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Research Paper: The Antibacterial Activity of a New Polymeric Local Drug Delivery System against an In-vitro Multispecies Pathogens Associated with Periodontitis

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ABSTRACT

Introduction: The purpose of this study was to evaluate the antibacterial properties of a local delivery system using an in vitro multispecies bacterial model associated with periodontal diseases.

Materials and Methods: Minimum inhibitory concentration (MIC) of effective drugs in periodontitis, doxycycline and metronidazole against Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Provetella intermedia and their multispecies mixture was determined. Drug-loaded polymeric films based on polycaprolactone and alginic acid with four different weight percentages for each drug were fabricated by solvent casting method. Antimicrobial properties of polymeric films against bacterial mixture were evaluated using disc agar diffusion test (DAD), planktonic cell count and biofilm formation. Data were analyzed by one-way analysis of variance (ANOVA), followed by a post-hoc Tukey test.

Results: MIC of metronidazole for A. actinomycetemcomitans, P. gingivalis, and P. intermedia strains were found 64, 2 and 0.25 µg/mL, while MIC of doxycycline were found 4, 0.25, and 4 µg/mL, respectively. DAD test exhibited that all polymeric films had an obvious inhibitory effect on bacterial mixture ranging from 16 to 30 mm. Drug-loaded polymeric films against the above multispecies bacterial model significantly reduced both planktonic cell count and biofilm formation compared to control ($p < 0.05$). Our data showed a reduction of ≥ 3 log CFU/mL in all treated groups until the end of experiment in 72h, showing the bactericidal efficacy.

Conclusion: All polymeric films followed an accepted antibiofilm activity and bactericidal action. Doxycycline-loaded polymeric films were more effective on multispecies bacteria, inhibiting both planktonic and biofilm growth at relatively low concentrations.

Keywords:
Periodontal Diseases,
Drug Delivery Systems,
Biofilms,
Doxycycline,
Metronidazole

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Introduction

It has been proven that there are up to 500 bacterial species in the oral cavity (1). These bacteria create an organized biofilm on the surfaces. Early colonizer species of this biofilm include streptococci, veillonellae and actinomyces (2). These bacteria form the commensal biofilm members in periodontically healthy individuals. When, due to poor oral health, biofilm biomass increases, situation become favorable for the growth and reproduction of anaerobic species. The presence of these bacteria is associated with misdirection of host immune response and increased inflammatory response (3, 4).

Bacteria in the oral cavity shift from commensal to pathogenic bacterial community, in periodontal diseases, and ultimately lead to the destruction of tooth supporting tissues (3). The basic therapy for periodontal disease is mechanical debridement. Although mechanical debridement of periodontal pockets plays an important role in diminishing bacterial load, because of inaccessible area at the depth of complex periodontal pockets, concavities, and furcations, some microorganisms and endotoxins are left behind. Failure in eradication of theses microorganisms may result in bacterial recolonization and treatment failure (5, 6).

Systemic administration of antibiotics appears to be effective in the periodontal therapy, but most studies reported findings showing the use of systemically and prolonged antibiotics, may have potential risks including; the formation of resistant strains and super-imposed infections (5). Consumption of systemic antibiotics may cause side effects such as gastrointestinal disorders (7, 8). In addition, the main problem with systemic antibiotics is that the concentrations obtained in periodontal pockets are too low to affect biofilms (9).

To optimize antibiotic therapy, new local drug delivery (LDD) systems have gained popularity in recent years to tackle these problems. Studies have revealed that the concentration of metronidazole released in gingival crevicular fluid (GCF) after systemic administration may be sufficient to control bacteria in planktonic form, but not enough to control biofilm of bacteria (9). Several experimental and commercial LDD products have been introduced. Most of these drug carriers are gel-based devices. But studies have shown uncertainty about stability of these gel-based devices (10, 11). The reason can be explained by rapid elimination of matrix due to gel form factor (9, 12).

To combat periodontal infections and their outcome for patients, in vitro artificial bacterial systems are used frequently by researchers to perform more specific and reproducible biofilm studies. To mimic the in vivo condition of oral cavity, such models should include an oral multispecies bacterial model (3). This study deals with fabrication of drug-loaded periodontal films using polymers that are biocompatible. Polycaprolactone (PCL) was selected as the main polymer in matrix due to its excellent mechanical properties and flexibility (13, 14). Two basic drugs in the treatment of periodontal diseases, metronidazole from the nitroimidazole family and doxycycline from the tetracycline family, were investigated in our study. Synthesis, characterization, physical properties, and drug release of prepared polymeric films were evaluated in the first phase of this study [15]. To the best of our knowledge, there is no report on the effect of LDD system on the complex of major periodontal pathogens. The aim of this study was to establish a multispecies bacterial model based on anaerobic frequent microorganisms in periodontitis. The anaerobically grown three-species model was used to estimate efficacy of local drug- delivery system.

Materials and Methods

Bacterial strains and culture conditions

Aggregatibacter actinomycetemcomitans ATCC 33384 strain, Porphyromonas gingivalis ATCC 33277 strain and Provetella intermedia ATCC 49046 strain were procured from the Institute of Microbiology (ETH Zurich, Switzerland). The bacteria were routinely cultured in brain heart infusion (BHI) agar (Merck KGaA, Darmstadt,

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Germany), supplemented with 5% defibrinated sheep blood (Sigma-Aldrich Co., Ltd., Dorset, United Kingdom), 5 mg/L of hemin (Sigma-Aldrich Co., Ltd., Dorset, United Kingdom), and 1 mg/L of vitamin K (Sigma-Aldrich Co., Ltd., Dorset, United Kingdom), 5 g/L of yeast extract (Merck KGaA, Darmstadt, Germany). A. actinomycetemcomitans was cultured in microaerophilic conditions (<20% O2) for 48 hours at 37°C. The other two bacterial strains; P. gingivalis and P. intermedia were cultured under anaerobic conditions (80% N2, 10% H2, 10% CO2) at 37°C. The optical densities of each of the three bacterial cultures were measured by spectrophotometer (Eppendorf BioPhotometer, Hamburg, Germany) and adjusted to (OD)600: 0.1 to final concentration of 1.0 ×108 colony forming units (CFU/ml). To obtain a multispecies bacterial model, suspension cultures of OD600 = 0.1 of three individual species, were mixed equally.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of antibiotics

Metronidazole and Doxycycline were purchased from Sigma-Aldrich (St Louis, MO). The MICs and MBC of each antibiotic solution of Metronidazole and Doxycycline were determined by broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) and International Organization for Standardization (ISO) (16, 17). Briefly, 50 µl of 2× BHI broth (Merck KGaA, Darmstadt, Germany) was added to each well of a round-bottom 96-well microplate (TPP AG, Trasadingen, Switzerland), and then 50 µl of final concentration of 512 µg/ml stock solutions of each antibiotic (doxycycline & metronidazole) was poured into the wells of the first column and each time was diluted two-fold stepwise from each column to the adjacent column to yield the concentration of 0.125 µg/ml. Finally, 50 µl of bacterial suspensions were inoculated in supplemented BHI broth to yield the concentration of 1.5×106 CFU/ml. Addition of 50 µl of bacterial suspensions to the antibiotic-containing wells resulted in bacterial density of 7.5×105 CFU/ml. The microplate was incubated at 37 °C under anaerobic condition for 48 h. In this study, the wells containing only bacterial suspension were used as positive control, and the wells containing supplemented BHI broth without bacterial suspension was considered as negative controls. The MIC was defined as the lowest concentration of antibiotic at which no visible growth could be detected in wells. 10 µl from the MIC wells and adjacent wells were spread across the surface of a supplemented BHI agar plate and incubated for 48 h. The lowest concentration of antibiotic at which no growth of the bacterial colony was observed at the surface of supplemented BHI agar plate, was considered as MBC.

Preparation of drug-loaded films

In this study, a new local drug delivery system based on polycaprolactone and alginic acid was designed for three periodontal bacteria. They were prepared using solvent casting technique. The system employed dichloromethane, dibutyl phthalate, and Pluronic F-127 as solvent, plasticizer, and surfactant, respectively. The details of synthesis and characterization of prepared polymeric films were explained in our previous study (15). According to the results of MIC and MBC that we evaluated in the initial step of study and the findings of Kulik et al. (18) and Kopytynska-Kasperczyk et al.(19) , higher value of metronidazole was considered for metronidazole-loading formulations versus doxycycline-loading formulations. Four formulations with different drug weight percentages (0.5, 1, 2 and 4%) were prepared for doxycycline which were named as D1-4, and four formulations with different drug weight percentages (3, 5, 9 and 13%) were prepared for metronidazole which were named as M1-4. The polymeric films were sterilized using gamma rays with dose of 25 kGy with 1.62 Gy/sec dose rate (Gamma Cell GC-220 Irradiator).

Evaluation of antibiotic-loaded film against multispecies model in planktonic form

1 ml of multispecies bacterial model were allo-

cated in each microtube and polymeric film of different groups were put in each microtube and placed at 37 ºC under anaerobic atmosphere. At various time points, 1, 3, 6, 12, 24, 48 and 72 hours, 10 µl of the culture medium was sampled and cultured on supplemented BHI agar plates and incubated at 37 ºC. The number of CFU/ml was evaluated after predetermined incubation time, based on a method demonstrated by Miles et al. [20]. Each experiment was performed in triplicate. Percent reduction of the bacteria was measured using the following equation;

$$\text{reduction}(\%) = \frac{N_{initial} - N_{\text{after each time-point}}}{N_{initial}} * 100$$

Where Ninitial is initial baseline amount of the bacteria, and Nafter each time-point is the number of bacteria after incubation under anaerobic atmosphere after predetermined time. Data were analyzed by one-way analysis of variance (ANOVA), followed by a post-hoc Tukey test.

Evaluation of antibiotic-loaded film in terms of biofilm formation ability against multispecies model

The biofilm formation on the surface of the polymeric films was evaluated for each film placed in the tubes containing bacterial multispecies suspensions. After 48h, polymeric films were aseptically removed from tubes and transferred to tubes containing 5ml of 0.9% normal saline and washed to remove non-adherent planktonic suspension of bacteria. Then, polymeric films were sonicated and vortexed in 1 ml normal saline for 15 seconds with high speed (100W,30KHz).

Aliquots of 10 µl of the resulting media were inoculated on BHI agar and incubated at 37 ◦C under anaerobic atmosphere for 48h. The number of Colony forming units was evaluated and expressed as CFU/ml according to method developed by Miles et al. [20]. Each experiment was run in triplicate. Data were analyzed by one-way analysis of variance (ANOVA), followed by a post-hoc Tukey test.

Evaluation of disc agar diffusion test (DAD) of antibiotic-loaded films against multispecies model

In this procedure, the agar plate surfaces were inoculated by spreading a volume (10 µl) of multispecies bacterial model over the whole supplemented BHI agar surface. After that, sterile drug-loaded films (discs with 5 mm diameter) were placed on supplemented BHI agar surface. The petri dishes were incubated at 37 ºC under anaerobic atmosphere for 48 h. Then, the diameters of inhibition growth zones were measured.

Results

MIC and MBC

The results of MIC and MBC tests are shown in table 1. The MIC of doxycycline (0.125 µg/mL) against P. gingivalis was 16-fold less than for metronidazole (2 µg/mL). The MIC of doxycycline (0.125 µg/mL) against A. actinomycetemcomitans was also 32-fold less than for metronidazole (64 µg/mL). whereas The MIC of doxycycline (2 µg/mL) against P. intermedia was 16-fold higher than for metronidazole (0.25 µg/mL). The MBC of doxycycline (8 µg/mL) against bacterial mixture was also 16-fold less than that of metronidazole (128 µg/mL).

Planktonic growth assay

Drug-loaded formulations significantly reduced log CFU/mL of planktonic multispecies culture compared to that of control group (no treatment) ($p<0.05$) (Table 2, Figure 1). Pairwise comparison revealed that metronidazole-containing formulations (M1-3) and doxycycline-containing formulations (D1-3) presented a significant reduction after 12h compared to control group. However, this significant reduction occurred after 6h for M4 and D4 compared to control group (Figure 2). Killing percentage of planktonic multispecies model by M1 was 90% at 12 h and reached to 99.9% (3 log reduction) at 72h. while M4 showed 90% reduction at first 6h and reached to 100% at 72h (Table 2). Percent reduction of planktonic multispecies model by D1 was approximately 90% at 12

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h and reached to 99.9% (3 log reduction) at 72h, while D2 showed 90% reduction at first 6h and reached to 99.99% at 72h. D3 and D4 showed 90% reduction approximately at first 6h, 99.9% at 24h and reached to 100% at 48h.

Table 1. MIC and MBC of Metronidazole and Doxycycline against periopathogenic bacteria

Bacteria	Metronidazole		Doxycycline	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
P. gingivalis (ATCC 33277)	2	4	0.125	0.25
A. actinomycetemcomitans(ATCC 33384)	64	128	2	4
P. intermedia(ATCC 49046)	0.25	0.5	2	4
Mixed	64	128	4	8

Table 2. Number of planktonic viable cells (log CFU/mL) and planktonic growth reduction percentage during exposure to drug-loaded polymeric film.

Groups	Mean of [CFU/ml ± SD] & [% reduction ± SD]							
	Time (hour)							
	baseline	1	3	6	12	24	48	72
M1 %reduction	6.64±0.02	6.61±0.02 6.87±0.24	6.41±0.06 40.30±9.39	6.18±0.09 64.87±6.86	5.54±0.03 92.04±0.85	4.31±0.06 99.53±0.05	4.04±0.07 99.75±0.03	3.22±0.17 99.96±0.01
M2 %reduction	6.64±0.02	6.60±0.02 9.17±0.32	6.48±0.04 31.12±8.40	6.28±0.05 56.49±4.28	5.71±0.03 88.25±0.68	5.32±0.09 95.21±0.89	4.51±0.04 99.26±0.05	2.65±0.16 99.99±0.003
M3 %reduction	6.64±0.02	6.60±0.02 9.10±4.44	6.48±0.04 31.36±4.09	6.28±0.05 56.52±3.78	5.71±0.03 88.21±1.33	5.23±0.07 96.09±0.75	4.51±0.04 99.26±0.08	2.44±0.42 99.99±0.01
M4 %reduction	6.64±0.02	6.59±0.01 11.39±3.61	6.42±0.05 38.75±8.96	5.50±0.05 92.76±0.66	4.41±0.07 99.40±0.10	3.41±0.07 99.94±0.01	1.16±0.28 99.99±0.0002	0.00 100.00
D1 %reduction	6.64±0.02	6.63±0.02 3.05±1.30	6.55±0.04 17.68±5.46	6.28±0.05 56.41±5.18	5.71±0.03 88.26±0.52	5.22±0.08 96.20±0.57	4.51±0.04 99.26±0.07	3.13±0.10 99.97±0.01
D2 %reduction	6.64±0.02	6.61±0.03 7.69±2.82	6.54±0.02 21.30±4.67	5.61±0.03 90.62±0.37	5.17±0.11 96.54±0.93	4.28±0.02 99.56±0.04	2.82±0.07 99.98±0.003	1.59±0.11 99.99±0.0002
D3 %reduction	6.64±0.02	6.61±0.02 7.60±3.40	6.47±0.03 32.67±6.94	5.59±0.05 91.12±1.39	4.54±0.03 99.21±0.03	3.45±0.07 99.93±0.01	1.10±0.17 99.99±0.0001	0.00 100.00
D4 %reduction	6.64±0.02	6.59±0.01 10.67±1.00	6.41±0.07 40.95±11.43	5.42±0.07 93.90±0.83	4.37±0.03 99.46±0.05	3.11±0.09 99.97±0.01	0.00 100.00	0.00 100.00

(D1-4), different formulations of doxycycline; (M1-4), different formulations of metronidazole.

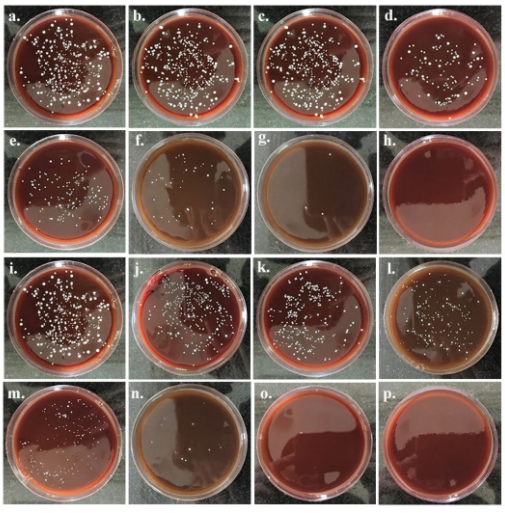

Figure 1. Photographs of plates in treated group. (a-h) related to M4 group; a) control, b) 1h, c) 3h, d) 6h, e) 12h, f) 24h, g) 48h, and h) 72h exposure to multispecies bacterial model. (i-p) related to D4 group; i) control, j) 1h, k) 3h, l) 6h, m) 12h, n) 24h, o) 48h, and p) 72h exposure to multispecies bacterial model.

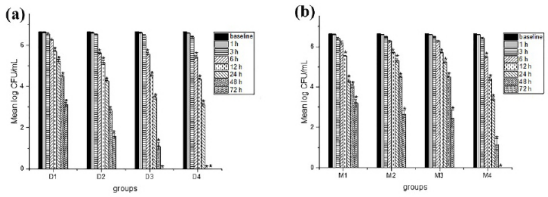

Figure 2. Antimicrobial effect of doxycycline-loaded formulations (a), and metronidazole-loaded formulations (b) against multispecies bacterial strains. *, Significantly different from control (no treatment).

Biofilm formation assay

The formation of mature multispecies biofilms on the surface of different drug-loaded polymeric films were compared with polymeric film without drug-loading as control group in the present study. One-way ANOVA followed by Tukey's post-hoc test showed a significant reduction of biofilm formation by incorporation of drugs to polymeric films in four percentages of both drugs ($p = 0.00$). However, the differences were not statistically significant for D1 and D2 ($P= 0.102$). Based on the results of this study, the efficiency of incorporation of 5% metronidazole (M2) to polymeric film was not significantly different from 9% (M3) in metronidazole group ($p = 0.791$). Furthermore, there was not found a significant difference between M3 and M4 ($p=0.337$).

In the biofilm model, drug-loaded polymeric film achieved >4.0 log reduction (>99.99%) for all four tested formulations in each antibiotic (Table 3). Bactericidal effect was highlighted for doxycycline compared to that of metronidazole.

Table 3. Biofilm formation ability and the results of disc agar diffusion test of different groups against multispecies bacterial model.

Groups	Biofilm formation after 48h		DAD test
	CFU/ ml	log	Inhibition zone (mm)
baseline	$4.40 \pm 0.30 \times 10^6$	6.64±0.03	-
M1	$2.11 \pm 0.15 \times 10^2$	2.32±0.03	16
M2	$1.56 \pm 0.09 \times 10^2$	2.19±0.02	20
M3	$1.33 \pm 0.08 \times 10^2$	2.12±0.03	20
M4	$1.06 \pm 0.09 \times 10^2$	2.03±0.04	28
D1	$1.96 \pm 0.10 \times 10^2$	2.29±0.02	17
D2	$1.45 \pm 0.11 \times 10^2$	2.16±0.03	23
D3	$0.91 \pm 0.09 \times 10^2$	1.96±0.04	25
D4	$0.44 \pm 0.13 \times 10^2$	1.63±0.13	30

DAD test

As shown in Table 3, all prepared drug-loaded films revealed an inhibition zone around polymeric film. D4 formulation presented highest inhibition zone followed in a descending order by M4, D3, D2, M3 = M2, D1, and M1.

Discussion

Mechanical debridement is considered as a first step for treatment of periodontal disease. Although this treatment has resulted significant clinical improvements, its success is limited by the inaccessibility to the depth of periodontal pockets and fistulas. The pathogenic microorganisms left behind after mechanical debridement can provide a potential source of recolonization in subgingival biofilms [21].

Local drug delivery (LDD) system has gained popularity in recent years. This system provides an alternative to systemic administration of antibiotics, decreasing side effects such as developing resistant strains, low drug level at the target site, gastrointestinal disorders, etc. Some commercial products have been introduced for treatment of periodontitis. Most of drug carriers are gel-based products. But studies have shown that gel matrix disappear within a few days and loses its ability to drug release. The reason can be attributed to the state of the topical gel shape (9, 12, 22). Furthermore, flow of gingival crevicular fluid (GCF) may also resulted in uncertainty about stability of gel-based systems.

We developed and characterized drug-loaded polymeric films, having acceptable physical properties and drug release in our previous study (15). The pre-existing situations of the oral cavity such as humidity, pH fluctuations, thermal alterations, and chewing forces require precise polymer selection. In the current study, polycaprolactone (PCL), which is a biocompatible polymer was selected as the main polymer in matrix due to its excellent mechanical properties, flexibility, and elongation at break (13, 14). Alginic acid was incorporated as the second hydrophilic antibacterial polymer to enhance water penetration, helping drug release. Having mechanical properties may help the device maintain itself during the experiment and does not disintegrate. According to the previous study, these polymers revealed higher tensile strength (15) than similar articles in this regard (23, 24).

P. gingivalis has been reported to be increased to a large extent in sites with periodontitis and the number of P. gingivalis was found very low in healthy periodontal tissues. This bacterium usually inhabits in deeper periodon-

tal pockets compared to shallow pockets (25).

There is evidence about A. actinomycetemcomitans that confirms its role as the causative agent of localized aggressive periodontitis. This gram-negative bacterium can colonize in the oral environment, invade the epithelial cells, initiate connective tissue destruction and interfere with tissue repair. A. actinomycetemcomitans is able to evade host defenses by means of modulating inflammation, inducing tissue destruction and interfering with tissue repair (26-28).

It has also been proven that P. intermedia may contribute to destruction of periodontium in chronic periodontitis by inducing human periodontal ligament cells to express matrixmetalloproteinase-9 (MMP-9) (29). In a study conducted by Ebadian et al., P. intermedia was recognized as one of the common species in chronic periodontitis (90.9%) in the Iranian population (30). Thus, these three bacteria were chosen for this project.

In the present study, MIC and MBC of each antibiotic against P. gingivalis, A. actinomycetemcomitans and P. intermedia and their mixture were calculated (Table 1). The observations were consistent with the study conducted by Jai et al. (31), which reported susceptibility of P. gingivalis to doxycycline was higher compared to A. actinomycetemcomitans. Moreover, Oettinger-Barak et al. (32) reported the most frequently prescribed antibiotic regimen, amoxicillin-metronidazole, was found less effective against both planktonic and biofilm cells of A. actinomycetemcomitans JP2 strain rather doxycycline. Doxycycline with a lower MIC and minimal biofilm inhibitory concentration could inhibit this bacterium. Our data revealed that susceptibility of A. actinomycetemcomitans to doxycycline was 32-fold higher compared to metronidazole antibiotic.

The present study established a multi species bacterial model based on anerobic periodontal pathogens. To the best of our knowledge, this is the first study that evaluated efficacy of a LDD system against a periodontopathic multi species model in terms of planktonic growth and biofilm formation assay. Other studies in this area have only limited to disc agar diffusion test in order to investigate the efficiency of the experimental drug carriers (31, 33-36).

Biofilms can complicate treating periodontal diseases by protecting bacterial species from the immune system, and reducing antibacterial effectiveness (32). Thus, investigation of biofilm formation against a LDD system is of great interest. This study addresses biofilm formation ability of drug-containing polymeric film as well as planktonic growth and inhibition zone.

The results of Phaechamud et al. (33) was in agreement with our study that Doxycycline-containing formulations revealed higher inhibition zone against P. gingivalis, P. intermedia and A. actinomycetemcomitans mixture compared to that of Metronidazole-containing formulations, even with lower drug loading. This is also consistent with the results of Table 1. Furthermore, Bottino et al. showed that metronidazole-containing fibrous matrices was inhibited A. actinomycetemcomitans by a dose-dependent process in disk agar diffusion test. (34). Oure data also followed a dose-dependent manner.

Another study published by Reise et al. (35) showed that similar drug-loaded electrospun fibers containing metronidazole are able to inhibit bacterial strains without showing a cytotoxic effect on gingival fibroblasts. The fibers loaded with metronidazole created a larger inhibition zone against the P. gingivalis compared to A. actinomycetemcomitans. This finding was in agreement with our study that metronidazole showed higher MIC against A. actinomycetemcomitans compared to that of P. gingivalis. Thus, a greater amount of this drug is needed to inhibit A. actinomycetemcomitans.

It has been reported that doxycycline is efficacious against most of periodontal pathogenic strains with a lower MIC (19, 37) in comparison with metronidazole (38). However, there are some concerns about cytotoxic effects of doxycycline in high concentrations on fibroblasts (39, 40). According to a study by Tsukuda et al., concentrations of doxycycline ≥ 25 µg/ml significantly decreased the number of adherent

fibroblasts to the protein-coated surface. Lactic dehydrogenase assay revealed concentration higher than 50 µg/ml of doxycycline resulted in detachment of fibroblasts from the surface and increased the risk of cytotoxicity (41).

Our previous study exhibited D3-4 formulations released very high amounts of drug and presented brittleness and reducing strength of doxycycline-containing films with higher drug concentrations (15). This unnecessary release, based on MIC of periodontal bacteria (Table 1) was also susceptible to cytotoxicity risks. Thus, regarding the increased risk of cytotoxicity as well as low MIC for doxycycline, the least amount of this drug is preferred in the formulation.

Jai et al. developed a doxycycline loaded electrospun membrane, which revealed inhibition effect on disk agar diffusion assay. Their results (diameter of inhibition zone) in 15% drug loading was comparable with our findings with 2% loading and higher (31). Thus, concentration of drug in eluent releasing from polymeric film during the experiment was definitely above 4 µg/mL to effectively inhibit the growth of multispecies bacterial model. With a lower drug loading percent, a larger inhibition zone was obtained in our study.

Reducing the number of live bacteria ≥3 log CFU / ml at the time of incubation is known as bactericidal activity (42). Therefore, according to table 2, bactericidal efficacy yielded after 48 h for M4 and after 72h for other metronidazole-loaded films. And about doxycycline-loaded films, bactericidal efficacy was obtained after 24 h for D3 & D4. Whereas this time was 48h for D2 and 72h for D1.

Our data showed a reduction of between ≈ 1 and 2.27 log10 CFU/mL in all treated groups at 12h, producing bacteriostatic action corresponding to ≥1 - <3 log10 reduction in colony count [43]. Polymeric films exhibited considerable antibiofilm ability rather control group. All of treated groups revealed acceptable antibacterial efficacy in terms of planktonically grown, biofilm formation, and inhibition zone. But, formulations D3-4 were ignored for clinical use due to their brittleness and decreased physical properties based on the previous study (15).

The present study showed the potential for application of drug-loaded periodontal films as a promising control release system, which can be used as an adjunctive therapy after mechanical debridement to reduce microbial load after mechanical debridement. The mechanical properties of this device enable it to be designed in the form of a ring around the tooth and then removed after 3-7 days.

The introduced system can tackle the problems of gel-based system due to rapid elimination. Considering that there is no drug interaction between these two drugs, it is recommended to evaluate combination of these substances to take advantage of both medications and/or possible synergic effect. Furthermore, cytocompatibility of this drug carrier system deserve further research and remain to be investigated in the future studies.

Conclusion

Based on the results of this study, exposure of multi species bacterial model (A. actinomycetemcomitans P. gingivalis, and P. intermedia) to drug-loaded polymeric films affect planktonic growth and biofilm formation ability in a dose dependent manner. This approach can be considered as useful can¬didate for periodontal strains eradication without the side effects of systemic antibiotic administration. Doxycycline-loaded polymeric films revealed greater impact against bacterial model rather those of metronidazole-containing films.

Conflicts of Interest

The authors of this manuscript declare that they have no conflicts of interest, in this article.

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