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Summary of the Ph.D. thesis

FORMULATION AND INVESTIGATION OF INNOVATIVE DRUG DELIVERY SYSTEMS FOR THE TREATMENT OF PERIODONTITIS

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1. INTRODUCTION

The plaque-induced forms of periodontal diseases are the most prevalent chronic inflammatory conditions seen in humans worldwide, affecting nearly half of the adult population. Periodontitis is a major health problem reducing the quality of life and causing tooth loss, disability, masticatory dysfunction, poor nutritional status and compromised speech. Periodontitis is also independently associated with systemic chronic inflammatory diseases including atherogenic cardiovascular disease, type 2 diabetes mellitus, rheumatoid arthritis, chronic kidney disease, obesity and chronic obstructive pulmonary disease.

Possible therapeutic methodologies include scaling and root planing and surgical intervention, all of which are supplemented by the administration of systemic antibiotics and local chlorhexidine-containing mouthwashes. By scaling and root planing, the debridement of deep pockets is often difficult, while recovery after surgical intervention is long. Using adjuvant systemic antibiotics burdens the patient as adverse events may be observed; and this does not provide a suitable concentration of the active agent in the periodontal pockets. Moreover, the administration of adjuvant antibiotics may contribute to the emergence of resistant bacterial strains.

In the last 10—15 years, local drug delivery systems received considerable attention. Numerous publications focused on local delivery systems containing antimicrobial drugs. It was established that the application of local drug delivery systems alone or in combination with other dental procedures may result in a more efficient treatment compared to the systemic administration of antimicrobial drugs. Therefore, local delivery systems with incorporated antibiotics is a promising approach to treating periodontitis. Subgingival administration of these systems is most prevalent in aiming at the increased bioavailability of drug formulations. Compared to systemic drug delivery, 100-fold larger concentrations of the antimicrobial agents can be achieved at subgingival sites. Locally administrable formulations may provide prolonged liberation of antimicrobial agents and protection from decomposition in hydrophilic media. These systems may also mask the unpleasant taste of drugs, and may prevent the emergence of antimicrobial resistance, and the appearance of side effects of oral antibiotic use.

Considering the expected properties of formulations, there is a great need for the development of topically used ideal carrier systems, which would allow a wider, safer and more promising application of active ingredients.

2. EXPERIMENTAL AIMS

The aim of my Ph.D. work was to develop and investigate innovative drug delivery systems containing antibiotics for the local treatment or adjuvant local therapy of periodontal disease. Two main research approaches were carried out during my work, the first is the development of lipid formulations, the second is the formulation and investigation of electrospun drug delivery systems for local therapy.

In the case of lipid-based system, components were selected, and the composition was optimized in order to prepare a delivery system which meets the following predefined requirements:

- To contain biocompatible and biodegradable materials.
- To soften at body temperature which allows the delivery system to accommodate to the shape of the periodontal pocket and to leave the periodontal pocket as the gingival tissue heals.
- To have a mucoadhesive feature which helps to maintain the delivery system in the subgingival area and, thus, prolong the effect of the active agent.
- To provide a non-aqueous system in which the incorporated active agent is protected against the effects of the environment (oxidization and humidity) and, thus, decomposition.
- To provide sustained release of the API which contribute to a longer antimicrobial effect.
- To be able to incorporate a wide spectrum of antibiotics or disinfectants to suit the individual needs of patients.

The second research line was focused on the development and characterization of nanofibrous electrospun drug delivery systems. The following requirements were set:

- To have a polymer base which is biocompatible and biodegradable
- To use an adequate polymeric base which provides sustained release of hydrophilic antimicrobial agents.

An important element of the work was the investigation of the relationship between the incorporated API and the bases of the drug delivery systems, and analysis of the antimicrobial effectiveness of the formulations.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Lipid-based systems

For the formulation of lipid-based drug delivery systems Suppocire BP (SBP) pellets, Methocel E4M, Kolliphor RH40 (KP) (polyoxyl 40 hydrogenated castor oil), amoxicillin (AMX), white beeswax (WB), cetostearyl alcohol (CA), metronidazole (MZ), zinc gluconate (ZnGlu), zinc hyaluronate (ZnHA), and chlorhexidine gluconate (CHX) 20% solution were used.

3.1.2. PLA-based nanofibers

For the PLA-based fibers polylactic acid (PLA), dichloromethane, dimethyl sulfoxide, and metronidazole was used.

3.2. Methods

3.2.1. Preparation of lipid-based systems

All the formulations were created by a melting and homogenation method. At first, the two lipophilic components (CA and SBP) and the surfactant (KP) were melted together at 70 °C on a hot plate, then the polymer (ZnHA or Methocel E4M) was suspended and homogenized with an overhead stirrer at 50 RPM. The antimicrobial agent(s) (AMX, MZ, ZnGlu, CHX solution) was/were dispersed and homogenized at 50 °C to avoid thermal decomposition. Delivery systems were created by molding the melted formulations into 1.5 mm high cylindrical shaped silicone molds with a diameter of 9 mm.

3.2.2. Differential scanning calorimetry of lipid-based formulations

The softening temperature and the melting point of the lipid formulations were determined by differential scanning calorimetry measurements with a Mettler-Toledo DSC 821e instrument in an argon atmosphere (100 mL/min). The temperature was raised from +5 °C to +100 °C by 5 °C per minute. Ten milligrams of the samples were put in 40 µL aluminum pans. The tops were holed, then the pans were sealed.

3.2.3. *In vitro* drug diffusion study of lipid-based formulations

The *in vitro* drug release profiles of the delivery systems were determined. The compositions were weighed with an analytical scale, put in 50-mm-long dialysis tubes (Spectra/Por® Standard RC tubing, MWCO: 12-14 kD) and sealed with closures. The tubes containing the formulations were placed in 7.5 mL of PBS solution (prepared by dissolving 8 g/dm^3 NaCl, 0.2

g/dm^3 KCl, 1.44 g/dm^3 $Na_2HPO_4 \cdot 2\ H_2O$ and 0.12 g/dm^3 KH_2PO_4 in distilled water and the pH was adjusted to 7.4 by adding an adequate amount of 0.1 M HCl) thermostated at 37 °C. Drug release was investigated for seven days, and three parallel measurements were carried out. One milliliter of samples was taken (at 0.5, 1, 2, 4, 6, 10, 24, 30, 48, 72, 96 and 168 h) and were replaced with 1 mL of fresh PBS solution thermostated at 37 °C.

CHX was quantified by UV spectrophotometric analysis at 250 nm, while AMX and MZ were analyzed with a Merck-Hitachi LaChrome Elite HPLC (Hitachi High Technologies America, Inc., Schaumburg, IL, USA) using UV detector at 230 nm. The column was a Kinetex 250 mm × 4.6 mm column packed with 53 µm EVOLuna C18, 100 Å (Phenomenex Inc., Torrance, CA, USA). Isocratic elution was performed with 20:80 (v/v) Methanol-NaH_2PO_4 (0.05 M) at a flow rate of 1 mL/min. Retention time for AMX and MZ was at 4.6 and 6.7 minutes, respectively, the resolution (R_S) was 3.10.

3.2.4. Electrostatic fiber spinning of PLA-based formulations

Electrostatic fiber spinning was carried out at the Budapest University of Technology and Economics by colleagues at the Laboratory of Plastics and Rubber Technology. For the PLA-based systems, spinning solution was made by dissolving polylactic acid (PLA) in a mixture of dichloromethane and dimethyl sulfoxide. For the spinning of fibers containing an active agent, MZ was also dissolved in the same solvent mixture in different concentrations.

3.2.5. Scanning electron microscopy of electrospun nanofibrous devices

The appearance of the disks and fibers were investigated by scanning electron microscopy using a Jeol JSM 6380 LA apparatus. The micrographs were recorded at different magnifications using 15 kV acceleration voltage. Before viewing, the fibers were sputtered with gold.

3.2.6. Antimicrobial effectiveness of formulations

Typical periodontopathogenic bacteria: *Fusobacterium nucleatum* (ATCC® 25586™), *Parvimonas micra* (ATCC® 33270™), *Eikenella corrodens* (ATCC® 23834™), *Porphyromonas gingivalis* (ATCC® 33277™), *Aggregatibacter actinomycetemcomitans* (ATCC® 29524™) and *Prevotella intermedia* (118710) control strains were used. A 1 McFarland standard concentration bacterial suspension of each bacterial strain was made separately with 0.9% NaCl solution (in suspension it is equivalent to approximately 3×10^8 colony forming units/mL). The suspension was spread onto a horse blood agar plate, where then formulations were placed on. After 24 hours of incubation in anaerobic conditions,

the diameter of the inhibition zones was measured. The formulations were then put on a new horse blood agar plate, also inoculated with 1 McFarland standard concentration freshly made bacterial suspension of each of the above-mentioned bacterial strains. The plates were then put in an anaerobic chamber for 24 hours. This was repeated until no inhibition zone could be detected.

3.2.7. *In vitro* drug diffusion study of electrospun nanofibrous devices

The in vitro drug release profiles of PLA nanofibers were determined by the measurement of dissolution followed by UV-Vis spectroscopy (Helios α Thermospectronic UV-spectrophotometer v4.55, Unicam: Thermo Fisher Scientific, Waltham, MA) at 318 nm. Disks (0.014—0.017 g) and neat fiber mats (0.012—0.016 g) were weighed and put into 7.5 mL of pH=7.4 PBS solution thermostated at 37 °C. Samples of 1.0 mL volume were taken at 0.5, 1, 4, 6, 10, 24, 30, 48, 72, 96 and 196 hours and replaced with 1.0 mL of fresh PBS solution. Drug release was followed for 7 days.

4. RESULTS AND DISCUSSION

4.1. Lipid-based systems

4.1.1. Differential scanning calorimetry

DSC measurements were used to analyze the effect of different structure-building components and their concentration and also the concentration of the surface-active agent on the melting point of the formulations. These measurements were also used to prove, that the incorporated active agents have no influence on melting point and softening temperature range of the formulations.

DSC measurements indicated that the best softening range of formulations may be achieved if the concentration of cetostearyl alcohol is fixed at 40 m/m%. The concentration of the surface-active agent did not influence the softening and melting point of formulations.

The lipid base of the compositions was SBP which has a melting point at 39.08 °C according to our measurements. CA, which was used as the structure-building component of the formulations, had a melting point at 61 °C. The results of DSC measurements show that KP has a congealing temperature approximately between 20—30 °C, which corresponds with the literature data. No sharp peaks could be perceived on the curves belonging to the polymers (zinc hyaluronate and Methocel E4M), and zinc gluconate in this temperature range (10—100 °C).

Formulations F1–F7 were examined to investigate the possible modifying effect of the incorporated drugs and excipients on melting point. Antibiotics or other suspended materials

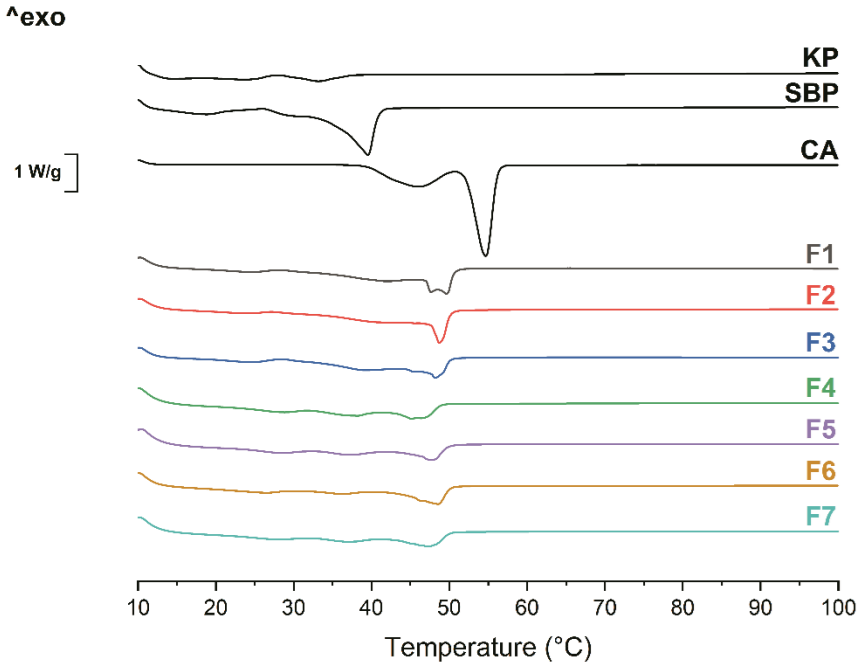

Figure 1. DSC curves of bases and different formulations F1—F7.

may partially dissolve in the lipid base, therefore, the effect of various incorporated components of different amounts on the melting point was also investigated. In all formulations, the sharp peaks of SBP and CA disappeared, even the two peaks of CA morphed into one, and shifted to temperatures between the melting points of the two pure components. At approximately 30 °C, a moderate melting can be observed, but total melting only occurs between 40 and 50 °C. A moderate melting starts at 30 °C, which supports the softening of the systems, but, as there are components with melting points at about 50 °C, which suggests the presence of a coherent structure at body temperature.

4.1.2. *In vitro* drug diffusion study

Drug dissolution measurements were carried out in order to evaluate the drug release profiles of the prepared formulations which contain MZ or CHX.

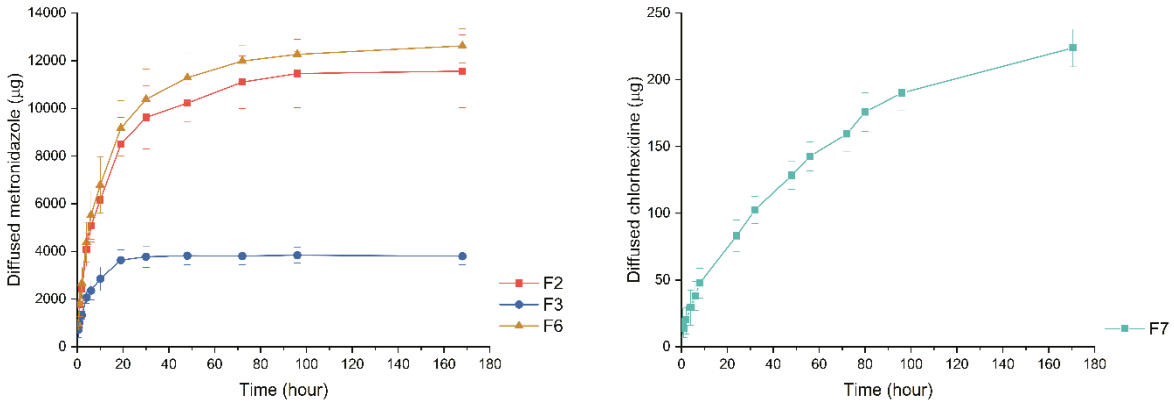

Figure 2. The amount (µg) of diffused drug (MZ and CHX) from formulations F2, F3, F6, and F7.

Unfortunately, the adequate drug release curve of AMX could not plotted, because of the degradation of the drug in the dissolution medium during the test.

The amount of released MZ or CHX from formulations is shown in Figure 2. Formulations F2 and F6 have shown similar drug release profiles. A plateau phase commencing at approximately the 100th hour is indicated in both cases, which is in accordance with a total drug release. However, the drug release curves of formulations F3 indicates that only one-third of the amount was liberated from the preparations even though half of the amount had been incorporated into the compositions.

MZ, which is a more soluble material in water compared to AMX, could be liberated more quickly from formulations creating capillaries, which contribute to a higher swelling and therefore, allow water to access all the suspended drug in the delivery systems. On the contrary, when smaller amounts of MZ are incorporated into the delivery systems with AMX – a substance with lower water solubility – fewer capillaries may be formed during drug release, permitting less water to penetrate the systems and resulting in non-complete drug dissolution. The application of ZnHA did not change the release profile, which can be explained by the similar swelling and degradation profiles of the formulation containing 15 w/w% MZ with and without ZnHA.

In the case of formulation F7, during the one-week investigation period, approximately 225 μg of active agent diffused from the delivery systems, which is nearly 50% of all incorporated drug. An average of 19% (83 μg) of all incorporated CHX diffused from the delivery systems during the first 24-hour period. A plateau phase cannot be observed during the measurement, which indicates that the release continues after 1 week.

According to Figure 2., the amount of drug diffused from formulation F7 during one week is approximately 50% of all incorporated drug, which indicates sustained release. Comparing the results to the CHX diffusion from PerioChip®, which is also a CHX-containing drug delivery system, it can be concluded that formulation F7 provides a continuous release, while in the case of PerioChip®, a burst release can be observed (40% of drug released during 24 hours). This burst release is followed by a slow release which results in 27% more released drug at the end of the 1-week observation period. This indicates that the lipid-based systems can provide a continuous release at a higher but steady rate.

Summarizing the results of drug dissolution testing, a sustained release of drugs could be achieved by incorporating them into these lipid-based compositions. The main factors affecting drug release are swelling (driven by the applied hydrophilic components such as polymer, active ingredients and their concentration) and the strength of the coherent lipid structure.

4.1.3. Antimicrobial effectiveness

In the microbiological investigation, where the antimicrobial activity of my formulations was measured six different strains of oral pathogen bacteria, which may contribute to the initiation of periodontitis, were used. These were the following: *E. corrodens*, *P. intermedia*, *P. micra*, *F. nucleatum*, *A. actinomycetemcomitans* and *P. gingivalis*. During the investigation the inhibition zone around the given formulation was measured using fresh agar plates day-by-day. The length of the inhibition was evaluated in case of the different formulations, the results can be found in Figure 3.

According to our measurements, *P. micra* was the most sensitive microorganism to AMX containing formulations as the compositions could provide 18 days of effective drug release, while the least susceptible pathogen was *E. corrodens* with only 9 days of growth inhibition.

MZ susceptibility was slightly lower, as the effect against the most sensitive bacterium *P. gingivalis* was only nine days. *E. corrodens* was the least susceptible to MZ as on the first 2 days no growth could be detected, but after two days there was no inhibition zone around the formulation. This result is in accordance with the literature data, where it was established that *E. corrodens* is resistant to MZ.

Formulation F3 did not provide larger inhibition zones than formulations containing only one antimicrobial agent as the antimicrobial effect lasted for a shorter time. *P. gingivalis* was an exception: this bacterial strain was the only one with higher susceptibility to the combination of AMX and MZ.

In most cases, the growth inhibition effect of combinations lasted longer than that of metronidazole. This could have been possible due to the more potent antimicrobial effect of AMX. Lower susceptibility to the combination of AMX and MZ compared to only AMX containing formulations may be due to the decreased concentration of AMX in the formulation F3 and the lower susceptibility of bacteria to MZ.

Susceptibility to ZnHA is different among various bacterial strains. *A. actinomycetemcomitans* and *E. corrodens* show resistance to ZnHA, but combined with ZnGlu, a longer effect can be observed in case of *A. actinomycetemcomitans*. *E. corrodens* remains unsusceptible to the combination of ZnHA and ZnGlu.

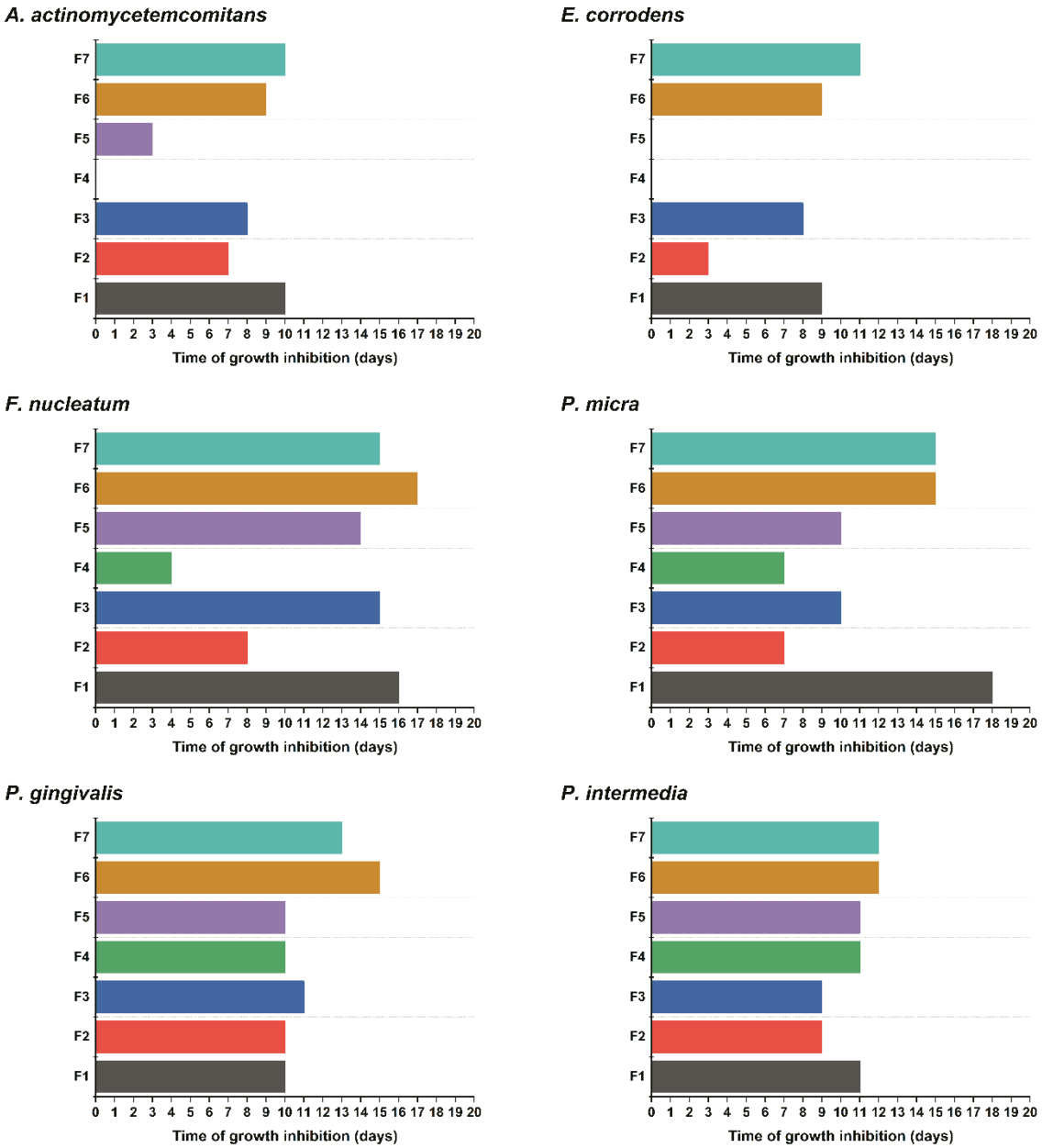

Figure 3. Time of bacterial growth inhibition of formulations F1—F7.

According to the results, the same susceptibility characterizes *P. gingivalis* and *P. intermedia* when using ZnHA alone or in combination with ZnGlu. In case of *P. micra* and *F. nucleatum*, the results show that higher efficiency may be achieved by administering a combination of ZnHA and ZnGlu instead of using only ZnHA.

4.2. PLA-based nanofibers

The metronidazole concentration of the fibers was 12.2 and 25.7 w/w%, the latter corresponding to the metronidazole concentration of the saturated spinning solution. Neat electrospun fiber mats taken directly from the aluminum collector film and round-shaped,

compressed disks were obtained by compressing approximately 10—15 mg of the neat fiber under 1 kN pressure for 30 seconds in a pellet die of 13 mm diameter.

4.2.1. Scanning electron microscopy

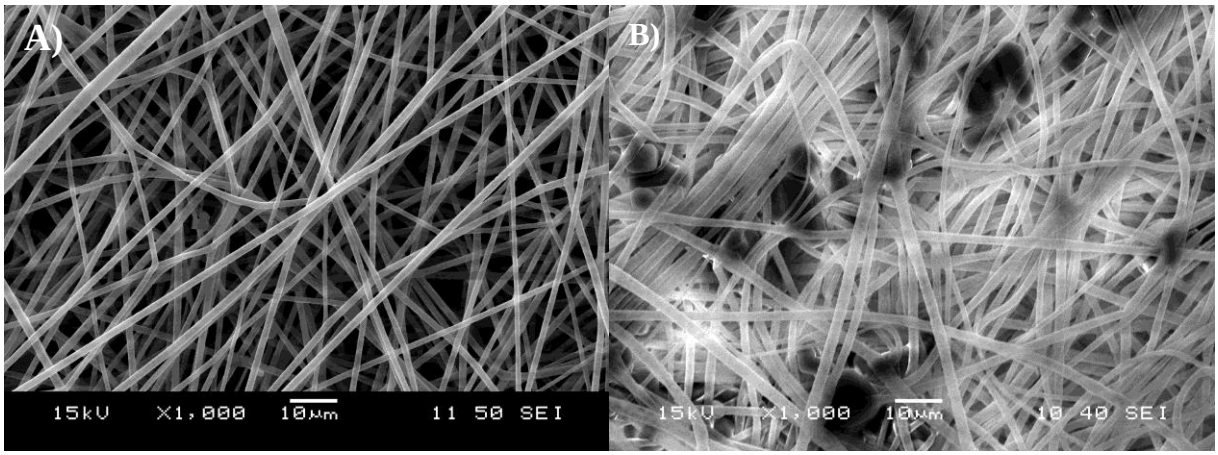

Figure 4. SEM micrographs recorded on the PLA devices studied. A) fiber mat, B) compressed disk. The black dots are crystalline metronidazole particles in picture B).

The structure of the fiber mat and the disks was also studied by scanning electron microscopy. Micrographs showing the differences in structure are presented in Figure 4. The mat consists of loose fibers with considerable space among individual fibers (Figure 4 A). One would expect fast penetration and easy flow of the fluid used for dissolution and thus very fast release of the drug. The scrutiny of micrographs recorded on mats reveals the presence of metronidazole crystals among the fibers. The structure of a disk is shown in the micrograph of Figure 4 B. The disk has a much more compact structure, voids are smaller, and the fibers are close to each other. The presence of metronidazole crystals among the fibers is more obvious in this case. The SEM study proves that a part of the drug is distributed in crystal form among the fibers.

4.2.2. *In vitro* drug diffusion study

The release of the drug incorporated into the devices prepared from electrospun fibers is a complex process and depends on several factors. The PBS solution must penetrate the device, dissolve the crystals and diffuse out into the surrounding medium. On the other hand, dissolved metronidazole must diffuse out of the PLA fibers into the surrounding medium. The solubility of metronidazole is small in PLA, and diffusion is driven by concentration difference, which is also small or even negative due to the large drug concentration of the surrounding solution because of the dissolution of the crystals. Consequently, the main factor determining the dissolution of metronidazole from the devices, i.e. drug release, is the penetration and flow of

the PBS solution. This is different for the two devices, mats and disks, thus dissimilar drug release is expected from them.

The time dependence of dissolution is presented in Figure 5 for the fiber mats and the disks at two different concentrations. In view of the observations presented, some of the results were expected. Compressed disks release the drug much faster than fiber mats because of the larger and faster penetration of the aqueous medium into the pores of the device. However, the fact that dissolution is independent of the initial concentration of metronidazole in the device is somewhat surprising. The fast penetration and dissolution of metronidazole in the PBS solution can result in the independence of concentration. The diffusion of the liquid containing the dissolved drug into the surrounding medium may be the rate-determining step of dissolution in this case.

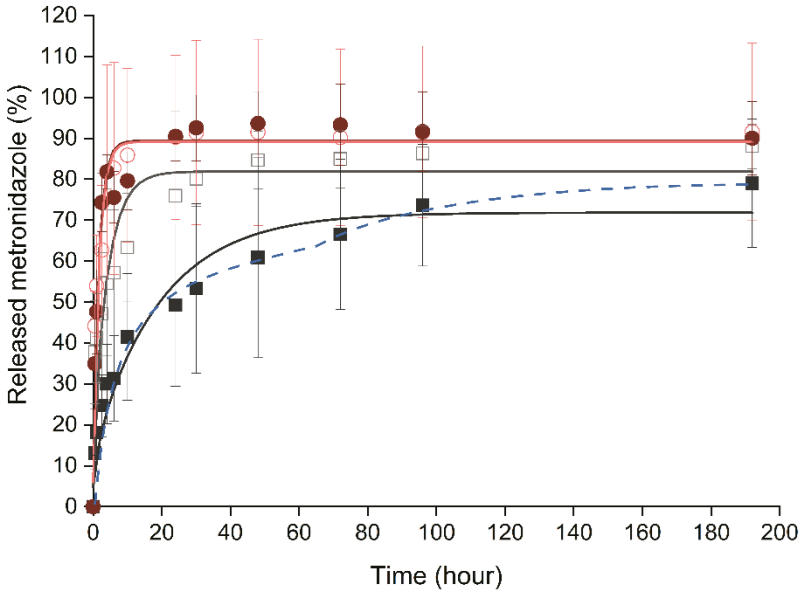

Figure 5. Dissolution of metronidazole from fiber mats and disks. Effect of drug content. Symbols: (□,■) Fiber mats, (○,●) Disks; empty symbols: 12.2 m/m% MZ, full symbol: 25.7 m/m% MZ.

In the case of the fiber mats, the rate of dissolution depends on concentration, however, not as expected, i.e. higher rate at larger concentration, but in the opposite way. The slower release of the drug from the mats can be understood easily if we consider the difference in the penetration of the aqueous medium. Obviously, the dissolution of the drug in the PBS solution and its diffusion into the surrounding medium are the rate-determining steps in this case. However, the presence of the drug cannot influence diffusion rate much, thus dissolution must be dissimilar at the two concentrations of metronidazole. The larger drug concentration probably results in larger precipitated crystals, which leads to slower dissolution and release.

The rate of dissolution can be estimated qualitatively from the correlations presented in Figure 5. According to this evaluation, plateau concentrations are reached after 24 hours for the

disks, and after 48 or 96 hours for the fiber mats. However, time dependence can be evaluated quantitatively if appropriate functions are fitted to the experimental data. The dissolution and the diffusion of the drug are determined by Fick's laws. Fick's equations can be solved numerically, or they can be expressed analytically using simplifications. Two main approaches are used in practice, those describing the first part of the function plotting experimental results as the function of the square root of time, or those which use an exponential function. This approach gives a more accurate estimate at long times and it allows the estimation of the overall rate of dissolution and the maximum amount of dissolved material at infinite time (if the shape and structure of the device do not change, e.g.: fiber degradation). We followed the latter approach and fitted the function of Eq. 1 to the experimental results:

$$M_t = M_\infty \left\{1 - \frac{8}{\pi^2}\left[exp(-at) + \frac{1}{9}exp(-9at) + \frac{1}{25}exp(-25at)\right]\right\} \quad (1)$$

where M_t and M_∞ are the dissolved amount of drug at time t and at infinite time, respectively, and a is the overall rate of dissolution. The parameters calculated from the fitting are collected in **Hiba! A hivatkozási forrás nem található.**.

Table 1. Parameters characterizing the kinetics of dissolution determined by fitting Eq. 1 to the experimental results.

Drug content (w/w%)	Form	a (1/h)	M_∞ (%)	R^2
12.2	mat	0.233	81.9	0.8855
25.7	mat	0.051	71.8	0.9634
12.2	disk	0.581	89.4	0.9449
25.7	disk	0.556	89.1	0.9650

Results presented in Table 1 confirm our qualitative evaluation and show that dissolution is much faster from the disk than from the fiber mat (see parameter a). It also confirms the composition dependence observed. The comparison of the predicted amount of drug dissolved at infinite time (M_∞) indicates that a considerable amount of drug, 10-30% remains in the devices after the dissolution experiment even in the case of the disks. Moreover, a closer comparison of the fitted lines and the measured values indicates that dissolution cannot be described with a single process; it consists of at least two steps, a faster one at the beginning of the experiment and another one proceeding at a slower rate. This two-step process is demonstrated especially well by the results obtained on the fiber mat containing 25.7 wt% metronidazole. The two steps demonstrated by the red broken line in the figure might be explained by the dissolution of the different forms of the drug; crystals located among the fibers dissolve much faster than dissolved metronidazole or crystals precipitated within the fibers. The different rates allow the regulation of the amount of the drug as a function of time and also the

active lifetime of the device. Controlling the form of the drug in the device and the rate of water diffusion into the electrospun porous fiber network might be an efficient strategy to control drug release

4.2.3. Antimicrobial effectiveness

Only disks were included in the microbiological study, as uniform shape, weight and thickness could not have been achieved with the fiber mats. The delivery systems evaluated contained 12.2 and 25.7 w/w% metronidazole. Five different bacterial strains were used in this investigation: *E. corrodens*, *P. intermedia*, *P. micra*, *F. nucleatum*, and *A. actinomycetemcomitans*.

The results of the microbiological study are presented in Figure 6. The duration of the antimicrobial effect is apparently independent of concentration; it is the same for disks with

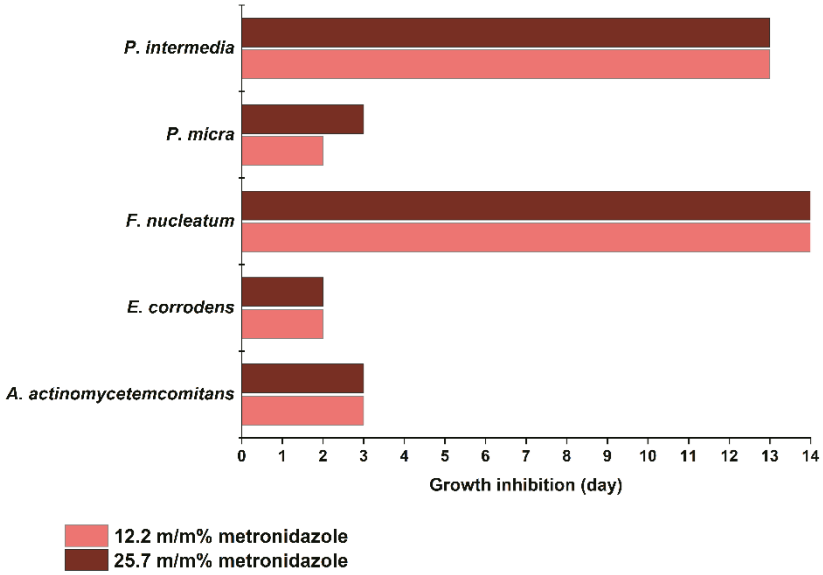

Figure 6. Inhibition of the growth of anaerobic pathogen bacteria. Time is shown in days.

12.2 and 25.7 w/w% metronidazole content in most cases. A one-day difference appeared in growth inhibition for *P. micra* at 12.2 and 25.7 w/w% metronidazole contents. In the other cases, disks with a larger metronidazole content provided slightly larger inhibition zones than systems containing less drug on most days. Bacterial growth inhibition is shorter, only 2—3 days for *A. actinomycetemcomitans*, *E. corrodens* and *P. micra*, while the growth of *F. nucleatum* and *P. intermedia* was affected for a longer time, for 13 days.

The results of our measurements agree well with those published in the literature, suggesting that *F. nucleatum* and *P. intermedia* are more susceptible to metronidazole than *A. actinomycetemcomitans*, *E. corrodens* and *P. micra*, which may be completely resistant or

minimally sensitive to the antimicrobial drug used. The diameter of the inhibition zone increases slightly with increasing metronidazole concentration, probably because of longer diffusion paths resulting in enhanced inhibition. The differences in the diameter of the inhibition zone are more pronounced for strains with larger susceptibility to metronidazole and they increase with time as well. Inhibition was observed in the growth of susceptible bacteria for as long as almost two weeks, indicating that our devices can be efficient for a long time. This fact, however, needs some consideration, since the dissolution study indicated that most of the drug is released from the disks in 24 hours. The contradiction might be explained by the difference in the conditions, but also in the presence of metronidazole located within the polymer in the form of dissolved molecules or precipitated crystals. The diffusion, thus the release rate of metronidazole is much slower in this latter case than for the drug located among the fibers in crystal form. A slower rate leads to prolonged inhibition times, which could result in greater patient compliance and better results of the periodontitis treatment.

5. SUMMARY

The aim of my Ph.D. work was the formulation and investigation of innovative drug delivery systems for the treatment of periodontal disease. Novelty of this work can be summarized as follows:

- The optimal composition of a local, swellable lipid-based drug delivery system was first described, which provides sustained release of incorporated antimicrobial agents and has a mucoadhesive feature.
- The developed lipid formulation enables incorporation of high dose of different antimicrobials with an easy preparation method; moreover, the diffusion of the hydrophilic drugs could be prolonged up to 2 weeks.
- The synergism between the antimicrobial effect of ZnHA and MZ on 6 bacterial strains was proven by bacterial growth inhibition measurements.
- The wettability and water penetration into PLA-based nanofibrous devices were modified by compression, therefore the drug release was also altered.

All of the results of the measurements performed excellently illustrated the potential of the application of the developed lipid-based delivery system, its sustained release and high and long effectiveness against the growth of bacterial strains responsible for the disease. The effectiveness of PLA-based nanofiber disks was also shown, and results indicate that this device is suitable for the incorporation of antimicrobial materials. It was also proven that compression of nanofibers results in a device with uniform shape and size, which is convenient for the local treatment of periodontal disease.

In conclusion, all of the devices described in the thesis have the potential to be used in the treatment of periodontal disease.

PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

I. **Attila Léber**, Mária Budai-Szűcs, Edit Urbán, Péter Vályi, Anita Kovács, Szilvia Berkó, Erzsébet Csányi: Formulation and Investigation of a Lipid Based Delivery System Containing Antimicrobials for the Treatment of Periodontal Disease, *CURRENT DRUG DELIVERY 15(6): 887—897., 2018*

IF: 1.645

II. **Attila Léber**, Mária Budai-Szűcs, Edit Urbán, Péter Vályi, Attila Gácsi, Szilvia Berkó, Anita Kovács, Erzsébet Csányi: Combination of Zinc Hyaluronate and Metronidazole in a Lipid-Based Drug Delivery System for the Treatment of Periodontitis, *PHARMACEUTICS 11(3): 142., 2019*

IF:4.773

III. Mária Budai-Szűcs, **Attila Léber**, Lu Cui, Muriel Józó, Péter Vályi, Katalin Burián, Balázs Kirschweng, Erzsébet Csányi, Béla Pukánszky: Electrospun PLA Fibers Containing Metronidazole for Periodontal Disease, *DRUG DESIGN DEVELOPMENT AND THERAPY 14: 233—242., 2020*

IF:3.208

ABSTRACTS RELATED TO THE SUBJECT OF THE THESIS

I. **Attila Léber**, Mária Budai-Szűcs, Edit Urbán, Péter Vályi, Erzsébet Csányi: Development of a lipid-based drug delivery system containing antibiotics for the treatment of periodontitis, *7th BBBB International Conference on Pharmaceutical Sciences*, 5—7 October 2017, Balatonfüred, Hungary (Poster presentation)

II. **Attila Léber**, Erzsébet Csányi, Mária Budai-Szűcs: Effectiveness of a lipid-based subgingival system for the treatment of periodontitis, *12th Central European Symposium on Pharmaceutical Technology and Regulatory Affairs*, 20—22 September 2018, Szeged, Hungary (Poster presentation)

III. **Léber Attila**: Fogágybetegség kezelésére szolgáló lipid alapú hordozórendszer fejlesztése és vizsgálata, *XIII. Clauder Ottó Emlékverseny*, 22—23 November 2018, Budapest, Hungary (Verbal presentation)

IV. **Attila Léber**, Erzsébet Csányi, Mária Budai-Szűcs: Lipid-based delivery systems for periodontitis treatment, *I. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science*, 31 January 2019, Szeged, Hungary (Verbal presentation)

V. **Léber Attila**, Budai-Szűcs Mária, Vályi Péter, Urbán Edit, Berkó Szilvia, Kovács Anita, Csányi Erzsébet: Antibiotikumot tartalmazó, lipid alapú, helyi hatóanyag-hordozó rendszer vizsgálata, *Gyógyszertechnológiai és Ipari Gyógyszerészeti Konferencia*, 26—28 September 2019, Siófok, Hungary (Poster presentation)

VI. **Attila Léber**, Erzsébet Csányi, Mária Budai-Szűcs: PLA-based nanofibrous systems for the treatment of periodontal disease, *II. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Science*, 23—24 January 2020, Szeged, Hungary (Verbal presentation)