DEVELOPMENT AND EVALUATION OF SOME METRONIDAZOLE-LOADED COLLAGEN SUPPORTS DESIGNED FOR PERIODONTITIS

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Periodontitis is one of the most common chronic disease in the world affecting both the adults and young people, having severe consequences at cardiovascular level, and causing the premature birth. Moreover, the untreated periodontal disease is the major cause for tooth loos. Thus, the purpose of this study was to design and investigate some collagen supports with metronidazole and strontium ranelate as a treatment option for bone dental regeneration and an effective way to remove the pathogen agents incriminated in the periodontal disease. Type I fibrillar collagen gel was extracted from calf hide. Collagen hydrogels with various ratios of metronidazole and strontium ranelate were rheologically tested at two temperatures: 23°C and 37°C. The collagenic supports obtained by hydrogels lyophilization were investigated by goniometric analysis. The in vitro metronidazole release from spongious matrices was conducted with a sandwich device adapted to a dissolution equipment. The hydrogels presented a pseudoplastic behaviour facilitating the formulation flow and their good manipulation. The Power law model fitted well the kinetic data indicating a non-Fickian drug transport mechanism. The physico-chemical properties were in relation with the drug release patterns from spongious supports. Based on the results obtained, we could conclude that the designed formulations are potentially usable as a favorable solution in periodontal disease.

Keywords: collagen supports, metronidazole release, strontium ranelate.

INTRODUCTION

Periodontal disease is a general term for a number of pathological conditions characterized by inflammation and degeneration of the gums (gingival), supporting bone (alveolar bone), periodontal ligament and cementum (Vyas *et al*., 2000). One of the most important clinical features of periodontitis is periodontal pocket (Divya and Nandakumar, 2006). The epithelium of the gingiva migrates along the tooth surface forming "periodontal pocket" that provides an ideal environment for the growth and proliferation of microorganisms (Vyas *et al*., 2000).

Antimicrobial agents have been suggested for use as an adjunctive therapy to eliminate pathogenic bacteria and improve the clinical outcome. The oral administration of an antimicrobial agent does not guarantee that an adequate concentration of drug is delivered to the periodontal pocket (Addy and Martin, 2003). Topical antimicrobial agents are more suitable because the drug can be delivered specifically to the action site at a high concentration, and patients have no systemic side effects (Southard and

Godowski, 1998). Metronidazole is a nitroimidazole compound, frequently used in the treatment of periodontal diseases since it can be effective against several Gram-negative anaerobic rods, the pathogenesis of periodontitis, by inhibiting bacterial nucleic acid synthesis (Pejcic *et al*., 2010). Many reports suggested that local delivery of metronidazole has been suitable for periodontitis treatment (Pichayakorn and Boonme, 2012). Collagen is one of the main components of the bone and teeth being one of the most used material for bone grafting scaffolds (Ficai *et al*., 2010). Strontium is an important trace element in human bone, stimulating bone formation and reduce bone resorption by increasing osteoblast activity (Wu *et al*., 2012; Er *et al*., 2008).

Thus, the purpose of this study was to design and investigate some collagen supports with metronidazole and strontium ranelate as a treatment option for bone dental regeneration and an effective way to remove the pathogen agents incriminated in the periodontal disease.

MATERIALS AND METHODS

Materials

Type I fibrillar collagen (C) gel having a concentration of 2.85% (w/w) and pH 2.5 was extracted from calf hide by the currently used technology as previously described (Albu, 2011). Metronidazole (MTZ) was supplied from Hubei Hongyuan Pharmaceutical technology Co., Ltd., China. Strontium ranelate was obtained from OSSEOR. Glutaraldehyde (GA) was purchased from Sigma-Aldrich (Germany). Sodium hydroxide, monobasic potassium phosphate and disodium hydrogen phosphate were obtained from Merck (Germany). All the chemicals were of analytical grade and the water was distilled.

Collagen Hydrogels Preparation

Reference hydrogel (Coll) having the concentration 1.1% and pH 7.4 was prepared from the initial collagen gel under stirring with distilled water and NaOH 1M solution. 2% metronidazol and 2% strontium ranelate reported to the collagen hydrogel was added and collagen according with composition from Table 1. The obtained hydrogels were then cross-linked with glutaraldehyde and stored for 24h at 4°C for cross-linking. The composition of the collagen hydrogels is given in Table 1.

Table 1. Composition of collagen hydrogels

Hydrogel	Collagen Coll (%)	Metronidazole MTZ (%)	Strontium ranelate Sr (%)	Glutaraldehyde GA (%)
Coll	1.1	0	0	0.0020
Coll-MTZ	1.1	2	0	0.0020
Coll-Sr	1.1	0	2	0.0020
Coll-Sr-MTZ	1.1	2	2	0.0020

*the amounts of Coll, MTZ, Sr and GA are reported to 100g hydrogel

The obtained hydrogels were then lyophilized using the Delta LSC 2-24 Martin Christ lyophilizer (Germany) and the method previously detailed (Albu, 2011) and the corresponding collagen spongious matrices were obtained.

Stationary Shear-Rheometry

The flow behaviour of the designed hydrogels was performed with a rotational viscometer MultiVisc-Rheometer (Fungilab) equipped with standard spindle TR 9 and an ultrathermostat ThermoHaake P5 to maintain constant the sample temperature during the measurements. All the experiments were carried out in triplicate at 23°C±0.5°C and 37°C±0.5°C.

Evaluation of Sponges Surface Wettability

The wetting behaviour of spongious matrices was determined with a KSV Scientific Instrument equipped with a video camera for images capturing and a CAM-101 software for data acquisition, using the pendant drop dynamic method. All the goniometric experiments were conducted in triplicate, on both sides of the porous supports.

In vitro Metronidazole Kinetics Release

The kinetic studies were carried out using a "sandwich" device adapted to a dissolution apparatus (EssaDissolver) as previously described in our studies (Ghica et al 2014). The receiving medium was the phosphate buffer pH 7.4, maintained at 37°C. The amount of metronidazole released at different period of time was spectrophotometrically assessed at 319 nm (Perkin-Elmer UV-Vis spectrophotometer).

RESULTS AND DISCUSSION

The flow patterns for the designed hydrogels tested at 23°C and 37°C and recorded as viscosity versus shear rate are presented for exemplification in Figure 1 a-b and Figure 2 a-b.

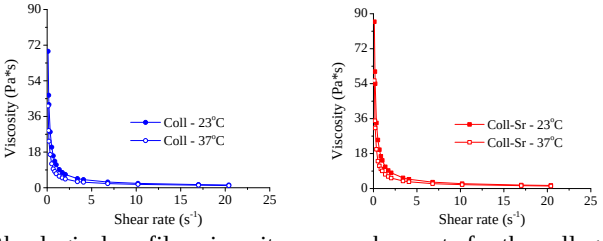

Figure 1. Rheological profiles viscosity versus shear rate for the collagen hydrogels: a) Coll; b) Coll-Sr, evaluated at 23°C and 37°C

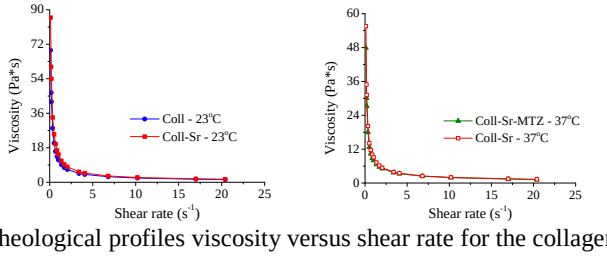

Figure 2. Rheological profiles viscosity versus shear rate for the collagen hydrogels: a) Coll and Coll-Sr analyzed at 23°C; b) Coll-Sr and Coll-Sr-MTZ analyzed at 37°C

As it can be remarked from the flow patterns given in Figures 1-2, all the tested samples presented a decrease of viscosity with the shear rate increase at both temperatures, indicating a shear thinning character which facilitates the formulations flow and their good manipulation.The above rheograms show both the influence of composition and work temperature on the hydrogels rheological behaviour.

The quantification of the stationary shear-rheometry data was realized through Power law rheological model (eq. 1):

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (1),$$

where, m and n are parameters correlated with the hydrogels composition (Ghica *et al*., 2015) and computed through the linearization of eq. (1) by double logarithmic method. m" parameter is associated with the viscosity obtained for the shear rate of $1 \cdot s^{-1}$. The values of the determination coefficients (R^2) were higher than 0.99 indicating that this model fitted well the experimental data. The values of the aforesaid parameters and of R^2 specific to Power law model are listed in Table 2.

Table 2. The m and n parameters values and the determination coefficients specific to the Power law rheological model applied to hydrogels tested at 23°C and 37°C

Hydrogel	Temperature, 23°C			Temperature, 37°C		
	m	n	R^2	m	n	R^2
Coll	12.171	0.756	0.9995	7.381	0.748	0.9983
Coll-MTZ	10.626	0.759	0.9994	6.845	0.687	0.9974
Coll-Sr	15.043	0.765	0.9984	8.752	0.795	0.9983
Coll-Sr-MTZ	12.007	0.778	0.9997	7.912	0.774	0.9980

As expected, the temperature increase conducted to a viscosity decrease for all hydrogels about 1.52-1.72 times. The presence of strontium ranelate in formulation determined an increase of m parameter about 1.13-1.24 times, while the addition of metronidazole led to a decrease about 8-10%.

The wettability characteristics of the spongious matrices surface was further determined. The surface properties allow a better understanding of the drug delivery patterns from collagen sponges (Ghica *et al*., 2013) and also represent an indicator of the interactions established between drug release support and gingival crevicular fluid.

The surface characteristics of the collagenic spongious matrices were quantified through contact angle (CA) and evaluated from Young-Laplace equation (eq. 2) (Popa et al. 2013):

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cdot \cos\theta \quad (2)$$

where, γ_{SG} is the solid-vapour interfacial tension, γ_{SL} – the solid-liquid interfacial tension, γ_{LG} – the liquid-vapor superficial tension, and θ – the contact angle.

The influence of the spongious matrices composition on surface wettability, as well the values recorded for the contact angle (expressed in degrees) are illustrated in Figure 3.

As it can be seen from Figure 3 a-d, for all the tested samples the contact angles are smaller than 90° indicating a good hydrophilicity of the spongious surfaces and consequently a proper wetting by the biological enviroment, favoring its diffusion in the porous structure.

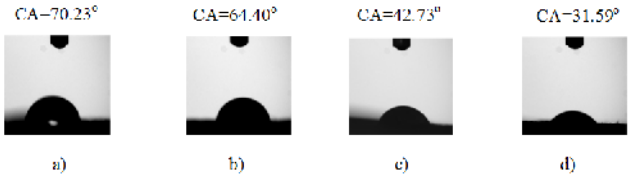

Figure 3. Images of the drop shape recorded at 0.064s for the collagen spongious matrices: a) Coll; b) Coll-MTZ; c) Coll-Sr; d) Coll-Sr-MTZ

The addition of metronidazole in both samples without (Coll-MTZ) and with strontium ranelate (Coll-Sr-MTZ) induced a decrease of the contact angle and consequently an increase of hydrophilicity in comparison with the samples without drug. Also, the presence of strontium ranelate in formulation determined an obvious increase of surface wettability comparing with the samples without mineral.

The influence of the formulation factors on the MTZ release from the spongious matrices was than evaluated and the kinetic patterns are presented in Figure 4.

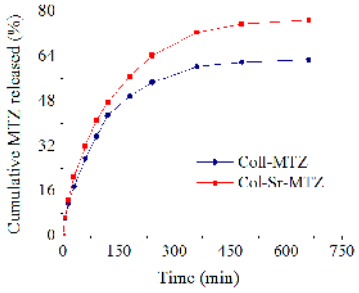

Figure 4. Cumulative release profiles of metronidazole from collagen spongious matrices as a function of time

The MTZ released percentage after 11 hours of experiments was higher for the sample with Sr, the increase being about 1.22 times (Table 3).

The Power law kinetic model (eq. 3) was applied to the experimental data and the fitting parameters are given in Table 3.

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

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

Table 3. Fitting parameters for the Power law kinetic model; MTZ released percentage

Collagen sponges	Correlation coefficient	Kinetic constant ($1/min^n$)	Release exponent	MTZ Released percent (%)
Coll-MTZ	0.9762	0.063	0.371	62.76
Coll-Sr-MTZ	0.9822	0.066	0.394	76.72

The values obtained for the release exponent indicated for both spongious supports a non-Fickian drug release mechanism.

The kinetic results are in line with the ones obtained for the goniometric studies, the samples proving a good wettability and facilitating the permeation of the receiving medium into the porous structures, and consequently the drug diffusion through the gel network.

CONCLUSIONS

The collagen hydrogels designed in this paper presented a non-newtonian pseudoplastic behaviour facilitating the formulation flow and their good manipulation. The spongious matrices composition influenced their surface wettability properties which were corelated with the drug release profiles. Based on the results obtained, we conclude that the designed formulations could represent a favorable starting solution for bone dental regeneration and an effective way to remove the pathogen agents incriminated in the periodontal disease.

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