COLLAGEN-DOXYCYCLINE SPONGIOUS FORMS FOR INFECTED TISSUES TREATMENT

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The purpose of the present work was to develop and characterize some spongious forms based on collagen and doxycycline, uncross-linked and cross-linked with glutaraldehyde, obtained by lyophilization. The prepared sponges were analyzed by FT-IR spectroscopy, water up-take and optical microscopy. The doxycycline release from collagen spongious forms was investigated and the kinetic mechanism was determined. The results of this paper indicated that the drug delivery is influenced by cross-linking degree and composition of spongious forms.

Keywords: collagen, doxycycline, drug delivery.

INTRODUCTION

Tissue infections can appear for different reasons, the most common being bacteria and medications (Christian *et al.*, 2007). The best way to effectively treat such an infection could be with antibiotics and pain medication (King, 2011).

Tissue regeneration approaches require a biocompatible material such as a scaffold to support cell proliferations as well as to deliver drugs needed for a proper recuperation. Many researchers study other alternatives such as natural polymers: chitosan, alginate or collagen, combined with ceramics for improved properties of obtained composite material (Oliveira *et al.*, 2010).

Among natural polymers, collagen is the most abundant protein in mammals and it is commonly extracted from bovine tissue. Collagen scaffolds are used in tissue regeneration, either in sponges, thin sheets (membrane) or gel / hydrogel forms (Albu *et al.*, 2011). Collagen has the proper properties for tissue regeneration such as pore structure, permeability, hydrophilicity and it is stable *in vivo*. Collagen scaffolds are also ideal for cells deposition, such as osteoblasts and fibroblasts and once inserted, growth is able to continue as normally as in the tissue (Trandafir *et al.*, 2007).

Collagen presents better biocompatibility and biodegradability when it is compared with other polymers and doxycycline is an antibiotic with both positive and negative spectra. The collagen-doxycycline spongious samples we prepared by lyopilization can be used for treatment or regeneration and prophylaxy of tissues that are damaged after an infection.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel having a concentration of 1.72% (w/w) was extracted from calf hide using technology currently available at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department (Albu, 2011). Doxycycline hyclate (DH) was purchased from Sigma-Aldrich, China. Sodium hydroxide and hydrochloric acid were of analytical grade. Type I collagenase obtained from *Clostridium histolyticum* was purchased from Sigma-Aldrich, Germany and glutaraldehyde (GA) from Merck (Germany).

Preparation of Collagen Scaffolds

The concentration of each collagen gel was adjusted at 1% and 7.3 pH using 1M sodium hydroxide (the pH of the physiological medium). 0.2% doxycycline hyclate was added to half of collagen gel (w/v), then the collagen gels were cross-linked with 0.25% glutaraldehyde (reported to collagen dry substance) as Table 1 presents.

Table 1. Composition and name of collagen gels

Code of gels	Col, %	DH, %	GA, %
Coll	1	0	0
Coll-R	1	0	0.25
Coll-DH	1	0.2	0
Coll-DH-R	1	0.2	0.25

The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) lyophilizer using the lyophilization program presented in Figure 1.

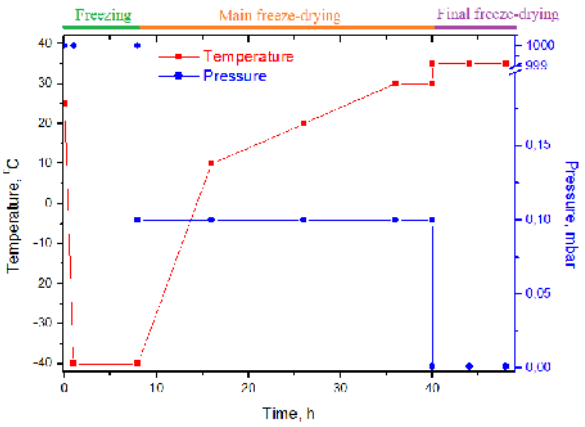

Figure 1. Graph chart of freeze-drying process

The resulting matrices were named as shown in Table 1.

FTIR-ATR Analysis

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

Water Absorption

In order to determine the water absorption, the scaffolds were first immersed in water at 36°C. At scheduled time intervals, the samples were withdrawn and weighed. The water absorption was calculated using the following equation:

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

where Wt denotes the weight of the swollen samples at immersion time t, and Wd denotes the weight of the dry samples. All the samples were studied in triplicate.

Optical Microscopy Study

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.

Doxycycline Hyclate *In Vitro* Release Kinetics Study

Doxycycline hyclate *in vitro* release evaluation from collagen sponges was conducted using a transdermal sandwich device adapted to a dissolution apparatus as previously described in our studies (Ghica *et al.*, 2013; Barbaresso *et al.*, 2014). In brief, doxycycline-collagen matrices un- and cross-linked with glutaraldehyde were fixed in the sandwich device and then immersed into the receiving medium (phosphate buffer of 7.4 pH, maintained at 37°C) from the release vessel. At different periods of time, samples of 5 mL were extracted from the release medium and replaced with an equal volume of fresh phosphate buffer solution, prewarmed at 37°C. The absorbances of the collected solutions were spectrophotometrically assessed at 347 nm, using a Perkin-Elmer UV-Vis spectrophotometer and the released doxycycline amount was evaluated based on the calibration curve, previously determined (Albu *et al.*, 2009). The Power law model was applied for the drug release kinetics investigation from collagen sponges (2).

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

where m_t/m_∞ represents the fractional release of drug at time t, k - the kinetic constant, n - the release exponent characteristic for the drug transport mechanism (Ghica *et al.*, 2013).

RESULTS AND DISCUSSION

After lyophilization the 3D porous collagen sponges based on collagen and doxycycline, cross-linked and uncross-linked, were obtained, with the appearance presented in Figure 2.

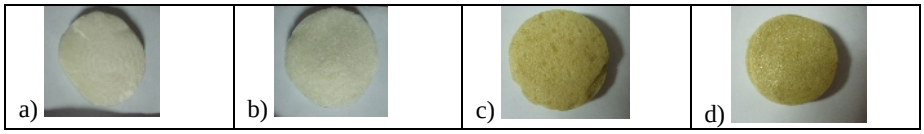

Figure 2. Collagen spongious forms: a) Coll; b) Coll-R; c) Coll-DH; d) Coll-DH-R

The samples from Table 1 were analysed by FT-IR spectroscopy, water absorbtion, optical microscopy and the samples with doxycycline were studied *in vitro* to establish the mechanism of drug release.

From the FT-IR spectra (Figure 3a) the typical bands from collagen can be observed: amide A, B, I, II and III (Albu, 2011).

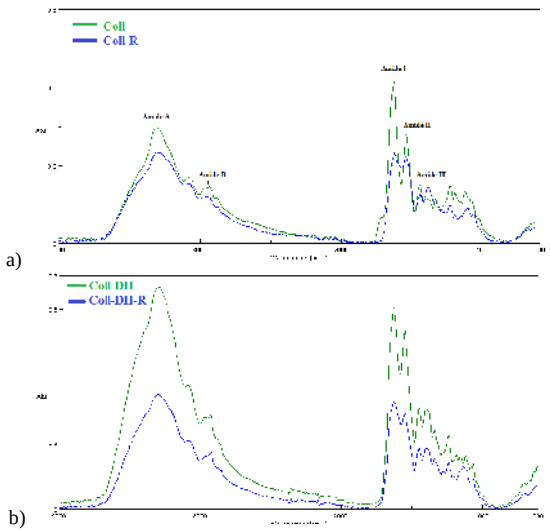

Figure 3. ATR-FTIR spectra of a) Coll and Coll-R; b) Coll-DH and Coll-DH-R

It is noticed that amide A shifts to lower wave number (from 3308 to 3305 cm^{-1}) to probably forming of hydrogen bonding. The amide B shift from 2937 to 2959 cm^{-1} when glutaraldehyde was added which indicates the cross-linking reaction. Moreover, this cross-linking is given by the ratio between areas of Coll and Coll-R. The lower area indicates the sample was cross-linked. There were no notable differences between wavenumbers of amides I, II and III, only between their intensities. Nevertheless, the triple helix of collagen kept its integrity, the value of A_{III}/A_{1451} being 1 for reference sample – Coll.

Comparing cross-linked and un-cross-linked reference collagen samples with ones with doxycycline we can notice that collagen kept his structure and the doxycycline presence are given by the following peaks: 1134, 1066, 1033 cm^{-1}.

The obtained sponges absorb an increased amount of fluid and swell, thus the drug will diffuse more easily.

The water up-take for all the studied samples is presented as kinetics during 72 hours in Figure 4.

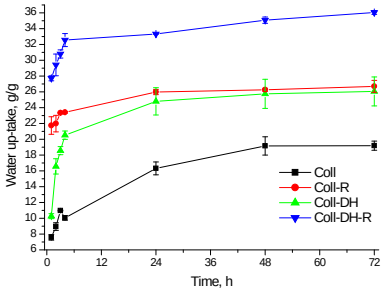

Figure 4. Kinetics of water up-take during 72 hours for spongious forms

Figure 4 presents the water up-take during 72 hours for the studied samples, swelling ratio after 4 hours for the designed sponges. The un-cross-linked samples absorbed a lower amount water than the cross-linked ones due to the porous structure formed during cross-linking. The spongious form with drug up-took a higher amount of water, doxycycline making them more hydrophilic. Thus, the spongious form Coll-DH-R which contains both DH and GA up-take about double amount of water compared with the reference one, Coll.

Optical image for a representative sample Coll-DH-R is shown in Figure 5.

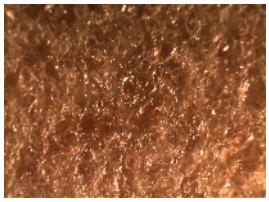

Figure 5. The optical microscopy image for Coll-DH-R

Figure 5 presents a denser structure with interconnected pores for Coll-DH-R.

The release kinetic profiles of doxycycline from un- and cross-linked collagen matrices were recorded as a function of time and presented in Figure 6. The released drug percent after 12 hours of experiment was about 1.3 times smaller in the case of cross-linked sponge due to the presence of cross-linking agent (Table 2).

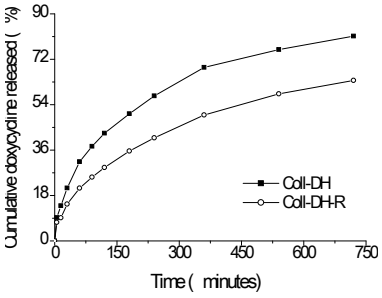

Figure 6. Cumulative release profiles of doxycycline from un- and cross-linked collagen sponges as a function of time

For the investigation of the kinetic mechanism the Power law equation was applied (eq. 2). The parameters specific to this model, the determination coefficient $(R)^2$ and the cumulative released drug percent are listed in Table 2.

Table 2. Values for determination coefficients and kinetic parameters specific to Power law model; released drug percent

Collagen sponges	Correlation coefficient	Kinetic constant ($1/min^n$)	Release exponent	Released percent (%)
Coll-DH	0.9904	0.059	0.405	81.18
Coll-DH-R	0.9966	0.032	0.455	63.61

The values obtained for the release exponent indicated for both matrices an anomalous drug diffusion mechanism, in accordance with our previous studies (Albu *et al.*, 2008).

CONCLUSIONS

Porous forms based on collagen and doxycycline with and without glutaraldehyde were prepared by lyophilization. The FT-IR analysis showed that collagen kept its structure in all the samples. The spongious form showed a porous structure with interconnected pores. The hydrophilicity was lowest for collagen reference spongious forms and highest for the sample which contains doxycycline and cross-linking agent, this form being a promising support for tissue regeneration.

Acknowledgements

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