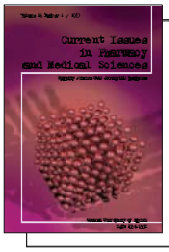

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Evaluation of the use of microcrystalline chitosan and collagen membranes as carriers for the platelet derived growth factor (PDGF-BB) in the presence of amoxicillin

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ABSTRACT

The aim of the present study was to evaluate the *in vitro* release of platelet-derived growth factor (PDGF-BB) from homogeneous microcrystalline chitosan (MCCh), collagen (Coll) and composite microcrystalline chitosan - collagen (MCCh-Coll) membranes in the presence or absence of amoxicillin (Am) and to select the most useful membrane for its practical application. The films were characterized by means of FTIR spectroscopy, swelling studies and SEM images. The kinetics of PDGF-BB release was evaluated by means of the ELISA immunoassay test. Amoxicillin concentration was determined spectrophotometrically at 273 nm. The process of the PDGF-BB growth factor and amoxicillin release from studied membranes was of two-phase nature. The first phase (during the 8 h) was characterized by rapid release whereas in the second phase (up to 10 days) the release was much slower. MCCh-Coll (M4) composite membrane appeared to be the most useful membrane because of the fast release of PDGF-BB as a significant angiogenic growth factor for tissue regeneration and the slow as well as gradual release of the antibiotic (amoxicillin), which carries protective role during regeneration process.

Keywords: microcrystalline chitosan (MCCh), collagen (Coll), platelet derived growth factor (PDGF-BB), amoxicillin (Am), kinetics of PDGF-BB and Am release

INTRODUCTION

In recent years tissue engineering (TI) has seen wide use in the treatment of injured bone, nerve and skin tissues. Natural, biodegradable polymers such as fibrin, gelatin, collagen, chitosan appeared to be the most useful, providing fully controlled biocompatible factor release [6, 9, 11]. Growth factors belong to the group of cytokines, which bind with specific cellular membrane receptors. A significant role in TI is played by the *platelet derived growth factor* (PDGF), which is a dimeric glycoprotein engaged in the regulation of cellular division, migration or cellular growth during angiogenesis [5]. Currently available evidence supports the use of PDGF-enhanced matrices to promote periodontal and peri-implant bone regeneration. The use of growth factors such as PDGF-BB with biocompatible matrices to promote tissue regeneration represent a promising approach in the disciplines of oral and maxillofacial surgery. The results of preclinical and clinical human studies evaluating the effectiveness of growth-factor-enhanced matrices confirmed the usefulness of PDGF in skeletal surgery, such as oral surgery [7, 12, 14].

The prevention of infection occurrence and pathological lesions resulting from membrane implantation inside an organism still remains an important problem. Previously data indicated that the prophylaxis of antibiotic usage in membranes decreases the risk of infection by even 81%. The drugs most frequently used in membranes are antibiotics and chemotherapeutics [2].

Collagen and chitosan are highly biocompatible and possess favorable physicochemical properties for this purpose. Molecular interactions between collagen and chitosan have the potential to produce biocomposites with novel properties [13]. Collagen and chitosan do not exist together as blends in nature, but the specific properties of

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each may be used to produce man made blends that confer unique structural and mechanical properties. The blending of collagen with chitosan gives the possibility of producing new materials for potential biomedical applications. Chitosan was chosen as a biomedicine because of its biocompatibility, biodegradability, and nontoxicity [4, 12]. In our study, we used microcrystalline chitosan (MCCh) in the form of hydrogel at a neutral pH of 6.8, which is used in the membrane as a carrier for growth factors [10]. MCCh is a special multifunctional polymeric material prepared by the aggregation of glucosamine macromolecules from an aqueous solution of organic acid [15]. It is the only form of polyaminosaccharide characterized by the presence of free unbound amine groups, which can be present in liquid dispersion form with direct film-forming behaviour.

The aim of our research was to assess the release of platelet derived growth factor (PDGF-BB) in the presence or absence of amoxicillin (Am) *in vitro* from homogeneous microcrystalline chitosan (MCCh), collagen (Coll) and composite microcrystalline chitosan – collagen (MCCh-Coll) membranes and to select the most useful membrane for its practical application, in view of the fast release of the growth factor and slow release of antibiotic.

MATERIALS AND METHODS

Reagents. Microcrystalline chitosan MCCh/LA Fg-90 (weight-average molecular weight $M_w = 2.8 \times 10^5$ Da) in the form of hydrogel of definite polymer content – 3.0 wt%, degree of deacetylation DD = 83.2% and water retention value of 587% was prepared with the previously published unconventional method [15] of the Institute of Biopolymers and Chemical Fibres, Łódź, Poland. The degree of DD, necessary to estimate the content of – NH_2 groups in the samples, was determined by the method of first derivative UV-spectrophotometry (1DUVS), according to Tan, Khor and co-workers [16]. Collagen platelet aggregation reagent and Amoxicillin A 8523 were supplied by Sigma-Aldrich Chemical Co.(St. Louis, MO USA). Platelet derived growth factor – BB (PDGF-BB) and Quantikine® Human PDGF-BB Immunoassay ELISA Kit were supplied by R&D System, Inc. 614 McKinley Place NE (Minneapolis, MN 55413 USA). Propylene glycol, Phosphate Buffered Saline, PBS pH 7.4 were supplied by Sigma.

Preparation of polymer carriers. Homogenous and mixed membranes M1–M6 with growth factor PDGF-BB and amoxicillin were prepared from biodegradable microcrystalline chitosan (MCCh) and collagen (Coll) polymers in aseptic conditions. For comparison, one set of polymer membranes was prepared with MCCh in the absence of Coll, whereas another was prepared with Coll in the absence of MCCh. The method of film preparation was modified in comparison with our previous publication [10]. The polymer hydrogel was introduced into the middle of the round metal disc (D = 40 mm, h = 2 mm) placed on a Teflon® plate. An amount of 100 μL of PDGF-BB (0.25 μg/mL) was introduced into M1, M3 and M5 membranes in aseptic conditions. Additionally,100 μL of amoxicillin (5.7 mg/mL) was introduced into M2, M4 and M6 membranes in aseptic conditions. M2', M4' and M6' membranes contained only 100 μL of amoxicillin (5.7 mg/mL), which was introduced in aseptic conditions (Table 1).

Polymer films (MCCh, Coll, and MCCh:Coll – 2:1) in the absence of active substance (PDGF-BB, Am) were prepared for use in FTIR.

Microcrystalline chitosan. Homogeneous MCCh films (M1, M2 and M2') (Table 1) were prepared by pouring MCCh hydrogel (1.33 g of hydrogel containing 40.0 mg of chitosan) with 20 μL of $CaCl_2$ (0.5 mol/L) and plasticizers, glycerol (G) and propylene glycol (GP), in equal amounts (25 mg) into the middle of the round metal disc. While the constituents were being stirred, 100 μL of platelet derived growth factor PDGF-BB (0.25 μg/mL) and/or 100 μL of amoxicillin (5.7 mg/mL) was quickly added in aseptic conditions. The solvent evaporated during incubation the hydrogel at 28 ± 2°C for 24 h and homogeneous MCCh membranes (M1, M2 and M2') were obtained (Table 1).

Microcrystalline chitosan – Collagen. To prepare a complex carrier containing chitosan and collagen, a mixture of these polymers in the form of microcrystalline chitosan hydrogel (3.0 wt.%) and collagen hydrogel (2.63 wt.%) was used (Table 1). Mixed microcrystalline chitosan-collagen membranes (M3, M4 and M4') were prepared by the addition of microcrystalline chitosan hydrogel (1.33 g containing 40.0 mg of chitosan) to collagen hydrogel (0.76 g containing 20.0 mg of collagen) with 20 μL of $CaCl_2$ (0.5 mol L^{-1}) and plasticizers, glycerol (G) and propylene glycol (GP), in equal amounts (25 mg) into the middle of the round metal disc. While the constituents were being stirred, 100 μL of PDGF-BB (0.25 μg/mL) and/or 100 μL of amoxicillin (5.7 mg/mL) was quickly added in aseptic conditions. When the films were desiccating for 24 h in an incubator at 28 ± 2°C, the solvent evaporated and the complex carriers were obtained.

Collagen. Collagen in the amount of 263 mg was dissoved in 10 mL of 0.1 mol/L CH_3COOH to give a hydrogel with a concentration of 2.63 wt.%. Homogeneous collagen films (M5, M6 and M6') (Table 1) were prepared by pouring collagen hydrogel (0.76 g containing 20.0 mg of collagen) with 20 μL of $CaCl_2$ (0.5 mol/L) and plasticizers, glycerol (G) and propylene glycol (GP), in equal amounts (25 mg) into the middle of the round metal disc. While the constituents were being stirred 100 μL of PDGF-BB (0.25 μg/mL) and/or 100 μL of amoxicillin

(5.7 mg/mL) was quickly added in aseptic conditions. When the solvent evaporated, a homogeneous collagen membrane was obtained (Table 1).

Table 1. Composition of membrane systems for determination PDGF-BB and amoxicillin release

Membrane	M1	M2	M2′	M3	M4	M4′	M5	M6	M6′
MCCh (40.0 mg)	+	+	+	+	+	+	-	-	-
Collagen (20.0 mg)	-	-	-	+	+	+	+	+	+
Amoxicillin (0.570 mg)	-	+	+	-	+	+	-	+	+
PDGF-BB (0.025 μg)	+	+	-	+	+	-	+	+	-

In all systems: $CaCl_2$ (1.11 mg), plasticizers: glycerol and propylene glycol in equal amounts (25 mg).

Dried membranes were removed from the interior of the metal disc and placed in a sealed tube. Then 1 mL of PBS (0.01 mol/L, pH 7.4) was added and membranes were eluted with continuous stirring using a mechanical shaker – Platform mixer type Y H DHN 24.

FTIR spectroscopy. The water dispersion of MCCh and a solution of coagulated collagen or coagulation mixture of these polymers were put on a Teflon plate and left to dry at room temperature. Then, the polymer film was removed and used in Fourier Transform Infrared (FTIR) measurements on an ATI Mattson Infinity Series FTIR spectrophotometer.

Swelling studies. The water sorption capacity of selected membranes MCCh (M2), MCCh-Coll (M4), Coll (M6) was determined by swelling the membranes in distilled water at room temperature. An exact weight of the membrane was placed in water for required period of time. The swollen membrane was weighed immediately on an electronic balance after removing the adsorbed water with filter paper. The swelling percentage of the membranes at various time periods was then calculated:

$$SI = \frac{w_t - w_o}{w_o} \cdot 100\% \qquad (1)$$

where: w_t – weight of the film at time t, w_0 – the weight at time 0.

Scanning Electron Microscopy. Scanning electron micrographs (surface and cross-section of the membrane) were taken for the MCCh-Coll (M4) membrane using an ESEM type Quanta 200 scanning electron microscope (SEM) from FEI.

Determination of PDGF-BB in vitro release. Platelet derived growth factor (PDGF-BB) release was performed both in the presence of 100 μL of amoxicillin (5.7 mg/mL) and without amoxicillin in six selected systems (Table 1) at room temperature [14]. For measurement of kinetics, the membranes (20 mg) were placed in tightly closed test tubes containing 1 mL of PBS buffer (0.01 mol/L, pH 7.40) and then agitated. Consecutive samples of 150 μL were periodically collected after 1, 5, 24, 48, 72, 96 and 120 h to determinate PDGF-BB concentration. The collected volume was always replaced with 0.01 mol/L of PBS, pH 7.4, buffer. The amount of PDGF-BB released was measured immunoenzymatically using ELISA assay (R&D System). The absorbance was measured at 450 nm using Elx800 ELISA Reader, BIO-TEK, Instruments, Inc. The concentrations of PDGF-BB were calculated from the regression equation: $y = (0.0012 \pm 0.0000198)x + (0.0848 \pm 0.00865)$ ($R^2 = 0.9987$), where y is the absorbance A and x is the concentration C of PDGF-BB in the tested samples (%). PDGF-BB release rate at a specified period was plotted as a release percentage versus time (h) curve, depicted in Figure 4.

In vitro amoxicillin release studies. Amoxicillin (Am) was released from the membranes into 1 ml of PBS buffer (0.01 mol/L, pH 7.4). Samples of 250 μL were periodically collected (after 1, 5, 24, 48, 72, 96 and 120 h) for amoxicillin determination. The collected volume was always replaced with 0.01 mol/L PBS, pH 7.4, buffer. The absorbance was measured at $\lambda = 273$ nm [1] with a Smart Spec TM Plus Spectrometer, Bio-Rad Laboratories Inc. in small quartz cuvettes (Helma, Light Path 10 mm). Amoxicillin concentration was calculated from the regression equation $y = (28.668 \pm 0.261)x$, where y is the absorbance A and x is the concentration C of amoxicillin in the tested samples (%). The standard calibration curve in dissolution medium was linear over the range of 1-100 g/mL ($R^2 = 0.9996$). All the experiments were carried out in triplicate.

Statistical analysis. The study was repeated in triplicate. The measurement error was less than 5%. Statistical analysis was performed using the Microsoft Excel Analysis Tool Pak in Microsoft Office Excel 2010 and Statistica 10.

RESULTS AND DISCUSSION

FTIR Spectroscopy Analysis

The FTIR spectrum of MCCh-Coll composite membrane consisting of chitosan and collagen in a 2:1 ratio by weight is shown in Figure 1, spectra 3. Characteristic bands in the spectrum included a peak at 1650 cm^{-1} which corresponds to amide I C = O stretching, a peak at 1550 cm^{-1} which corresponds to amide II N-H bending and C-N stretching, and a peak between 1150 and 1250 cm^{-1} which corresponds to amide III C-N stretching and N-H bending vibrations. Collagen displays bands at 1648, 1546 and 1236 cm^{-1}, which are characteristic of the amide I, II and III bands of collagen. The main amide III peak is observed at 1236 cm^{-1}. These absorption bands that are characteristics of native collagen revealed the intact structure of collagen in the composite membranes (spectra 2). The presence of chitosan in the composite is revealed by a strong absorption band between 800 and 1200 cm^{-1}, which is characteristic of the presence of pyranose rings (spectra 1).

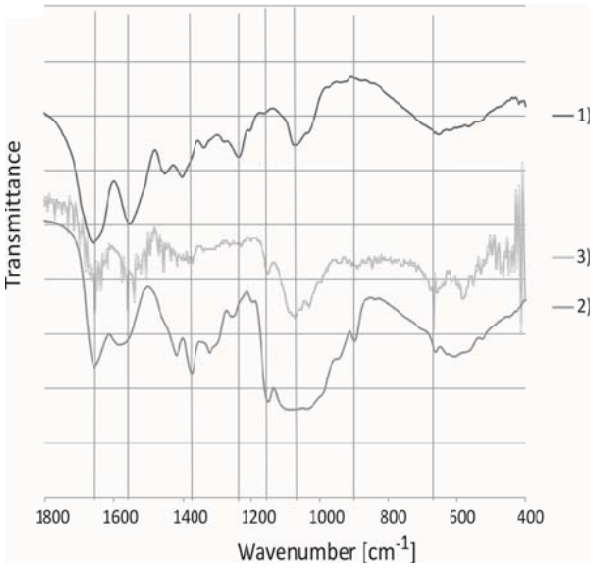

Fig. 1. FTIR spectra of homogeneous Coll film (1), MCCh film (2) and MCCh-Coll composite membrane (2:1) (3)

Swelling studies

Figure 2 demonstrates the swelling behaviour of composite MCCh-Coll (M4), homogeneous Coll (M6) and MCCh (M2) membranes. The weights of the membranes were observed after a specified time with respect to their initial dry weight. After 24 h marked swelling of membranes: 270% – Coll, 285% – MCCh and 295% – (MCCh-Coll) was observed. The results clearly indicated that MCCh-Coll in the composite can regulate the swelling properties, which in turn significantly influences the permeability properties of the membranes.

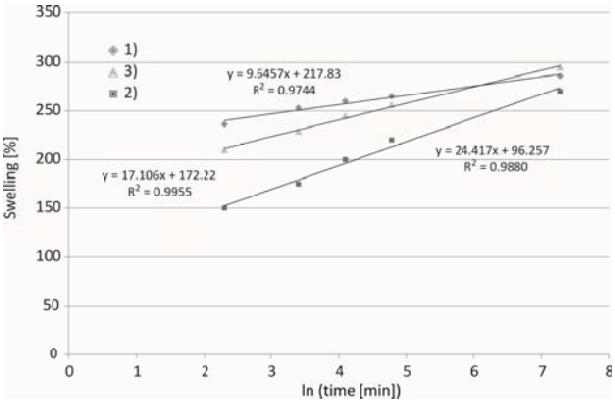

Fig. 2. Swelling percentage of 1) ♦ MCCh, 2) ■ Coll and 3) ▲ MCCh-Coll membranes in distilled water after 10, 30, 60, 120 and 1440 min. Values are average of six determinations

Scanning Electron Microscopy

Pictures of composite membrane MCCh-Coll obtained by the SEM (Quanta 200 SEM) are shown in Figure 3. M4 membrane with a well-developed surface is porous and coarse. Furthermore, aggregates or precipitated material may be seen on the surface of this membrane.

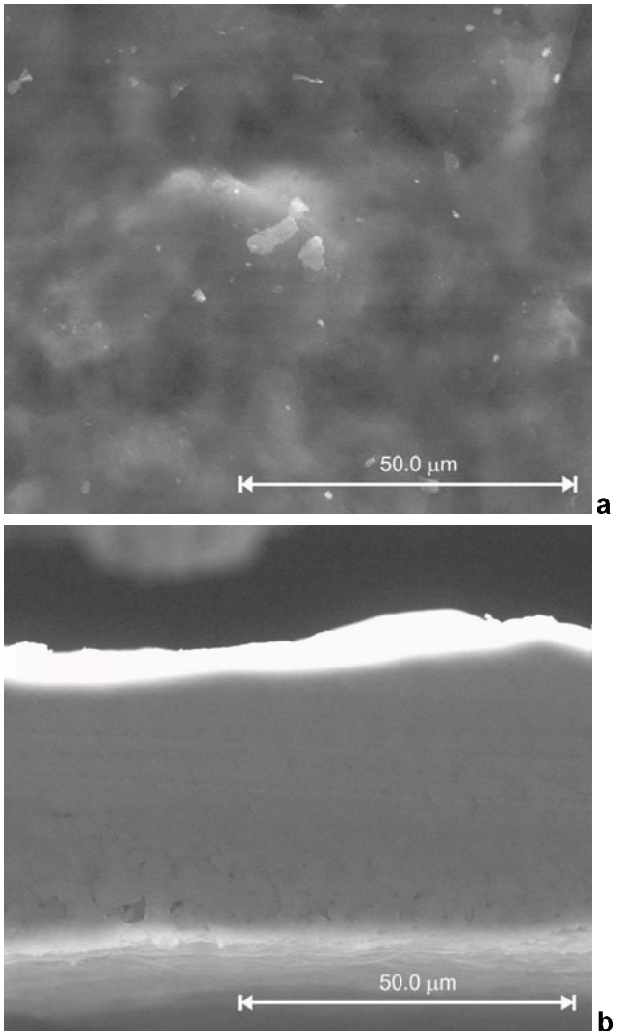

Fig. 3. SEM image of microcrystalline chitosan-collagen (MCCh-Coll) composite membrane a) membrane surface, b) cross-section – × 3000

In vitro release of PDGF-BB

Growth factors introduction into the membranes might significantly support and modify tissue regeneration. The use of growth factors such as PDGF-BB with biocompatible matrices to promote tissue regeneration represents a promising approach in the disciplines of oral and maxillofacial surgery [7]. The literature data [4, 11, 13] indicates that chitosan and collagen are the most often used polymers in TI as membrane carrier materials. The influence of chitosan on physicochemical and biochemical properties of collagen has been studied previously [13]. It has been shown that chitosan can modify the properties of collagen when the biological or mechanical properties are considered. The varied release of growth factors (PDGF-AB, TGF-ß, and b-FGF) from polymer carriers observed in our earlier studies was the reason for our continued interest in the kinetics of the release of selected *platelet derived growth factor* (PDGF-BB) from natural biodegradable polymers (MCCh and Coll) in MCCh-Coll membranes. PDGF-BB loaded chitosan may be beneficial

to enhance, for example, periodontal bone regeneration [12]. In our research, we applied PDGF-BB in six membranes (without M1, M3 & M5 and with M2, M4 & M6 amoxicillin). Table 1 shows the composition of membranes containing MCCh, MCCh-Coll and Coll. The amount of PDGF-BB released in time from these membranes is presented in Figure 4.

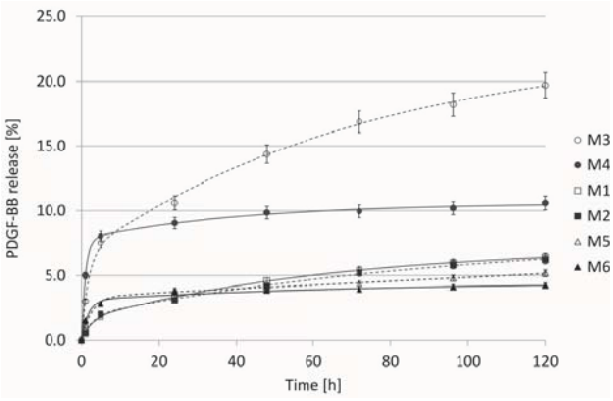

Fig. 4. Release profile of PDGF-BB from membranes: without M1 □ MCCh, M3 ○ MCCh-Coll, M5 △ Coll, and with amoxicillin M2 ■ MCCh, M4 ● MCCh-Coll & M6 ▲ Coll

Profiles of PDGF-BB release (Figure 4) especially from the M4 membrane (MCCh-Coll-Am) revealed that the growth factor release is two-stage process with the initial rapid effect and slower second stage [3, 8, 12]. In the first phase, this process is a function of change in drug concentration in surface layer, of which the total release of particles is more easily accessible. The second phase corresponds to the effective delayed release of drug substance from the deeper layers of the polymer membranes. It can be assumed that in this phase there is diffusion of drug substances from the deeper layers of the membrane.

Values of correlation coefficient R for the release profiles of the PDGF-BB membrane both with and without the presence of amoxicillin were significantly different.

The amount of PDGF-BB released from the MCCh membranes (M1 and M2) was low and significantly decreased (after 120 h up to 6.4%) both with and without the presence of amoxicillin (R = 0.9982) in compared with M3 and M4 composite membranes. The binding of MCCh with collagen (M3 and M4) increases the amount of factor released in compared with M1 and M2 homogeneous MCCh membranes. More PDGF-BB factor (20.0%) was released from composite MCCh-Coll M3 membrane without the presence of amoxicillin in compared with M4 membrane (11.0%), R = 0.8528. Amoxicillin insignificantly decreases (from 5.0% to 4.2%) the release of PDGF-BB from collagen M6 membrane in comparison with M5 (R = 0.9880).

Interpretation of kinetics data for PDGF-BB and amoxicillin release as zero order, first order as well as the assumed dependence of concentration changes with the square root of time, did not result in a straight line relation. In the case of first order kinetics was expressed as a log function of the remaining factor concentration in relation to time, curve lines corresponding to the initial phase of factor release were determined in all systems. Analysis of the diagrams depicted reveals that two different release phases may be found.

The obtained data indicates (Figure 4) that the release process of PDGF-BB from the membranes studied can be described with a first order equation with two exponential functions, as described previously [3]:

$$C_t = C_1 \times (1\text{-}\exp(-k_1 \times t)) + C_2 \times (1 - \exp(-k_2 \times t)) \qquad (2)$$

where:

C_t – percentage of substance released after time t

C_1,C_2 – percentage of substance released in the first and second phases

k_1,k_2 – rate constants for the first and second release phases

The values of k constants in equation (2) may play the role of kinetic constants; therefore they may be useful for comparison of drug release kinetics from various systems. The higher constant value of k_1 compared to k_2 indicates that the rate of release is larger than that of diffusion [8].

Rate constant values for the release process during first k_1 and second phase k_2 of the applied kinetic model are presented in Table 2.

Table 2. Constant values of kinetic equation (2) describing *in vitro* PDGF-BB release from the membranes; Means ± SD

Type of membranes	Phase I			Phase II			R^2
	C_1 (%)	k_1 (h^{-1})	$t_{0.5}$ (h)	C_2 (%)	k_2 (h^{-1})	$t_{0.5}$ (h)	
M1	1.544 ± 0.086	0.418 ± 0.048	1.66	5.710 ± 0.084	0.0156 ± 0.0009	44.4	0.9998
M2	1.833 ± 0.311	0.379 ± 0.133	1.83	6.292 ± 0.858	0.0102 ± 0.0034	67.9	0.9983
M3	6.536 ± 0.571	0.586 ± 0.126	1.18	16.435 ± 1.104	0.0134 ± 0.0025	51.7	0.9988
M4	7.741 ± 0.205	1.013 ± 0.068	0.68	2.850 ± 0.209	0.0245 ± 0.0057	28.3	0.9991
M5	3.365 ± 0.204	0.383 ± 0.054	1.81	45.310 ± 7.582	0.00033 ± 0.00060	2100	0.9980
M6	3.097 ± 0.289	0.576 ± 0.129	1.20	1.403 ± 0.569	0.0141 ± 0.0144	49.1	0.9933

In the first phase the half-life of PDGF-BB release is 1.18 h and 0.68 h for composite membranes (M3, M4). For homogeneous MCCh (M1, M2) membranes $t_{0.5}$ = 1.66 h and 1.83 h. In the second phase $t_{0.5}$ = 51.7 h and 28.3 h (M3, M4), while for MCCh membranes $t_{0.5}$ = 44.4 h (M1) and 67.9 h (M2). For collagen membranes in the first phase the half-life of release is 1.81 h (M5) and 1.20 h (M6); in the second phase 2100 hours (M5) and 49.1 h (M6).

The analysis of the data from Figure 4 and Table 2 indicated that the amount of PDGF-BB released from the membranes is depended on their properties and constituents.

In vitro release kinetics of amoxicillin

Previous study indicated that polymer membranes are often enriched with antibiotics, which allows to prevent infection and the inflammation process [2]. The implantation of a membrane into a body carries the risk of inflammation or immunogenicity. Many side effects can be avoided by local antibiotic release from membranes what results in the systemic administration of antibiotics in large amounts. In our research we applied amoxicillin in six membranes (in the presence of growth factor M2, M4, M6 and without growth factor M2', M4', M6'). The release of amoxicillin from the membranes in time (h) is presented in Figure 5.

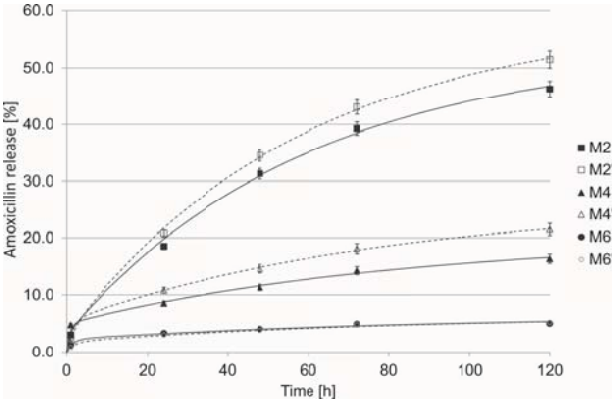

Fig. 5. Release profile of amoxicillin from membranes: without M2' □ MCCh, M4' ○ MCCh-Coll, M6' △ Coll, and with PDGF-BB – M2 ■ MCCh, M4 ● MCCh-Coll & M6 ▲ Coll

The amount of amoxicillin released (Figure 5) from the membranes in the presence (M2, M4, M6) and without growth factor PDGF-BB (M2', M4' M6') was different. As described previously, similar to PDGF-BB release, the obtained data shown on Figure 5 indicates that the release process of amoxicillin from membranes can be described with a first order equation with two exponential functions (Table 3) [3].

Table 3. Constant values of kinetic equation (2) describing *in vitro* amoxicillin release from the membranes; Means ± SD

Type of membranes	Phase I			Phase II			R^2
	C_1 (%)	k_1 (h^{-1})	$t_{0.5}$ (h)	C_2 (%)	k_2 (h^{-1})	$t_{0.5}$ (h)	
M2	3.124 ± 1.100	1.439 ± 1.608	0.48	50.59 ± 1.94	0.0167 ± 0.0018	41.5	0.9987
M2′	2.880 ± 1.127	0.646 ± 0.586	1.07	56.08 ± 1.39	0.0171 ± 0.0014	40.5	0.9996
M4	4.930 ± 0.435	1.290 ± 0.348	0.53	15.32 ± 1.50	0.0120 ± 0.0026	57.8	0.9979
M4′	5.297 ± 0.285	0.731 ± 0.102	0.95	20.70 ± 0.71	0.0129 ± 0.0011	53.7	0.9997
M6	2.243 ± 0.363	0.653 ± 0.262	1.06	4.22 ± 1.27	0.0112 ± 0.0070	61.9	0.9915
M6′	1.766 ± 0.087	0.555 ± 0.066	1.25	4.61 ± 0.19	0.0124 ± 0.0014	55.9	0.9997

The process of Am release from homogeneous MCCh membranes was quicker and higher in comparison with composite MCCh-Coll (52.0% for M2' and 22.0% for M4'after 120 h). In the first phase the half-life of Am release is about 0.50 h (M2, M4) and about 1.0 h (M2', M4'); in the second phase $t_{0.5}$ = 41.5 h (M2) and $t_{0.5}$ = 40.5 h for M2', while for the composite membranes (M4, M4') $t_{0.5}$ = 57.8 h and 53.7 h respectively. The lowest release degree of the Am (acc. 6.0% after 120 h) was observed for the homogeneous Coll membranes (during the first phase 2.24%, $t_{0.5}$ = 1.06 h for M6 and 1.77%, $t_{0.5}$ = 1.25 h for M6'; in the second phase 4.22%, $t_{0.5}$ = 61.9 h (M6) and 4.61%, $t_{0.5}$ = 55.9 h (M6').

The release of amoxicillin was significantly dependent on the character of the membranes as well as on the interaction between the constituents. The composite MCCh-Coll M4 membrane has promising properties because of the slow and gradual release of amoxicillin (Figure 5).

CONCLUSSIONS

The obtained results of the PDGF-BB and amoxicillin release from homogeneous microcrystalline chitosan, collagen and composite microcrystalline chitosan-collagen membranes indicated correlation between the level of release and composition of the membranes. The process of the PDGF-BB growth factor and amoxicillin released from the membranes studied was of a two-phase nature. The first phase (during the 8 h) was characterized by rapid release, whereas the release during second phase (up to 10 days) was much slower, which is positive from the point of view of the drug application assignment (prolonged therapeutic effect). The binding of MCCh with collagen increases the amount of PDGF-BB released (MCCh membranes M1 & M2 6.4%, MCCh-Coll membranes M3 20.0%, M4 11.0%) and decreases the level of amoxicillin released (MCCh membranes M2' 52.0%, M2 47%, MCCh-Coll membranes M4' 22.0%, M4 16.0%). MCCh-Coll M4 composite membrane has promising properties because of the fast release of PDGF-BB as a significant angiogenic growth factor for tissue regeneration as well as the slow and gradual release of antibiotic (amoxicillin), which carries protective role during regeneration. The porous surface of MCCh-Coll M4 membrane is crucial for the diffusion of growth factors and free oxygen penetration; these processes are essential in enhancing tissue growth and regeneration.

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