Amoxicilin and Clavulanic Acid Intercaled Nanostructures for Dentistry Uses

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Amoxicillin and clavulanic acid are two of the most commonly prescribed antibacterial worldwide for treating oral infectious diseases. Oral health is of big importance for well-being and general health. A few novel drug delivery systems were designed for oral treatment and prophylaxis of different diseases in the oral cavity. This work focused on the latest drug delivery development of the most common oral pathologies, namely, periodontitis, oral mucosal infections, dental caries and oral cancer. Herein we reveal the synthesis, characterization and application of chitosan nanoparticles for intracellular transport of the weakly cell-penetrating amoxicillin and clavulanic acid in order to improve their efficacy on bacterial infections.

Keywords: amoxicillin, clavulanic acid, nanostructures, particle size distribution, orthodontics, public health dentistry

The oral cavity represents the first section of the digestive system being consisted of various anatomical structures such as teeth, gum and their supportive tissues, tongue, hard and soft palate, lips and the mucosal membrane lining the interior surface of the cheek. Local therapy of most common oral problems has several advantages compared to systemic drug administration being targeted directly to the injured area while reducing adverse effects [1-3]. A healthy oral cavity is usually colonized by viruses, fungi and over 700 bacterial species of which some may be pathogenic, others are commensal or symbiotic. When ordinary flora of the mouth is destroyed, indigenous bacteria can transform to pathogenic ones leading to oral structures diseases. Oral cavity infections can be divided into odontogenic type such as dental caries and periodontitis and nonodontogenic infections [4-7]. One of the most prevalent disease in humans is dental caries being caused by the producing of acid resulted from bacteria fermenting carbohydrates which leads to demineralization of dental enamel followed by the formation of cavities as presented in figure 1. The amount of calcium and phosphate present in saliva can counterbalance the demineralization effect and decide whether a demineralization or a re-mineralization will take place [8-12].

The polymicrobial nature of odontogenic infections needs the therapy with antibiotic against aerobic and anaerobic bacteria respectively. The most commonly prescribed antibiotics for these affections are amoxicillin, penicillin, metronidazole as well as erythromycin with clindamycin as a choice in individuals which are allergic to β-lactam antibiotics. Adding a β-lactamase inhibitor such as clavulanic acid to amoxicillin gives resistance to β-lactamases thus extending the antibiotic spectrum to anaerobes. Several strudies described the efficacy of amoxicillin/clavulanic acid in the treatment of oral infections [13-15].

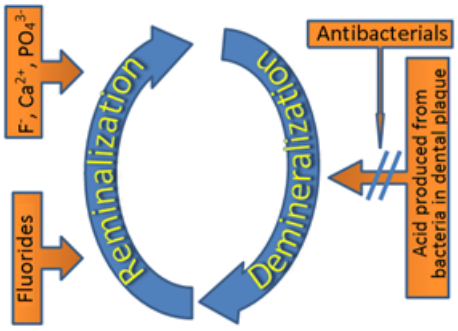

Fig. 1. The equilibrium between the re-mineraleralization and demineraleralization of the teeth

In last decade the use of nanodentistry has received considerable attention. Nanoparticles used in dentistry have a wide range of pharmaceutical uses since their phisico-chemical properties can be controlled accordingly to their target. Novel hybrid nanoparticulate formulations based on drug incorporated nanoparticles for local application in dentistry can be provided as an aqueous suspension or incorporated into a paste or gel creating nanoproducts with high patient approval and easy administration [16].

Chitosan is a multipurpose natural biomaterial being investigated for a several dental applications due to its numerous properties such as biocompatibility, biodegradability and broad antibacterial spectrum. Furthermore, chitosan appeared as a potential nano-material for dental uses due to its unique characteristic of antimicrobial agent being explored as a drug delivery system to ensure good public health dentistry. Therefore, antibiotics or other drugs used in oral diseses treatment can be loaded on chitosan nanoparticles having possible applications even in orthodontics as nano-materials deposited on mini-implants for a better osseointegration and prevention on microbial infections [17-24].

The aim of this work was to explore the possibility to improve the antimicrobial common treatment of oral infections by the incorporation of amoxicillin and clavulanic acid into chitosan nanoparticles.

Experimental part

Materials and methods

Ionic gelation method was used fpr preparing chitosan nanoparticles (fig. 2 - formation of the chitosan-tripolyphosphate complex by ionotropic gelation). By this

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way, chitosan powder was dissolved 0.2% (w/v) in 0.25M acetic acid then the solution was magnetically stirred overnight at 400 rpm at room temperature. Thus the amine group of chitosan molecule was protonated by the acetic acid for a stronger interaction with the crosslinking agent and the drugs. Sodium tripolyphosphate (TPP) was prepared in distilled water by dissolving the powder in 0.25M acetic acid then added to chitosan solution under stirring followed by 20 min of sonication so the resulting suspension being centrifuged at 15000 rpm for 10 min.

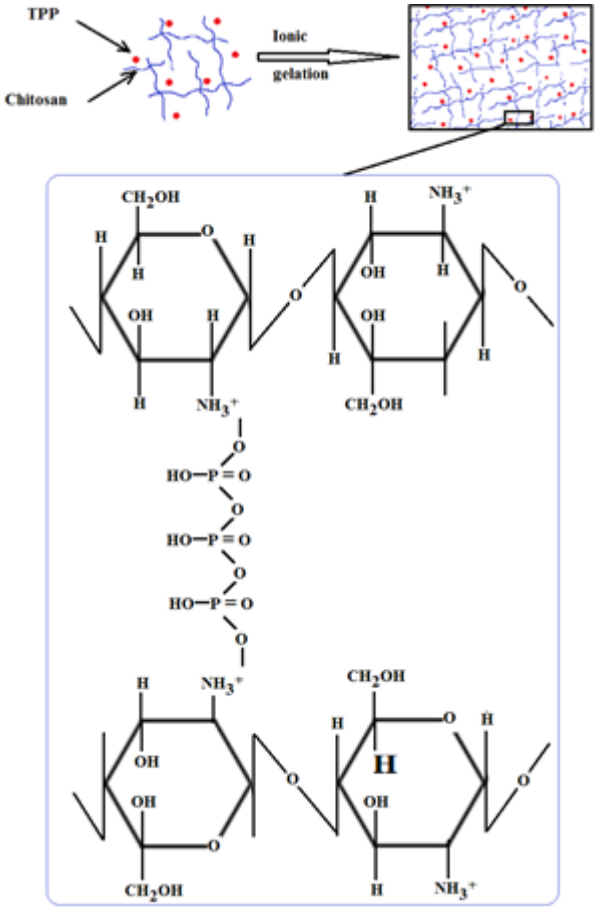

Fig. 2. General sketch of the chitosan-TPPcomplex

The obtained pallets were subsequently re-suspended in deionised water by sonication then centrifuged and dried at 25°C.

Drugs loaded nanoparticles were spontaneously formed by dropwise addition of 12 ml TPP 0,4% to 20 mL chitosan solution 0.35% (w/v) containing amoxicillin and clavulanic acid (5 mg/mL in a mass ratio of 7:1) under magnetic stirring conditions followed by sonication. The resulting nanoparticles suspension was centriguged at 15000 rpm for 4 times (15 minutes each), washed with distilled water and then dried.

Results and discussions

Drugs encapsulated chitosan nanoparticles prepared using 0,40% w/v TPP are denoted as Amox/Clav.ac.-CNP4 and those prepared using 0.60% w/v TPP are marked as Amox/Clav.ac.-CNP6.

Particle size distribution for drugs-loaded chitosan nanoparticles were determined using a Particle size analyzer which establishes dynamic light scattering by nanoparticles in suspension.

Figure 3 present amoxicillin and clavulanic acid-loaded nanoparticles with an average particle diameter of 45 nm having a wide particle size distribution feature. Amox/Clav.ac.-CNP4 nanoparticles are spherical in shape and smooth edges.

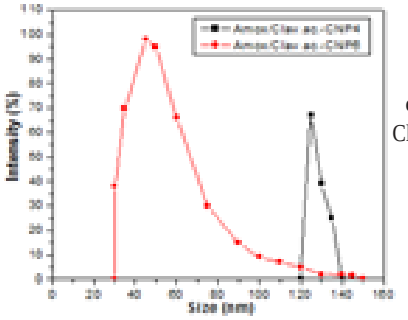

Fig. 3. Particle size distribution for Amox/Clav.ac.-CNP4 and Amox/Clav.ac.-CNP6

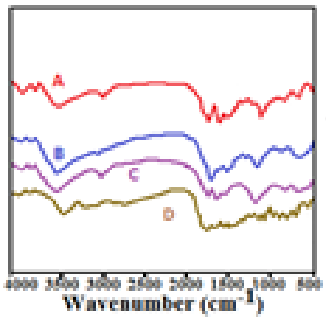

Fig. 4. FTIR spectra for drugs and loaded samples: A -Amoxicillin and clavulanic acid; B - Amox/Clav.ac.-CNP4; C - Amox/Clav.ac.-CNP6; D -chitosan nanoparticles;

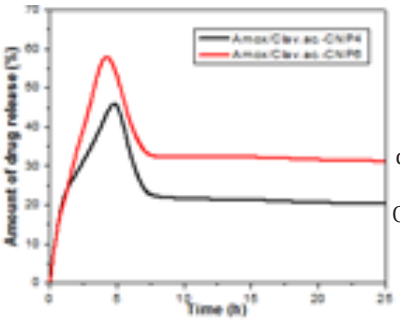

Fig. 5. The amount of drugs release over a 24-hour period for Amox/Clav.ac.-CNP4 and Amox/Clav.ac.-CNP6

Amox/Clav.ac.-CNP6 particles are an average diameter of 270 nm, spherical in shape and not so smooth edges. It can be presumed that the TPP concentration is important in particle size determination. Thereby, having a narrow particle size distribution, the drugs are equally distributed among the nanoparticles thus normalizing the role of drugs release.

FTIR analysis was used to detect chitosan nanoparticles, amoxicillin and clavulanic acid as well as drugs-loaded chitosan nanoparticles as shown in figure 4. From IR spectra it can be observed typical absorption peaks for drugs and chitosan nanoparticles respectively.

Drugs-loaded chitosan showds different IR spectra compared to chitosan nanoparticles spectrum proving that both amoxicillin and clavulanic acid were successfully encapsulated in chitosan nanoparticles.

Drug release profile presented in figure 5 reveals that both amoxicillin and clavulanic acid were released from drugs -loaded chitosan nanoparticles in a burst effect followed by a slow, sustained release manner. For Amox/Clav.ac.-CNP4, the release occurred within the first 5 h and for Amox/Clav.ac.-CNP6 within the first 4 h.

After that the amount of drugs release was reduced followed by a controlled release for the remaining time. By the end of the monitoring period, Amox/Clav.ac.-CNP6 released more total drugs than Amox/Clav.ac.-CNP4. It can be sustained that the burst effect followed by the slow controlled release is perfect for microbial infections treatment.

Conclusions

The oral cavity consisting of few distinct anatomical structures inhabited by a numerous varied microorganisms is a complex environment for drug delivery systems. The most ordinary diseases that can occur in the oral cavity

are oral infections such as dental caries, periodontitis or mucosal infections. Therapeutic efficacy at the targeted site of action can be improved by delivering drugs directly to the oral cavity. Therefore, systemic dose of the drug can be reduced thus decreasing adverse effects.

For oral diseases, drug delivery systems based on nanoparticles has higher efficacy by killing the pathogen bacteria in a sustained manner while reducing the cellular toxicity to non-bacterial cells, a normal issue when amoxicillin and clavulanic is used in common form. Chitosan is a biomaterial for dental uses having antimicrobial properties and continuously subjected to researches on clinical application.

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