Preliminary Studies on a Clindamycin-Loaded Hydrogel for Infection Control in Endodontics

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Introduction

In cases of infection, conventional endodontic therapies often fail to eliminate persistent biofilms and residual bacteria in root canals, highlighting the need for innovative drug delivery systems with potent antimicrobial capacity. In this context, clindamycin emerges as a promising antibiotic due to its proven efficacy against more than 70% of endodontic pathogens and its relatively low incidence of allergic reactions, offering a safer therapeutic profile. Moreover, nanoparticles (NPs) serve as ideal carriers for delivering antimicrobial agents, providing benefits such as controlled drug release, enhanced bioavailability, targeted delivery to infection sites, and reduced drug dosages required to achieve therapeutic effects. This study aimed to develop a chitosan (CS) and poly(vinyl alcohol) (PVA) hydrogel incorporating clindamycin-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles for endodontic applications, designed for administration through specific syringes and tips into root canals. To ensure the safety of the formulation, all reagents used are FDA-approved and have been extensively studied and validated for potential human use in comparable biomedical applications.

Methods

1. Nanoparticles formulation and characterization

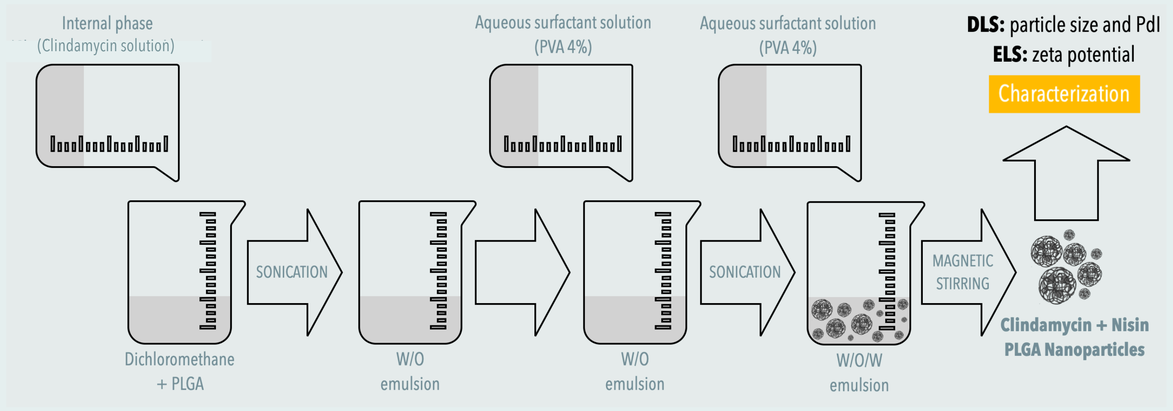
The PLGA NPs encapsulating clindamycin were synthesized using a double emulsion process and several physicochemical properties were analyzed, such as particle size (PS), polydispersity index (PdI), and zeta potential (ZP) **(Table 1)**. Empty NPs were also prepared as control **(Table 2)**.

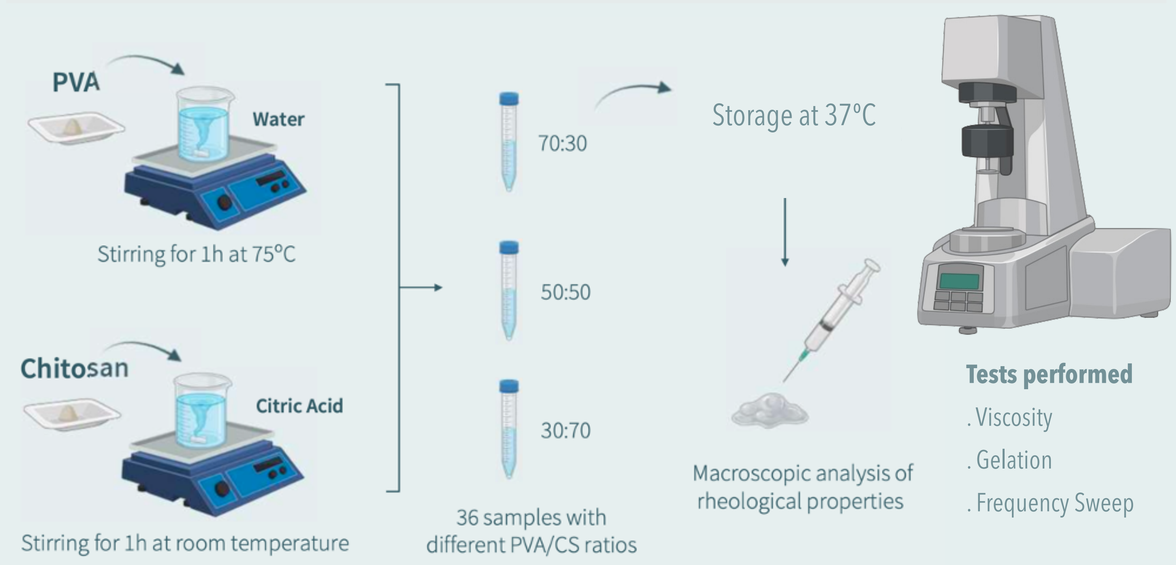
2. Hydrogel formulation and viscoelastic analysis

A solution of CS (5% w/w) in citric acid (CA) (15% w/w) was prepared, and hydrogels were formulated by mixing the NPs solution (PVA 4%) with CS/CA in 30:70, 50:50, and 70:30 ratios **(Figure 1)**. Samples were stored at 37°C, simulating human body temperature. Hydrogels underwent macroscopic and rheological analysis to assess viscoelasticity. Their applicability was tested by syringe delivery with endodontic tips **(Figure 2)**.

3. Preliminary antimicrobial activity evaluation

The antimicrobial activity was assessed following CLSI guidelines against *Pseudomonas aeruginosa* ATCC 1544, *Streptococcus mutans* ATCC 25176 and *Prevotella intermedia* DSM 20706 using the agar diffusion method. For *P. aeruginosa* Mueller-Hinton agar plates were used. *S. mutans* was inoculated on Mueller-Hinton agar supplemented with 5% sheep blood, and *P. intermedia* was grown on Wilkins-Chalgren agar or Brucella blood agar under anaerobic conditions. A bacterial suspension standardized at 10^8 CFU/mL was evenly spread onto the surface of the respective culture media, and the test compounds were applied directly to the inoculated plates. Sterile water was used as the negative control, while clindamycin disks served as the positive control for Gram-positive and anaerobic bacteria. The plates were incubated at 37 °C for 24 hours under appropriate atmospheric conditions for each microorganism. After incubation, the zones of inhibition were observed to evaluate the antimicrobial effectiveness of the tested compounds **(Figure 3)**.





Results

Table 1. Nanoparticles Zetasizer analysis

	PS (nm)	PdI	ZP (mV)
Empty NPs	194,99 ± 1,87	0,131 ± 0,02	-39,08 ± 0,97
Loaded NPs	207,00 ± 2,50	0,143 ± 0,18	-38,23 ± 1,13

Table 2. General comparison of physical characteristics of samples

PVA/CS ratio	T (°C)	Translucency	Viscosity
70:30	37	+	-
50:50	37	+	+
30:70	37	+	++

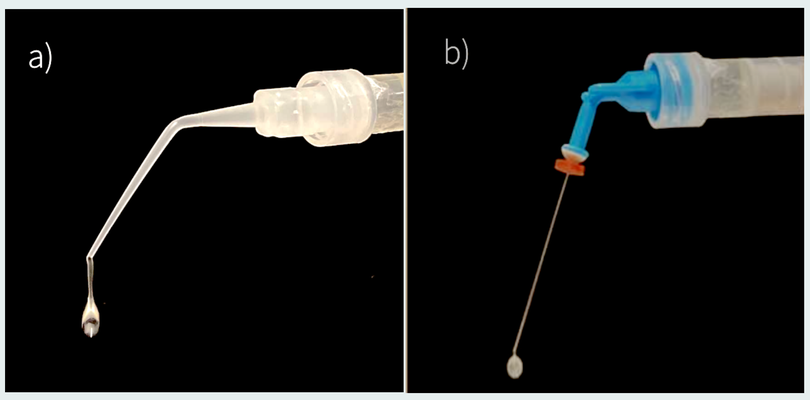


Figure 2. Rheological behavior test of the hydrogels using surgical syringes.
a) Standard tip; **b)** NaviTip

- The **viscosity** of the hydrogels increased proportionally with the **ratio of chitosan.**
- The gel maintains **optimal viscoelastic properties at 37°C**, ensuring clinical applicability.
- On frequency sweep test, storage (G') and loss (G'') moduli indicate **mechanical stability across frequencies and temperatures**, critical for sustained drug release.
- Viscosity reduction under pressure allows **syringe application**, with consistency recovery post-use.
- The **hydrogels offered resistance** when being injected in the syringes, proving its consistency.
- Viscoelastic properties enable **controlled drug diffusion**, ensuring prolonged therapeutic levels.
- Preliminary antimicrobial activity evaluation shows **promising activity against endodontic pathogens.**

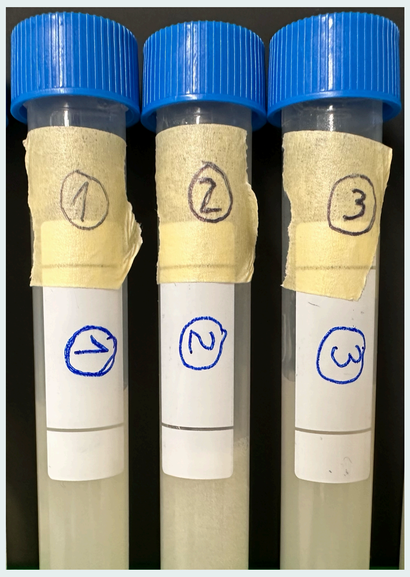


Figure 1. Hydrogel samples obtained

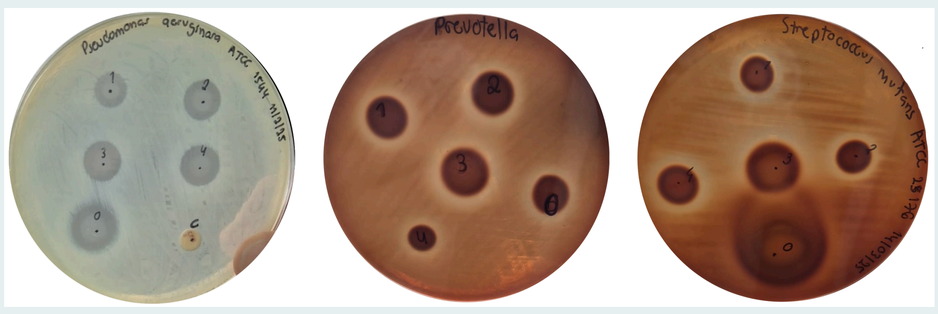


Figure 3. Microbiological evaluation

Conclusions and Future Perspectives

The developed **hydrogel**, based on poly(vinyl alcohol) and chitosan cross-linked with citric acid, showed **optimal physical and rheological properties**, particularly at a 30:70 polymer ratio and 37 °C, replicating physiological conditions. Its viscoelastic stability supports **sustained drug release**, while shear-thinning behavior allows **easy syringe application**. Clindamycin-loaded nanoparticles presented favorable physicochemical characteristics, enabling **efficient drug encapsulation** and **potential controlled release**. Preliminary microbiological assays revealed antimicrobial **activity against several endodontic pathogens**, namely *Prevotella intermedia, Pseudomonas aeruginosa,* and *Streptococcus mutans.* Despite promising results, further optimization may be needed to broaden antimicrobial coverage. The project now advances to *in vitro* studies, including cytotoxicity and drug release evaluations, essential to confirm the safety of the system and therapeutic potential for endodontic use.

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