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# Emerging liposomal therapies for diabetic retinopathy: a review of novel targeting approaches and advances in retinal health outcomes

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**ABSTRACT**

Diabetic retinopathy (DR), which affects over millions of individuals globally, is the leading cause of permanent visual loss. Current therapies, including as intravitreal anti-vascular endothelial growth factor (VEGF) medications and laser photocoagulation, are limited by frequent dosing and side effects. Liposomes, with their ability to encapsulate hydrophilic and hydrophobic medications, offer tailored delivery, prolonged release, and low systemic toxicity. This study looks at advances in liposomal formulations that address DR's multifactorial etiology, including as anti-angiogenic, anti-inflammatory, and antioxidant processes. We assess new preparation methods (e.g. supercritical $CO_2$, microfluidics) and clinical considerations, including stability and cost-effectiveness. To address the heterogeneity of DR, future endeavors will prioritize combinatorial medications and customized therapy.

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## 1. Introduction

The most frequent microvascular complication of diabetes is diabetic retinopathy (DR), which continues to be a major cause of blindness even though not all patients may experience noticeable vision loss. Nearly all Type 1 diabetes patients will have some retinopathy after 20 years of diabetes. Furthermore, after 20 years of diabetes, 80% of insulin-dependent Type 2 diabetic patients and 50% of Type 2 diabetic patients who do not require exogenous insulin will have retinopathy (Romero-Aroca et al. 2010). DR is a discrete, chronic, intricate, degenerative condition affecting the retina's neuro-vascular unit. It is clinically characterized by retinal neovascularization, the appearance of microaneurysms, a buildup of protein exudates in the vitreous humor, and ultimately a gradual decline in visual acuity in patients, and is estimated to affect approximately 13 million in developed nations (Cheung et al. 2010). The International Diabetes Federation (IDF) estimates that one in three diabetics suffer from DR, with proliferative DR (PDR) making about 25% of cases. It causes more than $500 million in medical expenses annually in the United States alone, and it is the primary cause of blindness in persons aged 20 to 74 (Thomas et al. 2019). The insidious nature of the disease typically results in asymptomatic progression, leading to vision impairment before it may be diagnosed. Therefore, regular surveillance to assess the severity and advancement of the condition is crucial to monitor its progression and early intervention (Jones and Edwards 2010). The degree of vascular anomalies in the retina is directly correlated with the clinical signs of DR (Cheung et al. 2015). The earliest and mildest stage of the illness is called non-proliferative DR (NPDR). Microaneurysms, intraretinal hemorrhages, hard exudates, cotton wool patches, and enhanced vascular permeability are biomicroscopic indicators of the non-proliferative phase. The more severePDR stage of the illness may then develop as a result of the retinal damage. The development of new blood vessels on the retinal surface, which have the potential to grow and infiltrate the vitreous, is what defines this phase. In the advanced and high-risk phase of PDR, the development of faulty new blood vessels can result in tractional retinal detachment, fibrous proliferation, retinal and vitreous hemorrhages, and, in more severe cases, neovascular glaucoma (Bandello et al. 2013).

## 2. Pathophysiology of DR

The progression of DR is a complex process that involves diverse molecular pathways. Microvascular changes, inflammatory responses, and oxidative stress are three key factors that are interconnected and contribute to the development of DR (Whitehead et al. 2018). Microvascular changes in the retina lead to the breakdown of the blood-retinal barrier (BRB) and the formation of acellular capillaries, which can cause retinal ischemia and hypoxia. Inflammatory responses are triggered by the accumulation of advanced glycation end products (AGEs) and other metabolic byproducts, which activate various signaling pathways that promote inflammation and leukostasis (Suryavanshi and Kulkarni 2017). Oxidative stress is also a major contributor to the pathogenesis of DR, as it leads to excessive production of free radicals in mitochondria, abnormal rheology, and activation of the renin-angiotensin system.

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All these three factors form a synergistic relationship and can amplify each other's effects leading to more severe outcomes. For example, oxidative stress can lead to increased production of inflammatory cytokines, which in turn can exacerbate microvascular changes. Similarly, inflammation can cause endothelial dysfunction and leukostasis, which can further increase oxidative stress (Biswas et al. 2018). In summary, microvascular changes, inflammatory responses, and oxidative stress are all important contributors to the progression of DR. Understanding their collective impact is crucial for developing effective treatments for this debilitating disease (Ola et al. 2012).

## 2.1. Microvascular changes

### 2.1.1. Capillary basement membrane thickening

In DR, the capillary basement membrane undergoes alterations that lead to thickening and impaired nutrient exchange. The thickening of the capillary basement membrane reduces the bioavailability of nitric oxide (NO) via disrupting endothelial tight junctions (claudin-5 and zonula occludens-1). As a result, the BRB degrades and vascular leakage develops due to poor vascular tone regulation and increased leukocyte adhesion caused by intercellular adhesion molecule-1 (ICAM-1) overexpression. Hypoxia increases VEGF expression even further, perpetuating a cycle of neovascularization and hyperpermeability. The thickened basement membrane also contributes to the formation of microaneurysms, which can rupture and cause hemorrhages (Kusuhara et al. 2018).

### 2.1.2. Pericyte loss

Pericytes are cells that wrap around the endothelial cells of capillaries and venules, providing structural support and regulating blood flow. They also play a crucial role in maintaining vascular stability by controlling endothelial proliferation. Pericyte loss is a hallmark feature of DR (Santos et al. 2018). This loss leads to microaneurysms, acellular capillaries, and increased vascular permeability. The resulting vascular instability can cause retinal ischemia, which can lead to neovascularization and PDR (Kusuhara et al. 2018; Santos et al. 2018).

### 2.1.3. Endothelial dysfunction

Endothelial cell dysfunction is a hallmark of DR. It is characterized by the accumulation of AGEs in the retinal blood vessel walls, which causes increased permeability of retinal endothelial cells (ECs) and induces vascular leakage. AGEs can also up regulate AGE receptor (RAGE) gene expression levels in pericytes and microvascular ECs (Semeraro et al. 2019). This leads to the breakdown of the BRB, which is essential for maintaining the homeostasis of the retina (Safi et al. 2014). Endothelial cell dysfunction also affects vascular tone and angiogenesis within the retinal microvasculature. It causes a decrease in NO bioavailability, which is essential for regulating vascular tone and growth, thrombosis, immune cell responses, and vascular barrier functions. The decrease in NO bioavailability leads to vasoconstriction and increased vascular resistance, which can cause hypoxia and ischemia in the retina. Endothelial cell dysfunction also impairs angiogenesis by reducing the ability of ECs to execute their functions in regulating vascular growth and remodeling (Whitehead et al. 2018). Moreover, the diabetic retina's endothelium promotes the expression of ICAM-1, which leads to a buildup of leucocytes around the retinal capillaries' vascular walls. This, in turn, causes the retina to release cytokines, chemokines, and proinflammatory and pro-angiogenic growth factors like VEGF, which cause neovascularization and a low-grade inflammatory states (Safi et al. 2014). The breakdown of the BRB and the development of intraretinal edema are caused by inflammatory mediators that break down the tight connections between endothelial cells and increase vascular permeability (Suryavanshi and Kulkarni 2017).

## 2.2. Oxidative stress

### 2.2.1. Mitochondria dysfunction

Under hyperglycemic conditions retina produces an excessive amount of reactive oxygen species (ROS), and the exacerbated oxidative stress causes mitochondrial malfunction. The essential components for transcription and regulatory sections for mitochondrial DNA (mtDNA) replication are found in the displacement-loop (D-loop), a sizable non-coding sequence and extremely susceptible unwinding region in mtDNA (Kang and Yang 2020). Compared to other regions of mtDNA, the D-loop experiences more mutations and impairments in diabetes, and its copy numbers are decreased. Furthermore, the hyperglycemia-induced hypermethylation of mtDNA in DR impacts its transcription and results in mitochondrial malfunction, ultimately encouraging capillary cell death (Kusuhara et al. 2018; Kang and Yang 2020). It was also established that the base mismatch of mtDNA in the pathophysiology of DR is a latent component caused by epigenetic change on mtDNA.

Proteins encoded by mtDNA are essential for maintaining mitochondrial homeostasis and the electron transport chain's (ETC) regular operations (Ola et al. 2012). As circular mtDNA lacks protective histones, it is more susceptible to more extensive and long-lasting oxidative stress-induced damage than nuclear DNA. Damaged mtDNA causes transcription and protein synthesis to malfunction, further impairing electron transport and exacerbating the production of ROS (Stitt et al. 2013). Subnormal transcriptional levels of mtDNA-encoded genes linked to the ETC system in DR, such as NADH dehydrogenase 1 and 6 of complex, have also been demonstrated (Safi et al. 2014).

Mitochondrial dysfunction in DR is also caused by the activation of gelatin matrix metalloproteinase (MMPs). Diabetes increases oxidative stress and upregulates MMP production by activating the NADPH oxidase (Nox) complex (Kang and Yang 2020). The translocation of MMPs into the mitochondria is facilitated by oxidative stress and diabetes. Redox-sensitive MMPs (MMP-2 and MMP-9) are transported and accumulate in the retinal mitochondria through this method, which depends on the regulation of chaperones Hsp60 and Hsp70. MMPs inside the mitochondria damage the mitochondria and enhance pore permeability by breaking down connexin. The apoptosome platform assembles and

the caspase cascade begins when cytochrome c (Cyt c) leaks from the mitochondria into the cytosol, resulting in larger mitochondria in the retinas of diabetic mice. This is caused by disrupted mitochondrial lipid membranes. Furthermore, superoxide and NO can combine to form the powerful oxidant peroxynitrite. Peroxynitrite oxidizes glutathione GSH, cysteine, and tetrahydrobiopterin, oxidizing membrane phospholipids, inactivating enzymes containing sulfhydryl moieties, nitrating tyrosine residues, and increasing DNA fragmentation (Stitt et al. 2013; Nebbioso et al. 2022). In addition to causing irreversible damage to mitochondria and calcium homeostasis, peroxynitrite also promotes the opening of the permeability transition pore, which ultimately leads to cell death (Kang and Yang 2020).

#### 2.2.2. Role of reactive oxygen species (ROS)

In response to the oxidative stress caused by hyperglycemia, the hexosamine/polyol pathway may be activated. As a result, ROS buildup and a drop in NADPH levels occur. Elevated glucose levels can cause glyceraldehyde 3-phosphate dehydrogenase to be inhibited, which results in elevated glucosamine (Whitehead et al. 2018). The ensuing rise in $H_2O_2$ generation causes angiogenesis, altered cell endothelium, increased vascular permeability, and enhanced oxidation. The pathophysiology of DR is mostly attributed to oxidative stress, which is exacerbated by an ischemia condition. Oxidative stress is a key factor in the initiation and progression of DR (Safi et al. 2014; Rübsam et al. 2018). In hyperglycemic states, different pathways are activated producing ROS which enhance inflammatory, apoptotic, and degeneration pathways, ultimately leading to the appearance of DR clinical characteristics. ROS can cause damage to macromolecules, cells, and tissues, leading to endothelial and neural damage. The mechanisms involved in DR development are interlinked, thus worsening the DR outcome. ROS can activate the NLRP3 inflammasome by several pathways such as ROS and ATP. The activation of NPRP3 leads to the secretion of inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18), and leads to pyroptosis, a rapid inflammatory form of lytic programmed cell death (Suryavanshi and Kulkarni 2017). Thus, ROS play an important role in DR complications such as inflammation and cellular degeneration leading to endothelial and neural damage (Feenstra et al. 2013).

#### 2.2.3. Antioxidant defense mechanism

The retina has a complex antioxidant defense system that includes enzymatic and non-enzymatic antioxidants, such as superoxide dismutase, catalase, glutathione peroxidase, vitamin C, vitamin E, and carotenoids. These antioxidants work together to neutralize ROS and prevent oxidative damage to the retina. In diabetic conditions, the retina is exposed to high levels of glucose, which can lead to the overproduction of ROS and the depletion of antioxidant defenses (Silva et al. 2010). This imbalance between ROS production and antioxidant defenses leads to sustained oxidative stress in the retina. Oxidative stress can cause damage to lipids, proteins, and DNA in retinal cells, leading to cell death and vision loss. By compromising the integrity of the BRB, oxidative stress sets the stage for DR (Silva et al. 2010; Kang and Yang 2020). The BRB is a specialized barrier that regulates the transport of nutrients and waste products between the retina and the blood vessels that supply it. Oxidative stress can cause damage to the cells that make up the BRB, leading to increased permeability and leakage of fluid into the retina. This can cause swelling of the retina and vision loss. In summary, endogenous antioxidant defense mechanisms in the retina play a crucial role in protecting retinal cells from oxidative damage. However, in diabetic conditions, these mechanisms are overwhelmed by sustained oxidative stress, leading to damage to retinal cells and vision loss (Kang and Yang 2020).

### 2.3. Inflammation

#### 2.3.1. Role of inflammatory mediators

In patients with diabetes mellitus, inflammation plays a significant part in the development of DR. The NF-κB pathway reduces the expression and alters the distribution of the tight junction proteins zonula occludens-1 and claudin-5 by promoting the expression of proinflammatory cytokines like tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8), IL-6, and other proapoptotic regulators in retinal endothelium (Suryavanshi and Kulkarni 2017). Retinal vascular permeability is raised as a result of this process. High levels of cytokines and proinflammatory mediators, as well as a significant leukocyte adhesion, are examples of inflammatory processes. Cellular damage and apoptosis are the outcomes of the ensuing rise in vascular permeability and ROS generation (Chu and Ali 2008). Additionally, diabetic animals' retinas exhibit elevated amounts of IL-1β due to increased caspase-1 enzyme activity. ICAM-1, cytokines, and inducible nitric oxide synthase (iNOS) are among the proinflammatory proteins whose transcription is triggered by IL-1β, one of the proinflammatory cytokines that activates NF-κB (Suryavanshi and Kulkarni 2017).

#### 2.3.2. Leukostasis and adhesion molecules

Leukostasis is a phenomenon where white blood cells (WBCs) adhere to the walls of blood vessels, obstructing blood flow and causing inflammation. In DR, leukostasis occurs in the retinal microvasculature, leading to vascular compromise and retinal damage. Adhesion molecules, such as ICAM-1, play a crucial role in leukostasis by promoting the adhesion of WBCs to the endothelial cells of blood vessels (Suryavanshi and Kulkarni 2017; Rübsam et al. 2018). Studies have shown that leukocytes, which are normally less deformable than erythrocytes, have decreased filterability under diabetic conditions. Activated monocytes and granulocytes are found in increased numbers in the diabetic environment (Semeraro et al. 2019). The diabetic milieu promotes a 'sticky' vascular endothelial phenotype, characterized by increased adhesion molecule expression, which underlies the observed leukostasis. In summary, leukostasis and adhesion molecules contribute to inflammation and vascular compromise in DR by obstructing blood flow and causing retinal damage. The role of adhesion molecules such as ICAM-1 is crucial in promoting leukostasis

by facilitating the adhesion of WBCs to endothelial cells of blood vessels (Chistiakov 2011).

#### 2.3.3. Microglia activation

Understanding the mechanism of microglial activation in response to retinal stress may provide insights into the DR. Microglia are the immune cells that dwell in the retina, and their chronic inflammation is known to contribute to the pathophysiology of DR (Rübsam et al. 2018). Uncontrolled microglial activation is probably a contributing factor to the diabetic retina's tissue destruction and neurotoxicity. However, little is known about the cellular and molecular processes that underlie microglial activation in the early stages of DR. Numerous investigations demonstrate that transcriptional alterations in activated microglia, facilitated by the extracellular signal-regulated kinase (ERK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) signaling pathways, cause the release of a variety of pro-inflammatory mediators, such as glutamate, cytokines, chemokines, and caspases (Feenstra et al. 2013; Suryavanshi and Kulkarni 2017). A detailed schematic diagram for pathogenesis of DR is depicted in Figure 1.

## 3. Conventional therapies: overview and limitations

### 3.1. Laser photocoagulation

Laser photocoagulation surgery, a widely adopted treatment for various eye conditions, involves delicately cauterizing ocular blood vessels with a laser. This technique is particularly effective in managing DR to reduce the risk of visual loss. In order to induce coagulation in the target tissue, a highly concentrated laser beam is directed toward it. Laser photocoagulation is recommended for the treatment of clinically substantial macular edema in NPDR (Lang et al. 2018).

The process of photocoagulation, which results from tissue absorption of radiant energy and conversion to heat, involves protein denaturation. It is important to distinguish it from photo-disruption and photo-ablation, which involve different chemical processes and are more frequently utilized in the anterior region and during refractive eye surgery. Proteins are denatured and leaky vessels are sealed by photocoagulation, which uses heat radiation (such as an argon laser). While photo-ablation (e.g. excimer laser) vaporizes tissue using high-energy photons, which is frequently employed in corneal refractive surgery, photodisruption (e.g. Nd:YAG laser) uses ultrashort pulses for non-thermal optical breakdown. Although photocoagulation with visible light is conceivable, the development of lasers has allowed for more accurate, dependable, and painless use of photocoagulation, transforming the retinal treatment. Laser photocoagulation is now the standard treatment for many retinal diseases, offering efficient and noninvasive application techniques (Sushma et al. 2015; Gawęcki et al. 2023).

Laser photocoagulation is a widely used therapeutic method for controlling DR and preventing further complications. This method uses light energy to inhibit neovascularization, which reduces the formation of new blood vessels and prevents vision loss. Although a one-time administration is feasible, spreading the treatment over several sittings reduces the likelihood of adverse effects. As medical treatment costs rise, laser therapy may become less common in affluent countries, but it will remain useful. Beyond the cost, laser photocoagulation increases the risk of peripheral vision loss (scotomas), choroidal effusion, and subretinal fibrosis. In advanced PDR, laser efficacy declines due to significant ischemia, necessitating additional therapy. Although the use of laser photocoagulation may decline in developed and high-income countries due to newer modalities like anti-VEGF medications, it will continue to be applicable in other parts of the world with limited access to advanced therapies. The inflammation caused by laser can be lessened with the use of steroids, thereby lowering the laser's intensity. In the past, laser photocoagulation was the main PDR therapy method; however, in diabetic macular edema (DME) care, it is gradually being replaced. If PDR patients receive laser therapy as soon as possible, vision loss can be prevented; however, once eyesight has been lost, there is almost no chance of recovery (Steijns et al. 2010; Evans et al. 2014; Zhang et al. 2021).

### 3.2. Anti-VEGF therapies

In the pathophysiology of DR, retinal ischemia stabilizes hypoxia-inducible factor-1α (HIF-1α), which translocates to the nucleus and binds to hypoxia-response elements (HREs) in the VEGF promoter. This stimulates VEGF transcription, resulting in endothelial proliferation, vascular permeability, and pathological angiogenesis. Chronic hyperglycemia increases HIF-1α activity by overproducing mitochondrial ROS and activating the polyol/hexosamine pathway, leading to VEGF overexpression in a feedback loop (Aiello and Wong 2000; Arrigo et al. 2022). It plays a significant role in the development of complications associated with DME and is a key mediator in the process of retinal neovascularization, which can lead to vitreous hemorrhage and tractional retinal detachment. Placental Growth Factor (PGF) and VEGF-A, B, C, D, E, and F are some of the isoforms of VEGF that are included in the term. The first isoform is one of the main pathogenic factors associated with DR (Mesquita et al. 2018).

The emergence of anti-VEGF intravitreal drugs substantially changed the course of DR and patient outcomes, leading to a significant reduction in the incidence of legal blindness. Nowadays, a number of anti-VEGF medications impact different VEGF isoforms and metabolic pathways. The benefits of anti-VEGF as a first-line treatment for DME are undeniable; nevertheless, there is significant controversy regarding the management of the proliferative form (Arrigo et al. 2022). Taking everything into consideration, the VEGF-A isoforms are the main therapeutic target of anti-VEGF medications. Endogenous anti-VEGF mechanisms are already present, although they are compromised in retinal disorders such as DR. Not much research has been done on the physiologic anti-VEGF pathways. The anti-VEGF effect of the VEGF165b isoform was one of the few findings from animal

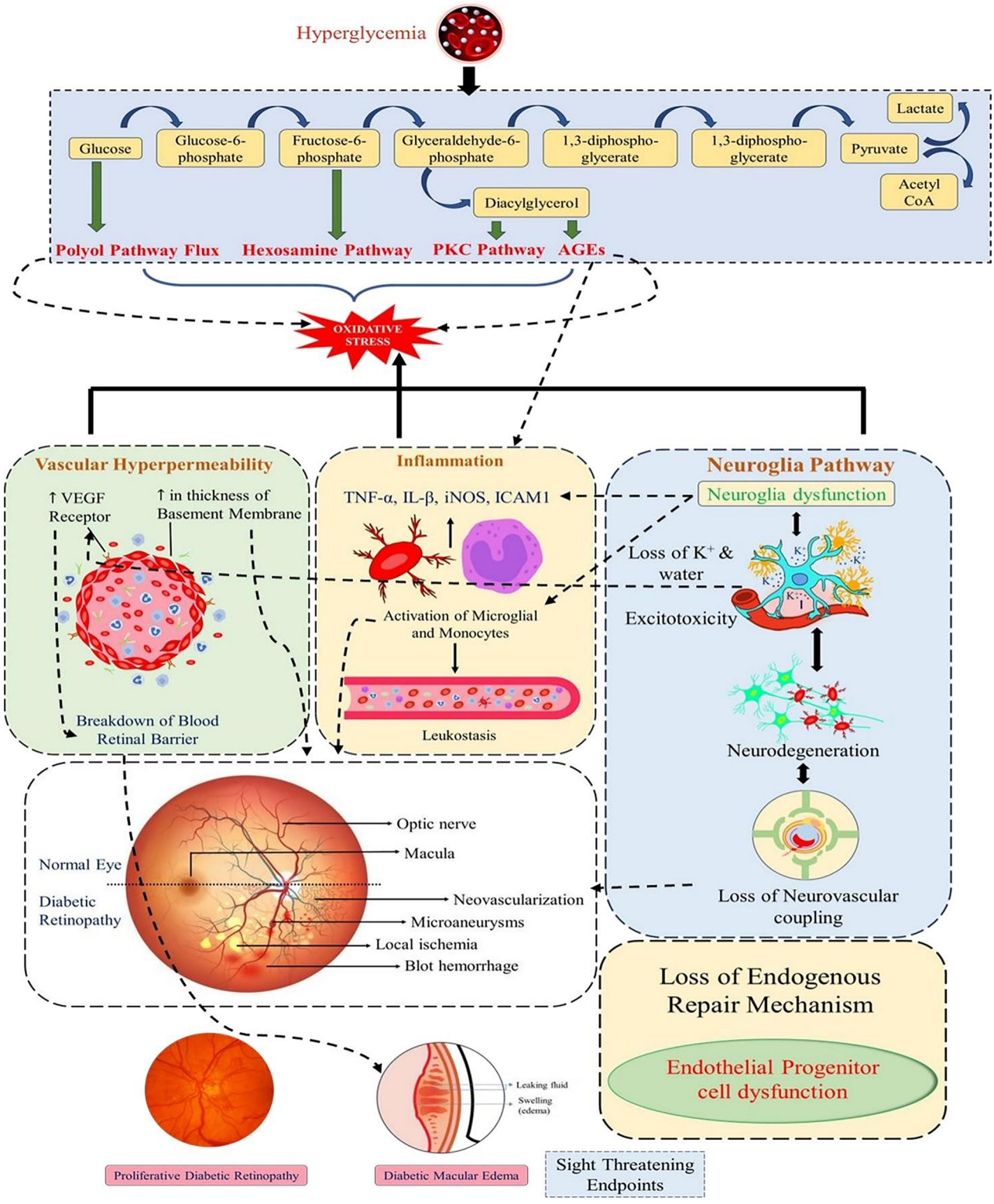


**Figure 1.** Schematic illustration of hyperglycemia-induced molecular pathways leading to diabetic retinopathy and other sight-threatening complications. (Self-prepared by authors).

models. More specifically, VEGF165b appears to block hypoxia and angiogenic stimulation brought on by VEGF overexpression, obstructing endothelial cell migration and proliferation. To better understand the endogenous anti-VEGF mechanisms, present in the retinas of humans and animals, more focused research on this subject is necessary (Zhang et al. 2006; Kociok and Joussen 2007). Aflibercept, Conbercept, Brolucizumab, Abicipar-pegol, Faricimab, Pegaptanib, Ranibizumab, and Bevacizumab are currently available as anti-VEGF medications (Arrigo et al. 2022). Anti-VEGF agents (e.g. ranibizumab, aflibercept) are administered via intravitreal injection monthly or as needed.

While effective, repeated injections risk endophthalmitis, retinal detachment, and patient noncompliance. A considerable proportion of patients do not exhibit a good response to the intervention. Resistance to the treatment becomes evident once it is administered repeatedly. Additionally, one of the potential risks of intravitreal injection practice is the development of corneal scarring (Blinder et al. 2017).

### 3.3. Corticosteroids

The anti-inflammatory and antiangiogenic properties of corticosteroids have led to their use in the treatment of DR and DME. Corticosteroids successfully modify several inflammatory mediators, including TNF-α, IL-1β, and VEGF, which are elevated in DR and contribute significantly to its pathogenesis. Reduction of vascular permeability, inhibition of leukocyte adherence to vascular walls, reduction of BRB disintegration, and inhibition of VEGF gene transcription and translation have all been demonstrated effects of these substances (Kaštelan et al. 2013; Tsai et al. 2018). A potential treatment for DME is intravitreal injection of the slow-releasing steroid triamcinolone acetonide (IVTA), commercially available as Kenalog-40®. This medication controls inflammation, lowers vascular leakage, and prevents the growth of new arteries. It reduces the thickness of the fovea in eyes with DME and, in many cases, improves visual acuity. For PDR, intravitreal TA injection is another successful therapeutic strategy (Gillies et al. 2006; Jonas 2007).

Although their impact is temporary and short-lived, corticosteroids quickly reduce macular edema. Sometimes, depending on the half-life of the steroid being administered, further injections are required at varied intervals after the antiedematous effects stop. Systemic adverse effects, including the aggravation of diabetes, as well as ocular problems such as cataract development, increased intraocular pressure, and glaucoma, preclude the use of systemic corticosteroids in DR treatment. These adverse effects are also present in intraocular formulations and may further restrict their use. Currently, triamcinolone, fluocinolone, and dexamethasone are the three different steroids utilized to treat DME (Cunningham et al. 2008; Stewart 2012).

### 3.4. Neuroprotective agents

DR is a complex disease that affects both neural and vascular tissue. A major contributing factor to the disease's pathogenesis is neurodegeneration. In addition to conventional vascular therapies, neuroprotection has become increasingly important as a therapeutic approach for DR, according to recent studies. Topical administration of brimonidine, nerve growth factor (NGF), and somatostatin (SST) has demonstrated neuroprotective effects; these neuroprotective agents have shown promise in animal models. By acting locally in the eye, these substances reduce systemic effects and have the potential to completely change the way diabetic patients are treated (Hernández and Simó 2014; Hernández et al. 2016). Neurodegenerative DR involves complex pathophysiological pathways. Diabetes-induced metabolic changes alter the expression of various mediators, causing vascular lesions and neuronal cell death. Neurodegeneration is now recognized as an early event in DR, which contributes to microvascular abnormalities. Retinal neurodegeneration can be detected through functional and structural changes, making it a potential early intervention target. Neurodegeneration in DR is caused by altered insulin signaling, decreased levels of neuroprotective factors such as pigment epithelial-derived factor (PEDF), and oxidative stress.

### 3.5. Vitrectomy

Vitrectomy is critical in the treatment of advanced DR, especially for significant vitreous hemorrhage and retinal detachment affecting central vision. The vitreous body influences DR advancement by structural alterations, angiogenic agents, and its role as a neovascularization scaffold (Hendrikse and Yeo 1993). In cases of severe vision loss caused by proliferative DR, vitrectomy can restore functional vision while lowering the risk of vision loss following traction retinal detachment (Arrigg and Cavallerano 1998). Since its inception in 1970, the surgery has progressed, with advances in instrumentation and procedures improving outcomes and reducing complications (El-Sabagh 2014). While vitrectomy is reserved for advanced cases where safer alternatives like photocoagulation are ineffective, future developments may include vitreous manipulation using enzymes and lasers for DR treatment and prevention (Hendrikse and Yeo 1993; El-Sabagh 2014).

## 4. Need for innovative drug delivery systems in DR treatment

Intravitreal injections, laser therapy, surgical techniques, and other options are currently available to treat DR. Although these therapies have been somewhat effective, there is still a need for novel strategies to improve therapeutic outcomes and mitigate the impact of this condition. The severity and progression of DR varies, making it a complicated disease with a wide spectrum, and this heterogeneity can be difficult to address with traditional therapies. Innovative approaches can improve treatment outcomes by providing tailored and focused therapy based on the patient's specific condition (Cho and Sobrin 2014; Nair et al. 2022). Early detection and regular surveillance are essential for effective DR management. Cutting-edge technologies such as telemedicine and artificial intelligence-based image analysis can aid in early diagnosis and remote monitoring, ensuring prompt action and lowering the risk of vision loss (Vujosevic et al. 2022; Land et al. 2023). Patients may find it difficult to endure the numerous injections or laser sessions required for current therapies. Novel drug delivery methods, such as gene therapies or sustained-release implants, can reduce treatment-related side effects and increase patient compliance (Ryan 2007; Simó and Hernández 2015). Novel approaches can focus on specific molecular pathways linked to DR, resulting in more specialized and effective treatments. This field of study has the potential to yield new treatment options and drug candidates (Dulull et al. 2019).

## 5. Liposomal drug delivery systems

Liposomes are biodegradable and biocompatible vesicular lipid assemblies where outer lipid bilayers entrap lipophilic drugs, while hydrophilic drugs are loaded within the aqueous core. Thus, it is capable of delivering both hydrophilic and lipophilic drugs (Parashar et al. 2024). Liposomes are emerging as a revolutionary platform for ocular drug administration, taking advantage of their unique structural features to enable medicines to be delivered precisely and consistently. Liposomes, by encapsulating pharmaceuticals within lipid bilayers and aqueous core, can increase the drug's contact time with the eye surface, boosting the possibility of diffusion through the ocular layers (López-Cano et al. 2021; Parashar et al. 2024). This strategic approach has been successfully used to treat a variety of ocular diseases, including age-related macular degeneration (AMD), where liposomes were used to deliver berberine hydrochloride and chrysophanol to the posterior chamber, resulting in increased bioavailability and protective effects (Lai et al. 2019). Furthermore, liposomes have been engineered to include positively charged chemicals, such as amines, to give mucoadhesive characteristics, extending formulation residence time and improving passive drug delivery. These novel formulations have been found to enhance the efficacy of topical ophthalmic antibiotics like besifloxacin by enhancing permeability and bioavailability (Dos Santos et al. 2020). Figure 2 depicts a schematic representation of the general structure of a liposome.

The composition of liposomes has a substantial impact on their size, polydispersity index (PDI), and zeta potential, and all these parameters influence colloidal stability, biodistribution, and cellular affinity. Liposomes can range in size from 0.025 µm to 2.5 µm, with either single or bilayer membranes (Akbarzadeh et al. 2013). The zeta potential is important for directing liposomes to specific cells and tissues because it affects electrostatic interactions and determines drug uptake (Dos Santos et al. 2020). Cholesterol, a fundamental component in liposomal compositions, is directly related to liposome size, with higher concentrations leading to larger vesicles. However, cholesterol improves liposome stability by boosting resistance to aggregation, decreasing bilayer permeability, and encouraging effective phospholipid packing (Nsairat et al. 2024). The nature and quantity of drug loaded into liposomes are important elements in determining their efficacy, and liposomes have been used to deliver a variety of pharmaceuticals, including peptides, proteins, hormones, and anticancer treatments.

Furthermore, liposomes have been designed to target specific ocular tissues, such as the cornea, by integrating specific lipids and surfactants, increasing their therapeutic potential (López-Cano et al. 2021). Schematic representation of (A) Conventional Liposomes, (B) Stealth Liposomes, (C) Targeted Liposomes is shown in the Figure 3. Overall, liposomes have proven themselves as a stable and versatile platform for ocular drug administration, offering a range of advantages including site-specificity, prolonged release, degradation protection, and decreased toxic side effects.

### 5.1. Liposomal formulations for targeting microvascular pathway (anti-VEGF)

Liposomal formulation has been widely explored for delivering anti-VEGF drugs in DR treatment, as they are less immunogenic, less toxic than polymeric nanoparticle-based delivery systems and provide distinct advantage of increased half-life and improved drug bioavailability in retina (Seah et al. 2020; Torkashvand et al. 2024). Liposome-polyethylenimine complexes (lipopolyplexes) were produced to deliver small interfering RNA (siRNA) to HuR, an RNA-binding protein that regulates VEGF expression. Size, zeta potential, serum stability, RNase stability, heparin stability, toxicity, and siRNA encapsulation efficiency were all investigated in the lipopolyplexes. *In vitro* and *in vivo* effectiveness experiments in human ARPE-19 cells and streptozotocin-induced diabetic rats indicated that intravitreal therapy with HuR siRNA employing lipopolyplexes as delivery vehicles lowers retinal HuR and VEGF levels (Supe et al. 2023). In another study, an

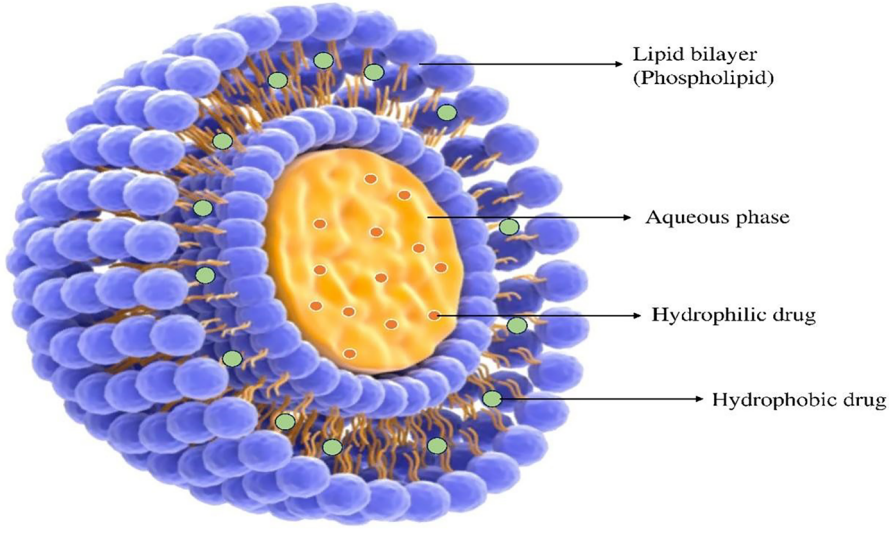


**Figure 2.** Schematic representation of the general structure of a liposome (Self-prepared by authors).

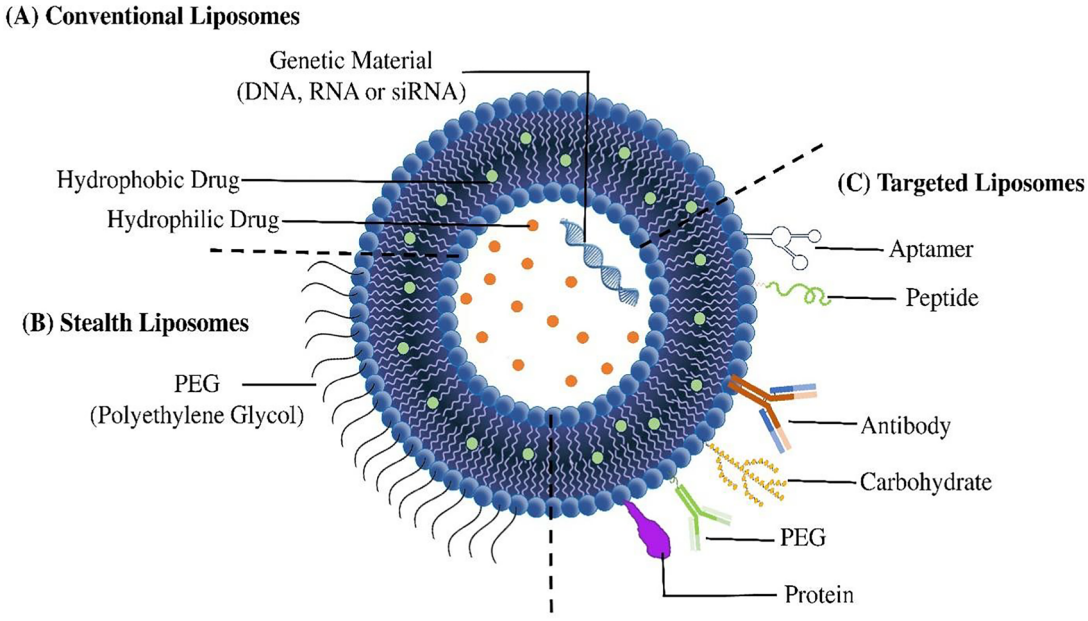


**Figure 3.** Schematic representation of (a) conventional liposomes, (b) stealth liposomes, (c) targeted liposomes (Self-prepared by authors).

efficient anti-neovascular effect was observed for 3 days after a single intravitreal injection of liposomes containing 1 µg of encapsulated sunitinib. At a comparatively high loading capacity, the liposomes (mean size 104 nm) were able to encapsulate sunitinib with an encapsulation effectiveness of around 95%. A laser-induced model of cerebrovascular accident (CNV) in mice demonstrated an inhibitory impact of intravitreal liposomes loaded with sunitinib on established neovascularization which suggests its potential ineffective DR treatment (Tavakoli et al. 2022).

These findings show that liposomal formulations have the potential to treat DR by delivering anti-VEGF medicines and other therapeutic substances to the retina. Animal models, such as DR mice models and diabetic rats generated by streptozotocin, have been invaluable in assessing the safety and effectiveness of these liposomal formulations *in vivo*.

### 5.2. Liposomal formulations for targeting inflammation

Anti-VEGF medications treat the vascular component of vision loss; however, inflammation still plays a major role. Chronic inflammation in diabetic patients is driven by overexpression of pro-inflammatory cytokines. Liposomal formulations have emerged as a viable strategy for delivering anti-inflammatory medicines to the retina. Targeting inflammation is critical for effective treatment of DR. Many vitreous and retinal problems are often treated with intravitreal injections (IVTs) of corticosteroids, specifically triamcinolone acetonide (TA). On the other hand, significant eye problems are linked to IVTs. A topical ocular TA-loaded liposome formulation (TALF) was designed to topically distribute TA in order to address the limitations of invasive routes of drug administration. The formulation improved visual acuity and decreased central foveal thickness in patients with DME and showed safety, acceptability, and biological activity in pre-clinical and clinical investigations (Navarro-Partida et al. 2021).

As a possible treatment for DR, a liposome-encapsulated bromfenac solution (100 µg/0.1 ml) has also been studied. In rabbit model of DME, the formulation was shown to be safe and nontoxic, indicating potential as a future alternative treatment for DR and warranting further studies (Sánchez-Santos et al. 2020). In another study, the potential of flavonoid-liposome formulations in the treatment of age-related macular degeneration (AMD) and DR has been studied. It has been demonstrated that these formulations contain anti-inflammatory and antioxidant qualities, which may aid in lowering oxidative stress and inflammation in the retina (Halevas et al. 2022).

Liposomal compositions have shown substantial potential in reducing inflammation in DR. The use of liposomes to transport anti-inflammatory medicines including citicoline, HuR siRNA, and diclofenac has been shown to reduce inflammation and improve retinal health.

### 5.3. Liposomal formulations for targeting antioxidant defense mechanism

Oxidative stress is a critical factor in the development of DR, and antioxidants have been studied as potential treatment agents to reduce damage. Liposomes offer a promising antioxidant delivery strategy, capable of encapsulating both hydrophobic and hydrophilic drugs, protecting them from potential degradation and enabling targeted delivery to specific tissues. Lisosan G (LG), a nutraceutical derived from fermented whole grains, encapsulated in liposomes (LipoLG) to increase its bioavailability and protect against DR in a mouse model has been reported (Amato et al. 2023). The study indicated that LG shields *Drosophila melanogaster* eyes from oxidative stress and neurotoxicity brought on by a high-sugar diet. Streptozotocin was used to develop diabetes in mice, who were then fed water, LG, or LipoLG for 6 weeks. Electroretinography and molecular

analysis revealed that, while the highest dose of LG only partially protected against DR-induced retinal damage, both LipoLG levels were extremely beneficial. These findings show that liposomal encapsulation greatly improves LG's efficacy in treating DR, emphasizing the benefits of employing liposomes as nanocarriers to improve the bioavailability and therapeutic potential of nutraceuticals for retinal diseases.

### 5.4. Liposomal formulations for targeting multiple pathways

Biacin, a multi-therapeutic flavonoid possessing anti-inflammatory, antioxidant, and anti-angiogenic activity was formulated into various vesicular delivery systems namely penetration enhancer vesicles PEVs, transfersomes, and liposomes to address its poor solubility and reduced stability in basic pH. Vesicular formulations outperformed baicalin solution in antioxidant potential and outperformed ascorbic acid in terms of sterilization durability and safety for ocular tissues. Pharmacokinetic tests found that transfersomes had the fastest onset of action and liposomes had the highest absorption, with $T_{max}$, $C_{max}$, and $AUC_{0-\infty}$ values indicating a 4–5 times improvement in bioavailability compared to the baicalin control. Baicalin vesicular systems show promise in treating eye illnesses like inflammation, cataracts, and DR (Ashraf et al. 2018).

Citicoline, an anti-inflammatory drug, was encapsulated in liposomes to target the retina and inhibit glial activation and neuronal death in DR patients. In experimental diabetes-induced retinal neurodegeneration in db/db mice, the liposomal formulation of citicoline was found to be beneficial in avoiding such effects (Bogdanov et al. 2018).

Naringenin, a flavanone found in citrus fruits, is an antioxidant, free radical scavenger, anti-inflammatory, and immunomodulator. It has been proven to improve DR by reducing angiogenesis and VEGF production while also providing antioxidant and anti-inflammatory benefits. Using nanoliposomes as nanocarriers for naringenin delivery improves its solubility, bioavailability, and controlled release, making it a potential strategy for treating retinopathy and other disorders in experimental rabbit models (Salimi et al. 2022) (Table 1).

### 5.5. Ideal liposome properties for posterior segment delivery

Effective liposomal delivery to the posterior eye segment (retina and choroid) requires careful optimization of physicochemical and functional properties to overcome anatomical barriers such as the BRB, vitreous humor, and rapid clearance mechanisms. The ideal liposomal characteristics include:

1. Particle Size (<200 nm): Liposomes that are smaller (50–200 nm) have better penetration into the layers of the retina and better diffusion through the vitreous, preventing trapping in the ocular matrix. Liposomes loaded with sunitinib (104 nm) effectively inhibited neovascularization in mouse models (Tavakoli et al. 2022).
2. Surface Charge (Slightly Positive): Positively charged liposomes (+10 to +30 mV) adhere to negatively charged mucin layers, prolonging ocular residence time. Neutral liposomes minimize nonspecific interactions with vitreal components. Cationic liposomes enhanced besifloxacin retention on the ocular surface (Dos Santos et al. 2020).
3. Sterility and Stability: Sterile formulations, produced through supercritical $CO_2$ or filtration, eliminate microbiological contamination. Lyophilization with cryoprotectants (e.g. trehalose) ensures long-term stability (Soares et al. 2019; Boafo et al. 2022).
4. Targeted Functionalization: Retinal specificity is improved by ligands (such as peptides or antibodies) that target overexpressed receptors in DR, such as integrins or VEGF receptors. For instance, in diabetic rats, transferrin-conjugated liposomes enhanced retinal absorption (Supe et al. 2023).
5. Sustained Release Profile: Stealth liposomes with PEGylation or cholesterol-rich bilayers slow down the rate of clearance and allow for a controlled release of the medicine over a period of weeks. PEGylated liposomes, for instance, prolonged the release of triamcinolone in DME (Navarro-Partida et al. 2021).

These criteria ensure efficient drug delivery to the retina while mitigating systemic exposure and off-target effects, addressing key challenges in DR therapy.

**Table 1.** Liposomal formulations for drug delivery in posterior segment diseases.

| Name of drug | Composition of liposomes | Vesicle size | Route of administration | Key outcomes | Reference |
|---|---|---|---|---|---|
| Sunitinib | DSPC/Cholesterol | 104 nm | Intravitreal | Inhibited choroidal neovascularization (CNV) in murine models | (Tavakoli et al. 2022) |
| Triamcinolone (TALF) | Phospholipids/ Cholesterol | 150 nm | Topical | Reduced central foveal thickness in DME patients | (Navarro-Partida et al. 2021) |
| Citicoline | Phospholipids | > 200 nm | Intravitreal | Prevented glial activation and neuronal death in diabetic mice | (Bogdanov et al. 2018) |
| Naringenin | DPPC/Cholesterol | 148 to 215 nm | Topical | Reduced angiogenesis and VEGF production in experimental rabbit models | (Salimi et al. 2022) |

## 6. Novel technologies for liposome preparation

There are substantial limitations with traditional methods to produce liposomes. One key challenge is the difficulty of scaling up the production process to handle huge quantities, which hinders the widespread adoption of these nanocarriers. Furthermore, current approaches struggle to achieve high encapsulation efficiencies, and these classic methods frequently fail to process many bio-molecules due to structural and functional changes caused by exposure to detergents, organic solvents, and high shear homogenization or sonication procedures. These changes could have a significant impact on the clinical use of liposomes. Novel liposome nano-formulation technologies have been developed to address these crucial challenges.

### 6.1. Freeze-drying (lyophilization) method

The fabrication of water-soluble pharmaceuticals using lipid-based nanoformulations is frequently hampered by leakage during preparation and storage. Furthermore, actives may degrade due to oxidation and other chemical processes prior to their usage in drug delivery systems. These problems provide significant barriers to the commercial development of liposome nanoformulations (Shah et al. 2020). To address these concerns, a potential solution is freeze-drying. This technique involves freezing the aqueous solution containing the liposome formulation and then removing the ice through sublimation, where the solid transitions directly to a gaseous state (Trenkenschuh and Friess 2021). The product is first frozen at atmospheric pressure, then placed in a deep vacuum below the water's triple point, and lastly heated to cause the ice to sublime. Upon primary drying (sublimation), vacuum drying is required to desorb unfrozen water, followed by evacuation of the dried product from the freeze drier (Ward and Matejtschuk 2021). This method allows the liposome products to be sealed while still under partial vacuum in the processing unit. Before sealing, a backfill of dry nitrogen is used. Although water is the principal solvent removed from the liposome solution during the freeze-drying process, some formulations necessitate the use of organic co-solvent systems (Lombardo and Kiselev 2022).

The freeze-drying method is ideal for drying thermo-labile liposome products that would otherwise deteriorate during heat-drying, conserving a wide range of heat-sensitive biomaterials. The lyophilized form of lipid-based pharmaceuticals extends shelf life, particularly for medications that are unstable in the aqueous phase. The addition of sugar macromolecules such as sucrose, lactose, and trehalose during the freezing stage of the lyophilization process aids in the cryoprotection of the liposome structure (Boafo et al. 2022). As the liposomes are rehydrated, water molecules replace the sugars, and they reconstitute without significant change in size. Certain sugars, such as trehalose, can simulate the presence of water, resulting in effective encapsulation and stability (Roque et al. 2022).

The freeze-drying process is critical for protecting the shelf stability of liposome systems because water can facilitate undesired chemical reactions that cause drug modification or degradation. This approach is especially effective for dry thermo-labile liposome products that would otherwise deteriorate during heat-drying processes, as it preserves a wide range of heat-sensitive biomaterials.

The degree of water absorption is heavily influenced by the hydrophilic nature of the phospholipid's head group, as well as the precise composition and length of the hydrocarbon chain. This approach produces small liposomes (<200 nm) with great encapsulation efficiency (80%), stability, and reproducibility when using appropriate cryoprotectants (Wang et al. 2006).

### 6.2. Supercritical fluid methods (SCF)

Supercritical fluids (SCF) are a revolutionary green technology that has been created as an alternative to address the drawbacks of standard liposome manufacturing methods, including toxicity and degradability. Supercritical reverse phase evaporation (SCRPE), supercritical assisted atomization, depressurization of an extended liquid organic solution suspension (DELOS), supercritical anti-solvent (SAS) approach, and supercritical assisted liposome synthesis (super Lip) are examples of SCF techniques (Maja et al. 2020). The most often utilized supercritical fluid is carbon dioxide ($CO_2$) because of its many benefits, including its non-flammability, low cost, non-corrosive nature, nontoxicity, and environmental friendliness. $CO_2$ is also useful for treating thermolabile materials (William et al. 2020).

#### 6.2.1. Supercritical anti-solvent method (SAS)

A new method for making liposomes is the supercritical anti-solvent (SAS) method. Using this technique, a supercritical fluid (SCF), such as supercritical carbon dioxide (SC-$CO_2$), is brought into contact with a solution that contains an organic solvent and the solute (lipids and active medications). Though it functions as an anti-solvent for the solute, SC-$CO_2$ is perfectly miscible with the organic solvent.

Lipidic nanoparticle precipitation is aided by the dissolution of SC-$CO_2$ in the liquid phase and the subsequent extraction of organic solvents. After processing, the solution is hydrated in an aqueous buffer solution, which causes liposomes to form. The last step is rinsing the mixture with pure $CO_2$ to get rid of any leftover organic solvent (Khan et al. 2024). In large-scale production, SAS method emerges as a safer option for thermolabile substances with controlled particle size and organic solvent free operations eliminating its toxicity.

#### 6.2.2. Supercritical $CO_2$ reverse phase evaporation process [SCRPE]

Otake et al. established the SCRPE method, which is a pioneering method for liposome preparation (Otake et al. 2006). In this process, the lipid, organic co-solvent, and compressed gas are combined in a stirred variable volume cell at

temperatures above the lipid phase transition temperature (60 °C) and pressures ranging from 10 to 30 bar. An aqueous solution is then slowly added into the cell, and the pressure is lowered by the release of compressed gas, resulting in liposomes with a mean diameter of 200–1200 nm. The size of the liposomes can be adjusted by changing the lipid concentration, with smaller sizes (100–250 nm) achieved at lower lipid concentrations (Huang et al. 2014). Overall, the SCRPE and ISCRPE approaches represent intriguing alternatives to standard liposome preparation methods, offering a more efficient, scalable, and environmentally friendly approach to manufacturing liposomes with regulated features.

#### 6.2.3. Rapid Expansion of supercritical solution [RESS] method

The Rapid Expansion of Supercritical Solutions (RESS) method is a two-step liposome production process. The solid substance is first dissolved in a supercritical fluid (SCF) at a specified pressure and temperature. The SCF then percolates and dissolves the solid substance in the extractor, forming a solution that is depressurized in a low-pressure chamber using a heated nozzle. When a single nozzle is used, the RESS procedure is simple and effective, reducing the usage of organic solvents and allowing the SCF to be reused indefinitely. However, the fundamental disadvantage is that most medicinal ingredients (e.g. polymers) are poorly soluble in SC-$CO_2$, necessitating huge quantities of fluid and raising the expense of liposome manufacturing (Kumar et al. 2021; Türk 2022).

The RESS procedure is a potential way for creating liposomes, but it has certain drawbacks. The procedure necessitates high pressure and temperature, which might be difficult to manage. Furthermore, the limited solubility of medicinal ingredients in SC-$CO_2$ can result in inefficient encapsulation and expensive production costs. Despite these obstacles, the RESS method has been found to be effective in manufacturing liposomes with regulated particle sizes and encapsulation efficiencies.

#### 6.2.4. Super-critical assisted liposome formation [Super Lip]

An innovative way for making liposomes is the Supercritical Liposome Formation (Super Lip) technique. This process creates an expanded fluid by dissolving the lipid in ethanol and then combining it with pure CO2 in a saturator. To create a supercritical fluid (SCF), thin bands are thermally heated in a saturator that is packed with baffles and kept at high pressure. The drug-containing aqueous solution is atomized after the mixture is run through a high-pressure formation tube. The formation vessel and saturator are operated at 40 °C and 100 bar of pressure, respectively. Following the collection of the liposome suspension from the vessel's bottom, $CO_2$ and ethanol are separated using a stainless steel separator kept at 30 °C and 10 bar pressure.

The Super Lip method's encapsulation effectiveness is dependent upon the flow rate of the aqueous solution. Reduced entrapment efficiency is the result of increasing flow rate. This technique has a number of benefits, such as little solvent residue, excellent encapsulation efficiency, and the capacity to encapsulate both lipophilic and hydrophilic compounds. It does have several drawbacks, though, namely the requirement for high pressure and the possibility of nozzle obstructions (Trucillo et al. 2017; Trucillo et al. 2019).

#### 6.2.5. Depressurization of an expanded liquid organic solution suspension [DELOS] method

Preparing liposomes using the Depressurization of an Expanded Liquid Organic Solution Suspension (DELOS) method is contemporary. Under particular pressure and temperature conditions, phospholipids are dissolved in an organic solvent using this process. The $CO_2$ serves as a co-solvent when the solution is combined with supercritical $CO_2$ in a vessel. Liposomal production occurs when the resultant mixture is depressurized via a nozzle.

Handling thermo-sensitive materials without the need for high temperatures is one of the main benefits of the DELOS process. At a working pressure of around 10 MPa at 35 °C, the DELOS process is conducted in comparatively mild conditions, in contrast to the Particles from Gas-Saturated Solutions (PGSS) method, which runs at high temperatures. This method is suitable for the synthesis of liposomes containing thermolabile chemicals, as it minimizes exposure to high temperature and pressure (Andra et al. 2022).

The DELOS approach's versatility and ease of usage in a range of environmental conditions are additional benefits. Nevertheless, there are a few drawbacks, such as the potential for nozzle blockage and the presence of residual organic solvent. Despite these limitations, the DELOS approach is still a practical way to synthesize liposomes, particularly for applications involving heat-sensitive materials.

### 6.3. Microfluidic method

Jahn et al. created the microfluidic approach for controlled liposome synthesis (Jahn et al. 2007). This technique involves dissolving lipids in isopropyl alcohol, and the resultant liquid passes through the center of two channels containing an aqueous medium. Subsequently, the stream of lipids in isopropyl alcohol is mixed to form liposomes. Lipid concentrations in microfluidic channels and laminar flow influence liposome size and distribution (van Swaay and DeMello 2013).

This method can be used to encapsulate drugs directly, resulting in self-assembling liposomes. The microfluidic approach is promising for a variety of applications because it allows for precise control of liposome size and distribution. Microfluidics is a one-of-a-kind approach that offers numerous advantages over traditional methods (Carugo et al. 2016).

### 6.4. Dual asymmetric centrifugation (DAC) method

DAC is a state-of-the-art centrifugation technique that sets itself apart from traditional approaches with its unique way of rotating samples. When employing DAC, vials are rotated around their axis as well as the main rotational axis; whereas with traditional methods, the vials are spun around their center axis. This bidirectional motion creates a unique mix of forces that help break down the sample material into smaller

particles, and produce nano-liposomes with a size distribution of about 60 nm (Massing et al. 2008).

The sample material is pulled in the opposite direction from the main rotation, which pushes it outward, by the revolution around its own axis due to the adhesion between the sample and the revolving vial. Mechanical turbulence and capitation generate a strong force that aids in the breakdown of the sample into smaller particles, resulting in nano-liposomes with a size distribution of roughly 60 nm (Hirsch et al. 2009).

DAC's unique sample rotation technique allows for the production of nano-liposomes with a size dispersion of around 60 nm, making it an excellent technology for a wide range of applications (Ali et al. 2024).

### 6.5. Membrane contactor method

The membrane contactor method is a modified ethanol injection procedure that produces liposomes utilizing a unique porous glass membrane. The apparatus consists of two pressure jars, one holding an aqueous phase and the other an organic phase containing lipids dissolved in alcohol (such as ethanol). Pressed through the porous membrane, the lipid phase is ejected into the aqueous phase, which moves tangentially toward the membrane surface. Lipid molecules self-assemble into liposomes at the membrane's exit portal when the organic solution comes into touch with the aqueous phase flow. Following the procedure, the produced liposomes can be stabilized by magnetic stirring, and the ethanol is eliminated by rotary evaporation at low pressure. The porous membrane module can be renewed by washing it in a water-ethanol solution. This procedure creates liposomes with controlled size and composition, making it a promising tool for a variety of applications (Jaafar-Maalej et al. 2011; Pham et al. 2012).

## 7. Post-processing of liposomes

### 7.1. Purification

Non-encapsulated substances, such as tiny molecules, toxins, or non-entrapped medications, are usually found in the external environment and need to be eliminated through a purification procedure, regardless of the liposome formation technique employed. Ultra-filtration (Yu et al. 2021), ultra-centrifugation (Dimov et al. 2017), dialysis (Roberts et al. 2018), and chromatography (Lin and Qi 2021) are some of the time-consuming procedures used in this process, which may also reduce the amount of liposomes that are produced at the end. Eliminating leftover organic solvents is another essential step (Suthar and Rathva 2019). These solvents help with lipid dispersion and prevent oxidation, but if left behind, they may cause the liposomes to become unstable (Filipczak et al. 2020; Xia et al. 2022). Usually, evaporation is used to remove these solvents; however, this method might concentrate pollutants and lipids, making subsequent removal of these substances challenging. The permissible safety levels and the amount of residual solvent in the finished product should be made abundantly evident by the manufacturers. Lipid peroxidation, a chemical process involving free radical reactions, is another way that liposome nanoformulations can be shielded against oxidation. Antioxidants such as butylated hydroxytoluene or alpha-tocopherol can be added to liposomes to reduce this, as can maintaining them in light-resistant containers or under inert gases (Lombardo and Kiselev 2022).

### 7.2. Sterilization

Sterilization of liposomes is a critical parameter as they are usually intended for parenteral administration and the presence of any viable microbes could significantly affect their properties. Various approaches for sterilization include steam heating (autoclaving), chemical, filtration, and ultraviolet and gamma ionizing irradiation (Delma et al. 2021). Lipid substances, a major constituent of liposomes, pose challenges in sterilization due to their thermolabile nature. Gamma radiation has high-energy ionizing power and may cause fragmentation or hydrolysis of lipid components, and degradation by peroxidation of unsaturated lipids. On the other hand, UV radiation is low-energy radiation and cannot penetrate inside liposomes, thus is ineffective. The use of chemical sterilization (ethylene oxide) is restricted due to its flammable and explosive nature and the carcinogenic, toxic, and mutagenic character of its residues (Toh and Chiu 2013). Filtration being the safest of all is limited by its time-consuming operation. Its other major limitations include being ineffective for liposomes greater than 0.2 µm (Goldbach et al. 1995). Considering these constraints, researchers are looking into a more effective and cost-efficient method of ensuring liposome sterility. One intriguing option is the single-step production and sterilizing of liposomes using supercritical carbon dioxide (SC-$CO_2$) technology (Soares et al. 2019). Standard biological indicators should be used for future research and optimization of this strategy. To ensure liposome sterility while decreasing the risks associated with standard therapies, it is critical to investigate innovative approaches.

### 7.3. Lyophilization

Freeze-drying has been widely employed in industry and research institutions to improve the stability and long-term storage capability of formulations like nanoparticles while also reducing the danger of contamination. In terms of liposomal formulations, multiple researchers have used freeze-drying to manufacture lipidic materials for liposomal transfection. However, the freezing procedure and vacuum cause liposomal dispersions to become unstable, breaking the vesicles and potentially resulting in drug leakage. Recent study suggests that adding cryo-protectants like trehalose to liposomal dispersions can help prevent these issues. Furthermore, encapsulating liposomes with smart polymers could overcome the stability and leakage difficulties, allowing researchers and industry to store them as dried powder (Yu et al. 2021).

## 8. Characterization of liposomes for critical quality attributes

### 8.1. Size and size distribution

The size of liposomes has a significant impact on their *in vivo* drug release profile. Average size of the liposomes is primarily influenced by the production method and the phospholipid composition. Liposome size and size distribution are measured using a variety of approaches, including microscopic, hydrodynamic, and diffraction light scattering methods. Microscopic methods that yield high-resolution images of liposomes, such as optical microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM), enable evaluation of the liposomes' shape, bilayer thickness, and inter-bilayer distance. TEM and SEM are especially helpful for displaying the liposome structure at the nanoscale (Guiot and Baudhuin 2019). Recently, even better resolution has been made possible by atomic force microscopy (AFM), which allows the examination of liposome shape, stability, size, and dynamic processes at the angstrom scale (Engelhardt et al. 2023).

Hydrodynamic methods are used to assess the size distribution, elution properties, and homogeneity of liposomes as well as to determine the molecular mass of substances. These methods include ultracentrifugation, field flow fractionation, gel exclusion chromatography, and analytical centrifugation. These techniques yield useful data regarding the distribution and total size of liposomal formulations (Egelhaaf et al. 1996).

### 8.2. Lamellarity determination

Lamellarity refers to the number of lipid bilayers surrounding the lipid vesicles. Cryo-electron microscopy (Tonggu and Wang 2020), 31 P-nuclear magnetic resonance (NMR) (Fröhlich et al. 2001), and small angle X-ray scattering (SAXS) (Pan et al. 2012) can assess liposomal lamellarity, revealing size, and homogeneity.

### 8.3. Zeta potential (mV)

The zeta potential is an important measure for assessing the colloidal stability of liposomes because it indicates the degree of electrostatic repulsion between nanoparticles in a dispersion. Nanoparticles with high (negative or positive) zeta potentials are electrically stable (and have high colloidal stability), whereas nanoparticles with low zeta potentials tend to agglomerate or flocculate (Yusuf and Casey 2020). The zeta potential of the liposomal dispersion is measured using the laser Doppler electrophoresis and Zetasizer. These instruments determine the zeta potential by applying an electric field and analyzing the scattering of an incident laser beam by the moving particles (Franzen et al. 2011).

## 9. Summary

As novel and biocompatible carriers, liposomes improve the absorption of drugs and successfully get past the ocular barriers that prevent conventional treatments. Notably, in preclinical models, biologics activating the mTOR signaling pathway have shown neuroprotective effects by lowering retinal ganglion cell (RGC) death and enhancing electrophysiological function. Moreover, lipid nanoparticles are ideal for clinical use and large-scale manufacture due to their exceptional stability and biocompatibility. However, several obstacles still exist despite the promising developments in liposomal technology. Developing stable liposomes that deliver directly to the retina is a major challenge. Furthermore, because patient reaction to various medications varies, more research into customized treatment plans is required. Comparing the cost-effectiveness of liposomal formulations to traditional medications is especially important because high production costs may restrict their availability in clinical settings.

In summary, liposomal treatments offer special advantages such as tailored drug delivery and improved therapeutic profiles, making them a suitable candidate for treating DR. A detailed study is required to meet the current issues with formulation stability, patient variability, and cost-effectiveness. All the related steps in resolving these challenges and ensuring that these cutting-edge treatments result in better clinical outcomes for patients with DR will require a cooperative strategy involving researchers, physicians, and regulatory agencies. The burden of vision impairment linked to this disorder can eventually be lessened with further investment in this field, which will also improve our understanding of liposomal technology and open the door to more potent treatments.

## 10. Conclusion and future perspective

Future research on liposomal therapies for DR should prioritize on several key areas to enhance efficacy and patient outcomes. Targeted delivery remains a crucial focus, necessitating the development of liposomal formulations that can selectively deliver therapeutic drugs to retinal tissues. Ligands that selectively bind to receptors overexpressed in DR, such as integrins and VEGF receptors, can be utilized to achieve this selectivity. Advancements in the realms of nanotechnology can further optimize the stability, release profiles, and pharmacokinetic properties of liposomes, potentially leading to improved patient adherence with decreasing dosing frequency. There may be synergistic advantages when liposomal drug delivery is combined with other modalities like gene or photodynamic therapy. Co-delivering neuroprotective elements and anti-inflammatory drugs within liposomes, for instance, may target multiple pathways involved in the course of DR. Rigorous clinical trials are imperative for assessing the long-term safety and effectiveness of these novel liposomal formulations in diverse patient populations. Stratifying clinical trials based on lifestyle, environmental, and genetic factors may facilitate customized treatment strategies. Patient engagement and education are essential to ensure optimal adherence and understanding the benefits and potential downsides of newly developed liposomal medications. Biomarkers to track response can help with treatment

personalization and long-term efficacy monitoring. A deeper understanding of the molecular interactions between liposomal carriers and retinal cells will inform future formulation strategies. Finally, addressing regulatory concerns associated with novel drug delivery systems is crucial to expedite the market entry of innovative liposomal therapies for DR.

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## Authors' contributions

CRediT: **Ravi Parashar**: Conceptualization, Methodology, Visualization, Writing – original draft, Writing – review & editing; **Preeti K. Suresh**: Funding acquisition, Project administration, Supervision, Writing – review & editing.

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