



## Original Article

# Development of a Curcumin-Clove Oil-Loaded Chitosan-Gelatin Dental Implant Coating (Curclodent)

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Received: 3 May 2026

Accepted: 26 June 2026

Published online: 30 June 2026

**Keywords:** chitosan, gelatin, curcumin, clove extract, bromocresol purple, dental implant coating, peri-implantitis, antimicrobial surface, pH-responsive biomaterial

Dental implants are often subjected to failure due to periapical disease, which is mostly caused by bacterial adherence to the implant surface and delayed identification of infection. In this study, we developed a multifunctional biopolymer coating called Curclodent, which provides not only antimicrobial protection but also allows for the detection of peri-implant inflammation based on pH. Curclodent is made up of a chitosan-gelatin matrix bridged with Tannic acid and contains curcumin and clove extract as natural antimicrobial agents. The incorporation of bromocresol purple as a pH indicator allows for visual recognition of acidic microenvironments associated with peri-implant inflammation. Aluminium substrates were used to prepare preliminary coating models by the use of dip-coating methods. The use of aluminium provided a cost-effective substrate for initial characterization, while the stainless-steel screw analogues served to represent the deposition of coatings onto implant-like geometries. FTIR analysis indicated successful polymer integration and spectral changes consistent with hydrogen-bonding interactions within the coating matrix. The Curclodent coatings demonstrated effective antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* through surface-mediated antimicrobial action, and therefore may help to increase the lifespan of the implant. Future biological validation studies need to be conducted to determine their usability in clinical settings.

## Introduction

Due to their long-term functionality, dental implants are a common way to replace a missing tooth. However, one of the major problems with dental implants is peri-implantitis. Peri-implantitis is defined as the inflammation of the soft tissues surrounding the implant; if left untreated, it can cause bone loss around the implant and eventually lead to implant failure [1]. The surface of conventional implants has been designed to enhance bone integration using surface roughening and/or chemical surface modifications, which promote osseointegration; however, these surfaces do not inherently provide an antimicrobial benefit or the ability to detect early infection [6].

Existing antimicrobial methods of surface modification, including antibiotic coatings or metallic nanoparticles (e.g., silver), have been demonstrated to be effective against oral bacteria, but cytotoxicity, the potential for resistance development to antibiotics, and non-standardised release characteristics have limited their use in clinical applications [7,8].

As a result of their generally lower risk profile than synthetic materials and their potential for antimicrobial activity, scientists are now investigating the use of natural bioactive materials for antimicrobial coatings of implant surfaces

There are two examples: curcumin (a polyphenolic substance derived from turmeric) exhibits antimicrobial and anti-inflammatory properties [2,3], and clove extract (containing eugenol) shows robust antimicrobial and anti-biofilm properties against oral pathogens [4,5]. When incorporated in a bio polymeric matrix it reduces the systemic side effects; these substances exert a local antimicrobial effect on the targeted tissue.

Biodegradable and biocompatible polymers such as chitosan and gelatin are commonly used for biomedical coatings [9,10]. Chitosan is inherently able to inhibit bacterial growth due to its positively charged (cationic) molecular structure, allowing it to interact with negatively charged (anionic) bacterial membranes [11,12]. Gelatin increases the flexibility of the coating film while also promoting the biological at-

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<https://doi.org/10.65795/m7sacs50>

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tachment of surrounding cells. Chitosan and gelatin form an effective, stable combination of two polymers that can be used together to create a composite surface coating.

Chitosan's antibacterial properties provide an important source of antimicrobial protection; however, another challenge facing implant manufacturers remains the need for early detection of microbiological infection. For example, localised acidic environments exist around implants due to bacterial metabolic activity and cause peri-implant disease. Incorporation of a pH-sensitive indicator (bromocresol purple) into a coating polymer may allow the physician to visually identify shifts in the pH surrounding an implant and detect a potential infection before the occurrence of major destructive changes in the adjacent tissue. As part of this work, a polymer-coated dental implant referred to as CurcloDent is being developed as a multifunctional coating by integrating antimicrobial activity with pH-sensitive detection using a combination of chitosan and gelatin cross-linked using tannic acid.

This study will involve the development of the coating formulations based upon structural characterisation, examination of the surface wettability, and evaluation for antibacterial activity before validating the intended application.

The main aim of this research is to develop and evaluate the dip-coated chitosan/gelatin implants, with the addition of antimicrobial agents and indicators of acidic pH level. This will enable the use of implants to prevent and detect the early onset of dental implant-related infections. And the main objectives of the study are a) Develop a uniform chitosan–gelatin composite coating on metallic substrates using the dip-coating technique for preliminary evaluation of coating performance. b) In addition to providing a physical barrier to protect against pathogenic organisms, incorporate curcumin/clove extract into the polymer matrix to provide effective antimicrobial activity against pathogenic oral organisms [2–5]. c) Incorporate bromocresol purple into the coating material to enable visual detection of pH changes associated with peri-implant inflammation. d) Confirm successful polymer blending/crosslinking of coating by using Fourier Transform Infrared Spectroscopy (FTIR). e) Assess the biological compatibility of the coating on the surface by measuring contact angles. f) Utilize a suitable microbiological assay method to evaluate whether or not the composite coating exhibits anti-microbial activity toward three selected strains of bacteria, namely *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. g) Gather preliminary data to assess the dual capabilities of the coating system as both a bactericidal agent and a pH-sensitive surface modifier to implanted metal implants.

## Materials and Methods

The primary structural polymer in the development of CurcloDent is chitosan. Chitosan functions as the film-forming material due to its natural antibacterial activity. Additionally, the cationic nature of chitosan interacts with bacterial cell membranes, presenting a surface-mediated method of antimicrobial action [11,12]. The use of gelatin helps in enhancing the flexibility and stability of the film. The blending of chitosan and gelatin produces a uniform composite suitable for the coating of metallic substrates [9,10]. Curcumin acts as a natural source of antimicrobial activity against both Gram-positive and Gram-negative bacteria, which enhances the overall effectiveness of localised antibacterial activity in the polymer matrix [2,3]. Eugenol is the primary component of clove extract, which contributes to the antimicrobial and antibiofilm properties of CurcloDent, working synergistically with curcumin and chitosan [4,5]. Bromocresol purple has been used as an embedded pH-sensitive indicator that exhibits a visible colour change in response to the acidic pH of infected tissues. Tannic acid is used as a crosslinking agent to provide structural stability and improved resistance to dissolution. Chitosan was dissolved and prepared for coating using 1% acetic acid as the solvent for the chitosan coating solution. Aluminium sheets were used as model substrates for initial characterization of the coating due to their low cost and ease of handling. In addition, stainless steel screws were used to demonstrate the coating deposition on structures similar to implants. The proposed substrates are only used as proof of concept and were not clinically optimised for use in implants.



**Figure 1: Dip-coated aluminium substrate**

### Preparation of chitosan solution

A small batch of 1% chitosan solution was prepared by dissolving 0.23 g of chitosan in 23 mL of 1% (v/v) acetic acid [13,14]. The solution was stirred continuously until a uniform viscous solution was obtained without visible aggregates. Small batches were prepared to minimize the possibility of contamination and to maintain the quality of the polymer solution before coating.

### Preparation of gelatin solution

A small batch of 1% gelatin solution was prepared by dissolving 0.10 g of gelatin in 10 mL of distilled water at 40–45 °C with continuous stirring until a clear and homogeneous solution was obtained [9]. The solution was allowed to cool slightly before blending with the chitosan solution.

### Chitosan-gelatin composite matrix formation

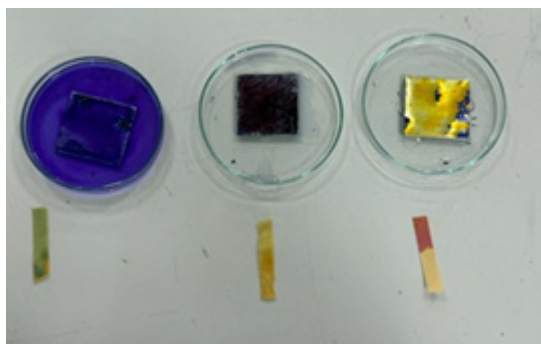
The prepared chitosan and gelatin solutions were combined in a 70:30 (chitosan:gelatin) ratio to obtain the composite polymer matrix. The gelatin solution was added gradually to the chitosan solution under continuous magnetic stirring for 30–45 minutes until a homogeneous blend was obtained. This ratio was maintained throughout the preparation of all coating batches. To minimise the possibility of contamination and maintain the consistency of the polymer blend, the coating solution was prepared in small batches immediately before use. Subsequently, tannic acid (0.5% w/v) was added as a crosslinking agent to improve the stability of the polymer matrix through hydrogen-bonding interactions [15].

### Incorporation of bioactive agents

The Curcumin was dissolved in a small amount of ethanol to create an even distribution for the addition of the Clove extract (0.05% v/v) to augment the antimicrobial properties of the two ingredients being combined into a composite matrix using the continuous stirring process (this would ensure that both of the extracts were evenly dispersed through the final coating formulation) [2–5]. After this was achieved, Bromocresol purple (0.03% w/v) was also added to the solution as a pH indicator

**Table 1: Antibacterial activity of curclodent against selected microorganisms**

| Indicator organism                          | Agar well diffusion | Spot assay |
|---------------------------------------------|---------------------|------------|
| <i>Staphylococcus aureus</i><br>ATCC 25923  | 6 ± SD mm           | ++         |
| <i>Enterococcus faecalis</i><br>ATCC 29212  | -                   | ++         |
| <i>Pseudomonas aeruginosa</i><br>ATCC 27853 | -                   | ++         |



**Figure 2: Visible pH based colour change of the coating**

and thoroughly mixed into the polymer solution until completely incorporated. Once completed, the resulting mixture was stirred until it forms a homogeneous coating without any visible evidence of phase separation.

#### Dip-coating procedure

Before coating, aluminium sheets and stainless-steel screw analogues were cleaned sequentially by means of deionised water and ethanol solution to ensure clean surfaces prior to application of coatings. The cleaned substrates were then submerged into the composite solution, and subsequently withdrawn from the solution at a fixed rate to keep the film thickness of the deposit uniform. After this process, the excess solution on the substrate was drained away, and then the substrate was allowed to dry by air. The dip-coated aluminium substrate is shown in figure 1.

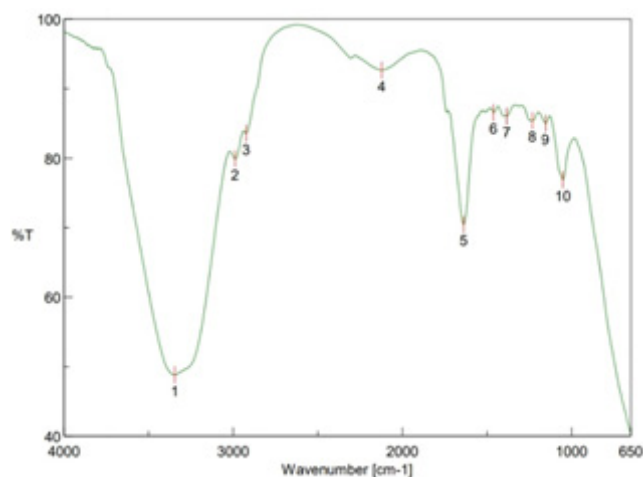
## Results and Discussion

#### pH-Test

Coated substrates with the CurcloDent coating were dipped into acidic and alkaline solutions having different pH values to see how their colors changed depending upon the pH of the solution. Coated substrates exhibited clear, repeatable colours at different pH values, which were as follows: purple for the alkaline (8-10) pH; yellow for the acidic (<5.5) pH; and a darker shade of colour for intermediate (7-7.5) pH's. This demonstrates that the bromocresol purple was successfully incorporated and maintained its function as a dye within the polymeric matrix. The visible pH-dependent colour changes of the coating are shown in figure 2. After films had dried, they still showed changes in colour when dipped into acid or alkaline solutions; therefore, the pH-sensitive response of the chitosan–gelatin network was not inhibited after drying of the films. The colour change observed in response to differing pH values is an indicator of the acidic microenvironment that exists due to bacterial activity during the inflammatory stage surrounding a dental implant. Although bromocresol purple showed a distinct colour change in response to pH variation, its release from the polymer matrix was not investigated in the present study. Since the coating is intended for bio-



**Figure 3: Antibacterial activity of curclodent against the different strains of bacteria**



**Figure 4: FTIR Spectrum of curclodent confirming polymer crosslinking**

medical applications, future studies should evaluate the release profile of the dye together with its *in vitro* cytotoxicity to better understand its long-term biocompatibility. Such investigations will provide additional evidence regarding the safety of the CurcloDent coating before clinical translation.

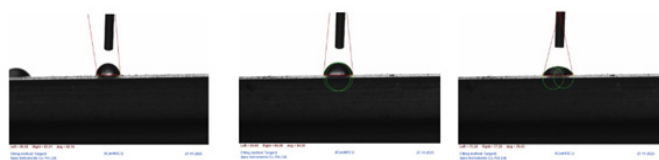
#### Antimicrobial test

The evaluation of antibacterial activity involved the use of three reference strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, and *Pseudomonas aeruginosa* (ATCC 27853).

The coatings tested positive for antibacterial activity against all three bacterial strains when assessed using the spot assay. The antibacterial activity of CurcloDent against the selected microorganisms is summarized in table 1. In addition, the agar well diffusion assay demonstrated the inhibition of the growth of *S. aureus* (where an inhibition zone of 6 mm was observed) while no inhibition zones were evident for either *E. faecalis* or *P. aeruginosa*. The antibacterial activity observed against the selected bacterial strains is shown in figure 3. The difference between the agar well diffusion assay and the spot assay suggests that the antimicrobial activity of CurcloDent is mainly surface-mediated rather than diffusion-dependent. The absence of inhibition zones against *Enterococcus faecalis* and *Pseudomonas aeruginosa* in the agar well diffusion assay may be due to the limited diffusion of the polymer-based coating through the agar medium, which was also observed during the microbiological evaluation. In contrast, positive results obtained in the spot assay indicate that the coating remained effective when in direct contact with the bacterial cells. The tannic acid crosslinked polymer matrix may also have limited the release of the active compounds while preserving their antibacterial activity on the coated surface. Such surface-localized activity is advantageous for implant coatings, where prolonged protection at the implant interface is generally preferred over rapid diffusion into the surrounding tissue [2-5,11].

#### FTIR analysis

Fourier transform infrared spectroscopy was used to confirm that the composite coating has been formed. The FTIR spectrum of the CurcloDent coating is shown in figure 4. The O–H and N–H stretching vibrations caused by hydrogen bonding interactions between chitosan, gelatin and tannic acid were observed as broad absorption bands at  $\sim 3345$   $\text{cm}^{-1}$ . The C–H stretching vibrations produced by the organic materials were also seen in the area of  $2923\text{--}2989$   $\text{cm}^{-1}$ . In addition, the presence of an amide I band at about  $1638$   $\text{cm}^{-1}$  indicates the presence of gelatin in the system. The glycosidic linkages characteristic of chitosan were observed in the region of  $1154\text{--}1052$   $\text{cm}^{-1}$ . Compared with the characteristic spectra of the individual polymers reported in the literature, the broadening of the O–H/N–H stretching band around  $3345$   $\text{cm}^{-1}$  to-



**Figure 5: Contact angle measurement at different time intervals**

gether with the slight shift of the amide I band is consistent with increased hydrogen-bonding interactions between chitosan, gelatin, and tannic acid rather than a simple physical blend. Although FTIR alone cannot conclusively confirm crosslinking, the observed spectral changes are consistent with the formation of a hydrogen-bonded polymer network and support the successful incorporation of tannic acid within the chitosan–gelatin matrix [15].

### Contact angle measurement

Time-dependent contact angle measurements were employed to assess the wettability of the coated surface. The initial contact angle after 0 min was measured at  $92.14^\circ$ , and the contact angle at 15 min was reduced to  $84.08^\circ$ . The contact angle decreased to  $76.43^\circ$  at 30 min. The time-dependent contact angle measurements are shown in figure 5. As time passes, the lowering of the contact angle shows that there is increasing ‘wetting’ of the surface, and the chitosan–gelatin matrix has become eluted and/or swollen as it absorbs moisture. From a biomechanical indicator, the formation and proof of a time-dependent change to a hydrophilic surface is beneficial in an environment that can help adsorb proteins to promote cell attachment but also reduce the degree of bacteria attached to the surface. The results suggest the CurcloDent coating undergoes a transition from relatively hydrophobic to moderately hydrophilic in a short period of time and may therefore improve biological integration with the host while still exhibiting antimicrobial properties.

### Conclusion

This study has created an anti-microbial active coating for use on dental implants called “CurcloDent.” It is made from chitosan and gelatin in the ratio of 70% chitosan and 30% gelatin and contains two bioactive agents: curcumin and clove oil. Tannic acid was incorporated to promote crosslinking within the polymer matrix, improving its structural stability and controlling the rate of swelling during hydration. Evidence of proper interaction between the polymer matrix and bioactive agents was determined through the use of Fourier-transform infrared spectroscopy (FTIR). Additionally, by measuring the contact angles over time, it was determined that the coating improved its wettability (relative to its original state) because it became more hydrated and swelled as a result. Improved hydrophilicity of the CurcloDent coating has significant implications for how well proteins attach to it, and, thereby, how well cells will attach to it. Cell attachment is critical for the success of implant integration. Preliminary tests for antimicrobial activity were conducted against representative bacterial species. The results indicated that curcumin in combination with clove oil has an effective bactericidal effect on the bacteria tested. The use of naturally-derived bioactive com-

pounds could potentially provide an effective replacement for commonly-used antibiotic kinds of coatings and may avoid the risk of developing resistant strains while retaining an equal level of effectiveness. The CurcloDent coating will likely prove to be a multifunctional antimicrobial coating with a wide variety of applications beyond dental implants. Its ability to provide enhanced wettability together with effective surface-mediated antimicrobial activity makes it a promising candidate for further investigation. Future studies should evaluate the long-term stability of the coating, the release profile of bromocresol purple, and its *in vitro* cytotoxicity to establish its safety and suitability for biomedical applications.

### Acknowledgments

We would like to express our sincere gratitude to Dr. T. Sudhakar, Dr. K. Gobi Saravanan, and Mr. Bharath Sankaralingam for their exceptional support, encouragement, and guidance throughout the entire project. We are grateful for the valuable insights and guidance throughout the project. We have greatly benefited from the guidance provided in the development and successful completion of this project. In addition, their willingness to mentor us and share their expertise has made our overall learning experience throughout this research project very impactful.

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