Nanocomposite based drug delivery for periodontal disease

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Abstract---Periodontal disease is the term used to describe some pathological conditions characterized by degeneration and inflammation of gums, periodontal ligaments, alveolar bone, and dental cementum. Periodontal disease involves destruction of alveolar bone around the teeth leading to defects or rather loss of the tooth if left untreated. This condition is caused by a chronic, mixed infection of Gram-negative and Gram-positive bacteria. Chlorhexidine (CHX) gluconate is an antiseptic, antifungal and bactericidal agent. CHX inhibits plaque formation by binding to anionic salivary glycoproteins and bacteria. Nano Hydroxyapatite (nHAP) is calcium phosphate in morphology and composition like the human hard tissues. Delivery of medications directly into the periodontal pockets alters the inflammatory response and reduces the pathogenic microbiota, an aspect which has attracted great interest. Result: The CHX Nanocomposites were non-toxic to human cells and promoted cell adhesion, proliferation, and spreading. The CHX Nanocomposites were found to be satisfactory in all respects, and CHX encapsulated in nanocomposites may be attractive candidates for the treatment of periodontal disease.

Keywords---Periodontitis, Nanocomposite, CHX, PGS, nHAP.

1. Introduction

Periodontal disease is a word that refers to a group of diseases marked by the degeneration and inflammation of the gums, periodontal ligaments, alveolar bone, and dental cementum. It’s a localized inflammatory reaction brought on by a
bacterial infection of a periodontal pocket and sub-gingival plaque. Periodontal disease involves destruction of alveolar bone around the teeth leading to defects or rather loss of the tooth if left untreated. [1]. Periodontal infections contain a broad scope of inflammatory circumstances that influence the supportive structures of the teeth, which could prompt tooth misfortune and add to fundamental irritation [2]. A study on periodontitis revealed that there are three types of disease progression: i) no progression of periodontal disease: in which around 10% of the population has very little or no disease that is of no particular consequence to the dentition; ii) moderate progression: affecting around 80% of the population and representing a very slowly progressing form of the disease that can generally be managed via routine therapies; and iii) rapid progression: a disease that progresses quickly and is difficult to manage[3]. A higher risk of heart disease has been related to this illness. According to several studies, periodontal disease is associated with an increased risk of diabetes, atherosclerosis, heart attack, and stroke. Several factors influence the clinical severity of periodontal disease defects: virulent pathogens must reach a critical threshold and overwhelm the host response, and environmental and acquired factors (such as smoking) can influence the clinical signs of disease and the magnitude of periodontal destruction [4]. Gram-negative bacteria such Porphyromonas gingivalis, Prevotella intermedia, Tannerella for synthesis, and Aggregatibacter (Actinobacillus) actinomycetemcomitans, as well as Gram-positive bacteria like Peptostreptococcus micros and Streptococcus intermedia, cause this disorder [5]. Periodontal disorders impact 20-50 percent of the global population in industrialized and developing countries. Periodontal disease is a public health concern due to its high prevalence in adolescents, adults, and the elderly [6]. In this regard, promising innovatively designed formulations such as chlorhexidine (CHX) nanocomposites are needed; researchers have discovered encouraging outcomes when using antibiotics or antibacterial medications that function as inhibitors of inflammatory damage in periodontal disease [7].

Antibiotics and antimicrobials, such as minocycline, doxycycline, or antimicrobials, such as CHX, are administered directly to the periodontal pocket via a powder, gel, chip, or fibre delivery method for targeted treatment. CHX is a common skin antisepsis agent that can also be found in toothpaste and mouthwash. When combined with booze, it is particularly successful germ-free. CHX gluconate is a bactericidal, antiseptic, and antifungal substance that can kill Gram-positive and Gram-negative bacteria. It also has a bacteriostatic effect, preventing germs from multiplying [8]. CHX works to reduce plaque formation by binding to anionic salivary glycoproteins and bacteria, preventing them from adhering to the tooth surface. CHX has an excellent affinity for mucosal membranes and inhibits the production of new plaque while being ineffective against preexisting plaque [9]. Scientists have produced a CHX gel in a skin model that displayed immediate and prolonged antibacterial action [10]. CHX was chosen as a rug for the current study since it is all above ground, and the polymers used were polyglycerol sebacate (PGS) and nanohydroxyapatite (nHAP). PGS is a biodegradable, low-cost, biocompatible elastomer that has been investigated for tissue engineering applications such as cardiac and vascular applications, as well as a tissue adhesive and nerve guide material [11]. In terms of form and chemistry, nanohydroxyapatite (nHAP) is a calcium phosphate that resembles human hard tissues [12]. When compared to other calcium
phosphates, one of the essential characteristics of HAP is its stability. Under physiological parameters such as temperature, pH, and bodily fluid composition, HAP is the thermodynamically stable calcium phosphate compound [13].

Nanocomposites are a type of delivery system that is non-toxic, biocompatible, stable, specific to a target and biodegradable. Due to their ease of formation via the introduction of therapeutic and imaging agents, nanocomposites are attracting much attention for target-specific diagnostic applications, which is a significant public problem worldwide [12]. Because of the outstanding features of dental nanocomposites, such as good translucency and contouring, it has been utilized to restore damaged or missing dental tissues, but it also demands many drug concentration concerns. By combining the medicine into controlled release delivery systems locally in the periodontal pockets, drug concentration in the periodontal tissue can be improved [14]. Nanocomposites generally improve barrier, flame resistance, structural, and thermal qualities while retaining their impact and clarity. The densely bound structure in a polymer matrix, on the other hand, is impermeable to gases and liquids and offers superior barrier qualities to the pure polymer, with surface dimensions reaching 1 micron. [15], [12].

**Materials & Methodology**

**Materials** - Chlorhexidine Gluconate Solution I.P. was received from Unilab chemical and pharmaceutical Pvt. Ltd, MS, India

**Preparation Nanocomposite**

In a round bottom flask (at least 2 necked), glycerol and sebacic acid are mixed in a 1:1 molar ratio. In an oil bath, heat the mixture until it liquefies, then begin stirring. In an oil bath, heat the mixture until it liquefies. Begin swirling the mixture in an inert atmosphere until it becomes a thick brownish semi-solid, the final mixture. 5g polymer + 100 mL methanol. To the weight of the polymer, add 10% Hydroxyapatite and sonicate to combine. To the weight of the polymer, add 1% CHX and sonicate to combine [16].
**Figure: Preparation of Nanocomposite**

**Formulation Table**  
Table No.2

<table>
<thead>
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<th>Sample code</th>
<th>Composition</th>
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<td>PGS 0.95 ALONE</td>
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<tr>
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<td>PGS 0.95</td>
<td>5%nHAP</td>
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<tr>
<td>NC25</td>
<td>PGS0.95</td>
<td>25%nHAP</td>
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</table>

*nHAP- Nanohydroxyapatite, PGS- Poly (Glycerol sebacate)*
**Rheology study**

Rheology study done at Brookfield viscometer (DV-III, 3.0) Cone type plate was connected with Bath thermostat at 37 °C.

<table>
<thead>
<tr>
<th>Shear Stress</th>
<th>PGS</th>
<th>PGS+5%HAP</th>
<th>PGS+10%HAP</th>
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<td>61</td>
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<td>101</td>
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</tbody>
</table>

**Table No. 3**

**Figure No. Viscosity Study**

Optimization of CHX Nanocomposite based on injectability & rheological behavior. Sample code NC15 got good results.

**Thermal analysis**

Thermogravimetric analysis and Differential Scanning Calorimetry (DSC) were used to assess the thermal stability and calculate the breakdown temperature of the drug-loaded nanocomposites [7]. Under inert nitrogen purge, a thermogram was obtained from 25 to 500 °C at a heating rate of 10 °C /min1 [17].
Chemical characterization by ATR-FTIR Characterization & evaluation of nanocomposites

**ATR-FTIR** in the range of 650–4000 cm⁻¹ was used to determine materials' chemical composition and molecular bond structure across 60 scans. PGS features ester crosslinks and hydroxyl groups directly attached to the backbone. The C=O stretch at 1740 cm⁻¹ in Fourier transformed infrared (FTIR) spectrum confirms the formation of ester bonds. FTIR also shows an O-H stretch at 3448 cm⁻¹, reflecting the molecule's presence of hydrogen-bonded hydroxyl groups. No peak shifting was observed in ATR-FTIR Scan in PGS+ nHAP and PGS with CHX in the presence of nHAP. Proton NMR was performed using CDCl₃.

**NMR-** ¹H-NMR (400 MHz; [D₂] CHCl₃; 25°C; tetramethylsilane): δ 1.1 ppm; δ 1.6 ppm; δ 2.3 ppm for -CO-CH₂- CH₂-CH₂- a group of sebacic acid and at δ 4.2 ppm; δ 5.2 ppm for -CH₂-CH₂- of glycerol.
Morphology of Nanocomposite

XRD (Bruker D-8 Advance; 3-60°; step size: 0.010°; step time: 1 s at 25°C) was used to determine the crystalline phase of the samples. Diffraction patterns were compared to the International Centre for Diffraction Data to determine phase (hydroxyapatite standard; JCPDS PDF card number 09-0432)
**SEM Analysis**

PGS (control), PGS+1 percent CHX composite, and PGS composite scaffolds have different surface morphologies (Fig.). Scaffolds have a porous appearance with a smooth surface shape. The composite scaffold is more permeable than the control scaffold. The pore size of composite scaffolds ranges from 100 to 150 nm [18].

![SEM Images](image)

**Injectability evaluation of Nanocomposite**

TA. XT2i texture analyzer with a 5-kg load cell was used to test the injectability of the CHX nanocomposite. This load was appropriate for testing injectable nanocomposites since it provided a constant force of 4.9 kgf, at which all samples demonstrated perfect injectability. This method maintained injectability while mechanical strength was maintained in the studied samples. In a nutshell, uncrosslinked PGS polymer/crosslinked nanocomposites were placed in a syringe, and a 16-gauge needle was used to inject them (1.6-mm internal diameter). Without any air retention, the syringe piston was placed in contact with the substance, and a steady force of 4.9 kgf (48 N) was exerted vertically on the top of the plunger for 2 minutes. The mass of the substance before and after injection was measured, and the ratio of mass discharged from the syringe to total mass before injecting was used to calculate the % injectability. Furthermore, the maximal propulsion required for injection was recorded as injection force [11].
Biocompatibility Test

The Institutional Animal Ethics Committee and the National Institute of Pharmaceutical Education and Research approved animal selection and care, surgical protocols, and biological evaluation experimental techniques (SAS Nagar, India). The biocompatibility of crosslinked PGS 0.95 and C30 specimens was assessed using MSCs extracted from rat bone marrow. MSCs were extracted from the femurs and tibias of recently deceased Wistar rats (150 g) and grown in StemlineTM media (Sigma-Aldrich) supplemented with 10% foetal bovine serum and 1% antibiotic. PGS 0.95 and C30 samples were applied to a sterile coverslip (Secure SlipTM glass coverslip, Sigma) and crosslinked with U.V. light before being sterilized for the biocompatibility testing. The sterilized samples and control (blank SecureSlip glass coverslip) were then seeded with 80,000 MSCs per coverslip on Costar® Ultra-Low attachment 12-well plates (Corning Lifescience Catalog, Product #3473). MSC adhesion and proliferation on PGS 0.95 and C30 samples were investigated using the MTT test at preset time intervals. The morphology of MSCs was investigated after they were fixed in a 2.5 percent glutaraldehyde solution buffered with 0.2 M cacodylate buffer (pH 7.4) at room temperature, then dehydrated in ethanol (50–100%). The fixed samples were air-dried and gold sputter-coated (30 s) before being examined with a JSM-6100 scanning microscope (JEOL, Tokyo, Japan). Alizarin red staining was also used to assess cell calcification.
Antimicrobial study

Chlorhexidine- Polyglycerol sebacate- Hydroxyapatite composite

Disc diffusion method was used for detecting the antimicrobial activity of the reagent with Penicillin as control: Initially, the zone of inhibitions observed were merged, probably because of higher concentration.

Antimicrobial activity of CHX gluconate nanocomposite against

A) *peptostreptococcus micros*  B) *Actinobacillus actinomycetemcomitans*

Therefore, 500X and 250X dilutions of the control, i.e. Penicillin, were used to obtain better results. The Zone of Inhibition observed was comparable for the 250X diluted Penicillin sample and the reagent. Calculations were made accordingly.

1. Sample X – chlorhexidine2%-PGS-HA
   C 500X – Penicillin 500X diluted
diluted
2. Sample chlorhexidine 1%-PGS-HA
   C 250X – Penicillin 250X
Observation:

<table>
<thead>
<tr>
<th>Concentration of Control</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>250X</td>
<td>Control (mm)</td>
</tr>
<tr>
<td>250X</td>
<td>9</td>
</tr>
<tr>
<td>500X</td>
<td>6</td>
</tr>
</tbody>
</table>

The control activity (Penicillin) used had 5000 Units/5ml. Since the Zone of Inhibition of the reagent sample and the 250X diluted control sample were found to be equivalent, for 250X dilution, the activity of control is 4 units/ml

**MTT assay**

MTT assay-Reagent preparation-MTT solution preparation-MTT was solubilized in culture media (5 mg/mL). After adding MTT, sterilize the solution by filtering it. The MTT solution was kept at -20°C. L929 cells were incubated in 96 healthy plates (10000 per well) for 24 hours. The media from 96 healthy plates is discarded with care. Each well received 50 µL of serum-free media and 50 L of MTT solution. For 3 hours, a 96-well plate was incubated at 37°C. Following incubation, 100 µL of MTT solvent was added to each well. Wrapped in foil, the plate was shaken for 15 minutes on an orbital shaker. At OD=590 nm, absorbance was measured [19, 20].

**Method of data analysis -Cell proliferation assays**

For each sample, take the average of the duplicate readings. Subtract the assay reading from the culture medium background. The amount of absorbance is proportional to the number of cells [21].

Extracts from material samples were prepared according to ISO specifications (10993–5) governing in-vitro tests. Each polymer nanocomposite was immersed in serum-free DMEM at a concentration of 0.2 g/mL for 24 hours at 37°C with constant agitation (200 rpm). The extracts were used undiluted and with a 10% FBS supplement. L929 cultured cells were seeded in 96-well plates (2.5105 cells/mL, 100µL/well) and allowed to adhere for 24 hours at 37°C in a humidified atmosphere of 5% CO2. The culture medium was replaced with the previously
prepared extracts, and the plates were incubated for an additional 24, 48, and 72 hours. L929 cells grown on tissue culture plastic supplemented with complete DMEM but not in contact with test extracts were used as positive controls. The negative control was 1% triton-X in PBS. After the incubation period, the extracts were removed, and each well was treated for 4 hours at 37°C with the MTT solution (10% 5mg/ml MTT in PBS). After adding DMSO to dissolve the formazan crystals, the microplate was shaken for 15 minutes before being read at 560 nm on a microplate reader [22]

**Results and Discussion**

The preparation and characterization of novel nanocomposites of Chlorhexidine gluconate using FTIR spectroscopy, NMR, X-ray diffractometry, and scanning electron microscopy. The Nanocomposite’s thermal stability was investigated, and a relatively stable formulation was discovered. The nanocomposites shown good injectability and biocompatibility has been described. Rheology study also carried out and optimization done on the basis of viscosity of formulation. Formulation Sample code NC15 got good results. The medication was distributed under strict monitoring. The antimicrobial test for antimicrobial activity and the MTT assay for cell viability was also done.

The CHX Nanocomposites were non-toxic to human cells and promoted cell adhesion, proliferation, and spreading. The CHX Nanocomposites were found to be satisfactory in all respects, and CHX encapsulated in nanocomposites may be attractive candidates for the treatment of periodontal disease.

**Summary and Conclusion**

This study aimed to develop a nanocomposite that could be used to treat periodontal defects. Local antimicrobial delivery systems based on CHX gluconate, PGS and nHAP Nanocomposite were designed to the nanoscale level. Nanocomposite inhibits the growth of *peptostreptococcus micros* and *A. actinomycetemcomitans*. However, Nanocomposite presents higher stability in saliva and exhibits a more controlled and sustained release of bactericidal CHX gluconate concentrations. The results of this work indicate that the CHX gluconate nanocomposite is a promissory and effective system for the future development of localized periodontal therapies.

Recent developments in nanomaterials and nanotechnology have provided a good insight into the commercial applications of nanomaterials in managing periodontal disease. It can be said that the antibiotic-free, mucoadhesive, biodegradable nanoparticle technology has an immense opportunity for designing a novel, low dose, and effective treatment method by the use of intra-pocket controlled devices that are more convenient, easy to use and more effective than the standard drugs and medicines which act systemically. It will become increasingly important to specifically develop nanocomposites as local drug delivery to manage periodontal disease.
Future

The development of injectable nanocomposites, which mimic the bone’s inherent composite nature and provide mechanical strength post-implantation, is an efficient strategy for enhancing bone regeneration. This approach is beneficial for load-bearing tissue engineering applications via minimally invasive surgery. However, one can hope to improve upon the properties of the final Nanocomposite in various ways.

References


