Published in final edited form as:

Proc IEEE Conf Nanotechnol. 2008 September 3; 2008: 822–825. doi:10.1109/NANO.2008.245.

Nanoparticles for Uveal Melanoma Treatment

Sheng Zhang¹, Jun Zhou², Zhibing Hu², Ashwin Nair¹, and Liping Tang¹

Liping Tang: ltang@uta.edu

¹Department of Bioengineering, University of Texas at Arlington

²Department of Physics, University of North Texas at Denton

Abstract

Chemotherapy is a common treatment for uveal melanoma. Rapid clearance of injected drug from the vitreous, necessitates repeated injections in to the eye. We have investigated the use of hydrogel particles to deliver chemotherapeutic drugs to the uveal melanoma site. Fluorescent dye-labeled nano- and micro-particles were injected intravenously into the animal. Particle accumulation in the uveal tissue was studied. The nanoparticles showed a much higher accumulation in the uveal tissue than the microparticles. The optimal particle size is between 100 and 300 nm. These particles could be decorated with targeting moieties and loaded with chemotherapeutic drugs to achieve sustained release at the cancerous site.

Keywords

uveal melanoma; NIPAM; nanoparticle; microparticle

I. Introduction

Uveal melanoma is the primary intraocular malignancy in Caucasians accounting for approximately 70% of all eye malignancies in adults. The estimated incidence is averaged six cases per one million subjects per year in the western world.¹ It occurs in all age groups, but its incidence in the general population increases considerably with age. The major cause of death by uveal melanoma is metastasis in which median survival is only 2~7 months. There is almost no effective treatment for primary uveal melanoma.² Over the past two decades chemotherapy has been widely used, but its efficiency has been severely affected by the difficulty in providing sustained drug concentration at the affected site. Also, many of the chemotherapeutic drugs cause systemic toxicity. Although the cause of these unsatisfactory results are still unclear, it is suggested that both uveal melanoma's high tolerance to anti-cancer drugs and the systemic toxicity associated with systemic high dose anti-cancer drugs is responsible for the failure of many uveal melanoma therapies.³ To overcome these drawbacks, it is important that anti-cancer drugs are targeted to the uveal melanoma tissue and released at the site to reduce systemic toxicity. Unfortunately, there is no such targeted delivery device available for uveal melanoma at the present time.

At the same time we know that tumor vasculature is extremely leaky,⁴ and that nanoparticles are used as diagnostic and therapeutic agents to treat tumors in other parts of the body.⁵ That accumulation of particles in tumor tissue is size dependent has been proven in an earlier study involving a breast cancer model.⁶ Also, mice treated with repeated intravenous injections of taxol-loaded nanoparticles showed a substantial accumulation of nanoparticles in B16F10 murine melanoma tissues, significant tumor regression and higher survival rates than mice treated with free taxol.⁷ Based on these facts and taking in to consideration that none of the studies so far have investigated the use of nanoparticles for uveal melanoma, we

have hypothesized that nanoparticles may be designed to target drug delivery for combating uveal melanoma.

Our recent work assessed the in vivo tissue compatibility of hydrogels and found that poly(N-isopropylacrylamide) (PNIPAM) nanoparticles elicited the least inflammatory response when compared to FDA approved Poly-L-Lactic acid (PLA) and poly-L-glycolic acid (PLGA) particles.⁶ In this study we have synthesized PNIPAM particles that were subsequently labeled with fluorescein-isothiocyanate (FITC) and injected systemically in a subcutaneous mice melanoma model. We compared the presence and distribution of these PNIPAM nanoparticles or microparticles in the uveal tissues and tumor tissue at different time points.

II. MATERIALS AND METHODS

A. Synthesis of NIPAM Nanoparticles & Microparticles

PNIPAM micro- and nano- particles were synthesized following the methods of Pelton and Chibante.⁹ Briefly, NIPA, Sodium Acrylate, N, N'-Methylene-bis-acrylamide were mixed together in deionized water. To control the particle size various amount of surfactant Sodium Dodecyl Sulfate (SDS) was used.¹⁰ The solution was stirred, deoxygenated and then heated to 70°C. Potassium per sulfate was added to initiate the polymerization. The synthesized particles were dialyzed to eliminate unreacted monomers and surfactants. They were then conjugated with FITC as previously published.⁷

B. Characterization of NIPAM Nano & Microparticles

Synthesized particles were characterized by observing the nanoparticles under the transmission electron microscope (Tecnai G2 Spirit, Oregon, USA) operating at 17 kV. Prior to analysis, a drop of NIPAM nanoparticle was stained with phospho-tungstic acid to enhance the contrast.

The NIPAM microparticles were dehydrated and sputter coated with silver for a minute and observed under the scanning electron microscope (Hitachi S-3000N VP-SEM, Hitachi, Pleasanton, CA, USA).

C. Animal Model & Particle Administration

FITC labeled PNIPAM micro- and nano-particles were intravenously injected in a murine model with/without subcutaneously implanted B16F10 melanoma. At the end of the study, all animals were euthanized. The eyes of control animals were enucleated and the subcutaneous tumor tissues were isolated from tumor bearing mice. The ocular and tumor tissue were frozen embedded, sectioned and used for the following analyses. To monitor the distribution of nano- and micro-particles in ocular and tumor tissues, tissue sections were directly observed under the fluorescence microscope (Leica DMLB, Leica Microsystems, Wetzlar, GmbH). The fluorescence intensity in the uveal tissue was determined using NIH ImageJ.¹¹

D. Statistical Analysis

Statistical analysis was done using Student's t-test. Differences were considered statistically significant at $p < 0.05$.

III. RESULTS & DISCUSSION

In our earlier study we had determined that NIPAM nanoparticles elicited the weakest foreign body reaction as compared to the commonly used polymers like PLLA and

polystyrene. In addition we also found that by varying the amount of surfactant and temperature used in the synthesis of NIPAM particles a range of particle sizes can be obtained.¹⁰ As such, a series of NIPAM particles bearing amine functional group with different sizes (10 μm and 100 nm diameter) were produced and characterized as shown in Figure 1 and Table 1.

To analyze the biodistribution of particles, we use both nano- and micro-particles conjugated with FITC. Following intravenous administration, we observed that FITC-labeled PNIPAM nanoparticles accumulated in the uveal tissue (Uv), but not in retinal pigment epithelium (abbreviated as Pe), sclera (Sc), or retina tissue (Rt) (Fig 2A). However, such preferential accumulation of fluorescence-labeled particles was not found when FITC- micro-particles (Fig 2B) were administered. We believe that the accumulation of PNIPAM nanoparticles in the uveal tissue depends, mainly on the size of particles and is independent of the FITC dye, since uveal tissues had no such signal following the injection of FITC-labeled microparticles or FITC alone (Figure 2C). Additional experiments have determined that the optimal particle size for such localized accumulation is between 100 and 300 nm diameter.

The fluorescence intensity in the uveal tissue was determined using NIH ImageJ (Fig 3). In the uveal tissue, we observed an almost five-fold increase in the fluorescence intensity in the case of the NIPAM nanoparticles labeled with FITC as compared to the microparticles and FITC dye alone.

In addition, no apparent foreign body responses associated with the accumulation of nanoparticles were observed. This is in agreement with our earlier studies where we found that hydrogel nanoparticles like PNIPAM and hydroxypropyl cellulose (HPC) elicited the least inflammatory response in a subcutaneous mice model.¹⁰

Although the mechanism governing the size dependent particle accumulation in uveal tissue is not clear, we believe that the ocular or tumor local vascular density and permeability plays an important role in determining the fate of nanoparticles. Therefore, particles with sizes > 700 nm stay in the capillary and do not escape into the target tissue while the smaller sized nanoparticles are able to escape and accumulate inside the uveal or tumor tissue.

Long term studies have uncovered that most of PNIPAM nanoparticles disappear from them in 3–5 days. Since the hydrolyzation of PNIPAM particles takes a much longer time, it is likely that the entrapment of PNIPAM nanoparticles in uveal or melanoma tissues may be associated with the slow nanoparticle movement and, potentially, slow blood flow rate outside capillary.

To investigate the preferential accumulation of nanoparticles in tumor tissue, similar particle accumulation studies were carried out on mice bearing subcutaneous B16F10 melanoma. As expected, IV injected PNIPAM nano-particles were found to have accumulated in the melanoma tissue near the capillary (400x, Fig 4A). However, such preferential accumulation of fluorescence-labeled particles was not found when FITC- micro-particles (Fig 4B) or FITC alone were administered (Fig 4C).

In an earlier study we conducted immunohistochemistry study on a panel of uveal melanoma cell lines and patient specimens. Our preliminary western blot studies have revealed that glycoprotein 100 (gp100) and melanoma antigen recognized by T cells 1 (MART-1 or Melan- A) are present on >95% of uveal melanoma samples tested (Fig 5). The abundant amounts of both gp100 and Melan-A surface antigens also suggest that both antigens are ideal targets for immunotherapy. The unique preferential accumulation of NIPAM nanoparticles that has been seen in this study can be combined with their targeting to the

uveal melanoma. This will facilitate the delivery of drugs directly to the uveal tissue. The envisioned strategy is depicted in Fig 6.

To summarize, both micro- and nano- particles circulate in the blood, however, nanoparticles can escape through the uveal and leaky tumor vasculature in to the uveal and melanoma tissue. The larger microparticles can only stay in the capillaries and circulation. We believe that, by conjugating the nanoparticles with ligands specific to uveal melanoma cells, drug-loaded nanoparticles would stay in the uveal melanoma tissue for a prolonged period of time which would lead to better therapeutic outcome.

Acknowledgments

This work was supported by NIH grant RO1 GM074021, Texas Higher Education Coordinating Board's Advanced Technology Program Grant 003594-0003-2006

References

1. Huerta C, Rodríguez LAG. Incidence of ocular melanoma in the general population and in glaucoma patients. *J Epidemiol Community Health*. 2001; 55:338–9. [PubMed: 11297655]
2. Coleman K, Baak JP, van Diest P, Mullaney J, Farrell M, Fenton M. Prognostic factors following enucleation of 111 uveal melanomas. *Br J Ophthalmol*. 1993; 77:688–92. [PubMed: 8280680]
3. Guo L, Wu Z, Zheng H, Zhang S. The expression and significance of the cell cycle related genes in uveal melanoma. *Chinese Journal of Ocular Fundus Disease*. 2001; 17:44–46.
4. Atmaca LS, Batioglu F, Atmaca P. Fluorescein and indocyanine green videoangiography of choroidal melanomas. *Jpn J Ophthalmol*. 1999; 43:25–30. [PubMed: 10197739]
5. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. *Adv Drug Deliv Rev*. 2002; 54:631–651. [PubMed: 12204596]
6. Grislain L, Couvreur P, Lenaerts V, Roland M, Deprez-Decampeneere D, Speiser P. Pharmacokinetics and distribution of a biodegradable drug-carrier. *Int J Pharm*. 1983; 15:266.
7. Sharma D, Chelvi TP, Kaur J, Chakravorty K, De TK, Maitra A, Ralhan R. Novel taxol formulation: polyvinyl-pyrrolidone nanoparticles-encapsulated taxol for drug delivery in cancer therapy. *Oncol Res*. 1996; 8:281–6. [PubMed: 8938791]
8. Haugland, RP. Handbook of fluorescent probes and research products. 9. 2002. p. 112-114.
9. Pelton RH, Chibante P. Preparation of Aqueous Latices with N-Isopropylacrylamide. *Colloids and Surfaces*. 1986; 20:247–256.
10. Weng H, Zhou J, Tang L, Hu Z. Tissue responses to thermally-responsive hydrogel nanoparticles. *J Biomat Sci Polym Ed*. 2004; 15:1167–1180.
11. Abramoff MD, Magelhaes PJ, Ram SJ. Image processing with ImageJ. *Biophotonics Int*. 2004; 11:36–42.

Fig. 1. Characterization of NIPAM particles. (A) TEM image showing the NIPAM nanoparticles. Scale bar = 2000nm; (B) SEM image showing the dehydrated NIPAM microparticles. Scale bar = 5 μ m)

Fig. 2.

Accumulation of intravenously administered (A) PNIPAM nanoparticle-FITC conjugates (100 nm diameter) in mice uveal tissue (green dots represent accumulated particles, pointed with arrow), (B) PNIPAM microparticle-FITC conjugates (10 μm diameter), and (C) FITC alone. Specific ocular tissues were labeled as follows -Sc: Sclera; Uv: Uvea; Pe: Retinal Pigment Epithelium; Rt: Retina

Fig. 3.

Comparison of the accumulation of nano-sized and micron-sized particles in uveal tissue was carried out by measuring the fluorescence intensity in the uveal tissue using NIH ImageJ. Significance of differences between NIPA nano-FITC vs NIPA micro-FITC & FITC alone; * $P < 0.05$

Fig. 4. Intra-tumoral accumulation of intravenously administered (A) PNIPAM nanoparticle-FITC conjugates (100 nm diameter) in tumor tissue (green dots represent accumulated particles, pointed with arrow) near the capillary. (B) Very few PNIPAM FITC-conjugated microparticles were found outside the capillary. (C) The administration of FITC alone yield very low background fluorescence.

Fig. 5.

Immunohistochemistry on uveal melanoma cell lines. (A) H&E staining of mixed cell melanoma ZEC4, $\times 200$; (B) Phase contrast microscope of mixed cell melanoma ZEC4, $\times 400$; (C) and (D), X400, two uveal melanoma cell lines (ZEC4 and ZEC7) stained for glycoprotein 100 (gp100) and >95% of cells in both uveal melanoma cell lines are gp100 positive. Both cell lines were isolated

Fig. 6.

Hypothetical (and simplified) strategy to target localized delivery of nanoparticles to uveal melanoma cell surfaces to specifically eradicating uveal melanoma with minimal systemic complications.

Table 1

NIPAM nano- and micro- particles size distribution

Particle Type	NIPAM Nanoparticle	NIPAM Microparticle
Size	100.1 ± 4.46 nm	10.4 ± 2.3 μ m