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An In Vitro And In Vivo Evaluation Of Novel Indigenously-Designed Controlled Release Flurbiprofen Gel System For Management Of Periodontal Diseases.

[1] Neeraj Deshpande
[2] K. M. Bhat
[3] G. S. Bhat
[4] Anshula Deshpande

[1] Associate Professor, Department of Periodontics
K.M.Shah Dental College,
Sumandeep Vidyapeth, Vadodara
[2] Professor
[3] Professor and Head
Department of Periodontics
Manipal College of Dental Sciences, Manipal
[4] Associate Professor, Department of Pedodontics
K.M.Shah Dental College,
Sumandeep Vidyapeth, Vadodara

Address For Correspondence:
Dr. Neeraj Deshpande
Associate Professor, Department of Periodontics
K.M.Shah Dental College, Sumandeep Vidyapeth,
Vadodara- 391760
Email: drneeraj78@rediffmail.com
Ph: +91 7600918500

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Abstract
Periodontal diseases are mainly the immune-inflammatory diseases caused by the microbial accumulations on the tooth surfaces. These microorganisms release number of chemical mediators which stimulates the series of host inflammatory response. Various Chemotherapeutic agents have been tried to modulate host response. Flurbiprofen is a non-steroidal anti-inflammatory drug widely researched as host modulating agent for periodontal diseases. A controlled release drug delivery system was designed for the effective delivery of flurbiprofen to the periodontal tissues with minimum side effects. The present study was undertaken to evaluate the in vitro and in vivo release of the drug from the novel indigenously prepared delivery system. The results of the study showed the sustained release of the drug from the system in the effective concentration for the period of 14 days.

Key Words
Host-Modulation therapy, periodontal diseases, Flurbiprofen, Local drug delivery, PLGA gel system

Introduction

Periodontal diseases are the inflammatory diseases caused by microbial plaque accumulated on the tooth surface. The severity as well as clinical manifestations of the disease depends on the immune-inflammatory reaction manifested by host defense mechanism.

The production of cyclooxygenase products of arachadonic within periodontal tissues may partly mediate the destructive process of the periodontal tissues[1],[2]. The ability of the non-steroidal anti-inflammatory drugs (NSAIDS) to block cyclooxygenase pathway and reduce the prostaglandin synthesis led to series of studies demonstrating inhibition of periodontal disease progression.

Flurbiprofen is one of the most widely studied NSAID due to its potent inhibition of alveolar bone loss in periodontal disease[3],[4]. Although relatively low dose systemic NSAIDS have been successfully used in periodontics, it is not without side-effects. The major among these was gastrointestinal tract irritation. It is therefore desirable to formulate an agent that will deliver an effective dose of NSAID into periodontal tissues with minimum side effects. In general, topical application of NSAIDs is possible because these drugs are lipophilic and are absorbed into gingival tissues.

In this regard, safe and intrinsically efficacious medications can be delivered into periodontal sites to suppress or modulate the inflammatory host response. Different forms of controlled-release systems are like solutions, pastes, hollow fibers, acrylic strips, monolithic fibers, resorbable cellulose, collagen and biodegradable gel. Using controlled release gel from within the pocket, a single administration of few milligrams of a chemotherapeutic agent can maintain therapeutic concentrations within crevicular fluid for long time than other mode of delivery[5].

The present study was undertaken to study the sustained release of the drug(Flurbiprofen) from the delivery system in in vitro conditions as well as to study the Gingival Crevicular fluid(GCF) concentrations of the drug(Flurbiprofen) from the respective gel systems at various intervals.

Material and Methods

Flurbiprofen is a NSAID and is potent cyclooxygenase inhibitor. We have instituted this study to evaluate the effect of flurbiprofen on gingival inflammation when placed locally as controlled release drug delivery system. It was also very important to the study the concentration of drug released from the delivery system over the period of time in vitro and then the concentration of the drug in vivo.

There are number of human studies and trials using flurbiprofen. Hence the safety of the drug is well proven. The drug used here was in concentration of 0.3%, which was least likely to cause any systemic

Table 1: Ingredients Of In Situ Gel

Material	
Flurbiprofen	BioChem, Hyderabad, India
Polymers	
Poly(lactide)-co-glycolide(75:25) (PLGA)	Birmingham Polymers Inc, USA
Chemicals	
Triacetin	Sigma Aldrich Inc, USA
N-methyl-2-pyrrolidone(NMP)	Sigma Aldrich Inc, USA
Instruments	
Sonicator	Sonics and Materials, USA
Shaker	Rimi Equipments

side effects. The study was reviewed and approved by the ethical committee of Kasturba Medical College, Manipal. The in vitro stud y was performed at College of Pharmaceutical Sciences, Manipal, India and the in vivo analysis was done at College of Dental Sciences, Manipal, India.

The ingredients of the gel are given in the **[Table 1]**. Accurately weighed amount of PLGA (copolymer ratio 75:25, having intrinsic viscosity 0.55 to 0.75dl/gm.) was placed in a glass vial and the required amount of biocompatible solvents was added. The vial was agitated using a mechanical shaker overnight to obtain clear solution. Weighed amount of drug was added to the polymer solution. Weighed amount of drug was added to the polymer solution, sonicated repeatedly to get uniform dispersion. The formulation contained 18% PLGA(75:25) in NMP, Flurbiprofen(15mg), PLGA (360mg), NMP(1.625gms).

The phosphate buffer saline was used to collect the gingival crevicular fluid samples for analysis of the drug levels.

In vitro evaluation of in situ gel

In vitro release studies of the drug delivery system is an important property of the characterization of the system. The in vitro drug estimation was performed in the simulated in vivo conditions so that the performance of the system in in vivo conditions can be predicted. The percentage cumulative drug release was estimated using UV spectrophotometer.

In vivo evaluation of drug concentration

The patients with localized periodontal pocket were selected for the study. Care was taken to ensure that patients do not have any systemic diseases, history of antibiotic or anti-inflammatory therapy in past 6 months. The patients who received gel were sub-divided into two categories viz., one with gel placement only (GO) and other with oral prophylaxis followed by gel placement(GOP).

The area of placement was dried properly and isolated. The pocket wall was then separated from the tooth surface with the help of an air syringe. 0.2ml of the gel was placed carefully in the pocket with the help of syringe and needle. When solution comes in contact with in vivo aqueous environment, it transforms into solid matrix that releases the loaded drug for prolonged period of time.

The gingival crevicular fluid was collected following gel placement and then at every recall visit i.e. on the 7 and 14 day after gel placement. Whatman No.1 filter paper discs of 6mm diameter were used to collect the samples. For collection of the samples the experimental tooth was cleaned and isolated. The filter paper disc was carefully placed into sulcus and left in the position for 3 minutes. Then it was removed and immediately transferred to a vial containing 5ml of isotonic phosphate buffer saline. The vial containing the sample was then closed and vigorously shaken for two minutes and then left undisturbed for 10 minutes. This was done so that the entire drug absorbed could be extracted from filter paper. At the end of 10 minutes this solution was filtered to remove any impurities like blood or plaque. The filtered solution was thus analyzed using UV spectrophotometer to obtain absorbance of the drug in 1ml of solution.

Results and Discussion

A sustained release delivery system consists of either non-resorbable or bioabsorbable matrices. They consist of a drug reservoir and a limiting element that controls the rate of medicament release. The present study was planned to develop a controlled release formulation of NSAID flurbiprofen to be used in the periodontal pocket as an adjunct to the conventional periodontal treatment.

As a first step, the PLGA copolymer in NMP containing 0.3% flurbiprofen was prepared. The in vitro release of the drug was then observed for 360 hours. The use of polymeric drug delivery devices in dentistry has added the new dimensions to the research in the field of locally

Table 2: In Vitro Drug Estimation

Time in hours	% Cumulative Drug Release
0	0
2	27.96 ± 0.58
8	30.62 ± 1.01
12	34.18 ± 0.87
24	42.75 ± 1.23
48	58.55 ± 0.86
72	65.73 ± 0.94
120	72.57 ± 0.34
240	79.61 ± 1.12
360	84.27 ± 0.94

delivering the desired drug for the effective results. PLGA is a biodegradable copolymer, widely used as a vehicle for controlled release of different drugs[6] as well as growth hormones[7] in the medical research.

The in vitro percentage cumulative release of the drug from the delivery system was found to be 27.96±0.58 on the first estimation at 2 hours. The drug was steadily releases from the in situ gel system over the period of time to 84.27±0.94 on 15 day **(Table 2)**.

Local drug delivery using NSAIDs have been evaluated in paste, gel form and mouth rinse form. The series on animal experiments have revealed the effectiveness of this form of drug delivery in periodontal diseases and stressed on the need for further research in this field[8],[9].

In this study flurbiprofen was selected to evaluate its host modulation effect in localized periodontitis. Various studies, utilized flurbiprofen in local delivery device in the form of toothpaste, local irrigation or in gel form[8],[9],[10]. Flurbiprofen is found to be a potent anti-inflammatory agent, showed significant decrease in alveolar bone loss as compared to other agents[11],[12].

The Delivery system used here was easy to dispense in the deep periodontal pockets. The gel solidifies as it comes in contact with aqueous environment and hence takes up the shape of the pocket. This helps in better retention of the delivery system without any need for the periodontal dressings.

In the in vivo study period was restricted to 2 weeks depending on the results of the in vitro study where the percentage

Table 3: Drug Concentration in GCF($\mu g/\mu l$)

Gel only group	Gel + Oral prophylaxis group	Mean
13.16 ± 2.42	11.86 ± 3.65	12.51 ± 3.09
6.48 ± 1.43	5.57 ± 2.26	6.09 ± 1.89
6.87 ± 1.32	6.20 ± 2.34	6.54 ± 1.89

cumulative drug release was calculated to be around 85% by 15th day. The similar rate of drug release was also found by Yewey et al (1991) in an animal experiment using flurbiprofen gel. The probing of the periodontal pocket for 2 weeks post-treatment is also not desirable.

In in vivo evaluation it was found that the mean drug concentration immediately after gel placement in GCF was 13.16±2.42µg/µl in GO group and 11.86±3.65µg/µl in GOP group. The drug concentration in GCF was eventually reduced to 6.87±1.32µg/µl in GO group on day 14, while the drug concentration was 6.20±2.34µg/µl.

It was also noticed that drug concentration decreased steadily in some of the subject on 7th and 14th day, while in some there was marginal increase in the drug concentration on day 14 as compared to day. The initial higher drug was probably due to the rapid release of drug from the fluid gel prior to its solidification within the periodontal pocket. **(Table 3)**.

This study was aimed at the development of controlled-release formulations of flurbiprofen suitable for use in the periodontal pocket and to our knowledge first of its kind where flurbiprofen controlled release system in gel form is used in humans. If and when successful controlled-release formulations of anti-inflammatory agents are developed, the inflammatory cytokines and enzymes derived from the host cells can be effectively controlled, eventually controlling periodontal tissue destruction.

In conclusion, the delivery system devised in this study was able to release the required drug in a sustained manner over the period of the study both in vitro and in vivo without the reports of any kind of side-effects or discomfort to the patient. Such novel vehicles of the delivering the desired drug to the desired site with definitely take the host-modulatory therapies in periodontal diseases to new heights.

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