

Thermoreversible gel for intrapocket delivery of green tea catechin as a local drug delivery system: An original research

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ABSTRACT

The periodontal therapies along with systemic antibiotic therapy aim at eliminating the subgingival microbiota to arrest the progression of periodontal diseases. The complete elimination is often difficult, and thus the probability of repopulation after periodontal therapy is also high. The objectives of the study are to develop *in situ* thermoreversible gelling system of green tea catechins suitable for periodontal pocket administration, which would act as an adjunct to mechanical periodontal therapy. Gel is prepared on a weight basis using a cold process. *In vitro* drug release pattern is observed through spectrophotometer analysis at 277 nm. The gel is subjected to serial dilution analysis to determine the minimum inhibitory concentration (MIC) and disc diffusion analysis to determine the *in vitro* antibacterial effectiveness. Release pattern studies showed a complete release of drug from gel occurred by 36 h. A volume of 1.25 mg/ml was determined as MIC required against the periodontal pathogens. Disc diffusion analysis showed a 14 mm zone of inhibition is present around the 75 μ l well for all the four species and 12 mm zone of inhibition around the 50 μ l well. The advantage of F-127 is its thermoreversible nature that used for *in situ* gel formulation. Pluronic gel proved to be a promising carrier for prolong and effective release of green tea catechin.

Key words: *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*

INTRODUCTION

Gram-negative microorganisms are responsible for the inflammation of supporting tissues of periodontium leading to the destruction of tissues resulting in periodontal disease.^[1-3] The periodontal therapies along with antibiotic therapy should aim at eliminating the microbiota to arrest the progression of diseases. Systemic antibiotics used

as an adjunct to mechanical therapy is questionable as the dosage attained in gingival crevicular fluid through such antimicrobial therapy does not maintain minimum inhibitory concentration (MIC) against periopathogens for a sustained period.^[4]

Sustained antimicrobial delivery systems incorporating various agents, such as doxycycline, metronidazole, and chlorhexidine, aimed at inhibiting the growth of pathogens thereby limiting the periodontal tissue breakdown.^[5-7]

Green tea (*Camellia sinensis*) has received considerable attention because of its numerable scientifically proven

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health benefits attributable to the presence of various polyphenols.^[8,9] Several epidemiologic and experimental observations in the field of medicine and dentistry have suggested that green tea catechins may exert cardioprotective, antioxidant, cholesterol-lowering, antiobesity, antidiabetic, anticancer, anti-inflammatory, anticaries, antibacterial, antifungal, and antiviral effects.^[10-12] Epigallocatechin gallate (EGCG), the most abundant polyphenol component in green tea, is responsible for all these diseases preventing properties.^[13-15] Some studies have reported that EGCG inhibits the activity of collagenase and gelatinase. In this study, an attempt was made to prepare a thermosetting gel system incorporating naturally occurring green tea extract and to evaluate its *in vitro* release pattern and antimicrobial efficacy over periodontal pathogens.

The thermoreversible gel is used more commonly and achieved success in treating periodontal diseases. Poloxamers form micelles when used in low concentrations, whereas in high concentrations it has the unique property of forming a thermoreversible gel. The gel forming solutions are formulated using F-127 and F-68 polymer by phase transition mediated by temperature. This helps in forming a gel in periodontal pockets at body temperature, leading to the availability of the drug in the pockets resulting in better therapeutic activity. The objectives of this study are to develop *in situ* thermoreversible gelling system of green tea catechins suitable for periodontal pocket administration.^[16,17]

MATERIALS AND METHODS

Green tea catechin is obtained from NESSO, Mysore. The extract consisted of about 90% catechins of which 96% consisted of EGCG. Polymer pluronic F-127 (PF-127) is obtained from JSS College of Pharmacy, Mysore, and in the same place, gel is formulated. A volume of 25 mg of green tea catechin is weighed and added sequentially to 5 g of PF-127 polymer. The weighed amount of PF-127 was added to cold water at 5°C and thoroughly stirred until 20% thermoreversible solution was prepared. The dispersion was then refrigerated until a clear solution is formed. MIC of green tea catechin is taken as 1.25 mg/ml slightly more than Hirasawa and Takada *et al.* study.^[18]

Estimation of drug content in gel formulations

Formulations containing 1 mg drug was taken in 10 ml volumetric flask, dissolved in ethanol, made up the volume to 10 ml with ethanol, and then filtered. Absorbance values were measured with suitable dilutions at 277 nm.^[19,20] Drug concentrations were calculated from the standard calibration curve prepared in ethanol.

In vitro drug release

With the 1 g of pluronic gel in a dialysis tube with 2.3 cm diameter was placed in a vessel containing 100 ml phosphate buffer at the pH of 7.4 and allowed to stir at 100 rpm.

Fresh medium of phosphate buffer at the pH 7.4 was used after collecting the samples. Whatman filter paper is used to filter, and then the green tea catechin was determined spectrophotometrically at 277 nm.^[21]

In vitro inhibitory effects of gel on periodontal pathogens

The inhibitory effect of formulated gel on growth of pathogens is identified by disc diffusion analysis. MIC against *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Actinobacillus actinomycetemcomitans* is determined by serial dilution analysis followed by disc diffusion analysis. Blood agar culture medium is used to culture the organisms.

Minimum inhibitory concentration procedure

Nine dilutions of each drug have to be done with thioglycollate broth for MIC. In the initial tube, 20 µl of drug was added to the 380 µl of thioglycollate broth. For dilutions, 200 µl of thioglycollate broth was added to the next nine tubes separately. Two hundred microliter mixture of drug and thioglycollate from the initial tube was transferred to the first tube in which 200 µl thioglycollate broth was already present. This was considered as 10⁻¹ dilution. From 10⁻¹ diluted tube, 200 µl was transferred to the second tube to make 10⁻² dilution. The serial dilution was repeated up to 10⁻⁹ dilution for each drug. From the maintained stock cultures of required organisms, 5 µl was taken and added to 2 ml of thioglycollate broth. Two hundred microliter of the culture suspension was added to all tubes which were diluted before. All the test tubes kept for incubation for 24 h and checked for turbidity.^[17]

Disc diffusion procedure

Brain heart infusion agar broth is used for analysis. Agar plates are brought to room temperature before use. Using a loop or swab, precultured colonies of microorganisms were transferred to the plates. Visually, turbidity is adjusted with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, standardize the suspension with a photometric device. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculum. Agar plates were rotated approximately at 60° in between streaking and see to that there is an even distribution on the entire surface after swabbing the plates thoroughly for at least three times. Allow inoculated plate to stand for at least 3 min but no longer than 15 min before applying disks. Take a hollow tube of 5 mm diameter, heat it. Press it on above inoculated agar plate and remove it immediately by making a well in the plate. Likewise, make five well on each plate. Add 75 µl, 50 µl, 25 µl, 10 µl, and 5 µl of compound into the respective wells on each plate. Incubate plates within 15 min of compound application. Invert plates and stack them no more than five high. Incubate for 18–24 h at

37°C in an incubator. Make sure that the plates are ready to read only when the growth is nearly confluent. With the help of measuring device, the inhibition zone is measured very precisely to the very nearest whole millimeter.^[21-23]

RESULTS

The drug is subjected to *in vitro* analysis to analyze the release pattern and antibacterial activity following which the drug is incorporated into periodontal pockets of periodontitis patients, and changes in clinical parameters are analyzed.

In vitro analysis

EGCG is identified as the major constituent in the gel. Release pattern studies showed a complete release of drug from gel occurred by 36 h [Charts 1 and 2].

Serial dilution analysis of the drug for each pathogen is done and 1.25 mg/ml is determined as MIC required against the pathogens. The clear fluid represents the sensitivity of pathogen toward the drug, whereas turbidity marks the resistance of the bacteria to the drug.

Serial dilution results showed that almost all the nine dilutions of gel were effective against *P. gingivalis* and *P. intermedia*. *A. actinomycetemcomitans* strain was sensitive up to six serial dilutions of the gel, whereas *F. nucleatum* was sensitive for five serial dilutions. After incubation with gel, all the four species showed an inhibition zone of about 12–14 mm around the 75 µl well on agar plates [Figures 1-4].

Disc diffusion analysis showed a marked inhibition in the growth of microorganisms. A 14 mm zone of inhibition is present around the 75 µl well, and 12 mm zone of inhibition is present for all the four species around the 50 µl well.

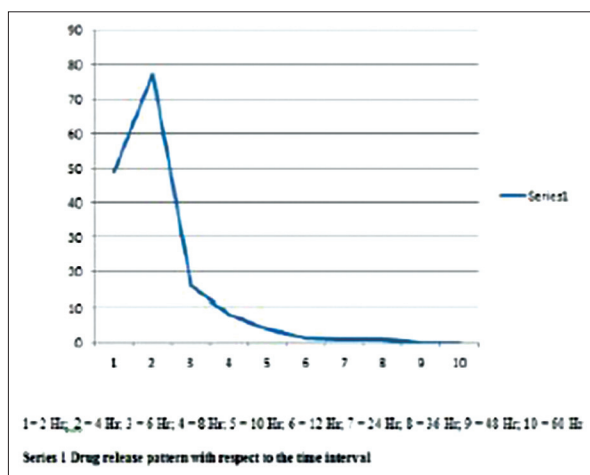


Chart 1: Drug release pattern with respect to time interval

In vivo analysis

The clinical parameters used were plaque index (PI), gingival index (GI), and pocket probing depth (PPD). There was no statistically significant difference between the test and control groups at baseline.

The mean GI, PI, and PPD scores were significantly reduced in both control (1.26 ± 0.562), (1.16 ± 0.375), and (4.37 ± 0.831) and test (0.58 ± 0.607), (0.32 ± 0.478), and (3.37 ± 0.597) group at 28th day. The intergroup comparison showed that the reduction in mean GI, PI, and PPD score at the end of 28 days was significantly higher in the test group (1.69 ± 0.96), (1.72 ± 0.75), and (1.68 ± 0.25) when compared to the control group (1.00 ± 0), (0.88 ± 0.85), and (0.88 ± 0.73), respectively.

DISCUSSION

The advantage of local drug delivery (LDD) system in the treatment of periodontal disease is controlled release of the drug with the help of drug reservoir and limiting element. The main aim of the drug is to maintain the needed concentration at the site for a longer duration. Sustained and controlled release devices are designed to provide drug release within 24 h and more than 1 day, respectively. The drug release in controlled delivery system takes over a period of time to achieve pharmacological objectives of a locally applied antimicrobial agent.^[24,25]

According to the *in vitro* studies on catechins, it is clear that EGCG has the function of inhibiting the growth of microorganisms.^[26] The use of degradable and bioresorbable polymers in drug delivery has gained attention in recent years. Delivery of catechin by one such polymer was documented by Hirasawa *et al.* In this study, hydroxypropyl cellulose which contains catechin in the form of strips was used as local drug delivery system. The study concluded that other modes of LDD with an improved retention period

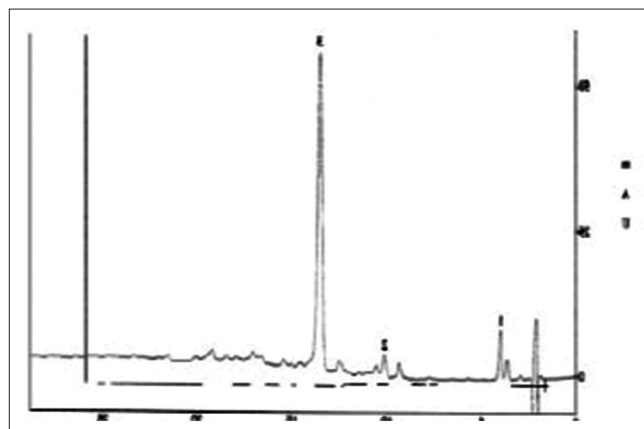


Chart 2: A complete release of drug by 36 h. The peak represents epigallocatechin with trace elements of gallic acid and epigallocatechin

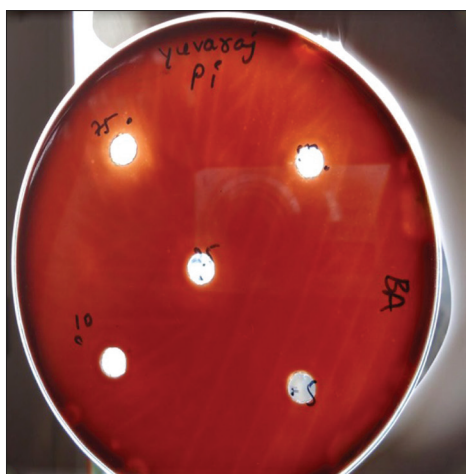


Figure 1: *Prevotella intermedia*

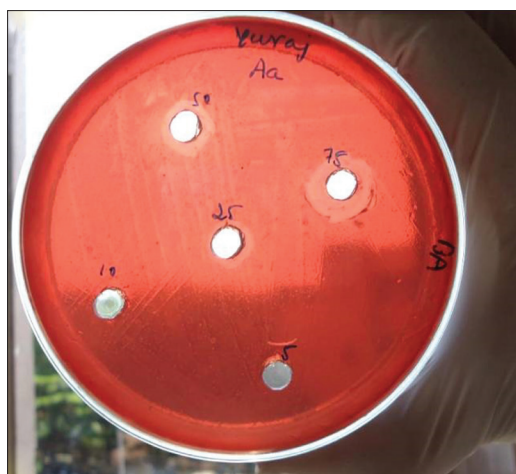


Figure 2: *Actinobacillus actinomycetemcomitans*



Figure 3: *Porphyromonas gingivalis*

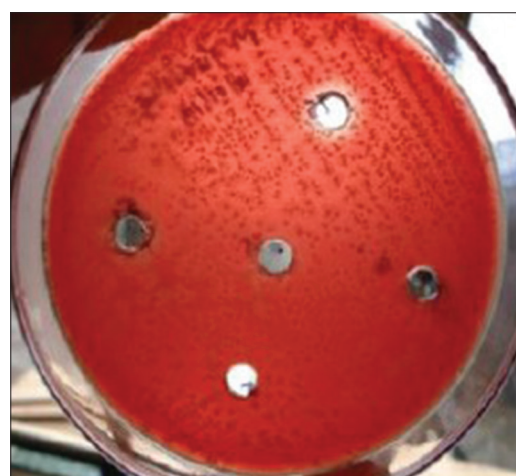


Figure 4: *Fusobacterium nucleatum*

and more controlled release was required to achieve better results.

In our study, PF-127 gel along with catechin is formulated as an LDD and *in vitro* analysis of the gel is carried over. Absorbance values at 277 nm showed a complete release of drug from the gel at 36 h. Hirasawa *et al.* (2002) in a similar type of *in vitro* analysis based on Noguchi *et al.* study (1988) analyzed the release of green tea catechin from HPC strips into the solution by monitoring the absorbance at 277 nm using a spectrophotometer at selected intervals. In a recent study by Chava and Vedula,^[27] the drug release from thermoreversible polymer was analyzed. The results showed a sustained release of 96.14% at 108 h. The variation in the release pattern of this study, when compared to our results can be attributed to the addition of carbopol 934 before addition of PF-127. Carbopol acts like a block copolymer and provides a sustained release pattern for over 4 days.

The concentration of catechins used in the preparation of gel was decided to be maintained at 1.25 mg/ml and

was prepared by JSS College of Pharmacy, Mysore, Karnataka. *In vitro* assay for the determination of MIC against specific pathogens is analyzed by serial dilution analysis and disc diffusion analysis. These *in vitro* antimicrobial sensitivity tests were carried over in Microbiology Department, Maratha Mandal Dental College, Belgaum, Karnataka. Strains tested include *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, and *F. nucleatum*. After incubation with gel, all the four species showed an inhibition zone of about 12–14 mm around the 75 μ l well on agar plates. In this study, inhibition was considered positive if there was an inhibition zone of 1 mm or more around the well.

The result of disc diffusion analysis showed that 75 μ l of gel was sufficient to produce a growth inhibition of microbial strains. Serial dilution results showed that almost all the nine dilutions of gel were effective against *P. gingivalis* and *P. intermedia*. *A. actinomycetemcomitans* strain was sensitive up to six serial dilutions of the gel, whereas *F. nucleatum* was sensitive for five serial dilutions of the gel.

CONCLUSION

Controlled drug delivery has become one of the important areas of research both in pharmaceutical and periodontal research. The thermoreversible gel PF-127 is the best choices as drug carrier when treating periodontal diseases by local drug delivery method. PF-127 is an excellent drug delivery system which is widely used by the researchers due to its characteristic cohesive, diffusive transport, ease of application, and cosmetic and compatible properties. Thermal gelation of PF-127 helps in increasing the drug contact time with the adjacent tissues in the pocket, thus prolonging the pharmacological action. Hence, with the above advantages, thermoreversible gel is used for intrapocket delivery of green tea catechin as a local drug delivery system.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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