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Research Article

Formulation and Evaluation of PLA and PLGA *In Situ* Implants Containing Secnidazole and/or Doxycycline for Treatment of Periodontitis

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Abstract. The purpose of this study is to formulate *in situ* implants containing doxycycline hydrochloride and/or secnidazole that could be used in the treatment of periodontitis by direct periodontal intrapocket administration. Biodegradable polymers [poly (lactide) (PLA) and poly (lactide-co-glycolide) (PLGA)], each polymer in two concentrations 25%w/w, 35%w/w were used to formulate the *in situ* implants. The rheological behavior, *in vitro* drug release and the antimicrobial activity of the prepared implants were evaluated. Increasing the concentration of each polymer increases the viscosity and decreases the percent of the drugs released after 24 h. PLA implants showed a slower drugs release rate than PLGA implants in which the implants composed of 25% PLGA showed the fastest drugs release. The *in vitro* drug release and antimicrobial activity results were compared with results of Atridox®. Results revealed that the pharmaceutical formulation based on 25% PLGA containing secnidazole and doxycycline hydrochloride has promising activity in treating periodontitis in comparison with Atridox®.

KEY WORDS: Atridox®; biodegradable polymers; doxycycline; implants; secnidazole.

INTRODUCTION

Periodontal diseases are a group of inflammatory conditions affecting the supportive structures of the teeth characterized by destruction of the periodontal ligament, resorption of the alveolar bone and the migration of the junctional epithelium along the tooth surface with the formation of a space (pocket) between the gum and the teeth and can eventually cause tooth loss. The formed pocket provides an ideal environment for the growth and proliferation of microbes (1).

Current periodontal therapy includes the removal of the bacterial deposits from the tooth surface and shifting the pathogenic microorganism to healthy one. Conventional root planning and scaling relies on the mechanical debridement of tooth surfaces as the primary antimicrobial measure (2). However, as specific bacteria are thought to play a major role in disease process, antimicrobial agents have been used as adjuncts to mechanical treatment (3). The potential side-effects in administering systemic antibiotics and the inability of antiseptic mouthwashes to penetrate the periodontal pocket have raised interest in the localized delivery of therapeutic agents within the periodontal pocket, thus ensuring a high effective concentration of antimicrobial agent at the site of infection.

The use of drug delivery systems based on biodegradable polymers that can achieve a sustained release of the drug over days had been used in the treatment of periodontal diseases (4,5), with the advantages of self-elimination, avoiding the need to remove the polymer system from the site of implantation after its use (6). Among the biodegradable polymers used, poly (lactic acid; PLA) and copoly (lactic: glycolic acid; PLGA) drug delivery systems are prepared by dissolving the polymers in biocompatible organic solvents. Drug is added into the polymer solution, where it forms a homogenous solution or a suspension depending on its solubility. The drug suspension or solution is injected subcutaneously, forming an "*in situ* implant", which slowly releases the drug over time. Organic solvents are fully or partially water-miscible, and hence the *in situ* implants were formed by a non-solvent induced phase separation mechanism (7).

Tetracyclines and metronidazole were reported to be effective for the treatment of periodontal diseases (8,9). Doxycycline hydrochloride is chosen for this study due to its activity against putative periodontal pathogens, and is the most potent for collagenase inhibition (10,11). Secnidazole is chosen due to its activity against anaerobic microorganisms, and has a longer terminal elimination half-life than metronidazole (12). The objective of this study is to formulate *in situ* implants containing both secnidazole and doxycycline hydrochloride to increase the spectrum of the antimicrobial activity against the microorganisms causing periodontal diseases (aerobic and anaerobic) in comparison to Atridox®.

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MATERIALS AND METHODS

Materials

Secnidazole (SC) was kindly supplied by EPICO, Cairo, Egypt. Doxycycline hydrochloride (DH) was kindly supplied by El-Nile pharmaceutical Company, Cairo, Egypt. Poly (D,L-lactide) (PLA; Mw=75,000–120,000) and poly (D,L-lactide-co-glycolide) (PLGA; lactide/glycolide ratio is 50:50, Mw=40,000–75,000), *N*-methyl-2-pyrrolidone (NMP) were purchased from Sigma Chemical Co., USA. Paper points were purchased from El Gomhorya, Cairo, Egypt, gas packs were purchased from Basingstoke, England. All other used materials were of analytical grades. Atridox® (doxycycline hyclate, 10% in the Atrigel® Delivery System; Atrix Laboratories) (13).

Methods

Preparation of PLA and PLGA In Situ Implants

The calculated amount of either PLA or PLGA to prepare 25% *w*/*w* and 35% *w*/*w* of each polymer were dissolved in *N*-methyl-2-pyrrolidone (NMP) by horizontal shaking of filled glass vials (20 ml) at 37°C, then 5% *w*/*w* of secnidazole and/or doxycycline hydrochloride was added to the formed solution and mixed. Solutions were stored overnight in refrigerator prior to experimentations (14). All preparations were evaluated freshly prepared to avoid the epimerization of DH and loss of activity (15).

Rheological Measurements and Data Analysis

Steady shear measurement was conducted where the rheograms of the prepared solutions was performed at 25± 0.1°C using cone and plate programmable viscometer (Brookfield Engineering Laboratories Inc., Model HADV-II, USA) connected to a digital thermostatically controlled circulating water bath (Polyscience, Model 9101, USA) with spindle 52, the shear rates range from 50 to 400 s^{-1} corresponding to 25 to 200 rpm with 10 s between each two successive speeds and then in a descending order. Equilibration of the sample for 5 min was made following loading of the viscometer. Ramp time for each viscosity stage was reading after 20 s. All studies were performed in triplicates and the average was taken.

Rheological data was fitted to different models (Bingham, Power law, Casson) to examine the pattern of flow and the presence of yield value (16).

- Bingham:

$$\tau = \tau o + \eta\gamma \quad (1)$$

- Power law:

$$\tau = \eta\gamma^n \quad (2)$$

- Casson:

$$\tau^{1/2} = \tau o^{1/2} + \eta^{1/2}\gamma^{1/2} \quad (3)$$

Where τ is the shear stress, τ_0 the yield value, η a constant called the apparent viscosity or the consistency index, γ the shear rate and n is the flow index. In case of Newtonian behavior $n=1$ and $\tau_o=0$, whereas in case of pseudoplastic (shear thinning) behavior $0<n<1$ and $\tau_o=0$; for plastic behavior it is the same as pseudoplastic but with $\tau_o>0$, while in case of dilatant flow (shear thickening) $n>1$. The software employed was Graph Pad Prism® version 4 and the level of significance was set at 5%.

In Vitro Drug Release from PLA and PLGA In Situ Implants

The *in vitro* release was performed using shaking water bath (Köttermann, Germany), in which 0.5 g of the prepared solutions were placed in 20-ml vials, then 10 ml phosphate buffer pH 6.8 (to simulate the gingival crevicular fluid) (15) was added thereafter. The sample vials were shaken in sealing condition to avoid partial evaporation of the fluids at 37°C± 0.1°C with a constant agitation (100 oscillation/min) (14).

One-milliliter samples were withdrawn and replaced with equal volumes of fresh buffer.

UV Measurements of SC and DH

Samples were diluted and measured spectrophotometrically (Shimadzu UV-1601 PC, double beam spectrophotometer, Kyoto, Japan) at λ_{max}=320 nm for SC and 274 nm for DH (when each drug used alone), Fig. 1. For implants containing the two drugs together, first derivative spectra was constructed, Fig. 2, in which SC can be measured at 350 nm and DH can be measured at 264 nm.

Drugs Release Data Analysis

Drug release data generated from the release study were fitted to the following general release equation (17) using logarithmic transformations and least squares regression analysis:

$$M_t/M_\infty = Kt^n \quad (4)$$

$$Log(M_t/M_\infty) = LogK + n\ Log\ t \quad (5)$$

Where M_t/M_∞ is the fraction of released drug at time t; k the release constant incorporating structural and geometrical

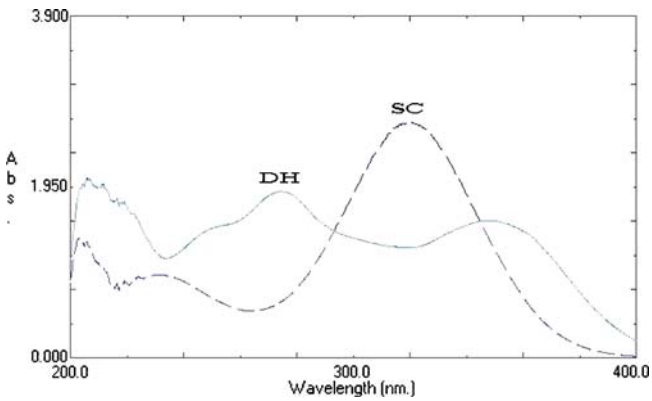

Fig. 1. Ultraviolet spectra of SC and DH in phosphate buffer (pH 6.8)

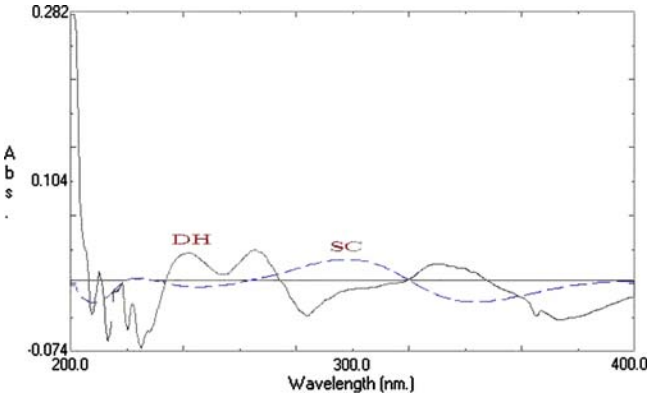

Fig. 2. First derivative spectra of SC and DH in phosphate buffer (pH 6.8)

characteristics of the delivery system; n is the release exponent, a measure of the primary mechanism of drug release.

Microbiological Evaluation of the Selected Formulations

Patient's specimen. This study included 20 patients suffering from periodontal diseases were randomly chosen from the flow of the Outpatient Diagnosis Clinic, Department of Oral Medicine, Diagnosis and Periodontology, Faculty of Dentistry, Ain Shams University. After removing of supragingival plaque, two paper points were inserted into a periodontal pocket for 10 s. The first paper point was inoculated on peptone water as transport medium for aerobes and the second paper point was inoculated on Robertson cooked meat broth, as transport medium for anaerobes (18).

Isolation and Identification of The Microorganisms. The samples were cultured aerobically from peptone water on Colombia blood agar plate and cultured anaerobically on Colombia blood agar plate with added kanamycin at concentration of 75 μg/ml (Kanamycin is selective for obligate anaerobes). The first plate was incubated aerobically at 37°C for 24 h while the second plate was incubated anaerobically in anaerobic jar at 37°C for 2–4 days. Identification of isolated colonies was done using Gram stain, morphology, UV lamp and biochemical reactions (19).

Antimicrobial Activity of the Selected Formulations of SC and DH on the Isolated Micro-organisms. Inoculums of 1.5 ml of a suspension containing each identified isolated organism was transferred to sterile Petri dish, then 30 ml of Mueller Hinton agar was added to the inoculums and mixed well and left to solidify (20). Wells were done by punching stainless steel cylinders (internal diameter 11 mm) onto the plate and removing the agar to form a well. Each well was aseptically filled with accurate weight of the selected tested formulation. For aerobic organisms; the Mueller Hinton plates were incubated aerobically at 37°C for 24 h. For anaerobic organisms the plates were incubated anaerobically in anaerobic jar at 37°C for 24 h. After incubation, the inhibition zone diameter was measured using a ruler. The extent of release (zone of inhibition) was measured by taking the average of three readings and compared with the inhibition zone formed by the global product Atridox®. Results were analyzed using one-way ANOVA followed by *post hoc* statistical analysis performed by Tukey–Kramer test for multiple comparisons. The software employed was Graph Pad Instat® V2.04 and the level of significance was set at 5%.

RESULTS AND DISCUSSION

Rheological Measurements and Data Analysis

The rheological parameters of the prepared formulations, namely, the flow index (n) the consistency index (η) and the apparent viscosity measured at shear rate 50 s^{-1} at 25°C are shown in Table I. The rheological data of the different formulations are best fitted to power law model. Based on the values of n, it is obvious that all the formulations exhibit nearly Newtonian behavior except 35% PLA solutions which have n values in the range of 0.8, indicating that 35% PLA solutions have a shear thinning (pseudoplastic) behavior. All formulations show a non-thixotropic behavior where the up and down curves coincide [examples of rheograms are shown in Figs. 3 and 4]

Table I. The Rheological Parameters of Different Prepared Formulations

Formula		Drug	Flow index (n)	Consistency index (K)	Regression coefficient (r^2)	Viscosity (P)[a]
PLA	25%	SC	0.997	7.4958	0.9999	743±34.80
		DH	0.992	7.5038	1	721±31.30
		SC + DH	0.995	7.4994	1	730±23.45
	35%	SC	0.811	55.116	0.9842	1940±70.50
		DH	0.818	55.717	0.9855	2044±55.20
		SC + DH	0.815	55.408	0.989	1850±40.54
PLGA	25%	SC	0.992	4.6207	1	442±16.80
		DH	0.965	5.4837	0.9998	455±21.70
		SC + DH	0.978	5.0401	1	445±18.76
	35%	SC	0.985	5.8748	1	542±34.50
		DH	0.990	5.3376	1	507±23.60
		SC + DH	0.987	5.6055	1	530±21.78

[a] Average of three determination ± standard deviation, measured at shear rate 50 s^{-1} at 25°C

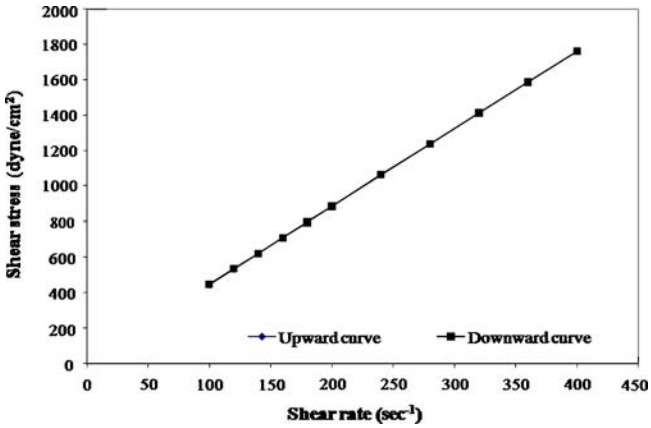

Fig. 3. Flow curves of 25% PLGA solution containing SC at 25°C

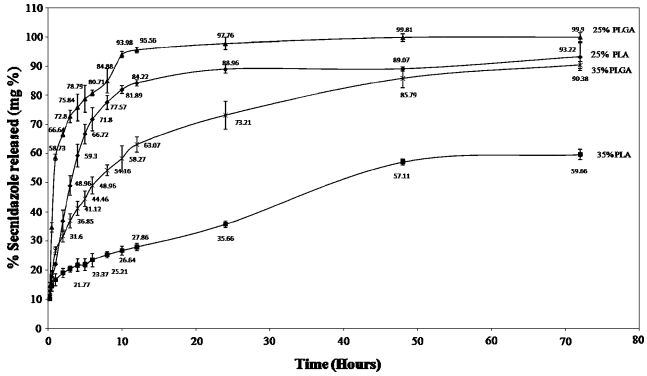

Fig. 5. *In vitro* release of SC from different concentrations of PLA and PLGA *in situ* implants

Increasing the concentration of PLA or PLGA polymer increases the viscosity of the solutions. However, solutions based on 35% PLA show the highest viscosity followed by 25% PLA, while 35% PLGA and 25% PLGA solutions have lower viscosity values. It is revealed that the incorporation of each drug or both together causes no change in the rheological properties of the prepared solutions.

In Vitro Release of SC or DH from PLA and PLGA *In Situ* Implants

The release of SC or DH from different formulations and Atridox® (25% PLA, 35% PLA, 25% PLGA and 35% PLGA) is illustrated in Figs. 5 and 6 respectively. All preparations show an initial burst effect after 15 min, which ranges from 10–15% of the drug for SC when used alone and from 4–12% for DH when used alone, while Atridox® shows a burst effect of 8% of DH. Same initial burst effect are observed upon the combination of the two drugs in which all *in situ* implants show an initial burst effect which ranges from 8–18% of the total percent drug released (figures not shown). *In situ* implants based on 25% PLGA containing SC or/and DH show a continuous fast rate of release, followed by 25% PLA implants with a slower release rate and same release profile. *In situ* implants based on 35% PLGA show slower rate of release, finally, *in situ* implants based on 35% PLA show the slowest rate of release followed by Atridox®.

An initial burst effect was observed by other workers as Schmidt *et al.* (21) and Yoo *et al.* (22). High release rates at the beginning of an antibiotic therapy are very important because the efficiency of antibiotics often depends on high drug concentrations at the beginning of a therapy at the site of infection (21,22). The initial drug release from conventional biodegradable systems is normally attributed to the surface-associated drug (23–25), which was in a good agreement of Lin *et al.* (26).

Increasing the concentration of the polymer decreases the rate of release of SC or DH, Figs. 5 and 6. This may be attributed to the nature of system, which is a solution of water insoluble polymer (PLA, PLGA) in biocompatible solvent (NMP). Such system precipitates upon contact with water as NMP diffuses into the dissolution media, which lead to the polymer precipitation forming *in situ* implant and entrapping the drug, which is released as the polymer degrades. Therefore, it is believed that increasing the concentration of the polymer will increase the time required

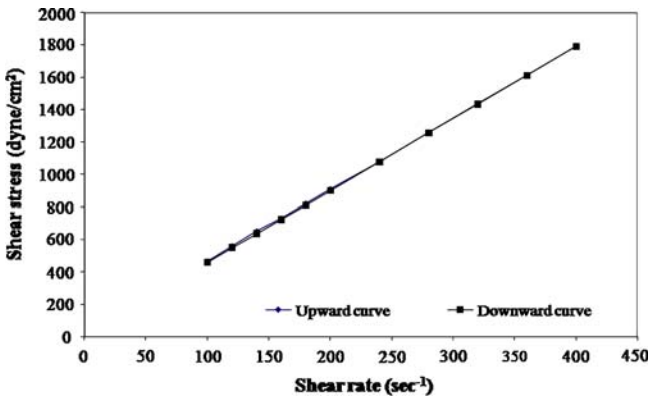

Fig. 4. Flow curves of 25% PLGA solution containing DH at 25°C

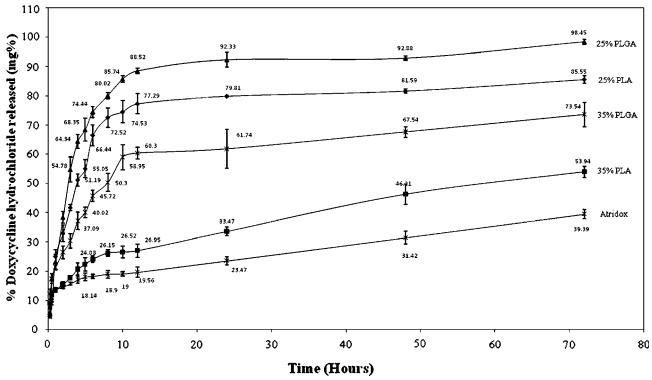

Fig. 6. *In vitro* release of DH from different concentrations of PLA and PLGA *in situ* implants

Table II. The Cumulative Drug Percent Released After 24 h and the Values of the Kinetic Release Parameters for Different *In Situ* Implants

Formula	Drug	Cumulative % drug released after 24 h	Release exponent (*n*)	Kinetic constant	Correlation coefficient
25% PLA + SC	SC	88.96±1.41	0.5929	25.14	0.9907
25% PLA + DH	DH	79.81±0.26	0.8087	22.52	0.9638
35% PLA + SC	SC	35.66±1.10	0.2889	15.15	0.9503
35% PLA + DH	DH	33.47±1.51	0.3065	13.46	0.9887
25% PLGA+ SC	SC	97.76±2.10	0.7373	48.31	0.9039
25% PLGA + DH	DH	92.33±2.51	0.8066	22.52	0.9638
35% PLGA+ SC	SC	73.21±4.75	0.4021	23.62	0.9918
35% PLGA+ DH	DH	61.74±6.63	0.4091	21.35	0.9890
25% PLA + SC + DH	SC	86.34±2.99	0.6864	23.60	0.9830
	DH	85.59±2.34	0.6901	24.55	0.9787
35% PLA + SC + DH	SC	43.60±3.61	0.3411	15.97	0.9748
	DH	45.32±3.12	0.3459	15.59	0.9797
25% PLGA + SC + DH	SC	98.76±3.45	0.6870	39.21	0.9290
	DH	95.65±0.76	0.5249	42.82	0.8817
35% PLGA + SC + DH	SC	67.98±0.86	0.3347	25.17	0.9926
	DH	61.84±0.68	0.3186	24.96	0.9685
Atridox®	DH	23.47±1.43	0.2350	12.19	0.9496

SC secnidazole, *DH* doxycycline hydrochloride

for degradation of the implant formed, as higher polymer concentration lead to the formation of a dense polymer matrix structure (27).

The release profile shown in Figs. 5 and 6 illustrates that implants formed of PLA have a slower rate of release when compared with others made from PLGA of the same concentration. Results are in a good agreement with previous study (28). PLGA polymers containing 50:50 ratio of lactic and glycolic acids are hydrolyzed much faster than those containing higher proportion of either of the two monomers. Polyglycolide acid is highly crystalline because it lacks the methyl side groups of the PLA, however, when it was prepared with D, L PLA it became amorphous so both PLA and PLGA are amorphous but PGA is more hydrophilic so it absorbs more water and degrade more quickly (29). Similar finding was obtained from Mundargi *et al.* (30), where the authors revealed that doxycycline release rate decreased as the ratio of PLGA decreased.

Previous studies have shown that the physico-chemical properties of the incorporated drug in the polymer matrix are important factors in polymer degradation and drug release (31,32). SC is a water soluble drug having the properties of weak base, while DH is an acidic drug. However no significant difference has been observed in the percent of drug released from the formulations containing each drug, as revealed in Table II, which shows the cumulative percent drug released after 24 h. Also there are no significant differences in the percent released after 24 h when each drug

Table III. The Microorganisms Isolated from 20 Patients Suffering from Periodontal Diseases and the Antimicrobial Susceptibility of Different Microorganisms to SC and DH

						% Antimicrobial susceptibility	
Category	Number[a]	Percent[a]	Organism	Number[b]	Percent[b]	SC	DH
Anaerobic Gram negative bacteria	7	50	*Porphyromonas*	4	28.57	95	70
			Prevotella	2	14.28	100	75
			Fusobacteria	1	7.14	100	Zero
Anaerobic Gram positive bacteria	4	28.57	*Peptostreptococci*	4	28.57	70–100	25
Total anaerobes	11	78.57	–	–	–	70–100	25–75
Aerobic bacteria	3	21.43	*Streptococci*	3	21.43	Zero	100
Total bacteria	14	100	–	14	100	–	–

SC secnidazole, *DH* doxycycline hydrochloride
[a] Number and percent of each category
[b] Number and percent of each organism

Table IV. Inhibition Zones Formed by the Antibacterial Activity of SC and/or DH in 25% PLGA *In Situ* Implants Using Isolated Anaerobes and *Streptococci* as Test Organisms

Formula	Zone of inhibition (cm) mean ± SD	
	Anaerobes	*Streptococci*
25% PLGA + SC	2.03±0.057	–
25% PLGA + DH	2.11±0.05	2.09±0.015
25% PLGA + SC +DH	2.07±0.015	2.10±0.05
Atridox®	1.93±0.057	1.97±0.057

was used separately or combined with the other drug upon statistical analyses at $p<0.05$.

Table II shows the release kinetics of SC or DH from different PLA and PLGA *in situ* implants when each drug used separately or combined together. Implants based on 25% PLA and 25% PLGA containing SC or/and DH show (n) values>0.5 indicating that the mechanism of release is anomalous diffusion (non-Fickian), while implants based on 35% PLA and 35% PLGA show (n) values of <0.5 which indicate that the mechanism of release also is non-Fickian diffusion, nevertheless these low values of (n) indicate a situation where drug release occur by means of diffusion, partially through a swollen matrix and partially through water filled spaces (17).

Microbiological Evaluation of the Selected Formulations

Identification of the Microorganisms

Table III shows the number and the percent of isolated microorganisms from the mouth of 20 patients suffering from periodontal diseases. It is revealed that anaerobic micro-organisms represent 78.57% of the total isolated organisms from different periodontal infections in which Bacteroid species represent 50% of these anaerobes. This is in agreement with Tomazinho and Avila-Campos (33), who reported that Bacteroid species as anaerobic bacteria are predominant components of the bacterial florae of mucous membranes and, therefore Bacteroid species are a common cause of endogenous infections.

From Table III, it is revealed that black-pigmented anaerobic rods such as *Prevotella* and *Porphyromonas* are involved in the etiology and perpetuation of endodontic infections. Of a total of 14 isolates from periodontal infections 28.57% have *Porphyromonas*, 14.28% have *Prevotella*, 7.14% have *Fusobacteria*, 28.57% have *Peptostreptococci* and 21.43% have *Streptococci*. Van Winkelhoff *et al.* (34), revealed that of 261 isolates from patients with periodontal infections 35% of cases had *Prevotella*, 37.5% had *Fusobacteria* and 65% had *Streptococci*. This difference in the percentage of isolates may be due to small number of the studied cases.

Antimicrobial Activity of the Selected Formulations of SC and DH on the Isolated Micro-organisms

The selected formulations were then tested for the antimicrobial activity against the isolated microbes using cup diffusion method, Table III. Results reveal that anaerobic Gram negative bacteria are highly sensitive to SC in which 95–100% of the inoculums are inhibited by SC, while Gram positive cocci (*Peptostreptococci*) show only 70% sensitivity to SC. These results agree with Behra-Miellet *et al.* (35). revealing that all strains of Gram negative anaerobes were inhibited by metronidazole while strains of *peptostreptococci* acquired resistance to metronidazole, while as expected SC shows no activity against aerobic bacteria.

Studying the susceptibility of different organisms to DH reveals that aerobic Gram positive cocci are highly sensitive to DH (100%), while anaerobic bacteria show some resistance, in which 25%, 70%, and 75% of *Peptostreptococci*, *Porphyromonas* and *Prevotella* respectively show sensitivity to DH, while *Fusobacteria* are resistant to it, Table III.

Table IV shows the antimicrobial activity of SC and DH (expressed as inhibition zone diameter) from the tested 25% PLGA *in situ* implants in comparison to the release of DH from Atridox®. Statistical analyses of the results at $p<0.05$ showed significant differences among the tested implants and Atridox®. As shown, implants based on 25% PLGA show a greater zone of inhibition than Atridox® and this appears in agreement with the release profile, also addition of SC to DH did not result in change in the extent of inhibition zones of when DH used alone. The pharmaceutical formulation based on 25% PLGA containing secnidazole and doxycycline hydrochloride has promising activity in treating periodontitis in comparison with Atridox®.

CONCLUSION

In conclusion, this study has shown that the combination of secnidazole and doxycycline hydrochloride results in no change in the flow pattern or viscosities of the *in situ* implants, also no change in the percent of the two drugs released from different poly (lactide) and poly (lactide-co-glycolide) implants after 24 h. Both drugs can be formulated in one drug delivery system to increase the spectrum of the antimicrobial activity against the microorganisms causing periodontal diseases and to decrease the therapy cost. Among formulations tested, 25% PLGA *in situ* implants containing secnidazole and/or doxycycline hydrochloride show the fastest rate of release with a promising antimicrobial activity against aerobic and anaerobic bacteria.

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