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**Original Article**

# Synthesis, Characterization and Drug Release Study of Novel Guided Tissue Regeneration Membranes Containing Drug Loaded Chitosan Nanoparticles

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## ABSTRACT

Periodontitis is an inflammatory disease which can cause the destruction of the supporting tissues of the tooth leading to tooth loss. The guided tissue regeneration is considered as a gold standard for its treatment but the re-infection of surgical site limits its overall success. **Objective:** To synthesize novel monolayer guided tissue regeneration (GTR) membrane containing drug loaded chitosan nanoparticles and to evaluate the drug release from the synthesized GTR membranes. **Methods:** The chitosan nanoparticles containing ciprofloxacin were synthesized by the ionotropic gelation method and these synthesized nanoparticles were added into chitosan GTR membrane fabricated by the freeze gelation method. For comparison GTR membrane was prepared as a control by freeze gelation method in which the drug was added directly. The prepared membranes were characterized by the SEM and FTIR. The drug release was measured from the membrane samples in the phosphate buffer saline (PBS) at 1, 3, 5, 7 and 9 days. **Results:** The GTR membrane containing the ciprofloxacin loaded chitosan nanoparticles showed fast drug release as compared to the membrane in which the ciprofloxacin was added directly. **Conclusions:** The inclusion of antibiotic loaded chitosan nanoparticles can increase the drug release from GTR membrane.

## INTRODUCTION

Periodontitis is the inflammation which involves the supporting structures of teeth and causes their destruction. This destruction of supporting tissues causes mobility and ultimately leads to loss of tooth [1]. Among humans, one of the most wide spread dental diseases are periodontitis and approximately 90% of people above the age of 70 years are affected by this disease with a varying severity [2]. The bacteria which cause the disease reside in the oral flora and involve *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythensis* [3]. Two types of methods are used for treatment of periodontitis i.e. non-surgical and surgical. These methods are aimed at curing the periodontitis by resolving the infection [4]. The non-surgical methods involve pharmacotherapy and scaling and root planing. The success of non-surgical treatment methods is limited because of the complex root anatomy and limited removal of calculus. The surgical treatment methods are used for eliminating the causative factors and for regenerating the lost periodontal structures. These involve grafting and guided tissue

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regeneration [5]. The application of grafts has been limited because of the risks of allergic reactions and foreign tissue rejection. The guided tissue regeneration (GTR) is considered a gold standard for treatment of periodontitis [6]. The GTR membranes used for regeneration of periodontal structures can be natural or synthetic in origin. These membranes are meant to prevent the migration of epithelial cells into the wound site and to provide the clot stabilization along with space maintenance [7, 8]. Earlier, the non-biodegradable membranes were used for this purpose but these membranes required a second surgery for their removal. This problem has been overcome by the development of biodegradable membranes [9, 10]. The biodegradable membranes are usually polymers of natural origin in which other additives can be included for enhancement of their antibacterial potential. The enhanced antibacterial activity of GTR membranes is beneficial to prevent the re-infection of surgical site and this can be achieved by loading of antibacterial agent into the membranes [11, 12]. One of the frequently used antibiotics is ciprofloxacin. This antibiotic is effective against a number of bacteria, especially the gram-negative bacteria residing in the oral cavity. The ciprofloxacin has also been reported to be effective against periodontal and endodontic infections [13]. The delivery of drug can be further improved by using the nanoparticles. The nanoparticles, because of their minute size, are able to penetrate into systems which otherwise cannot be accessed by conventional methods of drug delivery. The systems based on nanoparticles are useful because they reduce the number of times required for applying the drug. These also facilitate in delivering the drug in a controlled pattern [14]. The study was aimed at synthesizing a GTR membrane which can release antibiotic in a controlled pattern. The synthesized membrane was characterized and the drug release study (*in vitro*) was carried out for comparison between the direct and indirect drug release from GTR membranes.

## METHODS

The synthesis of chitosan nanoparticles (CSNPs) containing ciprofloxacin was carried out by using the modified ionotropic gelation method. The 1% (w/v) solution of chitosan was prepared by addition of 0.3g chitosan powder in 100ml of acetic acid (1%). This prepared solution was stirred at 600rpm for 30 minutes at room temperature. The tripolyphosphate (TPP) solution was prepared by dissolving 0.8g of Sodium tripolyphosphate in 10ml of distilled water. The liquid solution of ciprofloxacin (2µg/ml) was added into TPP solution. This TPP solution containing ciprofloxacin was poured into chitosan solution drop wise which was being stirred. The final solution was kept under stirring at 600 rpm for 30 minutes at room temperature. This solution was then ultrasonicated for 30 minutes by using Sonics CV334 ultrasonicator. The solution was then centrifugated at 10000 rpm for 5 minutes by using Centurion CSD-XT5 and a gel was collected followed by freeze drying at -80°C for converting the gel into powder form. The drug encapsulation efficiency of CSNPs was calculated by using an indirect method [15]. The drug loaded CSNPs were collected by centrifugation. The quantity of drug was calculated in supernatant. The absorbance of drug was measured in supernatant by using the UV-spectrophotometer (UV-Visible spectrophotometer Lambda 25). The drug loading efficiency was measured using the following formula. Drug loading efficiency = 100× (total drug – free drug)/total drug. The GTR membrane was synthesized by the freeze gelation method. The chitosan solution (2%) was prepared by dissolution of chitosan (500mg) in 1% acetic acid (25ml) at room temperature. After complete dissolution of chitosan was achieved, drug loaded CSNPs were added into the prepared solution (3 wt% of polymer). This solution was again stirred at 300 rpm for 5 minutes for uniform distribution of CSNPs in chitosan solution. Following this, the solution was transferred into plastic petri-plate and placed at 4ºC for 3 hours. The samples were refrigerated for 39 hours at -25ºC. A 3M solution of sodium hydroxide (NaOH) in Ethanol was prepared and was stored for 12 hours at -25°C. This super cooled solution was poured onto frozen membrane and the membrane was again placed at -25ºC. The washing of the membrane was done with ethanol (50%) after 21 hours. The washing was done three times and for 5 minutes each time. The pH of membrane was neutralized by placing membrane in distilled water overnight. After this the membrane was placed in plastic petri-plate and allowed to dry at room temperature. This group was named as GDNP. The drug containing membrane (control) was synthesized by the same freeze gelation method. The control was synthesized by directly adding the drug into membrane. The liquid drug was added after complete preparation of chitosan solution (3 wt %). Following the addition of drug, the solution was stirred at 300 rpm for 5 minutes for uniform distribution of drug. This group was nominated as GD. Following the synthesis of GTR membranes, samples of 8 x 8mm were cut from membranes using the cutter. These samples were sterilized by Gamma radiation from Pakistan Radiation Services (PARAS) Lahore. The morphology of samples was observed using FESEM (Nano Nova SEM 450) with built-in ETD and TLD detectors. The samples were scanned at different magnifications between 5,000 and 1,00,000 under the 3.0 kV voltages to analyze the distribution of drug loaded CSNPs in GDNP and the distribution of drug in GD samples. The FTIR of samples was done at resolution of 4

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$cm^{-1}$ and were scanned between ranges of 4000-400 $cm^{-1}$ by using FTIR (Thermo Nicolet 6700, Thermo Fisher Scientific, Waltham, MA, USA). Firstly, the calibration curve of Ciprofloxacin was plotted using various concentrations (0.05, 0.1, 1, 2, 3, 4 and 5 ppm) of Ciprofloxacin in Phosphate Buffer Saline (PBS). The drug release study was carried out for both membrane samples GDNP and GD. The membrane samples were placed in 5ml PBS in falcon tubes and placed at 37ºC in the incubator. The absorbance of the drug was measured by UV-Spectroscopy (UV-Visible spectrophotometer Lambda 25) at 271 nm in absorbance mode with slit width of 1 nm and scan speed of 960 in Quartz cuvette. The average drug release was measured at day 1,3,5,7 and, 9. The experiment was carried out in triplicates and each time the samples were placed in fresh PBS. The data was statistically analyzed by using the statistical package for social sciences (SPSS), version 22.0. The two-way mixed ANOVA test was applied to the drug release kinetics of GDNP and GD at day 1,3,5,7 and, 9 time points. The *p* value of less than *0.05* was taken significant.

## RESULTS

The FESEM image of ciprofloxacin loaded CSNPs and GTR membranes are shown in Figure 1. The SEM analysis (Figure 1a marked by white arrows) showed the CSNPs of varying shapes and sizes ranging from 75 to 175 nm. The images show two phases one might be the drug attached/adhered to nanoparticles. Aggregation was also seen in the SEM images. The SEM images of GDNP and GD membranes at 10,000 x are shown in Figure 1(b) and 1(c). The white arrows in Figure 1 (b) show the ciprofloxacin loaded CSNPs which are randomly distributed in the asymmetric membrane. Some agglomerates of CSNPs can also be seen randomly distributed in the membrane. The size of the nanoparticles and agglomerates was between 100nm and 1000nm.The Figure 1(c) shows the image of GD membrane in which white arrows indicates the drug particles which are also randomly distributed in the asymmetric membrane.

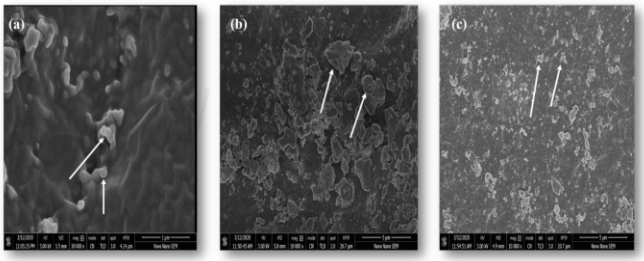


**Figure 1:** (a) FESEM image of ciprofloxacin loaded CSNPs. The arrows indicate the ciprofloxacin loaded CSNPs (b) FESEM image of GDNP. The arrows indicate the ciprofloxacin loaded chitosan nanoparticles in GDNP membrane (c) FESEM image of GD. The arrows indicate the drug particles in GD membrane

The FTIR spectra of ciprofloxacin loaded CSNPs is shown in Figure 2(a). This shows the peaks for both chitosan and ciprofloxacin. The peaks at 888 and 1631 $cm^{-1}$ show the chitosan ring and the N-H bending of chitosan, respectively, while the peak at 1020 $cm^{-1}$ shows the C-F stretching for ciprofloxacin. The 2950-3000 $cm^{-1}$ depression shows the presence of ν=CH & Ar-H for ciprofloxacin. The Figure 2 (b) shows the FTIR spectra of samples GDNP and GD.

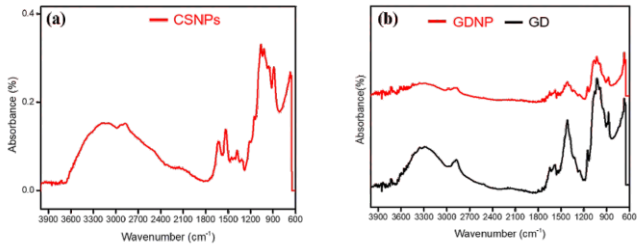


**Figure 2:** (a) FTIR spectra of ciprofloxacin loaded CSNPs (b) FTIR spectra of GDNP and GD membranes

The characteristic peaks for ciprofloxacin and chitosan for samples GDNP and GD are given in Table 1. The FTIR results of both membranes GDNP and GD are similar because both membranes have chitosan and ciprofloxacin in their composition. These membranes have shown the characteristic peaks for both chitosan and ciprofloxacin characteristic functional groups which show that both constituents co-exist without forming any byproduct.

**Table 1:** Characteristic peaks for sample GDNP and GD

| Peaks | GDNP | GD |
|---|---|---|
| Chitosan ring | 875 cm-1 | 875 cm-1 |
| ν= CH & Ar-H | 2950 cm-1 | 2950 cm-1 |
| C-H stretching vibration | 2904 cm-1 | 2975 cm-1 |
| N-H bending vibration | 1648 cm-1 | 1650 cm-1 |
| C-F stretching | 1031 cm-1 | 1027 cm-1 |

The drug encapsulation efficiency of drug loaded CSNPs was calculated to be 15%. The efficiency remained low because the yield of CSNPs was very low. The Figure 3 shows the average drug release from samples of GDNP and GD. The average drug release from GDNP was 2.84 µg/ml while from GD it was 0.762 µg/ml after 1 day. This initial burst release from both groups can be related to polymer swelling, pores formation or the diffusion of drug from polymer surface [16]. The increased release of drug from GDNP (8.02 µg/ml) as compared to GD (4.98 µg/ml) after 9 days can be related presence of ciprofloxacin loaded CSNPs because the nanoparticles increase the surface area available for interaction with functional groups and release of drug [17].

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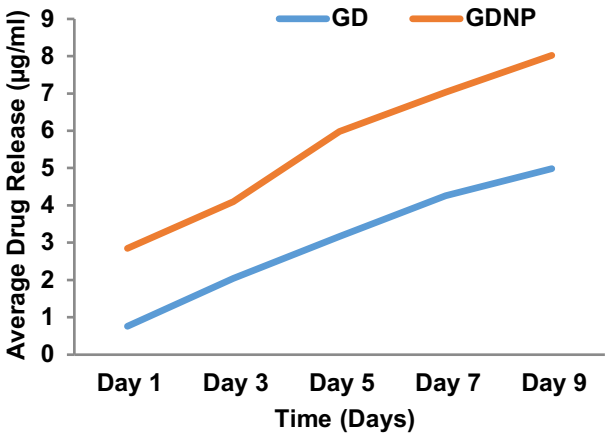


**Figure 3:** Average drug release kinetics of samples GDNP and GD (n=3)

Apart from surface area, polymer swelling and degradation and the diffusion of drug through the polymer matrix also play a major part in releasing the drug [18]. The solubility of chitosan depends upon the molecular weight of chitosan. The CSNPs synthesized from chitosan of low molecular weight are easy to be dissolved as compared to those synthesized from high molecular weight chitosan [19]. This can be related to the present study in which the low molecular weight chitosan was used for synthesis of both CSNPs and GTR membranes. The increase in drug release because of CSNPs is beneficial for increased bioavailability of the ciprofloxacin. The statistical comparison between the drug release kinetics of samples from groups GDNP and GD at day 1, 3, 5, 7 and 9 showed the statistically significant *p* values for all time points.

## DISCUSSION

The SEM analysis showed the CSNPs of varying shapes and sizes ranging from 75 to 175 nm which are in accordance with studies performed by Qashqoosh *et al.*, and Jamil *et al.*, [20, 21]. The SEM results of this study are in accordance with the studies previously performed by Ho *et al.*, and Ma *et al.*, [22, 23]. The 2950-3000 cm-1 depression in our study shows the presence of ν=CH & Ar-H for ciprofloxacin. The peaks and depressions of ciprofloxacin are in accordance with Ma *et al.*, [23]. In our study, the membranes have shown the characteristic peaks for both chitosan and ciprofloxacin characteristic functional groups which show that both constituents co-exist without forming any byproduct. The characteristic peaks of ciprofloxacin and chitosan are in accordance with the previous studies Qashqoosh *et al.*, and Sahoo *et al.*, [20, 24]. Our study find increase in drug release because of CSNPs which is beneficial for increased bioavailability of the ciprofloxacin. This finding can be related to the previous studies in which the nanoparticles were used to enhance the bioavailability and adsorption of drug [25-27]. The present study has shown that the drug loaded nanoparticles can be included in GTR membrane to improve its drug release kinetics and the drug release was measured till 9 days. A similar study was conducted by Owen *et al.*, in which the tetracycline drug was added directly into the GTR membrane but the membranes showed the initial burst release followed by full drug release in one day [28]. Another study was performed by He *et al.*, in which the metronidazole was added into the membrane for studying the drug release kinetics and the membranes showed a controlled drug release up to 6 days [29]. The same study was performed by Xue *et al.*, which included the addition of metronidazole loaded nanotubes into GTR membrane leading to a sustained release till 20 days [30]. A study involving the gentamicin loaded silica nanoparticles in GTR membrane was conducted by Chen *et al.*, which showed a drug release till 136 hours [31]. A similar study was conducted by Yar *et al.*, in which meloxicam was introduced into GTR membranes and the drug release was measured till 24 hours [32]. A study was performed by Zhang *et al.*, in which orindazole loaded composite membranes were fabricated which showed an initial burst release of drug for the first 4 hours and then showing a sustained release till 7 days [33].

## CONCLUSIONS

In this study, novel GTR membrane which contained ciprofloxacin loaded chitosan nanoparticles was prepared. The synthesis of ciprofloxacin loaded chitosan nanoparticles was done by ionotropic gelation method and these drugs loaded nanoparticles were added into GTR membrane which was fabricated by freeze gelation method. Apart from this, another GTR membrane was fabricated in which ciprofloxacin was added directly which was fabricated to act as a control during drug release study from novel GTR membrane. The drug release study showed the increase release of drug from the experimental membrane as compared to the control membrane which can be related to the presence of drug loaded nanoparticles leading to increased surface area and increased release.

## Authors Contribution

Conceptualization: MAG
Methodology: MN
Formal analysis: MK
Writing-review and editing: MA, ATS, SUK

All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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