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REVIEW

The Role and Advancement of Liposomes for Oral Diseases Therapy

Maya Nurul Rahma[1], Cecep Suhandi[1], Ahmed FA Mohammed[2], Ali El-Rayyes[3], Khaled M Elamin[4], Evi Sulastri[1,5], Nasrul Wathoni[1]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Universitas Padjadjaran, Sumedang, West Java, 45363, Indonesia; [2]Department of Pharmaceutics, Faculty of Pharmacy, Minia University, Minia, 61519, Egypt; [3]Center for Scientific Research and Entrepreneurship, Northern Border University, Arar, 73213, Saudi Arabia; [4]Graduated School of Pharmaceutical Science, Kumamoto University, Kumamoto, 862-0973, Japan; [5]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu, Central Sulawesi, 94118, Indonesia

Correspondence: Nasrul Wathoni, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, 45363, Indonesia, Tel +62-22-842-888-888, Email nasrul@unpad.ac.id

Abstract: As many as 48.0% of the global population suffers from disabilities caused by oral conditions. These conditions encompass dental caries, periodontal diseases, oral cancers, and other pathologies affecting the hard and soft tissues of the oral and maxillofacial regions. Topical drug treatments in the oral cavity are often ineffective due to the short contact time, which prevents the drug from reaching optimal concentrations necessary for therapeutic effect. Conventional liposomes have several limitations, including low stability, challenges in long-term storage, and rapid clearance by the reticuloendothelial system (RES). These factors significantly reduce their effectiveness in maintaining sustained drug delivery and achieving desired therapeutic outcomes. To overcome these challenges, advanced drug delivery systems have been developed. Among these systems, liposomes have been extensively explored as nanocarriers in targeted drug delivery systems, particularly in mucosal drug delivery, due to their biocompatibility and degradability, making them promising agents for the treatment of oral diseases. To address these issues, extensive research has been conducted to modify the surface of liposomes, optimizing their efficacy, and understanding their mechanisms of action. This review article discusses the role and recent advancements of liposomes in the treatment of oral diseases, highlighting their potential to revolutionize oral health care through improved drug delivery and therapeutic outcomes.

Keywords: oral diseases, nanocarrier, liposomes, targeted drug delivery, mucoadhesive polymers

Introduction

Nearly half of the global population suffers from disabilities caused by oral conditions (age-standardized prevalence: 48.0%), and this prevalence is on the rise, particularly in low-income and middle-income countries.[1] Oral diseases are chronic and progressive, affecting individuals from childhood to adulthood.[2] Those afflicted often experience a decline in their quality of life due to pain and discomfort, inadequate nutrition, diminished aesthetics, and disrupted social interactions.[3] Several treatments have been adopted to treat oral diseases, including surgery, radiation, and cancer immunotherapy. Although these developments aim to reduce the side effects of chemotherapy, issues related to the side effects and long-term effects of therapy persist.[4] The prognosis for this therapy is generally poor, with significant post-surgical impairments. Common side effects include difficulties in chewing, swallowing, and speaking, which are often exacerbated by chemotherapy and radiotherapy.[5] These treatments can lead to functional disabilities and a reduced quality of life, primarily due to the damage caused to both healthy tissues and vital structures during and after the therapeutic interventions.[6] Additionally, conventional topical medications for the oral cavity, such as gels,[7] mouth sprays,[8] mouthwashes,[9] and toothpastes,[10] have been utilized. The conventional use of drugs faces several challenges, including the ability to penetrate tissues, maintaining therapeutic concentrations at the mucosal surface, and avoiding systemic side effects, even in topical applications. These limitations often hinder the drug's efficacy and can lead to unintended adverse reactions, reducing the overall effectiveness of the treatment.[11] However, these methods often fail to

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maintain the optimal drug concentration necessary for pharmacological effects, due to insufficient contact time, leading to reduced treatment efficacy.[12] Therefore, advanced drug delivery systems have been developed to overcome these limitations.

Liposomes have been extensively explored as nanocarriers in targeted drug delivery systems such as mucosal drug delivery because they can be modified using mucoadhesive polymers, making them promising agents for the treatment of oral diseases.[13] In recent studies, liposomes conjugated with mucolytic enzymes have been shown to increase drug penetration into mucous membranes.[14] Liposomes consist of a phospholipid bilayer capable of encapsulating both hydrophobic and hydrophilic compounds in their lipid core and hydrophilic compartments, respectively, thereby enhancing the efficacy and therapeutic index of drugs.[15] They possess good biocompatibility because of their structural similarity to cell membranes in the body and can degrade naturally.[16] Therefore, this article aims to discuss the role and advancements of liposomes in the treatment of oral diseases, along with their associated challenges and future perspectives.

Drug Delivery via Liposomes

Innovations in drug delivery systems have been developed to provide local treatment and prevention in the oral cavity.[17] However, many therapeutic challenges remain, including low drug efficacy and retention at the target site.[18] While the oral mucosa has attractive properties for drug delivery, it also presents several challenges for researchers exploring new delivery techniques.[19] In drug delivery systems, the drug carrier plays a crucial role in transporting therapeutic agents to the targeted site.[20] Nanoparticle-based drug delivery systems formulated from lipids or polymers can be modified to enhance the therapeutic level of a drug as well as its pharmacological properties.[21] Effective drug carriers must possess several key characteristics,[20] including a surface charge that affects their interactions with biological membranes.[22] The hydrophilic–hydrophobic balance of the carrier affects its capacity, stability, and interactions with biological fluids.[23] Additionally, biocompatibility and biodegradability are critical factors that determine the performance of drug carriers.[16] Liposome-based products, such as drug delivery systems (DDS), have been developed to address the challenges in treating oral diseases.

Liposomes are spherical lipid vesicles with diameters ranging from 50 to 500 nm, and consist of a bilayer of lipids derived from natural or synthetic emulsified lipids in an aqueous medium[24] (Figure 1), they are capable of encapsulating both hydrophobic and hydrophilic compounds thereby enhancing the efficacy and therapeutic index of drugs[15] (Figure 2a). Various methods can be used to prepare liposomes, including thin-film hydration,[25] solvent injection,[26] and microfluidic channel methods.[27] Liposome formulations can reduce the toxicity of compounds in vivo by modifying their pharmacokinetics and biodistribution, thus, enhancing drug delivery to diseased tissues compared to free drugs.[28] Sterically stabilized liposomes, which use hydrophilic polymers such as polyethylene glycol (PEG), are considered the optimal choice for improving stability and circulation time.[16]

Liposome Internalization Mechanisms

Liposome-mediated internalization mechanisms can be classified into two main categories: endocytosis and membrane fusion (Figure 2b). Endocytosis refers to the process by which liposomes are engulfed by the plasma membrane, forming

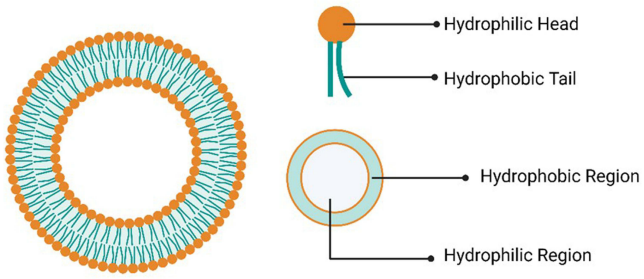

Figure 1 Liposome Structure. Created in BioRender. Suliman, K. (2024) BioRender.com/z34c794.

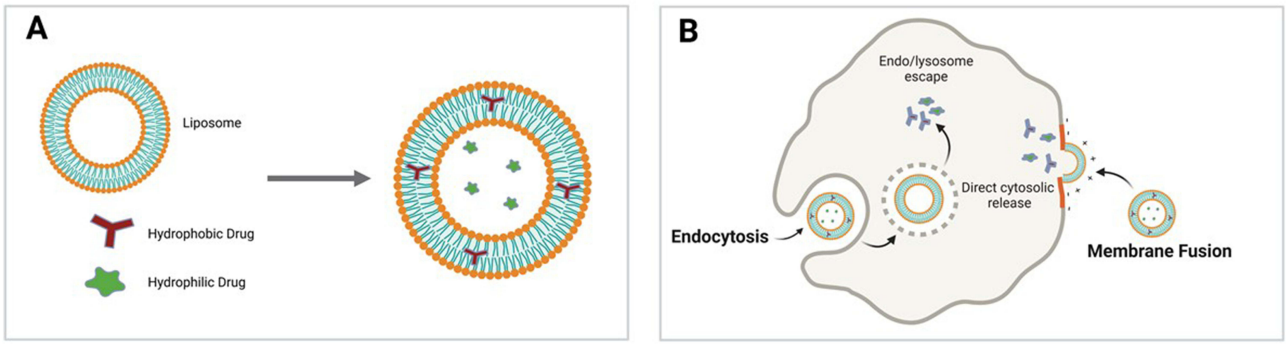

Figure 2 Liposome as Drug Delivery Carrier (**A**); Mechanism Action of Liposome (**B**). Created in BioRender. Suliman, K. (2024) https://BioRender.com/ x84r388.

endocytic vesicles. This pathway is commonly utilized for the delivery of therapeutic agents.[29] Liposome endocytosis follows several routes, including clathrin-mediated endocytosis, where clathrin-coated pits internalize the liposomes;[30,31] caveolin-mediated endocytosis, which involves caveolin proteins to facilitate vesicle formation;[32] micropinocytosis, driven by actin polymerization; and intracellular inflammatory pathways, where liposomes are directed to lysosomes for degradation after internalization.[33] Another critical pathway is direct cytosolic delivery, where liposomes bypass the endo-lysosomal route through direct membrane fusion, releasing their cargo into the cytosol, thereby enhancing therapeutic efficacy.[34] Various factors, such as the presence of specific receptors on the cell surface and the physico-chemical properties of liposomes, can influence these internalization mechanisms.[35] In membrane fusion, liposomes directly fuse with the cell membrane, allowing therapeutic agents to be delivered into the cytoplasm while bypassing the endo-lysosomal pathway. The occurrence of membrane fusion can vary depending on the cell type. Environmental acidity has been shown to enhance fusion activity, with acidic conditions leading to improved liposome fusion capacity. Furthermore, the lipid composition of liposomes can significantly impact their ability to merge with cell membranes, with certain lipids promoting higher fusion rates.[34]

Advancement of Liposomes for Drug Delivery in Oral Disease Therapy

Research on liposomes for the treatment of oral diseases has progressed significantly, evolving from conventional liposome formulations[36] for advanced designs such as polymer-grafted liposomes[37] and polymer-coated liposomes[13] (Figure 3) (Table 1).

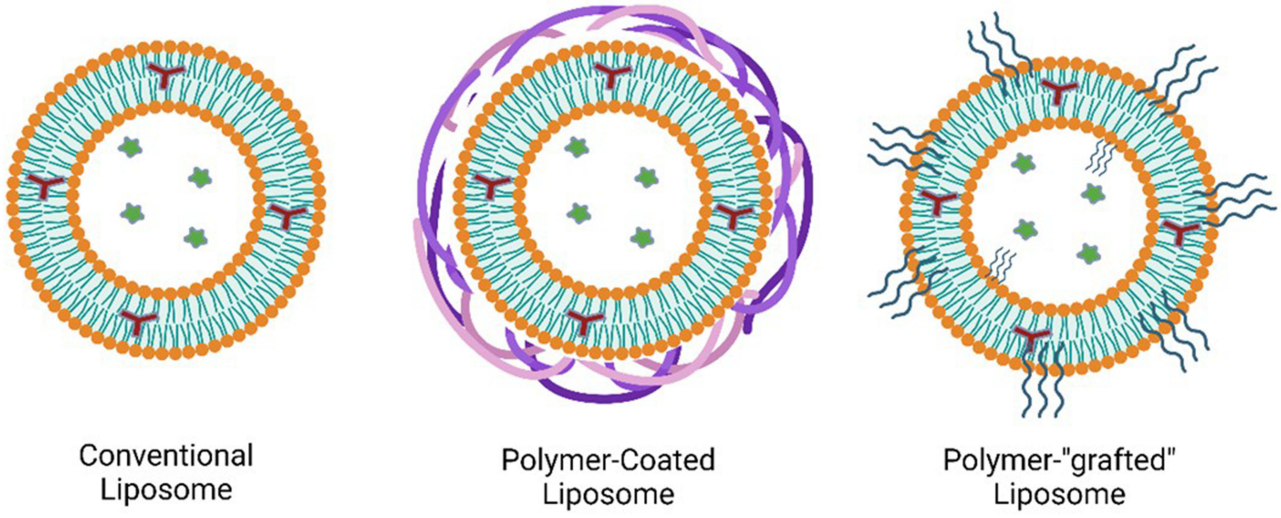

Figure 3 Advancement of Liposome. Created in BioRender. Suliman, K. (2024) BioRender.com/x30d074.

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Table 1 Advancement of Liposomes for Oral Diseases Therapy

Types of Liposomes	Liposome Composition	Active Substance	Indication	Result	Ref
Conventional Liposomes	Phosphatidylcholine, CHOL (ratio 0.3:5:1)	Betulinic Acid	Oral Cancer	The activity of the liposome-betulin complex can inhibit cell viability	[38]
	CHOL, phospholipid	Quercetin and Scaffolds Biocomposite	Oral Lesions	The increase in cell growth and migration appeared to be greater than the control	[39]
	Soybean lecithin CHOL (2:1 mass ratio)	Peptide (DK5) derived from histatin-1	Dental Carries	The lesion depth was shallower in the DK5-Lips group with good biocompatibility.	[40]
	Phospholipon 90G (2.0 μmol) and phytosphingosine (0.4 μmo)	Nisin	Dental Carries	Nisin-liposome prolongs the inhibitory activity of nisin on glucan-biofilm synthesis by S. mutans 10449	[41]
	Egg-PC, cholesterol	Insulin	Aphthous ulcers	Insulin-loaded liposomes exhibited significantly better results compared with control groups	[42]
	Phosphatidylcholine and cholesterol	Triamcinolone	Lichen Planus	0.1% triamcinolone acetonide with nanoliposomal carriers in Orabase is more effective than 0.1% triamcinolone in Orabase	[11]
	5 mg (DPPC), 3 mg of (DPPS), 1 mg of CHOL	Farnesol	Candidiasis	The incorporation of farnesol into liposomes significantly increased this activity.	[43]
	DSPC or DPPC, and CHO	Nystatin	Candidiasis	Antifungal activity of liposome encapsulation Nystatin was higher than free nystatin	[44]
	(DPPC), (DPPS) and CHOL	Trans-Caryophyllene (TC)	Candidiasis	The compound in the form of liposomes got the upper hand, because it inhibited all the strains tested	[45]
One Layer of Polymer Coated Liposomes	SL:Chol (80:20) 95 mg Tween 80 (%w/v) 0.1 to 1% Water 10 mL, chitosan 0.05%, 0.1% and 0.5%w/v	Erlotinib (ERB)	Oral Cancer	Tumor volume reduction for ERB Lipo gel was higher compared to regular ERB Gel	[46]
	PEG-DSPE, and CHOL in a molar ratio of 55:5:40, Chitosan	Doxorubicin	Oral Cancer	Significantly reduces tumor size	[47]
	DMPC and CHOL in molar ratio of 60:40, alginate	Doxorubicin	Oral Cancer	Very effective in inducing the death of cancer cells	[47]
	90 mol% of SoyPC and 10 mol% (DOTAP or EggPG), alginate and gellan gum, chitosan	Gellan gum	Xerostomia	Alginate- and chitosan-coated liposomes may be promising agents for use in the oral cavity	[13]
	Soya-PC: 89 DOTAP: 10 NBD-PC, (LM-pectin), (HM-pectin), alginate, chitosan and (HM-EHEC)	Pectin	Xerostomia	Liposomes with polymers significantly increase the water absorption capacity of the formulation	[48]
Cross-Linked Polymer-Caged Liposomes	Lipoid S100 2 or 4% w/v and CHOL or 0.5% w/v PG 20% w/v (Glut) (0.25%, 0.5%, 1% or 2% v/v)	Atorvastatin (ATV)	Candidiasis	ATV/PG-Lip3DP film is superior for managing oral candidiasis and overcoming antifungal resistance	[49]

(*Continued*)

Table 1 (Continued).

Types of Liposomes	Liposome Composition	Active Substance	Indication	Result	Ref
PEGylated Liposomes	HSPC/CHOL/mPEGDSPE2000 at a molar ratio of 63:32:5, metoksi polietilen glikol (mPEG)	Doxorubicin	Oral Cancer	Attachment of targeting 12 ligands and for active targeting of tumour cells through residual carboxyl groups on the surface of the liposome nanoparticles	[50]
	Cholesterol, HSPC, mPEGDSPE2000, F-PEG-CHEMS	Ursolic Acid	Oral Cancer	Encapsulation of ursolic acid in ligand-conjugated liposomes resulted in higher solubility and bioavailability	[51]
	(DPPC), (DPPS) and cholesterol, PEG, albumin	Amfoterisin B	Candidiasis	DEC3-AmB-LLs inhibited C. albicans and R. delemar more efficiently reducing the effective dose of AmB.	[52]
Targeted Liposomes	CHOL, DSPG, and DSPE-PEG (2:1:0.16)	Ligan c8, Dexamethasone	Oral Cancer	Liposomes mediated a significant reduction in cell viability of malignant UPCI-SCC-154 cells	[53]
	The cationic LPs, composed of DOTAP/CHOL, GRIM-19 gene	Cisplatin	Oral Cancer	Inhibits the proliferation of OSCC tumor cells and suppresses OSCC growth in vivo.	[54]
	(DPPC:CHOL:PE 10:2:1 molar ratio) 1:0.02 PE:WGA	Amoxicillin	Ulcerative Lesions	Great potential cell-binding drug delivery systems	[55]
	DPPC/CH/PG/ PE in 8:10:1:2, PE/ WGA 1:0.02 mol ratio	Cyclodextrin dan betametason	Oral Infection	Release significantly increases the viability of oral cells and synergistically reduces inflammation.	[56]
Stimuli-Responsive Liposome	(POPC), L-α-phosphatidyl-DL-glycerol (egg chicken, PG), (DOTAP) and cholesterol	2-(morpholin-4-yl)ethoxy phthalocyanines	Oral Cancer	Effective with oral cancer cell and is able to trigger photodynamic process	[57]
	DMPC, CHOL, or cardiolipin	Aluminum phthalocyanine Chloride	Tongue Tumor	Photodynamic therapy based and effective against chemically induced oral cancer	[58]
	70 mg lecithin, TMC (0.5 mg/mL)	Doxycycline	Periodontitis	TMC-Lip-DOX NPs have good potential for the treatment of periodontal diseases	[59]

Abbreviations: CHOL, Cholesterol; DSPG, 1,2-distearoyl-sn-glycero-3- phospho-(1′-rac-glycerol); DSPE-PEG, 1.2- distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino (polyethyleneglycol)-2000; DOTAP, N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride; POPC, 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; PDT, Photodynamic Therapy; AlPcCl, Aluminum-Phthalocyanine; TMC, N,N,N-Trimethyl chitosan; DPPC, dipalmitoylphosphatidylcholine; DPPS, dipalmitoylphosphatidyl-serine; SL, Soy Lecithin; DMPC, 1.2- dimyristoyl-sn-glycero-3-phosphocholine; EggPG, L-α-phosphatidylglycerol; NBD-PC, d 1-oleoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl}-sn-glycero-3-phospcholine; HM-EHEC, Hydrophobically modified ethyl hydroxyethyl cellulose; LM, low-methoxylated; HM, High-methoxylated; Glut, glutaraldehyde; PG, Propylene Glycol; WGA, Wheat germ agglutinin.

Conventional Liposomes

Oral diseases are typically treated using local therapeutic agents. However, conventional local treatments often face challenges owing to the low retention time in the oral cavity; which is affected by saliva secretion; swallowing; food intake; and abrasive actions on the soft tissues.[60] Bioadhesive formulations can enhance the retention time of active substances in the oral cavity, ultimately improving the treatment efficacy. Bioadhesion refers to the condition under which macromolecules or polymers can adhere or attach to biological surfaces through electrostatic interactions, hydrogen bonding, or mechanical attachment mechanisms.[61] In this context, bioadhesive nanosystems including liposomes have proven advantageous because of their small size; allowing them to reach locations inaccessible to other formulations and enabling targeted drug delivery.[62]

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Liposomes were first reported by Bangham et al in 1965. Initially; liposomes were lipid vesicles with a closed bilayer structure, primarily composed of phospholipids with or without cholesterol.[63] The composition of vesicles determines the physicochemical properties; surface structure; toxicity; efficacy; and biodistribution of the liposomes. Furthermore, the pharmacokinetics and in vivo performance of liposomes are influenced by their formulation.[64] Conventional liposomes consist mainly of zwitterionic phospholipids such as phosphatidylcholine; sphingomyelin; phosphatidylethanolamine; and cholesterol as a non-ionic lipid.[65] The addition of cholesterol in liposome preparation can affect liposome fluidity and stability as well as alter the rigidity of the bilayer.[66] Research by Jovanović et al confirmed that an optimal cholesterol concentration of 50 mol% is necessary to achieve the ideal balance of membrane fluidity and rigidity.[67]

Research on the use of conventional liposomes in the treatment of oral diseases has yielded promising results. Yamakami et al demonstrated that loading nisin into liposomes composed of phospholipase 90G and phytosphingosine enhanced the structural integrity and stability of the liposomes. These components facilitate the formation of multi-lamellar vesicles; which are crucial for effective encapsulation of nisin within liposomes. Nisin-loaded liposomes were found to significantly inhibit glucan biofilm synthesis by Streptococcus mutans, a major causative agent of dental caries.[41] In another study, Saadat et al formulated nystatin into liposomes; highlighting the critical role of lipid composition in determining liposome characteristic lipids such as dipalmitoylphosphatidylcholine (DPPC); distearoyl-phosphatidylcholine (DSPC); and cholesterol (CHO) were investigated. It was found that DPPC significantly improved the entrapment efficiency (%EE) of nystatin, with DPPC-containing formulations showing higher drug entrapment efficiency compared to those prepared with DSPC. The addition of cholesterol to DPPC formulations further increased drug entrapment efficiency, underscoring cholesterol's role in enhancing the %EE of liposomes. The optimal formulation identified in this study had a DPPC weight ratio of 50:10 (w/w) and a drug-to-lipid weight ratio of 2:5 (w/w).[44] However, conventional liposomes tend to have poor stability due to the absence of protective coatings on their surface; which can lead to vesicle fusion and leakage from the aqueous core.[68]

Polymer Coated Liposome

Several methods can be used to enhance liposome stability, including the application of protective layers using polymers.[69] Combining liposomes with mucoadhesive polymers offers the potency to increase the residence time of a formulation in the oral mucosa and provide prolonged moisture protection.[13] The outer structure of liposomes facilitates polymer adsorption through non-specific interactions.[70] Adsorbed polymers modify the interfacial and physicochemical properties of liposomes; resulting in colloidal; biological; and mechanical stability.[71] These enhancements include increasing circulation time, reducing lipid oxidation due to decreasing oxygen exposure and reducing liposome vesicle leakage.[72] Liposomes with polymer monolayer coating; layer-by-layer polymer coating; and cross-linked polymer coating are among the most commonly used approaches.

Liposomes Coated with One Layer of Polymer

In polymer-coated liposomal drug delivery systems, it is essential to achieve a sufficient concentration of polymer molecules (saturation concentration) to fully coat the liposome surface.[73] This coating ensures optimal protection and stability; allowing liposomes to effectively deliver therapeutic agents to target sites within the oral cavity. The polymers used to coat liposomes include pectin,[48] alginate,[47] and gellan gum.[13] Chitosan, in particular, has been extensively studied for its mucoadhesive properties. At low pH; the primary amine groups on chitosan are protonated, giving chitosan molecules a positive charge. This allows them to bind effectively to the negatively charged mucus layer, enhancing mucoadhesion.[74,75] Liposomes coated with chitosan have shown promise for drug delivery applications. This system's potency could be particularly beneficial for local drug delivery in the oral cavity, providing targeted and sustained therapeutic effects.[76]

In a study by Frigaard et al, liposomes coated with alginate and chitosan demonstrated excellent stability. Alginate-coated liposomes showed the highest stability in phosphate buffer, whereas chitosan-coated liposomes exhibited the most stability in glycerol phosphate buffer. Both formulations displayed low cytotoxicity, making them promising candidates for use in the oral cavity.[13] Further research on polymer-coated liposomes for oral cancer treatment was conducted by Hariharan et al, who aimed to deliver erlotinib (ERB) locally to tumors in the oral cavity. ERB was incorporated into

a liposome formulation (ERB-Lipo) and then coated with chitosan to obtain CS-ERB Lipo. Further characterization revealed that both ERB Lipo and CS-ERB Lipo had particle sizes less than 200 nm and a PDI of less than 0.4. The zeta potential was −50 mV for ERB Lipo and +25 mV for CS-ERB Lipo, indicating a stable formulation.[46]

Liposomes Coated with Layer by Layer of Polymer

In the formulation of liposomes using the layer-by-layer (LBL) coating technique; charged polymers are utilized to coat the liposome surface through ion exchange. During this coating process; ions bound to the substrate interact with the polyelectrolyte layer on the polymer. This process is driven by the entropy increase of the released ions. LBL coating leverages non-covalent interactions, primarily electrostatic interactions between oppositely charged polyelectrolytes, due to its straightforward design and procedure.[77] Polymers commonly used in this approach include chitosan; xanthan; alginate; dextran sulfate; and hyaluronic acid.[78]

Layer-by-layer liposomes exhibit important characteristics such as a hydrophilic surface, lipid bilayer structure, and biocompatibility. Furthermore, this method enhances protection against phospholipid oxidation and increases liposome stability.[79] In a study by Tan et al, the use of chitosan and alginate as polyelectrolytes in an LBL liposome system demonstrated superior stability compared to uncoated liposomes and monolayer-coated liposomes. Chitosan, with its positive charge, and alginate, with its negative charge; form a stable polyelectrolyte complex. Each polyelectrolyte layer provides additional protection to the active compounds encapsulated within the liposome.[80]

Cross-Linked Polymer-Caged Liposomes

Chemical crosslinking of polymers coating liposomes can effectively prevent polymer separation and enhance liposome stability.[81] It has been previously reported that nanoparticles contribute to the swelling of the polymer matrix; which influences the formation of physical crosslinks between polymer chains and liposomal vesicles, resulting in a denser and more compact structure.[82] The strength of vesicles is crucial for creating stable liposomes; which largely depends on the crosslinking applied to the polymer coating.[83]

Nour et al developed a buccal film containing atorvastatin liposomes for the treatment of oral candidiasis. The film was fabricated using 3D printing with chitosan polymer; and to maintain structural integrity; it was crosslinked in situ through a layered spraying method using glutaraldehyde to crosslink chitosan in the ink.[49] Glutaraldehyde functions as a crosslinker for chitosan by reacting with the amino groups in the chitosan chains. This process involves forming crosslinks between glutaraldehyde molecules and available amino groups; which can occur at neutral or slightly acidic pH. This reaction establishes connections between polymer chains, thus enhancing structural stability and resistance to degradation.[84] In this mechanism, glutaraldehyde acts as a linking agent with two aldehyde groups that can react with two amino groups to form imine bonds. This process not only strengthens the polymer network but also modifies the physical and chemical properties of chitosan, including increased solvent resistance and improved physicochemical characteristics.[85]

Polymer Grafted Liposome

Polymer-grafted liposomes are liposome formulations with polymers attached to their surface. This modification is undertaken to enhance liposome properties; improve stability; and increase drug-loading capacity, as well as to facilitate targeted delivery of compounds to specific cells.[86] Hydrophilic polymers are commonly used in this approach, as they adhere to the liposome surface to enhance interactions with the biological environment and reduce nonspecific binding.[87]

PEGylated Liposome

Local drug delivery for treating oral infections has fewer side effects compared to systemic drug administration.[61] However, current topical formulations have poor retention at the wound site requiring frequent dosing.[19] Liposomes as drug carriers are continually being developed for prolonged circulation by incorporating the synthetic polymer polyethylene glycol (PEG) into their composition. The presence of PEG on the surface of liposomal carriers extends the blood circulation time and reduces their uptake by the mononuclear phagocyte system.[88] Furthermore, to enhance liposome absorption; the lipid bilayer can be densely modified with PEG; which improves the permeability of the mucosal membrane in the oral cavity[37] and reduces the clearance rate by the reticuloendothelial system (RES).[89]

In the study by Yamazoe et al, liposomes were modified with PEG to enhance the penetration of active compounds into the oral mucosal membrane. PEGylation of liposomes was shown to improve their ability to traverse the mucosal layer. This study found that liposomes modified with 10% PEG effectively reached the lower layers of an artificial mucosal membrane, demonstrating increased mucus permeability compared to unmodified liposomes.[37] The incorporation of PEG into liposomes aims to enhance oral delivery of macromolecular drugs, representing a promising opportunity in the development of therapies for oral diseases.

Targeted Liposome

Targeted liposomes represent a third-generation liposome type, prepared by surface modification with specific ligands. This liposomal system can be utilized for both active and passive targeting. In the context of passive targeting, liposomal formulations exhibit enhanced drug accumulation in tumor cells due to the Enhanced Permeability and Retention (EPR) effect.[90] Liposomes targeting specific cells can be synthesized by conjugating selective ligands such as tumor-specific antibodies; peptides,[91] polysaccharides,[92,93] transferrin[94] and folic acid.[95] The folate receptor (FR) is frequently overexpressed in tumor cells, making it a promising strategy for tumor targeting. Previous research has shown that FR-mediated endocytosis can facilitate the delivery of macromolecules and liposomes into cells. Consequently, the use of folate-targeted liposomes for drug delivery has garnered significant interest among researchers.[96]

In a study by Yang et al, ursolic acid (UA) was encapsulated in folate-conjugated liposomes (FTL-UA) for the targeted treatment of oral cancer cells, showing enhanced solubility and bioavailability. Pharmacokinetic assessment revealed a significant systemic bioavailability advantage of FTL-UA (AUC = 218.32 mg/L·h; t1/2 = 7.61 hours) compared to free UA (AUC = 36.88 mg/L·h; t1/2 = 0.78 hours).[51] Wijetunge et al developed liposomes conjugated with wheat germ agglutinin (WGA) and grafted them with cyclodextrin on the liposome surface (WGA-liposome-CD) to serve as bioadhesive nanocarriers for oral cavity treatment. This study demonstrated controlled drug release in saliva over 24 h using an in vitro system.[56]

Stimuli-Responsive Liposomes

Stimuli-responsive systems can trigger drug release through destabilization mechanisms induced by internal or external stimuli, such as changes in pH, temperature, redox potential, enzymatic activity, and electrolyte concentration.[96,97] pH-sensitive liposomes are employed as an alternative to conventional liposomes for delivering antigens or other active compounds to target cells. These liposomes become unstable in the acidic pH of endocytic vesicles due to the pH-sensitive lipid components. Upon stimulation; the encapsulated material can be internalized into cells through destabilization or fusion with the endosomal membrane.[98]

In a study by Fang Hu et al, the pH-sensitive liposome mechanism was utilized for treating periodontitis. As pH decreases, as seen in inflamed periodontal tissues, the liposomes undergo structural changes that trigger the release of doxycycline. This process ensures that the drug is released at the desired location, maximizing therapeutic effects while reducing systemic exposure. The pH-responsive properties of the trimethyl chitosan-liposome-doxycycline nanoparticles (TMC-Lip-DOX NPs) not only facilitate drug delivery but also contribute to disrupting bacterial biofilms. These nanoparticles can damage bacterial membrane structures, thereby aiding in periodontal disease treatment.[59]

Role of Liposomes in Oral Disease Therapy

The role of liposomes in the treatment of oral diseases has been widely studied, including in the treatment of candidiasis,[45] oral cancer,[38] oral infections,[56] lesions in the oral cavity,[39] dental caries[41] and periodontitis[59] (Figure 4).

Treatment of Fungal Infections

Oral candidiasis is a common fungal infection of the mucosa that often occurs in immunocompromised patients, such as the elderly; newborns; HIV/AIDS patients; and those undergoing chemotherapy.[99] Fungal infections can spread to the esophagus; causing discomfort while chewing and hindering the patient's ability to swallow.[100] Nour et al conducted a study in which atorvastatin (ATV) was encapsulated in liposomes and incorporated into a 3D-printed polymer film for the local treatment of candidiasis. This method has proven to be superior in addressing resistance issues.[49] Utilizing

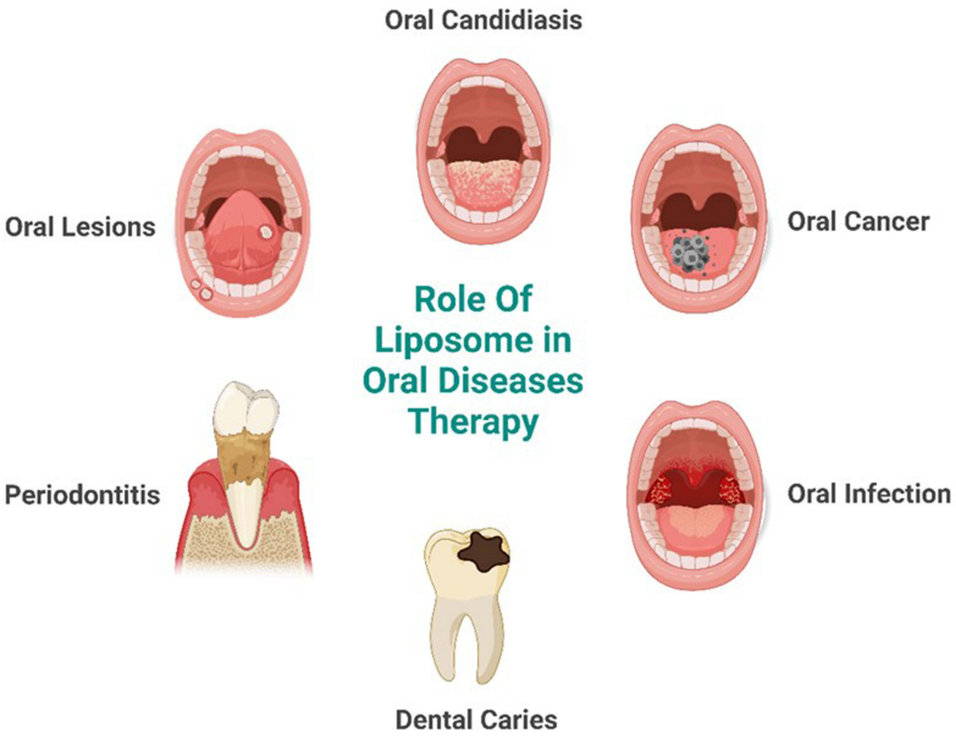

Figure 4 Role of Liposome in Oral Diseases Therapy. Created in BioRender. Suliman, K. (2024) BioRender.com/a09n546.

liposomes; ATV can sustain its release and prolong its antifungal activity. Liposomes worked by binding to the fungal cell wall and directly releasing drugs into fungal cells, thus enhancing drug penetration.[101] In addition to ATV, liposomal formulations have also been developed as drug carriers, including Amphotericin B,[52] farnesol,[43] nystatin,[102] and trans-caryophyllene.[45] In all these studies, the active compounds encapsulated in liposomes demonstrated superior antifungal activity compared to the free form of the active compounds.

Treatment of Bacterial Infections

Oral diseases caused by bacterial infections, such as caries and periodontitis, are prevalent and detrimental. The synthesis of insoluble glucans in teeth provides an environment conducive to bacterial colonization.[103] Bacteria in dental plaque metabolize carbohydrates and produce acids that lower the pH and dissolve minerals. This process ultimately leads to enamel demineralization and the formation of subsurface lesions known as early enamel caries.[104,105] One potential agent for inhibiting glucan biofilm synthesis and preventing dental caries is nisin. Yamakami et al encapsulated nisin in liposomes and found that nisin liposomes demonstrated a more sustained in vitro effect in reducing insoluble glucan synthesis by cariogenic streptococci (Streptococcus mutans 10449) than free nisin.[41]

In a recent study, Zhang et al developed a liposomal delivery system containing a novel peptide called DK5 derived from histatin-1 (DK5-Lips); to treat early enamel caries. DK5-lips release DK5 peptide continuously, maintain stability in saliva, and exhibit no significant toxicity to human gingival fibroblasts.[40] Chitosan-coated liposomes have also been developed by Hu et al. These pH-responsive nanoparticles (NPs) consisted of quaternary ammonium chitosan; liposomes; and doxycycline (TMC-lip-dox NP) and demonstrated strong antibacterial activity both in vitro and in vivo. These NPs effectively inhibited free bacteria and biofilm formation, showing promise for periodontal treatment.[59] Liposomes are capable in penetrating the biofilm matrix; disrupting cell membranes, causing leakage of cellular contents, and inducing bacterial cell death. This effect is crucial for suppressing bacterial biofilms; which are often resistant to traditional antibiotics.[104,106,107]

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Treatment of Oral Cavity Lesions

Ulcerative lesions in the oral cavity significantly diminish quality of life due to severe pain. The etiology of these lesions can be classified into three categories: acute oral ulceration; chronic oral ulceration; and recurrent oral ulceration.[108] These conditions can lead to serious complications, especially immunocompromised patients with an increased risk of infection.[109] Traditional topical agents such as antibiotics; analgesics; and corticosteroids do not always produce consistent and reproducible results.[110,111] In a study by Wakeel et al, wheat germ agglutinin (WGA)-conjugated liposomes loaded with amoxicillin were investigated for the treatment of oral ulcerative lesions. WGA-conjugated liposomes possess specific proteins that facilitate rapid adhesion to oral cells, resulting in significantly reduced oral cell damage compared with untreated controls and free amoxicillin.[56]

Treatment of Oral Cancer

Oral cancer is the sixth most common cancer globally, characterized by high mortality rate.[112] Traditional treatment methods such as surgery; chemotherapy; and radiation therapy can cause significant side effects and damage to healthy tissues.[113] Liposomes have emerged as promising drug carriers, encapsulating therapeutic agents and delivering them directly to cancer cells.; thereby minimizing negative effects on healthy tissues and enhancing therapeutic efficacy. Additionally,; liposomes can provide controlled release and protect drugs from biodegradation, improving the overall effectiveness of cancer treatment.[114] Lopes et al developed RNA-coated liposomes for the treatment of oral cancer, demonstrating their potency as effective drug carriers for oral cancer treatment by affecting cancer cell viability and inducing cytotoxicity effects.[50,53] These RNA-coated liposomes enter cells through a process called clathrin-mediated endocytosis; with RNA facilitating their uptake by various cells, including cancer cells, such as A549.[115] In another study, Hariharan et al developed a liposomal gel formulation containing erlotinib (ERB Lipo) and chitosan coated variant (CS-ERB Lipo) for local delivery to treat oral squamous cell carcinoma (OSCC). In vivo studies revealed that both ERB Lipo gel and CS-ERB Lipo gel significantly reduced tumor volume compared to the standard ERB gel, with CS-ERB Lipo gel showing the highest efficacy, achieving a 55.27% reduction in tumor size.[46]

Clinical Trials

El-Wakeel et al conducted a study to evaluate the effectiveness of topical insulin liposome gel for the treatment of aphthous ulcers. Eighty participants with minor aphthous ulcers received either topical insulin liposome gel or placebo gel (once daily) for six days. The participants were treated with insulin-liposome gel; which was reported to reduce pain associated with recurrent aphthous ulcers. Outcome measures were divided into a visual analog scale (VAS) assessment on days 1, 2, 3, 4, and 6, and an Oral Health Impact Profile-14 (OHIP-14) assessment on the 6th day. The test group demonstrated a significantly shorter average duration than the placebo group ($P < 0.001$). After six days, OHIP-14 scores indicated that the test group had significantly lower scores compared to the placebo ($P < 0.001$). This reduction in pain scores indicated that the insulin liposome formulation was effective in managing discomfort typically associated with oral ulcers.[42] Azizi et al compared the effectiveness of two formulations of 0.1% triamcinolone acetonide, liposomal and non-liposomal, for the treatment of oral lichen planus (OLP). Sixty patients with erosive-ulcerative OLP participated in a clinical trial, receiving 0.1% triamcinolone acetonide with or without a nanoliposomal carrier in Orabase three times daily. The results demonstrated that both formulations led to a significant reduction in pain intensity and oral lesion size ($P < 0.001$). Additionally, after two and four weeks of treatment, the nanoliposomal carrier formulation was significantly more effective than the formulation without it ($P < 0.05$).[116]

Clinical Applications

Ambisome® (Gilead Sciences; Foster City; CA; USA) represents an example of a liposome-based drug that has successfully reached the market. Ambisome® encapsulates Amphotericin B, an antifungal agent, within conventional liposomes composed of neutral or negatively charged lipids along with cholesterol. Nyotran® (Aronex Pharmaceuticals; The Woodlands; TX; USA) contains Nystatin A1, an active polyene membrane antifungal, formulated in multilamellar liposomes consisting of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG). Both

Ambisome® and Nyotran® are utilized for treating fungal infections in immunocompromised patients. However, Nyotran® is currently undergoing Phase II/III clinical trials.[88] Although research on liposomes continues to advance, the clinical application of liposomal formulations remains hindered by various factors, including discrepancies between experimental study results and clinical trials. To address this issue, utilizing appropriate animal models or multiple models may be beneficial for enhancing the efficient translation of liposomal systems into clinical testing environments and improving the quality of treatment for oral diseases.[117]

Challenges in Liposome Development

There are widespread concerns regarding the physical stability of the liposomes. Instability; which leads to instantaneous degradation due to payload leakage stemming from disturbances in the liposome structure; has been reported.[118] Meanwhile, in the current regulations; especially for liposomal products; stability testing needs to be carried out, including stability after exposure to high and low temperatures; light; pH and oxygen; which can cause lipid degradation.[119] Although there were efforts to develop polymer-coated liposomes to mitigate vesicle leakage,[120] challenges have arisen as liposome formulations have become more complex. The integration of multiple components, such as polymers and ligands; into a single nanosized carrier necessitates chemical synthesis steps and formulation modifications. This complexity poses issues for large-scale manufacturing under Good Manufacturing Practice (cGMP) guidelines, including increased production costs and complex product evaluation.[121,122] The inherent complexity of liposomal formulations presents greater challenges in characterization and regulatory review compared to conventional drugs, potentially slowing the development process and increasing costs. Current FDA guidelines do not sufficiently address the clinical efficacy and safety of liposomal products, as they primarily focus on Chemistry Manufacturing and Controls (CMC). This gap highlights the need for a more detailed regulatory framework. Currently, in vitro/in vivo correlation (IVIVC) is highly recommended by the FDA.[119]

Other challenges in liposome development specific to the oral cavity include mucosal permeability,[123] limited surface area,[124] and the abundance of enzymes within the oral environment.[125] Polymer coatings have also been utilized to enhance liposome retention time in the oral cavity.[126] However, continuous mucosal turnover and saliva flushing reduces the residence time of these polymers in the oral cavity, thereby limiting their therapeutic efficacy.[127]

Future Directions

Future advancements in liposome development as targeted drug delivery systems for oral diseases are expected to improve the vesicle stability. Liposomes can be modified with mucoadhesive polymers to enhance the efficacy of drug delivery into the oral mucosa. Moreover, liposomes exhibit good biocompatibility and natural degradation, making them a favorable option for oral disease treatment. With ongoing research advancements in this field, liposomes are anticipated to become an innovative and effective solution for treating oral diseases in the future, with the aim of improving patients' quality of life and reducing the negative impact of oral diseases.

Conclusion

The increasing prevalence of oral diseases; particularly in developing countries; poses a global health challenge. Oral diseases can lead to chronic and progressive effects on an individual's quality of life, including pain; nutritional disturbances; aesthetic deterioration; and social impairment. Various treatments have been developed to address oral diseases; however, their significant limitations often render them ineffective. In this context, liposome research continues to develop through modifications to conventional liposomes; polymer-grafted liposomes; and polymer-coated liposomes; demonstrating promising results in the treatment for candidiasis, oral cancer, oral infections, oral lesions, dental caries, and periodontitis. Based on numerous studies; the use of liposomes as drug carriers has shown potential to enhance the effectiveness of oral disease treatments.

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Disclosure

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