

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

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The effect of homogeneous fibrin (Fb), collagen (Coll) and composite fibrin-heparin (Fb-Hp), fibrin-collagen (Fb-Coll) membranes on *in vitro* release of platelet-derived growth factor (PDGF-BB) was evaluated in the presence or absence of amoxicillin using of the ELISA immunoassay test. Amoxicillin concentration was determined spectrophotometrically at 272 nm. The process of the PDGF-BB growth factor and amoxicillin release from the studied membranes was of a two-phase nature in the majority of the systems analysed. The PDGF-BB was released in the highest amount from the Coll membrane (M7) without the presence of amoxicillin – 546.2 ± 7.47 pg, $t_{0.5} = 0.88$ h and 202.5 ± 6.83 pg, $t_{0.5} = 26.65$ h during the first phase and second phase, respectively. The lowest PDGF-BB release was observed from composite M4 (Fb-Hp) membrane – 5.88 ± 0.81 pg, $t_{0.5} = 1.69$ h; and 110.2 ± 6.48 pg, $t_{0.5} = 855.6$ h during first and second phase respectively. An optimal release of amoxicillin was observed in the case of the composite M6 (Fb-Coll) membrane – only in the second phase: 64.2 ± 7.8 µg, $t_{0.5} = 83.5$ h. The lowest and delayed amoxicillin release was achieved for M4 membrane (approx. 17.1 ± 1.12 µg, $t_{0.5} = 46.5$ h). The results of the PDGF-BB release and amoxicillin from membranes indicated a correlation between the level of release and composition of the film. Our results suggested that fibrin and collagen membranes may be beneficial to enhance periodontal bone regeneration.

Keywords: PDGF-BB release, Amoxicillin, Fibrin-collagen membranes

Tissue engineering (TI) is a modern method with a wide range of applications in the case of bone, skin, muscles, blood vessels, nerves and internal organ damage. The biodegradable membrane (*scaffold*) used for tissue regeneration must fulfill very specific requirements^{1,2}. In recent years, there has been a steep increase in the amount of research on appropriate carrying material for membranes for fully controlled and optimal growth factor release³⁻⁶. An ideal membrane should imitate the natural environment in the area of membrane implementation and should be biodegradable, having no unwanted side effects. Natural, biodegradable polymers, such as collagen (Coll), fibrin (Fb) and gelatin appear to be the most useful for providing fully controlled biocompatible factor release^{4,7}.

Growth factors (GFs) belong to the group of cytokines, which bind with specific cellular membrane

receptors. The platelet-derived growth factor (PDGF), which is a dimeric glycoprotein engaged in the regulation of cellular division, migration or cellular growth during angiogenesis plays a significant role in TI⁸. Currently available evidence supports the use of PDGF-enhanced matrices to promote periodontal and peri-implant bone regeneration⁹.

The prevention of infection occurrence and pathological lesions, resulting from membrane implantation inside an organism still remains an important problem¹⁰. Literature data show that 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^{11,12}.

Fibrin gel rich in growth factors can be used for such release and can be injected around the infected tissue, simultaneously decomposing and releasing a growth factor in the meantime¹³. Fibrin is natural polymer, which takes part in the regeneration of many tissue types (nerves, blood vessels and bone). This protein has a three-dimensional structure, which allows adhesion and cellular migration during the healing process. Fibrin membranes show a fast regeneration action, unlike collagen membranes, which tend to join

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Abbreviations: Coll, collagen; Fb, fibrin; Fb-Hp, fibrin-heparin; GAG, glycosaminoglycan; GFs, growth factors; Hp, heparin; Fb-Coll, fibrin-collagen; PDGF-BB, platelet-derived growth factor-BB; TI, tissue engineering.

with cells engaged in tissue regeneration processes^{14,15}. Membranes currently applied frequently contain a combination of fibrin and collagen and for this type of membrane, presence of heparin is required, which significantly increases the level of binding between collagen and PDGF¹⁶.

The heparin (Hp) is used to produce membranes in our experiments because it is a strongly anionic glycosaminoglycan (GAG) a potential wound healing modulator, exhibiting a significant effect on fibroblast proliferation^{17,18}. It could act by interacting with cell surface proteins in a manner that affects biosynthesis pathways of growth factors. Among the many materials used for making membranes (natural or synthetic polymers, inorganic materials), natural collagen and fibrin have low risk of inflammation, no immunological response, low toxicity and an increased ability to promote cellular adhesion^{16,19,20}.

In our previous reports, fibrin, microcrystalline chitosan and methylcellulose hydrogels for bFGF in the presence of ketoprofen⁵ and fibrin, microcrystalline chitosan membranes for TGF- β ⁶ as carriers have been studied. This study has been aimed to assess the release of platelet-derived growth factor (PDGF-BB) *in vitro* in the presence or absence of amoxicillin from homogeneous fibrin, collagen and composites fibrin-heparin, fibrin-collagen membranes and to select the most useful membrane for its practical application, in view of fast release of the growth factor and slow release of antibiotic.

Materials and Methods

Fibrinogen, fraction I, F-8630 and type I-S from bovine plasma (9001-32-5), phosphate buffered saline (PBS, pH 7.4), amoxicillin A 8523 and collagen platelet aggregation reagent were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO USA). Heparin (*Heparinum natricum*), solution for intravenous administration of 25,000 IU/5 mL was obtained from Polfa (Warsaw, 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).

Preparation of polymer carriers

Homogenous and mixed membranes M1 to M8 with growth factor PDGF-BB were prepared from biodegradable fibrin, heparin and collagen polymers. 100 μ L of PDGF-BB (0.25 μ g/mL) was introduced into membranes in aseptic conditions. For comparison, one set of polymer membranes was prepared with fibrin in the absence of collagen, whereas another was prepared with collagen in the absence of fibrin. 100 μ L of amoxicillin (5.7 mg/mL) was added into M2, M4, M6 and M8 in aseptic conditions. The method of film preparation was modified in comparison with our previous report⁶. The polymer hydrogel was introduced into the middle of the round metal disc (D = 40 mm, h = 2 mm) placed on a Teflon[®] plate. Table 1 presents the composition of membranes containing fibrin, collagen and heparin.

Fibrin

Fibrin film (Table 1) was prepared by pouring 1 mL of fibrinogen solution (4 mg/mL) in PBS buffer (0.01 mol/L, pH 7.40) with an addition of 100 μ L of thrombin (40 NIH U/mL) and 100 μ L of PDGF-BB (0.25 μ g/mL) into the middle of the round metal disc. The water evaporated during incubation the solution at 28 ± 2 °C for 24 h and homogeneous fibrin membranes (M 1 and M 2) were obtained (Table 1).

Fibrin-Heparin

For samples 3 and 4 (Table 1), 1 mL fibrinogen solution (concentration 4 mg/mL), 50 μ L (250 IU) heparin, 100 μ L PDGF-BB (0.25 μ g/mL) and 100 μ L thrombin (40 NIH U/mL) were applied. The fibrin-heparin mixture was then introduced into the middle

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

Membranes	M1	M2	M3	M4	M5	M6	M7	M8
Fibrinogen (4 mg)	+	+	+	+	+	+	-	-
Heparin (250 IU)	-	-	+	+	-	-	-	-
Collagen (mg)	-	-	-	-	2.0	2.0	4.0	4.0
Amoxicillin (570 μ g)	-	+	-	+	-	+	-	+
PDGF (25 ng)	+	+	+	+	+	+	+	+
Thrombin (4 NIH)	+	+	+	+	+	+	-	-

of the round metal disc. Mixed fibrin-heparin membranes (M3 and M4) were obtained after evaporating the solvent.

Fibrin-Collagen

To prepare a complex carrier (M5 and M6) containing fibrin and collagen, a mixture of these polymers in the form of fibrinogen solution (4 mg/mL) and collagen hydrogel (2.63 wt. %) was used (Table 1). Composite fibrin-collagen membranes (M5 and M6) were prepared by the addition of 1 mL fibrinogen solution in PBS buffer (0.01 mol/L, pH 7.4) to collagen hydrogel (0.08 g containing 2 mg of collagen). While the constituents were being stirred, 100 μ L of PDGF-BB (0.25 μ g/mL) and 100 μ L of thrombin (40 NIH U/mL) were quickly added in aseptic conditions. The complex carriers were obtained after desiccating the films for 24 h in an incubator at $28 \pm 2^\circ\text{C}$ during which the solvent got evaporated.

Collagen

Collagen (263 mg) was dissolved in 10 mL of 0.1 mol/L CH_3COOH giving a hydrogel with a concentration of 2.63%. Homogeneous collagen films (M7 and M8) (Table 1) were prepared by pouring collagen hydrogel (0.16 g of hydrogel containing 4 mg of collagen) with 100 μ L of PDGF-BB (0.25 μ g/mL) into the middle of the round metal disc. While the constituents were being stirred, 100 μ L of PDGF-BB (0.25 μ g/mL) and/or 100 μ L of amoxicillin (5.7 mg/mL) was quickly added in aseptic conditions. When the solvent evaporated, a homogeneous membrane was obtained (Table 1).

Methods

Determination of PDGF-BB *in vitro* release

The release of PDGF-BB was performed both in the presence of 100 μ L of amoxicillin (5.7 mg/mL) and without amoxicillin in eight selected systems (Table 1) at room temperature²¹. For measurement of kinetics, the membranes (10 mg) were placed in tightly closed test tubes containing 1 mL of PBS buffer (0.01 mol/L, pH 7.4) and were then agitated using a mechanical shaker-Platform mixer type Y H DHN 24. Consecutive samples of 150 μ L were periodically collected after 1, 5, 12, 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 (R&D System). The absorbance was measured at 450 nm using an 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 the concentration C of PDGF-BB in the tested samples (pg/mL). PDGF-BB release rate at a specified period was plotted as a release amount of substance vs. time (h) curve as depicted in Fig. 1.

In vitro amoxicillin release studies

Amoxicillin was released from the membranes into 1 mL of PBS buffer (0.01 mol/L, pH 7.4) at room temperature. Samples of 250 μ L were periodically collected (after 1, 5, 12, 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 $\lambda = 272 \text{ nm}$ ²² 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: $y = (2.867 \pm 0.261)x$, where y is the absorbance A and x the concentration C of amoxicillin in the samples tested (μ g). Standard calibration curve in dissolution medium was linear over the range of 1.0-1000 μ 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 2007 and Statistica 10.0.

Results

In vitro release of PDGF-BB

Figure 1 demonstrated the release of PDGF-BB from 8 kinds of carriers: fibrin (Fb), fibrin-heparin (Fb-Hp), collagen (Coll) and fibrin-collagen (Fb-Coll) membranes (without M1, M3, M5, M7 and M2, M4, M6, M8 with amoxicillin).

Profiles of PDGF-BB release have revealed that the growth factor release is two-stage process with the initial rapid effect and the slower second stage^{23,24}. The highest amount of PDGF-BB was released from homogeneous membranes containing only collagen (M7, M8) or fibrin (M1, M2). From the composite

membranes containing fibrin and collagen (M5, M6) or fibrin and heparin (M3, M4), the amount of released PDGF-BB was significantly lower in comparison with M7 and M8, especially for membrane with amoxicillin (M4).

In the case of fibrin (M2) and fibrin-collagen (M6) membranes, the presence of amoxicillin increased the amount of growth factor released. However, the presence of amoxicillin in collagen (M8) and fibrin-heparin (M4) membrane reduced the amount of growth factor released.

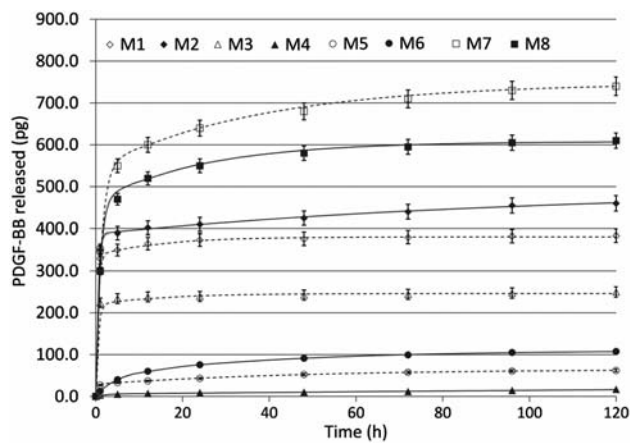


Fig. 1—Release profiles of PDGF-BB from membranes without Fb (M1 $\diamond$), Fb-Hp (M3 $\triangle$), Fb-Coll (M5 $\circ$), Coll (M7 $\square$) and with amoxicillin (Am) Fb (M2 $\blacklozenge$), Fb-Hp (M4 $\blacktriangle$), Fb-Coll (M6 $\bullet$), and Coll (M8 $\blacksquare$)

The obtained data indicated (Fig. 1) that the release of PDGF-BB from the membranes studied can be described with a first order equation with two exponential functions, as described previously²⁵:

$$C_t = C_1 \times (1 - \exp(-k_1 \times t)) + C_2 \times (1 - \exp(-k_2 \times t)) \dots (1)$$

Where C_t – amount of substance released after time t ; C_1 , C_2 – amount of substance released in the first and second phases; and k_1 , k_2 – rate constants for the first and second release phases.

The first phase was characterized by rapid release, whereas the release during second phase was much slower. The values of k constants in Eqn. 1 may play the role of kinetic constants; therefore, they may be useful for comparison of drug release kinetics from various systems. 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.

The half-life of release was within the range of about 0.3-3.0 h in the case of the first phase, while in the second phase was in the range of about 10.23 h up to approx. 855.6 h. In the first phase, the half-life of PDGF-BB release was 0.294 h and 2.88 h for the composite membranes (M5, M6), while for the homogeneous membranes (M1, M2, and M7, M8) was about 0.30 h up to 0.88 h. In the second phase, the half-life was about 30 h for M5, M6, 10.23 h for M1 and 68.61 h for M2 membranes. The PDGF-BB

Table 2—Constant values of kinetic Eqn. 1 describing *in vitro* PDGF-BB release from the membranes [Standard deviations in parentheses]

Type of membranes	Phase I			Phase II			R^2
	C_1 (pg)	k_1 (h ⁻¹)	$t_{0.5}$ (h)	C_2 (pg)	$k_2 \times 10^3$ (h ⁻¹)	$t_{0.5}$ (h)	
M1	336.5 (4.96)	2.19 (0.11)	0.316	44.7 (4.75)	67.73 (1.33)	10.23	0.9998
M2	386.8 (2.51)	2.31 (0.09)	0.300	106.0 (14.70)	10.1 (2.72)	68.61	0.9998
M3	219.7 (6.52)	1.80 (0.15)	0.385	26.5 (1.23)	63.9 (2.87)	10.85	0.9991
M4	5.88 (0.81)	0.41 (0.02)	1.69	110.2 (6.48)	0.81 (0.05)	855.6	0.9912
M5	29.2 (0.47)	2.36 (0.21)	0.294	36.0 (0.60)	21.14 (1.20)	32.78	0.9997
M6	47.0 (2.49)	0.24 (0.02)	2.888	63.4 (1.86)	24.88 (1.96)	27.85	0.9998
M7	546.2 (7.47)	0.79 (0.03)	0.877	202.5 (6.83)	26.0 (3.10)	26.65	0.9997
M8	466.2 (5.78)	1.02 (0.03)	0.679	141.8 (5.24)	39.5 (3.83)	17.54	0.9998

was released in the highest amount from the collagen membrane (M7) without the presence of amoxicillin – 546.2 ± 7.47 pg, $t_{0.5} = 0.88$ h during the first phase and 202.5 ± 6.83 pg, $t_{0.5} = 26.65$ h in the second phase. The lowest PDGF-BB release was observed from composite M4 membrane (during first phase 5.88 ± 0.81 pg, $t_{0.5} = 1.69$ h; in the second phase 110.2 ± 6.48 pg, $t_{0.5} = 855.6$ h).

Table 2 demonstrates values of kinetic constants. The higher kinetic constant value of k_1 compared to k_2 indicated that the rate of release was larger than that of diffusion²⁵. The data reported in Fig. 1 and Table 2 indicated that the amount of PDGF-BB release from the membranes was dependent on their properties and constituents.

For each pair of membranes (M1 and M2, M3 and M4, M5 and M6 or M7 and M8), the Student's t -test parameter was determined (Table 3).

From comparing the profiles of PDGF-BB release on the basis of the parameter designated ($t_{\text{calculated}} = 11.64$), it could be concluded that the presence of

amoxicillin had a significant influence on PDGF-BB release only for membranes M3 and M4 at a level of significance of $p = 0.05$. For other pair (M1 and M2, M5 and M6, M7 and M8) of membranes at a level of significance of $p = 0.05$ ($t_{\text{theoretical}} = 2.145$), we can accept the hypothesis that the PDGF-BB release profiles had the same distribution ($t_{\text{calculated}} < t_{\text{theoretical}}$).

In vitro release kinetics of amoxicillin

The amoxicillin release kinetics from four membranes (M2, M4, M6, M8) is illustrated in Fig. 2 and Table 4. Similar to PDGF-BB release, the data given in Fig. 2 and Table 4 indicated that the release of amoxicillin from membranes Fb (M2) and Coll (M8) could be described by a first order equation with two exponential functions²⁵. In the case of composite Fb-Hp (M4) and Fb-Coll (M6) membranes, only one phase was observed. The process of amoxicillin release from the Fb-Coll (M6) membrane was quicker in comparison with other films.

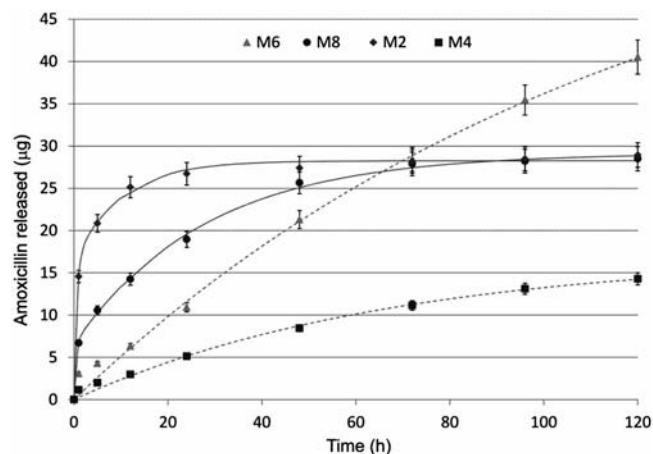


Fig. 2—Release profiles of amoxicillin from membranes [Fb (M2 ◆), Fb-Hp (M4 ■), Fb-Coll (M6 ▲), and Coll (M8 ●)]

Table 3—Student's t -test parameter values for the membrane systems

Basic carriers	System	PDGF-BB	Am	Student's <i>t</i> -test	
				Calculated	Theoretical
Fibrin	M1	+	-	1.23	2.145
	M2	+	+		
Fibrin + Heparin	M3	+	-	11.64	
	M4	+	+		
Fibrin + Collagen	M5	+	-	1.98	
	M6	+	+		
Collagen	M7	+	-	1.06	
	M8	+	+		

Student's t -test values for $p = 0.05$ and $n_1 + n_2 - 4$; $n_1 = n_2 = 9$; Am, amoxicillin

Table 4—Constant values of kinetic Eqn. 1 describing *in vitro* amoxicillin release from the membranes

[Standard deviations in parentheses]

System	Phase I			Phase II			R^2
	C_1 (µg)	k_1 (h ⁻¹)	$t_{0.5}$ (h)	C_2 (µg)	$k_2 \times 10^{-3}$ (h ⁻¹)	$t_{0.5}$ (h)	
M2	16.4 (0.17)	1.75 (0.04)	0.396	11.9 (1.70)	99.4 (2.1)	6.97	0.9979
M4	-	-	-	17.1 (1.12)	14.9 (2.0)	46.5	0.9934
M6	-	-	-	64.2 (7.80)	8.30 (1.56)	83.5	0.9945
M8	6.57 (0.70)	2.30 (0.11)	0.301	22.6 (0.64)	35.7 (3.0)	19.4	0.9987

The amount of amoxicillin released from the membranes (M2, M4, M6 and M8) was different. The greatest amount of amoxicillin was released from composite M6 membrane, which contained Fb and Coll – in one phase the release was $64.2 \pm 7.8 \mu\text{g}$, $t_{0.5} = 83.5 \text{ h}$. The lowest and delayed amoxicillin release was achieved for membrane M4 containing fibrin and heparin (approx. $17.1 \pm 1.12 \mu\text{g}$, $t_{0.5} = 46.5 \text{ h}$). Another mechanism of release was observed for homogeneous membranes; during the first phase c.a. $16.4 \pm 0.17 \mu\text{g}$, $t_{0.5} = 0.396 \text{ h}$ (M2) and $6.57 \pm 0.70 \mu\text{g}$, $t_{0.5} = 0.301 \text{ h}$ (M8) and in the second phase $11.9 \pm 1.70 \mu\text{g}$, $t_{0.5} = 6.97 \text{ h}$ (M2) and $22.6 \pm 0.64 \mu\text{g}$, $t_{0.5} = 19.4 \text{ h}$ (M8), respectively. The release of amoxicillin was significantly dependent on the character of the membranes, as well as on the interaction between the constituents.

Discussion

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, the variety and concentration of the growth factors incorporated and adding factors (heparin)^{16,26}. The literature data^{15,19,20,27,28} indicate that fibrin and collagen are the most often used polymers in tissue engineering as membranes carrier materials.

In this study, we applied PDGF-BB in fibrin (Fb), fibrin-heparin (Fb-Hp), collagen (Coll) and fibrin-collagen (Fb-Coll) membranes (without M1, M3, M5, M7 and with M2, M4, M6, M8 amoxicillin). The M7 and M8 membranes gave the highest level of PDGF-BB release, while much lower of release was observed from fibrin membranes (M1 and M2). In the presence of amoxicillin, an insignificant increase in PDGF-BB release was noticed for M2. On the other hand, the fibrin and collagen combination (M5 and M6) contributed to a decrease in the release of PDGF-BB (Fig. 1).

The process of PDGF-BB and amoxicillin release from the majority of the polymeric membranes analysed was in two phases. In the first phase, this process was a function of changes in drug concentration in the surface layer, of which the total release of particles was more easily accessible. The second phase corresponds to the effective delayed release of drug substance from the deeper layers of the polymer membranes. It could be assumed that in this phase, there was diffusion of drug substances from the

deeper layers of the membrane; the release rate was greater than that of diffusion, and the results obtained ($k_1 > k_2$) satisfied this assumption²⁹. In the majority of the experiments, in the second phase the membranes showed steady-state diffusion-controlled release.

Achieving successful tissue regeneration following traditional therapeutic protocols, combining bone grafts and barrier membranes may be challenging in certain clinical scenarios. A deeper understanding of periodontal and peri-implant wound healing and recent advances in the field of tissue engineering have provided clinicians with novel means to obtain predictable clinical outcomes⁹. 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. Polymer membranes are often enriched with antibiotics, which allow preventing infection and the inflammation process¹². The implantation of a membrane into a body carries the risk of inflammation or immunogenicity. However, the system of antibiotic local delivery to the wound (i.e. membrane) is superior over its systemic application, due to the reducing of a dose and decreasing probability of side effects development.

In our study, we applied amoxicillin in four membranes. The best release parameters were achieved with the fibrin-collagen (M6) and collagen (M8) membranes, unlike the M4 membrane (fabricated from fibrin and heparin), which provided the lowest release. The most useful, delayed and gradual amoxicillin release was achieved with fibrin membrane M2 (Fig. 2).

The results of preclinical and clinical human studies evaluating the effectiveness of growth-factor-enhanced matrices have confirmed the usefulness of PDGF in skeletal surgery, such as oral surgery^{9,10}.

The heparin is the supporting and modifying factor for the slow release of the growth factor from membranes. The increased affinity of heparin to growth factors assures it's binding with the inside of the membrane and allows for gradual release during degradation. Covalent bonds which appear between the heparin and growth factors permit permanent incorporation of growth factors inside the membranes. Due to its high affinity to many growth factors, in this study, we used heparin as one of the ingredients in two membranes (M3 and M4). According to the results obtained by other authors^{30,31}, heparin binds the growth factor (PDGF-BB) and slows down its

release from the membranes, especially in the presence of amoxicillin (M4).

The results of the PDGF-BB release and amoxicillin from fibrin (Fb), fibrin-heparin (Fb-Hb), collagen (Coll) and fibrin-collagen (Fb-Coll) membranes indicated a correlation between the level of release and composition of the membrane. The PDGF-BB release was highest from the Coll membrane (M7) in the absence of amoxicillin. An optimal release of amoxicillin was observed in the case of the fibrin-collagen membrane (M6).

The process of PDGF-BB and amoxicillin release from the studied membranes was of a two-phase nature in the majority of the systems analysed. The first phase was characterized by rapid release, while in the second phase it was much slower, which was positive from the point of view of the drug application (prolonged therapeutic effect). The collagen (M8) and fibrin membranes (M2) showed promising properties due to 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 has protective role during regeneration process. Our results suggested that fibrin and collagen membranes may be beneficial to enhance periodontal bone regeneration.

Acknowledgments

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