Dextran-Based Nanoparticles for Encapsulation of Ciprofloxacin

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Abstract. Key success for regenerative endodontics is an effective removal of bacteria inside the root canal during the treatment. Ciprofloxacin (CIP) is one of the triple antibiotics commonly used as the root canal medicament. However, the rapid clearance of antibiotics causes bacterial reinfection. Nanoparticles (NPs) provide an alternative approach for antibiotics delivery system to improve the drug stability and release control. In the previous study, modified dextran was successfully formed the ester linkage with vinyl decanoate via lipase-catalyzed transesterification. The amphiphilic dextran, dextran-decanoate (Dex-D), has been synthesized with the degree of substitution of 83-88% and were applied for CIP-loaded nanoparticles. The suitable technique for producing antibiotic carriers considering by size, shape, and drug encapsulation efficiency was investigated in this work. The preparation methods were compared between nanoprecipitation and solid-in-oil-in-water (s/o/w) ion pairing technique. We found that the obtained Dex-D nanoparticles had stable monodispersed with spherical shape in both techniques. However, Dex-D nanoparticles formed by s/o/w ion pairing technique provided smaller size with better encapsulation efficiency. The antimicrobial activity of CIP-loaded Dex-D nanoparticles against oral pathogens showed satisfactory outcome with the MIC equal to 0.7 µg/mL. Also, the compatibility of nanoparticles and dental stem cells was observed in this work.

1. Introduction

The consideration of successful regenerative endodontic treatment is the complete elucidation of bacterial residues in the infection pulp [1]. However, the using of ciprofloxacin (CIP), hydrophilic antibiotic drugs, in the treatment has some drawbacks including rapid clearance and poor pharmacokinetics [2]. Also, high concentration of drug provides acidic environment that affects stem cells growth and differentiation [3]. To improve the drug activity, nanoparticle has become an alternative approach to control the drug release, prolong the drug stability, and reduce toxicity to stem cells.

Dextran-decanoate (Dex-D), amphiphilic dextran, has been developed in the previous report [4]. This polymer was applied for nanoparticles formation in this work due to biocompatibility and biodegradability of dextran [5]. Two different methods including nanoprecipitation and Solid-in-Oil-in-Water (s/o/w) ion pairing methods were performed. Nanoprecipitation method is the frequently used technique to prepare nanoparticles by the rapid self-assembly of amphiphilic polymer in aqueous phase, and it was considered as reproducible and controllable method [6]. Solid-in-Oil-in-Water (s/o/w) ion...
pairing has developed to improve the encapsulation efficiency of hydrophilic drug by the complexation with dextran sulfate via electrostatic interaction [7].

In this work, Dex-D polymer was synthesized and used for CIP-loaded Dex-D nanoparticles preparation based on two mentioned methods. Size, shape, and encapsulation efficiency of nanoparticles prepared by both techniques were compared. The obtained nanoparticles were further investigated their antibacterial activity against oral bacteria, Enterococcus faecalis, and the effect on the cell viability of dental stem cells.

2. Experimental Procedure

2.1. Synthesis of modified dextran
Modified dextran was prepared by transesterification reaction according to the previous report [4]. Dextran T40 (Phamacosmos) and Vinyl decanoate (Sigma-Aldrich, Germany) with the molar ratio of 1:4 was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Poland) following by pH-adjusted lipase AY with additional 18-crown ether [8]. The reaction was continuing for 32 h at 50°C. Modified dextran was purified by dialysis method using dialysis membrane (MW cut-off = 6000-8000, Spectra/Por, Thomas Scientific, USA). Polymer was lyophilized (Freeze One6 Plus, LABCONCO, USA) and further dissolved in DMSO-d6 to investigate the structure by 1H NMR spectroscopy (UltraShield™ 500 MHz NMR, USA). The quality of Dex-D reported as %degree of substitution (%DS) meaning that the percentage of decanoate groups in dextran. The equation is shown below [8].

\[
\text{% Degree of substitution} = \left( \frac{I_1}{\frac{I_3}{3} + \frac{(I_2 - I_1)}{4}} \right) \times 10
\]  

When \(I_1\) refers to the area under curve of methylic protons of decanoate (0.84 ppm) and \(I_2\) refers to one proton of anomeric carbon and three protons from three hydroxyl groups of glucopyranoside subunits in dextran (4.91 – 4.51 ppm).

2.2. Preparation of CIP-loaded Dex-D nanoparticles

2.2.1. Nanoprecipitation technique. Distilled water or CIP (Cayman Chemical company, USA) at the concentration of 0.0, 0.7, and 7.0 %Wdrug/Wpolymer was added drop-wise into 10 mg/mL of Dex-D in dimethyl formamide (DMF, MP Biomedicals, LLC, France) under magnetic stirring. The ratio of water:DMF was 2:1. Nanoparticle suspension was dialyzed against distilled water to remove solvent and free drug.

2.2.2. Solid-in-oil-in-water (s/o/w) ion pairing technique. Ciprofloxacin 0.07 mL at the mentioned concentration was poured into 10 mg/mL of Dex-D in DMF. 0.03 ml of daxtran sulfate sodium salt (MW > 500,000 g/mol, Sigma-Aldrich, Germany) at the concentration of 80 mg/mL was added afterward. The mixture was then poured into distilled water under magnetic stirring [7]. The solution was purified by dialysis method.

2.3. Characterization of CIP-loaded Dex-D nanoparticles
Size distribution of nanoparticles was investigated by dynamic light scattering (Malvern Zeta Nanosizer ZS, Malvern Instruments Ltd). The observation of morphology was carried out by transmission electron microscopy (JEM-1400, JEOL Ltd., Japan).

Drug concentration in nanoparticles was determined by dissolving dried nanoparticles in 0.05N HCl, DMSO and measured by UV-spectrophotometer at a wavelength of 275 nm. The encapsulation efficiency (%EE) was calculated according to this following equation [9].
Encapsulation efficiency = \frac{\text{Weight of drug in nanoparticles}}{\text{Initial weight of drug}} \times 100 \quad (2)

The antibacterial activity of CIP-loaded Dex-D NPs against *Enterococcus faecalis* (ATCC® 19433™) compared with free CIP and empty Dex-D NPs were reported as minimal inhibitory concentration (MIC), while cytotoxicity of CIP-loaded Dex-D NPs on stem cell from apical papilla (SCAPs) was performed by using methylthiazolyldiphenyl-tetrazoliumbromide (MTT) assay. Empty Dex-D NPs (the polymer concentration of 1, 0.1, and 0.01 mg/mL), CIP-loaded Dex-D NPs and free CIP (the CIP concentration of 10, 1, and 0.1 µg/mL) were treated with the cells and incubated for 24 h before determining the cell viability.

3. Results and Discussion

3.1. Synthesis of modified dextran

Dextran-decanoate (Dex-D) was synthesized via lipase-catalysed transesterification reaction. The grafting of hydrocarbon chain of decanoate to the hydroxyl group of dextran was confirmed by \(^1\)H NMR spectroscopy (Figure 1). The %DS was 83 – 88 which is considered to be sufficient for preparing nanoparticles with size ranged from 80 to 200 nm according to previous report [10].

![Figure 1 \(^1\)H NMR (500 MHz) spectra of Dextran-decanoate (Dex-D)](image)

3.2. CIP-loaded Dex-D nanoparticles

Dex-D NPs prepared by nanoprecipitation method had mean diameter of 180-200 nm and PDI lower than 0.1 (Table 1). However, the encapsulation efficiency of CIP-loaded Dex-D NPs (0.7 %w/w) was low (%EE = 1.6 shown in Table 1) due to the rapid diffusion of soluble drugs during the formation step [11]. Increasing of CIP concentration to 7.0 %w/w did not improve encapsulation efficiency. Furthermore, the size of nanoparticles dramatically increased to 817.6 nm and PDI raised over than 0.5. The similarly results were observed in previous research which suggested that increasing of hydrophilic drug caused osmotic pressure between inner core and outer aqueous phase resulting in pores formation and leakness of drugs [12].
Therefore, s/o/w ion pairing was applied. This method was developed to improve the encapsulation efficiency of minocycline HCl [7]. Addition of dextran sulfate improved %EE to 23.46% when the initial concentration of CIP was 7.0 %w/w (Table 1). It was suggested that the ionic interaction between polyanionic dextran and CIP reduced the solubility of CIP in aqueous phase [13]. The complex prevents drug lost during the nanoparticles formation due to their hydrophobicity [14]. With the method similarly to nanoprecipitation, s/o/w ion pairing provided a better stability. The results also suggested that the presence of CIP did not affect size and PDI of nanoparticles. The size was in the range of 90-130 nm with PDI not over than 0.22 (Table 1). TEM imaging showed Dex-D NPs and CIP-loaded Dex-D NPs with spherical shapes. CIP-loaded Dex-D NPs (7.0 %w/w) prepared by nanoprecipitation method showed large aggregates and uncertain morphology (data not shown). According to the results, CIP-loaded Dex-D NPs (7.0 %w/w) prepared by s/o/w ion pairing provided the satisfy quality in size, shape, and encapsulation efficiency among tested conditions and were applied for determination of antibacterial activity and cytotoxicity experiment.

Table 1 Characterization of CIP-loaded Dex-D nanoparticles

| Preparation method | CIP conc. | Z-average (nm) | PDI   | %EE  | MIC (µg/mL) |
|--------------------|-----------|--------------|-------|------|-------------|
| Nanoprecipitation  | 0         | 184.8 ± 9.3  | 0.060 | -    | n/a         |
|                    | 0.7       | 203.3 ± 18.6 | 0.050 | 1.61 | n/a         |
|                    | 7.0       | 817.6 ± 111.4| 0.690 | -    | n/a         |
| s/o/w ion pairing  | 0         | 100.7 ± 2.6  | 0.220 | -    | n/a         |
|                    | 0.7       | 92.9 ± 2.8   | 0.150 | 7.66 | n/a         |
|                    | 7.0       | 129.1 ± 1.8  | 0.100 | 23.46| 0.7         |

a = Initial concentration of ciprofloxacin (%W\text{drug}/W\text{polymer})

Figure 2 TEM images of nanoparticles prepared by nanoprecipitation; empty Dex-D NPs (A) and CIP-loaded Dex-D NPs (0.7 %w/w) (B) (at 10,000x magnification) and s/o/w ion pairing; empty Dex-D NPs (C) and CIP-loaded Dex-D NPs (7.0 %w/w) (D) (at 40,000x magnification)
The properties of CIP-loaded Dex-D NPs as disinfection agent for regenerative endodontic treatment were evaluated. Antibacterial activity against *E. faecalis*, gram-positive bacteria frequently found in re-infection of the treated root canal teeth [15], was performed. The MIC suggested that CIP-loaded Dex-D NPs (0.7 µg/ml) provided inhibitory effect on bacterial growth which was comparable with free CIP (0.8 µg/ml), while the activity of empty Dex-D NPs was not detected (Table 1). The results indicated that encapsulation of CIP had no effect on antibacterial activity.

The biocompatibility of empty Dex-D NPs and CIP-loaded Dex-D NPs with dental stem cells is considered to be a criteria for applying in regenerative endodontic. Stem cells from the apical papilla (SCAPs), isolated and cultured as described in previous report [16], were incubated with nanoparticles for 24 h. The cell viability of SCAPs after treated with empty Dex-D NPs (Figure 3A) and CIP-loaded Dex-D NPs (Figure 3B) were similar to the negative control (untreated SCAPs). 10 µg/ml of free CIP had slightly affected with SCAPs as cell viability was decreased to 80%. However, cell viability over 70% is considered to be non-cytotoxic potential due to the international organization for standardization (ISO10993-5) regulation. Therefore, Dex-D NPs could be applied as drug delivery system in regenerative endodontic treatment to maintain the activity and stability of antibiotics. Also, it could provide a better environment for dental stem cells as low cytotoxicity was observed.

![Figure 3](image.png)

**Figure 3** Cell viability of SCAPs determined by MTT assay after treated with empty Dex-D NPs (A), CIP-loaded Dex-D NPs, and free CIP (B) compared with positive control (10 %v/v DMSO) and negative control (untreated SCAPs)

4. Conclusion
Dex-D polymer was successfully synthesized with %DS of 83- 88%. CIP-loaded Dex-D NPs prepared by both nanoprecipitation and s/o/w ion pairing were spherical shape with good size. However, s/o/w ion pairing provided encapsulation efficiency at sufficient dosage to inhibit bacterial growth when tested with *E. faecalis*. Even the antibacterial activity was similarly to the free drug, CIP-loaded Dex-D nanoparticles showed more biocompatibility with SCAPs. According to the results, Dex-D NPs provided the suitable nanoparticles system as they exhibited good conditions in both characteristic and application, and CIP-loaded Dex-D NPs could be considered as a good candidate for disinfection agent and could be further applied in the endodontic treatment.
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