

SPONGIOUS COLLAGEN-MINOCYCLINE DELIVERY SYSTEMS

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Abstract

The purpose of the present work was to develop and characterize some spongy delivery systems based on collagen and minocycline, uncross-linked and cross-linked with glutaraldehyde, obtained at different freezing temperatures during lyophilization. The prepared sponges were analysed by scanning electron microscopy and swelling ability. The minocycline release from collagen spongy systems was investigated and the kinetic mechanism was determined. The results of this study indicated that the sponges porous architecture, their swelling and also the percentage of drug released are influenced by both sponges composition and their obtaining process.

Rezumat

Scopul prezentei lucrări a fost dezvoltarea și caracterizarea unor sisteme de cedare spongioase pe bază de colagen și minociclină, nereticulate și reticulate cu glutaraldehidă, obținute la diferite temperaturi de înghețare în timpul liofilizării. Bureții spongioși preparați au fost analizați prin microscopie electronică de scanare și capacitatea de absorbție. A fost evaluată cedarea minociclinei din formele spongioase colagenice și s-a determinat mecanismul cinetic. Rezultatele din acest studiu au arătat că arhitectura poroasă a bureților spongioși, capacitatea lor de gonflare și de asemenea procentul cedat de substanță medicamentoasă sunt influențate atât de compoziția bureților spongioși cât și de procesul de obținere al acestora.

Keywords: collagen, minocycline, spongy delivery systems, freeze-drying

Introduction

During the past two decades, researchers focused their activity to develop topical drug delivery systems usually based on polymers (either biopolymers or synthetic polymers) [1, 2]. Although there are many natural polymers and their synthetic analogues used as carriers for drug delivery, collagen is distinct mainly in its way of interaction with the body [3]. Collagen-based biomaterials offer several advantages: they are biocompatible and non-toxic and have well-known structural, physical, chemical, biological and immunological properties. Additionally, the drug release kinetics from collagen carriers can be influenced by the modification of the drug delivery system characteristics [4, 5]. The main applications of collagen as drug delivery system are films/membranes used in ophthalmology and dentistry [3], sponges (porous matrix) for burns/wounds [6], mini-pellets and tablets for protein delivery, gels/hydrogels for transdermal and local drug delivery [7], spongy composites for orthopaedy [8, 9], polymeric matrices for tissue engineering [10]. Among the collagen drug delivery systems, the spongy ones have been very useful in the treatment of damaged tissues because they

have the ability to easily absorb large amount of tissue exudates, smooth adherence to wet wound bed and rapid recovery due to intense penetration of cells into the sponge as experiments demonstrated [11]. Many methods including fiber bonding, solvent casting/particulate leaching, three-dimensional printing, gas forming, freeze-drying, and phase separation have been developed to generate porous 3D matrices from natural and synthetic polymers [12]. Freeze-drying is an important method compared to other drying techniques ensuring the long-term stability of liable pharmaceuticals [13]. Lyophilization, also known as freeze-drying, is an important and well-established process to improve the long-term stability of liable drugs, especially therapeutic proteins [14].

The main objective of this study was to prepare and characterize three-dimensional spongy (3D) drug delivery systems based on collagen and minocycline. The spongy systems with different concentration of minocycline and cross-linking agent, obtained at different freezing temperatures during lyophilization were characterized by scanning electron microscopy (SEM) and swelling capacity. The minocycline release from collagen

spongy forms was examined and the kinetic mechanism was discussed.

Materials and Methods

Materials

Type I collagen of bovine origin was extracted by the currently used technology in the Collagen Department of Division Leather and Footwear Research Institute as previously described [15]. The collagen (Coll) was obtained as gel in native form with fibrillar structure with an initial concentration of 2.54%, pH 2.6 and free of fat and ash. Minocycline hydrochloride (MH) was purchased from Sigma (Germany), glutaraldehyde (GA) was supplied by Sigma-Aldrich (Germany), sodium hydroxide, monobasic potassium phosphate and disodium hydrogen phosphate were purchased from Merck (Germany). All the chemicals used in this work were of analytical grade and the water was distilled.

Collagen-minocycline sponges preparation

Collagen gel – reference (Coll) – with initial concentration of 2.54% and pH 2.6 (adjusted to pH 7.4 with NaOH 1M solution under mechanical stirring) was the base material for obtaining the spongy drug delivery systems. Minocycline in different concentrations (0.2% and 0.4%) was embedded in the collagen gel and gel dispersions with 0.9% collagen (w/w) and pH 7.4 were obtained. Then, some of them were cross-linked with GA (0.0015%) at 4°C for 24 hours. The gel dispersions were named G1, G2 and G3 and had the following compositions: G1 – Coll : MH : GA = 0.9 : 0.2 : 0.0015, G2 – Coll : MH : GA = 0.9 : 0.4 : 0.0015 and G3 – Coll : MH : GA = 0.9 : 0.4 : 0.0000. The amounts of Coll, MH and GA are reported to 100 g gel. The sponges were obtained by freeze-drying of G1, G2 and G3 gel dispersions using a LSC Delta 2-24 freeze-dryer (Martin Christ, Germany), following the program presented in the chart from Figure 1.

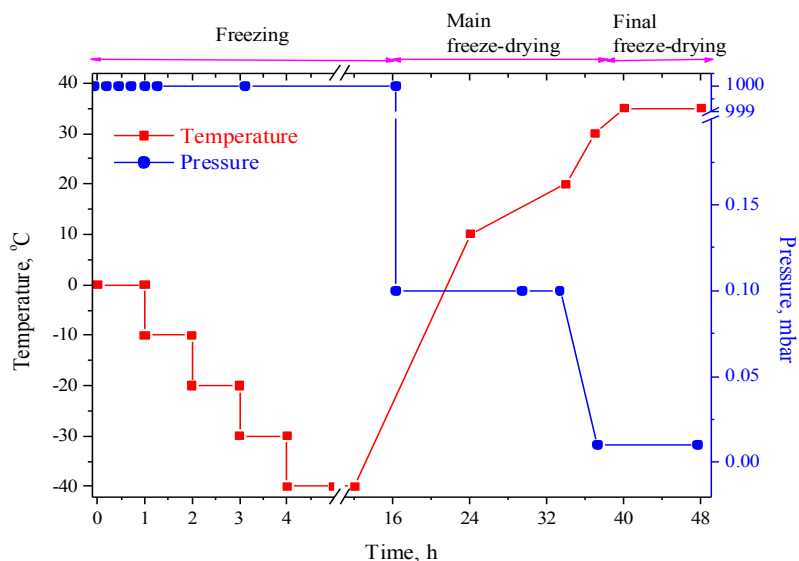


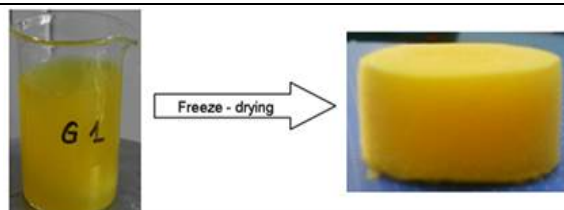
Figure 1.
Freeze-drying chart of spongy Coll-MH systems obtaining

The freezing step is of paramount importance with regard to the biological activity and stability of the active pharmaceutical ingredients and proteins [14]. The process started with freezing of each gel dispersion (G1, G2, G3) for one hour at the following temperatures: 0; -10; -20; -30 and -40°C. The temperature was maintained for an additional 8 hours after reaching -40°C. Ice morphology is directly correlated with the sublimation rate in primary and secondary drying [16]. So, this process continues with main freeze-drying at 0.12 mbar reaching +10°C in 12 hours, then the temperature

increased reaching 20°C during 10 hours, then 30°C during 3 hours and then 35°C during other 3 hours. The last step, final freeze-drying lasted for 8 hours at 0.01 mbar maintaining the same temperature, 35°C. After 48 hours of freeze-drying the sponges in form of disk having 3 cm diameter and 0.3 mm height were obtained from the gel dispersions.

The prepared sponges were named depending on the freezing temperature and their composition as we presented in Table I.

An example of the obtained sponge by freeze-drying of a gel dispersion is given in Figure 2.

**Figure 2.**

Spongy Coll-MH system (M1-40) after freeze-drying of gel dispersion (GI)

Table I.

The code of spongy Coll-MH systems (sponges)

Freezing temp. during freeze-drying, °C	Composition of gel dispersions	Code of obtained sponges	Composition of gel dispersions	Code of obtained sponges	Composition of gel dispersions	Code of obtained sponges
0	G1	M1-0	G2	M2-0	G3	M3-0
-10		M1-10		M2-10		M3-10
-20		M1-20		M2-20		M3-20
-30		M1-30		M2-30		M3-30
-40		M1-40		M2-40		M3-40

Sponges swelling evaluation

In order to determine the swelling capacity, the sponges were studied using a conventional gravimetric procedure [17]. The swelling behaviour of the collagen-minocycline systems was determined by immersing samples in the absorption medium represented by phosphate buffer pH 7.4 at 37°C. At scheduled time intervals, the samples were withdrawn, wiped (to remove the liquid excess from the surface) and weighed in the swollen state [18]. The experiments were repeated three times under the same conditions and average values were reported. The swelling ratio (SR) was calculated using the following equation (eq.1):

$$SR = \frac{W_t - W_0}{W_0} \quad (1),$$

where W_t is the weight of the swollen samples at immersion time t , and W_0 is the weight of the dry samples.

Morphological characterization

The morphology of the spongy delivery systems was observed using a Hitachi S-2600N scanning electron microscope with EDX spindle and a resolution of up to 4 nm (at 25 kV, in high vacuum when secondary electron detector was used) and a 15 – 300 x magnification (accelerating voltage from 0.5 kV to 30 kV).

In vitro drug release studies

The release behaviour of MH from uncross- and cross-linked collagen sponges was determined in triplicate at 37°C±0.5°C using a sandwich device adapted to a paddle dissolution apparatus (Essa Dissolver, Italy). The stirring paddle was rotated with a speed of 50 rpm. The phosphate buffer having the pH of 7.4 was used as release medium.

Aliquots of 5 mL were collected at different times from the release medium, which was completed with the same volume of fresh pre-heated phosphate buffer. The samples were spectrophotometrically analysed at 348 nm (Perkin-Elmer UV-Vis spectrophotometer), using the calibration curve previously determined [7]. Each kinetic experiment was performed in triplicate and the results are presented as mean.

Results and Discussion

The collagen and drug concentrations, cross-linking density, porosity, freeze-drying process etc. are among various deciding factors that can be tailored to regulate transport of drug/bioactive molecules from three dimensional matrices [1, 11].

The fluid absorption ability of sponges is essential in functional tissue regeneration and affects not only its morphology and structure but also the release of bioactive components into solvent [19]. As the sponge absorbs an increased amount of fluid and swells more, the drug will diffuse more easily.

All the studied spongy Coll-MH systems presented in Table I imbibed phosphate buffer more rapidly at the initial stage (the first several minutes) reaching equilibrium within 4 hours. Figure 3a-c presents the swelling ratio after 4 hours for the designed sponges.

The swelling ratio was influenced by the freezing temperature during the obtaining process, decreasing with the freezing temperature (Figure 3a-c) being also strongly influenced by both MH concentration and cross-linking agent. A lower amount of water was loaded for the cross-linked samples (M2 series) than for the uncross-linked one (M3 series). Comparing the sponges with different

percentage of MH (M1 and M2 series) we can observe that the higher amount of drug leads to higher water absorption which is due to the minocycline content, a drug with hydrophilic properties. For example, as Figure 3d shows, M3-30 sponge (uncross-linked and with maximum MH

concentration) absorbed 44.50 g/g liquid during 4h, while M2-30 sponge (cross-linked and with the same MH concentration) up took 42.35 g/g. Also, the sponge M1-30 (cross-linked and with a lower MH concentration) up took 37.54 g/g, recording a decrease of about 11.35% compared to M2-30.

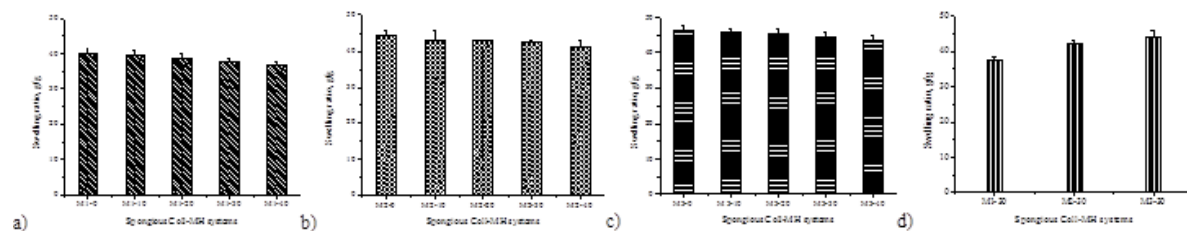


Figure 3.

Swelling ratio for sponges: a) M1 series, b) M2 series, c) M3 series and d) M1-30, M2-30 and M3-30

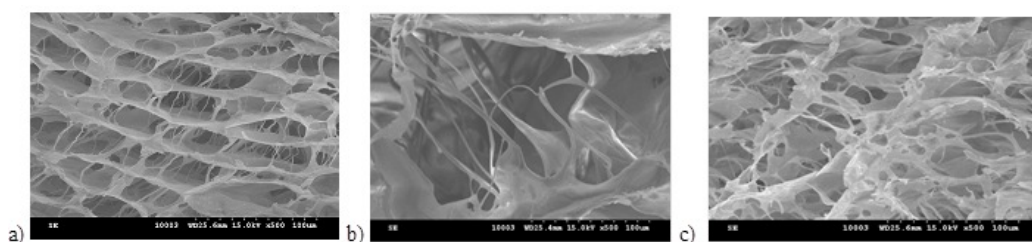


Figure 4.

SEM images for sponges: a) M3-40; b) M3-0 and c) M2-40

The pore sizes depend on the growth rate of ice crystals during the freeze-drying process which is in correlation with the freezing temperature during this process. The spongy Coll-MH systems show specific structure with different pore sizes as Figure 4 shows. Generally, according to the literature, a porosity higher than 90% is specific to the porous structures [20].

Figure 4a presents the M3-40 sponge obtained at -40°C with pore sizes between 10-50 µm, while the M3-0 sponge with the same composition, but frozen at 0°C during freeze-drying has pores larger than 100 µm (Figure 4b). The porous structure was also influenced by the cross-linking agent, as the Figure 4c shows. Thus, although the pores are interconnected for both sponges M3-40 and M2-40, the cross-linked sponge M2-40 shows a denser structure with thick walls pores. The morphologies of sponges are in good correlation with the swelling analyses.

The influence of freeze-drying process as well as of the sponges composition on the drug kinetic release were then analysed.

The percentages of minocycline released from the studied sponges are presented in Figures 5a-d. According to Figure 5 we can observe that similar profiles are recorded for matrices having different compositions, noting for all formulations a burst release effect which is more evident for an increased freeze-drying temperature. This burst release was followed by a period where the drug was released more slowly until a plateau was reached after 240 minutes of experiment, obvious in the case of sponges prepared at the freeze-drying temperatures of 0, -10 and -20°C respectively. For this reason we consider a release period of 4h for all sponges in order to compare the kinetic results obtained. The cumulative MH release percentage after 4 hours varied between 83.73% (M1-40) and 99.05% (M3-0) as shown in Table II.

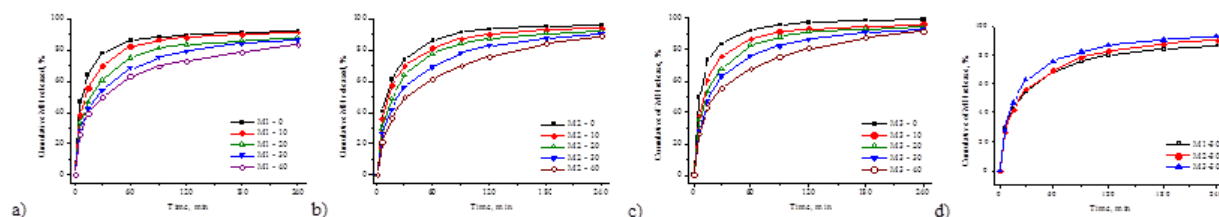


Figure 5.

Cumulative release of MH from sponges:

a) M1 series, b) M2 series, c) M3 series and d) M1-30, M2-30 and M3-30

Table II.

The correlation coefficients for MH release from collagen sponges using the Power law and Higuchi models; the kinetic parameters for the Power law model and the percentage of drug released

Sponges	Power law model	Higuchi model	Kinetic constant (1/min ⁿ)	Release exponent	Percentage released (%)
M1-0	0.9864	0.8376	0.433	0.149	92.16
M1-10	0.9855	0.8848	0.342	0.192	91.49
M1-20	0.9878	0.9161	0.273	0.225	87.78
M1-30	0.9926	0.9438	0.221	0.259	86.46
M1-40	0.9955	0.9547	0.194	0.272	83.73
M2-0	0.9854	0.8811	0.367	0.188	96.17
M2-10	0.9860	0.8964	0.328	0.204	93.87
M2-20	0.9850	0.9220	0.265	0.240	92.19
M2-30	0.9910	0.9497	0.211	0.276	90.30
M2-40	0.9955	0.9732	0.157	0.323	88.90
M3-0	0.9851	0.8281	0.480	0.143	99.05
M3-10	0.9804	0.8763	0.362	0.191	95.57
M3-20	0.9832	0.9086	0.298	0.224	94.38
M3-30	0.9866	0.9325	0.246	0.255	92.84
M3-40	0.9949	0.9578	0.203	0.282	91.58

Comparing the sponges with the same composition but prepared at different freezing temperature we can see that sponges obtained at -40°C released with 7.54%-9.15% MH less than sponges obtained at 0°C (Table II). This is in accordance with the sponges swelling and morphology: the lower freezing temperature results in smaller fluid uptake and smaller pore sizes and consequently in smaller amounts of MH released.

Analysing the release of minocycline from spongy systems obtained for the same freeze-drying parameter (freezing temperature: -30°C) we can see in Figure 5d that there are differences between them due to the cross-linking agent and drug content. Thus, at the end of 240 min the percentage of MH released is 86.46% for M1-30, 90.30% for M2-30 and 92.84% for M3-30. The uncross-linked sponge released the highest percentage of MH, while the lowest amounts were released from M1-30 which is a cross-linked sponge and contains 2 times less minocycline.

Although an increased freeze-drying temperature of the collagen gel dispersions leads to the obtaining of sponges with increased porosity and subsequently to a higher fluid absorption which determines a high burst release effect (desirable in the case of MH sponges for the prophylaxis against bacterial invasion), a progressive antibiotic release should however be ensured over a longer period of time in order to get a protective effect against the infected wounds. Consequently, lower freeze-drying temperatures are preferred for the preparation of spongy carriers for antibiotic drug delivery.

To determine the drug release mechanism from the spongy Coll-MH systems, the data presented in

Figure 5 were fitted according to the Power law model (eq.2) [21]:

$$\frac{m_t}{m_\infty} = k \cdot t^n \quad (2),$$

where m_t/m_∞ is the fraction of drug released at time t , k is the kinetic constant and n is the release exponent indicating the drug transport mechanism. The correlation coefficients and the values for the kinetic parameters specific to this above mentioned model are summarized in Table II.

The Power law model gained popularity in the last years for the assessment of the drug release from the formulations for which several processes are involved in the kinetic mechanism [22].

Thus, the release exponent varied between 0.143 and 0.323, showing a non-Fickian transport for MH delivery from sponges due to the complex processes that occur for the drug porous systems: fluid absorption through sponge pores followed by its swelling and transformation into gel and the drug diffusion through gel network. This complex mechanism associated with drug delivery from sponges explained the deviation from the Higuchi model (the correlation coefficients for this model are presented in Table II).

Conclusions

Minocycline-loaded collagen matrices, uncross-linked and cross-linked with glutaraldehyde were obtained by corresponding hydrogels lyophilization at different freezing temperatures.

The water uptake ability, the morphological characterization and the drug kinetic release studies were performed. The results of this study showed

that the sponges composition as well as their preparation process influenced the swelling behaviour, the released drug percentage and the porous architecture.

The kinetic mechanism was also investigated and correlated with the processes involved in the drug delivery from spongy forms.

The most suitable sponge for topical drug delivery in infected wound healing applications could be the one with 0.4% minocycline, uncross-linked or cross-linked with 0.0015%GA, obtained at a lower freezing temperature and further studies are recommended for the assessment of the *in vivo* efficacy.

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