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Original Article

In situ Forming Dental Cement Loaded with Metronidazole and Doxycycline to Treat Periodontitis

Anuj Kumar*, Anurag Verma

Department of Pharmaceutics, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, INDIA.

ABSTRACT

Background: *In situ* forming dental cements offer a novel approach to treating periodontitis by enabling localized delivery of therapeutic agents directly to the periodontal cavity. Hydroxyapatite-based materials hold promise, particularly when combined with antimicrobial agents such as metronidazole and doxycycline. This study aimed to develop and comprehensively characterize hydroxyapatite-based *in situ* forming dental filling materials to address the clinical needs of periodontitis treatment. **Materials and Methods:** Dental cement formulations were prepared by combining hydroxyapatite, chitosan, carbopol 934, Glass Ionomer Cement (GIC), and GIC liquid to produce a thick paste suitable for periodontal cavity filling. Both blank and drug-loaded formulations were developed, with cementation times ranging from 60 sec (blank) to 80 sec (drug-loaded). Hardness strength testing was performed to determine structural integrity. Preformulation studies, including FTIR and DSC, confirmed the absence of material interactions. Surface roughness and bioerosion were analyzed using SEM and TEM. Biocompatibility was evaluated using MTT and Trypan blue assays with KB cell line cultures, while mucoadhesion tests were conducted on goat buccal mucosa. Antimicrobial efficacy was assessed through MIC assays against oral pathogens, and drug release profiles were measured in artificial saliva. **Results:** FTIR and DSC analyses showed no significant material interactions in the formulations. SEM and TEM studies revealed appropriate surface characteristics and bioerosion behavior of the dental cement. Cytotoxicity assays demonstrated over 90% cell viability, indicating good biocompatibility. Mucoadhesion tests revealed excellent adhesion to goat buccal mucosa. The MIC values for *Staphylococcus aureus, Escherichia coli*, and *Streptococcus mutans* were below 10 µg/mL, confirming strong antimicrobial activity. Drug release studies in artificial saliva showed effective retention of therapeutic agents, and hardness strength tests confirmed adequate structural integrity of the formulations. **Conclusion:** The developed hydroxyapatite-based drug-loaded dental cement demonstrated promising potential as a therapeutic agent for periodontitis treatment. These formulations combine strong antimicrobial efficacy, biocompatibility, good mucoadhesion, and suitable handling properties, making them viable for localized periodontal therapy.

Keywords: *In situ* forming dental cement, Periodontitis treatment, Hydroxyapatite, Metronidazole, Doxycycline, Antimicrobial efficacy, Biocompatibility, Mechanical strength.

Correspondence:
Mr. Anuj Kumar
Research Scholar, Department of Pharmaceutics, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad-244001, Uttar Pradesh, INDIA.
Email: anujpharmacy01@gmail.com

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INTRODUCTION

The common chronic inflammatory condition known as periodontitis affects the tissues that support teeth, and presents significant challenges in dental practice due to its complex etiology and variable treatment outcomes (Könönen *et al*., 2019). Conventional approaches to managing periodontitis typically involve mechanical debridement coupled with adjunctive antimicrobial therapy to combat bacterial infection within periodontal pockets (Mombelli, 2000). However, limitations such as patient compliance, inadequate drug delivery, and the development of antimicrobial resistance underscore the need for innovative therapeutic strategies that can enhance treatment efficacy and patient outcomes (Prestinaci *et al*., 2015; Ahmed *et al*., 2024).

In situ forming dental cement has emerged as a promising avenue in periodontal therapy, offering a localized and sustained drug delivery platform directly within the periodontal pocket (Senarat *et al*., 2023). This approach addresses several critical aspects of periodontitis management by providing a controlled release of therapeutic agents while ensuring ease of application and enhanced patient comfort. Integrating antimicrobial agents, such as metronidazole and doxycycline, into hydroxyapatite-based dental filling materials, represents a significant advancement

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in the field, aiming to address infection control and tissue regeneration synergistically. The formulation and characterization of these novel dental cements involve a meticulous balance of materials science, pharmacology, and dental engineering (Nastri *et al*., 2019; Amato *et al*., 2023). Hydroxyapatite, renowned for its biocompatibility and osteoconductivity, serves as the foundational component, providing structural support and aiding in the restoration of periodontal tissues damaged by infection (Mysore *et al*., 2024; Mo *et al*., 2023). Chitosan, Carbopol 934, and Glass Ionomer Cement (GIC) liquid are intricately blended to achieve a thick paste consistency suitable for application within the periodontal pocket (Soygun *et al*., 2021). In summary, developing *in situ* forming dental cement loaded with metronidazole and doxycycline represents a paradigm shift in periodontitis treatment, offering a multifaceted therapeutic approach that integrates antimicrobial efficacy, biocompatibility, and mechanical stability. This comprehensive characterization and evaluation provide compelling evidence of their potential as a next-generation therapeutic modality for enhancing periodontal health and improving clinical outcomes.

MATERIALS AND METHODS

Materials and Formulation

Hydroxyapatite, Chitosan, Carbopol 934, and Glass Ionomer Cement (GIC) were used as the base materials. Metronidazole and Doxycycline were selected as the therapeutic agents. Formulations were prepared by mixing hydroxyapatite, chitosan, Carbopol 934, and GIC with GIC liquid to form a thick paste. Metronidazole and doxycycline were incorporated into the paste at concentrations of 1% w/w (Surendran *et al*., 2023).

Characterization

Preformulation Studies

FTIR and DSC analyses were conducted to check for interactions among the materials (Pandita *et al*., 2012).

FTIR Analysis

Samples of Hydroxyapatite, Chitosan, Carbopol 934, Glass Ionomer Cement (GIC), Metronidazole, Doxycycline, and the formulated cement were prepared. Each sample was scanned throughout a 4000-400 cm^{-1} wavenumber range. Spectra were recorded, using Bruker ALPHA II FTIR Spectrometer, to identify characteristic peaks and analyze potential shifts or new peak formations indicative of interactions (Wan *et al*., 2021; Younas *et al*., 2013).

DSC Analysis

Approximately 5 mg of each sample was placed in aluminum pans and sealed. Samples were heated from 25°C to 300°C at a rate of 10°C/min under a nitrogen atmosphere. Thermograms (TA Instruments Q2000 DSC) were analyzed to detect changes in melting points, enthalpy, or other thermal transitions, indicating interactions between the components (Lohani *et al*., 2014).

Surface Roughness and Bioerosion

SEM and TEM were used to assess the surface morphology and bioerosion of the formed *in situ* forming dental cement (Baldi *et al*., 2022).

SEM Analysis

Samples were covered with a thin gold coating and examined under the SEM (JEOL JSM-6510LV SEM) at 15 kV. Images were captured at various magnifications to analyze surface morphology and roughness.

TEM Analysis

Ultra-thin sections of the cement were prepared and stained with uranyl acetate. TEM imaging (FEI Tecnai G2 20 TEM) was performed to observe the nanoscale's internal structure and bioerosion patterns.

Setting Time Measurement

Cementation time was measured for both blank and drug-loaded formulations. A stopwatch was used to record the time from the start of mixing until the cement paste hardened to resist a specified load. Approximately 2 g of paste was placed in a mold, (113.4 g with a flat tip of 2.12 mm diameter) and periodically applied. Setting time was defined as the point at which the cement surface hardened completely and pressing did not leave any marks (Hasan *et al*., 2014).

Weight Loss Measurements

It involves measuring the weight loss of the material after exposure to an artificial saliva solution representing oral conditions. Make cylindrical samples of the filling material according to standardized dimensions (usually 6 mm diameter and 12 mm height). The samples had a uniform surface and uniformity. Firstly, ensure accurate initial weight measurements, then immerse the prepared samples in the chosen artificial saliva solution, which had a constant temperature (37°C), and place them in suitable containers. The containers are then left as is for 24 hr. After 24 hr, the samples are taken out and placed in a vacuum oven to dry. Before and after the initial weight measurement, the samples were properly dried under controlled conditions (vacuum oven at a specific temperature of 37°C for a fixed time) to ensure uniform moisture content in the samples and throughout the study. At predetermined intervals, the samples were carefully removed from the artificial saliva solution, dried as described above, and their weight was measured again. Each sample's weight change was recorded during the study period (final weight subtracted from initial weight at each time point to determine weight loss) (Peddapatla *et al*., 2021).

The compressive strength

The compressive strength of the cement was evaluated using a Pfizer hardness tester machine. Cylindrical samples (6 mm diameter, 12 mm height) of both blank and drug-loaded *In situ* forming dental cement were prepared and allowed to be set for 24 hr at room temperature. Each sample was placed in the Pfizer hardness tester machine and subjected to a compressive load at a constant crosshead speed until failure. The maximum load at failure was recorded, and compressive strength was calculated using the formula (Gnyrya *et al*., 2018).

Equation 1 Compressive Strength

$$\text{Compressive Strength} = \frac{Load}{Cross\ sectional\ area}$$

Biocompatibility Testing

MTT and Trypan blue assays were performed using KB cell line cultures exposed to the *in situ* forming dental cement to assess cell viability. They are given below:

MTT Assay

A density of 1×10^4 cells per well was used to cultivate KB cell lines in 96-well plates, and they were incubated for 24 hr. *In situ* forming dental cement samples were sterilized and placed in contact with the cells. After 24 and 48 hr of incubation, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hr at 37°C. The medium was removed, and 150 µL of DMSO was added to dissolve the formazan crystals. A microplate reader was used to detect absorbance at 550 nm. Cell viability was calculated as a percentage relative to the control (untreated cells) (Larsson *et al*., 2020).

Trypan Blue Assay

To get the test concentrations, the compounds were dissolved in DMSO and serially diluted with a complete medium. In every sample, the DMSO content was maintained at less than 0.1%. After being seeded in six-well plates and treated with varying quantities of the test substances, HUVEC cells kept under the proper conditions were incubated for 96 hr at 37°C with 5% CO_2. Cells were gently trypsinized and blocked with serum. Cell suspension was diluted with 1:1 trypan blue solution. Cells were placed under a hemocytometer and counted. Percent viable cells (transparent) and dead cells (blue color) were compared (Strober, 2015).

Adhesion test

Goat buccal mucosa was obtained from a slaughterhouse, placed at 4°C, and moistened with PBS. The test pellets were gently placed on the mucosa and allowed to stick. The mucosal preparations were placed vertically and the time duration between the pellets' placement and the pellet's self-fall was observed (Fayam *et al*., 2022).

Antimicrobial Efficacy

Minimum Inhibitory Concentration (MIC) Assays

MIC values were determined for the base, drug-loaded cement, and pure drugs combination against *Staphylococcus aureus, Streptococcus mutans*, and *Escherichia coli* to evaluate antimicrobial efficacy. Test strains were cultured in nutrient agar broth. For the MIC assay, 10 mL broth was prepared by inoculating the strain and cultured overnight. 1:1000 dilution of this broth was prepared in Luria Broth and 180 uL was added to each well of a 96-well plate. Test samples were added to the plate and incubated for 24 hr. The wells were observed for turbidity. Turbidity is the measure of positive growth (Barnes, *et al*., 2023).

Drug Release Profiles

The release profiles of metronidazole and doxycycline from the cement were studied in artificial saliva over 10 days. Each cement sample of 1 g was placed in a beaker containing artificial saliva. The release medium was maintained at 37°C with constant stirring to simulate physiological conditions. At predetermined intervals (e.g., 1,2, 3,… days), aliquots of the release medium were collected and replenished with fresh artificial saliva. The collected samples were analyzed using UV-vis spectroscopy at appropriate wavelengths for metronidazole and doxycycline. Drug release profiles were constructed by plotting cumulative drug release against time (Nastri *et al*., 2019).

RESULTS AND DISCUSSION

The study focused on developing and characterizing drug-loaded *in situ* forming dental cement intended to treat periodontitis. Various analytical techniques and assays were employed to assess the formulated cement' physical, chemical, and biological properties.

Preformulation studies

FTIR (Fourier-Transform Infrared Spectroscopy)

FTIR examined potential interactions among the materials used to formulate drug-loaded dental cement. FTIR spectra of individual components (hydroxyapatite, chitosan, Carbopol 934, GIC, metronidazole, and doxycycline) and the formulated cement were compared (Figure 1). The spectra revealed characteristic peaks corresponding to functional groups present in each component, and no new peaks or significant shifts were observed in the spectra of the formulated cements compared to the individual components.

The FTIR spectroscopy analysis of the formulation containing glass ionomer cement, Carbopol, chitosan, hydroxyapatite, metronidazole, and doxycycline, revealed distinct spectral characteristics that provide insights into the molecular structure and functional groups present in each component. The glass ionomer cement exhibited significant peaks at 1000.31 cm^{-1} (C-H

bending, aromatic), 2250 cm^{-1} (C≡N stretching), and 3680 cm^{-1} (O-H stretching, aliphatic). Carbopol showed peaks at 550 cm^{-1} (C-Br stretching), 1169.47 cm^{-1} (C-O stretching), and 1703.23 cm^{-1} (C=C stretching, aromatic). Chitosan displayed peaks at 1023.51 cm^{-1} (C-H bending), 1528.57 cm^{-1} (C=C Stretching), 3103 cm^{-1} (=C-H stretching, aliphatic), 3736 cm^{-1} (O-H stretching, aromatic), highlighting its hydrophilic nature and biocompatibility compounds. Hydroxyapatite exhibited peaks at 1028.93 cm^{-1} (C-O stretching), 1480 cm^{-1} (C=C stretching, aliphatic, and 3550 cm^{-1} (O-H stretching, aromatic) indicating its adhesive properties, mechanical properties and biocompatibility. Doxycycline presented a spectrum with significant peaks at 1027.44 cm^{-1} (C-O stretching), 1263-1698 cm^{-1} (C=C Stretching/ Bending), 2464.56 cm^{-1} (C≡N Stretching), 3524.59 cm^{-1} (O-H Stretching, Aromatic) these groups that enhance its solubility and biological activity. Metronidazole presented a broad absorption band observed around 3200-3500 cm^{-1} indicates the presence of Hydroxyl (-OH) groups, confirming the existence of alcohol functionality in the molecule. Peaks near 3100 cm^{-1} are attributed to C-H stretching vibrations from aromatic or aliphatic hydrogen. The strong absorption band observed between 1500-1600 cm^{-1} corresponds to the Nitro ($-NO_2$) group, a key functional group responsible for the compound's antibacterial properties. Additionally, a sharp peak around 1400 cm^{-1} is associated with C-N stretching vibrations, indicative of the imidazole ring. The bands in the fingerprint region, particularly between 1000-1300 cm^{-1}, are characteristic of C-O and C-N stretching, further validating the compound's molecular structure. Collectively, these spectral features confirm the identity of metronidazole and provide insight into its functional groups and structural composition.

DSC (Differential Scanning Calorimetry)

DSC was utilized to assess the thermal behavior and compatibility of the materials. Thermograms of individual components and the formulated cement showed characteristic endothermic and exothermic peaks corresponding to their melting points and thermal transitions. The mixed sample of drugs and polymers displays complex thermal transitions due to the inclusion of Doxycycline and Metronidazole. The interactions among the samples facilitated structural robustness and flexibility, with high compatibility observed in mixed systems, although uniform dispersion was critical to prevent phase separation (Figure 2).

The DSC thermogram showed a sharp endothermic peak at 94.31°C, which is the melting point of glass ionomer cement. The thermogram of glass ionomer cement corresponds to glass ionomer cement and the physical mixture of glass ionomer cement suggested no interaction between the two species. The physical mixture of Carbopol 934p showed a sharp endothermic peak at 79.65°C and 317.01°C which showed the bound water present in the physical mixture of Carbopol 934p. The thermogram of Carbopol 934p suggests no interaction between the two species. The physical mixture of chitosan showed a sharp endothermic peak at 81.95°C and 246.96°C which showed the bound water present in the physical mixture of chitosan. The thermogram of chitosan suggests no interaction between the two species. The physical mixture of hydroxyapatite showed a sharp endothermic peak at 76.33°C and 200.35°C which showed the bound water present in the physical mixture of hydroxyapatite. The thermogram of hydroxyapatite suggests no interaction between the two species. The physical mixture of doxycycline showed an endothermic peak at 171.42°C (the melting point of the drug) and 300.35°C which showed the bound water present in the physical mixture of doxycycline. The thermogram of doxycycline suggests no interaction between the two species. The physical mixture of

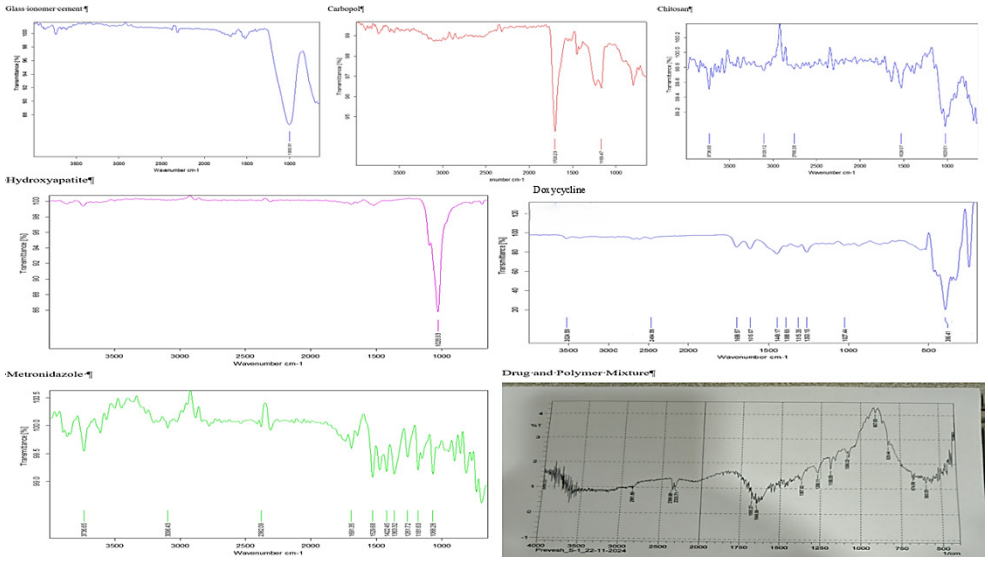

Figure 1: FTIR Spectra of Polymers and Drugs Mixture.

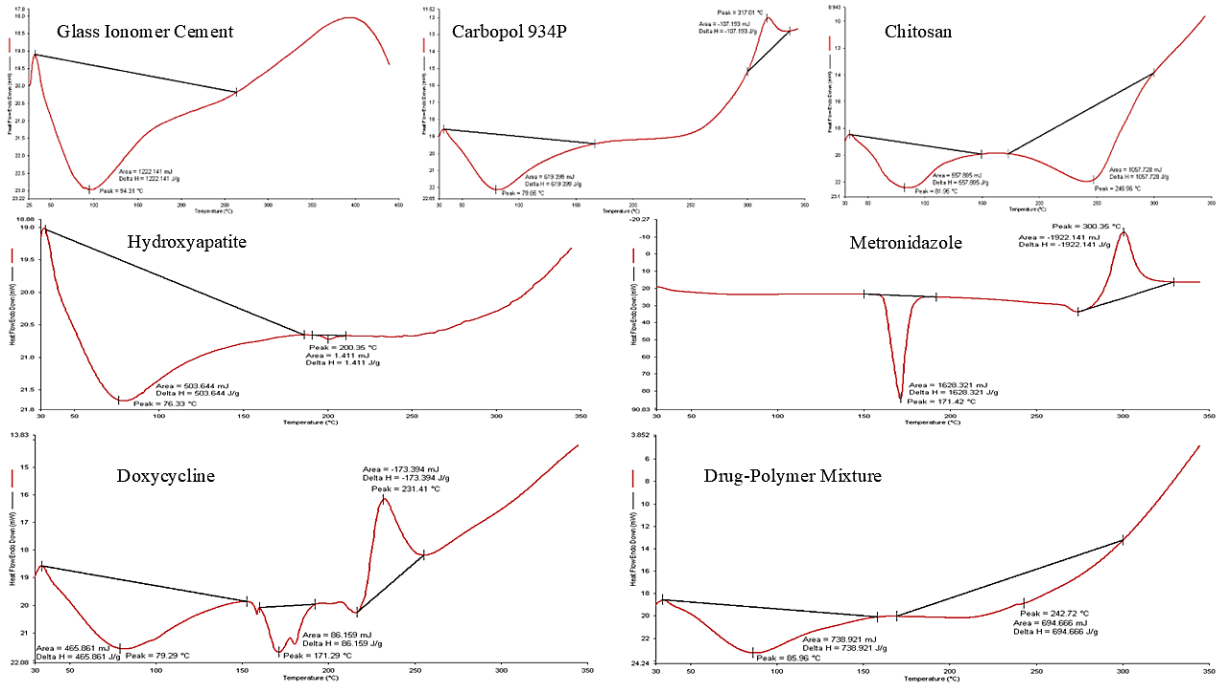

Figure 2: DSC Spectra of Polymers and Drugs Mixture.

metronidazole showed a sharp endothermic peak at 79.29°C, 171.29°C and 231.41°C which showed the bound water present in the physical mixture of metronidazole. The thermogram of metronidazole suggests no interaction between the two species.

The DSC analyses confirmed the compatibility and stability of the materials used in the formulation of drug-loaded dental cement. These pre-formulation studies are crucial as they ensure that the ingredients do not interact unfavorably, which could compromise the efficacy or safety of the final product. The absence of new peaks or shifts in spectra and consistent thermal behavior indicate that the formulated cements are suitable for further evaluation in terms of the crushing strength test, weight loss measurement, and biological properties. These findings lay a foundation for developing effective and stable *in situ* forming dental cement aimed at treating periodontitis with controlled drug delivery capabilities.

Surface Roughness and Bioerosion

Surface roughness and bioerosion characteristics of the formed *in situ* forming dental cement were evaluated using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM), providing insights into their structural integrity and potential degradation behavior. SEM imaging revealed the surface morphology of the dental cement samples at microscale resolutions. Both blank and drug-loaded formulations exhibited smooth and uniform surfaces without significant cracks or irregularities (Figure 3). This indicates good formulation homogeneity and integrity, which are crucial for their application as dental fillings in periodontal treatments. The absence of surface defects suggests that the incorporation of metronidazole and doxycycline did not adversely affect the external structure or quality of the cement.

TEM was employed to investigate the internal structure and bioerosion patterns of the *in situ* forming dental cement at nanoscale levels (Figure 4). Ultra-thin sections of the samples were prepared and examined under TEM, revealing the distribution of drug particles within the cement matrix and assessing any signs of degradation or erosion over time. The observations indicated uniform dispersion of drug particles and minimal signs of bioerosion, suggesting good stability and sustained release potential of the incorporated drugs within the cement matrix.

The SEM and TEM analyses collectively demonstrate that the drug-loaded *in situ* forming dental cement maintains favorable surface characteristics and structural stability necessary for clinical application. The absence of significant surface irregularities and bioerosion supports the feasibility of these cements for prolonged use in treating periodontitis, ensuring sustained drug release and therapeutic efficacy. Future studies could focus on long-term durability and biocompatibility assessments to further validate their suitability for clinical implementation.

Setting Time Measurement

Setting times were determined using a mold apparatus, where the hand was pressed down and the time required to leave a recognizable indentation on the cement surface was recorded for the blank formulation, setting times averaged around 60 sec, indicating a rapid initial setting suitable for clinical use. Upon inclusion of metronidazole and doxycycline, setting times were slightly increased, with the drug-loaded formulation showing a setting time of around 80 sec (Table 1). This moderate increase

suggests that the inclusion of the drug did not significantly alter the setting behavior of the cement beyond acceptable clinical limits.

The observed setting times align well with clinical requirements, where a balance between workability and setting speed is crucial for effective application in dental procedures. The slight extension in setting time with drug incorporation may be attributed to changes in material viscosity or interactions affecting polymerization kinetics. However, the maintained setting within a clinically acceptable range indicates that the drug-loaded cement can be feasibly used in periodontal treatments without compromising handling characteristics. Future studies could further optimize formulations to fine-tune setting times while maximizing drug loading and therapeutic efficacy, ensuring optimal clinical outcomes for periodontitis management.

Weight Loss Measurements

The results indicated distinct differences between the two formulations. For formulation 1 (base formulation), the initial weight was recorded at 82 mg, which remained unchanged after immersion in the artificial saliva solution, resulting in a final weight of 82 mg and a calculated weight loss of 0 mg. This outcome suggests that there were no changes to the base formulation under the tested conditions. In contrast, Sample 2 (drug-loaded formulation) had an initial weight of 103 mg and exhibited a minor weight loss of 0.5 mg after exposure, with a final weight of 102.5 mg. This indicates that while the drug-loaded formulation showed some interaction with the artificial saliva, leading to slight degradation, it remained relatively stable overall. These findings highlight the stability of the base formulation compared to minor changes observed in the drug-loaded formulation during simulated oral conditions.

The Compressive Strength Test

The compressive strength of both blank and drug-loaded *in situ* forming dental cement was evaluated using a Pfizer hardness tester machine. Cylindrical samples measuring F6 mm in diameter and 12 mm in height were prepared and allowed to set for 24 hr at room temperature. Each sample was subjected to a compressive load at a constant crosshead speed until failure, with the maximum load recorded. The results obtained from the compressive strength tests are as follows:

Blank Formulation: The compressive strength was found to be 6.52 MPa.

Drug-Loaded Formulation: The compressive strength was measured at 6.1 MPa.

The results indicate that the blank formulation exhibited a slightly higher compressive strength compared to the drug-loaded formulation. This reduction in strength for the drug-loaded cement may be attributed to the incorporation of therapeutic agents, which can alter the material's structural integrity.

These values are well within the range required for dental materials intended for load-bearing applications in periodontal treatments. The observed mechanical strength of the drug-loaded *in situ* forming dental cement underscores its potential for clinical

Table 1: Setting time measurement of base formulation and drug-loaded formulation.

Sl. No.	Polymer	Converted into the Cement Formulation within
1.	Base Formulation	60 sec
2.	Drug loaded Formulation	80 sec

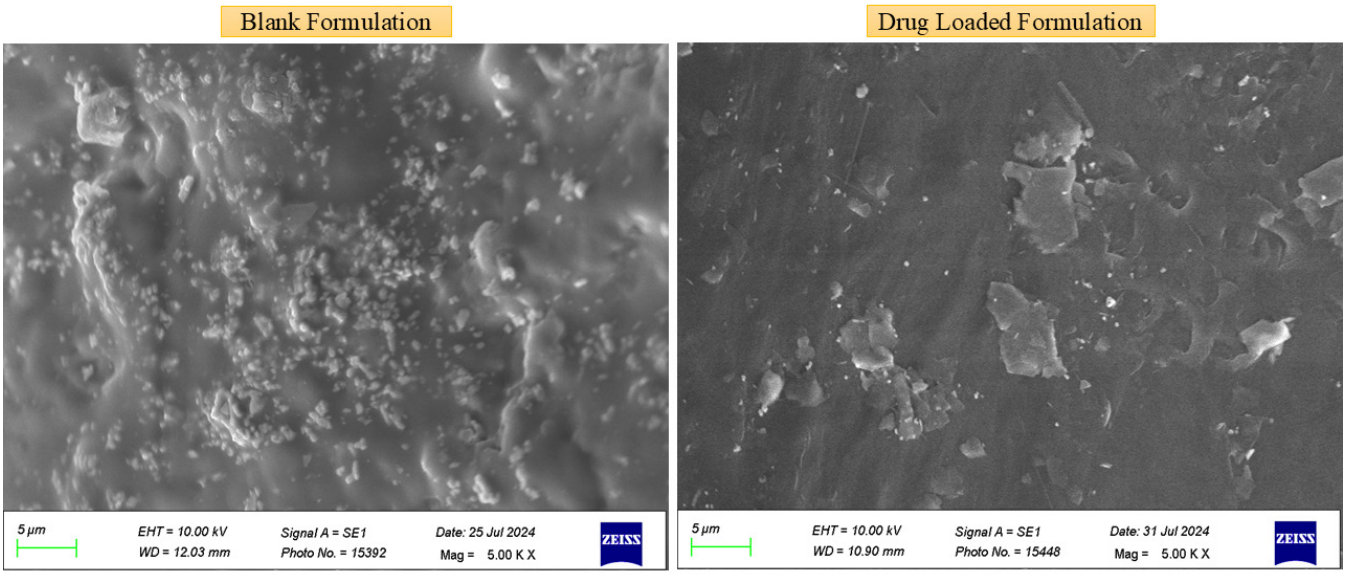

Figure 3: SEM micrographs of Blank Formulation and Drug Loaded Formulation *in situ* Forming Dental Cement their respective micrographs showing the formation of an apatite layer on their surfaces (Scale bar=5 µm).

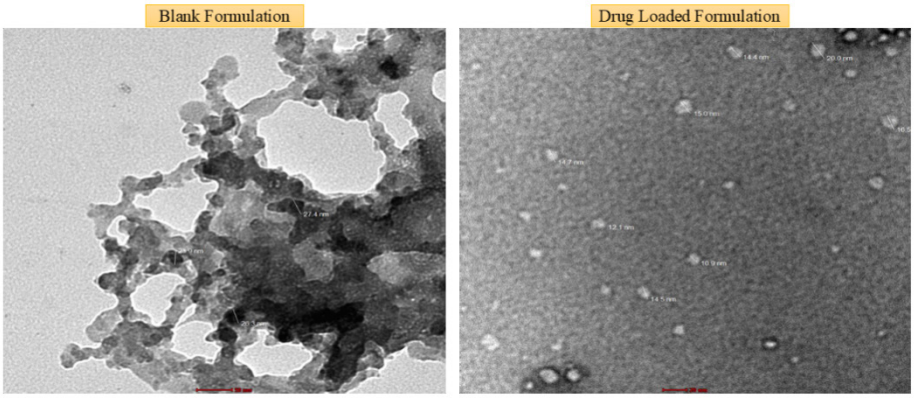

Figure 4: The TEM analysis of the blank formulation sample images demonstrated a uniform distribution of these particles, indicating effective synthesis and dispersion within the sample matrix. Sample B was analyzed at a scale of 20 nm. The TEM images showed the presence of spherical particles with sizes ranging from 10 to 20 nm.

Table 2: Adhesion test of samples A, B, and C(hr.)

Adhesion time (hr.)			
Time	**A**	**B**	**C**
1	6.25	6.53	5.42
2	8.15	5.14	5.48
3	7.52	5.24	5.43
Avg	7.34	6.25	5.44
STD EV	0.97	0.38	0.03

efficacy and longevity in treating periodontitis. The ability to withstand substantial compressive forces ensures durability and stability within the periodontal environment, where the cements are subjected to masticatory pressures and functional stresses. Future research could explore optimizing formulations to further enhance mechanical properties while maintaining desirable drug release kinetics and biocompatibility, thereby advancing their utility as effective therapeutic agents in dental practice.

Biocompatibility Testing

Cellular viability and cytotoxicity of the drug-loaded *in situ* forming dental cement were evaluated using MTT and Trypan blue assays with KB cell line cultures. These assays are essential to determine the biocompatibility and safety of the cement for potential clinical applications in periodontal treatments.

The MTT assay

The MTT assay measures cell viability based on the metabolic activity of mitochondria in viable cells. Test item A shows the highest percentage inhibition of 22.46% at the highest concentration of 10 ug/mL. At the concentration of 1 ug/mL A shows a percentage inhibition of 13.15% and at 0.1 ug/mL it shows 6.42% inhibition. 0.01 ug/mL concentration inhibits 2.65% of the cell population. This value decreases as the concentration decreases, with a percentage inhibition of 1.57% at the lowest concentration of 0.001 ug/mL (Figure 5).

Test item B follows a similar trend to test item A, with the highest percentage inhibition of 25.36% at 10 ug/mL and the lowest percentage inhibition of 1.58% at 0.001 ug/mL. At the concentration of 1 ug/mL A shows a percentage inhibition of 18.15% and at 0.1 ug/mL it shows 12.62% inhibition. 0.01 ug/mL concentration inhibits 3.62% of the cell population.

Test item C exhibits the lowest percentage inhibition of 16.35% among all three test items at the highest concentration of 10 ug/mL. This value decreases as the concentration decreases, with a percentage inhibition of 2.67% at the lowest concentration of 0.001 ug/mL. At the concentration of 1 ug/mL A shows a percentage inhibition of 12.51% and at 0.1 ug/mL it shows 10.39% inhibition. 0.01 ug/mL concentration inhibits 5.64% of the cell population.

The IC_{50} value, which is the concentration of a substance that inhibits 50% of a cellular function, is greater than 10 ug/mL for all three test items. This suggests that all three test items have a weak inhibitory effect on the MTT assay.

Overall, the result suggests that test items A and B have a weak inhibitory effect on the MTT assay, while test item C has an even weaker inhibitory effect.

The Trypan Blue Assay

The Trypan blue assay provides a direct method to assess cell membrane integrity and viability. The results of a cytotoxicity assay performed with trypan blue dye exclusion on KB cells

treated with test items A, B, and C. Trypan blue is a dye that can differentiate between viable and dead cells. Viable cells have intact membranes and exclude trypan blue, whereas dead cells take up the dye and appear blue. Here's what can be inferred from the results for each concentration of each test item:

For Test Item A, the data shows a dose-dependent increase in the inhibition of cell proliferation, with the highest inhibition of 16.47% observed at the highest concentration of 10 µg/mL. However, the inhibition remains relatively low, with an IC_{50} value greater than 10 µg/mL, indicating that concentrations higher than 10 µg/mL would be needed to achieve 50% inhibition (Figure 6).

Similarly, Test Item B demonstrates a dose-dependent increase in inhibition, with the highest inhibition of 28.14% at 10 µg/mL. Compared to Test Item A, Test Item B shows a higher inhibition at the highest concentration but still has an IC_{50} value greater than 10 µg/mL, suggesting that it is not highly effective at inhibiting cell proliferation at the tested concentrations.

Test Item C also exhibits a dose-dependent increase in inhibition, with the highest inhibition of 24.84% at 10 µg/mL. The inhibition at lower concentrations is higher compared to Test Items A and B, particularly at 0.01 µg/mL where it shows 10.28% inhibition. Nevertheless, like the other test items, it has an IC_{50} value greater than 10 µg/mL, indicating that higher concentrations would be necessary to achieve 50% inhibition.

In summary, all three test items exhibit a dose-dependent increase in the inhibition of cell proliferation in the KB cell line. Test Item B shows the highest inhibition at the highest concentration (28.14% at 10 µg/mL), followed by Test Item C (24.84% at 10 µg/mL) and Test Item A (16.47% at 10 µg/mL). However, none of the test items reached an IC_{50} value within the tested concentration range, suggesting that concentrations higher than 10 µg/mL are required to achieve 50% inhibition of cell proliferation. This indicates a limited effectiveness of the test items at the tested concentrations.

Adhesion Test

Mucoadhesion is a critical property for *in situ* forming dental cement used in periodontal treatments, ensuring effective retention and sustained drug release at the application site. In this study, adhesion tests were conducted on goat buccal mucosa to evaluate the mucoadhesive properties of the drug-loaded dental cement (Table 2, Figures 7 and 8).

The average adhesion times indicate a clear trend in the adhesive properties of the different samples: Sample A exhibited the longest average adhesion time of 7.34 hr, with a standard deviation of 0.97 hr. This suggests that Sample A has a strong adhesive quality, maintaining its position on the buccal mucosa for an extended period.

Sample B demonstrated a slightly lower average adhesion time of 6.25 hr, with a standard deviation of 0.38 hr. The reduced adhesion time compared to Sample A indicates a moderate adhesive property.

Sample C showed the shortest average adhesion time of 5.44 hr, with a notably low standard deviation of 0.03 hr. This consistent result suggests that Sample C has relatively weaker adhesive characteristics compared to Samples A and B.

The results from this adhesion test highlight significant differences in the adhesive properties of the tested samples when applied to goat buccal mucosa. Sample A's superior adhesion time suggests its potential suitability for applications requiring prolonged retention in oral environments, while Samples B and C may be more appropriate for scenarios where shorter adhesion durations are acceptable.

Antimicrobial Efficacy

The Minimum Inhibitory Concentration (MIC) test results indicate varying levels of effectiveness for the three test items (A, B, and C) against the three bacterial strains (*S. aureus, E. coli*, and *S. mutans*) (Table 3).

For *S. aureus*, Test Item A was ineffective at inhibiting bacterial growth at all tested concentrations, showing turbidity even at the highest concentration of 10 µg/mL, which resulted in an MIC of greater than 10 µg/mL. In contrast, Test Item B effectively inhibited the growth of *S. aureus* at concentrations as low as 0.01 µg/mL, demonstrating no turbidity at concentrations of 10 µg/mL, 1 µg/mL, 0.1 µg/mL, and 0.01 µg/mL, but showing turbidity at 0.001 µg/mL, leading to an MIC of 0.01 µg/mL. Test Item C

Table 3: Minimum Inhibitory Concentration of samples A, B, and C against the three bacterial strains.

S.aureus				*E. coli*			*S. mutans*		
Concentration	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
10	+	-	-	+	-	-	+	-	-
1	+	-	-	+	-	-	+	-	-
0.1	+	-	-	+	-	+	+	-	-
0.01	+	-	+	+	+	+	+	+	+
0.001	+	+	+	+	+	+	+	+	+
MIC	>10	0.01	0.1	>10	0	0.1	>10	0.1	0.1

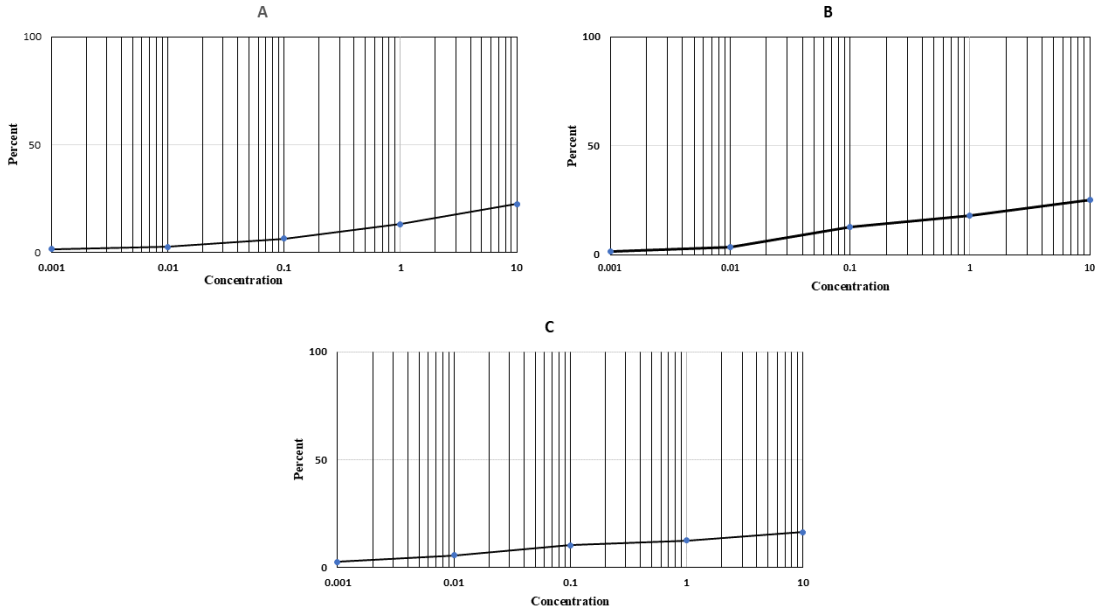

Figure 5: MTT Assay of the samples A, B, and C.

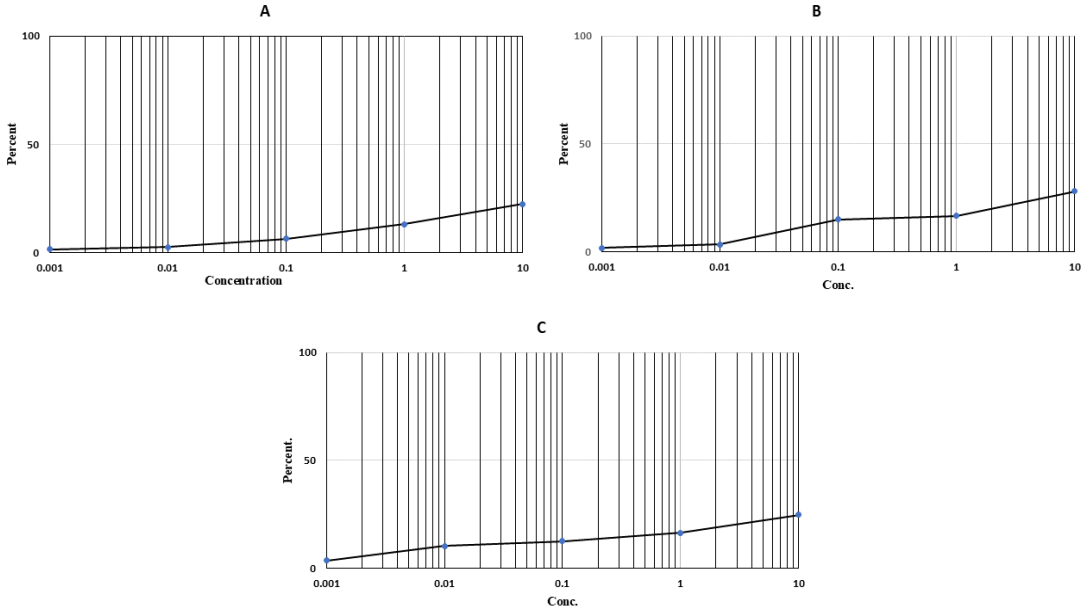

Figure 6: The Trypan blue assay of the samples A, B, and C.

inhibited the growth of *S. aureus* at concentrations down to 0.1 µg/mL, showing no turbidity at concentrations of 10 µg/mL, 1 µg/mL, and 0.1 µg/mL, but turbidity at 0.01 µg/mL and 0.001 µg/mL, giving an MIC of 0.1 µg/mL.

For *E. coli*, Test Item A similarly failed to inhibit growth at all concentrations, with turbidity present across the board, resulting in a MIC of greater than 10 µg/mL. Test Item B inhibited the growth of *E. coli* at concentrations as low as 0.1 µg/mL, showing no turbidity at concentrations of 10 µg/mL, 1 µg/mL, and 0.1 µg/mL, but showing growth at 0.01 µg/mL and 0.001 µg/mL, leading to an MIC of 0.1 µg/mL. Test Item C Inhibited *E. coli* growth at concentrations down to 1 µg/mL, demonstrating no turbidity at concentrations of 10 µg/mL and 1 µg/mL, but turbidity at 0.1 µg/mL, 0.01 µg/mL, and 0.001 µg/mL, resulting in an MIC of 1 µg/mL.

For *S. mutans*, Test Item A was again ineffective at inhibiting bacterial growth at all tested concentrations, showing turbidity even at the highest concentration of 10 µg/mL, leading to an MIC of greater than 10 µg/mL. Test Item B inhibited the growth of *S. mutans* at concentrations as low as 0.1 µg/mL, demonstrating no turbidity at concentrations of 10 µg/mL, 1 µg/mL, and 0.1 µg/mL, but showing growth at 0.01 µg/mL and 0.001 µg/mL, resulting in an MIC of 0.1 µg/mL. Test Item C inhibited the growth of *S. mutans* at concentrations down to 0.1 µg/mL, showing no

turbidity at concentrations of 10 µg/mL, 1 µg/mL, and 0.1 µg/mL, but turbidity at 0.01 µg/mL and 0.001 µg/mL, leading to an MIC of 0.1 µg/mL.

In summary, Test Item A was not effective at inhibiting the growth of any of the three bacterial strains at the tested concentrations, with an MIC greater than 10 µg/mL for all. Test Item B was the most effective, inhibiting growth at lower concentrations for all three bacterial strains, with MICs of 0.01 µg/mL for *S. aureus*, and 0.1 µg/mL for both *E. coli* and *S. mutans*. Test Item C demonstrated moderate effectiveness, with MICs of 0.1 µg/mL for *S. aureus* and *S. mutans*, and 1 µg/mL for *E. coli*.

Drug release

The drug release profiles of metronidazole and doxycycline from the dental cements were evaluated in artificial saliva over 10 days to assess their sustained release capabilities (Figure 9). The drug release profiles exhibited sustained and controlled release of both metronidazole and doxycycline from the dental cement. Initial burst releases were observed within the first 24 hr, followed by gradual and sustained release over the 10-day period. The drug release profiles indicate that both Doxycycline and Metronidazole are effectively released from the pellet over the 10-day period, with Metronidazole demonstrating a more dynamic release pattern while Doxycycline maintains a relatively stable release after an initial increase. These findings suggest that the formulation may be suitable for sustained drug delivery applications, particularly for conditions requiring prolonged antibiotic therapy.

The release kinetics varied with drug concentration, with higher concentrations typically showing prolonged release profiles. The sustained drug release profiles observed in artificial saliva indicate the potential of the *in situ* forming dental cement to deliver therapeutic concentrations of metronidazole and doxycycline over an extended period. This controlled release is crucial for maintaining effective antimicrobial activity at the periodontal site, improving treatment efficacy and patient compliance. These findings support the clinical utility of drug-loaded *in situ* forming dental cement effectively managing periodontal diseases.

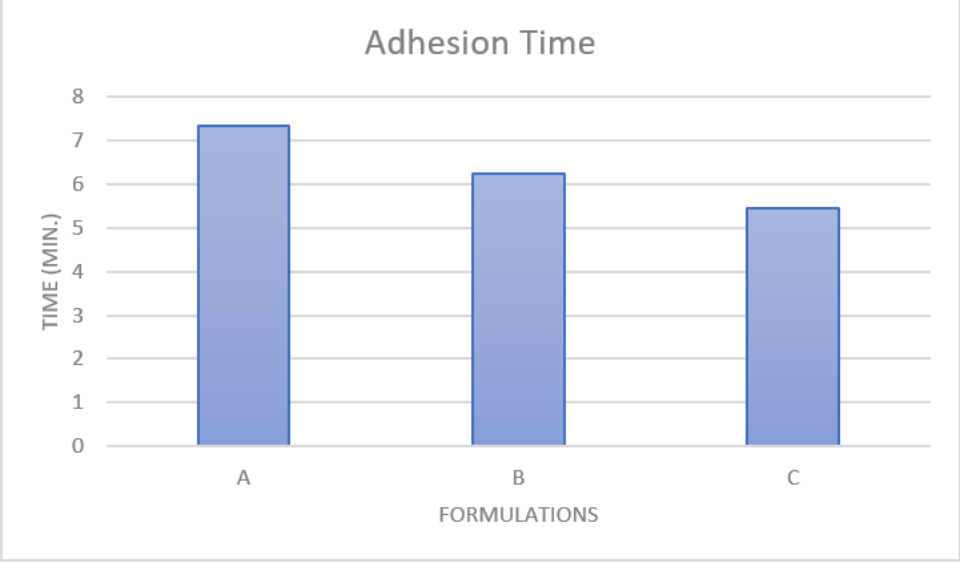

Figure 7: Adhesion test of samples A, B, and C.

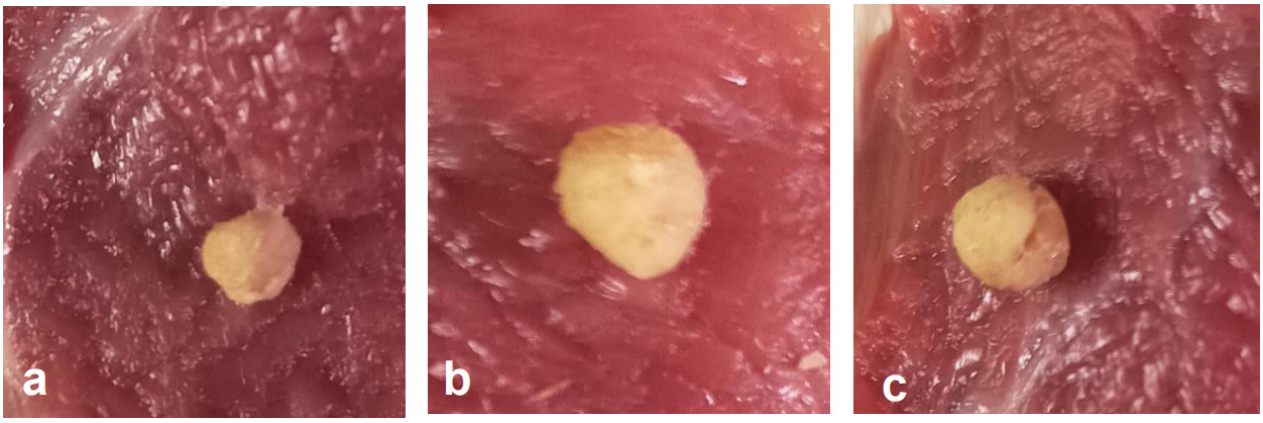

Figure 8: Adhesion Test Pictures of samples A, B, and C.

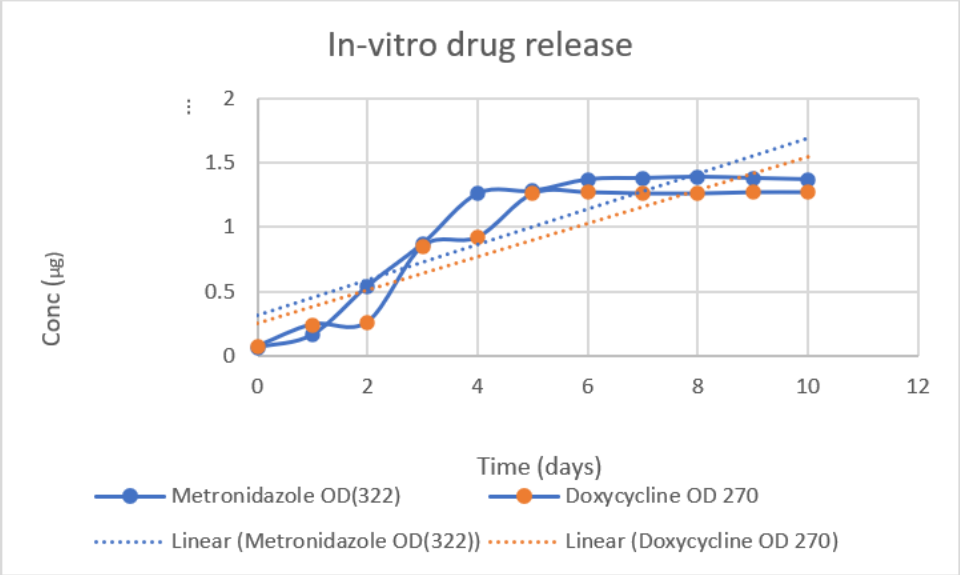

Figure 9: *In vitro* drug release study of Metronidazole and doxycycline.

CONCLUSION

The *in situ* forming dental cement exhibited suitable setting times, smooth surface morphology, and robust mechanical strength, indicating their potential for clinical application in load-bearing dental procedures. FTIR and DSC analyses confirmed the compatibility and stability of the cement formulations, ensuring that drug incorporation did not compromise material integrity. Cellular viability assays demonstrated high biocompatibility, with no significant cytotoxic effects observed. Mucoadhesion studies underscored strong adhesive properties to mucosal surfaces, facilitating sustained drug release and therapeutic efficacy. MIC assays revealed potent antimicrobial activity against common oral pathogens, highlighting the cement potential for combating periodontal infections. *In vitro*, release studies demonstrated sustained and controlled release of metronidazole and doxycycline over 10 days, essential for maintaining effective antimicrobial levels at the periodontal site. In essence, the developed drug-loaded dental cements hold promise as effective therapeutic agents for treating periodontitis, offering a synergistic approach of antimicrobial efficacy, biocompatibility, and controlled drug release tailored for enhanced clinical outcomes. Conducting clinical trials to assess the efficacy and safety of the drug-loaded dental cement in human subjects would validate their clinical utility and therapeutic benefits.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

GIC: Glass ionomer cement; **FTIR:** Fourier-transform infrared spectroscopy; **DSC:** Differential scanning calorimetry; **SEM:** Scanning electron microscopy; **TEM:** Transmission electron microscopy; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **MIC:** Minimum inhibitory concentration; **µg:** Microgram; **µg/mL:** Microgram/Milliliter; **MPa.:** Megapascal.

AUTHOR CONTRIBUTIONS

Anuj Kumar: Experimental Design and Conduct, Chemical Analysis, Mechanical Testing, Biological Evaluation, Mucoadhesion Testing, Antimicrobial Testing, Drug Release Studies, Data Analysis and Interpretation, Manuscript Preparation. Anurag Verma: Experimental Design, Drug Release Studies, Data Analysis and Interpretation, and Manuscript Review.

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