http://dx.doi.org/10.1590/1678-4162-12586

Arq. Bras. Med. Vet. Zootec., v.74, n.4, p.641-648, 2022

Delivery of active minocycline hydrochloride by local sustained-release system of complex and thermoresponsive hydrogel for dogs

[*Entrega de hidrocloreto de minociclina ativo pelo sistema local de libertação sustentável de hidrogênio complexo e termorresponsivo para cães*]

Tingting *Yi*[1]*, Guiyu *Zhang*[2], Yanling *Wang*[3]*

[1]Shandong Agricultural University-Tai'an, CHN
[2]Qingdao West Coast New Area Agricultural and rural bureau-Qingdao, CHN
[3]Qingdao Vland biological Limited co., LTD.-Qingdao, CHN

ABSTRACT

The objective of this study was to develop a novel subgingival sustained-release system for local delivery of bioactive minocycline hydrochloride for periodontal disease treatment in dogs. The system incorporated the Minocycline hydrochloride-Calcium-Dextran sulfate sodium into a thermoresponsive Pluronic F127 hydrogel. Minocycline hydrochloride was sustained release from the system for up to 10 days and the release kinetics fit the power law model. The release medium had a significant statistical difference in antimicrobial activity to Aggregatibacter actinomycetemcomitans. The results showed the system was a promising subgingival sustained-release minocycline hydrochloride delivery system for periodontal disease treatment in dogs.

Keywords: dogs, sustained-release, thermosensitive hydrogel, minocycline hydrochloride, periodontal disease

RESUMO

O objetivo deste estudo foi desenvolver um novo sistema subgengival de liberação sustentada para administração local de cloridrato bioativo de minociclina para tratamento da doença periodontal em cães. O sistema incorporou o cloridrato de minociclina-cálcio-dextrano sulfato de sódio em um hidrogel Pluronic F127 termorresponsivo. O cloridrato de minociclina foi liberado do sistema por até 10 dias e a cinética de liberação se ajustou ao modelo da lei de potência. O meio de liberação apresentou uma diferença estatística significativa na atividade antimicrobiana para Aggregatibacter actinomycetemcomitans. *Os resultados mostraram que o sistema foi um promissor sistema subgengival de liberação sustentada de cloridrato de minociclina para o tratamento da doença periodontal em cães.*

Palavras-chave: cães, libertação prolongada, hidrogel termossensível, cloridrato de minociclina, doença periodontal

INTRODUCTION

Periodontal disease (PD) is a chronic inflammatory condition in the periodontium, which includes early gingiva inflammation named gingivitis and advanced periodontium inflammation named periodontitis (Albuquerque *et al.*, 2012). It is the most common dental disease in dogs. In prevalence studies, the incidence is ranging from 44 to 100%. The small breeds and old dogs are more vulnerable than large breeds and young dogs (Marshall *et al.*, 2014). For example, the incidence is almost 100% in poodles of more than 4 years old (Marshall *et al*., 2014).

The origin and development of PD are determined by multiple risk factors, which includes microbiological, behavioral, environmental, systemic/metabolic, immunological, anatomical, and genetical factors (Albuquerque *et al*., 2012). The main causes of PD are oral bacterial infection and dental plaque. Periodontal disease related to

*Corresponding author: yitingting_0@outlook.com; ylwang33@126.com
Submitted: October 22, 2021. Accepted: February 18, 2022.

many severe systemic diseases in dogs (Albuquerque *et al.*, 2015; Shirai *et al.*, 2015; Semedo-Lemsaddek *et al.*, 2016; Cunha *et al.*, 2017). Therefore, the development of effective therapeutics is critical for improving the health of dogs.

Ultrasonic scaling and root planning is the most common approach to treat PD (Albuquerque *et al.*, 2012). Besides, local antibiotics administration is used as an adjunct method. Many formulations, including fibers, strips, films, implants, gels, and microspheres, were used for treating PD (Kenawy El *et al.*, 2002; Kelly *et al.*, 2004; Jain *et al.*, 2008; Yao *et al.*, 2014; Rajeshwari *et al.*, 2019). However, they are often technically challenging. For example, fibers and strips need to be removed by professionals after release. Gels need to be frequently administered because of burst release and rapid clearance.

Minocycline hydrochloride (MH) is a tetracycline derivative antibiotic. It can be used as an adjunct for scaling and root planning procedures to reduce the pocket depth. However, current drug delivery systems are not ideal for sustained-release bioactive MH in the periodontium. Minocycline hydrochloride is not stable in aqueous solution, especially at body temperature (Soliman *et al.*, 2010). It also releases very rapidly from hydrophilic systems because of small molecule and high water solubility (Jones *et al.*, 2008; Soliman *et al.*, 2010; Sung *et al.*, 2010). Arestin® is commercially available to treat PD for humans. It is MH microspheres served as dry powder and very hard to be administered. Besides, the degradation product of microspheres can reduce local tissue pH and cause inflammation (Martin *et al.*, 1996).

Ion pairing complexation can increase the therapeutic effect by affecting the solubility, stability, release rates, and bioactivity (Kashi *et al.*, 2012). It is an expecting method for drug release and delivery (Ruan *et al.*, 2016; Oliveira *et al.*, 2017). Minocycline hydrochloride can chelate with Ca^{2+}or other multivalent metal ions to form a positively charged complex without affecting its biological activity (Zhang *et al.*, 2015; Holmkvist *et al.*, 2016; Wu *et al.*, 2018; Zhang *et al.*, 2019). Studies have shown that Ca^{2+} enhances the stability of MH by forming more stable Ca^{2+}-MH chelates (Chow *et al.*, 2008; Soliman *et al.*, 2010). Wu *et al.* studied that the Minocycline hydrochloride-Calcium-Dextran sulfate sodium (MH-Ca^{2+}-DS) sustained release MH for more than 9 days at pH 7.4 and 18 days at pH 6.4 in PBS, respectively. The release medium had antibacterial effects and it was potential to use MH-Ca^{2+}-DS for periodontitis treatment (Wu *et al.*, 2018). Zhang *et al*. studied that MH-Ca^{2+}-DS had a long-term stable release over 71 days, and the release medium had potent anti-biofilm activities (Zhang *et al.*, 2015). However, the periodontal tissue local administration of MH-Ca^{2+}-DS powder is inconvenient and has a short retention time. Therefore, a better formulation is needed.

Thermoresponsive hydrogels are attractive carriers in PD treatment. It can be easily injected into periodontal pockets due to liquid form at low temperatures. After touching the body, the liquid can rapidly change into gel and form a depot at the administration site (Ji *et al.*, 2010; Nasra *et al.*, 2017). In this study, we developed a novel formulation as a subgingival sustained-release system for local delivery of bioactive MH for PD treatment in dogs. In this system, MH-Ca^{2+}-DS was incorporated into the Pluronic F127 thermoresponsive hydrogel (Fig. 1). The MH-Ca^{2+}-DS can improve stability and achieve extended- release of MH. The hydrogel made it more convenient for local delivery and further improved the drug release.

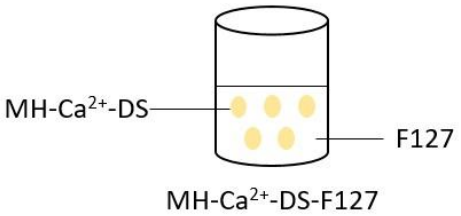

Figure 1. Schematic representation of MH-Ca^{2+}-DS-F127 formation. MH-Ca^{2+}-DS complex was incorporated in thermoresponsive hydrogel Pluronic F127 to form MH-Ca^{2+}-DS-F127 as a novel subgingival sustained-release system for local delivery of bioactive minocycline hydrochloride for periodontal disease treatment in dogs. F127: Pluronic F127; MH-Ca^{2+}-DS: Minocycline hydrochloride-Calcium-Dextran sulfate sodium; MH-Ca^{2+}-DS-F127: Minocycline hydrochloride-Calcium-Dextran sulfate sodium-Pluronic F127.

MATERIAL AND METHODS

Minocycline hydrochloride was purchased from Acros Organics. Dextran sulfate sodium (average molecular weight > 500,000) and Pluronic® F127 were got from Sigma. $CaCl_2$ was got from Alfa Aesar. Thiazolyl Blue Tetrazolium Bromide (MTT) was purchased from Biosynth. Phosphate-buffered saline (PBS) was got from Mediatech. Dimethyl sulfoxide was got from EMD. Potassium metabisulfite was purchased from Beantown Chemical. Aggregatibacter actinomycetemcomitans (ATCC® 29522) strains were purchased from American Type Culture Collection (ATCC, Manassas, VA). Brain heart infusion (BHI) broth was got from Hardy diagnostics. Nutrient agar was purchased from Ward's science.

The MH-Ca^{2+}-DS-F127 was prepared as follows: 5 mg MH powder was dispersed in 9.5 ml Pluronic F127 (25% w/w) solution and stirred for 3 mins. Then 0.25 ml $CaCl_2$ (16 mg/ml) and 0.25 ml dextran sulfate sodium (48 mg/ml) were added to the above mixture and stirred for 5 mins to form MH-Ca^{2+}-DS-F127. The MH-F127 was prepared as a control by mixing MH with Pluronic F127.

Two milliliters of MH-Ca^{2+}-DS-F127 and MH-F127 were loaded into 5 ml centrifuge tubes respectively. One milliliter PBS (containing 0.015% Potassium metabisulfite) was added as the release medium. Tubes were kept in a 37℃ shaker at a speed of 20 rpm. At a specific time, release medium samples were collected and centrifuged at 10 000 rpm for 10 mins. The supernatant was analyzed with UV-VIS spectrometer at 346 nm to determine the MH concentration. Power law model (Eq. 1), first order model (Eq. 2), zero order model (Eq. 3), and Higuchi model (Eq. 4) were used to analyze the release kinetics of MH from MH-Ca^{2+}-DS-F127 and MH-F127. For the four models, where M_t *and* M_∞ are the mass fractions released at time t and infinity, respectively. Where k is a kinetic constant.

$$M_t/M_\infty = kt^n \quad \text{Eq. 1}$$

Where n is an exponent characterizing the diffusional mechanism.

$$ln(1-M_t/M_\infty) = -kt \quad \text{Eq. 2}$$

$$M_t/M_\infty = kt \quad \text{Eq. 3}$$

$$M_t/Mt_\infty = kt^{1/2} \quad \text{Eq. 4}$$

Aggregatibacter actinomycetemcomitans (A.a) is a pathogenic bacterium of periodontitis and thus was used to test the antimicrobial activities. The A.a was cultured in BHI in a 37℃ shaker with a speed of 220 rpm. The resulting suspension in the exponential phase was diluted using fresh BHI to A.a working suspension with an absorbance of 0.1 at 600 nm. The A.a working suspension was freshly prepared in the study. The antimicrobial activities of release medium were tested with agar disk diffusion assay and biofilm assay. The sterile PBS (containing 0.015% Potassium metabisulfite) was used as a control.

The BHI agar plates were evenly spread with 150μL of A.a working suspension. Three wells were drilled and 20 μl test sample was added to each well. Then, plates were kept in the clean bench for 20 mins to make the wells dry. These plates were incubated at 37℃ in a 5% CO_2 incubator. The inhibition zones were measured after 3 days.

Two hundred microliter of A.a working suspension was seeded in 96 well plate and kept at 37℃ in a 5% CO_2 incubator for 3 days. Then, 100 μl of test samples were added into each well. After 24 h, the medium in the well was carefully removed without disrupting the biofilm. The biofilms were stained with MTT reagent and Calcein-AM fluorescent dye, respectively.

One hundred microliter of MTT (0.5mg/mL) was added into each well and incubated in 37℃ incubator for 4h. Then, the medium was replaced by 100μL of dimethyl sulfoxide to dissolve the formazan. The absorbance was measured at a wavelength of 570nm and a reference wavelength of 670nm. The biofilm viability was calculated with Eq. 5.

Biofilm viability (%) =

$$(A_{\text{test sample}} / A_{\text{control}}) \times 100\% \quad \text{Eq. 5}$$

Calcein-AM was used for fluorescent staining. Non-fluorescent calcein-AM is high lipophilicity and can pass through the cell membranes. The AM group can be removed by intracellular esterase in live cells and convert to green fluorescent calcein dye. Therefore, the live bacteria cells can be detected with green fluorescence. In this study, 100 μl of 3 μM/ml calcein-AM was added into each well to treat biofilm and incubated in a 37℃ incubator for 30

mins. The live bacteria in the biofilm were imaged with the microplate spectrophotometer (cytation5).

The results were shown as mean ± standard deviation. All data were analyzed with Graphpad Prism 8.3.0. The results were considered statistically significant at *p < 0.05 and **p < 0.01.

RESULTS

In this paper, the MH-Ca^{2+}-DS-F127 (8d) refers to the release of the first 8 days, and the MH-Ca^{2+}-DS-F127 (10d) refers to the release of all 10 days. Figure 2 is the in vitro release of MH from MH-Ca^{2+}-DS-F127 and MH-F127 in PBS at 37℃ and the simulation with the power law model. The release of MH from MH-Ca^{2+}-DS-F127 lasted for 10 days, and the release from MH-F127 lasted for 8 days. The MH-Ca^{2+}-DS-F127 showed slower release of MH than MH-F127, indicating that the complex formation could slow down drug release. The release profiles fit well with the power law model.

Table 1 is the simulated results obtained by studying the release of MH from MH-Ca^{2+}-DS-F127 and MH-F127 with the power law model. Table 2 is the correlation coefficient of the four models. Among the four different models (Power law, First order, Zero order, Higuchi), the release profile fit best with the power law model (with largest R^2).

Figure 2. The in vitro release profiles of MH from MH-Ca^{2+}-DS-F127 and MH-F127 in PBS at 37℃ and the simulation with the power law model. The release of MH from MH-Ca^{2+}-DS-F127 lasted for 10 days, and the release from MH-F127 lasted for 8 days. The MH-Ca^{2+}-DS-F127 showed slower release of MH than MH-F127. The release profiles fit well with the power law model. The MH-Ca^{2+}-DS-F127 (10d) referred to the release of all 10 days. MH-F127: Minocycline hydrochloride-Pluronic F127; MH-Ca^{2+}-DS-F127: Minocycline hydrochloride-Calcium-Dextran sulfate sodium-Pluronic F127.

Table 1. Fitting equation and parameter in power law model (Eq. 1) for the in vitro release profiles of MH from MH-Ca^{2+}-DS-F127 and MH-F127 in PBS at 37℃. MH-Ca^{2+}-DS-F127: Minocycline hydrochloride-Calcium-Dextran sulfate sodium-Pluronic F127. The MH-Ca^{2+}-DS-F127 (10d) referred to the release of all 10 days. The MH-Ca^{2+}-DS-F127 (8d) referred to the release of the first 8 days. MH-F127: Minocycline hydrochloride-Pluronic F127

Sample	Equation	R^2
MH-Ca^{2+}-DS-F127(10d)	$M_t/M_\infty=0.0298t^{1.4749}$	0.9940
MH-Ca^{2+}-DS-F127(8d)	$M_t/M_\infty=0.0281t^{1.5416}$	0.9994
MH-F127	$M_t/M_\infty=0.1716t^{0.8579}$	0.9917

Table 2. The correlation coefficient R^2 of four models for the in vitro release profiles of MH from MH-Ca^{2+}-DS-F127 and MH-F127 in PBS at 37℃. Among the four different models (Power law, First order, Zero order, Higuchi), the release profile fit best with the power law model (with largest R^2). MH-Ca^{2+}-DS-F127: Minocycline hydrochloride-Calcium-Dextran sulfate sodium-Pluronic F127. The MH-Ca^{2+}-DS-F127 (10d) referred to the release of all 10 days. MH-F127: Minocycline hydrochloride-Pluronic F127

Sample	Power law	First order	Zero order	Higuchi
MH-Ca^{2+}-DS-F127(10d)	0.9940	0.8964	0.9686	0.7718
MH-F127	0.9917	0.9843	0.9312	0.9483

Figure 3a showed that the diameters of inhibition zones in the agar disk diffusion assay. The 29, 59, and 89 μg/ml of release medium from MH-Ca^{2+}-DS-F127 were selected as the test samples. No inhibition zones were observed in the sterile PBS used as the control. There were significant statistical differences between test samples with the negative control. Figure 3b showed the anti-biofilm activity of MH from MH-Ca^{2+}-DS-F127 in the in vitro A.a biofilm model in the MTT assay. The release medium containing 91 μg/ml MH showed anti-biofilm activity and the relative

viability is 57%. The sterile PBS was used as the control. There was a significant statistical difference between the test samples with the PBS control.

Figure 4a showed the image of the live bacteria in the biofilm treated with sterile PBS in fluorescent staining assay. Figure 4b showed the image of the live bacteria in the biofilm treated with 92 μg/ml release medium from MH-Ca^{2+}-DS-F127 in fluorescent staining assay.

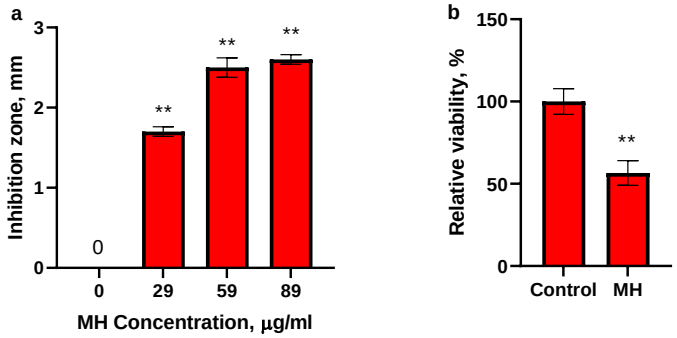

Figure 3a. The release medium from MH-Ca^{2+}-DS-F127 inhibited the growth of A.a on the agar plate. The inhibition zone was shown as the diameter of the inhibition zone. The concentrations of release medium were 29, 59, and 89μg/mL, and the diameters of inhibition zones were 1.7, 2.5 and 2.6cm, respectively. No inhibition zone was observed for the sterile PBS used as the negative control. Figure 3b. It showed the anti-biofilm activity of MH from MH-Ca^{2+}-DS-F127 in the in vitro A.a biofilm model in the MTT assay. The concentration of release medium was 91μg/mL and the relative viability is 57%. The sterile PBS used as the negative control. Significant statistical difference between the test samples and the negative control was marked with *p < 0.05 and **p<0.01. MH: Minocycline hydrochloride. MH-Ca^{2+}-DS-F127: Minocycline hydrochloride-Calcium-Dextran sulfate sodium-Pluronic F127.

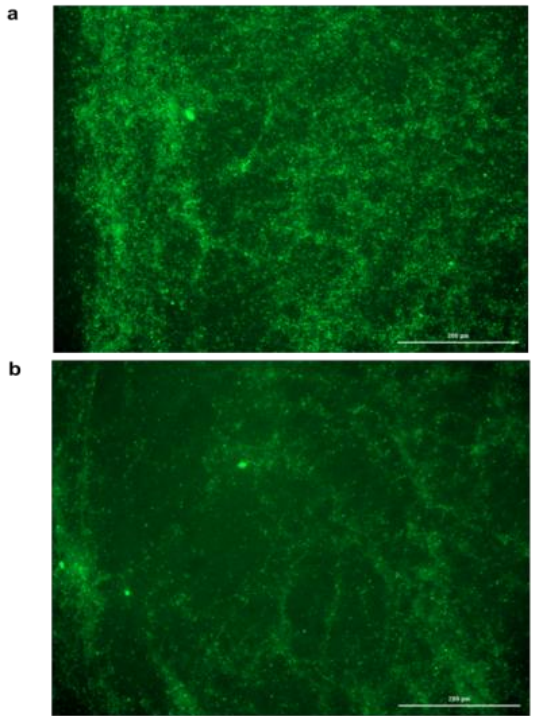

Figure 4a. The image of the live bacteria in the A.a biofilm treated with sterile PBS in fluorescent staining assay. Figure 4b. The image of the live bacteria in the A.a biofilm treated with 92μg/mL release medium from MH-Ca^{2+}-DS-F127 in fluorescent staining assay. The live bacteria in the biofilm were imaged with the microplate spectrophotometer (cytation5), and the live bacteria cells can be detected with green fluorescence. MH-Ca^{2+}-DS-F127: Minocycline hydrochloride-Calcium-Dextran sulfate sodium-Pluronic F127.

DISCUSSION

In the in vitro release studies, release medium from MH-Ca^{2+}-DS-F127 and MH-F127 were detected over days. The release of MH from MH-Ca^{2+}-DS-F127 lasted for 10 days, and from MH-F127 lasted for 8 days. The release time of MH from MH-Ca^{2+}-DS-F127 was two days more than from MH-F127. Although two days was not a big difference, MH in the complex was more stable than free MH (Chow *et al*., 2008; Soliman *et al*., 2010), which meant it was necessary to prepare MH-Ca^{2+}-DS.

The cumulative release of MH from MH-Ca^{2+}-DS-F127 and MH-F127 was 75% and 86% respectively, due to the sampling loss. It was inevitable that a small number of complex precipitates were extracted whenever the samples were taken from the top of the gel. After centrifugation of the samples, complex precipitates with light yellow were observed at the bottom of the tubes. When injected into the canine periodontal pockets, there is no sampling loss. There would be more complex left in the pockets after the gel was completely dissolved. Minocycline hydrochloride could be released from MH-Ca^{2+}-DS-F127 for more days than in vitro.

The thermoresponsive hydrogel Pluronic-F127 in MH-Ca^{2+}-DS-F127 disappeared completely on the eighth day, but a small amount of complex remained at the bottom of the tubes. For MH-Ca^{2+}-DS-F127, the MH was dissociated from the complex and then released from gel to PBS in the first eight days. In the last two days, the MH released to PBS just after dissociating from the complex, due to no effect of the gel. The release of MH in the first eight days and the last two days were affected by different factors. In the power law model, the correlation coefficient R^2 of MH-Ca^{2+}-DS-F127 (8d) was higher than the R^2 of MH-Ca^{2+}-DS-F127 (10d).

The A.a is a common pathogenic bacterium of periodontitis. To find out the possibility of MH-Ca^{2+}-DS-F127 for periodontitis treatment, agar disk diffusion assay and biofilm assay were done to examine antibacterial activity against A.a. The MH-Ca^{2+}-DS was complex with low water solubility. The dissociation and binding of MH, Ca^{2+}, and DS were reversible. The release mediums were taken and centrifuged, then the supernatants were used for antibacterial experiments. Thus, the antibacterial effect was mainly attributed to MH released from the MH-Ca^{2+}-DS-F127.

In the agar disk diffusion assay, increasing the concentration of release medium from 29μg/ml to 59μg/ml, the diameters of inhibition zones increased from 1.7 to 2.5cm. Increasing the concentration from 59μg/mL to 89μg/mL, the diameters slightly increased from 2.5 to 2.6cm. It showed a possible trend to level off with a further increase in concentration.

Compared with planktonic bacteria, biofilm makes residual bacteria have higher antimicrobial resistance. Thus, bacteria on the plaque are less sensitive to antibiotics. The MTT assay and fluorescent staining assay were done in the biofilm experiment. Two release mediums of nearly 90μg/mL were chosen as the test samples. One was 91μg/mL for MTT assay, and the other was 92μg/mL for fluorescent staining assay. Before adding 100μL test samples, there were 200μL working suspensions in each well. Thus, the test samples were diluted to nearly 30μg/mL, which was the true concentration to interact with the bacteria in the biofilm. The results of the biofilm experiment showed that the release medium from MH-Ca^{2+}-DS-F127 had an anti-biofilm activity to A.a.

CONCLUSION

The results showed that MH-Ca^{2+}-DS-F127 sustained release MH for up to 10 days and the release medium had antibacterial effects against A.a. Thus, the MH-Ca^{2+}-DS complex incorporated in thermoresponsive hydrogel Pluronic F127 could be developed as a local delivery system for canine periodontitis treatment.

ACKNOWLEDGMENTS

We thank Qingdao postdoctoral applied research project. We thank National Natural Science Foundation of China (31602111).

REFERENCES

ALBUQUERQUE, C.; MORINHA, F.; MAGALHAES, J. *et al*. Variants in the interleukin-1 alpha and beta genes, and the risk for periodontal disease in dogs. *J. Genet.*, v.94, p.651-659, 2015.

ALBUQUERQUE, C.; MORINHA, F.; REQUICHA, J. *et al*. Canine periodontitis: the dog as an important model for periodontal studies. *Vet. J.*, v.191, p.299-305, 2012.

CHOW, K.T.; CHAN, L.W.; HENG, P.W. Formulation of hydrophilic non-aqueous gel: drug stability in different solvents and rheological behavior of gel matrices. *Pharm. Res.*, v.25, p.207-217, 2008.

CUNHA, E.; TROVÃO, T.; SANTOS, R. *et al*. Canine periodontal disease and its systemic implications–a review. *Rev. Port. Cienc. Vet.*, v.113, p.12-22, 2017.

HOLMKVIST, A.D.; FRIBERG, A.; NILSSON, U.J.; SCHOUENBORG, J. Hydrophobic ion pairing of a minocycline/Ca(2+)/AOT complex for preparation of drug-loaded PLGA nanoparticles with improved sustained release. *Int. J. Pharm.*, v.499, p.351-357, 2016.

JAIN, N.; JAIN, G.K.; JAVED, S. *et al*. Recent approaches for the treatment of periodontitis. *Drug Discov. Today*, v.13, p.932-943, 2008.

JI, Q.X.; ZHAO, Q.S.; DENG, J.; LU, R. A novel injectable chlorhexidine thermosensitive hydrogel for periodontal application: preparation, antibacterial activity and toxicity evaluation. *J. Mater. Sci. Mater. Med.*, v.21, p.2435-2442, 2010.

JONES, D.S.; LORIMER, C.P.; MCCOY, C.P.; GORMAN, S.P. Characterization of the physicochemical, antimicrobial, and drug release properties of thermoresponsive hydrogel copolymers designed for medical device applications. *J. Biomed. Mater. Res. B Appl. Biomater.*, v.85, p.417-426, 2008.

KASHI, T.S.; ESKANDARION, S.; ESFANDYARI-MANESH, M. *et al*. Improved drug loading and antibacterial activity of minocycline-loaded PLGA nanoparticles prepared by solid/oil/water ion pairing method. *Int. J. Nanomedicine*, v.7, p.221-234, 2012.

KELLY, H.M.; DEASY, P.B.; ZIAKA, E.; CLAFFEY, N. Formulation and preliminary in vivo dog studies of a novel drug delivery system for the treatment of periodontitis. *Int. J. Pharm.*, v.274, p.167-183, 2004.

KENAWY EL, R.; BOWLIN, G.L.; MANSFIELD, K. *et al*. Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend. *J. Control. Release*, v.81, p.57-64, 2002.

MARSHALL, M.D.; WALLIS, C.; MILELLA, L. *et al*. A longitudinal assessment of periodontal disease in 52 Miniature Schnauzers. *BMC Vet. Res.*, v.10, p.166, 2014.

MARTIN, C.; WINET, H.; BAO, J.Y. Acidity near eroding polylactide-polyglycolide in vitro and in vivo in rabbit tibial bone chambers. *Biomaterials*, v.17, p.2373-2380, 1996.

NASRA, M.M.; KHIRI, H.; HAZZAH, H.A.; ABDALLAH, O.Y. Formulation, in-vitro characterization and clinical evaluation of curcumin in-situ gel for treatment of periodontitis. *Drug Deliv.*, v.24, p.133-142, 2017.

OLIVEIRA, M.S.; GOULART, G.C.A.; FERREIRA, L.A.M.; CARNEIRO, G. Hydrophobic ion pairing as a strategy to improve drug encapsulation into lipid nanocarriers for the cancer treatment. *Expert Opin. Drug. Deliv.*, v.14, p.983-995, 2017.

RAJESHWARI, H.; DHAMECHA, D.; JAGWANI, S. *et al*. Local drug delivery systems in the management of periodontitis: a scientific review. *J. Controlled Release*, v.307, p.393-409, 2019.

RUAN, H.; YU, Y.; LIU, Y. *et al*. Preparation and characteristics of thermoresponsive gel of minocycline hydrochloride and evaluation of its effect on experimental periodontitis models. *Drug Deliv.*, v.23, p.525-531, 2016.

SEMEDO-LEMSADDEK, T.; TAVARES, M.; SAO BRAZ, B. *et al*. Enterococcal infective endocarditis following periodontal disease in dogs. *PLoS One*, v.11, p.e0146860, 2016.

SHIRAI, M.; NOMURA, R.; KATO, Y. *et al*. Short communication: distribution of porphyromonas gulae fimA genotypes in oral specimens from dogs with mitral regurgitation. *Res. Vet. Sci.*, v.102, p.49-52, 2015.

SOLIMAN, G.M.; CHOI, A.O.; MAYSINGER, D.; WINNIK, F.M. Minocycline block copolymer micelles and their anti-inflammatory effects on microglia. *Macromol. Biosci.*, v.10, p.278-288, 2010.

SUNG, J.H.; HWANG, M.R.; KIM, J.O. *et al*. Gel characterisation and in vivo evaluation of minocycline-loaded wound dressing with enhanced wound healing using polyvinyl alcohol and chitosan. *Int. J. Pharm.*, v.392, p.232-240, 2010.

WU, L.; CHEN, W.; LI, F. *et al*. Sustained release of minocycline from minocycline-calcium-dextran sulfate complex microparticles for periodontitis treatment. *J. Pharm. Sci.*, v.107, p.3134-3142, 2018.

YAO, W.; XU, P.; PANG, Z. *et al*. Local delivery of minocycline-loaded PEG-PLA nanoparticles for the enhanced treatment of periodontitis in dogs. *Int. J. Nanomedicine*, v.9, p.3963-3970, 2014.

ZHANG, T.; NONG, J.; ALZAHRANI, N. *et al*. Self-assembly of DNA-minocycline complexes by metal ions with controlled drug release. *ACS Appl. Mater. Interfaces*, v.11, p.29512-29521, 2019.

ZHANG, Z.; WANG, Z.; NONG, J. *et al*. Metal ion-assisted self-assembly of complexes for controlled and sustained release of minocycline for biomedical applications. *Biofabrication*, v.7, p.015006, 2015.