·Review·

Nanomedicine in the application of uveal melanoma

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Abstract

• Rapid advances in nanomedicine have significantly changed many aspects of nanoparticle application to the eye including areas of diagnosis, imaging and more importantly drug delivery. The nanoparticle-based drug delivery systems has provided a solution to various drug solubility-related problems in ophthalmology treatment. Nanostructured compounds could be used to achieve local ocular delivery with minimal unwanted systematic side effects produced by taking advantage of the phagocyte system. In addition, the *in vivo* control release by nanomaterials encapsulated drugs provides prolong exposure of the compound in the body. Furthermore, certain nanoparticles can overcome important body barriers including the blood-retinal barrier as well as the corneal-retinal barrier of the eye for effective delivery of the drug. In summary, the nanotechnology based drug delivery system may serve as an important tool for uveal melanoma treatment.

• **KEYWORDS:** nanomedicine; nanoparticles; gene delivery; drug delivery; uveal melanoma
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INTRODUCTION OF UVEAL MELANOMA

Uveal melanoma is the most common adult primary tumor of the eye. The pace of incidence increase of melanoma as a whole group in the USA has surpassed all other types of tumors within the last 20y [1]. The liver is the sole or initial site of tumor spread in more than 80% patients with uveal melanoma; once liver metastases have been diagnosed, the mean survival time drops to only 6-9mo, with a 5-year survival rate <40%[2-3].

Although traditionally, eye removal and plaque radiotherapy have been standard approaches to control the primary uveal melanoma, these procedures lead to cosmetic defects and eventual loss of vision. Chemotherapeutic agents have been widely studied for treating the liver metastases of uveal melanoma, yet they are ineffective and does not prolong the survival rate. Overall, the mortality rate of uveal melanoma remains unchanged over the past decades in request of improving therapeutic outcome for patients[4-9].

The ineffectiveness of therapeutic agents may be associated with the incapability of delivering a large enough concentration of the compound into the local tumor area in the eye. Studies have found that most systemically administered drugs are consumed by other organs/tissues prior to accumulation in cancer tissues [10]. Such deficiency cannot be resolved by dramatically increasing the amounts of drug administered, as it would likely cause severe systemic side-effects [11]. Instead, local delivery and specifically targeting the tumor cells at the ocular site may achieve improved efficacy along with reduced systemic toxicity.

However, effective drug delivery remains to be a big obstacle of treating uveal melanoma and other intraocular diseases [12-15]. The blood-retinal barrier along with aqueous and corneal barriers can restrict the access of drug in the eyes. Due to the structural peculiarities of the posterior segment, it remains as one of the most challenging barrier to overcome by drug delivery [14]. Interestingly, recent progress in nanotechnology provided novel inspirations to the drug delivery in uveal melanoma and other ophthalmological diseases.

THERAPEUTIC NANOPARTICLES

Nanoparticle delivery of drugs can achieve the following objectives [16]: 1) enhances drug permeability; 2) controls the drug stability and release rate; and 3) target delivery of drug into specific tissues [17-19]. The main nanoparticles used for therapeutic drug delivery includes: lipid-based nanopartic1les, polymer-based nanoparticles, inorganic

nanoparticles, polyion complex micelles, hybrid nanoparticles, poly lactic-co-glycolic acid (PLGA) based nanoparticles[20], as well as peptide based nanoparticles. Lipid-based nanoparticles are comprised of cationic lipid nanoparticles, cationic liposomes, and cationic emulsions. Polymer-based nanoparticles can be made up from poly-L-lysine, polyethylenimine (PEI), chitosan, dendrimers, or cyclodextrin. Hybrid nanoparticles include multilayered nanoparticles and also liposome-polycation-DNA nanoparticles while quantum dots and magnetic nanoparticles are in the category of Inorganic nanoparticles. Several studies have applied nanomaterials for uveal melanoma chemotherapy, gene therapy, radiotherapy, and photodynamic therapy (PDT). The outcomes of these works are summarized below.

Nanoparticalized Chemotherapeutic Agents Researchers have developed and are in the process of developing a variety of nanoparticles which can be structurally customized to deliver chemotherapeutic agents to treat a variety of cancers [18-19,21-26]. Compared to conventional delivery of chemotherapy, nanoparticles can transport larger amounts of a payload as they contain high surface area compared to their volume. Solid tumors show much higher uptake and prolonged retention of nanoparticles in contrast to normal tissues, known as the enhanced permeation and retention effect (EPR)[27]. This is due to the cancerous lesions have leaky and deficient vasculatures with large pores which result in higher uptake of the drug [28-31]. In addition, the reduced blood flow through the neoplasm and an impaired lymphatic system within the tumor leads to very little clearance of the nanoparticles accumulated in the tumor[32-34]. In the last ten years, favorable ocular distribution of nanoparticle packaged drugs led to encouraging progress been made in the field of ocular drug delivery. In particular, dendrimers and cyclodextrins have been used for anterior chamber drug-delivery [35-38]. Dendrimers can easily get in or go out from a cell as they are 2 to 20 nm in size with characteristics of narrow polydispersity. It has an advantage of a higher drug payload when comparing to linear polymers as their highly tailorable surface functional groups allows multiple attachment of the compounds [39]. Enhanced ocular bioavailability was observed when polymer nanoparticles were applied [40]. Intravitreal injection of Polylactic acid (PLA) and PLGA nanoparticles can leak to the retinal pigment epithelium [41-42] as the particles can go through the retinal layers [43]. A strategy of using PLGA nanoparticles to encapsulate dexamethasone acetate was effective for treating choroidal neovascularization (CNV) [44-46]. Albumin nanoparticles can be used to carry both positive and negative charge drugs such as ganciclovir or oligonucleotides due to their abundance of charged amino acids [47]. This characteristic make albumin nanoparticles ideal for delivering drugs to the posterior part of the eye, sutable for treating diseasing such as cytomegalovirus retinitis. Elbialy *et al* [48] have found an important factors to improve the efficiency of ocular drug delivery for prednisolone acetate is to induce the positive charge. With good affinity to the conjunctival surface and corneal, chitosan along with other natural polymer nanoparticles are also efficient to penetrate into the eye [49]. The electrostatic interactions between the positive amine groups of chitosan and negative sialic acid groups of mucins present in the tear film, which is intact with the corneal epithelium, enabled an increase in corneal residence time, and increased penetration of drug- loaded nanoparticles into the intact corneal epithelium [50-51]. Adhesion properties of the nanoparticles can be improved with the help of coating various polymers. When we further coat chitosan on the poly epsilon-caprolactone (PECL) encapsulated indomethacin nanoparticle, we observe a significant increase in bioavailability [52-53]. If we further coat polyethylene glycol (PEG) onto PECL particles, we could also increase the compound's ability to penetrate the corneal[54]. Recently, hydrogel nanoparticles have been investigated local delivery of chemotherapy compounds to treat uveal melanoma [55]. High concentration of poly N-isopropylacrylamide (PNIPAM) was detected in the uveal tissue after systemic injection of fluorescein-isothiocyanate (FITC) labeled PNIPAM nanoparticle. These findings demonstrated that nanoparticles as a novel carrier has strong potentials to be utilized in the treatment of uveal melanoma. Furthermore, it is possible to deliver more than one chemotherapy drugs at the same time using advanced nanoparticle delivery system to simultaneously target several important tumor signaling pathways. This would result in better treatment efficacy while lowering the drug cytotoxicity in cancer patients [56-58]. Currently, several combo drug formulations using such technology are under clinical trials testing. Acute leukemia patients whom received a formulation called CPX-351 which is a cytarabine and daunorubicin molar mixture of 5 to 1 formulated in liposome responded well as the formulation produced a nice synergistic effect[59-61]. Another combo nanoparticle drug CPX-1 which is a mixture of irinotecan HCl and floxuridine formulated in loposome also demonstrated synergistic anti-tumor effect againsted late stage solid tumors in a Phase 1 clinical trail[62]. Dendrimers, polymer-drug conjugates, iron oxide particles, nanoemulsions as well as silica particles have all been tested as an attempt to formulate and carry out combination drug delivery to improve therapeutic efficacy[63-68]. These particles are promising to be decorated with multiple chemotherapeutic drugs, and may be applied in ocular melanoma to achieve better cytotoxic effects.

Gene Delivery with Nanoparticles Nanomedicine has also been recognized by the National Institutes of Health (NIH) as it released a roadmap for it. Nanoparticles are ideal tools

for gene therapy into a single delivery system as it is possible to engineer the desired characteristics to be not susceptible to degradation, not mount an immune response, gain prolonged circulation time, exhibit increased specificity to target tissue, and ultimately deliver the genetic material into the target cells. A variety of cancers has been treated with experiments of nanoparticle gene delivery. Major focuses are in using gold, magnetic nanoparticles, liposome and carbon nanotubes for delivery [69-77]. Cationic polymer has already found to be a promising reduction-responsive gene carrier with low cytotoxicity and high transfection ability in melanoma cells [78]. Magnetofection, a method without using virus for transfection has shown to be effective *in vitro* for treating the B16F1 melanoma cells [79]. The eyes are special sites where foreign antigens are tolerated instead of rejected, making it a great place for taking advantage of gene therapy. The other advantage for gene therapy for the eye is that the eye is a closed organ with limited space. This would limit the local delivered drug diffusing into the body blood circulation because of the physical barrier structures. Therefore, more and more experiments with nanoparticle gene therapy focusing on treating eye diseases are conducted [80-82]. As an example, Farjo *et al* [83] performed subretinal or intravitreal injections delivering CMV-EGFP DNA nanoparticles into the mice eye. Farjo *et al* [83] showed that the transfection efficiency of the nanoparticles was very high along with nice target gene expression. Vectosome formulation of antisense oligonucleotides were used to study melanoma using a light induced system. In rat experiments, intravitreal injection of vectosomes resulted in a fast transetinal migration followed by uptake from the melanoma cells [84]. The vectosome treatment potently inhibited the cell growth of OCM-1 melanoma cells by 60% compared to the control group. More recently, Wang *et al* [85] has reported that after transfection with recombinant DNA plasmids such as pEgr1-TNFα, and pEgr1-TNFα-TK with dendrimer nanoparticles as vectors, and then combined with iodine-125 (125I) radiation, the gene expression and protein level of TNFα and HSV1-TK in OCM-1 melanoma cells was increased, cell proliferation was significantly decreased, and the cellular morphology altering, apoptosis and necrosis was observed. The current study suggests that combining gene therapy with nanoparticles may provide a new way of treating uveal melanoma.

Nanoparticles for Brachytherapy Brachytherapy, where localized radiotherapy is delivered directly to the tumor, is currently a commonly used treatment method for uveal melanoma therapy. However, as the energy absorption dose of normal and tumor tissue are quite similar, the maximum radiation dose is limited to the normal tissue which surrounds the tumor. The use of radiosensitizing agents may address this problem and overcome hypoxia mediated heterogeneity response of the tumor [86]. Fullerene and lipid nanoparticles have been explored as strategies to deliver effective brachytherapy and longitudinal imaging in brain tumors [87-89]. More commonly, gold nanoparticles (GNanoparticles, AuNanoparticles) have been applied as radiosensitizing agents due to their high atomic number and strong photoelectric absorption coefficient [90-96]. Studies have shown by combining brachytherapy with gold nanoparticles, it could induce apoptosis of melanoma *in vitro* and *in vivo* [86,97-99]. Chang *et al* [97] revealed that gold nanoparticles can sensitize melanoma B16F10 cells to radiation and showed that the nanoparticles can accumulate within the tumor cells. The radiation and nanoparticle combo treatment strategy has also significantly prolonged mice survival while potently inhibiting tumor growth in a B16F10 mice model. Further evidence of apoptosis induction was found in the combination group *vs* control[97]. Also, gold nanoparticles has additional vasculature disruption properties with combined with brachytherapy [100-103]. Berbeco *et al* [101] found that even low concentrations of AuNanoparticle has vasculature disruption effects to the tumor endothelial cells. As the nanoparticles can target both induce apoptosis of the tumor cells and disrupt its supporting vasculature when combined with radiation, it could potentially be used to support brachytherapy for treating uveal melanoma.

Nanoparticles for Phototherapy Photodynamic therapy, also known as phototherapy is a treatment method of using special light which are called photosensitizers to activate the drug for cancer therapy. The compound to be delivered is active only after light activation. In tumors, photosensitizers may have multiple effects including direct killing of tumor cells, suppressing tumor vasculature and also activating the body's immune system [104]. As visible light is the most common activator of conventional photosensitizers, it could not infiltrate through very thick tissues. As a result, tissue depth remains one of the major limiting factor for effective photodynamic therapy. In the case of uveal melanoma, the quantity of melanin in the tumor could also affect light absorption and treatment efficacy [105-108]. Nanoparticles has unique advantages for phototherapy as it can act as a transducer for converting light with deep penetration ability into light within visible wavelength. It can also be used to carry photosensitizers for treating tumors [73,109-112]. It has been reported that the use photosensitizer loaded-magnetic nanoparticles (MNPs) and/or polyethyleneimine (PEI) are novel attempts to improve phototherapy to treat melanoma [113-116]. As single excitation wavelength has multicolor-emission capability, multifunctional nanoplatform as potential dual carrier system has been developed [109,117]. Makky *et al* [118] have developed prophyrin-based glycodendrimers with the mannose-specific ligand protein concanavalin A conjugated on to their surface, to specifically target the tumor cells in

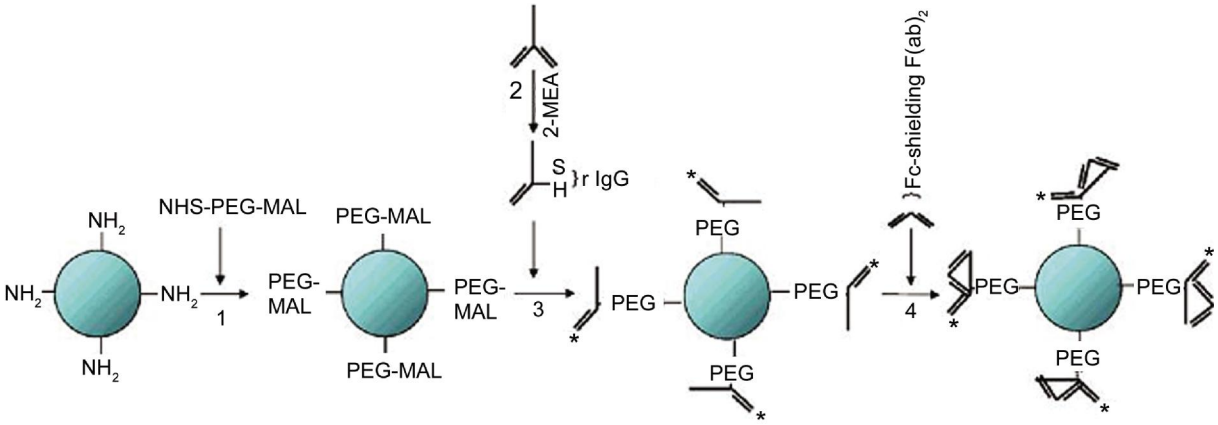

Figure 1 Steps of making an antibody labeled quantum dots (QD) PEG is used to decrease nonspecific binding and decrease the clearance rate while attaching the IgG fragments can lead to increase in affinity[129].

the retina. These hybrid dendrimers are designed as photosensitizers for preferential accumulation in malignant ocular tissue, for enhancing the effectiveness of PDT. The mannosylated dendrimers demonstrated specific interactions with the receptors in the lipid bilayer inducing protein channel rearrangement favoring the entry of the dendrimers into the cell. Wang *et al* [119] synthesized α-mannosyl dendrimeric porphyrins, which exhibited good photo efficiency, superior cellular uptake, and significant photo toxicity in retinoblastoma cells.

NANOPARTICLES FOR IMAGING OF UVEAL MELANOMA

Nanoparticles and nanotechnology has great potential in the field of eye disease and uveal melanoma imaging for early detection and diagnosis [120]. They can be developed into non-invasive biomarkers for detection of the disease [14,121]. Tari *et al* [122] invented a nanoparticle which can distinguish early and late stage of retinal vascular diseases. Their proposal is based on a simple matter of particle size. Bigger particles would stay in the blood circulation while smaller particles may manage to get out of the circulation in the early stages of the disease. By labeling the small and big size particles with two separate color dye, we gain the ability to track and monitor the diseases status. Currently, a variety of imaging modalities can reach the eye vasculature. So developing nanoparticle contrast agent to identify different tissues are possible. By the help of nanoparticles as contrast agents, the imaging sensitivity and quality can be improved[123]. For ocular vascular imaging, nanotechnology is mainly used in ophthalmoscopy as well as optical coherence tomography. Both of the application takes advantage of the optical contrast property of the light based imaging modalities. Interestingly, progress has also been made in developing contrast agents in the application of ultrasound, MRI to better monitor the structure of the retinal vasculature. The use of highly specific and highly sensitive quantum dots label is one of promising approaches. Anti-GFAP functionalized quantum dots (QDs) have been optimized for specific labeling and robust imaging of intermediate filaments in astrocyte and Müller glial cells in rat neural sensory retina [124]. Yamamoto *et al* [125] used a new quantum dot based method to detect vitreous lesions and even demonstrated that this nanotechnology method to stain the vitreous can guild surgeons to do vitrectomy surgeries. Another study conducted by Takeda *et al* [126] showed that *in vivo* quantum dot imaging can be used to detect spontaneous CNV of age-related macular degeneration (AMD) before it invades the retina. As CNV can lead to vision loss in AMD patients, the significance of this study is that it brings hope to early detect and diagnose AMD and save the patient's vision. QDs can also be applied into the ocular lymphatic pathway for imaging and may be useful in glaucoma to monitor eye pressure [127]. Recently, antibody-conjugated QDs have also found to be a high-throughput screening system and effective strategy for detection of melanoma [128]. In a melanoma-melanocyte co-culture *in vitro* model, QDs were constructed and conjugated with antibodies that could recognize melanoma cells. Data shown that these QDs can specifically detect melanoma cells only. Furthermore, PEG can be used to decrease nonspecific binding and decrease the clearance rate of the QDs product (Figure 1)[129]. Chemotherapeutic agents formulated with QDs and liposomes are also used for treating melanoma. Up until now, QDs have been used extensively *in vivo* to detect tumor vasculature [130-132]. Gold nanocages and nanoshells have been used in optical coherence tomography and other imaging approaches [133-139]. Iron oxide nanoparticles can be used to target certain cell surface receptors as well as macrophages [56-58]. Iron oxide particles with some modifications in size can also be used to detect the integrity of the retinal vessel structure. Manganese oxide particles were developed into the first T_1 nanoparticulate contrast agent which is biocompatible [140]. Also, iron oxide particles are developed into novel MRI contrast agent for detecting uveal melanoma in rabbit models [141]. The nanoparticle contrast agent increased the

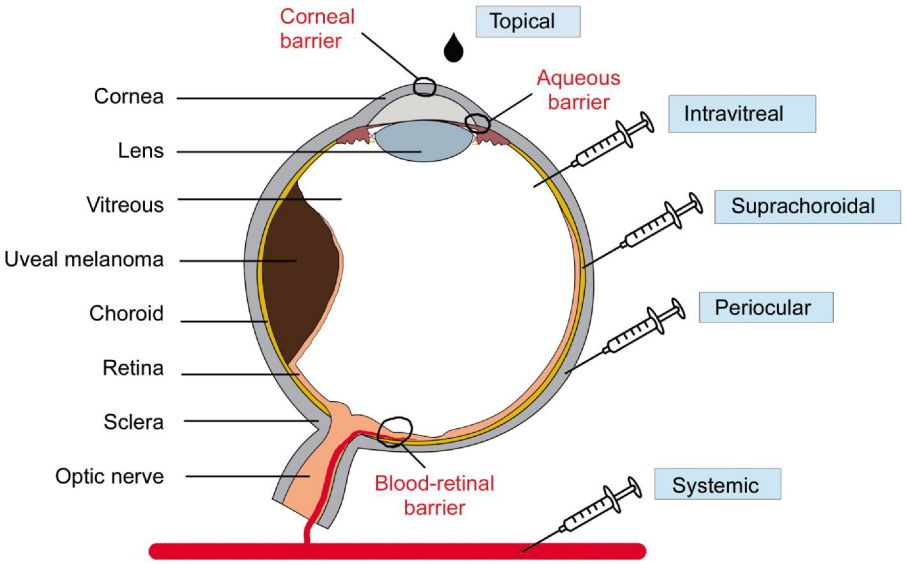

Figure 2 Diagram of the eye with uveal melanoma and routes of drug delivery to the eye (systemic, periocular, suprachoroidal, intravitreal and topical).

ratio of the T_1 to T_2 signal intensity in all of the ocular tissue. These findings indicate that nanoparticles are promising to provide noninvasive technique for the diagnosis of ocular melanoma and evaluation of tumor viability following treatments.

TOXICITY AND BIOCOMPATIBILITY

Toxicology analysis of nanoparticles are similar to the pharmacokinetics concept in pharmacology. Four key components for toxicity analysis of nanoparticles are looking at their adsorption, distribution, metabolism, and excretion inside the body. The toxicity of nanoparticles can be affected by many factors. The particle shape, charge, solubility, dosing frequency as well as particle size, route of injection and patient individual variation can all play a role in formation of the particle toxicity [142-150]. For nanoparticles to be used in human eyes, the toxicity of particles particularly need to be tested because if the particles damage the ocular barriers it would likely affect patient vision [43,151-152]. Unfortunately, the journey of using nanoparticles to treat eye diseases has just began. Therefore we currently lack the important local and systematic toxicity data for the particles used for eye imaging, diagnosis and also drug delivery. A few recent studies of toxicity and biocompatibility of nanoparticles in ophthalmology are briefly discussed below. Allergic or hypersensitivity reactions have been reported from the use of titanium dioxide, dendrimers and polystyrene nanoparticles in certain animal models and even in humans [153-155]. Carbon nanotubes have the potential of disruping and altering cell membrane [156-157]. Made out of the natural lipid, liposome nanoparticles are considered nontoxic and biodegradable. Intravitreal injection of liposome-loaded tacrolimus for suppressing experimental autoimmune uveoretinitis (EAU) caused no side effects on retinal function or systemic cellular immunity[158]. There is also data showing intravenously administered gold nanoparticles can penetrate the blood-retinal barrier without damaging the retina tissue [159]. The potential toxicity of cationic lipids, PEI and polyamidoamine dendrimers was also evaluated by experiments [160]. The results showed that PEI were the least toxic compared to the other two. Silicon QD by intravitreal injection was shown to be well tolerated by rats. No toxicity was observed in the experiment yet the therapeutic effect was obvious for treating retinal degeneration [161]. No toxicity to the retina was observed in another CCR3-targeting QD imaging mice experiment [126]. A detailed review paper summarizing approximately 30 types of nanoparticles was published by Dr. Prow[162].

EYE DRUG DELIVERY ROUTES

There are five main routes to deliver drugs into the eye, including systemic, periocular, suprachoroidal, intravitreal and topical injection. Figure 2 summarizes the above delivery methods which can also be applied to inject nanoparticles. Topical application is useful for treating disorders affecting the anterior segment of the eye. However, it has a big disadvantage of reaching a high ocular bioavailability as a large portion of the compound applied would be lost due to dilution of tears and lacrimation. Usually, the amount of drug that reaches aqueous humor is <5% . Systemic injection also has disadvantages as the amount of drug that would get to the vitreous cavity is quite low [163-166]. Lipophilic drugs are more in favor of going through the blood-retinal barrier and reaching the posterior part of the eye. If the compound is less lipophilic, then more frequent systemic injection is needed to reach and maintain an effective dose. This would very likely lead to higher incidence of systemic side effects to the body. The periocular injection route could have multiple meanings. It may refer to posterior juxtascleral, subconjunctival, retrobulbar, peribulbar

or subtenon injection. There are associated risks for periocular injections as well. Common complications include hyphema, increase in intraocular pressure, corneal decompensation and even strabismus [167-169]. Intravitreal injections are becoming more popular choice for ocular drug delivery. By micro-needle injection of the compound directly into the vitreous, intravitreal injection could offer a higher drug load in the retina and vitreous compared to other delivery methods. The drug molecular weight is the major factor to affecting drug elimination for intravitreal injection. Disadvantages of intravitreal injection includes development of certain complications such as intravitreal hemorrhages, endophthalmitis and retinal detachment [170-177]. Also, patients with the diseases affecting posterior segment usually need multiple intravitreal injections and following careful monitor.

MAJOR CHALLENGES AND FUTURE DIRECTIONS

Although significant progress has been made in the application of nanotechnology-based diagnostics and therapy for ocular tumors, formal approved particles for clinical application remains low in numbers. Currently, a solo nanoparticle delivery method cannot provide a conclusion to all the challenges we face in ocular drug delivery. In order to overcome such challenges, we need to invent and discover novel routes for delivering the drug and also creating novel delivery systems. Our aim is to create an efficient and non-invasive way to better delivery drugs to the patients to improve therapeutic efficacy. Recent discovery of the possibility of injecting drugs into the suprachoroidal space provides a new way of treating posterior eye diseases[16,178-182]. By using micro-needle injection into the space between the choroid and the sclera, suprachoroidal space injection can bypass the optical pathway while effectively delivery drugs into the choroid and ciliary body. Therefore, this injection technique may provide new possibilities to ocular melanoma treatment. Another major challenge in the treatment of uveal melanoma is that primary ocular tumor may be radiated or surgically removed, but the liver metastases is hard to treat. Nanoparticles may be useful to early detect and diagnosis ocular melanoma before the liver metastasis develops. Nanoparticles can be also applied for detection of circulating tumor cells, which play vital roles in the uveal melanoma metastasis to the liver.

CONCLUSION

The recent advance in nanotechnology has provided new opportunities in uveal melanoma and other ocular diseases treatment and diagnosis. By utilizing nanotechnology drug delivery systems, we can achieve higher efficacy, less toxicity, prolonged activity and less invasive administration of the drug for treating uveal melanoma. Challenges remains warranting further scientific research.

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