Hybrid Collagen-NaCMC Matrices Loaded with Mefenamic Acid for Wound Healing

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Fire and burns represent the fourth cause of death in the world. Numerous options for dressings exist, but their selection should be based on several factors such as burn severity, wound location and water retention. Collagen (COLL) is the most common protein in the human body and, due to its biocompatibility, is the main component in biomaterials development. Mefenamic acid (MA) is a non-steroidal anti-inflammatory drug with analgesic properties, and carboxymethylcellulose (NaCMC) is a biocompatible and biodegradable polymer that is commonly used in biomedical field. Collagen - carboxymethylcellulose - mefenamic acid hydrogels, developed in order to be used in burn treatments were lyophilized and the corresponding spongious matrices were investigated by optical microscopy, FT-IR spectroscopy, water absorption, enzymatic degradation and drug release kinetics studies. All tests revealed proper morphological structure, favourable release patterns, convenient swelling capacity and degradation profiles, indicating the possibility of their use for medical applications.

Keywords: collagen, mefenamic acid, carboxymethylcellulose, spongious matrices

Fire and burns represent the fourth cause of death in the world. Moreover, in United States, in 2012 more than 136,000 children, of which over 67,000 with age under 5, arrived in emergency rooms of hospitals with severe injuries caused by burns [1]. Numerous options for dressings exist, but their selection should be based on several factors such as burn severity, wound location and water retention [2]. Materials used for dressings development must fulfil some prerequisites for a faster healing, namely: to maintain local humidity, to protect the wound against infection, to absorb fluids and wound exudates, to minimize surface necrosis, to prevent wound drying and to be flexible, non-toxic, non-allergenic, biocompatible and biodegradable [3].

A wound appears when a part of the body is damaged by different sources. The local response to injury generated by living organisms is inflammation and it appears in order to limit or eliminate the injurious agent [4, 5]. The dermal wound healing starts by progressive increase of biomechanical strength of the tissue with the aid of biomaterials [6].

Collagen is the most common protein in the human body and has the ability to take over the biological tissue function and shape. Due to its biocompatibility collagen is the main component in biomaterials development for applications such as dressings, devices for tissue engineering like scaffolds or controlled delivery systems [7, 8].

Wounds from various sources are a real opportunity for the microorganisms invasion that could cause infections, causing a slower healing. Inflammatory agents increase vascular permeability leading to fibrin matrices generation and causing exudates formation and redness. In such cases an anti-inflammatory drug can be orally administered. But the best option is the development of bioactive dressings that can be used topically for wound healing, containing active substances that will facilitate and promote healing, **inhibit infections** by eliminating pathogens and antibiotics release [9].

Mefenamic acid (MA) is a nonsteroidal anti-inflammatory drug with analgesic properties. It is beneficial for premenstrual syndrome, pain syndrome, musculoskeletal injury, back pain, and is generally used to treat moderate pain. It can be easily used in wound dressings in order to reduce inflammation generated by different skin lesions [10].

Carboxymethylcellulose (NaCMC) is a biocompatible and biodegradable polymer commonly used in biomedical field. The main characteristics of NaCMC are, firstly, the high water uptake and secondly, the high compatibility with the skin [11]. It has the capability to maintain a moist environment to the wound, helping in this way the extracellular matrix formation and re-epithelialization [12].

An interesting study subject is the use of these materials in the wound healing after extensive surgery, especially in the oncologic head and neck surgery after radiotherapy. When surgery is performed for malignant tumors and the body lacks the proper means of healing, the wound will behave as if burned. This is especially true for patients that are diagnosed in late stages, their general state of health already affected and the tumor of large dimensions. Although we have extremely sharp diagnosis tools, such as videocontact endoscopy, narrow band imaging or different staining tests that facilitate early diagnosis [13-15], unfortunately many of these patients still present with a locally advanced disease. In such patients, any aid in wound healing could be extremely valuable, but we must still further investigate the use of such substances in oncologic patients. Also, in such cases we always advice to investigate further, as far as the cells are concerned, as

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this influences greatly the prognosis [16] and implicitly the capacity of the body to heal.

The aim of this study was to develop and to characterize some collagen - carboxymethylcellulose - mefenamic acid matrices usable in burn treatments.

Experimental part

Materials and methods

Type I fibrillar collagen gel (COLL) having a concentration of 2.37% (w/w) was extracted from calf hide using the technology developed at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department [17]. Carboxymethylcellulose (NaCMC), was purchased from Fluka, glutaraldehyde (GA) from Merck (Germany) and mefenamic acid from MP Biomedicals (USA).

The collagen gel with the initial concentration of 2.37% and acid pH was adjusted using 1M sodium hydroxide at *p*H 7.3 for a better biocompatibility. The final concentration of collagen gel used was 1% (w/v). NaCMC was then added in a concentration of 2% and MA in a concentration of 0.5%, according to the compositions shown in table 1. For cross-linking 0.0025% glutaraldehyde solution was added in all samples.

In order to be analysed, the collagen gels were freeze-dried using a Delta 2-24 LSC (Martin Christ, Germany) lyophilizer, using a 48 hours lyophilisation program, presented in figure 1.

Table 1
COMPOSITION OF COLLAGEN HYDROGELS

	COLL, %	NaCMC, %	MA, %	GA, %
CC1	100	-	-	0.0025
CC2	85	15	-	
CC3	70	30	-	
CC4	55	45	-	
CCM5	100	-	0.5	
CCM6	85	15	0.5	
CCM7	70	30	0.5	
CCM8	55	45	0.5	

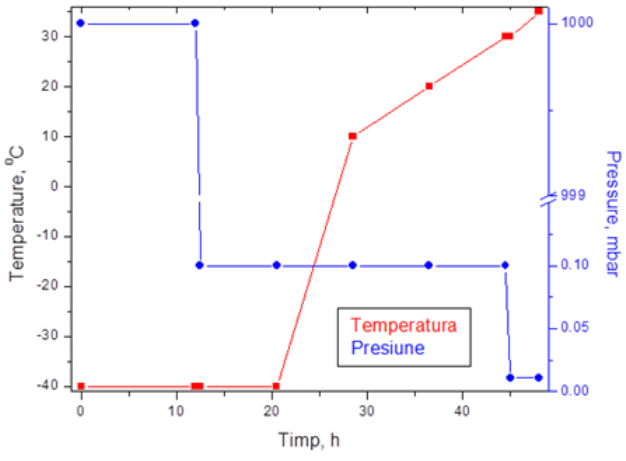

Fig. 1. Freeze - drying process graph chart process

Water absorption

In order to determine the water absorption, collagen matrices were firstly immersed in water. At scheduled time intervals, the samples were withdrawn and weighed. The water absorption was calculated using the following equation:

$$\%\ Water\ up\text{-}take = (Wt - Wd)/Wd\ (g/g) \quad (1)$$

where Wd denotes the weight of the dry samples and Wt denotes the weight of the swollen samples at immersion time t.

Enzymatic degradation

Enzymatic degradation of collagen scaffolds was investigated by monitoring the weight loss depending on exposure time to collagenase solution. At regular time intervals, the swollen scaffolds were withdrawn and weighed. The percentage of hydrogel degradation was determined by the following relation:

$$\%\ Weight\ loss = (Wi - Wt)/Wi * 100\ (g/g) \quad (2)$$

where Wi is the initial weight and Wt is the weight after time t.

FTIR-ATR analysis

FT-IR spectral measurements were recorded by spectrophotometer Jasco FT/IR-4200. All spectra were recorded at the following parameters: spectral range 4000-600 cm^{-1}, resolution 4 cm^{-1} with 30 acquisitions per each sample.

Optical microscopy

All images were captured with a Leica Stereomicroscope model S8AP0, 20-160x magnification capacity. For better evaluation of the samples, a 20x magnification and incident external cold light were used and optical images were obtained.

Drug release kinetics study

The *in vitro* release of mefenamic acid from the designed spongious matrices was conducted with a transdermal sandwich device adapted to a paddle dissolution equipment (Essa Dissolver). The protocol was detailed in our previous works [17, 19]. The amount of mefenamic acid released in the receiving medium (phosphate buffer solution, *p*H 7.4) at predetermined time intervals during 12 h was spectrophotometrically assessed to a wavelenght of 285 nm (Perkin-Elmer UV-Vis Spectrophotometer), using the calibration curve ($A_{1\%}^{1cm} = 414$, $R^2 = 0.9993$) (fig. 2).

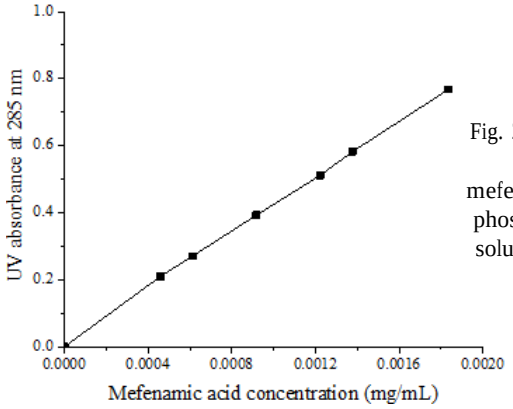

Fig. 2. Calibration curve of mefenamic acid in phosphate buffer solution (*p*H 7.4)

The experimental kinetic data were fitted using the general equation of Power law model (eq. 3) and its particular case, Higuchi model (eq. 4):

$$\frac{m_t}{m_\infty} = k \cdot t^n \quad (3)$$

$$\frac{m_t}{m_\infty} = k \cdot t^{0.5} \quad (4)$$

where m_t/m_∞ represents the fraction of drug released at time t, k - the kinetic constant, n - the release exponent characteristic for the drug release mechanism.

Results and discussions

After lyophilisation all the samples were tested from different points of views. The first analysis performed was the water absorption capacity in order to correlate the results with the matrices exudates absorbance properties. It is very important to maintain the wound clean and moisturized for a proper and faster healing. Therefore the results of water uptake test are presented in figure 3.

From figure 2 it can be noticed that the samples with mefenamic acid absorbed a higher amount of water compared to the ones where the drug is not present. The sample CCM5, containing only collagen and mefenamic acid recorded a swelling capacity around 45 g/g, which is normal due to the porosity of collagen matrix. By addition of NaCMC the samples become denser compared to those without synthetic polymer, the highest amount of water absorbed being around the value of 43 g/g in case of CCM8 sample, containing 45% NaCMC and 55% collagen. If only the samples with collagen and NaCMC are compared, it can be remarked that the samples become denser by lowering the amount of carboxymethylcellulose, therefore the samples with the lowest amount, namely CC2 and CCM6, recorded a swelling capacity around 27 g/g. The samples CC2, CC3 and CC4, without drug, showed almost the same swelling capacity, so it can be concluded that this property is influenced by the presence of mefenamic acid.

After the water absorption capacity analysis, the samples were immersed in collagenase solution in order to test their degradation capacity, the results being presented in figure 4.

The samples with mefenamic acid recorded a weight loss higher than the ones without drug. In the same time, the percentage of weight lost is higher for a higher amount of NaCMC. It can be noticed that the sample CC4, consisting of 55% collagen and 45% carboxy-methyl-cellulose, completely degraded after 48 h and also the same sample CCM8 with mefenamic acid recorded a weight loss around 75%.

The spectroscopy analysis results are presented in fig.5.

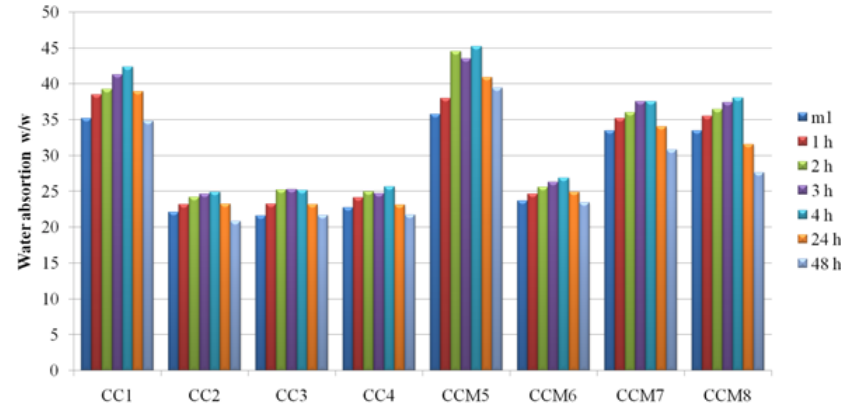

Fig. 3. Water uptake capacity for samples obtained

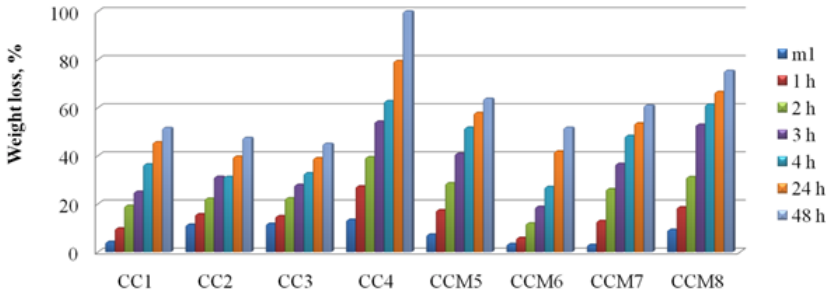

Fig. 4. Weight loss recorded in collagenase solution

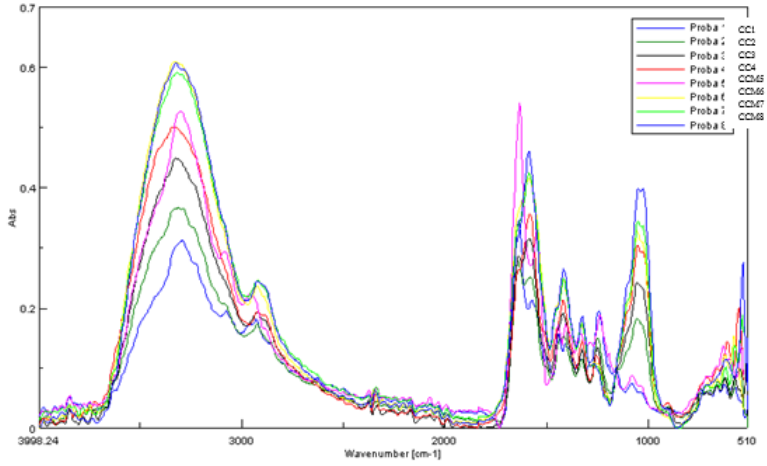

Fig. 5. FT-IR spectra of spongious matrices

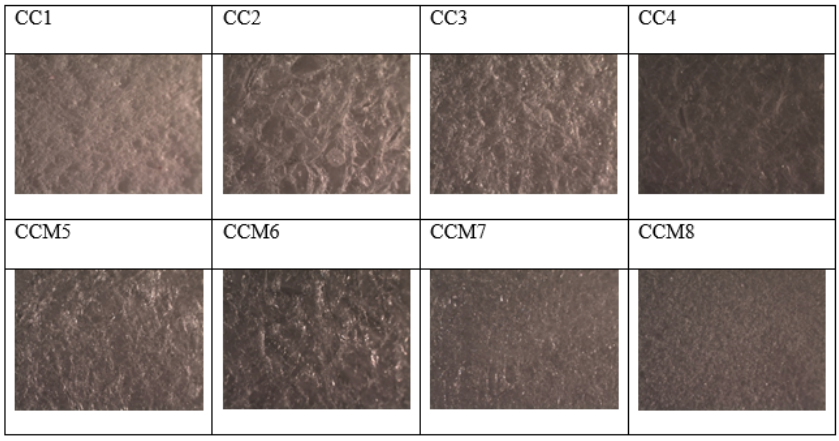

Fig. 6. Optical microscopy images for obtained samples (x20).

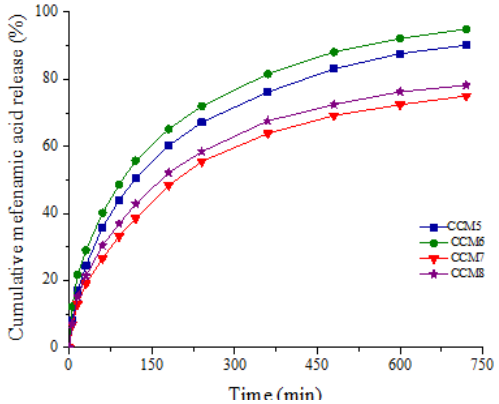

Fig. 7. Cumulative release patterns of mefenamic acid from the designed matrices as a function of time

Table 2.
DETERMINATION COEFFICIENTS FOR MEFENAMIC ACID RELEASE FROM THE DESIGNED COMPOSITES DETERMINED BY APPLICATION OF HIGUCHI AND POWER LAW MODELS; KINETIC PARAMETERS SPECIFIC TO POWER LAW MODEL; DRUG RELEASED PERCENT AFTER 12 h

Composites	Kinetic constant ($1/min^n$)	Release exponent	Determination coefficient		Drug released percent (%)
			ower law model	Higuchi model	
CCM1	0.074	0.38	0.9863	0.9678	90.17
CCM2	0.094	0.36	0.9890	0.9613	95.01
CCM3	0.052	0.41	0.9865	0.9760	75.05
CCM4	0.063	0.39	0.9861	0.9694	78.27

From figure 5 it can be noticed that the spectrum of collagen matrix showed typical amide bands of proteins i.e. 3299 cm^{-1} and 2928 cm^{-1} for amide A and B respectively, 1636 cm^{-1} was ascribed to amide I (C=O stretching), 1570 cm^{-1} to amide II (N-H deformation) and 1242 cm^{-1} to amide III (N-H deformation) [17,19]. When mefenamic acid was added, amide II shifted to 1548 cm^{-1} maybe because of its interaction with collagen**.** Also, the specific peaks for NaCMC are present in FT-IR spectra: 1582 cm^{-1} (stretching vibration of carboxylate group, 2925 cm^{-1} (C–H stretching vibration), 1420 cm^{-1} (–CH_2 scissoring vibration), 1324 cm^{-1} (–OH bending vibration), 1050 cm^{-1}, (>CH–O–CH_2 stretching vibrations), respectively.

The morphological structure was also investigated by optical microscopy and the corresponding images are presented in figure 6.

From optic microscopy images it can be observed firstly, the specific structure of collagen, with interconnected pores with sizes around 200 nm, and secondly, a denser structure with NaCMC amount increase. For samples CC4 and CCM8, containing the highest amount of carboxymethylcelullose, the pore size is smaller than for the other samples.

The kinetic profiles presented in figure 7 highlighted the influence of collagen:NaCMC ratio on mefenamic acid release from the spongious matrices.

The kinetic profiles in figure 7 presented a similar shape with an initial rapid drug release stage, followed by a gradual release. Thus, in the first 120 min the drug released percent varied between 38.50% (CCM7) and 55.74% (CCM6). In the next 10 h of experiment, the cumulative mefenamic acid release ranged between 75.05% (CCM7) and 95.01% (CCM6).

It can be seen that the drug released percent depends on the various collagen:NaCMC ratios (table 2). Thus, a collagen:NaCMC ratio of 85:15 (CCM6) leads to a higher drug released percent after 12 h of experiments, about 5% higher comparing to the sample without NaCMC (CCM5),

while the composite with a 70:30 ratio conducts to the smallest drug release percentage (CCM7).

The drug fast release is important for reducing the inflammation and pain associated to a cutaneous lesion, while the gradual and sustained release ensures a protective anti-inflammatory and analgesic action for a longer period of time.

Applying Power law and Higuchi kinetic models, mentioned in *Materials and Methods Section*, to the release data, the drug mass transfer mechanism was determined. The determination coefficients characteristic for the above models are given in table 2.

We can see that the highest values for the determination coefficients were obtained for Power law model, indicating a non-Fickian drug release mechanism from collagen composites. The kinetic parameters specific to Power law model are listed in table 2.

In another paper were studied the biochemical effects of collagen suports-coated with stem cells on experimental skin wound healing [20].

Conclusions

Considering that sample CCM6 presents favourable release patterns, being able to release 95% from the drug quantity bound in the matrix, and the fact that swelling capacity and degradation profiles were also convenient, we propose CCM6 sample for future analysis, such as *in vitro* and *in vivo* testing in order to determine its capacity of burns healing.

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