IBUPROFEN-COLLAGEN SPONGES FOR WOUND HEALING

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The aim of this paper was to design and characterize some collagen-ibuprofen sponges, potentially usable in the treatment of inflammation associated to cutaneous lesions and subsequently to the post-lession pain, the *in vitro* drug release evaluation and *in vivo* wound healing test. The collagenic matrices, obtained by collagen-ibuprofen hydrogels lyophilization, uncrosslinked and crosslinked with glutaraldehyde, were characterized by morphological (water absorption), goniometric (contact angle), and biological analysis (enzymatic biodegradation). *In vitro* ibuprofen release was performed with a transdermal sandwich device adapted to a dissolution apparatus. The *in vivo* wound healing test was determined using experimental animals (small rodents) with lesions induced with a special metallic device. Similar release profiles were obtained for the matrices with different composition and the kinetic mechanism was set. The matrices swelling capacity, surface wettability and resistance at enzymatic degradation are in accordance with kinetic results. The animal groups treated with collagen sponges and drug-loaded collagen sponges indicated a much faster wound healing effect compared to a non-treated control group. The study results showed that physical-chemical, biological and biopharmaceutical characteristics, and *in vivo* sponges efficiency are strongly influenced by their composition, the determination of the optimum formulation parameters for the new drug supports being possible by modulating the matrices composition.

Keywords: collagen sponges, ibuprofen delivery, anti-inflammatory effect.

INTRODUCTION

An important aspect to be considered in the healing of acute or chronic lesions with low, moderate or high exudate is the control of the post-lesion inflammatory response and implicitely of the associated pain which is a major discomfort for the patients, influencing markedly their life quality (Arapoglou *et al.*, 2011; Shemesh and Zilberman, 2014). Among the pain-killing agents, a special attention is given to the non-steroidal anti-inflammatory drugs (NSAIDs) which generally possess analgesic, anti-inflammatory and antipyretic properties. But, due to the side effects induced by these drugs, especially at gastro-intestinal level, an alternative to the oral administration route was studied (Albu *et al.*, 2012; Komatsu and Sakurada, 2012). Thus, the healing of cutaneous wounds of different etiologies may be optimized and supported through a rational approach based on the use of topical drug delivery systems with biopolymeric supports (Ghica *et al.*, 2012). NSAIDs release directly at lesion level, in a controlled manner to maintain a sufficient and effective drug concentration, is essential to combat the inflammation and subsequently the pain that occur during the healing process.

Collagen, a natural biopolymer, processed as spongious form is useful in the healing of different cutaneous wounds, as it absorbs large exudate quantities, preserves a moist environment, and enhances the formation of new granulation and epithelium tissue (Albu *et al.*, 2011; Ghica *et al.*, 2013; Lu *et al.*, 2014).

The goal of the present work was the design and characterization of some collagen-based spongious matrices ibuprofen-loaded as a NSAIDs drug model, the *in vitro* drug release evaluation and *in vivo* wound healing test using experimental animals (Aoyagi *et al.*, 2007; Sung *et al.*, 2010; Ramli and Wong, 2011; Xingang *et al.*, 2013).

MATERIALS AND METHODS

Materials

Type I fibrillar collagen (C) gel having a concentration of 2.11% (w/w) and pH 2.5 was extracted from calf hide as previously described (Albu 2011). Ibuprofen (IBU) was purchased from ICN Biomedicals Inc. (USA) and glutaraldehyde (GA) was supplied from Sigma-Aldrich (Germany). Sodium hydroxide, monobasic potassium phosphate and disodium hydrogen phosphate were obtained from Merck (Germany). All the reagents used were of analytical grade and the water was distilled.

Preparation of Collagen Sponges and Ibuprofen-Loaded Collagen Sponges

Collagen hydrogels were obtained by adjusting initial collagen gel to 1.0% and 1.2% and 7.4 pH, adding 0.5% ibuprofen, reported to the amount of collagen gel, cross-linked with glutaraldehyde. The composition of the designed collagen formulations is presented in Table 1.

Table 1. Composition of collagen-based hydrogels

Hydrogel	Collagen, C (g%)	Glutaraldehyde, GA (g%)	Ibuprofen, IBU (g%)
G1	1.0	0	0
G2	1.2	0	0
G3	1.0	0.0025	0
G4	1.2	0.0025	0
G5	1.0	0	0.5
G6	1.2	0	0.5
G7	1.0	0.0025	0.5
G8	1.2	0.0025	0.5

*the amounts of C, IBU and GA are reported to 100g hydrogel

The obtained hydrogels were then lyophilized using the Delta LSC 2-24 Martin Christ lyophilizer (Germany) using the method previously described (Lungu *et al.*, 2011) and the corresponding M1-M8 collagen sponges were obtained.

Water Absorption and Enzymatic Biodegradability Studies

The obtained collagen sponges were assessed by water absorption and enzymatic biodegradation according with the methods previously described (Lungu *et al.*, 2013).

Contact Angles Determination

The wettability capacity evaluation of sponges surface was quantified by the contact angle measurement, using a KSV Scientific Instrument (Finland) equipped with a video camera for images capturing and a CAM-101 software for data acquisition. For the determination of contact angle the pendant drop dynamic method was applied, and for its computing the Young-Laplace equation which describe the drop shape was used.

***In vitro* Ibuprofen Release Analysis**

Ibuprofen *in vitro* release from collagen spongious forms was performed using a transdermal sandwich device adapted to a dissolution apparatus as previously reported in our studies (Ghica *et al.*, 2013). Briefly, ibuprofen-loaded collagen sponge samples were placed into the release vessels, using as the release medium the phosphate buffer of 7.4 pH, maintained at 37°C. The absorbance of the solution extracted at predetermined period of time was spectrophotometrically determined at 264.4 nm (Perkin-Elmer UV-Vis spectrophotometer), using the calibration curve ($A_{1\%}^{1cm} = 19$, R = 0.9999). The Power law equation was applied for the ibuprofen release kinetics assessment from sponges as previously described (Ghica *et al.*, 2013).

***In Vivo* Wound Healing Test**

Experiments were performed on Wistar rats weighing 230±10g purchased from The Animal Biobase of The "Carol Davila" University of Medicine and Pharmacy, Bucharest. All animals used in the study were kept in standard laboratory conditions. They received water *ad libitum* and were not fed for 12h before the experiment. All experiments were performed in compliance with European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from February 2nd, 2002. The rats were distributed in 9 groups of 3 individuals each and were anesthetized with ether ethylic. The hair was removed from the dorsal area of the animal and an experimental wound was induced using a special metallic device of 1cm diameter. The device was heated in boiling water and applied on the shaved dorsal area for 25 seconds. The severe burns measuring 1cm diameter were treated with collagen sponges as it follows: Group 1 to Group 4 with M1-M4 collagen sponges, Group 5 to Group 8 with M5-M8 ibuprofen-loaded collagen sponges, and Group 9 is control group, no dressing applied on the wounded area. The experimental wounds were sterilized and the collagen scaffolds were applied and fixed with a plaster. The surface morphology of the wounds was recorded using a digital camera and the wound diameter was measured every two days for about 2 weeks. Any aspects of inflammation or infection of the wounds, as well as any modification on the animal health status were also monitored.

RESULTS AND DISCUSSION

The water absorption of spongious matrices was dependant on collagen / crosslinking agent concentration and drug content. Thus, the high concentrated matrices absorbed less water than the ones with 1% collagen (29% for M4 and 37% for M3) while the cross-linked sponges absorbed more water than the un-cross-linked ones (19% for M1 and 37% for M3). The drug content decreased the water absorption for all the samples with 1-2% biopolymer. Enzymatic degradation showed a good resistance to

collagenase for all the cross-linked samples. Moreover, samples M7 and M8 were degraded 38.85% and 33.79% respectively after 12 days.

The values recorded for the contact angle indicate a surface hydrophilicity both for collagen and drug-loaded sponges (45.55^{o}-88.21^{o}), which favors the wetting by the biologic fluids.

The released ibuprofen percent from M5-M8 sponges during 8 hours of experiment was plotted against time and the kinetic patterns of the swellable systems un- and cross-linked with glutaraldehyde are shown in Figure 1. The released drug percent varied between 54.59% (M8) and 72.99% (M5) (Table 2). A biopolymer concentration increase determines a released ibuprofen percent decrease of 1.16 – 1.21 times while the cross-linking leads to a decrease of 1.11 – 1.15 times respectively.

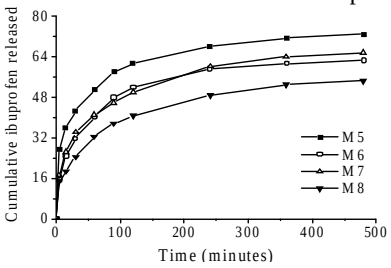


Figure 1. Cumulative release profiles of ibuprofen from M5-M8 collagen sponges as a function of time

The application of Power law model to the kinetic data indicated a non-Fickian drug diffusion mechanism, specific to the drug porous matrices for which various factors are involved: sponges wetting and swelling after contact with biological fluid and their transformation into gel, in the same time with drug diffusion through the gel formed, and eventual erosion of the polymeric gel matrix. The values recorded for the correlation coefficient (R) as well as the values obtained for the kinetic characteristics specific to the above model are listed in Table 2.

Table 2. Values for correlation coefficients and kinetic parameters characteristic for Power law model; released drug percent

Collagen sponges	Correlation coefficient	Kinetic constant ($1/min^{n}$)	Release exponent	Released percent (%)
M5	0.9942	0.219	0.202	72.99
M6	0.9859	0.135	0.260	62.67
M7	0.9948	0.137	0.262	65.81
M8	0.9947	0.098	0.285	54.59

After the treatment with the burning device, the affected skin area appeared as a white eschar with a hyperemic area on the periphery evolving in the following days to a full hyperemic area. The re-epithelialization process was higher in Group 7 followed by Groups 5, 6 and 8 (Figure 2). After the treatment with the collagen sponges, a small increase of the wound diameters was observed in Groups 1-4 and Group 9 in the first two days due to the inflammation of the local tissues. During the following days the wound diameter decreased in all treated groups and the re-epithelialization was faster compared to the control group which needed more than 25 days for a complete healing.

Collagen itself offers the advantage of a natural biomaterial with wound healing effect, explaining its own pharmacological action on the experimental animals. Also, in combination with ibuprofen a sinergic action occurs during lesion healing process.

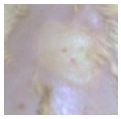
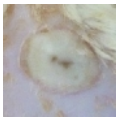
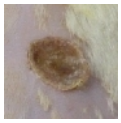
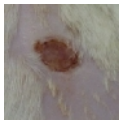
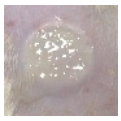
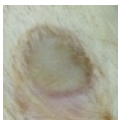
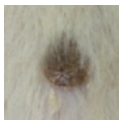
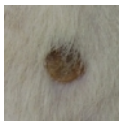
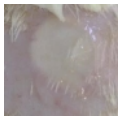
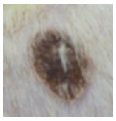
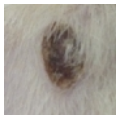
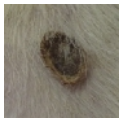
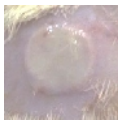
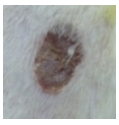
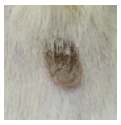
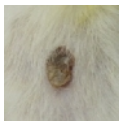
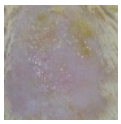
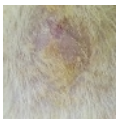
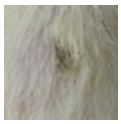
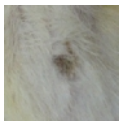
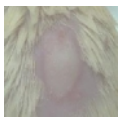
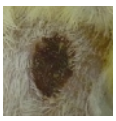
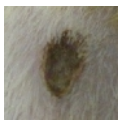
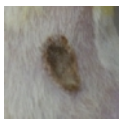
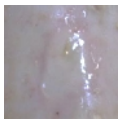
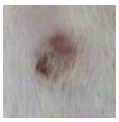
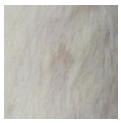
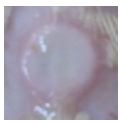
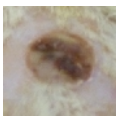
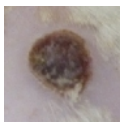
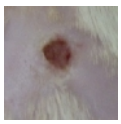
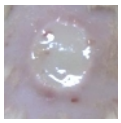
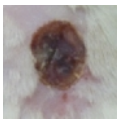
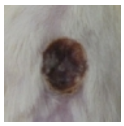
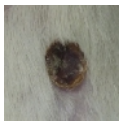
	DAY 1	DAY 4	DAY 8	DAY 12
Group 1				
Group 2				
Group 3				
Group 4				
Group 5				
Group 6				
Group 7				
Group 8				
Group 9				

Figure 2. The evolution of re-epithelialization process after the sponge application on wounds induced to the experimental animals

CONCLUSIONS

The study results showed that physical-chemical, biological and biopharmaceutical characteristics, and *in vivo* sponges efficiency are strongly influenced by their composition, the determination of the optimum formulation parameters for the new drug supports being possible by modulating the matrices composition. The ibuprofen-loaded sponge cross-linked with glutaraldehide and with 1% collagen (M7) could be potentially usable in the treatment of inflammation associated to cutaneous lesions and subsequently to the post-lession pain.

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