26.1 ± 0.6	22.0 ± 0.8	25.5 ± 2.2	23.1 ± 0.6	25.0 ± 1.1	26.4 ± 0.4	25.5 ± 0.5	23.4 ± 0.5	23.8 ± 0.4
S. mutans (C.I.)	16.1 ± 0.5	21.0 ± 0.8	16.2 ± 0.3	20.4 ± 1.0	15.9 ± 0.4	20.0 ± 0.8	21.2 ± 0.7	18.9 ± 0.7	18.2 ± 0.4	21.3 ± 0.6
P. aeruginosa (ATCC 27853)	18.7 ± 0.6	31.7 ± 0.6	20.0 ± 1.0	28.7 ± 0.6	20.3 ± 0.7	27.3 ± 0.6	31.3 ± 0.2	29.0 ± 0.6	27.6 ± 0.8	21.0 ± 0.5
P. aeruginosa (C.I.)	16.7 ± 0.3	28.9 ± 0.7	19.1 ± 0.8	27.7 ± 0.4	19.3 ± 0.4	27.4 ± 0.9	28.2 ± 0.4	27.2 ± 0.3	26.0 ± 0.6	20.6 ± 0.5
E. coli (ATCC 25922)	25.3 ± 0.4	32.8 ± 0.4	26.2 ± 0.4	31.5 ± 0.3	26.6 ± 0.1	29.1 ± 0.2	32.5 ± 0.4	30.8 ± 0.5	28.6 ± 1.0	25.0 ± 0.2
E. coli (C.I.)	23.3 ± 0.2	23.7 ± 0.3	23.5 ± 0.5	23.3 ± 0.9	23.8 ± 0.8	22.9 ± 0.2	24.8 ± 0.3	24.5 ± 0.4	23.8 ± 0.3	21.2 ± 0.2
C. albicans (C.I.)	9.3 ± 0.6	27.3 ± 0.6	8.7 ± 0.6	27.0 ± 1.0	8.7 ± 0.6	27.0 ± 1.0	19.6 ± 0.6	19.1 ± 0.5	18.3 ± 0.4	14.2 ± 0.1

[a] C.I. = clinical isolate.

prepared with 2% chitosan displayed the best results in terms of zones of inhibition (Table 6). Likewise, the results indicated that the chips are comparatively better than the pure CHX solution at similar concentrations against the test isolates except *S. mutans* (Table 6). The paired sample two-tailed *t*-test revealed a significant difference ($p < 0.05$) between the antimicrobial activity of pure CHX and formulation chips, except for the ATCC culture of *S. aureus* against formulation F3 ($p = 0.408$). In the case of *S. mutans*, the clinical isolate showed no significant difference against formulation F1 ($p = 0.225$), whereas the pure CHX showed statistically significant differences against formulations F2 ($p = 0.000578$) and F3 ($p = 0.001385$).

CHX possesses the property of substantivity, which enables it to coat the tooth surface and delays the decay of the tooth,[82] thereby maintaining its antimicrobial efficacy over time. This thick layer of CHX (approximately 25 μm) acts as a reservoir, helping to eliminate microbes and prevent the formation of biofilm.[82] This prolonged retention is a significant factor behind its long-standing and widespread use in the field of dentistry. The concentration of CHX used is also vital, as a previous study observed that a 2% CHX solution showed weaker substantivity compared to its 0.2% solution.[83] This study demonstrates that cementum also possesses the property of substantivity, which is beneficial for the healing of periodontium. If these chips are used as an adjunct to routine treatment, as suggested by Ma and Diao,[13] they will coat the cementum with CHX, which will exert its antimicrobial action and maintain a pathogen-free environment for a prolonged period.

4. Conclusion

In developing countries, the burden of chronic diseases has unfavorable effects on limited health resources, which can further worsen the condition due to noncompliance by the patients. This study has provided valuable data that could help in the development of a cost-effective, simple, and efficient CHX-based drug delivery system. The formulation was developed with an emphasis on economy, ease of production, patient safety, and the use of minimal excipients, thereby creating a clean and compliant alternative with prolonged action. The formulated CHX dental chips, due to their enhanced antimicrobial activity and slow release, could facilitate the desired therapeutic effects for an extended period. The broad-spectrum antimicrobial activity of the formulated chips, targeting both Gram-positive and Gram-negative organisms, combined with their substantivity, makes CHX highly effective in preventing and managing periodontal disease. Since CHX degrades rapidly at higher temperatures, the formulated chips should always be stored in a cool environment protected from light and humidity or prepared extemporaneously. Further investigations into its clinical applications, antimicrobial testing against a larger number of isolates, and formulation improvements, such as the addition of stabilizers, additives, or cross-linking agents to enhance stability or alter release, could help pharmaceutical scientists gain a deeper understanding of the CHX-loaded dental chips.

Conflicts of interest

The authors declare that there is no conflict of interest.

Data availability

Data will be available on reasonable request to the corresponding author.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d5pm00086f.

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