COLLAGEN BASED SPONGES FOR VETERINARY APPLICATIONS

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In the last decades, periodontal and peri-implantation research on animals has been focused on different types of treatments. Oral diseases are very common in small and big animals like cats dogs, equines, bovines and other ruminants, and it should get a lot more attention in veterinary practice particularly because there are diseases specific to the oral cavity that pose a great risk to the health of these animals. This study proposes new materials based on collagen for the treatment of different animal diseases. The results concerning the characterization of these new collagen-based materials will be presented and discussed in view of their possible oral applications on mammals such as felines, and other animals.

Keywords: Collagen based materials, piroxicam, collagen sponges.

1. Introduction

In the last decades, periodontal and peri-implantation research on animals has been focused on different types of treatments. A recent article shows that for two specialized journals, the number of articles dedicated to research related to animals has increased significantly, research that includes results from the pathogenesis to the therapy of human and animal diseases [1].

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The important inflammation of the mucosa membranes of the mouth, the stomatitis, could be found often between the animals. Depending on the place of the lesions the malady is called glossitis (tongue), cheilitis (lips), buccostomatittis (inner cheek), pharyngitis (pharynx), faucitis (fauces – glossopalatine folds or angles of the mouth), palatitis (hard or soft palatitis due to the palate lesions), ginigivtis (gingiva), tonsilititis (tonsilar), as periodontitis (periodotium – periodontal membrane, ginigiva, and alveolar bone).

Diseases like neoplastic disease, autoimmune disease, toxic disease, specific diseases, periodontal diseases, and miscellaneous diseases could have as consequence the inflammatory lesions of the oral cavity of domestic cats and this inflammation could be due to viral, fungal, mycoplasma or bacterial agents. Thus, there are diseases specific to the oral cavity that pose a great risk to the health of cats and other animals, and several studies have been carried out to apply to animals what has already been discovered from the counterparts of human diseases and vice versa [2, 3].

Oral diseases are very common in cats and other animals, and it should get a lot more attention in veterinary practice. For example, there were specific studies on the young population of cats, where one of four cats had resorptive lesions, and one out of five had advanced periodontal disease. However, the periodontal disease can be prevented therefore important attention has to be paid by the veterinary medicine. In addition, the oral diagnosis and treatments of animals have to include besides the scaling and polishing as routine the radiographic examination and possibly dental extraction [4].

Feline chronic gingivostomatitis is a painful, often debilating, condition which is characterized by protracted oral inflammation typically lasting month to years. A good medical or surgical management of such an inflammation could have as a final result the clinical remission [5].

For equines one can meet diseases as retained deciduous teeth, periodontis, periapical infection, gingival hyperplasia, tumors. The old horses can face ameloblastomas which involves the mandible while in young horses it can be found odontomas a disease involving the maxillary [6].

The most used reconstructive procedure in case of a bone deficiency is sinus floor elevation with lateral approach. Different graft materials as well as different dental implants was shown that can successfully be used in patients suffering different systemic diseases as well as deficient bone condition. The bone augmentation, sometimes, represents the unique option that allows the replacement of missing teeth with dental implants. Therefore, this kind of therapeutically management may easily be used also in animals like equine and maybe felines as well [7].

Piroxicam exhibits anti-inflammatory, analgesic, and antypyretic activities in animal models and in humans. Piroxicam is a nonsteroidial anti-inflammatory

drug (NSAID) of the oxicam class, which is nonselective and usually used in diseases like arthritis and musculoskeletal conditions. In animals piroxicam is generally used to reduce pain, fever, and inflammation, but in some cases is used also in the rheumatoid disorders and mastitis treatment management. The main advantage of this drug is represented by the high half-life of 50 h, and by the response which increases over several weeks [8].

Y-P. Hung and collab. proposed a treatment using a combination of bovine lactoferrin (bLf) and piroxicam oral spray for cats with caudal stomatitis. It was observed that the bLf and piroxicam treatment leads to a significantly better result in terms of improvement of clinical signs and oral lesions [9].

C. W. Park et collab. revealed that delivering IA (intra-articular) an NSAID has as consequence a better pain relief, indicating a peripheral analgesic effect. The local NSAID administration leads to high concentrations of NSAIDs at the site of cell injury and at the same time to clinical benefits (use of lower doses, lower subsequent systemic exposure, and a reduced frequency of adverse events). They concluded that the rate and extent of systemic absorption of piroxicam following IA injection is rapid and substantial [10].

Other specific study related to the management procedure (considered one of the most important welfare priorities in livestock production) showed that the combination between piroxicam and ascorbic acid is very effective in the management of postsurgical pain of orchidectomised Savannah Brown goats [11].

In addition, due to its properties, collagen which is mostly found in various tissues such as tendon, ligaments and skin and in other body structures such as cornea, cartilage, bone, blood vessels and dentin in teeth could be a valuable support for drug release and contribute to enhance the treatment benefits [12 – 14].

The present study is focused on the synthesis and characterization of collagen-based sponges with piroxicam in view of their use in animal treatment. Due to this our experiments were performed using collagen-based sponges with a high dose of piroxicam which is indicated in bovines, or other animals, including horse oral implants. A high dose of piroxicam represents a contraindication in humans where a much lower dose is required [15].

2.Materials and methods

The collagen, type I, used in the synthesis of materials was extracted from bovine skin according to the internal procedure [16] at the INCDTP-Division Leather and Footwear Research Institute and it was in the form of a gel with a content of 2.82% collagen. The collagen gel was transformed in a solution with 1% collagen and pH = 7.4 with distilled water and NaOH 1M. To this solution piroxicam (Alfa Aesar) was added. The piroxicam content was calculated with respect to the dry collagen and the concentrations were 50% and 80%. Finally,

glutaraldehyde (Merck) was added in order to obtain gels containing 0.5% glutaraldehyde reported to dry collagen. The role of glutaraldehyde is to cross-link the collagen. The sample with content only in collagen was coded as C1, and the samples with collagen and piroxicam were coded as C2 and C3 respectively.

The crosslinked gels C1, C2 and C3 were poured into glass Petri dishes and lyophilized for 48 hours according to the following procedure. Firstly, the Petri dishes were placed on the shelves of the lyophilizer cooled in advance at - 40°C to obtain a constant porosity of the samples and kept for 10 hours. Next the samples undergo the lyophilization (water extraction under vacuum) at a pressure of 0.12 mbar and a temperature of -40°C. During freeze-drying, the temperature gradually increases, reaching +10°C in 10 hours, +20°C in 8 hours and +35°C in 12 hours at same pressure. The final stage lasted 8 hours, at a pressure of 0.01 mbar, and the final temperature of the shelves of 35°C. The samples were obtained as spongious forms.

Optical microscopy images were registered using a ZEISS Scope.A1 digital microscope equipped with a Canon camera (Taiwan) to confirm the fibrillar structure of the collagen and to highlight the presence of the drug.

The obtained sponges were then investigated with respect to the water absorption capacity, enzymatic degradation and drug release kinetics.

The water absorption percentage was calculated at different immersion time up to 192 h using Equation 1. The experiments were performed in triplicate, on sponges cubes (1 cm x 1 cm x 0.5 cm) immersed in distilled water at 25 ^{0}C.

$$\%\ water\ absorption = \frac{w_1 - w_0}{w_0} \times 100 \quad (1)$$

where w_0 is the dry weight and w_1 is the wet weight.

Collagen sponge degradation under enzymatic environment was estimated using collagenase of *Clostridium histolyticum* from Sigma-Aldrich (USA) and a phosphate buffer solution (PBS) with pH 7.4. The tests were performed by immersing samples in PBS and incubating them at 37 °C over night. The next step was to add collagenase (10 μg/mL) and maintaining the test tube at 37 °C. At different time period, the remained sponge was squeezed and weight. The degradation was assimilated to the collagen mass degradation and calculated according to Equation 2:

$$\%\ collagen\ mass\ degraded = \frac{w_i - w_t}{w_i} \times 100 \quad (2)$$

where w_i is the initial weight and w_t is the weight after time t. Each biodegradation test was made in triplicate.

The *in vitro* drug release measurements were performed using a sandwich device adapted to a paddle dissolution equipment, as previously reported [12]. The quantitative evaluation of piroxicam released at different time intervals from the designed sponges was monitored by UV spectroscopy (λ=353 nm) [16], and the cumulative kinetic profiles were built. The kinetic experimental data were fitted according to general Power law model and its two particular cases for n=0 (Zero-order model) and n=0.5 (Higuchi model), and the drug transport mechanism was set up.

$$\frac{m_t}{m_\infty} = k \times t^n \tag{3}$$

where m_t/m_∞ is the fractional release of drug at different periods of time t, k – the kinetic constant, and n – the drug release exponent indicating the release mechanism.

3. Results and discussion

3.1. Optical measurements

In Figure 1 are presented the microscopic images for collagen-based sponges. It can be seen the fibrillar structure with interconnected pores, in all obtained sponges. The luminescent spots that are seen in Figure 1b are due to the piroxicam presence, showing that these new materials contain the drug.

a **b**

Fig. 1 - Optical microscopy images of the collagen sponges surfaces at 100 X magnification, a) C1 - Collagen sponge; b) C3 - Collagen sponge with the highest content of piroxicam (80%)

3.2. Water absorption measurements

The water absorption results of collagen sponges without or with different concentration of piroxicam are shown in Figure 2. As expected, in time, the water content in sponges is increasing and the presence of the drug has as consequence the decreasing of the absorbed water content. For example, after 24h C2 sponge has absorption of 34% compared to collagen sponge C1 (57%) and C3 sponge (22%). However, after 24h, the sample containing the highest drug content disintegrated, while the C2 sponge continued to absorb water until 168h and then disintegrate.

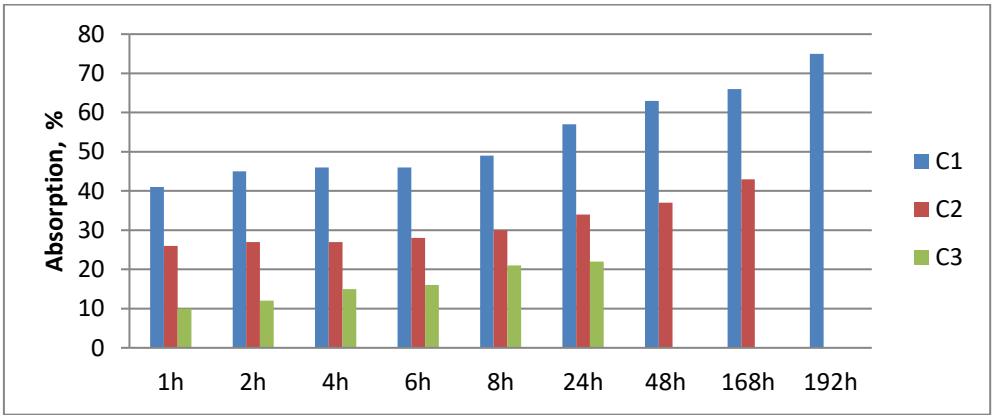

Fig. 2. Water absorption of collagen sponges: C1 – collagen sponge, C2 – collagen sponge with 50% piroxicam, C3 – Collagen sponge with the highest content of piroxicam (80%)

3.3. Enzymatic degradation

Despite the fact that piroxicam decreases the water absorption, the collagen sponges immersed in PBS undergo during one night incubation some damages and the collagenase addition lead to the sponge disintegration. Collagen sponge itself, as expected, is degraded slowly, after 24 h about 40% being degraded.

3.4. In vitro drug release

The kinetic experimental data were graphically illustrated as drug cumulative release (%) versus time (Fig. 3).

From Figure 3 a rapid drug release effect is obvious in the first hour, recording a value of about 31% for sponge C2 and 41% for sponge C3, respectively, followed by a gradual piroxicam delivery in the next 10 hours of experiment up to 69.96% (formulation C2) and 79.83% (formulation C3). It can be noticed that a higher content of piroxicam led to an increase of drug release percentage about 1.14 times.

The drug release behaviour comprising two phases is beneficial to atenuate and control the local inflammation and pain specific for the oral diseases with different ethiology [16]. To set up the piroxicam release mechanism from the designed collagen sponges, different kinetic models (Power law, Zero-order and Higuchi) were tested. The highest values for the the correlation coefficients, R (Table 1) were recorded for the Power law model showing a non-Fickian drug release transport. The kinetic parameters specific to the above model are listed in Table 1.

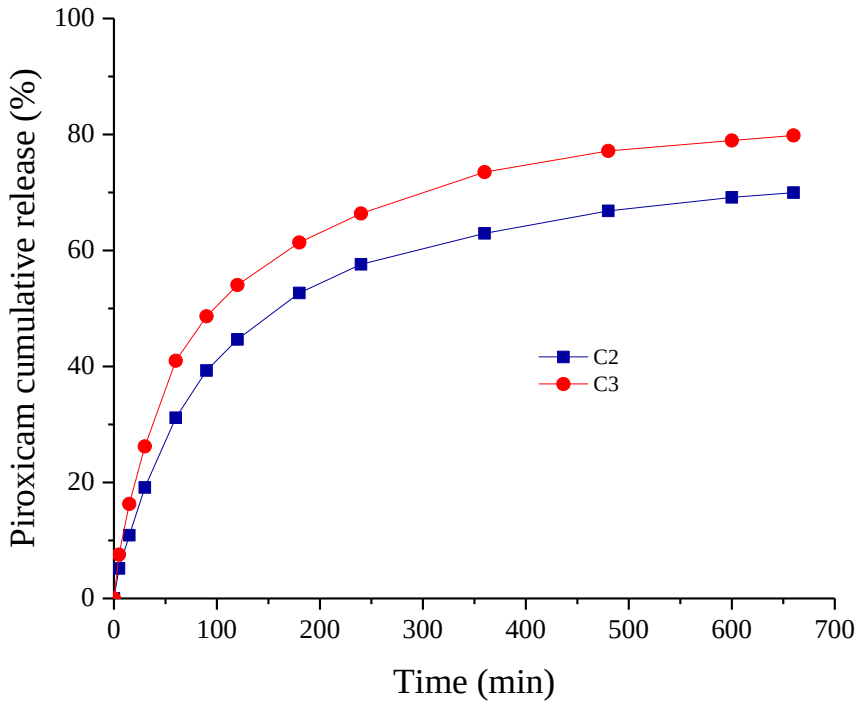

Fig. 3. Cumulative piroxicam release patterns from collagen sponges versus time; C2 – collagen sponge with 50% piroxicam and C3 – collagen sponge with 80% piroxicam

Table 1

Kinetic parameters and correlation coefficient characteristic to Power law model; correlation coefficients specific to Higuchi and Zero-Order models

Collagen-piroxicam sponges	Kinetic constant ($1/min^n$)	Release exponent	Correlation coefficient R		
			Power law model	Higuchi model	Zero-Order model
C2	0.067	0.37	0.9811	0.9665	0.8712
C3	0.097	0.34	0.9815	0.9577	0.8526

4. Conclusions

The present study allowed us to lay down the following conclusions:

- The collagen solution with or without piroxicam were chemical cross-linked with glutaraldehyde and prepared in the form of sponges by lyophilization process;

- The optical microscopy images displayed a fibril structure with interconnected pores in all obtained sponges but with piroxicam aggregations in samples C2 and C3;

- The collagen sponge with no piroxicam content absorbed an important quantity of water, and when drug was added into the collagen sponge composition the water absorption rate decreased. It can be concluded that the water absorption for the studied sponges is decreasing with the drug dose increasing;

- A high content of drug results in the sponge disintegration. It cannot be stated that the samples are totally degraded in the sense of mass loss, they disintegrate and practically fibers of collagen with piroxicam are presented in the solution (PBS with collagenase);

- The kinetic studies indicated a byphasic drug delievry, favorable for ensuring an adequate piroxicam concentration at the application site to manage the inflammation and consecutive pain associated with different oral disease;

- These preliminary results suggest that such kind of sponges with high dose of piroxicam could be used for different treatments in big animals like equines and bovines.

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