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Research Article

Evaluation of Microcrystalline Chitosan and Fibrin Membranes as Platelet-Derived Growth Factor-BB Carriers with Amoxicillin

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The aim of this study was to describe the mechanical and sorption features of homogeneous and composite membranes which consist of microcrystalline chitosan (MCCh) and fibrin (Fb) in various proportions as well as the *in vitro* kinetics of platelet-derived growth factor-BB (PDGF-BB) released from ten types of membranes in the presence or absence of amoxicillin (Am). The films were characterized by Fourier transform infrared (FTIR) spectroscopy, mechanical tests: breaking strength (Bs) and elongation at break (Eb), as well as SEM images, and swelling study. The influence of the form of samples (dry or wet) on Young's modulus (E) was also examined. The homogeneous MCCh (M1) and composite M3 and M4 (MCCh : Fb = 2 : 1 and 1 : 1) membranes were characterized by good sorption properties and higher mechanical strength, when compared with Fb (M2) membrane. Connecting MCCh with Fb decreases release of PDGF-BB and increases release of Am. The most efficient release of PDGF-BB was observed in the case of M4 (the optimum MCCh : Fb ratio was 1 : 1) membrane. It was found that the degree of PDGF-BB release from the membrane is influenced by the physicochemical and mechanical characteristics of the films and by its affinity to growth factor PDGF-BB.

1. Introduction

The tissue engineering approach to repair and regeneration is founded upon the use of polymer scaffolds which serve to support, reinforce, and in some cases organize the tissue [1–3]. This process can be accelerated by membranes soaked with different protein factors. A significant role in tissue regeneration 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 [4]. Three PDGF isoforms were identified as disulfide-linked dimers, namely, PDGF-AA, PDGF-BB, and PDGF-AB. PDGF-BB is reported as a potential mediator of bone 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 [5, 6]. The use of growth factors can significantly improve wound healing with more rapid bone formation and maturation [7, 8]. However, little information is available regarding the mechanisms of PDGF-BB regulating bone turnover. Until now, there have not been any studies published on the combination of PDGF-BB and amoxicillin carriers for surgery. Besides the role of membrane as a growth factor delivery carrier, it provides structural support for directing cells and helps to launch the healing of tissue in the bone defect by encapsulating it with a new bone tissue.

The prevention of infection occurrence and pathological lesions resulting from membrane implantation inside an organism still remains an important problem, crucial during the guided bone regeneration procedures. For these reasons, many laboratories were established to develop infection-preventing membranes after implantation. The objective of previous study [9] was to evaluate cellulose membrane

impregnated by tetracycline hydrochloride. In the work of Park et al. [10] tobramycin or ciprofloxacin, as an antimicrobial agent, was loaded into a cross-linked porous collagen-hyaluronic acid (HA) matrix. In the *in vivo* experiment platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) were also incorporated into tobramycin containing collagen-HA matrices in order to further enhance wound healing.

The importance of natural polymers, such as microcrystalline chitosan, and minerals, such as hydroxyapatite (HAp) and tricalcium phosphate (TCP), has grown significantly due to their renewable and biodegradable source, increasing the knowledge and functionality of composites in technological and biomedical applications. The excellent biocompatibility, biofunctionality, and nonantigenic property make the chitosan (Ch) and its derivatives, such as a microcrystalline chitosan (MCCh), an ideal material for tissue regeneration [11–16].

Our interest in the use of microcrystalline chitosan (MCCh) and fibrin (Fb) as a carrier (scaffold) of PDGF-BB was first roused by the wide range of practical applications of membranes in tissue engineering described in the work of Cartmell [1], which also inspired further research. In another study of Park et al. [6] chitosan sponge containing platelet-derived growth factor-BB (PDGF-BB) was developed as an osteoconductive material which stimulates bone formulation.

In our study, we measured the rate of release of PDGF-BB from membranes containing MCCh, Fb, or mixtures of the two with various polymer contents.

The natural polymers are preferred because the synthetic origin lacks cell-recognition signals [17]. Therefore, MCCh and Fb, which are natural materials, are selected for fabrication as the membranes (scaffolds) in this study.

Chitosan was chosen as a biomaterial because of its biocompatibility, biodegradability, and nontoxicity [18, 19]. It can be used as an individual compound in a carrier [13, 14] or in combination with other polymers (fibrin, gelatin, collagen, and heparin) [20–24] and inorganic substances [15, 16]. Ch is a biodegradable cationic polysaccharide composed of $\beta(1 \rightarrow 4)$-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) which is known to accelerate wound healing and bone formation [15]. The strong functionality of chitosan (two hydroxyl groups (C3, C6) and one primary amine group (C2) per repeat unit) gives it a considerable opportunity of chemical modification.

Polysaccharides, such as chitosan and its derivatives, have some excellent properties for medical applications: nontoxicity (monomer residues are not harmful to health), water solubility or high swelling ability after simple chemical modification, stability to pH variations, biocompatibility, high adhesiveness, and extensive chemical reactivity. Moreover, these materials evidence a strong ability to create hydrogen and ionic bonds and biostimulation of natural resistance by controlling and improving bioactivity [16, 25]. The physical properties of chitosan recommend it for use in many types of devices. Both chitin and chitosan have demonstrated antiviral, antibacterial, and antifungal properties and have been explored for many agricultural uses. Apart from their applications in the medical field, chitin and chitosan fibers have potential applications in wastewater treatment where the removal of heavy metal ions by chitosan through chelation has received much attention. An interesting area of research, however, has been generated by the ability of chitosan to remove metal ions from wastewaters by the process of adsorption [25–27].

In recent years, there has been increasing interest in the pharmaceutical field in discovering new excipient materials of natural origin. One such material is chitosan, a monograph (chitosan hydrochloride) on which was included in the fourth edition of the European Pharmacopoeia in 2002 and sixth edition of the Handbook of Pharmaceutical Excipients in 2009. This nontoxic, biodegradable, and biocompatible material has attained great interest in pharmaceutical applications, as versatile drug delivery agent. Chitosan has been investigated as an excipient in the pharmaceutical industry to be used in direct tablet compression, as a tablet disintegrant for the production of controlled release of solid dosage forms or for the improvement of drug dissolution. Chitosan membranes to provide an alternative means of evaluating transdermal drug delivery systems were developed and evaluated. Chitosan has been used as an aid to transdermal drug delivery both *in vitro* and *in vivo* in numerous studies. Chitosan has been associated with other biopolymers and with synthetic polymer dispersions to produce wound dressings. Chitosan-calcium phosphate composites were investigated as injectable resorbable scaffolds for bone tissue regeneration [28].

Both chitin and chitosan possess properties characteristic of polycations. Like other natural polysaccharides, chitin and chitosan are biocompatible and can therefore be used as bioengineering material, are biodegradable by lysozymes, and show low toxicity. The properties and applications of amino saccharides have been described in the books of Muzzarelli [29], Zikakis [30], and Roberts [31] as well in the articles dealing with associations of chitosan [25–28]. The important work of Austin, Tokura, and Hirano [26], who have contributed to the applications development of chitin, especially in fiber form. Chitosan, which is soluble in acidic aqueous media, is used in many applications (food, cosmetics, and biomedical and pharmaceutical applications). Other reviews deal with the antimicrobial activity of chitosans and DNA-chitosan complexes [28].

A valuable physicochemical modification of chitosan, used among others as excipient in drug formulations, is microcrystalline chitosan (MCCh), obtained in the form of a suspension, powder, or granules [32, 33]. A polymer of desired chemical properties is prepared by appropriate aggregation of the macromolecules from aqueous solutions of organic acids (via neutralization, coagulation, and then precipitation of microcrystalline chitosan) [34]. During this process, the polymer is also refined from low-molecular weight side products. MCCh shows a number of valuable features as compared with unmodified chitosan: higher absorptivity, higher crystallinity, chelating capability, and higher bioactivity. We could observe an increase in optical density of absorption band at about 1380 cm^{-1} as in comparison with initial chitosan. This can be explained by a corresponding rise of the product crystallinity. It should be noticed that an

increase in the crystallinity index of a product is accompanied by a decrease in water retention value (WRV). MCCh is a safe and effective biopolymer for the achievement of hemostasis at puncture sites [35].

The most important and extraordinary property of MCCh seems to be its direct-film-forming behaviour just from aqueous dispersion. Film obtained in this process shows excellent adhesion to different types of surface and water resistance [36]. The above properties present several possibilities for pharmaceutical as well as medical uses of microcrystalline chitosan. Microcrystalline chitosan, especially in the form of gelatinous dispersion, can be effectively used as an auxiliary agent with bioactive action for the modification of cosmetic preparations. Microcrystalline chitosan is also characterized by unique miscibility with several polymers, substances, and solvents. High affinity of MCCh for the bonding of protein has been applied in the preparation of a MCCh complex with casein or keratin using various MCCh: protein ratios [36]. These complexes, especially with casein, show an increase in sorption of water and improvement of direct-film behaviour. In this study, we applied connection MCCh with fibrin (Fb) in different proportion.

Fibrinogen [37, 38] is a soluble plasma glycoprotein synthesized by the liver, which, in the presence of thrombin and calcium ions, is converted into Fb monomers. Fb has been used extensively as a biopolymer scaffold in tissue engineering. It show great potential in tissue regeneration and wound healing and can be used in surgery for patients with hemophilia. Fb is an effective hemostatic and wound dressing material that can be made in the form of a sponge, film, powder, or sheet. Fibrin gels, prepared from fibrinogen and thrombin, the key proteins involved in blood clotting, were among the first biomaterials used to prevent bleeding and promote wound healing. This protein has a three-dimensional structure, which allows adhesion and cellular migration during the healing process [39, 40]. Fb, either alone or in combination with other materials, has been used as a biological scaffold for stem or primary cells in regeneration of tissue and bone.

In our previous research Fb, MCCh, and methylcellulose (MC) hydrogels for bFGF in the presence of ketoprofen [41], Fb, and MCCh membranes for TGF-β [42], MCCh, and collagen (Coll) membranes for PDGF-BB in the presence of amoxicillin (Am) [43] as carriers were studied. Previous studies did not include the impact of membrane structure on the sorption property and degree of release of growth factors. Therefore, it was advisable to produce membranes that differ in their preparation and percentage of MCCh and Fb in the membrane. The applied polymers MCCh and Fb are commonly used well-tolerated and biocompatible polymers. Preliminary experiments revealed that matrices containing only the above-mentioned polymers were too fragile, hard, and brittle; for this reason, the addition of elasticizing agents proved necessary. Thus, propylene glycol as elasticizing agent and calcium chloride as a cross-linking factor were added.

In this paper, an assessment of the impact of the physicochemical (sorption) and mechanical properties of MCCh-Fb composite membranes consisting of MCCh and Fb at different proportions on the release of PDGF-BB in the presence or absence of Am *in vitro* from homogeneous MCCh, Fb and composite MCCh-Fb membranes as the polymer carriers is presented.

2. Experimental

2.1. Materials. Fibrinogen, fraction I, F-8630, type I-S from bovine plasma (9001-32-5), was supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Microcrystalline chitosan MCCh/LA 0171 (weight-average molecular weight M_w = 150 kDa) in the form of hydrogel of definite polymer content — 2.57 wt%, degree of deacetylation DD—79.8%, and water retention value of 760% was prepared with the previously published unconventional method [34] of the Institute of Biopolymers and Chemical Fibres (Łódź, Poland).

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). Thrombin EC 3.4.4.13 was supplied by Biomed (Lublin, Poland). Factor XIIIa (Fb-stabilizing factor, FSF), fragment 72–97, aprotinin from bovine lung 5 TI U mg^{-1} protein, amoxicillin A 8523, propylene glycol, and phosphate buffered saline, PBS pH 7.4, were supplied by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of Polymer Carriers. Homogeneous (M1, M2) and composite (M3–M5) membranes with growth factor PDGF-BB and additionally with amoxicillin (M1′–M5′) were prepared from biodegradable microcrystalline chitosan (MCCh) and fibrin (Fb) polymers at the following ratios MCCh : Fb (2 : 1, 1 : 1, and 1 : 2) in aseptic conditions. For comparison, one set of polymer membranes was prepared with MCCh (M1) in the absence of Fb, whereas another was prepared with Fb (M2) in the absence of MCCh. The method of film preparation was modified in comparison with our previous publication [42]. The polymer hydrogel was poured into the middle of the round metal disc (D = 40 mm, h = 2 mm) placed on a Teflon plate. 100 μL of PDGF-BB (0.25 μg mL^{-1}) was added to all of the membranes (with and without amoxicillin) in aseptic conditions. 100 μL of amoxicillin (1.1 mg mL^{-1}) was additionally added to M1′, M2′, M3′, M4′, and M5′ membranes in aseptic conditions (Table 1).

2.2.1. Microcrystalline Chitosan. Homogeneous MCCh film (M1) (Table 1) was prepared by adding MCCh hydrogel (1.20 g of hydrogel containing 30.0 mg of chitosan) with 20 μL of $CaCl_2$ (0.5 mol L^{-1}) and plasticizer, propylene glycol (GP) (50 mg) onto the middle of a round metal disc. While the constituents were stirred, 100 μL of platelet-derived growth factor PDGF-BB (0.25 μg mL^{-1}) was quickly added in aseptic conditions. The water evaporated during the incubation of the hydrogel at 28 ± 2°C for 24 h and homogeneous MCCh membrane (M1) was obtained (Table 1).

Table 1: Component of fibrin, microcrystalline chitosan, and fibrin-microcrystalline chitosan membrane systems.

Components	Systems									
	M1	M1′	M2	M2′	M3	M3′	M4	M4′	M5	M5′
Microcrystalline chitosan (mg)	30.0	30.0	–	–	40.0	40.0	30.0	30.0	20.0	20.0
Fibrinogen (mg)	–	–	30.0	30.0	20.0	20.0	30.0	30.0	40.0	40.0
Amoxicillin (110 μg)	–	+	–	+	–	+	–	+	–	+
PDGF-BB (0.025 μg)	+	+	+	+	+	+	+	+	+	+
Aprotinin (20 μg)	–	–	+	+	+	+	+	+	+	+
Thrombin (4.0 NIH)	–	–	+	+	+	+	+	+	+	+
FSF (1.0 μg)	–	–	+	+	+	+	+	+	+	+

In all systems: $CaCl_2$ (1.11 mg), propylene glycol (50 mg).

2.2.2. Microcrystalline Chitosan-Fibrin. To prepare a complex carrier (MCCh-Fb) containing MCCh and Fb, a mixture of these polymers in the form of a microcrystalline chitosan hydrogel (2.57 wt%) and fibrinogen solution (15.0 mg mL^{-1}) was used (Table 1).

Composite MCCh-Fb membranes (M3, M3′, M4, M4′, M5, and M5′) were prepared by adding fibrinogen solution in PBS buffer (0.01 mol L^{-1}, pH 7.4) (1.33 mL, 2.0 mL, or 2.77 mL containing adequately 20.0 mg, 30.0 mg, or 40.0 mg of fibrin) to MCCh hydrogel (1.60 g, 1.20 g, or 0.80 g containing adequately 40.0 mg, 30.0 mg, or 20.0 mg of chitosan) with 20 μL of $CaCl_2$ (0.5 mol L^{-1}), 50 mg of GP, 20 μL of aprotinin (1 mg mL^{-1}), 10 μL of FSF (0.1 mg mL^{-1}), and 0.4 mL of thrombin (40 NIH U mL^{-1}) onto the middle of the round metal disc. While the constituents were stirred, 100 μL of PDGF-BB (0.25 μg mL^{-1}) and/or 100 μL of amoxicillin (1.1 mg mL^{-1}) were quickly added in aseptic conditions. While the films were drying in an incubator at 28 ± 2°C for 24 h, the solvent evaporated and the complex carriers were obtained (Table 1).

2.2.3. Fibrin. An Fb film (M2) (Table 1) was produced by adding 2.0 mL of fibrinogen solution (15 mg mL^{-1}) in PBS buffer (0.01 mol L^{-1}, pH 7.40), 10 μL of FSF (0.1 mg mL^{-1}), 20 μL of aprotinin (1 mg mL^{-1}), and PG (50 mg) onto the middle of the round metal disc. While the constituents were stirred, 100 μL of PDGF-BB (0.25 μg mL^{-1}), 20 μL of $CaCl_2$ (0.5 mol L^{-1}), and 100 μL of thrombin (40 NIH U mL^{-1}) were added. The solvent evaporated during an incubation of the hydrogel at 28 ± 2°C for 24 h and homogeneous fibrin membrane (M2) was obtained (Table 1).

2.3. Methods

2.3.1. Swelling Studies. The sorption capacity of homogeneous MCCh (M1), Fb (M2), and composite at different ratios MCCh : Fb = 2 : 1 (M3), 1 : 1 (M4), and 1 : 2 (M5) membranes was determined by making the membranes swell in phosphate buffer solution, pH 7.40, at 37 ± 0.5°C in incubator with occasional shaking. For this, a known weight of the membrane was placed in a glass vial containing 50 mL of phosphate buffer solution, pH 7.40 for required period of time. The weights of the membranes were observed after a specified time with respect to their initial dry weight at 65°C. The swollen membranes were periodically removed and blotted with filter paper and their changes in weights were measured during the swelling until equilibrium was attained. Finally, the weight of the swollen membranes was recorded (on an electronic balance) after a period of 24 hours. The swelling index (SI) of the membranes at various time periods was then calculated using the formula:

$$\mathrm{SI} = \frac{w_t - w_0}{w_0} \times 100\%, \quad (1)$$

where w_0 is the initial weight of the dry membrane at 65°C and w_t is the weight of the swollen membrane at equilibrium swelling (E_{sw}) in the medium. The tests were carried out three times.

After removing the membranes from medium (phosphate buffer solution, pH 7.40), the pH of the solution was measured at 37 ± 0.5°C by means of a Multifunction Meter CX-501 ELMETRON with the calibrated electrode of ERH-111 Hydromet type (Gliwice, Poland). This experiment was made to check the influence of soluble membrane components on the change in physiological pH value of phosphate buffer.

2.3.2. Investigation of Mechanical Parameters of the Membranes. The mechanical properties of the membranes prepared were determined at the Accredited Metrological Laboratory, Institute of Biopolymers and Chemical Fibres (Łódź, Poland), which holds an accreditation certificate number AB 338. The basic mechanical parameters of the polymer materials, which were in the form of dry and wet membranes, were estimated according to appropriate standards:

(i) thickness of the film (mm): PN-EN ISO 4593: 1999,

(ii) mechanical properties: breaking strength (MPa) and elongation at break (%) according to PN-EN ISO 527-3: 1998.

The film samples were tested using Instron 5544 tensile tester. The film samples tested were 10 mm long and 15 mm wide, and the elongation rate was 10 mm min^{-1}. The thickness of dry samples, measured by a micrometer screw, was in the range of 0.024 mm Fb (M2) to 0.077 mm MCCh : Fb = 2 : 1 (M3). For selected samples, the elastic modulus (Young's modulus, E) was tested according to PN-EN ISO 6383-12005.

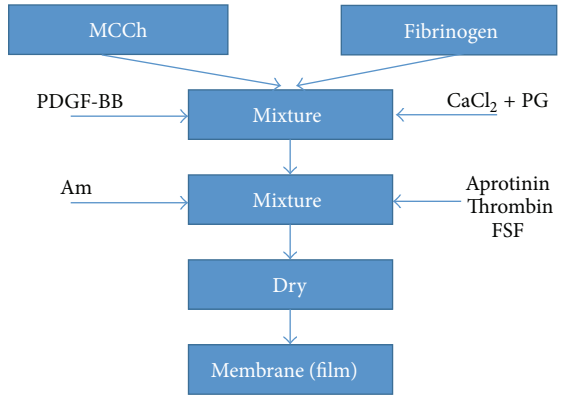

Figure 1: Diagram of the membrane preparation.

All mechanical tests were carried out in an air-conditioned room at 65 ± 4% RH and 20 ± 2°C. Testing in "wet conditions" was performed for some of the samples. For this purpose, prior to testing, the film samples were immersed in phosphate buffer, pH 7.40, for 10 min after conditioning at 65 ± 4% RH and 20 ± 2°C. The thickness of wet samples was larger: 0.310 mm MCCh (M1) and 0.100 mm MCCh : Fb = 2 : 1 (M3) [44].

2.3.3. Scanning Electron Microscopy. Scanning electron micrographs (surface and cross-section of the dry and wet membrane) were taken for the membranes studied using an ESEM type Quanta 200 (W) scanning electron microscope (SEM) from FEI Co. (Hillsboro, OR, USA).

2.3.4. FTIR Spectroscopy. The water dispersion of MCCh (M1) and a solution of coagulated fibrinogen (M2) or a coagulation mixture of these polymers (M3, M4, and M5) were placed on a Teflon plate and left to dry at room temperature (25 ± 2°C). Then, the polymer film was removed and used in Fourier transform infrared (FTIR) measurements performed using an ATI Mattson Infinity Series FTIR spectrophotometer. A total of 10 scans were collected. Spectral resolution was ±2 cm^{-1}. For comparison, homogeneous MCCh (M1′) and complex MCCh-Fb (M4′) membranes in the presence of Am and PDGF-BB were prepared as well. Separately, the IR spectra of PDGF-BB and Am were taken in KBr pellets.

2.4. Determination of PDGF-BB In Vitro Release. Platelet-derived growth factor (PDGF-BB) release was performed both in the presence and absence of 100 μL of amoxicillin (1.1 mg mL^{-1}) in ten systems selected (Table 1) at 37 ± 0.5°C. For measurement of kinetics, the membranes (20 mg) were placed in securely closed test tubes containing 1 mL of PBS buffer (0.01 mol L^{-1}, pH 7.40) and then agitated using a mechanical water bath shaker of 357 $ELPIN_{sc}$+ type, Conbest (Kraków, Poland). Consecutive samples of 150 μL were periodically collected after 5, 24, 72, 120, 264, and 432 h to determinate PDGF-BB concentration. The collected volume was always replaced with 0.01 mol L^{-1} PBS, pH 7.40. The amount of PDGF-BB released was measured immunoenzymatically using ELISA assay (R&D System). The absorbance

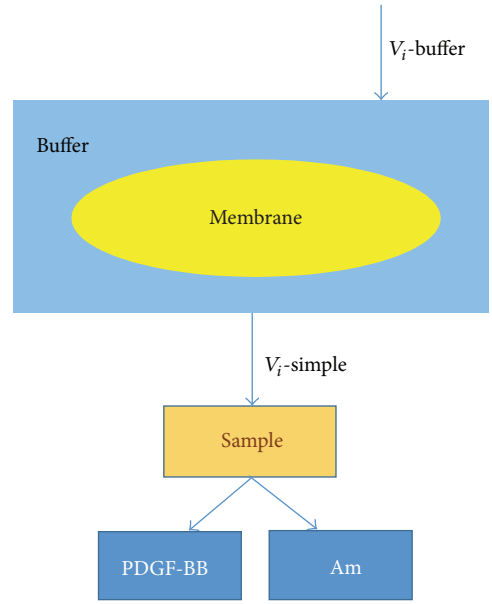

Figure 2: Diagram of the release of PDGF-BB and Am from membrane.

was measured at 450 nm using Elx800 ELISA Reader, BIO-TEK, Instruments, Inc. The concentrations of PDGF-BB were calculated from the regression equation: $y = (0.00673 \pm 0.00097)x + (0.2543 \pm 0.0076)$ ($R^2 = 0.9861$), where y is the absorbance A and x is the concentration C of PDGF-BB in the tested samples (%). All the experiments were carried out three times.

2.4.1. In Vitro Amoxicillin Release Studies. Amoxicillin (Am) was released from the membranes into 1 mL of PBS buffer (0.01 mol L^{-1}, pH 7.40) (Figure 2). Samples of 250 μL were periodically collected (after 5, 24, 72, 120, 264, and 432 h) for amoxicillin determination. The collected volume was always replaced with 0.01 mol L^{-1} PBS, pH 7.40. The absorbance was measured at $\lambda = 272$ nm [45] with a Nicolet Evolution 300 Spectrometer (Spectro-Lab, Warsaw, Poland) in small quartz cuvettes (Helma, Light Path 10 mm). Amoxicillin concentration was calculated from the regression equation $y = (3.234 \pm 0.027)x$, where y is the absorbance A and x is the concentration C of amoxicillin in the samples tested (%). The standard calibration curve in dissolution medium was linear over the range of 0.01–0.80 mg mL^{-1} ($R^2 = 0.9998$). All the experiments were carried out three times.

2.4.2. Statistical Analysis. The study was repeated two times. 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.

3. Results and Discussion

3.1. Swelling Studies. Water absorption ability and retaining this ability are an important factor in determining the usefulness of the biomaterials. The ability of swelling is an essential characteristic to analyze this kind of formulations.

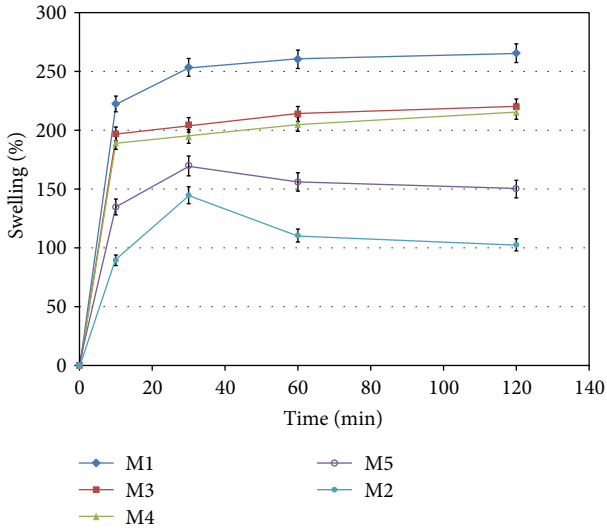

Figure 3: Swelling percentage of homogeneous MCCh (M1), Fb (M2), and composite: MCCh : Fb = 2 : 1 (M3), 1 : 1 (M4), and 1 : 2 (M5) membranes in phosphate buffer solution. pH 7.40 at 37°C from 24 h. The values are an average of three determinations.

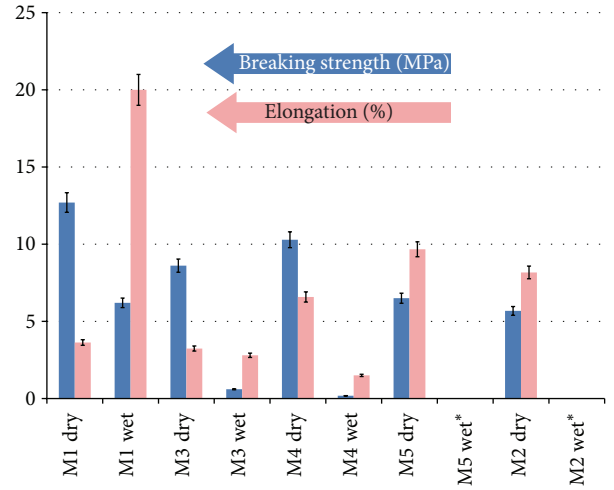

Figure 4: Influence of MCCh and Fb content on breaking strength (Bs) and elongation at break (Eb) of membrane systems.

The swelling of the polymeric membranes is shown in Figure 3.

The absorption ability of the MCCh-Fb membranes was measured in terms of degree of swelling at equilibrium. It was found that the degree of swelling of the membranes was in range of 260–100% of their dry weight and relatively correlated with the MCCh content (Figure 3). The homogeneous MCCh (M1) and composite MCCh : Fb = 2 : 1 (M3), MCCh : Fb = 1 : 1 (M4) membranes present high stability and good swelling values from 225% after 10 min to 260% after 120 min (M1) and ca. 200% after 10 min, 220% after 120 min (M3, M4). Figure 1 shows that degree of swelling of these membranes was quickly increasing during first 10 min, which could be caused by a large amount of microcrystalline chitosan characterized by high ability to absorb liquid.

The composite MCCh : Fb = 1 : 2 (M5) membrane (of a higher concentration of fibrin) shows less swelling ability (150% after 120 min) and stability. The smallest swelling capacity was found for homogeneous Fb (M2) membrane (Figure 3). An increase in weight of the membrane (% swelling) at the beginning was fast (up to 148% after 30 min), but then it decreased to 100% after 120 minutes. Further measurements are less accurate due to the degradation of this membrane. Biodegradation of biomaterials is a promising function of a carrier. However, materials with rapid degradation are not suitable for application in tissue engineering.

Measurements of pH of the acceptor fluid after swelling of membranes were conducted in order to check the influence of the soluble membrane components on the acceptor fluid. Aqueous extracts of membranes M1, M3, and M4 remain clear and transparent. The studies show that membranes have a slight impact on the change in physiological pH value (7.40) of phosphate buffer (M1 pH 7.38, M2 pH 7.48, M3 pH 7.41, M4 pH 7.44, and M5 pH 7.45).

3.2. Investigation of Mechanical Parameters of the Membranes. Two kinds of biomaterials (dry and wet samples) were tested in order to evaluate their mechanical properties such as breaking strength (Bs), elongation at break (Eb), and elastic modulus (Young's modulus, E). Only for the following membranes: homogeneous MCCh (M1) and composite MCCh : Fb = 2 : 1 (M3), MCCh : Fb = 1 : 1 (M4), testing in "wet state" was performed. These membranes showed the highest stability under conditions of the experiment. The other membranes, Fb (M2) and MCCh : Fb = 1 : 2 (M5) only dry samples, were taken into consideration (wet membranes were not evaluated because of their degradation). The mechanical properties such as breaking strength (Bs) and elongation at break (Eb) were tested at 65 ± 4% RH and 20 ± 2°C.

Propylene glycol and calcium chloride were added to the hydrogel of MCCh (Table 1) in order to improve the film formation and mechanical properties. The thickness of dry samples was as follows: 0.024 mm Fb (M2), 0.069 mm MCCh : Fb = 1 : 2 (M5), 0.074 mm MCCh : Fb = 1 : 1 (M4), 0.077 mm MCCh : Fb = 2 : 1 (M3), and 0.059 mm MCCh (M1). Figure 4 depicts the mechanical properties (Bs and Eb) of the membranes. The results are presented as combined value of 10 independent samples.

From the results presented in Figure 4 it was concluded that the membranes made of the MCCh (M1) and MCCh : Fb = 2 : 1 (M3 with the highest amount of MCCh) are characterized by a breaking strength of about 12.7 MPa and 8.6 MPa and the least elongation at break of about 3.6% and 3.2%. Membrane M4 made of the MCCh : Fb = 1 : 1 is characterized by breaking strength of about 10.3 MPa and elongation at break of about 6.6%. The breaking strength of the membranes made of the Fb (M2) and MCCh : Fb = 1 : 2 (M5 with the highest fibrin concentration) showed lower level of 5.7 MPa and 6.5 MPa and greatest elongation at break of about 8.2% and 9.7%. Moisture content of the sample had a significant effect on the breaking strength of the films. M1, M3, and M4 membranes tested in "wet conditions" are characterized by a breaking strength of 6.2 MPa, 0.6 MPa, and 0.2 MPa. The

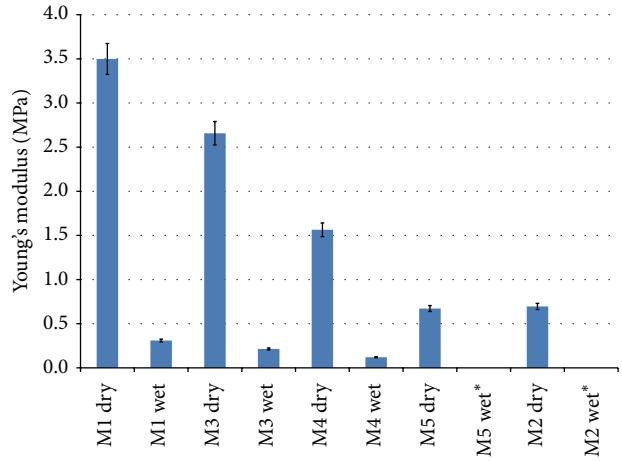

FIGURE 5: Influence of MCCh and Fb content on Young's modulus (E) of membrane systems.

elongation at break of "wet" form is the greatest for M1—20.0%, 2.8% for M3, and 1.5% for M4 membranes. As can be seen the "dry" MCCh membrane has stiffer structure of the surface, which was caused by a loss of its gel-like elastic structure. Membranes of mechanical strength >10 MPa may be applied in practice.

Besides the main properties, namely, thickness, breaking strength (Bs), and elongation at break (Eb), another parameter, Young's modulus (elasticity modulus, E), was tested according to the methods described [44]. The results are presented in Figure 5.

From the results shown in Figure 5 it can be noticed that the highest strength values were found for M1 and M3 membranes and that Young's modulus (E) decreased with an increase in concentration of fibrin. E of membrane systems in "dry conditions" decreased as follows: 3.5 MPa for M1 > 2.66 MPa for M3 > 1.56 MPa for M4 > 0.75 MPa for M2 > 0.70 MPa for M5. In "wet conditions" the elasticity modulus for M2 and M5 membranes was zero, while for other membranes E > 0.1 MPa (0.31 MPa for M1, 0.21 MPa for M3, and 0.12 MPa for M4).

3.3. Scanning Electron Microscopy. The porous surface of a membrane is crucial for the diffusion of growth factors and free oxygen penetration; these processes are essential in enhancing regeneration. Pictures of homogeneous MCCh (M1), Fb (M2), and composite MCCh-Fb (M4) membranes obtained by the SEM (Quanta 200 SEM) are shown in Figure 6. The photomicrographs of the films were obtained by SEM for the upper surface and for a cross-section. The dry membranes showed heterogeneous surfaces (Figures 6(a), 6(c), and 6(d)), but after wetting (Figure 6(b)) the membrane (M1) shows smooth surface. However, aggregates or precipitated material may be seen on the surface of membrane. In cross-section of dry MCCh-Fb (M4) membrane a solid layer from the bottom can be seen (Figure 6(e)), above which it depicts nonuniform pores, but after wetting the membrane is significantly thicker and cross-section is more uniform (Figure 6(f)). In addition, the cross-section shows homogeneity between the MCCh and Fb phases and absence of pores.

3.4. FTIR Spectroscopy Analysis. The FTIR spectra of homogeneous MCCh (M1), Fb (M2) and of related composite MCCh-Fb (M3, M4, and M5) membranes without the presence of Am are shown in Figure 7. An additional FTIR spectra of pure Am and growth factor PDGF-BB as well as homogeneous MCCh (M1′) and complex MCCh-Fb (M4′) membranes with the presence of Am and PDGF-BB are shown in Figure 7.

The spectra of MCCh from thin films (Figure 7 spectrum M1, M1′) showed both the amide I band at ca. 1650 cm^{-1} (which is essentially the C=O stretching vibration of the –NHOC–CH_3 group) as well as the –NH amide II band and the deformation scissoring mode, $\delta(NH_2)$, of the primary amine groups –NH_2—ca. 1560 cm^{-1} and amide III at 1320 cm^{-1}. In addition, the spectrum showed absorption bands at ca. 1380 cm^{-1}, referring to the crystalline area of the polymer, characteristic absorption bands of high intensity (800–1200 cm^{-1}) due to the repeating MCCh pyranose rings, and bands at ca. 1150 and 1050 cm^{-1}, corresponding to the interring ethereal bond C–O–C [46, 47]. The spectral analysis shows that in the FTIR spectra of MCCh M1′ and MCCh-Fb M4′ films in the presence of bioactive substances (AM and PDGF-BB) predominated bands characteristic of MCCh; therefore, it is difficult to determine the appearance or disappearance of any of the characteristic peaks of pure substance in film, although disappearance of the bands at ca. 1080 cm^{-1}, 980 cm^{-1}, and 860 cm^{-1} characteristic for PDGF-BB and Am can be observed. In presence of Am, spectra M1′ and M4′ do not show any visible shift of the bands characteristic for MCCh, but a weak new band at ca. 1520 cm^{-1} characteristic of Am [48] can be observed, which confirms the weak intermolecular interaction between drug and polymer.

The FTIR spectrum of MCCh-Fb composite (Figure 7) contains the main characteristic absorption peaks of fibrin and chitosan. Since fibrin is a protein, FTIR spectrum (M2) exhibits the amide absorption bands at around 1650 cm^{-1}, 1550 cm^{-1}, and 1240 cm^{-1}.

Analysis of the FTIR spectroscopy for the MCCh-Fb composite (Figure 7, spectra M3, M4, and M5) shows that the –NH amide II band at ca. 1560 cm^{-1} is present in spectrum M3, whereas in the spectrum of M4 and M5 it is shifted to 1548 cm^{-1}. A band at ca. 1650 cm^{-1} is still visible; however, it is less distinct and of lower intensity when compared with the characteristic amide I band (C=O). The bands at ca. 1150, 1320 cm^{-1}, and 1260 cm^{-1}, characteristic for MCCh, are still visible but are of lower intensity. Although the band at 1380 cm^{-1}, related to the crystal structure of the polymer, disappears, a new broad band at 1400 cm^{-1} appears. However, the bands at ca. 800 cm^{-1}, 760 cm^{-1}, and 700 cm^{-1}, characteristic for Fb, are absent in the spectrum of MCCh-Fb. The disappearance of these bands and clear shift of the amide II peak at ca. 1560 cm^{-1} show the weakening of chemical bonds and may also suggest that linkages between the MCCh-NH_2 and Fb-COOH groups are formed.

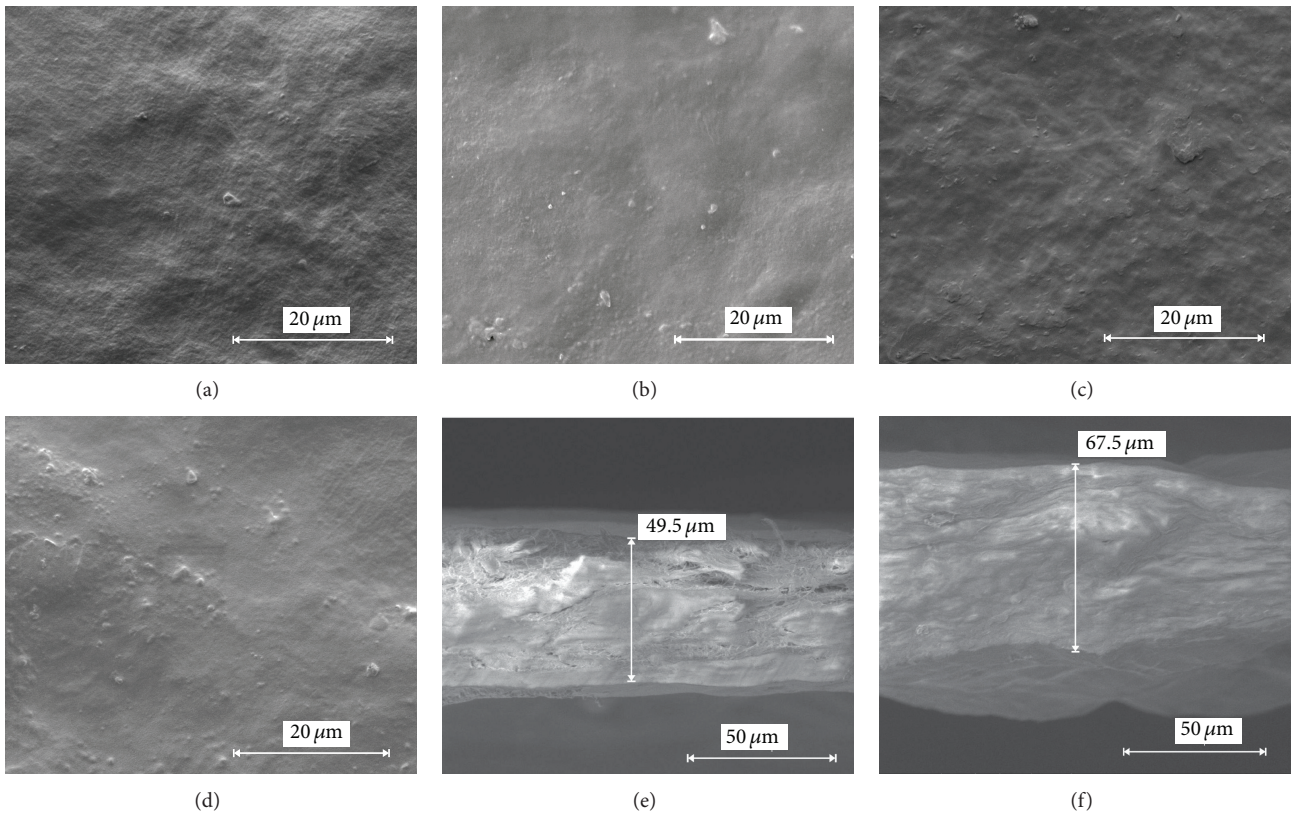

Figure 6: SEM images of MCCh : Fb = 1 : 1 composite membrane (M4). (a) Dry membrane surface—×5000, (b) wet membrane surface – ×5000 and (c) dry cross-section—×2000, (d) wet cross-section—×2000.

3.5. In Vitro Release of PDGF-BB. The release profile of PDGF-BB was determined for ten kinds of carriers (with M1′, M2′, M3′, M4′, and M5′ and without M1, M2, M3, M4, and M5 amoxicillin), which contain MCCh and Fb in various proportions (Table 1). The amount of PDGF-BB released in time from these membranes is presented in Figures 8 and 9.

Figure 8 demonstrated the release of PDGF-BB from homogeneous membranes: with M1′ MCCh, M2′ Fb and without M1 MCCh, M2 Fb amoxicillin.

Figure 9 demonstrated the release of PDGF-BB from six different composite carriers: with M3′ MCCh : FB = 2 : 1, M4′ MCCh : Fb = 1 : 1, and M5′ MCCh : Fb = 1 : 2, and without M3 MCCh : Fb = 2 : 1, M4 MCCh : Fb = 1 : 1, and M5 MCCh : Fb = 1 : 2 amoxicillin.

Values of correlation coefficient R for the release profiles of the PDGF-BB from membranes both with and without amoxicillin were significantly different. The greatest amount of PDGF-BB was released from homogeneous membranes containing only microcrystalline chitosan (M1) or fibrin (M2) (Figure 8). More PDGF factor (7.0%) was released (after 120 hours) from homogeneous Fb (M2′) membrane in the presence of amoxicillin in comparison with M2 membrane (6.8%), (R = 0.9922). PDGF-BB release level from M1 and M1′ membranes, which contained only MCCh, was relatively low: from 5.6% in the presence of amoxicillin (M1′) up to 6.2% in the absence of amoxicillin (M1) (R = 0.9992). The binding of MCCh with fibrin decreases the amount of factor released. The amount of PDGF-BB released from the composite membranes containing MCCh and Fb in different proportions (M3, M4, and M5) was low and significantly decreased both with and without the amoxicillin, especially from MCCh : Fb = 1 : 2 (M5) membrane (from 0.4% after 120 h up to 0.7% after 432 h (18 days) in comparison with M1 and M2 homogeneous membranes (Figure 8)). Amoxicillin insignificantly increases (from 0.65% after 120 h up to 0.78% after 18 days) the release of PDGF-BB from M5′ membrane in comparison with M5 (R = 0.9434). More PDGF-BB factor was released from composite MCCh : Fb = 1 : 1 (M4) membrane (0.9% after 120 h up to 1.5% after 18 days) in the absence of amoxicillin, while in the presence of amoxicillin (M4′) it was 0.65% after 120 h up to 0.85% after 18 days (R = 0.9476). Similarly, the amount of platelet growth factor released from M3 varied from 0.65% after 120 h up to 1.2% after 18 days, while with the presence of amoxicillin (M3′) it was 0.60% after 120 h up to 0.65% after 18 days (R = 0.9104).

Profiles of PDGF-BB release (Figures 8 and 9) revealed that the growth factor release is two-stage process with the initial faster effect and slower second stage [6, 43, 49].

In the first phase, this process is 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

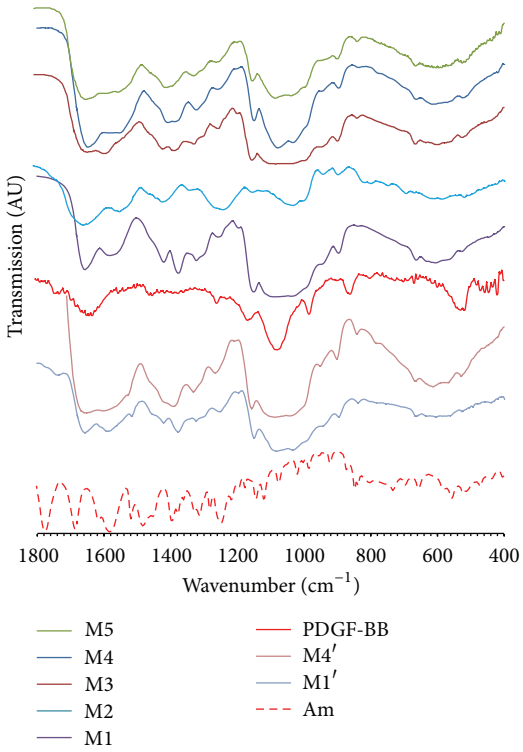

Figure 7: FTIR spectra obtained in thin polymer films of homogeneous: MCCh, Fb, and composite. MCCh : Fb = 1 : 1 membranes with (M1′, M4′) and without (M1, M2, and M4) Am. Comparative spectrum for Am was taken in KBr pellets.

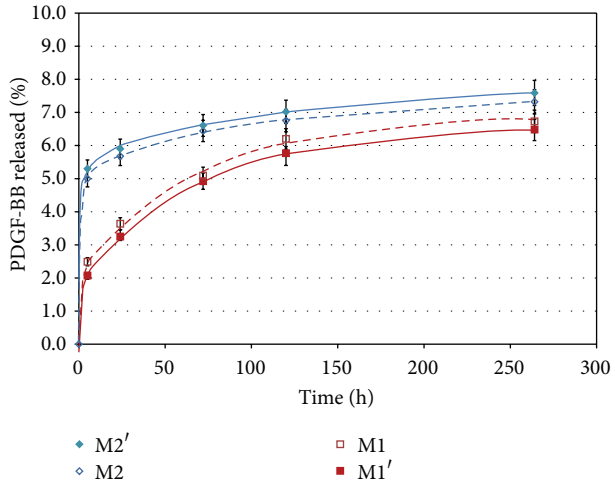

Figure 8: Release profile of PDGF-BB from homogeneous membranes, with amoxicillin M1′ MCCh, M2′ Fb and without amoxicillin M1 MCCh, M2 Fb.

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.

Interpretation of kinetics data for PDGF-BB release as zero order and first order as well as the assumed dependence of concentration changes with the square root of time did

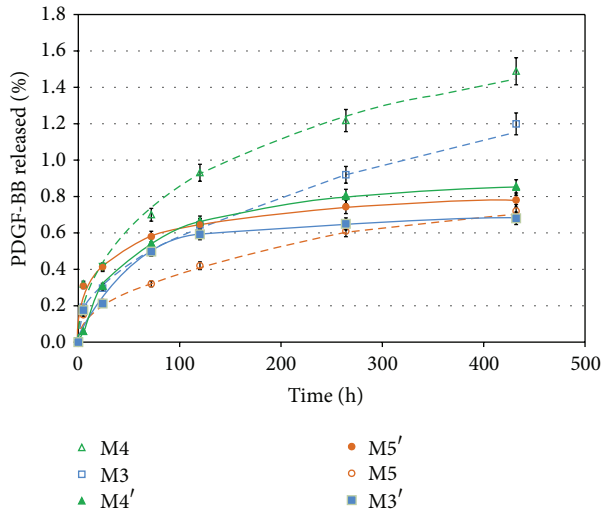

Figure 9: Release profile of PDGF-BB from composite membranes: with amoxicillin M3′ MCCh : Fb = 2 : 1, M4′ MCCh : Fb = 1 : 1, and M5′ MCCh : Fb = 1 : 2, and without M3 MCCh : Fb = 2 : 1, M4 MCCh : Fb = 1 : 1, and M5 MCCh : Fb = 1 : 2.

not result in a straight line relation. In the case of first order, the kinetics was expressed as a log function of the remaining factor concentration in relation to time, and 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 (Figures 8 and 9) that the release process of PDGF-BB from the membranes as described previously [43] can be written as the first order equation with two exponential functions:

$$C_t = 100 - \left(C_1 \times \exp\left(-k_1 \times t\right) + C_2 \times \exp\left(-k_2 \times t\right)\right), \quad (2)$$

where C_t is percentage of substance released after time t, C_1, C_2 are percentage of substance released in the first and second phases, and k_1, k_2 are rate constants for the first and second release phases.

The values of k constants in (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 [49]. In the majority of the experiments, in the second phase the membranes showed steady state diffusion-controlled release.

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

In the first phase, the half-life of PDGF-BB release is 3.27 h, 3.98 h, and 2.77 h for composite membranes (M3, M4, and M5). For homogeneous (M1, M2) membranes $t_{0.5}$ = 1.46 h and 0.88 h. In the second phase, $t_{0.5}$ = 214 h, 126 h, and 145 h (M3, M4, and M5), while for homogeneous membranes $t_{0.5}$ = 53.3 h (M1) and 66.6 h (M2). For composite membranes with amoxicillin in the first phase the half-life of release is 28.9 h (M3′), 18.3 h (M4′), and 2.66 h (M5′) and in the second phase 231 h (M3′), 91.5 h (M4′), and 61.9 h (M5′).

Table 2: Constant values of kinetic equation describing *in vitro* PDGF-BB release from the membranes, mean ± SD.

Type of membranes	Phase I			Phase II			R^2
	C_1 (%)	k_1 (h^{-1})	$t_{0,5}$ (h)	C_2 (%)	$k_2 \times 10^3$ (h^{-1})	$t_{0,5}$ (h)	
M1	2.43 ± 0.11	0.476 ± 0.086	1.46	4.45 ± 0.10	13.0 ± 0.76	53.3	0.9998
M1′	2.01 ± 0.07	0.416 ± 0.047	1.67	4.57 ± 0.06	13.9 ± 4.82	49.9	0.9999
M2	5.20 ± 0.11	0.792 ± 0.217	0.88	2.26 ± 0.11	10.4 ± 1.5	66.6	0.9998
M2′	4.56 ± 0.08	0.743 ± 0.128	0.93	2.30 ± 0.19	5.86 ± 1.29	118	0.9999
M3	0.244 ± 0.020	0.212 ± 0.045	3.27	1.20 ± 0.068	3.24 ± 0.043	214	0.9994
M3′	0.572 ± 0.020	0.0240 ± 0.0076	28.9	0.146 ± 0.014	3.00 ± 0.15	231	0.9973
M4	0.317 ± 0.035	0.174 ± 0.044	3.98	1.24 ± 0.038	5.50 ± 0.060	126	0.9992
M4′	0.352 ± 0.015	0.0378 ± 0.0014	18.3	0.518 ± 0.013	7.57 ± 0.29	91.5	0.9991
M5	0.132 ± 0.009	0.250 ± 0.045	2.77	0.650 ± 0.014	4.79 ± 0.321	145	0.9997
M5′	0.309 ± 0.022	0.259 ± 0.041	2.66	0.468 ± 0.020	11.2 ± 1.10	61.9	0.9993

Table 3: Student's *t*-test parameter values for the membrane systems.

Membrane	Student's *t*-test	
	Calculated	Theoretical
M1 M1′	0.271	2.178
M2 M2′	−0.561	
M3 M3′	0.724	
M4 M4′	1.079	
M5 M5′	−1.055	

t values of Student's *t*-test for $P = 0.05$ and $n_1 + n_2 - 4$; $n_1 = n_2 = 7$.

The analysis of the data from Figures 8 and 9 and Table 2 indicated that the amount of PDGF-BB released from the membranes is dependent on their properties and components. The composite MCCh-Fb membranes showed optimal release parameters, except the M5 membrane, fabricated from MCCh and Fb in ratio 1 : 2, which caused the lowest degree of release. An optimal release of PDGF-BB was observed in the case of MCCh : Fb = 1 : 1 (M4) membrane without amoxicillin.

For each pair of membranes (M1 and M1′, M2 and M2′, M3 and M3′, M4 and M4′, and M5 and M5′) Student's *t*-test parameter was determined (Table 3).

Comparing the profiles of PDGF-BB release on the basis of the parameter determined ($t_{\text{calculated}}$ acc.mod.1.0), it can be concluded that, for each pair of membranes at a level of significance of $P = 0.05$ ($t_{\text{theoretical}} = 2.178$), we can accept the hypothesis H_0 that the PDGF-BB release profiles have the same distribution ($t_{\text{calculated}} < t_{\text{theoretical}}$).

3.6. In Vitro Release Kinetics of Amoxicillin. 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 [10, 11]. In our research, we applied amoxicillin in five membranes (M1′, M2′, M3′, M4′, and M5′). The release of amoxicillin from the membranes in time (h) is presented in Figure 10.

The kinetics of amoxicillin release from selected membranes was evaluated on the basis of the amount of drug substance released to phosphate buffer at pH 7.4 in the time of the experiment (Figure 10). The greatest amount of amoxicillin was released from composite M3′ and M4′ membranes: the release percentage was 65% after 120 h and 88% after 432 h (18 days). Lower level of release was assigned to homogeneous MCCh (M1′) membrane—ca. 51% after 120 h and approximately 78% after 18 days. The delayed amoxicillin release was achieved for homogeneous Fb (M2′) membrane (60% after 120 h and approximately 75% after 18 days).

The data obtained, shown in Figure 10, indicates that the release process of amoxicillin from homogeneous MCCh M1′ and composite membranes takes place in one phase. In the case of Fb M2′ membrane, similar to PDGF-BB release, two phases were observed [38]. The values of k, $t_{0.5}$, and the coefficient of determination (R^2) are listed in Table 4.

In the first phase the half-life of amoxicillin release is 5.09 h for homogeneous Fb (M2′) membrane, while in the second phase $t_{0.5} = 441.4$ h. For homogeneous MCCh (M1′) and composite membranes (only in the one phase) the half-life of Am release is 8.15 h (M1′), 60.58 h (M3′), 65.19 h (M4′), and 74.76 h.

Different physicochemical properties of membranes have an effect on various release profiles of amoxicillin, especially the swelling ability and stability, as it was previously shown (Figure 3). The release of amoxicillin was significantly dependent on the character of the membranes as well as on the interaction between the components. The most useful, delayed, and gradual amoxicillin release achieved was with Fb M2′ membrane (Figure 10). Long-term antibacterial protection can be expected in the cases of this membrane. Its effect can last until primary wound healing is finished. Homogeneous MCCh (M1′) and composite MCCh-Fb (M3′, M4′, and M5′) membranes can also be considered as a clinically effective material, because the speed of amoxicillin release during the most critical first postoperative day is the highest in this experiment.

Table 4: Constant values of kinetic equation describing *in vitro* amoxicillin release from the membranes, mean ± SD.

Type of membranes	Phase I			Phase II			R^2
	C_1 (%)	k_1 (h^{-1})	$t_{0.5}$ (h)	C_2 (%)	$k_2 \times 10^3$ (h^{-1})	$t_{0.5}$ (h)	
M1′	—	—	—	80.54 ± 7.25	85.0 ± 12.3	8.15	0.9924
M2′	51.97 ± 4.23	0.136 ± 0.021	5.09	47.99 ± 3.34	1.57 ± 0.027	441.4	0.9932
M3′	—	—	—	88.78 ± 3.31	11.44 ± 1.33	60.58	0.9911
M4′	—	—	—	89.76 ± 4.52	10.63 ± 1.63	65.19	0.9854
M5′	—	—	—	83.93 ± 2.73	9.27 ± 0.89	74.76	0.9944

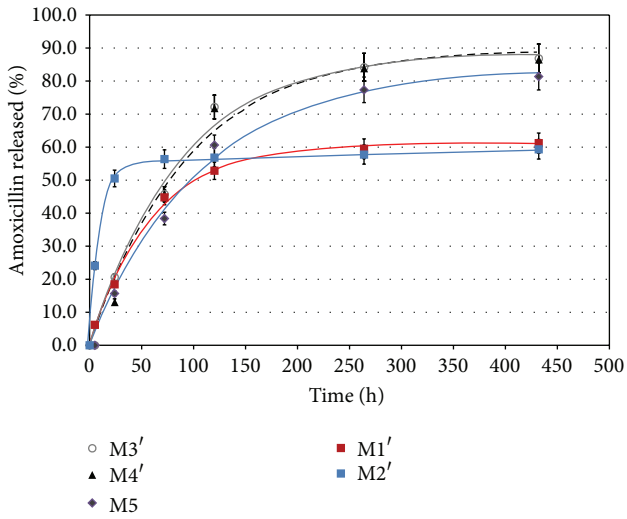

Figure 10: Release profile of amoxicillin from membrane systems: M1′ MCCh, M2′ Fb, and M3′ MCCh : Fb = 2 : 1, M4′ MCCh : Fb = 1 : 1, and M5′ MCCh : Fb = 1 : 2.

Insertion of growth factors in 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 [5]. The literature data [13, 14, 20–22, 41, 42] indicates that chitosan and fibrin are the most often used polymers in tissue regeneration as carrier materials. Chitin and chitosan enhanced the release of the platelet-derived growth factor-AB and transforming growth factor-β1 [27, 28]. Nevertheless, there is a lack of reports concerning scaffolds for *platelet-derived growth factor* PDGF-BB when using the more advantageous microcrystalline form of chitosan (MCCh).

The varied release of growth factors (b-FGF, TGF-β, and PDGF-BB) from polymer carriers observed in our earlier studies [41–43] has been the reason of the interest in the kinetics of the release of PDGF-BB selected from natural biodegradable polymers (MCCh and Fb) in MCCh-Fb membranes. PDGF-BB loaded chitosan may be beneficial to enhance, for example, periodontal bone regeneration [6]. Growth factors applied in the membranes might significantly support and modify tissue regeneration. Of utmost importance is the selection of appropriate material as a membrane carrier. In our research, we applied PDGF-BB in microcrystalline chitosan (MCCh), fibrin (Fb), and MCCh-Fb membranes (without M1, M2, M3, M4, and M5 and with M1′, M2′, M3′, and M4′ amoxicillin). The homogeneous MCCh (M1) and Fb (M2) membranes provided the highest levels of PDGF-BB release. Binding of MCCh with fibrin decreases the release of growth factor PDGF-BB and at the same time increases the release of amoxicillin.

The results of the PDGF-BB and amoxicillin release from homogeneous MCCh and Fb and composite MCCh-Fb membranes indicated a correlation between the level of release and composition of the membrane. The platelet growth factor was released in the highest amount from the Fb membrane (M2′) with the presence of amoxicillin. An optimal release of amoxicillin was observed in the case of the MCCh-Fb membrane (M3′ and M4′).

The process of the PDGF-BB growth factor release from the membranes studied was of a two-phase nature. The first phase was characterised by rapid release, while in the second phase it was much slower, which is positive from the point of view of the drug application assignment (prolonged therapeutic effect). The faster effect indicates rapid water uptake of polymer membrane and dissolution of the exposed PDGF-BB particles at the surface of the membrane. The second process is slower and it is caused by the swelling of the inner matrix layer and the acceptor fluid absorption, together with the active substance.

The microcrystalline chitosan (M1) and fibrin membranes (M2) have promising properties because of the fast release of PDGF-BB as a significant angiogenic growth factor for tissue regeneration. Moreover, the slow and gradual release of antibiotic (amoxicillin) from Fb membrane may have protective role during the regeneration process. Our results suggested that MCCh and Fb membranes may be beneficial to enhance periodontal bone regeneration.

4. Conclusions

The obtained results of the PDGF-BB and Am release from the membranes indicated a correlation between the level of the release and their composition. The platelet growth factor-BB was released in the highest amount from the MCCh and Fb membranes (M1 and M2). The profiles of PDGF-BB growth factor release from the homogeneous MCCh (M1) and Fb (M2) membranes are suitable for oral surgery. Connection of MCCh with Fb decreases the release of PDGF-BB and increases the release of Am. The results reveal that MCCh-Fb films demonstrate higher efficiency in binding PDGF-BB and, at the same time, are less effective in their

release. The PDGF-BB release was lower with increasing of Fb concentrations in the membrane. The results indicate that factor PDGF-BB is released *in vitro* from MCCh-Fb membranes with different kinetics. Assessment of the angiogenic PDGF-BB release profile from a polymer base may be valuable in choosing a proper carrier for a growth factor. The homogeneous MCCh (M1) and composite M3 and M4 (MCCh : Fb = 2 : 1 and 1 : 1) membranes were characterized by good sorption properties and high resistance to physiological liquid (0.1 mol L^{-1} phosphate buffer, pH 7.40). Moreover, these membranes show higher mechanical strength, when compared with the Fb (M2). Membranes of mechanical strength >10 MPa may be applied in practice. The membranes with incorporated PDGF-BB may prove to be a very useful scaffold in the tissue regeneration process.

Abbreviations

Ch:	Chitosan
MCCh:	Microcrystalline chitosan
Fb:	Fibrin
Am:	Amoxicillin
PDGF-BB:	Platelet-derived growth factor-BB
FTIR:	Fourier transform infrared
M:	Membrane
HAp:	Hydroxyapatite
TCP:	Tricalcium phosphate
WRV:	Water retention value
M_w:	Weight-average molecular weight
DD:	Degree of deacetylation
FSF:	Fb-stabilizing factor
PBS:	Phosphate buffered saline
SI:	Swelling index
E_{sw}:	Equilibrium swelling
E:	Young's modulus
Bs:	Breaking strength
Eb:	Elongation at break
SEM:	Scanning electron microscope.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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