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LIPOSOMAL ENCAPSULATION OF DOXYCYCLINE FOR LOCAL PERIODONTAL DELIVERY

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Abstract

This review brings together published work on encapsulating doxycycline in liposomes for local delivery into the periodontal pocket, and it reports no new data. It covers the biological reasons for treating periodontitis locally, the pharmacology of doxycycline as both an antibacterial and a host-modulating drug, and the lipid compositions, preparation routes and loading methods used for liposomal carriers. Characterisation tools, release testing and the clinical record of existing local doxycycline products are compared. Liposomes can hold the drug at the pocket for longer and soften its burden on tissue, yet most evidence is preclinical, and release methods differ so widely that results from different laboratories are hard to set side by side.

1. INTRODUCTION

Periodontitis is a chronic inflammatory disease in which the tissues that anchor the tooth are slowly broken down, and it is one of the commonest reasons adults lose teeth. Severe forms affect a sizeable minority of the world's population, and the burden climbs with age [1, 2]. The present classification separates disease by stage and grade, so that the extent of attachment loss and the likely speed of progression are both recorded [3]. Treatment starts with mechanical removal of plaque and calculus from the root surface,

which works well in shallow pockets. Deeper sites are harder to clean, and residual pockets often bleed and keep losing support afterwards.

The reason lies partly in the microbial community. Subgingival plaque is not a scatter of free-floating bacteria but a structured biofilm, with groups of species that tend to appear together and that become more pathogenic as pockets deepen [4]. Biofilm bacteria tolerate antibiotics far better than planktonic cells of the same species, and they recolonise cleaned root surfaces within weeks [5]. The host response matters just as much. The inflammatory reaction to a dysbiotic community, rather than the bacteria alone, drives most of the tissue destruction, which is why the disease is now described as a failure of immune balance and not simply as an infection [6].

Placing an antimicrobial directly into the pocket is attractive because the gingival crevicular fluid washes drug away quickly. A systemic course exposes the whole body to an antibiotic in order to treat a site a few millimetres wide, and it adds to the pressure for resistance [7, 8]. A local product could reach a high concentration where it is needed and keep it there. Several local systems have been marketed, mainly fibres, gels and microspheres. Liposomes, which are closed phospholipid vesicles able to carry both water-soluble and lipid-soluble drugs, offer a different route to the same aim [9, 10]. This review looks at what has been published on doxycycline in such carriers.

Outcomes in this field are measured in a few standard ways, and they shape how any new product is judged. Probing depth records how far a graduated probe slides into the pocket, clinical attachment level tracks the true position of the supporting tissue, and bleeding on probing serves as a marker of active inflammation. Differences between treatments are often measured in fractions of a millimetre. A reduction of around half a millimetre in probing depth is statistically detectable in a large trial, yet whether a patient or dentist would notice it is another matter [3, 11]. Any liposomal product will therefore be assessed against modest margins, which is a reason to design preclinical work carefully before moving to people.

Cost and access matter more than they first appear. Periodontitis is common in regions where specialist care is scarce, so a local product that needed refrigeration, trained handling or repeated visits would reach few of the people who might benefit. Formulation choices made early, such as the use of freeze-dried powder reconstituted at the chairside, can decide whether a laboratory concept is ever used outside a research clinic. Where such products have been introduced, uptake has depended as much on price and storage as on clinical effect. Training of dental teams in handling a new carrier, and clear instructions on when to apply it, would also have to be part of any rollout plan.

2. DOXYCYCLINE IN PERIODONTAL THERAPY

Doxycycline is a semi-synthetic tetracycline that blocks the bacterial 30S ribosomal subunit and so halts protein synthesis. It is lipophilic compared with older tetracyclines, is absorbed well by mouth, and has a long half-life that permits once- or twice-daily dosing [12, 13]. These properties explain why it enters gingival tissue and crevicular fluid readily after a tablet is swallowed. They also explain why it is a sensible candidate for local use, since it diffuses through biofilm and into the surrounding tissue reasonably well. Its spectrum covers many of the anaerobic Gram-negative rods linked with periodontitis, though susceptibility varies between sites and patients.

The chemistry of the molecule matters for formulators. Doxycycline carries several ionisable groups, so its charge and solubility shift with pH, and it binds calcium, magnesium, iron and zinc through its beta-diketone and phenolic regions. This chelation underlies both the enzyme inhibition described below and the tendency of the drug to form complexes with divalent cations in buffers and on tooth surfaces. It is also photosensitive and can epimerise in solution. Concentrations in crevicular fluid after oral dosing have been reported to exceed those in serum, as the drug is concentrated by inflamed tissue and by fluid flow, but the figures differ between studies and patients [12, 13]. A local product has to keep drug above the effective level while avoiding degradation during storage.

A second line of work began with the observation that tetracyclines reduced collagen-degrading activity in inflamed gingiva even in animals without bacteria. Early work reported lower collagenase activity in the gingival tissue of diabetic animals treated with a tetracycline analogue, even though the animals were not being treated for infection [14]. Later work showed that the drugs inhibit matrix metalloproteinases, partly through chelation of calcium and zinc at the enzyme's active site, and that they also dampen osteoclast activity and some oxidative pathways [15, 16]. These effects do not depend on killing bacteria. They gave rise to the idea of host modulation, in which a drug is used to limit the tissue breakdown that follows inflammation.

The distinction led to a low-dose oral doxycycline regimen that works below the antibacterial threshold. Trials in patients with chronic periodontitis found that the 20 mg twice-daily dose added to scaling and root planing gave a modest further gain in attachment level and a fall in probing depth compared with scaling alone [17, 18]. Because plasma concentrations stay far below those needed to affect bacteria, the regimen did not appear to select for resistant organisms in the studies that checked. The result tells a formulation scientist something useful: a local carrier for doxycycline could aim at two targets, bacterial load and enzyme activity, at concentrations that need not be high.

3. LIPOSOME DESIGN AND PREPARATION

Liposomes were first described in the 1960s, when swollen phospholipids were seen to form concentric closed bilayers that behaved as semi-permeable compartments [19]. Over the following decades they moved from a laboratory curiosity to licensed medicines, mostly for cancer and fungal infection [9, 20]. A vesicle can be unilamellar or multilamellar, and its size spans from tens of nanometres to several micrometres. The bilayer can carry a lipophilic drug, while the water core can hold a hydrophilic one. Doxycycline, which is soluble in water as its hyclate salt and still has some affinity for membranes, sits across both regions to a degree that depends on pH and ionic strength.

Composition decides most of what a liposome does. Phosphatidylcholine from egg or soya, or a synthetic equivalent such as dipalmitoyl or distearoyl phosphatidylcholine, forms the bulk of the bilayer. Cholesterol is added to tighten packing and cut leakage, and charged lipids such as phosphatidylglycerol or stearylamine alter surface charge and aggregation behaviour [21, 22]. Lipids with saturated chains and a transition temperature above body temperature give stiffer, less leaky vesicles, while unsaturated lipids are softer and more prone to oxidation. A formulation intended to stay in a pocket at 37 °C must be chosen with these trade-offs in mind, and published doxycycline systems have explored several of them (table 1). How those entries divide between drug rationale, preparation, characterisation and clinical evidence is shown in fig. 1.

Preparation routes fall into a few families. In thin-film hydration, lipids are dissolved in organic solvent, dried to a film and rehydrated with an aqueous drug solution, which gives multilamellar vesicles of mixed size. Reverse-phase evaporation produces vesicles with a large aqueous space and good capture of water-soluble drugs [23]. Extrusion through polycarbonate membranes then narrows the size distribution and yields large unilamellar vesicles [24]. Sonication and microfluidic mixing are alternatives. Each route has consequences for yield, residual solvent and scalability, and the choice made in a research laboratory is often not the one that would be used in a pilot plant.

The phase behaviour of the lipid is worth a paragraph of its own. Below the transition temperature, a saturated phospholipid sits in a tightly packed gel state that holds water-soluble solutes well; above it, the chains melt and the membrane becomes far more permeable. Cholesterol smooths out this transition and broadens the temperature range over which a bilayer is stable [21, 22]. For a pocket product the target is a membrane that stays reasonably ordered at 37 °C but is not so rigid that nothing is released in the time available. Drug interaction with the bilayer further complicates matters, since

amphipathic compounds can lodge among the lipids and alter packing. These effects are rarely measured in doxycycline studies, which tend to report size and entrapment only [25].

Drug loading is a separate issue from vesicle formation. Passive loading, in which doxycycline is simply present in the hydration medium, is easy but often captures only a small share of the drug because the aqueous volume enclosed is small. Active or remote loading uses a transmembrane gradient, typically of pH or ammonium sulfate, to pull a weak base inside and trap it as a charged species [26, 27]. Remote loading is best known for doxorubicin. For tetracyclines, which carry several ionisable groups and chelate divalent cations, the approach is harder to apply, and reported entrapment depends strongly on the buffer and on any metal ions in the system [25].

Surface modification adds another layer. Coating vesicles with polyethylene glycol prolongs circulation after injection, which is irrelevant in a pocket but may reduce clumping in storage [28]. Of more interest in the mouth are mucoadhesive coatings such as chitosan, which carry a positive charge and cling to the negatively charged surfaces of tissue and bacteria, and which may help a liposome stay in place against the outflow of crevicular fluid [10]. A cationic surface can also bind to bacterial membranes. These benefits are plausible and have some experimental support, but most reports come from in vitro work on a single formulation rather than head-to-head comparisons.

Embedding vesicles in a second matrix is a frequent proposal for periodontal use. A liposome suspension alone is likely to be cleared from a pocket quickly, so authors have suggested dispersing vesicles within a thermosensitive or ionically gelling vehicle that thickens after placement. The gel would act as a reservoir and the vesicles as a second barrier to diffusion, giving two stages of release [10, 29]. The idea is logical and has precedent in other mucosal sites, but it adds complexity. The vesicles must survive mixing with the polymer, the gelling process must not rupture them, and the sum of two release barriers can slow delivery below the level needed to act on bacteria. Direct comparisons with a plain gel are needed.

Table 1: Published evidence relevant to liposomal doxycycline for periodontal use

Topic	Reported finding	Ref.
Antibacterial action 30S ribosome binding	Semi-synthetic tetracycline with good tissue penetration and a long half-life.	[12, 13]
Collagenase inhibition host modulation	Reduced collagenolytic activity in inflamed gingiva independent of bacterial killing.	[14, 15, 16]
Sub-antimicrobial dose 20 mg twice daily	Modest added gain in attachment level when given with scaling and root planing.	[17, 18]
Thin-film hydration multilamellar vesicles	Simple preparation giving mixed sizes; usually followed by extrusion or sonication.	[21, 22]
Reverse-phase evaporation large aqueous core	High capture of water-soluble drugs in large unilamellar vesicles.	[23]
Extrusion polycarbonate membranes	Narrows size distribution and gives reproducible unilamellar vesicles.	[24]
Remote loading pH or ammonium gradient	Efficient trapping of weak bases; harder for chelating tetracyclines.	[26, 27]
Size and zeta potential light scattering	Narrow polydispersity and adequate surface charge indicate stable dispersions.	[30, 31]
Release modelling Higuchi and Korsmeyer-Peppas	Burst then diffusion-controlled phase; fit does not prove mechanism.	[32, 33]
Doxycycline polymer gel two multicentre trials	Greater pocket depth reduction than scaling alone or placebo.	[34]
Meta-analysis local antimicrobials	Small average adjunctive benefit; guideline restricts use to selected sites.	[11, 35]

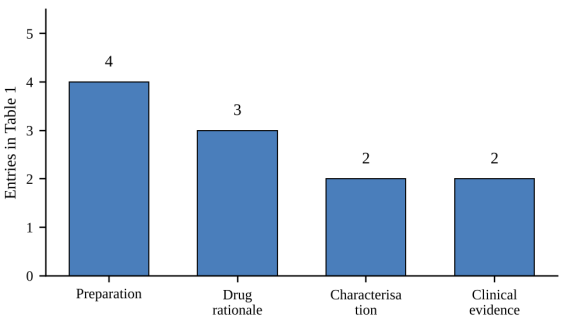


Fig. 1: Number of entries in table 1 by category of evidence

4. CHARACTERISATION AND RELEASE

Characterisation of liposomal doxycycline follows the usual pattern for lipid nanocarriers. Mean vesicle size and polydispersity index are measured by dynamic light scattering, and a low polydispersity value indicates a narrow, reproducible population [30]. Zeta potential reports surface charge and is used as a rough guide to colloidal stability, with larger absolute values generally resisting aggregation [31]. Both measurements are sensitive to dilution medium and ionic strength, so a result in water may not carry over to a saline or gel base. Electron microscopy, particularly cryogenic imaging, gives shape and lamellarity directly and is a useful check on light-scattering data.

Entrapment efficiency is the share of drug that ends up inside the vesicles. It is calculated after unencapsulated drug has been separated by centrifugation, dialysis or gel filtration, and the drug in the vesicles is then measured after lysis with a detergent or solvent. The weak point is the separation step, because a dialysis membrane that holds back vesicles may also slow free drug, and ultracentrifugation can rupture fragile bilayers. Reported entrapment values for doxycycline vary widely across studies, from low to moderate, and differences in method make it hard to say how much comes from the formulation and how much from the assay [21, 25].

Release testing is where comparisons between papers become hardest. Many laboratories use a dialysis bag in phosphate-buffered saline, others use a diffusion cell, and a few use sample-and-separate methods. The choice affects the apparent rate, since the membrane itself can limit transport [36]. A pocket holds only a few tens of microlitres of crevicular fluid, so large-volume sink conditions in a beaker are far from what happens at the site. Some groups therefore use small-volume apparatus or artificial pocket models. Whichever is chosen, the temperature, pH, agitation and membrane need to be reported, because without them a profile cannot be reproduced.

Antibacterial testing is the other half of characterisation, and it is done with less consistency than physical testing. Minimum inhibitory concentrations against planktonic cultures of periodontal species give a baseline. Agar diffusion zones are easy to measure but depend on how far the formulation diffuses through agar, and so they rate release as much as potency. More relevant are biofilm assays, where bacteria grown on discs or in flow cells are exposed to the product, because biofilm cells tolerate much higher drug levels than free cells [5]. Relatively few liposomal doxycycline reports include such assays, and fewer still include anaerobic species of the kind found in deep pockets [4].

Release profiles are commonly fitted to simple models. Diffusion from a matrix is described by Fickian kinetics, in which the amount released scales with the square root of time, and a power-law expression separates Fickian from non-Fickian release [33]. Liposomal systems often show a quick initial burst, from drug adsorbed on or near the surface, followed by a slower phase governed by diffusion across the bilayer and, later, by lipid breakdown. First-order and Korsmeyer-Peppas fits are also used, and the exponent indicates whether transport is diffusional or more complex [32]. A good fit does not prove the mechanism, however, and the model should be treated as a description, not as a test.

Sterility and endotoxin limits are sometimes forgotten in early reports. A formulation placed in a pocket with ulcerated epithelium should be free of viable organisms, and lipids from some suppliers carry detectable endotoxin. Filtration through a membrane of 0.22 micrometres works for small unilamellar vesicles, yet sheds larger ones and may reduce drug content. Recording these details early makes later scale-up less of a shock. Recording these details early, together with the supplier and grade of each lipid, makes later scale-up far less of a shock and helps other groups reproduce a batch. Endotoxin testing of the final product is straightforward and should be routine.

A short note on analytical practice belongs here too. Doxycycline is light-sensitive,

chelates metals and epimerises in solution, so the chromatographic or spectrophotometric method needs to be shown to separate the parent drug from its degradation products. Validation should cover the accepted parameters of specificity, linearity, accuracy, precision and range, as set out in regulatory and pharmacopoeial requirements [37]. Several published formulation studies rely on ultraviolet absorbance at a single wavelength without demonstrating this, which means that lipid components or degradants may inflate apparent drug content. Without such checks, entrapment values in the literature are best read with caution.

5. LOCAL DELIVERY AND CLINICAL EVIDENCE

Local delivery into the periodontal pocket has a longer history than liposomes in dentistry. In the late 1970s, tetracycline held in a hollow fibre was placed in pockets and was shown to give very high crevicular concentrations compared with those after oral dosing [38]. Monolithic ethylene vinyl acetate fibres followed and offered controlled release over several days [39]. These early reports set the pattern: the idea works in principle, the clinical gain beyond scaling is real but modest, and the delivery vehicle decides how long the drug stays. In that sense the field has been learning the same lesson for more than forty years.

Reviews of controlled-release products have repeatedly made the same two points. First, a device must hold drug concentrations above the minimum inhibitory level for long enough to matter, which in a pocket flushed by fluid means days rather than hours [29]. Second, any product is an adjunct to mechanical debridement and not a replacement for it. A systematic review of local anti-infective agents concluded that tetracycline fibres, minocycline microspheres and doxycycline gel each gave small average improvements in probing depth over scaling alone, with considerable differences between studies [7]. The same pattern appears in later syntheses of the topic.

The best-known doxycycline product is a biodegradable polymer gel containing doxycycline hyclate, which is applied as a liquid that sets in the pocket. Two multicentre trials in patients with moderate to severe periodontitis compared the gel with scaling alone and with placebo and found larger reductions in probing depth and gains in attachment with the active treatment [34]. The result supports the principle that local doxycycline helps. It also gives liposome developers a clear benchmark, because any new carrier must show something beyond what a polymer gel already achieves, whether that is longer retention, gentler tissue effects or easier handling.

Trial design itself affects what can be concluded. Many local-delivery studies use a split-mouth arrangement, in which different pockets in one patient receive different treatments. This removes some between-patient variation but invites carry-over, because drug released at one site may have effects elsewhere through saliva. Placebo vehicles are not always inert; a gel or a fibre can alter local flow and plaque retention in its own right. Follow-up periods range from a few weeks to a year, and early gains in probing depth sometimes fade as bacteria return [7, 29]. A fair assessment of a new carrier would use a parallel design, a vehicle control and follow-up long enough to see whether recolonisation occurs.

Pooled data have tempered earlier enthusiasm. A meta-analysis of locally delivered antimicrobials found a statistically significant but small additional benefit in pocket depth, and the clinical importance depends on the starting depth and on how the sites were selected [35]. Current European guidance advises against routine use of locally delivered antimicrobials, reserving them for selected residual pockets after proper instrumentation [11]. For liposomal doxycycline the practical reading is that a carrier is more likely to find a place as a targeted adjunct for stubborn sites than as a general-purpose treatment, and the clinical question needs to be framed that way.

Microbiological outcomes deserve separate mention. Subgingival sampling after local doxycycline has generally shown reductions in the proportion of pathogenic anaerobes in the first weeks, followed by gradual return of the community towards its previous

composition, particularly where the biofilm is not disrupted mechanically [4, 40]. This is consistent with the biofilm tolerance described earlier and with the view that antimicrobials are adjuncts. It also suggests a possible role for the host-modulating action of the drug, which does not depend on the composition of the flora, and which a sustained-release liposome could in theory prolong. Direct evidence for that benefit in local products is still thin.

A distinct possibility is combining the antibacterial and host-modulating roles in a single local product. If liposomes hold doxycycline at a concentration low enough to dampen metalloproteinases without the peaks associated with a gel, the formulation might reduce tissue breakdown for longer. That idea rests on mechanistic data and on the oral low-dose studies, and it has not been tested in controlled trials of a local liposomal product to the best of the literature available [16, 17]. Such a trial would need both microbiological and enzymatic endpoints to show the dual action.

6. SAFETY, STABILITY AND REGULATION

Liposomes made from natural phospholipids are generally well tolerated, and several licensed products show that the class can meet regulatory expectations [20]. Local application brings its own concerns: irritation of gingival tissue, taste, staining of teeth, and the effect of residual solvents or charged lipids. Cationic lipids can be cytotoxic to fibroblasts at higher concentrations, which matters in a pocket where regeneration is the goal. Tetracyclines are linked to tooth discolouration in developing teeth, so any product would carry restrictions in children and in pregnancy, as the oral drugs do.

Biocompatibility testing for a periodontal product usually begins with cell culture. Gingival and periodontal ligament fibroblasts exposed to the formulation give a first indication of cytotoxicity, and a scratch or proliferation assay shows whether wound healing is affected. Tetracyclines at modest concentrations are generally tolerated by these cells and may even encourage attachment of fibroblasts to root surfaces, while at higher levels they inhibit proliferation. The balance depends on concentration and exposure time, and so on the release profile of the carrier. Systemic exposure after placement is expected to be low, though mucosal absorption through ulcerated pocket epithelium is not zero, and resistance monitoring remains advisable given the findings in plaque and saliva after sustained-release doxycycline [40].

Physical and chemical stability are major hurdles. Liposomal dispersions in water are prone to aggregation, fusion, leakage and lipid hydrolysis or oxidation, and doxycycline itself degrades under light and heat. Stability studies need to cover the chemical stability of the lipids, which hydrolyse and oxidise on storage, as well as physical stability, with storage at defined temperature and humidity conditions and testing of size, entrapment, drug content and degradation products over time [41]. Freeze-drying with a cryoprotectant such as a disaccharide is the usual route to a longer shelf life, though the drying step can itself change vesicle size and cause leakage if the process is not well controlled.

Regulatory classification depends on how the product is presented and on the claims made. A drug-containing product placed in a pocket is a medicinal product in most jurisdictions, and it would need quality, nonclinical and clinical data accordingly. Liposomal products also attract particular attention to critical quality attributes such as size distribution, lamellarity and release, and regulators have issued guidance on how such features should be controlled [20]. For a small developer the cost of this package can be large, which partly explains why so few dental liposome products have reached the market despite a steady stream of laboratory papers.

One practical advantage of a liposome in the mouth is that its excipients are familiar, which may ease the toxicology package. Phosphatidylcholine and cholesterol are physiological, and degradation products are natural lipids. A lower administered dose of doxycycline than in a systemic course also reduces the chance of systemic adverse effects, although some drug will reach the circulation through inflamed pocket

epithelium. That said, nobody should assume a liposomal product is free of safety questions merely because its lipids are natural. Local tolerance still needs to be shown in studies of the finished product, not inferred from the excipients alone.

7. LIMITATIONS AND FUTURE DIRECTIONS

The literature on liposomal doxycycline for the periodontium has clear limitations. Most studies are in vitro or in small animal models, many use a single lipid composition, and few compare against a marketed polymer gel under the same conditions. Release testing is inconsistent, as discussed above, and the small volume of crevicular fluid is rarely reflected in the apparatus. Reports often give entrapment efficiency and release but not stability on storage, so it is difficult to judge whether a laboratory batch would survive a supply chain. Very few papers measure the antibacterial effect against biofilm instead of planktonic cultures [5].

Translation faces non-scientific barriers too. A dental product is sold in a market where the comparator, scaling and root planing, is cheap, and where payers and clinicians judge adjuncts by cost as well as effect. A liposomal system that requires cold storage, special mixing or a long preparation step at the chairside will struggle against a ready-to-use gel. Development plans should therefore consider shelf life at room temperature, ease of application through a fine cannula and compatibility with the instruments already in the surgery. Better laboratory data alone will not change practice if the product is awkward to use [20, 35].

Several directions look worthwhile. Standardised small-volume release methods that mimic the pocket would make results from different groups comparable. Liposomes embedded in an in situ gelling vehicle could combine retention with sustained release, building on the experience of polymer gels [34]. Studies in biofilm models and in animals with induced periodontitis should measure both bacterial and enzymatic outcomes, since the dual role of the drug is what sets it apart [16]. Finally, early randomised trials in residual pockets, with attachment level as the primary outcome, would show whether any of this changes practice.

8. CONCLUSION

Liposomal doxycycline is a reasonable idea built on solid foundations. The drug is well characterised, its two actions on bacteria and on tissue-destroying enzymes are documented, and local delivery into the pocket has decades of clinical precedent [14, 17, 38]. Liposomes add a way to hold the drug at the site, to protect it from rapid washout and to tune release through lipid choice, and the preparation and loading methods are mature enough to be applied to a molecule as awkward as a tetracycline [9, 24, 26]. The combination is therefore scientifically credible and technically feasible.

The weak points lie in the evidence rather than the concept. Most published work is preclinical, release and entrapment methods differ from one laboratory to the next, and a polymer gel already offers a clinical benchmark that is hard to beat [34, 35]. Progress will need pocket-relevant release tests, stability data, biofilm models and, eventually, controlled trials in residual pockets, in line with guideline advice on where local agents belong [11]. Until then liposomal doxycycline remains a promising formulation, not a proven treatment. Careful, comparable studies would settle the matter.

9. ABBREVIATIONS

DLS-Dynamic light scattering, EFP-European Federation of Periodontology, ICH-International Council for Harmonisation, MMP-Matrix metalloproteinase, PDI-Polydispersity index, PEG-Polyethylene glycol, SRP-Scaling and root planing, MIC-Minimum inhibitory concentration, UV-Ultraviolet, GCF-Gingival crevicular fluid

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11. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

12. CONFLICT OF INTERESTS

Declared none

13. REFERENCES

1. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers 2017;3:17038.
2. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005;366:1809–20.
3. Papapanou PN, Sanz M, Buduneli N, *et al.* Periodontitis: consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol 2018;89(Suppl 1):S173–82.
4. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol 1998;25:134–44.
5. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;284:1318–22.
6. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol 2015;15:30–44.
7. Hanes PJ, Purvis JP. Local anti-infective therapy: pharmacological agents. A systematic review. Ann Periodontol 2003;8:79–98.
8. Slots J, Ting M. Systemic antibiotics in the treatment of periodontal disease. Periodontol 2000 2002;28:106–76.
9. Allen TM, Cullis PR. Liposomal drug delivery systems: from concept to clinical applications. Adv Drug Deliv Rev 2013;65:36–48.
10. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 2005;4:145–60.
11. Sanz M, Herrera D, Kebschull M, *et al.* Treatment of stage I–III periodontitis: the EFP S3 level clinical practice guideline. J Clin Periodontol 2020;47(Suppl 22):4–60.
12. Agwuh KN, MacGowan A. Pharmacokinetics and pharmacodynamics of the tetracyclines including glycylcyclines. J Antimicrob Chemother 2006;58:256–65.
13. Saivin S, Houin G. Clinical pharmacokinetics of doxycycline and minocycline. Clin Pharmacokinet 1988;15:355–66.
14. Golub LM, Lee HM, Lehrer G, *et al.* Minocycline reduces gingival collagenolytic activity during diabetes: preliminary observations and a proposed new mechanism of action. J Periodontal Res 1983;18:516–26.
15. Golub LM, Ramamurthy NS, McNamara TF, Greenwald RA, Rifkin BR. Tetracyclines inhibit connective tissue breakdown: new therapeutic implications for an old family of drugs. Crit Rev Oral Biol Med 1991;2:297–321.
16. Golub LM, Lee HM, Ryan ME, Giannobile WV, Payne J, Sorsa T. Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. Adv Dent Res 1998;12:12–26.
17. Preshaw PM, Hefti AF, Jepsen S, Etienne D, Walker C, Bradshaw MH. Subantimicrobial dose doxycycline as adjunctive treatment for periodontitis: a review. J Clin Periodontol 2004;31:697–707.
18. Caton JG, Ciancio SG, Blieden TM, *et al.* Treatment with subantimicrobial dose doxycycline improves the efficacy of scaling and root planing in patients with adult periodontitis. J Periodontol 2000;71:521–32.
19. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 1965;13:238–52.
20. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. Pharmaceutics 2017;9:12.
21. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, *et al.* Liposome: classification, preparation, and applications. Nanoscale Res Lett 2013;8:102.
22. Vemuri S, Rhodes CT. Preparation and characterization of liposomes as therapeutic delivery systems: a review. Pharm Acta Helv 1995;70:95–111.
23. Szoka F, Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proc Natl Acad Sci USA 1978;75:4194–8.
24. Hope MJ, Bally MB, Webb G, Cullis PR. Production of large unilamellar vesicles by a rapid extrusion procedure: characterization of size distribution, trapped volume and ability to maintain a membrane potential. Biochim Biophys Acta 1985;812:55–65.
25. Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomedicine 2015;10:975–99.
26. Mayer LD, Bally MB, Cullis PR. Uptake of adriamycin into large unilamellar vesicles in response to a pH gradient. Biochim Biophys

Acta 1986;857:123–6.

27. Haran G, Cohen R, Bar LK, Barenholz Y. Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. Biochim Biophys Acta 1993;1151:201–15.
28. Immordino ML, Dosio F, Cattel L. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. Int J Nanomedicine 2006;1:297–315.
29. Greenstein G, Tonetti M. The role of controlled drug delivery for periodontitis. J Periodontol 2000;71:125–40.
30. Egelhaaf SU, Wehrli E, Müller M, Adrian M, Schurtenberger P. Determination of the size distribution of lecithin liposomes: a comparative study using freeze fracture, cryoelectron microscopy and dynamic light scattering. J Microsc 1996;184:214–28.
31. Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems: a review (part 1). Trop J Pharm Res 2013;12:255–64.
32. Siepmann J, Siepmann F. Modeling of diffusion controlled drug delivery. J Control Release 2012;161:351–62.
33. Peppas NA, Sahlin JJ. A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. Int J Pharm 1989;57:169–72.
34. Garrett S, Johnson L, Drisko CH, *et al.* Two multi-center studies evaluating locally delivered doxycycline hyclate, placebo control, oral hygiene, and scaling and root planing in the treatment of periodontitis. J Periodontol 1999;70:490–503.
35. Herrera D, Matesanz P, Martín C, Oud V, Feres M, Teughels W. Adjunctive effect of locally delivered antimicrobials in periodontitis therapy: a systematic review and meta-analysis. J Clin Periodontol 2020;47(Suppl 22):239–56.
36. D'Souza SS, DeLuca PP. Methods to assess in vitro drug release from injectable polymeric particulate systems. Pharm Res 2006;23:460–74.
37. Thompson M, Ellison SLR, Wood R. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC technical report). Pure Appl Chem 2002;74:835–55.
38. Goodson JM, Haffajee A, Socransky SS. Periodontal therapy by local delivery of tetracycline. J Clin Periodontol 1979;6:83–92.
39. Goodson JM, Holborow D, Dunn RL, Hogan P, Dunham S. Monolithic tetracycline-containing fibers for controlled delivery to periodontal pockets. J Periodontol 1983;54:575–9.
40. Walker CB, Godowski KC, Borden L, *et al.* The effects of sustained release doxycycline on the anaerobic flora and antibiotic-resistant patterns in subgingival plaque and saliva. J Periodontol 2000;71:768–74.
41. Grit M, Crommelin DJA. Chemical stability of liposomes: implications for their physical stability. Chem Phys Lipids 1993;64:3–18.