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Research Article

Polymer – flufenamic acid delivery systems for injured skin

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ABSTRACT

Collagen, the main protein of the body, is extracted in different forms and used as reservoir for drug delivery. The aim of this work was to obtain a drug delivery system based on collagen-dextran matrices cross-linked with glutaraldehyde as support and flufenamic acid and/or microcapsules with flufenamic acid as drug. The flufenamic acid was encapsulated in polymeric microcapsules consisting in gelatin, alginate, and sodium carboxymethyl cellulose. The morphology of matrices was determined by water absorption and contact angle. The biodegradation was performed in collagenase solution. In vitroflufenamic acid release profiles were built and the kinetic mechanism was set according to different mathematical models. The pharmacological studies followed the effect of collagen formulations treatment on the healing process of Wistar rats which were induced experimental wounds. The studied matrices proved that flufenamic acid delivery can be controlled, and the healing can be completed using the designed spongious matrices.

Keywords: Collagen sponges, microcapsules, flufenamic acid, kinetic release, wound healing.

Doku yaralanmaları için polimer-flufenamik asit salınım sistemleri

ÖZ

Vücudun ana proteini olan kolajen, farklı formlarda ekstrakte edilmekte ve ilaç salınımı için rezervuar olarak kullanılmaktadır. Bu çalışmanın amacı, destek olarak glutaraldehit ile çapraz bağlanmış kolajen-dekstran matrislerine ve ilaç olarak flufenamik asit ve/veya flufenamik asitli mikrokapsül içeren ilaç dağıtım sistemleri elde etmektir. Flufenamik asit, jelatin, aljinat ve karboksimetil selülozdan oluşan bir polimerik mikrokapsül içinde kapsüllenmiştir. Matris morfolojisi, su emme ve temas açısı ölçümleriyle belirlenmiştir. Biyolojik bozunma kolajenaz solüsyonunda gerçekleştirilmiştir. İn vitro flufenamik asit salımı, USP aparatına uyarlanmış bir sandviç cihaz kullanılarak belirlenmiş, kinetik mekanizma ise ilaç salımı için mevcut matematiksel modellere göre belirlenmiştir. Hayvan çalışmalarında wistar sıçanlarında deneysel olarak indüklenen yaralarda kolajen formülasyonlarının iyileşme süreci üzerindeki etkileri izlenmiştir. Çalışmada elde edilen matrisler incelendiğinde, flufenamik asit iletiminin kontrol edilebileceğini ve iyileşmenin tamamen bu süngerimsi matrisler kullanılarak yapılabileceğini kanıtlamıştır.

Anahtar Kelimeler: İlaç salımı, kolajen, sünger, flufenamik asit.

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1. INTRODUCTION

Worldwide, the most common type of injury are skin lessions, such as burns.[1] In the past, the treatment of burns was rudimentary, and a lot of patients died because of hypervolemic shock immediately after they suffered burns.[2]

So far the progress of burn therapy, regenerative medicine and pharmacotherapy is high. However, treating burn injuries continue to be a challenge for researchers, despite the numerous materials available for the patients.[3] In the last years many biomaterials for skin regeneration based on natural and synthetic polymers and loaded with drugs have been developed. In order to design the most suitable biomaterial for the treatment of burn wounds, several natural polymers were selected for this research.

Collagen represents the major fibrous protein component in connective tissues like skin, tendons, ligaments, cartilage, cornea etc. Having exceptional characteristics, collagen is used in varied scaffolds as wound dressings, antithrombogenic surfaces, ophthalmologic shields, artificial blood vessels, bone substitutes and valves, and drug delivery systems.[4-6] Gelatin is obtained by collagen denaturation and, in the medical field, is used for the manufacture of hydrogels, nano and microsphere particles, nanofibers, pharmaceutical additives and drug delivery carries for bioactive substances.[7-9]

Alginate is a natural polymer from polysaccharides class, extracted from a diversity of brown algae such as *Macrocystis pyrifera*, *Laminaria hyperborea*, and *Ascophyllum nodosum*.[10-12] This natural polymer, due to its good properties like adequate bioadhesion and mucoadhesion, and biocompatibility is used in medical applications mostly for drug delivery systems and microencapsulation of cells.[13,14]

Dextran is a polysaccharide produced by lactic acid bacteria or their enzymes in the presence of sucrose.[15,16] The main properties of dextran such as anticoagulant, antithrombotic, osmotic agent, and intravenous plasma lubricant, also cryopreservative for vaccines and organs[17] allow it to be a good candidate in medical field. Sodium carboxymethyl cellulose is furthermost a promising cellulose byproduct. Due to its representative characteristics like mechanical strength, tunable hydrophilicity, viscous properties,low price it can be used in food industry, functionalisation of textiles, pharmaceutical industries and biomedical area.[18]

An ideal biomaterial for burn injuries must present proper biocompatibility, absorbability, antimicrobial properties, ease of use and personalised tailored size.[19] A scaffold based only on natural polymers can not reach such requirements so an ideal wound dressing needs to be combined with some anti-inflammatory drugs. For this reason, flufenamic acid was selected. Flufenamic acid is a non-steroidal anti-inflammatory drug (NSAID) from the anthranilic group with analgesic, anti-inflammatory and antipyretic characteristics.[20]

In our previous work we studied similar spongious matrices but with different concentrations of polymers and cross-linking agent.[21] The aim of this study was the development of a multiparticulate drug delivery system based on a polymeric matrix, with controlled delivery of an anti-inflammatory drug loaded with polymeric microparticles and also with anti-inflammatory drug incapsulated, and the investigation of their biocompatibility, release kinetics and efficiency using preclinical studies involving animal models.

2. MATERIALS AND METHODS

2.1. Materials

Type I fibrillar collagen gel (Coll) of 1.92% (*w*/*v*) and gelatin (Gel) with 2.5% were obtained from calf hide using technology developed in Collagen Department of INCDPT Division ICPI.[22] Collagen matrices consisted in collagen gel, dextran sulfate sodium salt (Dex) and flufenamic acid (FA). The microcapsules consisted in gelatin, sodium carboxymethyl cellulose (CMCNa) and sodium alginate salt. The crosslinking was performed with glutaraldehyde (GA) for matrices and calcium chloride ($CaCl_2$) for microcapsules. Sodiumhydroxide was of analytical grade and the water was distilled.

2.2. Preparation of microcapsules and matrices

The microcapsules and matrices were prepared according with the method previously described[21] and have the compositions presented in the Table 1 and Table 2.

Table 1. Composition of microcapsules (MC).

Gelatin (g%)	CMCNa (g%)	Flufenamicacid (g%)	Sodium alginate (g%)
7.50	0.75	2.00	1.00

Two types of hydrogels were prepared with the following compositions: hydrogel H1 which consists in collagen (1.2%), dextran (0.6%) and glutaraldehyde (0.012%) and hydrogel H2 which consists in same composition as H1 with 0.5% flufenamic acid. The matrices were prepared by lyophilisation of hydrogels and their compositions are the following:

The obtained matrices named M1-M4 were than characterize by physical-chemical, biopharmaceutical and pharmacological analysis.

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Table 2. Composition of matrices.

Matrices	M1	M2	M3	M4
Ratio between hydrogels and microcpsules	H1 : MC 70 : 30	H1 : MC 85 : 15	H2 : MC 70 : 30	H2 : MC 85 : 15

2.3. Determination of water uptake capacity

The water up-take was determined in triplicate on matrices using the methods previously described[24] at different intervals of time. The following equation (*Equation 1*) was used to measure the water uptake capacity of matrices:

$$Water\ Uptake = \frac{Ww - Wd}{Wd}\ g/g \qquad (Equation 1)$$

Where W_w represents the weight of wet matrices at immersion time, and W_d– the weight of dry scaffolds.

2.4. Enzymatic degradation

Enzymatic degradation of matrices was measured in triplicate using collagenase solution as previously described[24]. The following equation (*Equation 2)* was used to measure the amount of degraded matrices:

$$\%weight\ loss = \frac{Wi - Wt}{Wi}\ x\ 100 \qquad (Equation\ 2)$$

where Wi represents the initial weight, and *Wt*– the last weight.

2.5. Goniometric analysis

The monitoring of the sponges porous surface properties, quantified by contact angle (CA°) values, was carried out with CAM 101 (KSV Instruments), using the pendant drop dynamic method, as reported in our previous paper.[23] Using the Young equation (*Equation3*) the drop shape was fitted and the contact angle was evaluated.[24]

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cdot cos\theta \qquad (Equation\ 3)$$

Where γ_{SG} is the interfacial tension S/G, γ_{SL} – the interfacial tension S/L, γ_{LG}– the superficial tension L/G, and θ– the contact angle.

2.6. Flufenamic acid in vitro release

The flufenamic acid *in vitro*delivery from the spongious collagen-basedmultiparticulate systemswas conducted with a paddle dissolution apparatus equipped with a sandwich device, as mentioned in our previous Works.[21] The amount of drug released at different periods of time was spectrophotometrically assessed using the calibration curve ($A_{1\%}^{1cm}$ = 534, λ = 288 nm), and the drug cumulative release (%) was determined.

The experimental kinetic results were quantified using the general model – Power law(*Equation4*), also applying two particular cases for n=0.5 (Higuchi model) and n=1 (Zero-Order), and the flufenamic acid transport mechanism was established.

$$\frac{m_t}{m_\infty} = k \times t^n \qquad (Equation\ 4)$$

where m_t/m_∞ represents thefraction of the drugdelivered at different time *t, k* – the kinetic constant, *n* – the release exponent correlated with the drug transport mechanism.

2.7. The animal model for the induced burns

The pharmacological experiments were performed as we previously described.[21,25] Briefly, experiments were conducted using Wistar rats (200±10g weight), obtained from The Animal Biobase of “Carol Davila” University of Medicine and Pharmacy, Bucharest. The rats were kept in laboratory standard conditions. The pharmacological experiments were conducted according to the Directive 2010/63/EU of the European Parliament, respecting the national legislation. Five groups of five animals each were used. Thermic lesions were induced under anaesthesia with a special device of 10mm diameter on dorsal area. The animals were than treated with multiparticulate collagen-basedsystemsin the form of spongeas follows: M1-M4 sponges were used for the Group 1 – Group 4, and the control group (no dressing on the wounded area) was Group 5. A digital camera was used to evaluate the evolution of wound surface morphology, and the wound diameter was measured every two days during 17 days. In the following equation (*Equation5*) the healing processesis described according to the size profile of wound:

$$Healing\ process\ \% = \frac{(Wound\ diameter\ at\ t = 0) - (Wound\ diameter\ at\ t)}{Wound\ diameter\ at\ t = 0} x100 \qquad (Equation\ 5)$$

where the wound size is evaluated as an average measurement of the longest and shortest dimensions of the affected area. When of the crust of the lesion fell of, the wound was considered healed.

For all animals, two aspects were monitored: lesion inflammation or infection, and modification on the animal health status.

For statistical interpretation of data, GraphPad Prism 6

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was used. All the results were presented as mean with standard deviation. The Kolmogorov–Smirnov test was applied for normal distribution calculation and the statistical significance was calculated using ANOVA, *t*-student and Bonferroni tests. For the $p<0.01$ the results were highly significant, for the $p<0.05$ – significant, and for the $p>0.05$ – not significant, respectively.

3. RESULTS AND DISCUSSION

A primary indicator for the surface wettability of spongious matrices was considered the drop shape, recorded with a digital camera, corresponding to the contact angle value, as presented in Figure1.

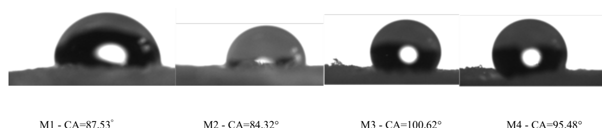

Figure 1. Images of the drops shape for the spongious matrices and the associated contact angle values.

The contact angle values are in the range 84.32 to100.62°, being strongly influenced by the way of drug incorporation. The highest values of CA were reported for M3 and M4collagen sponges with the drugincorporated in both forms (free and encapsulated), while for M1 and M2 sampleswith drug incorporated only in encapsulated form the CA was smaller, the decrease being about 1.09-1.19 times. It seems that the presence of the FA in free form in the designed multiparticulate systems led to a decrease of the sponge surface hydrophilicity. On the other hand, a smaller amount of microcapsules (M2 and M4) determined a decrease of CA values, corresponding to a higher spongious matrices surface wettability. The ability of water up-take of collagen matrices with free and encapsulated FA was determined. The swelling degree after 2 and 72 hours is presented in Figure 2. The matrices M1 and M2 presented higher up-take ability after 2 and 72 hours. The matrices with higher content of flufenamic acid (M3 and M4) (both free and encapsulated) are more hydrophobic than the others (M1 and M2). The results of water up-take are in correlation with contact angles values and show that both drug and amount of microcapsules influence the wettability properties of spongious matrices.

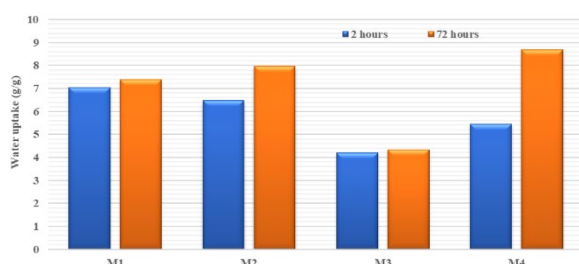

Figure 2. Water up-take for spongious matrices.

Enzymatic degradation of matrices was tested at different time intervals. All matrices presented good biodegradability, losing less than 20% mass in 24 hours (Figure 3).

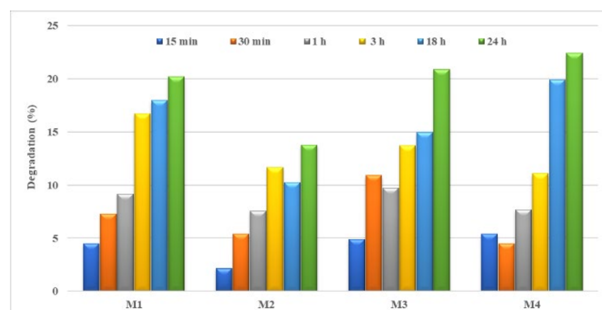

Figure 3. Enzymatic degradation of spongious matrices.

The most degradable matrix was M1 with lowest amout of flufenamic acid and without microcapsules. After 72 hours the matrices with flufenamic acid in free form were totally degraded and the ones with microcapsule content were degraded about 70% during same time.

The *in vitro* flufenamic acid kinetic profiles from collagen spongious matrices were built as drug cumulative released (%) as a function of time (Figure 4).

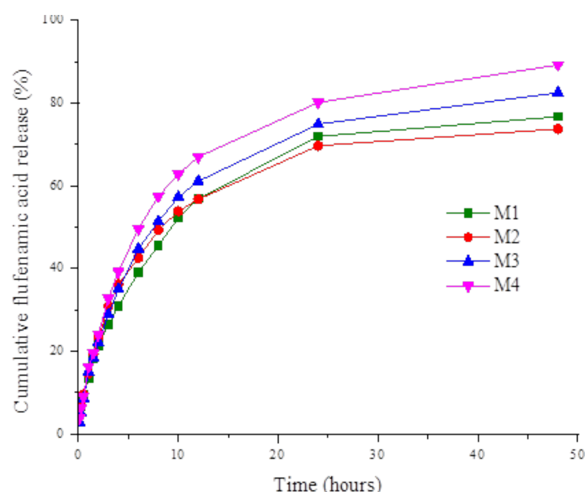

Figure 4. Time-dependent cumulative release patterns of flufenamic acid from spongious matrices collagen-based.

As seen in Figure 4, comparable kinetic profiles for all delivery systems with FA incorporated in various forms were obtained, and described by two stages: in the first hour, the initial drug released was between 13.52% (M1) and 16.37% (M4), followed by a gradual and slower release for a prolonged period up to 48hours.The cumulative flufenamic acid released percentage after 48 hours has varied between 76.77% (M1) and 89.22% (M4) (Table 3). It can be noticed that the samples with FA incorporated in free and encapsulated forms presented a higher burst release due to the presence of drug available in free form, near to the surface and delivered through desorption. After 48 hours, the released drug percentage

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for the sponges with drug incorporated in both forms is higher compared to sponges with the drug presented only in encapsulated form, the increase being about 1.07-1.20 times.The kinetic data are in line with sponges surface wettability (contact angles values around 90º or higher than 90º) and absorption capacity, respectively. As we underlined in our previous works,[21,26] this kinetic behaviour is desired for the local inflammation control as well as for monitoring the pain associated to a cutaneous wound lesion taking into account that the first 12-48 hours are important inhealing process of a wound. The experimental data were fitted with different kinetic models, obtaining the highest correlation coefficient (R) for the the Power law model. The respect of this model indicates a non-Fickiandrug transport mechanism, the release exponent being between 0.37 and 0.40.The parameters specific to this model (the kinetic constant and the release exponent) are listed in Table 3.

Table 3. Correlation coefficients for drug delivery from spongious matrices collagen-based obtained by application of various kinetic models; kinetic parameters specific to Power law model; drug released (%).

Collagen spongious matrices	Higuchi model	Zero-order model	Power law model	Release exponent	Kinetic constant ($1/min^n$)	Drug released (%)
M1	0.9684	0.8420	0.9805	0.40	0.036	76.77
M2	0.9523	0.8051	0.9750	0.37	0.045	73.79
M3	0.9630	0.8289	0.9767	0.39	0.039	82.55
M4	0.9590	0.8211	0.9747	0.39	0.044	89.22

3.1. The effect of collagen formulations treatment on the healing of induced wounds, using the animal model

In the case of treatment with collagen sponges (Figure 5, Figure 6, Figure 7 and Table 4), we observed that when applying spongious matrices M3 and M4 (drugincorporated in both forms), the lesiondiameter was maintained constant during the first 3 days of treatment. Starting from day 5, a significant decrease ($p<0.01$) of approximately 30% in lesion diameter was recorded in the case of the M4 multiparticulated system, followed by formulation M2 (flufenamic acid incorporated only in encapsulated form) with a 10% decrease of diameter compared to first day of treatment. From the applied treatments, the M4 formulation (flufenamic acid incorporated in the collagen spongious matrices both in free and encapsulated form) presentedthe most favorable dynamics of the healing process in the induced lesions throughout the 17 days of monitoring. The results, in this case, were statistically significant in the first 10 days compared to control group, not treated ($p<0.05$).

The physical-chemical, biopharmaceutical and preclinical studies involving animal models obtained in the present work are in line with the ones presented in our previous studies for similar spongious matrices but with different concentrations of polymers and cross-linking agent, conducting to flufenamic acid controlled delivery systems which proved that the healing processes can be completed using the designed multiparticulate formulations.[21,26]

Table 4. The evolution of lesion (mm) diameter in experimentally induced woundsafter the treatment with collagen formulations.

Collagen Sponges	Day 1	Day 3	Day 5	Day 7	Day 10	Day 12	Day 14	Day 17
	Mean±DS	Mean±DS	Mean±DS	Mean±DS	Mean±DS	Mean±DS	Mean±DS	Mean±DS
M1	10.00±0.00	10.75±0.50	10.5±0.60	8.50±0.60	6.75±1.00	4.00±2.80	0.50±0.60	0.00±0.00
M2	10.00±0.00	10.83±0.25	9.00±0.25	7.75±1.00	5.75±1.00	4.00±0.80	0.75±1.50	0.00±0.00
M3	10.00±0.00	10.00±0.80	10.25±1.30	7.25±1.30	5.50±0.60	4.00±0.80	0.25±0.50	0.00±0.00
M4	10.00±0.00	9.86±0.40	7.00±2.20	5.33±1.00	3.14±1.90	1.71±1.70	0.29±0.50	0.00±0.00
Control	10.00±0.00	11.75±0.40	10.75±0.40	8.67±1.20	7.83±1.10	5.50±0.50	2.00±2.00	1.00±1.40
ANOVA (p)	NS	p<0.01	p<0.01	p<0.01	p<0.01	p<0.05	NS	NS

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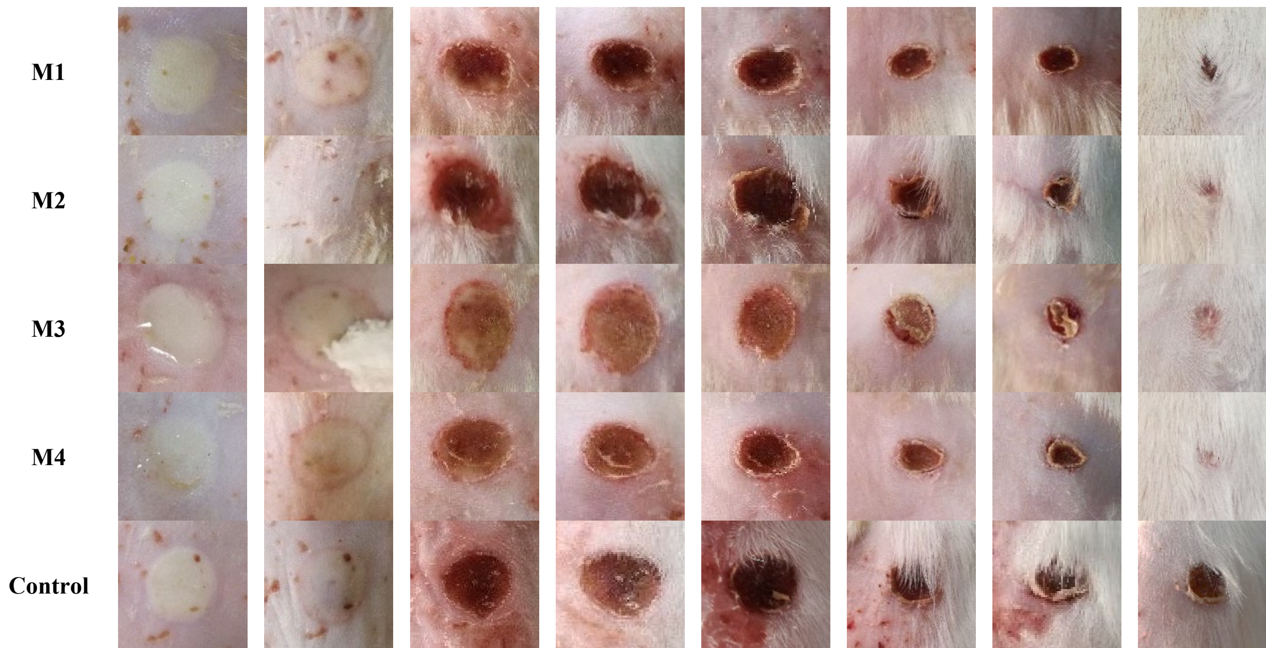

Figure 5. The healing process evolution for the induced wounds treated with collagenic sponges based on flufenamic acid in comparison with control group (not treated).

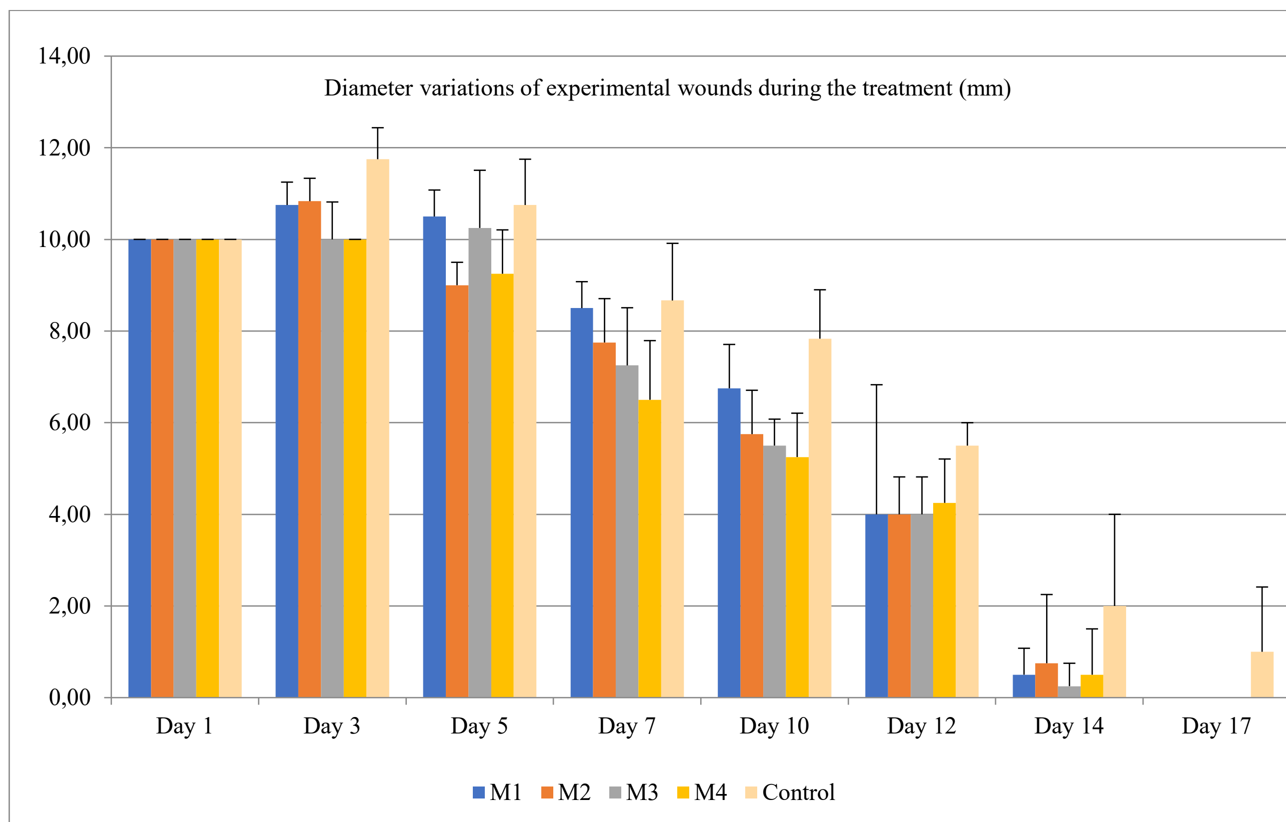

Figure 6. The diameter evolution of experimentally induced wound on animals after the treatment with collagenic formulations based on flufenamic acid (M1-M4) and control group.

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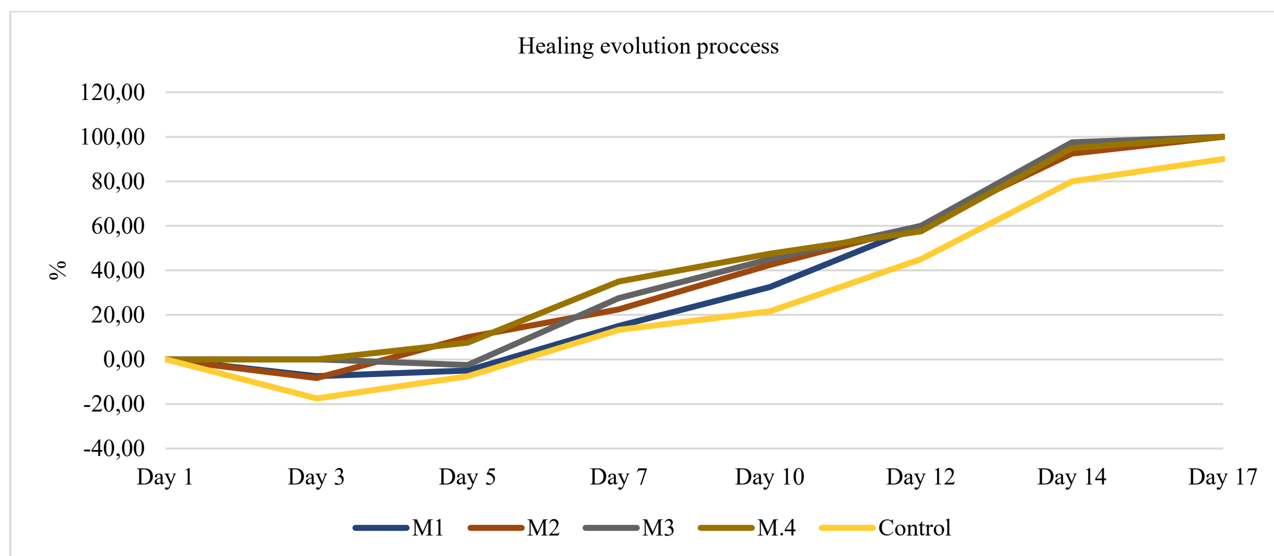

Figure 7. The healing process dynamics after the treatment with collagen formulations (M1-M4) and control group (not treated) in wounds induced on animals.

4. CONCLUSIONS

Drug delivery systems based on polymeric supports consisting in collagen and dextran, and flufenamic acid as drug were developed to be used in different skin lesions. To perform a controlled drug release, the flufenamic acid was presented both free in polymeric support and encapsulated. The microcapsules with flufenamic acid were embedded in spongious matrices in order to be slowly released for a longer period of time. Both water uptake and contact angle studies showed that the drug incorporated in both forms (free and encapsulated) influences the properties of spongious matrices, making them more hydrophobic. The stability to collagenase showed a slow biodegradation, of less than 20% mass loss in 24 hours. The release profiles indicated a non-Fickiandrug transport mechanism. The kinetic patterns obtained are suitable for the local inflammationand pain control specific to a cutaneous wound.The presence of flufenamic acid both in free and encapsulated form, respectively only in encapsulated form in the polymer matrix demonstrated a positive effect on the evolution of the healing process from the first days without topical and systemic side effects. The use in burnstreatment justified by the *in vivo* results of multiparticulate systems with anti-inflammatory drug showed significant pharmacological effects and no side effects with the acceleration of the healing process in case of medium severity burns.

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Conflict of interests

I declares that there is no a conflict of interest with any institute, person, company, etc.

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