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Chloramphenicol collagen sponges for local drug delivery in dentistry

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ABSTRACT

Drug delivery systems based on natural drug carriers have become important due to their non-toxicity and biodegradability. We report here the synthesis and characterization of new biomaterials like sponges containing collagen, chloramphenicol and glutaraldehyde for dentistry. All sponges favour water absorption, showing that increasing the glutaraldehyde content leads to an increase in water uptake. The sponges showed resistance to collagenase degradation and strong activity against the tested bacteria. Kinetic data showed non-Fickian diffusion behaviour with a slow release rate. Taking into account that dental drug delivery systems exhibit low water absorption, slow drug release, high content of drug delivery, good antimicrobial activity, and resistance to enzymatic action, the results obtained in this study indicate the optimal content of glutaraldehyde for the sponge as being 0.5%. The properties of the designed formulations demonstrate that these sponges could be adequate for the treatment and/or the prophylaxis of infected lesions at the dental level.

1. Introduction

Over the past two decades, an increasing interest in how the drugs are administered to patients was observed, this playing an important role in their effectiveness [1]. Such studies included systems in which the drug delivery degree is controlled by the rate at which the drug is osmotically pumped from a reservoir. Also, for more prolonged drug availability and sustained action, drug release through a membrane or sponge is revealed as a function of the amount of cross-linking agent or it is depending on drug or polymer concentration [2].

Current treatments for diseases and trauma of dental structures are based on carriers used for the local drug-delivery system [3]. Among carriers classified as non-biodegradable or biodegradable, polymeric biodegradable ones have widespread use in drug delivery because they can be degraded to non-toxic monomers inside the body. Several advantages like excellent biocompatibility, non-toxicity, as well as the fact they are well documented in terms of structural, physicochemical, biological, and immunological properties, made collagen the favourable matrix in medicine [4,5].

Previous studies [5–7] have revealed that the biological activities of collagen and its derivatives could be very

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useful for the treatment of diseases and trauma of dental structures. In many studies, collagen has been treated with glutaraldehyde, the widely used reagent for proteins [8], knowing the chemistry of glutaraldehyde cross-linking and its effect on the biological performance of a number of biomaterials, including drug delivery systems [9,10]. According to Kohlpaintner et al. [11], a 0.1% to 1% glutaraldehyde solution may be used for system disinfection and as a preservative for long-term storage, while 2% glutaraldehyde is the reference disinfectant for hospital instruments [12].

Additionally, different drugs are used for local delivery, including chloramphenicol and some of the most preferred ways by which drugs are loaded into this system based on collagen are hydrogen bonding, covalent bonding, or simple entrapment.

Chloramphenicol, an effective bacteriostatic antimicrobial against a wide variety of Gram-negative and Gram-positive bacteria, including most anaerobic microorganisms, is currently used for ocular and dermal local treatment [13–16]; moreover, due to its broad spectrum of activity, its use in infections and other sites is totally justified. Thus, several studies demonstrating its antibiotic application in gynaecological diseases have been reported (local treatment of bacterial vaginosis) [13,17].

Taking into account these considerations, chloramphenicol was chosen in this study as an antibiotic drug for the treatment and/or the prophylaxis of infected lesions in various dental structures. Its selection is supported by previous studies that have shown that this drug [18] does not produce chromosomal changes after direct administration in the oral cavity [19].

Moreover, there is currently a revival of the use of already consecrated drugs, the older generation, introduced in therapeutics for a long time, whose side effects and adverse reactions are well known. This has the advantage of already knowing the risk/benefit ratio of these drugs with established actions on patients [20–23].

In order to evaluate the *in vivo* stability of the collagen sponges, the *in vitro* enzymatic degradation is studied. Degradation of collagen can occur through either extracellular or intracellular collagenases, which are the only enzymes that completely cleave collagen. The rate of collagen digestion can be controlled by changing the degree of cross-linking of the collagen matrix [24].

Because the collagen is the primary resource in biomedical applications, this study was focused on the preparation and the characterization of new types of collagen matrix in sponge form for drug delivery systems. The obtained sponges based on collagen gel, chloramphenicol and different concentrations of glutaraldehyde as cross-linking agent were characterized by spectroscopic studies, water absorption, drug release test, and enzymatic degradation. Also, the new drug delivery sponges were tested for their antimicrobial susceptibility against Gram-positive or Gram-negative bacteria. Particularly, the prepared sponges were tested against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Escherichia coli* [25], knowing that these microorganisms are usually found in the oral cavity environment and, on the other hand, these bacteria are important for clinical purpose due to their association with endodontic infections [26,27].

2. Materials and methods

2.1. Materials

For the obtained collagen sponges devoted to biomedical applications, especially as drug delivery systems in dentistry, the following materials were used: a natural polymer-like type-I collagen gel (2.11% dry substance/collagen gel) extracted from bovine skin (The National Research and Development Institute for Textiles and Leather, Bucharest) [28]; a bacteriostatic and antimicrobial drug such as chloramphenicol (CP) purchased from the Sigma Chemical Co., and a cross-linking agent as glutaraldehyde (GA) provided by Merck (Germany). Sodium hydroxide 1 M (NaOH) of analytical grade was used as a solution for pH adjustment.

For enzymatic degradation, test collagenase of *Clostridium histolyticum* from Sigma-Aldrich (USA) and a phosphate buffer solution (PBS) (pH 7.4) were used.

In order to evaluate the antibacterial activity of the sponges, the following human pathogenic microbial strains were tested: *Escherichia coli ATCC 8738*, *Staphylococcus aureus ATTC 25923* and *Enterococcus faecalis ATCC 29212* (facultative anaerobe). The bacterial strains were grown in Luria Bertani Agar (LBA) plates at 37 °C with the following composition: peptone (Merck), 10 g/L; yeast extract (Biolife) 5 g/L, NaCl (Sigma-Aldrich) 5 g/L and agar (Fluka) 20 g/L. The stock culture was maintained at 4 °C.

2.2. Preparation of collagen sponges containing chloramphenicol

Collagen gels prepared as a 1% water solution with a pH adjusted to 7.4 by adding a 1 M NaOH solution were mixed with a water solution of a 1 g/L active substance. After stirring, 1% GA solution is added for cross-linking and let for mixing for 10 min. The obtained homogenous mixture in the form of a gel was placed in a refrigerator for 24 h. The final step in the preparation method was a freeze-drying process using the freeze-dryer Delta 2-24 LSC equipment (Martin Christ, Germany) [29]. The resultant collagen sponges have a concentration of 4% CP relative to the dry substance (0.1% relative to the collagen gel), and a concentration of glutaraldehyde ranging from 0.25, 0.5, 0.75 to 1% related to the dry substance.

2.3. Characterization techniques

Our ATR/FT–IR study was performed using a Perkin Elmer Spectrum 100 FT–IR spectrophotometer in attenuated total reflection mode (ATR). The IR spectra were registered between 4000 and 600 cm^{-1} as a result of the average of four scans with a resolution of 4 cm^{-1}.

The UV–VIS–NIR spectroscopic analysis was realized using a Jasco UV–VIS–NIR spectrophotometer, model V 670 in the 200–2000 nm spectral region, with a step of 0.5 nm.