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Review Article

Recent Trends in Nano Delivery of siRNA to the Eye

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Abstract

The discovery of RNAi and the regulatory role that these small molecules play in the silencing of certain genes has captured the imagination of scientists and clinicians. siRNA delivery has been able to help address certain genetic mutations leading to altered gene expression or the accumulation of abnormal proteins- both of which can be associated with human diseases. Nanocarriers and siRNA can be used to create a targeted therapy that can aid in angiogenic ocular diseases such as age-related macular degeneration (AMD) and diabetic macular edema (DME). AMD and DME are ocular diseases that are due to the neovascularization in or underneath the retina- most often in the macula, the area of central high acuity vision. This can cause blurriness, distortion (metamorphopsia), and vision loss. Current treatments to decrease neovascularization are the application of anti-vascular endothelial growth factor (VEGF) antibodies which neutralize VEGF's biological activity. Understanding both the siRNA biology of the eye along with nanoparticle delivery systems will help in improving target tissue delivery of these molecules and could make for a more clinically impactful therapy. Current studies have demonstrated the successful use of intravitreous Ets1 siRNA as a pro-angiogenic treatment in pro-inflammatory conditions. This improves the leakage and restricts the dysfunction of retinal epithelial (RPE) cells. Also, administering HuR siRNA intraocularly leads to decreases in the retinal HuR in diabetic rats which can be useful in diabetic retinopathy.

Keywords: *RNAi; Age-Related Macular Degeneration (AMD); Diabetic Macular Edema (DME); Anti-Vascular Endothelial Growth Factor (VEGF); Retinal Epithelial (RPE)*

Introduction

In continuation of the published review article in 2015, this review paper is an updated version of the review and have included the recent trends in siRNA delivery especially for ophthalmic drug delivery [1].

The use of nanotechnology for drug delivery to assist with systemic ocular diseases.

Systematic ocular diseases

Over the past few decades, there has been significant advancements in the understanding of the molecular biology and biochemistry of ocular diseases. Due to the high prevalence of hyperglycemia, diabetes and various other causes. There have been numerous studies

addressing the relationship between the eye and systemic disease all over the body. The eye contains delicate blood vessels, which are connected to the brain and are separate from the rest of the body. The condition of these vessels can present markers for certain diseases and conditions such as atherosclerosis, diabetes, hyperglycemia, and smoking. The main systemic ocular diseases that have severe effects in the eye are age-related macular degeneration (AMD) and diabetic retinopathy (DR). AMD is a neurodegenerative macular disease in which inflammation and metabolic stress damage can occur to the macula, resulting in blurriness or a loss of central vision. AMD can progress rapidly due to high inflammation and lifestyle choices. DR is a retinal neurodegenerative disorder, which is a complication of diabetes mellitus. The progression of DR can be due to large fluctuations in blood glucose levels. DR can cause diabetic macular edema (DME), the accumulation of fluid in the macula. Central to the development of DR and DME is the elevated retinal levels of Vascular Endothelial Growth Factor (VEGF), a protein that under normal conditions triggers the formation of new blood vessels to support the tissues and organs**.** However, in disease states like, neovascular AMD and DME, VEGF can cause abnormal new blood vessels to grow within the retina or render the existing blood vessels to be more permeable to intravascular fluid and proteins. AMD and DR are systemic ocular diseases, which need to be studied more regarding their impact on other diseases or conditions that involve various organs in the body.

Drug delivery

Treatment for neovascular AMD and proliferative DR have been proven to temporarily improve symptoms of the disease. With a combination of a healthy diet and repeated injections, the inflammation and leaky blood vessels associated with the diseases can be reduced. The reduction allows increase in oxygen flow in the retina. Currently, the clinical treatment for AMD and DR is anti-VEGF therapy, a treatment approved by the FDA to intravitreally inject anti-VEGF agents to inhibit the growth of new blood vessels. The 3 main anti-VEGF drugs currently being used are bevacizumab (Avastin, UK, Roche), Ranibizumab (Lucentis, UK, Novartis), and Aflibercept (Eylea) [2,3].

Use of nanotechnology

Targeted gene therapy and the application of nanotechnology has piqued the interest of many in the medical world. The emergence of nanotechnology has started to develop in different fields, from nano medicine, dentistry, and ophthalmology. The possibility of creating an alternative approach to specific gene therapy has interested many. The use of nanotechnology such as siRNA has been shown to achieve more of an effective therapeutic approach in treating certain diseases. Studies have shown that the use of siRNA and other nanotechnologies have a higher effectiveness of drug delivery than conventional methods. Being able to utilize nanotechnology has allowed for the detection of cancer cells, kidney damage and drug delivery to certain areas in a higher concentration with minimal side effects as compared to normal drug delivery.

siRNA functions, mechanisms, barriers, platforms

Functions and mechanism of siRNA

siRNA is a double stranded RNA molecule, which stands for small interfering ribonucleic acid. This small ribonucleic acid contains small 20 - 25 nucleotide noncoding RNAs that regulate genes. The strand contains 5' phosphate groups and 3' hydroxy groups. siRNA is produced from a viral double-stranded RNA (dsRNA) and is cleaved by RNAase III family enzyme Dicer, producing 19 - 27 base pairs [4]. These base pairs allow for long molecules containing a complementary middle region with a 2-nt overhanging on both of the 3' hydroxy groups [5]. The short-stranded RNAs causes specific post-transcriptional gene silencing. This process begins with the RNA-induced silencing complex (RISC), allowing for gene expression to occur by cleaving the mRNA to code targeted genes. The process includes the siRNA being associated with the RISC complex and being unwounded to form a single strand. This will allow the siRNA to be scanned and analyzed through RISC to find the complementary mRNA sequence. The siRNA and mRNA's pairing leads to the enzymatic cleavage and degradation of the mRNA molecule. This is a one specific way to engineer a pathway or get rid of a specific gene. The biological functions of siRNA are still being researched due to the wide range of therapeutic uses varying from in gene silencing, defense of viral infections, gene

expression, and autoimmune disorders like retinal degeneration [4,6]. siRNA is a factor of the RNAi pathway, a cellular mechanism that inhibits gene expression in targeting and neutralizing mRNA molecules. Previously researched, the RNAi pathway was found to be in the suppression of desired genes [7]. The functions can help aid in many genome functions due to the interfering of either strand individually. These functions can include RNA stability, chromosome segregation, transcription, transcription, translation, and chromatin structure [4].

siRNA barriers

siRNAs have been delivered by two major delivery systems: viral vectors and non-viral biopolymers. These delivery systems are dependent on the target site and the intended use of the siRNA [4]. The polymer-mediated system has previously been used for plasmid DNA. It has been used for siRNA as it can aid in the protection of contents from degrading, participate in targeted delivery, and control release of the released agent [8]. This polymer is advantageous because of its flexible chemistries and characteristics allowing it to be modified to change the physicochemical properties [9]. It has been reported that the chitosan polymer is a successful vector because of its biocompatible materials and versatile chemical, physical and biological properties. This polymer is a natural polysaccharide has been researched and tested with other synthetic materials to lower the toxicity, a beneficial factor for *in vivo* and *in vitro* results [10]. The peptide-base delivery system has been used in the delivery of siRNA due to the efficient cross over the cell membrane permeation. There has been evidence the cell-penetrating peptides (CPPs) have high potential in oligonucleotide delivery of nanoparticles [11]. CPPs are short peptides that help to promote the covalent and non-covalent interactions within the cell through endocytosis. One of the main advantages is its ability to translocate in the plasma membrane and aid in the movement of molecular interactions to organelles or cytoplasm [12]. This polymer has been found to be more suitable because of the design versatility and the sequence and function diversity [10]. The lipid-based delivery system has been developed specifically for *in vivo* applications. Similar to the other delivery systems, lipid-based systems are beneficial in targeted delivery, specifically in oral delivery systems [13]. These delivery systems have been used because of their hydrophilic and lipophilic properties, aiding in the use of those types of drugs, proteins and nucleic acids to produce a target delivery [14].

Understanding the benefits

In past decades, siRNAs have been developed for therapeutic applications. They can be easily modified. in either creating a pathway through RNAi or inhibiting a specific gene expression. Since the discovery of the siRNA mechanism, it has been used a delivery pathway to target a specific gene expression, especially in drug delivery for ophthalmic applications [14]. The benefits of siRNA allow for a more specific target delivery preventing DNA modifications in the genome. The delivery to certain tissues, such as in the eye are a challenge, but can be extremely beneficial by the use of target delivery. The use of siRNA can silence gene expression and be used to target certain disease-related genes, such as age-related macular degeneration (AMD). Within certain tissues and cells, it can provide a high local concentration without using more drugs. In ocular tissues, it has been found that a siRNA-based anti-angiogenic agent drug, Bevasiranib, has been used in treating wet AMD [14]. The use of Bevasiranib helps to eliminate repeated intra-vitreal injections that could cause complications, such as retinal tears, intravitreal bleeding or lens injury [16]. The use of siRNA to target a gene will allow for less complications, bursting of blood vessels and repeated injections [14].

Non-viral vectors

Non-viral delivery systems are chemical and physical systems. These systems can be used together or separately to deliver siRNA. Non-viral systems reduce the immune response and have no size limit for the transgenic DNA [15]. Non-viral delivery systems have effects in the use of *in vivo* and *in vitro* delivery, based on cell membrane penetration. The most common non-viral deliveries are divided among the chemical and physical systems. The chemical systems consist of cationic lipids, cationic polymers and lipid polymers [17]. The physical systems consist of naked DNA, DNA Bombardant, electroporation, hydrodynamic, ultrasound, and magnetofection [17]. The lipid-substituted polyethylene imines chemical system of non-viral delivery of siRNA has been targeted to acute Myeloid Leukemia cells [18].

Retina- Structure, function and pathogenesis

Structure and function

The retina, the photographic film of the eye, is attached to the inner wall of the back of the eye. It is no thicker than tissue paper. The various layers of the retina are responsible for capturing light and processing this input thereby turning photons into an electrical signal that is relayed to the occipital cortex where visual perception occurs. The retina is the main ocular connection to the brain. It contains 10 different layers that work in parallel to produce a visual output. The order of the layers, from furthest to closest in relation to the vitreous humor will be described.

Underneath the retina is the retinal pigment epithelium (RPE). It is a single layer of densely packed hexagonal post-mitotic cells containing granules pigment. These cells are crucial in creating a barrier and maintaining the photoreceptor layer [19]. Each cell contains 2 layers, the outer non-pigmented and the inner pigmented. The RPE is placed in between the blood supply of the choroid and the light-sensitive segments of the photoreceptors forming a part of the blood barrier [20]. The role of the RPE is to nourish and protect the retina. This is done by transporting ions, water, nutrients, absorbing light and protecting against photooxidation, isomerization of *trans*-retinal into *cis*-retinal, maintaining the integrity of the retina [20,21]. The photoreceptor layer containing a specialized neuroepithelial cell that is prominent in the sensory transduction. This sensory transduction is important for converting light into electrical signals for the rod, cone and the retinal ganglion cells. Once the photons have been absorbed, the rod and cone photoreceptors will release the glutamate neurotransmitter to the bipolar cells. The photoreceptors are released in the dark since it depolarizes. The effect of the glutamate will be different in the bipolar cells, dependent on the cell membrane's receptor. This allows for the bipolar cells to get excited by light allowing to detect a difference between color and edges. This layer can allow light to be sensed in a visual field [22]. The outer limiting membrane is not a true membrane. It contains a network-like structure of rods and cones which make it appear similar to a membrane. The function is to protect the inner layers by forming the blood-retina barrier and to prevent any harmful properties from blood circulation and help maintain the integral structure of the retina. The outer plexiform layer contains many synapses of the rod and cone cells. This layer is interlaced with the dendrites of the ganglion cells in the inner nuclear layer. The inner nuclear layer is made up of bipolar, amacrine, and horizontal cells which are closely packed together. The retinal vasculature is located within this layer. The inner plexiform layer which contains interlaced retinal ganglion cells' (RGC) dendrites and cells of the inner nuclear layer. The RGC project axons towards the optic disc. The ganglion cell layer which contains the retinal ganglion cells (RGC). These neurons receive visual information from the bipolar and amacrine cells. This layer is closest to the macula and the optic disc. It processes electrical signals by sending signals through the axons to the optic nerve and subsequently the brain. The nerve fiber layer (RNFL) which contains ganglion cell axons that run parallel to the optic disk. It is an extension of fibers from the optic nerve. The nerve fibers from this layer will lose their medullary sheaths as they pass through the lamina cribrosa sclerae, a mesh-like structure in which the nerve fibers from the optic nerve exit through the sclera, as they move on to the retina and choroid. The layer is important because it is a consolidating center that sends electrical signals from the outer layer of the retina. These signals are sent to the occipital cortex for visual perception. The inner limiting membrane is the top-most layer separating the vitreous body from the retina. The membrane is formed by astrocytes and Müller cells. The astrocytes are star-shaped glial cells that help to support the blood-brain barrier endothelial cells. This allows for proper nutrients to the nervous system [23]. The Müller cells are another type of glial cells that aid in supporting the neuron cells in the retina. This cell plays a role in the integrity of the structure and the stability of the cells.

Pathogenesis

The retina is the center of ocular diseases such as age-related macular degeneration (AMD) and diabetic retinopathy (DR) that can lead to conditions such as vision impairment or irreversible blindness. Understanding the pathogenesis and development of these disorders is crucial to allow for prevention and treatment. Many ocular disorders such as AMD and DR start in the retina. Currently, studies

have shown that the Interleukin-7 (IL-7) pathway is involved in the pathogenesis of AMD [24]. This is a crucial discovery because IL-7 is a crucial protein that contains a non-hematopoietic cell-derived cytokine that has seen to have a role in the immune system, specifically T-cells and B-cells [24]. This connection shows that the pathway of IL-7 in the retina can allow for a more complete understanding of the pathogenesis of AMD.

Being able to understand the pathogenesis of ocular disorders will allow for a more suitable targeted gene therapy treatment. Gene therapy is currently in the experimental stages, but certain human clinical trials have shown to be promising in treating patients with AMD, glaucoma, choroideremia or retinitis pigmentosa [25]. The trend of understanding pathogenesis can be extremely important for the delivery of the certain drugs to the eye. There is a current trend of using nanoplatforms to deliver drugs for a higher concentrated targeted therapy.

Ocular disorders

Age-related macular degeneration (AMD)

Age-related macular degeneration (AMD) is an incurable neurodegenerative ocular medical condition when parts of the retina and the macula starts to deteriorate, resulting in blurriness or a loss of vision in patients over 60 years. AMD is the leading cause of blindness worldwide. The macula is a part of the retina is no bigger than 5mm and is responsible for central vision. The central vision includes fine detail and color vision. The macula contains a high concentration of photoreceptor cells that identifies light, which sends signals through the optic nerve to the brain to be able to interpret the signals into images. The macula is crucial for the ability to perform daily tasks such as reading, driving, recognizing images, and seeing fine details. AMD affects the retinal pigment epithelium (RPE), a layer of post-mitotic cells that functions in a barrier and a regulator of the photoreceptor layer. There is no specific reasoning for the cause of the AMD but there are many lifestyle risks factors that have been linked such as diet, genetic history, smoking, high blood pressure, or high amount of inflammation within the body [26].

AMD is divided into two main types, atrophic (dry) and neovascular (wet) degeneration. The two types are classified by blood vessels being present or absent which disrupt the retina. The dry form is the most common version as it affects 85 - 90% of the population [27]. Dry AMD is detected by drusen, small yellow deposits, between the RPE and the Bruch's membrane detected on an ocular coherence tomography (OCT) scan. These deposits are detected under the retina and can vary in different sizes. Over time these deposits can cause the macula retinal cells to die. This form of AMD leads to fewer symptoms, such as a gradual vision loss, and have no form of treatment. The wet form is an advanced version of dry AMD, affecting 10 - 15% of patients. This form is caused by a leaky blood vessel or the formation of new blood vessels into the macula. Due to this, new blood vessels start to branch below and above the other layers of the retina and macula [26]. For the wet version there are medications that may be used to block the growth of blood vessels and prevent the formation of new blood vessels. Such medications are given through intravitreal injections. The injections are anti-vascular endothelial growth factor (anti-VEGF), which blocks the endothelial vascular growth and is shown to be beneficial leaving high concentrations in the eye [28]. The ophthalmologist would inject the medication into the affected eye. A variety of different medications have been proven to reduce blood vessel presence and allowing for partial vision to recover. The reduction of blood vessels aids in the recovery of retinal cells to gain function. This treatment can be given repeatedly overtime dependent on the progression of the AMD.

Diabetic retinopathy (DR)

Diabetic retinopathy (DR) is a progressive microvascular disease that affects the retina and a common complication to those who have type 1 and 2 diabetes mellitus. DR affects over 100 million people worldwide [29]. DR is similar to AMD and diagnosed by manifestations of vascular abnormalities in the retina. This microvascular disease is directly correlated to hyperglycemia pathways. There have been many pathways that have been detected to increase the oxidative stress, such as vascular occlusion and inflammation leading to DR [30].

DR is associated with the damage neurons and blood vessels in the retina causing a reduced blood flow and dysfunction of the inner retina. The outer retina dysfunctions start to occur after the inner when the ocular blood-retinal barrier, which contain retinal epithelial cells that are held tightly together preventing substances from entering the retina, contains leaky blood vessels [31]. These leaky blood vessels effect the retinal neuropil, which is an area in the nervous system that connects the brain. When theses blood vessels start to thicken, the vascular smooth muscle cells and the pericytes start to degenerate. When the neurons start to get damaged, the barrier starts to leak causing the retinal neuropil to be affected with toxins. This process can lead to a loss of blood flow, microaneurysms, capillary bulges, attracting inflammatory cells, and dysfunction of the retina [32]. The causes of the DR vary but are affected due to the high fluctuations of blood glucose levels, causing the blood vessels to restrict causing retinal vessel occlusion [33]. A common cause of DR is diabetic macular edema (DME). This is due to the swelling and thickening of the macula from the accumulation of fluid and protein deposits in the macula, usually detected on an OCT [34]. The severe swelling can lead blurred or double vision, and/or eye floaters, which are deposits of the eye's vitreous humor that has become transparent [35].

DR is divided into two broad main forms non- proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) [34]. There are many stages of NPDR before leading to the PDR. The first stage is usually detected with microscopic changes of the blood vessels. When detecting NPDR, microaneurysms are present that do not affect the vision and usually go unnoticed [36]. After this stage, without proper maintenance of the glucose levels, blood vessels can start to become swollen and block the sustenance to the retina. There are more visible changes, such as blurry vision, vitreous floaters and DME. Without proper blood flow, the neurons in the retina will send signals to create new blood vessels [37]. PDR is the advanced version of NPDR, resulting in a loss of oxygen flow to the retina. Due to this oxygen loss, new abnormal neovascular blood vessels will be formed on the optic nerve [38]. These blood vessels are fragile, causing for more of a risk of bleeding to occur into the vitreous hemorrhage. This can result in a retinal ischemia. There is no cure for the DR, but can be treated with intravitreal injections, similar to the treatment of neovascular AMD. The injections are anti-vascular endothelial growth factor (anti-VEGF), blocking endothelial vascular growth and appear to beneficial in having a pathway of high concentration to the eye [28].

Major retinal angiogenic diseases

Vascular endothelial growth factor (VEGF)

Vascular Endothelial Growth Factor (VEGF) is an angiogenic factor and main mediator of neovascularization, which is involved in the pathogenesis of certain diseases [39]. This signal protein stimulates the formation of new blood vessels in vasculogenic and angiogenesis. VEGF is mitogen specific to endothelial cells. While angiogenesis is a complex process involving the activation of a series of receptors, VEGF signaling is a rate limiting step process. Pathological angiogenesis is a factor in supporting malignancies. The endothelial cell specific mitogen VEGF has been extensively documented to promote the growth of endothelia cells sourced from various vascular tissue [40]. The VEGF family contains 5 main members: VEGF-A, VEGF-B, VEGF-C, VEGF-D and PIGF. All of these proteins stimulate the enzyme tyrosine kinase receptors located on the cell surface. This enzyme transfers a phosphate group from ATP to the cell's protein. Usually aiding in interactions and communication within the cell. These receptors dimerize though transphosphorylation to stimulate the new blood vessel formations [41]. VEGFs have crucial roles within maintaining and forming the vasculature [42]. VEGF and its receptor (VEGFR) have been shown to play a role in other pathological forms of angiogenesis, such as cancer, neovascular (wet) AMD and DME [43]. VEGF-A has been shown to have pro-angiogenic activity to help and stimulate the cell migration in endothelial cells [44]. Studies have shown that the use of VEGF-A has been effective to create anti-VEGF therapy drugs to minimize the neovascularization in wet AMD and DME in improving vision and decreasing the extra fluid build-up [45]. VEGF is a key mediator for angiogenesis which regulates the receptor in maintaining the cell's homeostasis and regulating the expression of the blood vessels [46]. The protein also has mitogenic effects on retinal epithelial, pancreatic duct, and Schwann cells. The human VEGF-A gene is localized on chromosome 6p21.3. It is a heparin-binding homodimeric glycoprotein. The isomers of VEGF vary in pH and affinity for binding to heparin. The expression of VEGF mRNA may be induced through

hypoxic conditions. It is also induced by other growth factors, such as epidermal growth factor, TGF-α, TGF-β, keratinocyte growth factor, IGF-I, FGF, and PDGF [40].

Diabetic macular edema- BRVO and CRVO

Diabetic Macular Edema (DME) is a development of diabetic retinopathy in which there is a buildup of fluid in the macula due to the neovascularization. Due to high fluctuations in the blood glucose levels of a patient with diabetes mellitus, there may be fluid leakages of retinal capillaries and microaneurysms. Central Retinal Vein Occlusion (CRVO) and Branch Retinal Vein Occlusion (BRVO) are associated with macular edema and vision loss, causing them to be the second leading cause of retinal blindness after diabetic retinopathy. CRVO is when the central retinal vein, running through the optic nerve that drains blood from the retina to the cavernous sinus or superior ophthalmic vein, suffers vascular occlusion. This occlusion can cause severe damage to the retina, resulting in vision loss, ischemia, and edema due to interrupted blood flow [47]. BRVO is another version of retinal vein occlusion when one of the branched veins of the central retinal vein is blocked [48].

Neovascular (Wet) macular degeneration

Neovascular, otherwise known as wet, Age-Related Macular degeneration (AMD) is the more advanced version of dry AMD. Wet AMD is found in 10 - 15% of patients who had the dry type. The wet form is more deleterious because it stimulates the formation of new blood vessels and neovascularization. When the macula, the center of vision of the retina, gets damaged, it causes the symptoms of AMD to emerge. This causes vision loss due to choroidal neovascularization (CNV) and polypoidal choroidal vasculopathy (PCV) in the choriocapillaris. This layer of capillaries is adjacent to the Bruch's membrane. The Bruch's membrane is crucial because with age this membrane starts to thicken, slowing metabolites transport causing poor circulation of oxygen to the macula and retina and stimulating the VEGF, a protein that triggers for the formation of new blood vessels. The overexpression of VEGF can cause retinal ischemia, resulting in the release of VEGF-A, causing an imbalance of the pro-angiogenic protein [49].

Being able to target and regulate the pathological angiogenesis of AMD has been a new field to study. Angiogenesis is the equilibrium between anti-angiogenic and proangiogenic factors. The ocular angiogenesis of the retina and the choroid are crucial and should be controlled as it can progress to vision loss. The sprouting of blood vessels from endothelial cells in avascular tissue can cause the degradation of the Bruch's membrane. Understanding that the degradation of the membrane would result in the death of retinal pigment epithelium (RPE) and photoreceptor cells. The photoreceptor cells and RPE work together, and if one is affected, the other one is affected. When the photoreceptor cells start to die off when they are separated from the RPE and the choroidal vessels, resulting in the crucial cells for visual acuity to die [49].

When the RPE cells start to degenerate, the neovascularization of the choroid occurs. The result of the death would be that the cells will have gone through autophagy, degradation cellular components, taking away the essential nutrition and energy necessary of the cell to function and homeostasis [50]. Currently, it has been tested that anti-VEGF drugs have been affective in regulating ocular angiogenesis, but there has been a resistance in patients that are presented with worsening eye diseases [51]. This suggests that other vascular mediators are contributing to ocular angiogenesis. Understanding the current angiogenesis therapies will aid in developing new targeted drugs to avoid the resistance of anti-VEGF drugs to reduce the long-term treatment of neovascular AMD [52-54].

Non-viral carries and barriers of siRNA

Non-viral carriers

Studies have shown that non-viral carriers of siRNA have been proven to be effective in therapeutic uses [55]. Non-viral carriers are DNA plasmids, polymer and liposome-DNA complexes that can target certain drugs as naked DNA. The use of non-viral carries is effective

in giving *in* vivo targeted drug delivery. Non-viral carriers have been more of an intriguing topic of research. Previously, non-viral carriers were used to test DNA delivery, but have started to make an emergence with being a carrier of siRNA [56]. The emergence of non-viral carriers has been proven to be more effective and express on the surface of the targeted cells. The advantages of non-viral carriers are that is it safe, stable, biocompatible, high gene capacity, high transfection efficiency, chemical design flexibility and low immunogenic response [57]. Despite the use of non-viral carriers being new, strategies have been developed to improve the outcome of drug delivery. By increasing the toxicological profile, reducing the pathogenicity, the delivery efficiency will increase [58].

Effective siRNA delivery

One of the most significant recent discoveries in RNA molecular biology has been small noncoding RNAs about 20 - 30 nucleotides in length, which regulate genes and genomes. Out of these small RNAs, small interfering RNA (siRNA) have the ability to induce RNA interference. This is a biological mechanism that silences genes through targeting and degradation of complementary mRNA. The siRNAs are formed through the cleaving of longer, double-stranded DNA. They form multiprotein complexes known as RNA-induced silencing complexes (RISC), which are guided by siRNA to the target mRNA. Once aligned on the target, the RISC protein cleaves the mRNA. siRNAs are very promising in the treatment of chronic conditions. There are ongoing clinical trials for the application of siRNA in respiratory and ocular diseases. One of the main factors limiting the clinical application of siRNA is the delivery system. Due to their negative charge and density, siRNA requires a carrier to cross cell membranes to the desired site of action. Viral vectors are possible; however, not very scalable for widespread clinical application. Non-viral vectors are a focus of extensive research. Polymer-mediated delivery systems such as cyclodextrin are quite versatile in their simple modification to become specialized for specific tasks. Peptides are another excellent candidate for siRNA delivery because of they are easily modified and can readily penetrate the cell membrane. Peptides of varying charge, conformation, polarity, and hydrophilicity can be produced from an array of amino acid combinations. Lipid-based systems are another promising non-viral siRNA delivery system. Lipids such as liposomes are naturally and efficiently taken up by cells. These have been used for many years in the delivery of siRNA into cells. These delivery systems can be used in conjunction with one another to transport siRNA into cells effectively. In ophthalmic applications, the use of nanocarrier assisted siRNA delivery has the potential for positive clinical impacts.

Due to challenges in drug delivery to the posterior segment of the eye, conventional delivery such as topical application and particular instillation are of low effectiveness. Currently, the most effective mode of delivery is intravitreal, through the use of a small gauge needle to inject therapeutics into the vitreous. While this is effective in creating high concentrations of the drug near the retinal tissue, there are limitations. These high concentrations quickly permeate through the blood-retinal barrier (BRB). This requires repeated injections commonly in 4 - 6-week intervals to keep the concentrations of the drug at optimal levels. The complications of repeated injections in such short periods include raised intraocular pressure, hemorrhage, retinal detachment and/or tears, endophthalmitis, cataracts, floaters, and blurry vision. Therefore, the use of siRNA delivery has great clinical potential in treating ocular conditions. The current limitation of siRNA delivery is the carrier. As the body of research surrounding the field grows and new delivery methods and carriers are developed, siRNA treatments could provide favorable outcomes for patients.

Main advantages of siRNA nano delivery for ocular use

AMD and siRNA

With the advancements of siRNA and its applications for specific disorders, the use of siRNA has prompted researchers to look at the implications of it within AMD. Previously studied, VEGF has been associated with the choroidal neovascularization (CNV) associated with AMD, which can cause a loss of vision in the elderly population [59]. In mouse studies, it was identified that Intravitreal Ets1 siRNA could play a pro-angiogenic role in the pro-inflammatory function. Ets1, otherwise known as transcription factor E26 transformation specific-1,

has been associated with regulating gene expression through different growth factors, adhesion molecules, and chemokines [60]. In this specific study, the Intravitreal Ets1 siRNA was localized to the CNV laser-region of the macrophage and microglia region in the mouse. The Ets1 siRNA was injected in the CNV region, improving the leakage and restricting the dysfunction of RPE cells and activation of macrophages/microglia. First, a CNV mouse model was developed using laser photocoagulation. The Ets1 was injected intravitreally and was tracked over a 7-day period. After the injections and laser injury, it was detected that the Ets1 protein level in the RPE-choroid-retina had drastically decreased when compared to vehicle and scramble siRNA groups. When the fundus fluorescein angiography and the indocyanine green angiography were performed, there was a decrease in the leakage in the CNV area. The study has highlighted the effectiveness of using Ets1 siRNA as a targeted drug for CNV by reducing the choroidal flat mount.

DR and siRNA

Diabetic retinopathy (DR) is one of the most common complications due to diabetes and the leading cause of blindness worldwide. Although both types of diabetes do not have a direct cure, intravitreal injections can be given to patients to reduce the leaky blood vessels. A recent study has targeted the human antigen R (HuR) that can be a practical therapeutic approach for diabetic retinopathy [61]. The HuR is a human protein encoded in the *ELAL1* gene, containing 3'-untranslated regions of mRNA binding domains, involving in inflammation and cell growth. This allows for the gene expression to be regulated and transformed into the according stability and translation that is necessary [1]. It was shown that when specific nanosystems are loaded with siRNA to silence the HuR expression. This expression contained solid lipid nanoparticles (SLN) and liposomes (SUV). The siRNA was able to transmit to the rat's retina using lipid-based nanocarriers and lipoplexes in order to silence the expression of the retinal HuR and the VEGF. The study demonstrated that when the HuR is targeted, the overexpression of the VEGF is reduced. Then when administering intraocular siRNA, the HuR expression is silenced, allowing for a discovery of a new pathway for treating DR. In this particular study, the use of siRNA nanocarriers were used to improve the retinal penetration instead of using naked siRNA, as it did not protect the retinal layers. It was previously demonstrated that the HuR protein is able to bind to the VEGF-encoding mRNA to improve protein expression. Causing an increase in the VEGF of the retina in diabetic rats. When the HuR siRNA intraocular was administered, there was a decrease in the retinal HuR in the diabetic rats. This was due to the post-transcriptional level for the HuR-mediated regulation dealing with the VEFG expression. It was discovered that the liposome binding to the lipoplexes (SLN- based lipoplex L4 with liposomes with the same N/P ratio of L1 and L3) resulted in a positive charge allowing for a uniform surface coverage of the lipid matrix. This proved that the residual charge affected the lipoplex L4 to appropriately interact with the targeted cells to induce a proper delivery to manage retinal diseases.

Conclusion

The retina is a thin layer of tissue, near the optic nerve, located at the back of the eye. The function of the retina is to convert light from the lens and create neural signals to be sent to the brain for visual recognition. Over time, the macula, the center of good vision, can deteriorate due to age or altered glucose metabolism. Neovascular age-related macular degeneration (AMD) and diabetic retinopathy (DR), causing diabetic macular edema (DME), are two primary neurodegenerative ocular diseases that can lead to the blindness. In both disorders, the macula in the retina begins to lose oxygen and metabolites transport slows down, causing the Bruch's membrane to thicken. This thickening causes the vascular endothelial growth factor (VEFG) protein to trigger the formation of new blood vessels in avascular areas in the eye. The growth of abnormal new blood vessels can cause the blood flow to the eye to be sluggish and obstructed. Currently, anti-VEGF therapies are being used to obstruct the growth of new blood vessels.

With the progression of recent trends and modernization of siRNA, nano delivery to the eye can aid in the advancement of preventing and facilitating ocular diseases. The development of nano delivery to the eye can be useful in preventing repeated procedures and the risk of complications. Further studies must be done to understand the effects. Studies have shown to be successful in using intravitreal Ets1 siRNA to play a pro-angiogenic role in the pro-inflammatory function to improve the leakage and restricting the dysfunction of retinal epithelial (RPE) cells as well as administering HuR siRNA intraocularly to decrease in the retinal HuR in the diabetic rats. Futures studies

must be done to see the effects of using siRNA to deliver anti-VEGF to the eye to reduce the neovascularization due to the angiogenesis of ocular disorders.

Future Trends

The next step would be to further study to understand the therapeutic and side effects of nano delivery of siRNA to the eye. Studies have seen to be promising to deliver anti-VEGF using siRNA to reduce the neovascularization and edema of the eye. VEGF is a crucial mediator in the angiogenesis of neovascular Age-Related Macular Degeneration and Diabetic Macular Edema and has been targeted by anti-VEGF drugs to decrease the formation of the blood vessels within the eye. Being able to target VEGF and signaling will allow for more insight into drug therapy, such as combining and delivering anti-VEGF drugs via siRNA. This would allow for a higher concentration delivery to the macula to increase the effectiveness and decrease the need for additional injections and side effects as previous studies have shown, using lipid-based nanocarriers and lipoplexes to silence the expression of the retinal HuR and the VEGF. If these siRNA nanocarriers, could be used to deliver anti-VEGF to silence the retinal VEGF, then it would be a novel therapy used for patients with wet age-related macular degeneration and diabetic macular edema. The use of the nanocarriers would allow for a higher concentration of the anti-VEGF to be delivered to the retina. This would reduce the resistance of anti-VEGF drugs to reduce the long-term treatment of neovascular AMD.

Preventing the angiogenesis and the subsequent effects caused by the protein VEGF, anti-VEGF suppressors, such as injections, may be used. To deliver them will require research on the utilization of siRNA as a carrier to the site of action. Ideally, the effect of anti-VEGF at the target site will be long-lasting at a controlled dose. Delayed-release forms would be a potential area for future research as siRNA is versatile in functions and the ability to target specific sites of action. Also, more scalable and clinically applicable methods of siRNA delivery are becoming areas of extensive research.

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