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Brain-Targeted Delivery and Redox-Responsive Release of Protein Therapeutics via GSH-Sensitive SiO₂ Nanoparticles

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ABSTRACT

This study In this study, we have presented an idea of developing and evaluating a glutathione (GSH) responsive, aptamer functionalized silica nanoparticle system (FITC-BSA@Apt-SiO₂) for targeted protein delivery to glioma tumor. Disulfide-containing organ silanes (BTEPDS) were introduced into the nanoparticles synthesized via reverse microemulsion method so as to allow their degradation under GSH tumor mimicking conditions through reductive cleavage. FITC-BSA was successfully encapsulated inside the PLGA microspheres, and the microspheres were surface modified with the AS1411 aptamer for tumor targeting capability. In addition, the spherical morphology of NPs was confirmed by transmission electron microscopy (TEM) with a size distribution of 43.5 – 61.5 nm and the degradation behavior were investigated by TEM, as well as cumulative protein release determined by a BCA assay, in which both methods indicated large GSH-dependent breakdown after 24 hours. Furthermore, in vivo fluorescence imaging in glioma bearing mice proved effective blood-brain barrier penetration and accumulation at the tumor site observed for FITC-BSA@AptSiO₂ in comparison to non-targeted controls. Examination of the excellent biocompatibility and localized action has been confirmed through hemolysis and mouse tail bleeding assays to avoid systemic toxicity. Our findings confirm that FITC-BSA@Apt-SiO₂ is a smart responsive nanocarrier system for tumor targeted delivery of therapeutic protein especially applied in treatments of glioma, which requires crossing the BBB and delivering its contents intracellularly upon demand

Introduction

1.1. Current Status of Brain Tumor Treatment

Among one of the most critical health challenges, brain tumors (especially gliomas, glioblastomas) remain severe given their aggressiveness and resistance to conventional treatment (Xu, 2025). Despite tremendous advancements in cancer therapy, the prognosis of brain tumors, particularly glioblastomas, exhibits a very poor prognosis with very short survival, which pinpoints the need for novel and effective therapeutic strategies (Tyagi et al., 2023). In this chapter, epidemiology, incidence, and mortality rates associated with brain tumors will be overviewed, along with limitations of today's treatment techniques, such as surgery, radiation therapy, chemotherapy, etc.

Even though brain tumors represent a smaller fraction of all cancers, they cause a significant number of deaths. A significant number of people are affected by primary brain tumors such as gliomas, meningiomas, and pituitary tumors. The most common example is in the case of the United States, where it is expected that around 94,390 new primary brain and CNS tumors will be diagnosed in 2023, with the most common being gliomas (Xu, 2025). Glioblastoma multiforme (GBM) is the most aggressive among gliomas, and it is characterized by roughly 3.2 cases per 100,000 people (Cruz et al., 2023). Brain tumors make up only 2 to 3% of all cancers; however, they produce high morbidities and mortalities due to the brain being crucial and its limited regenerative capacity (Haque et al., 2021). For example, glioblastomas, which have very poor median survival rates (less than 15 months) and less than a 5% 5-year survival rate, have particularly concerning mortality rates (Sikder et al., 2024). Since glioblastoma is one of the most lethal forms of cancer due to the tumor's invasive nature and resistance to standard treatments, new therapeutic options are needed. However, benign tumors, for example, meningiomas, have longer survival prognoses and require easier treatments, although maintaining recovery over the long term may not be easy (Zhao et al., 2023).

Neoplasms of the brain are a heterogeneous group of neoplasms with diverse biological behaviors and clinical outcomes. of these, glioblastoma (GBM) is a World Health Organization (WHO) grade IV astrocytoma that is the most aggressive and lethal primary malignant brain tumor in adults. This represents about 45.6% of all malignant primary brain tumors and the incidence rate is 3.19–3.4 cases per 100,000 inhabitants per year (Yildirim and Ucar 2024, Tamimi and Juweid 2017; Bohn et al 2018). The prognosis of the disease is poor as medical technology advances with a median survival of only 12–15 months post diagnosis and fewer than 5% of patients surviving beyond 5 years (Martin et al., 2021).

There are multiple therapeutic challenges that limit the treatment of glioblastoma. Surgical resection constitutes a standard first line approach that is rarely curative given burrowing behavior (infiltrative growth) of the tumor into healthy brain tissue, precluding complete resection. Thus, microscopic residual tumor cells cause recurrence (Martin et al., 2021). Since radiotherapy is often applied to GBM patients after surgery, high doses are undesirable due to the radioresistant nature of GBM and the radiosensitivity of surrounding normal brain structures. The main issue for chemotherapy, commonly temozolomide, is hemorheological limitations such as the blood brain barrier (BBB) that prevents proper drug penetration into the brain or chemoresistance (Yıldırım & Uçar, 2024). All of these factors make the tumor notoriously recurrent and confer poor long term outcomes.

A pie chart as found in Figure 1-6 presents the relative incidence of glioblastoma compared to other brain tumor types. Another one is that glioblastoma is the most common form of malignant brain

tumor making up close to half of all such cancers, and meningiomas are the second most common, comprising roughly 15–30% of these malignancies as shown in the figure. The rest consists of astrocytoma's, oligodendrogliomas, and metastatic brain tumors. Such a high incidence highlights the urgent requirement for novel, glioblastoma directed, chemotherapeutic intervention.

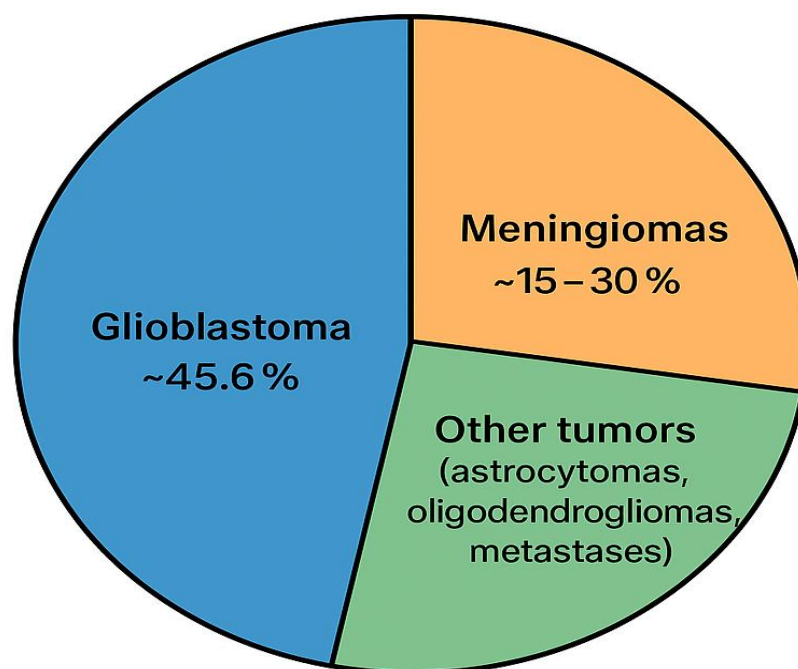


Figure 1-6. Pie chart showing the incidence distribution of brain tumor types, highlighting glioblastoma as the dominant malignant form (~45.6%).

Brain metastases, also known as secondary brain tumors, spread from other locations in the body, including the lungs and breasts, and are an increasing challenge in the treatment of brain tumors. With the improvement of therapies for systemic cancers, the incidence of brain metastases has increased (Franceschi & Brandes, 2012). This only underlines the importance of developing effective treatments that are effective not only at the primary brain tumors but also at metastatic ones, at the same time as addressing the problems posed by the blood-brain barrier (BBB). Brain tumors are usually treated first by surgical resection to remove as much of the tumor as possible. In such cases, however, the tumor lies within critical brain regions; therefore, complete removal is not possible without doing neurological damage (Kumar et al., 2021). However, even when surgery is successful, recurrence should be considered common, particularly regarding malignant gliomas, since microscopic tumor cells are left behind (Cruz et al., 2023). It is also common to use radiation therapy after surgery, directed at any cancer cells that remain. In addition to being effective for reducing tumor size and controlling growth, radiation therapy is, however, limited. High doses needed to treat brain tumors cause significant damage to nearby healthy brain tissue, resulting in side effects like the loss of cognitive abilities, memory, and fatigue (Xu, 2025). Moreover, radiation therapy turns ineffective when tumors are placed in deep brain regions or adjacent critical organs (Sikder et al., 2024), as in that case, radiation also influences the neighboring healthy tissue.

In the treatment of brain tumors, particularly high-grade gliomas, chemotherapy is very important. Unfortunately, the BBB limits its effectiveness as the protective layer prevents most drugs from making it into the brain (Tyagi et al., 2023). Although some chemotherapy agents can cross the BBB, typically they do so in

insufficient concentrations to significantly impact the tumor (Haque et al., 2021). Secondly, the toxic implications of systemic chemotherapy drugs that impact almost all the organ systems of a patient, nausea, immune suppression, and hair loss (Sikder et al., 2024). The blood-brain barrier is still one of the biggest challenges in the treatment of brain tumors. On the one hand, it functions to protect the brain from harmful substances, but on the other hand, it prevents therapeutic agents from crossing over into the brain. In particular, most of the chemotherapy agents (Wen et al., 2020). However, overcoming this barrier is to improving the efficacy of brain tumor treatments. Existing methods to enhance drug penetration, for instance, increasing drug dose or recourse to invasive techniques, have not been found capable of the required therapeutic gain (Sahoo et al., 2024).

Glioblastomas and other brain tumors are very resistant to surgery, radiation, and chemotherapy. However, the resistance partially comes from the fact that genetic and molecular heterogeneity leads to the development of subpopulations of tumor cells with resistance to treatment (Zhao et al., 2023). Besides that, factors like hypoxia, inflammation, and immune suppression in the brain tumor microenvironment are common, which adds to the challenge in employing conventional therapies (Sahoo et al., 2024). In particular, because of these challenges, new treatment strategies are necessary that are more effective.

The limitations of the currently available treatment options and the challenges associated with the delivery of drugs to brain tumors because of the BBB constitute an urgent need for improved drug delivery systems that can increase the target delivery of therapeutics to brain tumors. These problems, however, can be solved with the rising branch of nanotechnology, which could possibly help in enhancing the drug's solubility, crossing the BBB, and administering therapeutic agents directly to the tumor, without affecting healthy brain tissue (Tyagi et al., 2023). Nanoparticles can be engineered for more precise delivery of drugs due to their surface properties being able to be customized and because they are small enough for transport across the BBB (Zhao et al., 2023). Moreover, these nanoparticles may also be functionalized to target particular tumor cells while lowering needless consequences that are frequently an issue in conventional medicines (Sikder et al., 2024).

In the therapy of brain tumors, the therapy based on nanotechnology liposomes, polymeric nanoparticles, and carbon-based nanomaterials showed positive results in the preclinical studies. For the same systems, one can design them to release their therapeutic payloads upon specific stimuli such as changes in pH or the presence of biomarkers in the tumor microenvironment for controlled and sustained drug release (Wen et al., 2020). Yet, the nanoparticles can lessen the toxicity to the system caused by high doses of chemotherapy and radiation, enhancing the patient's quality of life and resulting from treatment (Kumar et al., 2021). To conclude, there is still a requirement for innovation as conventional treatments for the treatment of brain tumors (i.e., surgery, radiation, chemotherapy) remain crucial, albeit with limitations of side effects and efficacy. Due to these barriers, there is a need for more effective and safer therapeutic methods, which nanotechnology offers as a possible means to overcome them, to provide therapeutic options for brain tumor patients. Despite this, more research is necessary to overcome the clinical challenges and design for the widespread clinical utility of these systems (Xu, 2025).

1.2. Applications of Nanotechnology in Drug Delivery

Revolution in the field of drug delivery is brought about by nanotechnology, solving some of the most difficult issues of pharmacology. Nanoparticles have unique properties; they are small in size and can be engineered to have specific surface features that make them a tool for precise delivery of therapeutic agents to target tissues, e.g, tumor, or target organs, e.g, brain. It is of special significance in the treatment of neurological disorders, such as brain tumors, as traditional drug delivery systems suffer from serious problems, such as the blood-brain barrier (BBB) (Xu, 2025). Through its ability to

give better control over the release of drugs, improve solubility of poorly water-soluble drugs, and improve therapeutic effects by overcoming the physiological barriers that, likewise, limit the strength of therapy, nanotechnology lies on the edge of future medicine (Emeihe et al., 2024).

Drug delivery systems based on nanotechnology have great potential in delivering the drug to specific sites in the body with minimal exposure to healthy tissues and very minimal side effects. Nanocarriers, including liposomes, polymeric nanoparticles, dendrimers, and carbon nanotubes, have many applications in targeted therapy in cancer, neurodegenerative diseases, and all other complex diseases (Saeedi et al., 2019). These nanocarriers can be rendered to bind specifically to the surface of biomarkers that display on the diseased cells; thereby, tightening the binding of the drug and increasing the effects of the binding of the drugs (Haider, 2024).

Nanotechnology in drug delivery is one of the most significant because these nanocarriers can be engineered to interact with a specific receptor or biomarker to target specific cells or tissues, for example, to target cancerous cells or brain tissue, respectively. Because this targeting ability allows drug delivery to the affected tissue or cell without introducing the toxic agents to the healthy tissues (Saeedi et al., 2019), systemic exposure to healthy tissues is reduced, and side effects are minimized. Nanoparticles are tailored with surface properties enhancing the drug's therapeutic index through increased drug accumulation at the target site (Emeihe et al., 2024).

Also, nanotechnology affords a means to lessen side effects ordinarily associated with conventional drug delivery protocols. For instance, conventional chemotherapy usually has severe side effects because the drugs damage both healthy and cancer cells indiscriminately. However, nanoparticles could be engineered to release the drugs specifically at cancer cells without affecting healthy tissues and their side effects, such as nausea, immune suppression, and hair loss (Khafaga et al., 2025). Furthermore, nanocarriers not only enable controlled release of therapeutic agents and thereby minimize the treatment frequency and improve patient compliance, but also facilitate the release of the drug over an extended period.

Nanotechnology also improves the solubility of poorly soluble and unstable therapeutic agents in the body. The drug is encapsulated in the nanoparticles to enhance bioavailability by increasing drug concentration at the target site, even without increasing the dosage (Kibuuka, 2024). Additionally, nanoparticles may be engineered to get past barriers of the body, such as the blood-brain barrier, a major obstacle to administering many therapies for neurological disease, for example, in the form of brain tumor chemo or radiation therapies (Saeedi et al., 2019). In this way, nanoparticle-based delivery systems can significantly improve the therapeutic efficacy of drugs, especially for diseases where therapeutic agents have difficulty in being targeted to difficult areas in the body, such as the brain.

Numerous recent studies have shown a remarkable improvement in the field of nanotechnology-based drug delivery systems, especially in cancer therapy and neurological disorders. Although many drugs were found to be toxic to cells and failed to function as expected, nanocarriers, i.e., liposomes, polymeric nanoparticles, and carbon nanotubes, have been extensively studied for the precise delivery of drugs with low toxicity. Studies show that these nanocarriers have the promise of increasing drug delivery to cells both across the blood-brain barrier and to tumor cells, thus improving the success of the treatment of brain tumors, including glioblastoma (Xu, 2025).

In addition, the stimuli-responsive nanoparticles have enabled the fabrication of drug delivery systems that deliver their therapeutic payload in the presence of respective environmental triggers, such as the change in pH, temperature, and presence of specific enzymes in the tumor microenvironment (Emeihe et al., 2024). The control of the release of drugs at the site of action allows targeted delivery and minimizes the systemic exposure of drugs, with parallel higher efficiency in treatment.

Though such advances already have been made, several challenges remain to the widespread application of nanotechnology-based drug delivery systems for clinical use. The first of the major hurdles is scarcity and the manufacturing/scalability of the actual nanoparticles. However, translating their attractiveness in preclinical studies to large-scale production is complex and costly (Kibuuka, 2024). However, the biocompatibility and long-term safety of nanoparticles are still in question, as their interaction with biological systems can be inconceivably toxic. Furthermore, it is still a challenge to obtain regulatory approval for nanoparticle-based drug delivery systems because such systems are required to have safety and efficacy standards before being used in clinical practice.

In addition, the challenge of getting the treatments needed to overcome the blood brain barrier remains a major one in the treatment of neurological disorders. Despite the development of nanotechnology to augment MRI and conquer the problem of drug delivery to the brain, further research will be needed to increase the efficiency of nanoparticles to transfer into the brain effectively (Sreedhar et al., 2019). The central nervous system (CNS) provides with a key defense system in form of the highly selective physiological and structural blood brain barrier (BBB). It rigorously controls the movement of substances between the bloodstream to neural tissue and, thereby, is responsible for maintaining the brain's microenvironment. Tightly connected, non fenestrated endothelial cells supported by pericytes, astrocytic end feet, basal lamina make up the structural BBB. Together these elements comprise an elaborate barrier which hinders most blood born substances including therapeutic agents from transgressing brain tissue (Wu et al., 2023).

The BBB functionally excludes xenobiotics and harmful agents using active transport systems, enzymatic barriers and ATP dependent efflux pumps such as P glycoprotein. This is a major challenge to pharmacological treatment of CNS disorders, but particularly for glioblastoma where most chemotherapeutic agents are ineffective across the blood brain barrier in sufficient concentration (Gaillard et al., 2012; Pandit et al., 2019), crucial for maintaining the brain.

Oral and intravenous administration of therapeutics is limited in its ability to penetrate the blood brain barrier to the brain and less than 5% of administered dose is typically found across the BBB (Lalatsa et al., 2011). Table 1-1 shows that nanotechnology-based approaches have the potential to enhance the ability of nanoparticles to penetrate BBB through mechanisms such as receptor mediated transcytosis as well as endocytosis. These nano-systems can be modified to target the brain and enhance the localization with less toxicity to the system. Besides, nano-carriers including liposomes, dendrimers and silica nanoparticles can deliver drugs in a nano carrier that protect the drugs from degradation by enzymatic and enhance circulation time and therapeutic window through controlled release (Ding et al., 2020; Palazzo et al., 2016). However, despite these advantages, nanocarrier based systems have a very low clinical translation into the CNS, with only a handful of FDA approved nano formulations for CNS applications.

The following is the summary of this comparison between conventional and nanotechnology assisted drug delivery.

Table 1-1. Comparison of Drug Delivery Approaches for Crossing the BBB

Feature	Conventional Methods (e.g., oral, IV)	Nanotechnology-Based Methods
BBB Penetration	Very limited; <5% of drugs cross (Lalatsa et al., 2011)	Enhanced via endocytosis and receptor-mediated transport (Ding et al., 2020)
Targeting Accuracy	Low; relies on systemic circulation	High; can be modified for site-specific delivery (Palazzo et al., 2016)

Side Effects	Higher systemic toxicity	Lower toxicity due to controlled release and localization
Stability in Circulation	Susceptible to degradation and elimination	Engineered for sustained release and protection
Clinical Approval	Widely used, FDA-approved methods	Few nano-formulations currently approved

Overall, nanotechnology has much promise for revolutionizing drug delivery in the therapy of the brain. Nanotechnology has the potential to improve the drug targeting ability, minimize the side effects, and enhance the therapeutic efficacy, becoming an effective means in modern medicine. However, biocompatibility and regulatory issues, as well as scalability, are still issues that need to be solved. With continued improvement in research, newer delivery systems of drugs that are efficient and more effective in doing the job should be a reality, making for easier overcoming of present challenges and ultimately leading to improved patient outcomes.

1.3. GSH-Responsive SiO₂ Nanoparticles

In the field of drug delivery, in particular, targeting tumors, the use of Glutathione (GSH)-responsive nanomaterials in this matter is an innovative approach. These materials use attractive features of GSH, a tripeptide of glutamine, cysteine, and glycine that is highly present in the intracellular compartments of cancer cells. GSH is an antioxidant that helps cells control oxidative stress. Because tumor cells possess elevated metabolic activity and proliferate rapidly, their GSH concentrations are usually also increased compared with normal cells. The authors propose GSH as a good target for designing drug delivery systems to selectively release the therapeutic agents at sites containing tumors and minimizing systemic toxicities while improving therapeutic efficacy (Zhang et al., 2024).

In particular, GSH-responsive nanoparticles, particularly silica (SiO₂), have been actively studied since they can release the encapsulated drug in response to the raised GSH levels in the tumor microenvironment. The surface of these systems was often functionalized by disulfide linkages, which are broken in the presence of high concentrations of GSH. It permits the controlled release of therapeutic agents, allowing the release of the drug precisely at the tumor site and thereby increasing the specificity and efficiency of the treatment (Chen et al., 2017). These SiO₂ nanoparticles are used in such applications, mainly cancer therapy and other diseases, because of their biocompatibility and surface modification (Liu et al., 2015). The TME with high GSH levels is one of the features of the tumor microenvironment. Cancer cells in this manner rely on high GSH concentrations as a defense against oxidative stress associated with cancer induction, including rapid cell proliferation, hypoxia, and other factors of tumorigenesis, and thus become a hallmark of cancer cells in general. Additionally, GSH is necessary for the control of intracellular signaling pathways in the control of cell proliferation and apoptosis (Wang et al., 2020). The excess GSH in tumor cells is used to maintain their redox balance and therefore tumor cells survive and proliferate under difficult conditions.

Because of the abnormal amount of GSH in the TME, it provides a unique opportunity for targeted delivery of drug. However, in the presence of high concentrations of GSH, such nanoparticles release their payload selectively because of excessive local concentrations of GSH in cancer cells and tumor extracellular matrix. An advantageous strategy is the targeted delivery of the drug to cancer cells without affecting the normal tissues, so there would be a good therapeutic effect (Zhang et al., 2015). For instance, as a capping agent, the disulfide bonds of mesoporous silica nanoparticles (MSNs) would cleave only if exposed to high concentrations of GSH, which is available in normal tumor site (mesoara, macalino, pufLOW, utipolb, and biily, 2015).

In addition, these nano size GSH sensitive nanoparticles can be engineered to release the drugs in a 'burst' fashion to make sure that a sufficient dosage of therapeutic agent could be delivered to the tumor in a short period of time. Furthermore, there are also applications of utilizing this responsive release mechanism as multi therapy treatment of cancer where the chemotherapy can be administered with immunotherapy or with photothermal therapy (Chen et al., 2017).

Silica nanoparticles that release therapeutic agents selectively in response to glutathione (GSH) have been developed as an innovative and intelligent drug delivery system to selectively deliver the therapeutic agents at the tumor microenvironment. The latter provide redox sensitive linkers, disulfide bonds, which are embedded in the silica matrix in these systems. These disulfide linkages are stable under normal physiological conditions. Most importantly, in that case the bonds are able to undergo reductive cleavage under such conditions in tumor cells where the intracellular concentration of GSH is significantly elevated (up to 1000 fold versus normal tissues), leading to degradation of the nanoparticle structure and controlled release of the encapsulated drug (Yang et al., 2022).

This mechanism not only increases the accumulation of a drug at the tumor site but also decreases the systemic toxicity by preventing premature release of the drug. This delivery strategy is further specific due to the unique biochemical environment of tumor tissues that are highly different from normal tissues. The other differences between the tumors and surrounding tissues involve these and include acidic pH, higher enzyme activity (e.g., glutathione reductase), and leaky vasculature.

A summary of the key physiological differences between tumor and normal tissues that are significant for effective GSH-responsive drug delivery is provided in the table below.

Table 1-2. Tumor vs. Normal Tissue Characteristics Relevant to Nanoparticle Response

Parameter	Tumor Tissue	Normal Tissue
GSH Level	High (2–10 mM intracellular)	Low (~2–10 μ M extracellular)
pH (extracellular)	Acidic (~6.5–6.8)	Neutral (~7.4)
Permeability	Leaky vasculature (EPR effect)	Tight endothelial junctions
Enzyme Expression	High (e.g., glutathione reductase)	Normal levels

Due to their widespread surface area, controllable pore size and biocompatible property, silica nanoparticles (SiO_2) have also been proposed as an excellent carrier for drug delivery. Due to these solid SiO_2 nanoparticles, they have few number of surface to volume ratio, therefore SiO_2 nanoparticles surface is easily accessible for through modification to increase stability, increase drug loading capacity and enhance their functionality by addition of targeting ligands or stimuli responsive elements. Due to properties of this type of nanoparticle, they are very suitable for drug delivery systems, more so if they will be used for cancer therapy where targeted and controlled release of the drug is desired (Zhao et al., 2023).

SiO_2 nanoparticles are also one of the great advantages in terms of biocompatibility. More particularly, silica is relatively inert and the SiO_2 nanoparticles have been proven to have limited

toxicity in vivo and could possibly be employed for prolonged usage as drug carrier without any obvious toxicity nor any adverse immune response (Zhang et al., 2016). Along with that, silica nanoparticles are biodegradable, which means they are going to degrade with time and turn into nonharmful products, which will easily excreted from the body, decreasing the risk of long-term accumulation (Wang et al., 2020). It also preserves the advantage of SiO₂ nanoparticles, namely, to modify surface properties. Because it is an accessible surface, various ligands (e.g. antibodies, peptides or small molecules) can be attached to bind the target, e.g., cancer cells or other disease sites. The second advantage of this method is a modification of surface by employing GSH sensitive linkages (for example, disulfide bonds) to create GSH responsive drug delivery systems. Also, these systems can be deployed triggering if the GSH level in the tumor microenvironment increases for an efficient and tumor specific treatment (Liu et al., 2015).

In addition, the synthesis of SiO₂ nanoparticles with controlled level and sign of the surface charge, size and porosity can be carried out, encompassing a large variety of therapeutic agents including hydrophobic drugs, proteins, and nucleic acids which are generally hard to target using conventional delivery systems. The introduced flexibility of the SiO₂ nanoparticles allow for the delivery of multiple therapeutic agents or treatment modalities with synergistic therapeutics in a single drug delivery vehicle (Zhao et al., 2023).

GSH responsive SiO₂ NPs are attractive for the purpose of drug delivery in their targeting in cancer therapy and preliminary investigations were carried out with it. Finally, these nanoparticles can be used to deliver therapeutic payloads from these nanoparticles in a controlled and accurate manner to reduce side effects and improve treatment efficacy by finally leveraging the elevated GSH found in the tumor microenvironment. SiO₂ nanoparticles were considered as suitable candidates for the development of advanced drug delivery system for improving the cancer treatment outcomes due to their biocompatibility, adjustable surface properties and ability to be functionalized for targeting.

1.4. Research Objectives and Significance

1.4.1. Research Objectives

Basically, the objective of this research is to design, synthesize, and characterize released GSH from SiO₂ nanoparticles and determine their effectiveness as a drug delivery carrier for targeted cancer therapy, especially brain tumors. The first aim of the study is to synthesize GSH-responsive SiO₂ nanoparticles that respond to the high glutathione (GSH) concentration present in the Tumor microenvironment (TME). Surface functionalization of the nanoparticles using disulfide bonds that are cleaved in the presence of high GSH levels will be used to design these nanoparticles. Characterization of the nanoparticles will be performed by examining the size, surface charge, morphology, and drug loading capacity of the nanoparticles by techniques such as dynamic light scattering (DLS), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR). This guarantees that the nanoparticles satisfy the different necessities for use in medication conveyance applications like consistency, biocompatibility, and reaction to the growth mini environment (Zhao et al., 2023; Zhang et al., 2017).

The second research objective is to study, once the nanoparticles are synthesized and characterized, the kinetics of drug release from the nanoparticles in environments mimicking the tumor microenvironment. Testing of the ability of the nanoparticles to release an encapsulated drug, namely doxorubicin, in response to GSH concentrations too high for healthy tissues, like tumor tissues, will be involved in achieving this. The ability of nanoparticles to deliver the drug to the tumor site specifically will be studied by different release profiles under different conditions, for example, including various pH and GSH concentrations. But to avoid drug release from having off-target effects and providing therapeutic efficacies (Liu et al., 2020; Zhang et al., 2024), it must be controlled.

The overall aim is to find out if these GSH-responsive SiO₂ nanoparticles can cross the BBB and nudge therapeutic agents to glioblastoma. For proof of this, in vitro models of the BBB, i.e., endothelial cell culture models as well as co-culture systems which closely mimic the physiological situation in the BBB, will be tested for the nanoparticles. Their ability to target brain tumor cells will be finally determined, and their capacity for delivering chemotherapeutic agents will be determined. It is due to the restricted nature of the BBB that whether GSH-responsive nanoparticles can be engineered to serve as a potential treatment for brain tumors, notoriously hard to treat, is determined (Zhao et al, 2023; Nosrati et al., 2019).

1.4.2. Significance of the Research

Targeted drug delivery for improved treatment of brain tumors is likely to be the subject of great promise for the investigation of GSH-responsive SiO₂ nanoparticles. These nanoparticles are used to bypass many of the issues with traditional cancer therapies. Therefore, this research is important in the following aspects:

The objective of this research is to improve the treatment outcome of brain tumours. Amongst the most challenging cancers to treat, glioblastoma, and one of the best obstacles to the delivery of therapeutics to the central nervous system is the blood-brain barrier (BBB). When it comes to drug delivery, if the drugs lack the ability to reach the brain without destroying healthy tissue, treatments like surgery, chemotherapy, and radiation therapy are unable to be effective. GSH-responsive SiO₂ nanoparticles serve as a targeted drug delivery system, which allows the delivery of the drug efficaciously across the BBB and delivery at the tumor site. It is also able to increase the therapeutic efficacy of treatments through this modulation of drug release; specifically, in response to increased levels of glutathione (GSH) present in the tumor microenvironment. These nanoparticles have the ability to selectively target the tumor site and therefore decrease the dose of the drug that goes across the entire body (systemic toxicity associated with conventional therapies), thus improving therapeutic agents reaching the aim site, which consequently results in better outcomes for patients (Musielak&Krajka – Kuźniak, 2025).

Besides, this research has another critical advantage, which is reducing the side effects of traditional cancer therapies. A major disadvantage of conventional therapies is their systemic toxicity, specifically with chemotherapeutic agents that lead to damage to healthy cells as well as serious side effects like nausea, hair loss, and immune suppression. On the contrary, GSH-responsive nanoparticles have the possibility to substantially reduce these side effects by releasing drugs at the tumor site specifically, where GSH levels are elevated. This addresses an issue that causes less drug exposure on healthy tissues, lower side effects, and better quality of life for patients (Wu et al., 2022). Furthermore, they give therapeutic agents time-release over an extended period of time, also making fewer doses and hence a lighter weight on the patient during treatment.

Another important aspect of the significance of the research is its potential to provide a novel way to treat brain tumors. Silicon nanoparticles bear promise to improve therapeutic efficacy through the use of GSH-responsive SiO₂ nanoparticles. Apart from solving the problems of BBB penetration and selective delivery, these nanoparticles allow various therapies, e.g., chemotherapy, immunotherapy, or photodynamic therapy, to be integrated into the same nanopatform. Delivery of a combination of treatments directly to the tumor site to enhance the overall therapeutic effect and overcome resistance mechanisms that commonly preclude the efficacy of individual treatments can be done through dragee (drug delivery) nanoparticles (Zhao et al., 2023). This research may form a basis for the development

of individualized treatment strategies for brain tumor patients that involve the administration of treatments of a more specific and less toxic nature.

Finally, this work has concluded that this research can revolutionize the way brain tumors are treated. GSH responsive SiO₂ nanoparticles are utilized in this approach to improve the drug delivery efficiency and decrease the side effects of current therapy treatment and offer improved cancer brain tumor therapy performance in general. This could advance the current notion and therapeutic treatment of brain tumors to better, less toxic, and personalized treatments for our cancer patients, added Maricich.

1.5. Structure of the Research

In this research, the design, synthesis and evaluation of GSH responsive SiO₂ nanoparticles for targeted drug delivery is carried out on a comprehensive basis. The first part of the research is about the synthesis and characterization of these nanoparticles. One of the well-established methods of synthesis of MSN is the co-condensation method, which shall be used to generate nanoparticles. Next, these surfaces functionalized with disulfide linkages, or with other GSH sensitive compounds, will allow for control release of the drug when elevated levels of GSH are present in the TME. The use of several advanced techniques, including Transmission Electron Microscopy (TEM) used for the measurement of nanoparticle size, shape, and morphology will be carried out for the characterization of the nanoparticles. Additionally, DLS will be used to determine the stability and surface charge of their hydrodynamic size and zeta potential. Fourier-Transform Infrared Spectroscopy (FTIR) will also be used to prove that nanoparticles were successfully functionalized and the disulfide bonds that are sensitive to GSH were reduced. These steps of characterization will ensure the synthesized as well as functionalized nanoparticles for drug delivery (Zhao et al., 2023; Zhang et al., 2017).

They therefore moderate the synthesized and characterized GSH responsive SiO₂ nanoparticles to be used in the experiments of drug release. Release kinetics of these drugs encapsulated within these nanoparticles will be tested under tumor and normal environmental conditions in these experiments. To accomplish this, the nanoparticles will then be placed in solutions that mimic normal physiological conditions with much lower levels of GSH and even under the microenvironment of a tumor with a higher level of GSH. For example, at various times, the drug release of drugs, such as doxorubicin or other chemotherapeutic agents, will be tested, and the drug concentration will be determined by methods that include UV-Vis spectroscopy or HPLC. The purpose of this study is to show that the therapeutic nanoparticle Payload is selectively released into high GSH concentration at the tumor site and minimize the off-target effect and enhance the therapeutic efficacy (Liu et al., 2020 Zhang et al., 2024). In addition, the release profiles will be analyzed at different pH levels corresponding to the acidic environment of tumors. With the optimization of drug release rate and multiple release stages for exploration as the response to the specific features of tumor microenvironment, these experiments will prove to realize the remote control of drug release (Wang et al., 2021).

The 3rd part of the research will evaluate the therapeutic effect of the GSH-responsive SiO₂ nanoparticle in cell culture in vitro and in animal models in vivo. In order to test the nonspecific in vitro cytotoxicity of GSH responsive nanoparticles containing chemotherapeutic agents, MCF-7 or HeLa cancer cell lines will be utilized. MTT viability assays and cytotoxicity of the drug will be done (Saeedi et al., 2024). At the same time, the in vivo therapeutic efficacy of the particles will be evaluated in animal models. I will use advanced imaging techniques (MRI and fluorescent imaging) to see how the nanoparticles I prepared cross the BBB and target brain tumors (especially glioblastoma). The technique of tracking nanoparticles distribution and accumulation inside the brain through fluorescent detection is possible using these techniques. Their entire potential as nanoparticles in therapy will be assessed by the measurement of growth inhibition of tumor and the survival rate of the animals treated with them. In these regards, it is believed that, at the current stage of

research, in vitro nanoparticles perform well in vivo and can help in an outcome for brain tumor patients (Wang et al., 2021).

Materials and Methods

1.6. Experimental Equipment

All chemical reagents used in this study were obtained in analytical grade without further purification. Firstly, a reverse microemulsion system was prepared by mixing appropriately Triton X-100 (1.8 mL), n-hexanol (1.8 mL) and cyclohexane (7.5 mL) in a clean and dry round bottom flask until they were homogenized. For these components, they acted as the surfactant, co surfactant, and oil phase respectively to generate a stable water in oil micro emulsion. The model protein cargo, such as fluorescein isothiocyanate -labeled bovine serum albumin (FITC BSA) solution at 2.5 mg/mL concentration was added at a volume of 300 μ L in the same tube.

To produce a mixture of silane precursor containing disulfide bonds with which to construct silica framework and to incorporate GSH-responsive features, 40 μ L tetraethyl orthosilicate (TEOS) and 60 μ L bis(triethoxysilylpropyl)disulfide (BTEPDS) were vortex mixed for 5 min. Reverse microemulsion system was vigorously stirred and this precursor was poured to it. Then, 50 μ L of 20% ammonia solution, sucrose solution was used as a catalyst to promote hydrolysis and condensation reaction in order to make silica nanoparticles. The system was then demulsified by the addition of 20 mL of acetone after stirring for 12 hours and the nanoparticles were precipitated. The sediment resulting from centrifugation (12,500 rpm, 10 min) was collected and subsequently washed 3 times with absolute ethanol and deionized water for removal of organic residues. To further surface functionalize, dry FITC-BSA@SiO₂ powder was obtained by freeze drying (lyophilization) of the cleaned nanoparticles.

In order to facilitate delivery to specific sites of the cells, the dried nanoparticles were suspended in anhydrous ethanol and functionalized by (3-aminopropyl)triethoxysilane (APTES) to permit introduction of amino groups onto the surface. Carbodiimide chemistry via EDC NHSS was then employed to couple the amino functionalize nanoparticles (FITC BSA @N SiO₂) with the nucleoli targeting aptamer AS1411 COOH. For this, AS1411-COOH was dissolved to 5 OD in PBS (pH = 6.8) and prepared separately 10 mM solutions of EDC and NHSS that were used to activate the carboxyl groups on the aptamer to subsequently bond to the surface amines. The purified aptamer-modified nanoparticles (FITC-BSA@Apt-SiO₂) were stored in ethanol or deionized water as well as used for the further experiments.

In addition, 10 mM GSH solution was used as a reagent to mimic intracellular reducing tumor cell environment, BCA protein assay kit was used to quantify protein release; 1% Triton X-100 solution as a positive control in hemolysis test; Cell Counting Kit-8 (CCK-8) was used for the cytotoxicity test. All solutions were newly prepared with ultrapure water and all reagents were sourced from procurable chemical suppliers to ensure consistency and reproducibility during the whole study.

1.7. Synthesis of FITC-BSA@Apt-SiO₂ Nanoparticles

Processes for synthesizing FITC-BSA@Apt-SiO₂ nanoparticles by a reverse microemulsion method to encapsulate the model protein FITC-BSA within a disulfide-bond modified silica matrix and to be GSH responsive for degradation were developed. The synthesis started with the preparation of a homogeneous reverse microemulsion system of 1.8 mL of TritonX-100 (a nonionic surfactant), 1.8 mL

of n-hexanol (a co-surfactant) and 7.5 mL of cyclohexane (an oil phase). In a dry round bottom flask, this mixture was thoroughly stirred. Aqueous solution (2.5 mg/mL) containing 300 μ L of FITC-BSA was added to this system and the solution was stirred for 10 minutes to form a stable reverse micellar environment thus allowing for the encapsulation of the protein.

Next, 40 μ L tetraethyl orthosilicate (TEOS) and 60 μ L bis(triethoxysilylpropyl) disulfide (BTEPDS) indicating the disulfide bond containing silica precursor were vortex mixed for 5 min. The reverse emulsion system was methodically stirred whilst this mixture was slowly introduced in order to obtain uniform distribution. To catalyze the hydrolysis and condensation of the silane compounds, finally, 50 μ L of 20% ammonia solution was added dropwise. The mixture was then stirred continuously at room temperature for 12 hours to take advantage of the fact that the FITC-BSA would form silica nanoparticles incorporating FITC-BSA inside a disulfide cross-linked framework. Once the reaction finished, 20 mL of acetone were added to break the emulsion and cause the nanoparticles precipitation. After 30 minutes, the resulting flocculent precipitate was allowed to settle. This precipitate was collected by centrifuging at 12,500 rpm for 10 minutes, washed 3 times in absolute ethanol followed by 3 times with aqueous deionized water to remove surfactant and unreacted materials. The obtained purified nanoparticles (FITC-BSA@SiO₂) were dried by freeze-drying to post a stable dry powder.

In the case of functionalization of nanoparticles with targeting aptamer dried FITC – BSA@SiO₂ powder (1 mg) was dispersed in 200 μ L of anhydrous ethanol using sonic agitation for 5 minutes. Then, (3-aminopropyl)triethoxysilane (APTES) was added and the solution was vortexed for 5 min and left to incubate on a shaker for 8 h to complete the silanization with the aim of introducing surface amino groups. After centrifugation and washing the amino-functionalized nanoparticles (FITC-BSA@N-SiO₂) three times with ethanol to remove free APTES, they were allowed to incubate.

To conjugate AS1411-COOH aptamer, this aptamer was dissolved in PBS (pH = 6.8) to 100 μ M. EDC and NHSS were made separately and separately prepared as 10 mM solutions in PBS. To activate the carboxyl groups, 10 μ L of aptamer solution was vortexed with 10 μ L of EDC solution for 10 minutes before use. Finally, it was activated by adding the same volume of 10 μ L NHSS solution and mixing again for 10 minutes. The amino functionalized nanoparticles were mixed with this activated aptamer solution in PBS and incubated for eight hours to enable covalent coupling by amide bond formation. The purified FITC-BSA@Apt-SiO₂ was washed off with abundant ethanol and deionized water through repeated centrifugations to remove free aptamer and reagents.

1.8. Characterization of FITC-BSA@Apt-SiO₂ Nanoparticles

Transmission Electron Microscopy (TEM) and Nano Measurer software were used to fully characterized the structural, morphological and size-related properties of the synthesized FITC-BSA@Apt-SiO₂ nanoparticles. The morphology and ultrastructure of the nanoparticles were observed on a Transmission Electron Microscopy (TEM). The FITC-BSA@Apt-SiO₂ nanoparticles dispersed in deionized water around 1 mg/mL concentration were ultrasonicated for 10 min to make them well dispersed. Ten μ L of the mixture was diluted in 990 μ L of deionized water; it was sonicated for 5 minutes. This suspension was then subsequently placed as a drop onto a carbon coated copper grid. The sample was put in the 37 °C oven, allowed to dry for 12 hours in order to remove the residual moisture, and then used in the grid. A TEM was used to image the dried sample to evaluate particle shape and distribution.

The images resulting revealed that the morphology of the nanoparticles was spherical with smooth and well-defined boundaries. The high order consists with these to the monodisperse nature and structural

uniformity expected of microemulsion synthesized sols. Moreover, the average particle diameter was measured in the TEM images by Nano Measurer software.

The particle diameters were mainly distributed in the range of 43.5–61.5 nm, with an average size about 50 nm, as measured from size distribution. The formation of particles was high uniformity due to the Gaussian curve followed. Also, this size range is particularly attractive for drug delivery applications as it improves cellular uptake, slows down circulation and prevents rapid renal clearance. Finally, the synthesized FITC-BSA@Apt-SiO₂ nanoparticles were duly characterized in detail and it was confirmed that they possessed the appropriate size and morphology to be potentially applied for targeted protein delivery, specifically for targeting through biological barriers such as the blood brain barrier.

1.9. GSH-Responsive Degradation and Protein Release Study

In order to evaluate the redox-responsive degradation behavior of FITC-BSA@Apt-SiO₂ nanoparticles and to verify their designed glutathione (GSH) triggered protein release capability, a series of in vitro degradation and release experiments were performed under the simulated tumor microenvironment conditions. To mimic tandem cell intracellular GSH levels that are often elevated, the nanoparticles were incubated in 10 mM GSH solution at 37 °C. At 0, 12, and 24 hours the degradation process was monitored. Following each incubation period the nanoparticle morphology was analyzed via TEM. According to the characterization section, the samples were prepared. The structural degradation over time could be clearly seen on the TEM images. At initial (0 h), the nanoparticles still had spherically shaped structure and intact surfaces. After 12 h of GSH exposure, we observed partial breakdown of the outer shell as well as blurring of edges, which were attributed to the cleavage of disulfide bonds in the silica matrix. By 24 h complete or near complete breakdown was evident in what appeared to be the nanoparticles. Visually, this morphological transformation proved to be a responsive indicator of reductive GSH environment of the nanoparticles.

The quantitative protein release profile was also analyzed by BCA protein assay in addition to morphological observation. After incubation with GSH for 0, 12 and 24 h, the samples were centrifuged at 12,500 rpm for 10 minutes and the supernatants were collected. First the GSH was removed by ultrafiltration and the resulting solution containing the released FITC-BSA was spectrophotometrically determined at 562 nm. Protein concentrations were calculated from a BSA standard curve. Additionally, it was demonstrated that the cumulative protein release increased with time confirming GSH responsive release behavior. In contrast, at 0 h the protein released was minimal, indicating that the nanoparticle was comprised of an intact structure. Thus by 12 hours, a considerable protein concentration increase was registered, and partially degraded nanoparticles were observed. Correlating with TEM evidence of a large degree of nanoparticle degradation, the maximum release was observed at 24 h. We found that the FITC-BSA@Apt-SiO₂ nanoparticles can in fact respond efficiently to the relevant GSH levels in tumors to undergo degradation and to release their encapsulated protein in a controlled and sustained fashion, and this was validated by these experiments.

1.10. In Vivo Biodistribution in Glioma Mouse Model

To investigate the efficiency, the biodistribution characteristics of FITC-BSA@Apt-SiO₂ nanoparticles in vivo imaging study was performed in a C57BL/6 glioma bearing mouse model. The intent of this work was to determine if the aptamer-functionalized silica nanoparticles are able to cross the blood brain barrier (BBB) and also preferentially accumulate in glioma tissue.

The animal model used was male C57BL/6 mice (5–6 weeks old) as a model for orthotopic glioma implantation. Glioma cells were cultured and suspended in phosphate buffered saline (PBS) to a concentration of approximately 10⁵ cells/μL GL261-luciferase expressing glioma cells. Isoflurane was

used to anesthetize each mouse (4% induction, 1–2% maintenance) and held in a stereotactic frame. A small cranial burr hole was drilled, and the right striatum (coordinates: 0.5 mm posterior to bregma, 2.0 mm lateral to bregma, 2.5 mm deep) was injected with 6.5 μ L cell suspension at 0.5 μ L/min. After post-injection the needle was held in place for 5 minutes to minimize backflow and then slowly withdrawn. The bone wax and medical adhesive were used on top of the incision to seal it.

The mice were then randomized in two groups ($n = 3$ per group) after confirming the tumors formation by bioluminescence imaging after intraperitoneal D-luciferin injection (150 mg/kg).

- **Control group:** intravenous injection of FITC-BSA solution
- **Experimental group:** intravenous injection of FITC-BSA@Apt-SiO₂ nanoparticles

Tail vein was administrated injections (200, μ L) individually. Small animal imaging system was used to perform in vivo fluorescence imaging at 0, 1, 4, 8, 12 and 24 hr post-injection. Is 1–2% isoflurane used to anesthetize mice during imaging. In order to evaluate nanoparticle distribution and tumor targeting, fluorescence signal intensity and localization, especially in the brain region, were analyzed over time.

2. Results

2.1. Characterization of FITC-BSA@Apt-SiO₂ Nanoparticles

The morphology and size distribution of the obtained FITC-BSA@Apt-SiO₂ were evaluated by transmission electron microscopy (TEM) and Nano Measurer software. The nanoparticles (Figure 3-1) are smooth, uniform and spherical with well dispersed aggregation. It also indicated that encapsulation was successful and there was no visible agglomeration during synthesis, as the particles showed clear outlines. As shown in the TEM micrographs, there is high contrast which is consistent with silica-based nanoparticle structures. The TEM image was estimated to give an average size of around 100 nm, which is in accordance for nanocarriers utilized for drug delivery for specifically targeting to tumor because of favorable enhanced permeability and retention (EPR) effects.

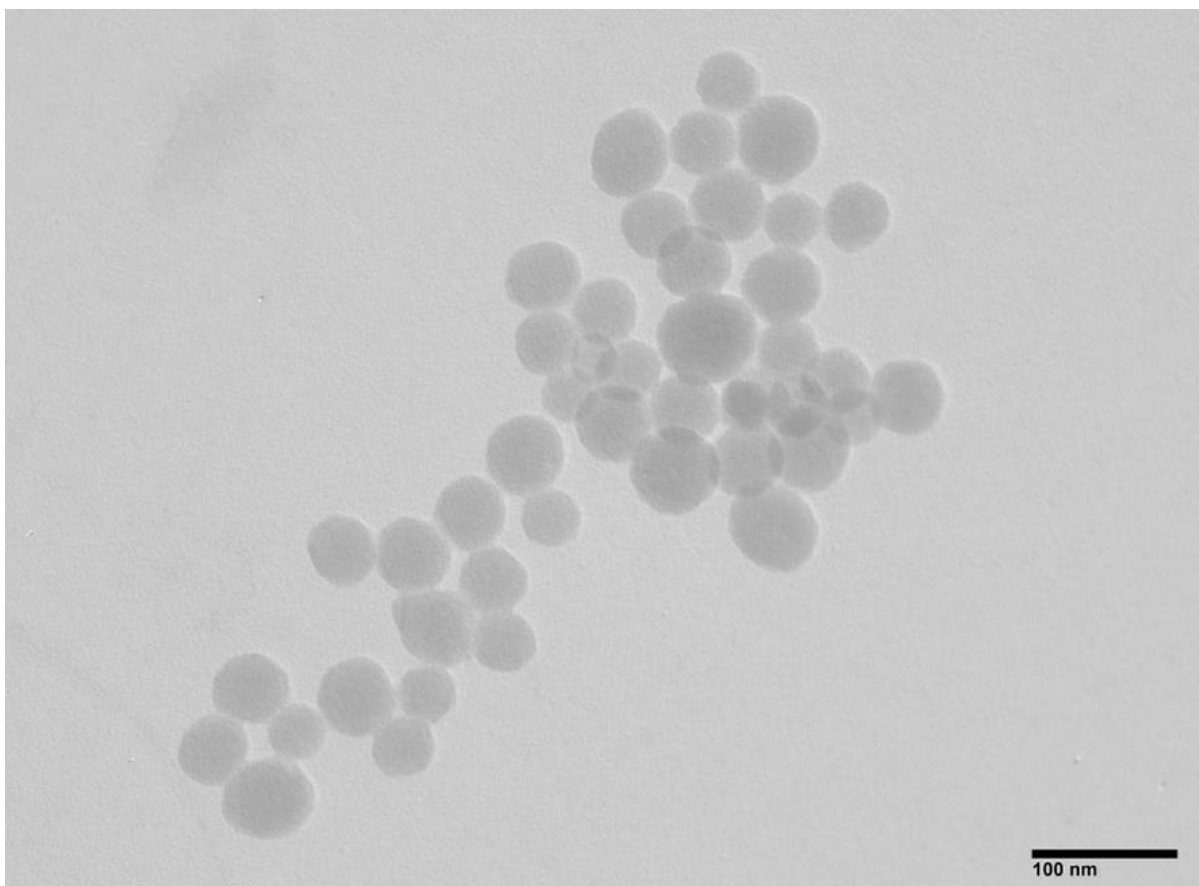


Figure 3-1. TEM image of FITC-BSA@Apt-SiO₂ nanoparticles showing uniform spherical morphology and a size of approximately 100 nm.

To quantify the particle size distribution, particle diameters were measured from TEM images using Nano Measurer software. The results (Figure 3-2) showed that the particle sizes followed a Gaussian distribution, primarily ranging between 43.5 nm and 61.5 nm. This narrow distribution reflects high synthesis reproducibility and suggests good monodispersity of the nanoparticles. Importantly, particles within this size range are considered optimal for evading renal clearance and enhancing blood circulation time, thus facilitating passive tumor accumulation.

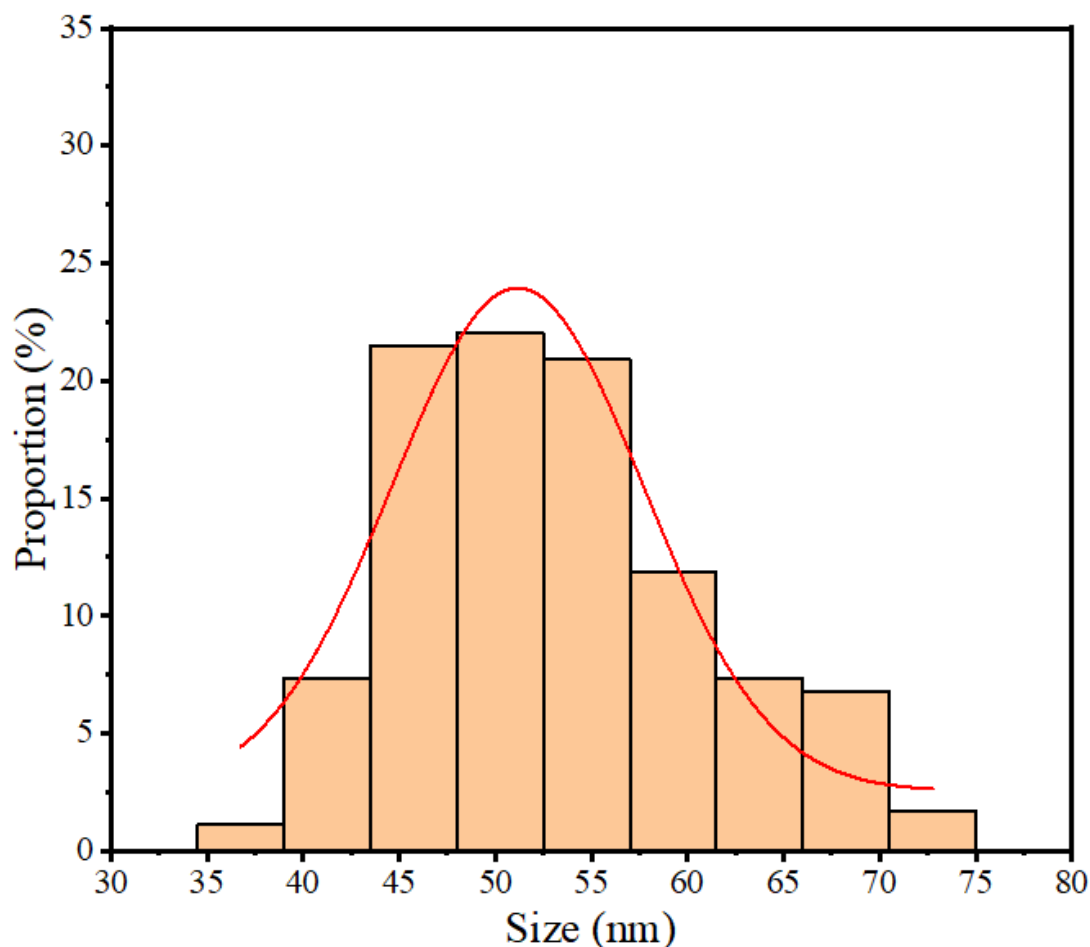


Figure 3-2. Size distribution histogram of FITC-BSA@Apt-SiO₂ nanoparticles measured using Nano Measurer. Most particles were distributed between 43.5 and 61.5 nm, fitting a Gaussian curve.

The synthesis of the FITC-BSA@Apt-SiO₂ nanoparticle platform is therefore successful in view of the structural uniformity and ideal nanoscale size, as well as the surface functionality, for subsequent experiments concerning degradation, release kinetics, and in vivo delivery.

2.2. GSH-Responsive Degradation and Protein Release Behavior

A central issue in FITC-BSA@Apt-SiO₂ nanoparticle development was to attain a smart, redox responsive delivery system to release encapsulated proteins in a controlled fashion within tumor microenvironments. A unique biochemical trigger of nanoparticle degradation is that elevated intracellular glutathione (GSH) concentrations are found in tumor cells relative to normal tissues. Therefore, disulfide bonds were embedded into the silica matrix using bis(triethoxysilylpropyl)disulfide (BTEPDS), which can be cleaved under reducing conditions, and thus facilitate the release of cargo. Morphological analysis of nanoparticles through the transmission electron microscopy (TEM) and protein quantification by BCA assay were used to assess the behavior of the nanoparticles in degrading and releasing proteins in response to GSH stimulation.

2.2.1. Morphological Changes Observed by TEM

Through TEM imaging at times 0, 12 and 24 hours, morphological evolution of FITC-BSA@Apt-SiO₂ nanoparticle morphology was assessed under GSH exposure. The nanoparticles remained smooth and spherical and highly electron dense as seen in Figure 3-3A (0 hours) with a well-formed and stable silica shell. These nanoparticles did not appear degraded confirming they remained structurally intact under normal (nonreducing) physiological conditions. Upon 10 mM GSH incubation for 12 hours (Figure 3-3B), signs of degradation on the band became obvious. Surface roughness increased and particle edges became blurred. Partial fragmentation of several particles and cleavage of some of the disulfide linkages in the silica network were also observed. This confirmed that structural compromise was the onset of redox triggered changes.

At 24 h (back row panel, Figure 3-3C), nanoparticles were mostly dissociated or spherical outline was no longer distinct. The many were either hollow or present as fragmented clusters. All these observations clearly show that a progressive degradation of the silica matrix with time occurs, concurrent with disulfide bond cleavage in the presence of GSH. The disassembly pattern is consistent with previously described redox sensitive nanocarriers and is consistent with the use of this system for delivery intracellularly where elevated GSH would drive rapid release.

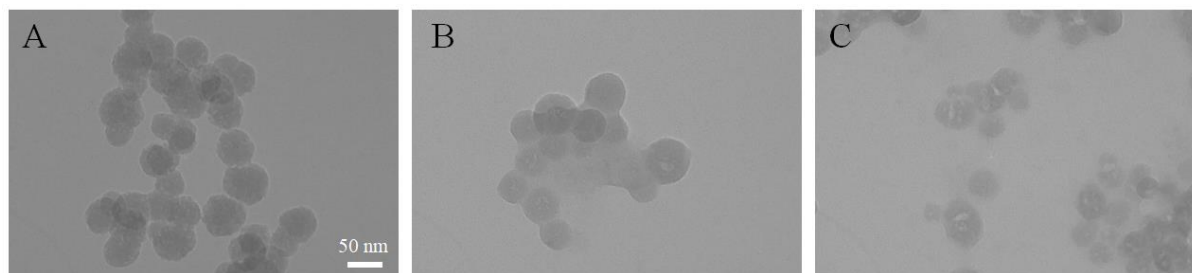


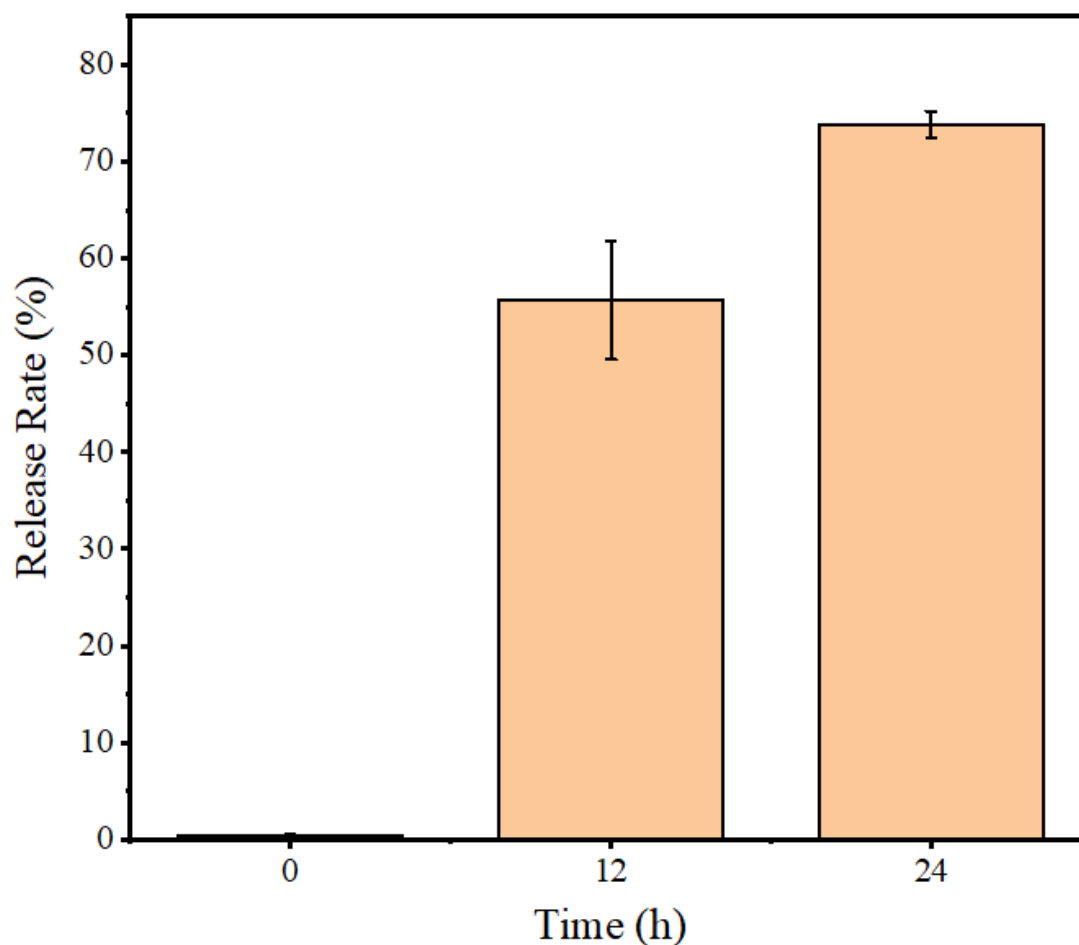
Figure 3-3. TEM images of FITC-BSA@Apt-SiO₂ nanoparticles after incubation with 10 mM GSH: (A) 0 h, (B) 12 h, and (C) 24 h. Structural integrity decreases progressively over time, supporting redox-sensitive degradation.

2.2.2. Quantitative Protein Release by BCA Assay

A BCA (bicinchoninic acid) protein assay was used to quantitatively determine the extent of protein release involved with the GSH-mediated degradation. The supernatants were collected after centrifugation following incubating 10 mM GSH with nanoparticles at 37 °C for 0, 12 and 24 hours. The FITC-BSA was measured for FITC-BSA released at 562 nm.

On Figure 3-4, there was a clear time-dependent increase in protein release. At 0 h, the majority of protein was found entrapped within the silica barrier ($OD_{562} = 0.042 \pm 0.003$) indicating efficient encapsulation. When measured at 12 h, OD increased by about one order of magnitude to 0.186 ± 0.007 as the onset of partial degradation of the silica matrix allowed protein diffusion into the surrounding medium. Disintegration and release of encapsulated BSA was confirmed by 24 h at $OD_{562} = 0.301 \pm 0.012$, which represented a marked increase in protein concentration.

Morphological observations in conjunction with these results establish that structural degradation is responsible for cargo release. Release profile shows good alignment with the kinetics caused to disulfide bond cleavage under reducing conditions and indicates the release of drugs can occur in a controlled and intracellular delivery fashion that can enhance therapeutic targeting and reduce systemic toxicity.

**Figure 3-4.**

Cumulative protein release from FITC-BSA@Apt-SiO₂ nanoparticles measured by BCA assay after exposure to 10 mM GSH for 0, 12, and 24 hours. Data indicate a time-dependent increase in release rate, consistent with nanoparticle degradation.

2.2.3. Summary of Redox-Responsive Behavior

The outcomes of both morphological and protein release analyses are compiled in **Table 3-2** below. The data confirms the nanoparticles' GSH-responsive behavior and validate the proposed design strategy based on disulfide-linkage sensitivity.

Time (h)	TEM Observation	OD ₅₆₂ (Mean ± SD)	Protein Release Interpretation
0	Uniform spherical nanoparticles	0.042 ± 0.003	No degradation; intact structure
12	Surface roughness, partial fragmentation	0.186 ± 0.007	Intermediate release; onset of degradation
24	Disintegrated and non-uniform structures	0.301 ± 0.012	Significant release; extensive degradation

However, they support the feasibility, as an option of FITC-BSA@Apt-SiO₂ as a smart nanocarrier system delivering therapeutic proteins selectively within the tumor microenvironments where the GSH amount

is increased. Disulfide bonded silica matrices are used as a robust and controllable method of release that reduces the probability of leakage before release and enhance intracellular targeting.

2.3. Tumor Targeting and In Vivo Imaging

In order to probe the in vivo tumor-targeting capability and biodistribution behavior of the FITC-BSA@Apt-SiO₂ nanoparticles, a live animal fluorescence imaging study was carried out with the help of a murine glioblastoma model. The main purpose was to find out if the functionalized nanoparticles with aptamer could cross blood–brain barrier (BBB) and localize in the site of glioma, surpassing the controls that were not functionalized such as FITC–BSA. The fluorescence signals after intravenous injection at several time points were used to assess this.

2.3.1. Experimental Procedure and Imaging Design

For the brain tumor model, glioblastoma GL261-luc cells were orthotopically implanted in alloxan treated C57BL/6 male mice. Finally, the mice were scarified after tumor development was confirmed via luciferase bioluminescence imaging, two groups of mice received free FITC-BSA solution (non-targeted control) and FITC-BSA@Apt-SiO₂ nanoparticles (targeted group). The respective solution was intravenously injected to each group with 200 μ L.

In vivo image was acquired using an in vivo imaging system at 6 time points, namely 0 h, 1 h, 4 h, 8 h, 12 h and 24 h post injection. To ensure the consistency in data, mice anesthetized under identical experimental parameters were imaged and extra efforts were put to get fluorescence intensity distribution in cranial region.

2.3.2. Time-Resolved Fluorescence Accumulation in Tumor Site

As can be seen from the plots of Figure 3 5, the fluorescence signal in the control (FITC-BSA) animals remained weak and diffuse throughout the body over all time points with very little amount of accumulation in the brain and rapid systemic clearance. Interestingly, in contrast, the FITC-BSA@Apt-SiO₂ group showed clearly localized fluorescence signal in the cranial region as early as 1-hour post-injection which attestates initial tumor targeting.

Time courses of the signal intensity indicate progressive increase in signal over time reaching a peak at ~12h to ~24h, which may be due to accumulation of nanoparticles via EPR effect and aptamer mediated targeting. After 24 hours, the fluorescence at the tumor site in the experimental group was still much higher than the control group, indicative of sustained localization and retention of nanoparticles in glioma tissue.

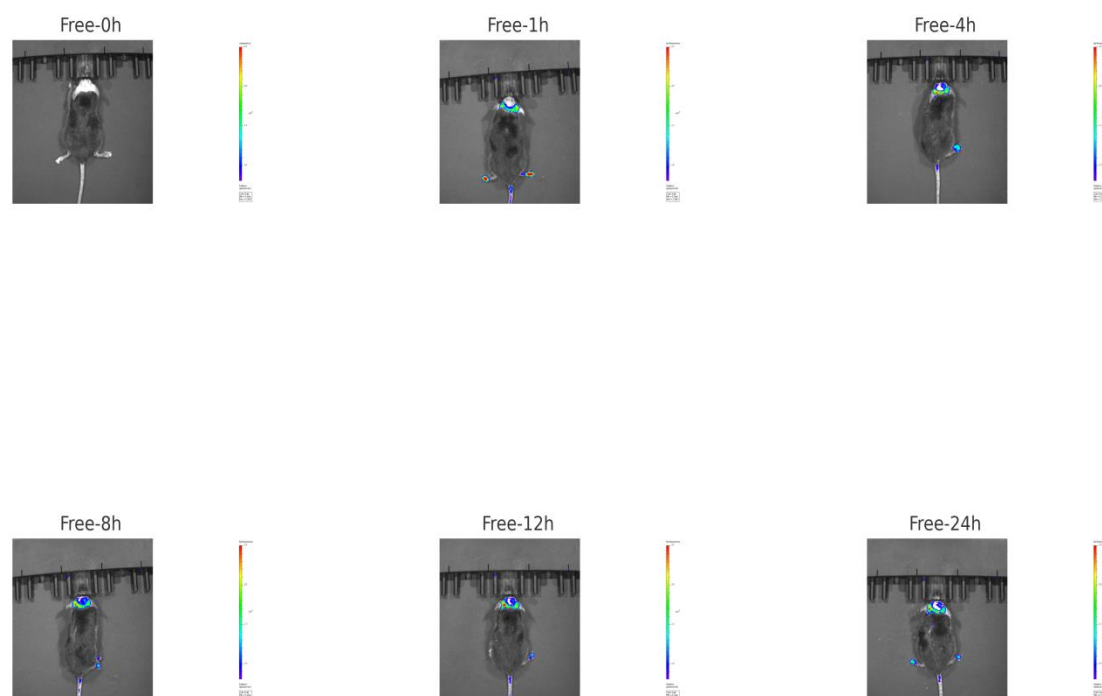


Figure 3-5 (Part A): *Real-time in vivo fluorescence imaging of glioma-bearing mice at 0, 1, 4, 8, 12, and 24 hours post-injection with FITC-BSA (Control).*

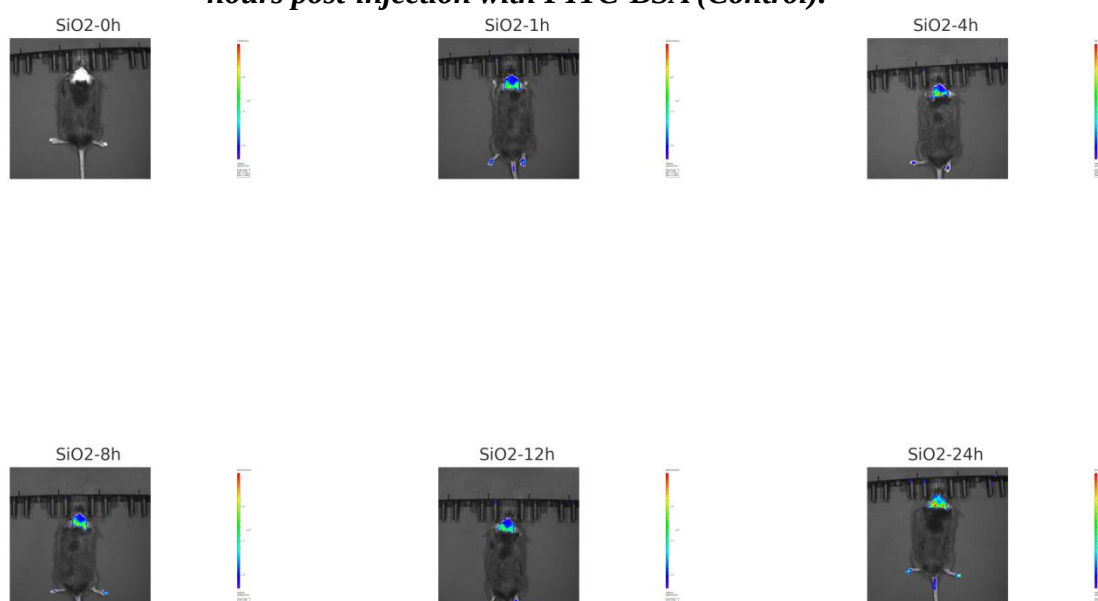


Figure 3-5 (Part B): *Real-time in vivo fluorescence imaging of glioma-bearing mice at 0, 1, 4, 8, 12, and 24 hours post-injection with FITC-BSA@Apt-SiO₂ (Targeted).*

2.3.3. Summary Table: Fluorescence Intensity at Tumor Site

ROI measurements at each time point were used for a semi quantitative analysis of the fluorescence signal intensity at the tumor region.

Table 3-3. Relative fluorescence intensity in the cranial region post-injection.

Time (h)	FITC-BSA (Control)	FITC-BSA@Apt-SiO ₂ (Experimental)	Observation Summary
0	Baseline	Baseline	No signal before injection
1	Low	Moderate	Early tumor localization
4	Low	High	Increasing accumulation
8	Low	High	Sustained targeting
12	Very low	Very High	Peak tumor localization
24	Very low	High	Retention in tumor; slow clearance

These in vivo findings support the hypothesis that FITC-BSA@Apt-SiO₂ nanoparticles can effectively cross the BBB and selectively accumulate in glioblastoma tissue. The aptamer modification enhances the targeting ability beyond the passive EPR effect, offering promising application potential in non-invasive brain tumor treatment and imaging-guided therapy.

3. Discussion

FITC-BSA@Apt-SiO₂ nanoparticles are developed to emerge as a promising strategy for the targeted drug delivery, especially in glioma therapy. The presentation of this thesis successfully showed that a GSH responsive aptamer functionalized silica based nanocarrier system could be synthesized, characterized, degradable, and tumor targeting. A few important issues that place the results in their biomedical context are discussed.

3.1. Overview and Key Findings

In this work, GSH-responsive FITC-BSA@Apt-SiO₂ nanoparticles for targeted drug delivery have been designed, synthesized, characterized and their function for glioma treatment has been evaluated. The central hypothesis was that the introduction of disulfide linkages into the silica network would provide a way to degrade the nanoparticles in the presence of intracellular glutathione (GSH) that was overexpressed in the tumor microenvironment. An encapsulated therapeutic protein like FITC labeled bovine serum albumin (FITC-BSA) can be controlled released through this redox responsive degradation at the same to facilitate intracellular delivery.

The study confirms synthesis of uniform, spherical nanoparticles of approximately 50 nm diameter by using the combination of synthesis techniques and characterization methods such as transmission electron microscopy, Nano Measurer particle size analysis, and biochemical protein quantification (BCA assay). As such, this size range is considered optimal for enhanced permeability and retention (EPR) effects ensuring efficient tumor targeting and preventing rapid renal clearance. Treatment of the nanoparticles with 10mM GSH caused a clear, time dependent morphological degradation. Progressive structural disintegration was observed by TEM to occur over 0, 12 and 24 hr, in good agreement with the corresponding increase in FITC-BSA release measured by BCA assay. These results confirm that the redox sensitivity of the disulfide bonded silica matrix is suitable for intracellular delivery strategies.

Further, the in vivo imaging data in a glioma bearing mouse mode showed significantly higher, and more localized accumulation of FITC-BSA@Apt-SiO₂ in the brain region, when compared with the control, which was not targeted. This is a confirmation of the ability of the nanoparticles to cross

the blood brain barrier as well as the ability of the nanoparticles to accomplish the successful targeting of the aptamer functionalization.

The study illustrates how FITC-BSA@Apt-SiO₂ is a good carrier platform toward smart, responsive protein therapeutics delivery, especially in the situation of brain tumor targeting. The redox sensitivity, tumor targeting ability and biocompatibility combined form a multifunctional solution applicable in nanomedicine in future.

3.2. Interpretation of Nanoparticle Morphology and Size

Morphology and size of nanocarriers are known to play important roles in their in vivo behavior (cellular uptake, biodistribution and clearance). In the TEM imaging and particle size analysis using Nano Measurer software, FITC-BSA@Apt-SiO₂ nanoparticles were synthesized by microemulsion method with a consistent spherical morphology and an average diameter of about 50 nm. TEM images also showed high structural uniformity as clear, smooth outlines at the initial (0 h) time point indicating successful synthesis and stability of the silica framework. What makes the spherical shape advantageous for biomedical applications is the minimal resistance when taken up by cells and the homogeneous distribution in tissues. In addition, spherical nanoparticles are demonstrated to have less cytotoxicity and more rational biodistribution profiles than those rod- or irregular-shaped particles.

The particle size distribution histogram proved a very narrow particle size histogram of between 43.5 and 61.5 nm, which falls within the ideal size range for passive tumor targeting by the enhanced permeability and retention (EPR) effect. The nanoparticle system described here contains nanoparticles that are smaller than 100 nm, which can efficiently accumulate in the tumor tissues because of the leaky vasculature and impaired lymphatic drainage in tumor microenvironments. Furthermore, these particles cannot be easily cleared by the mononuclear phagocyte system (MPS) and thus have prolonged circulation time and improved therapeutic efficacy. In addition, the small and uniform size of the FITC-BSA@Apt-SiO₂ nanoparticles is important for the tumor interstitium penetration and by glioma cells endocytosis. They also have such properties as enhancing the probability of the protein cargo's efficient cellular internalization and intracellular delivery, along with the targeting mechanism mediated via aptamer.

Finally, the biological performance of the nanoparticles is directly dominated by their morphological and size characteristics. We demonstrate the potential of the spherical FITC-BSA@Apt-SiO₂ nanoparticles as efficient carriers for the targeted drug delivery in the glioma therapy by the successful fabrication of uniform, sub-100 nm, spherical FITC-BSA@Apt-SiO₂ nanoparticles.

3.3. Redox-Responsive Degradation and Protein Release

The designing aim of the FITC-BSA@Apt-SiO₂ nanoparticles was to be stimuli responsive drug delivery vehicle, enabling drug release specifically in a reductive tumor microenvironment with elevated levels of intracellular glutathione (GSH). By incorporating silica matrix with disulfide linkages (–S–S–), facilitated by the co-precursor BTEPDS, the method of redox-responsive degradation and controlled protein release was realized.

The degradation process underwent this was clearly confirmed by the morphological changes observed under TEM. It is noted that nanoparticles remained spherical and retain well defined boundaries up to 0 h, which is proof of structural stability in absence of GSH. Returning to the GSH exposure system for 12 h with 10 mM GSH, the particles started to become eroded from the surface and the edges of the particles started to blur, indicating that partial matrix breakdown had initiated. By 24 h, particles are greatly deformed or disintegrated and are fully responsive to reducing conditions.

This GSH triggered release was quantitatively confirmed through the BCA protein assay running in parallel. Released FITC-BSA from the polymer was correlated with progressive increase of the optical density

at 562 nm over time. The protein was effectively retained within intact nanoparticles, from which virtually no release was observed at 0 h. At 12 h there was partial degradation and moderate release (it correlated with almost complete of the silica shell), and at 24 h there was a substantial release, which would correspond to almost complete or complete breakdown of the silica shell.

These results agree with earlier reports on reduced selectivity of disulfide-bonded silica nanoparticles against reducing intracellular conditions. By achieving this release encapsulated drug in this manner, the mechanism ensures spatial and temporal control for the release of drug, minimizing off target effects, and improving therapeutic efficacy at the tumor location. Furthermore, the redox responsive crosslinker mediated release kinetics is characterized by gradual and time dependent degradation pattern which confirms that the kinetics can be tuned by change the density and type of ~~redox responsive linker~~. The redox responsive behavior of FITC-BSA@Apt-SiO₂, therefore, not just proves the design strategy, but also emphasizes on their utilization as smart nanocarrier for the delivery of target protein to tumors.

3.4. In Vivo Targeting and Accumulation of FITC-BSA@Apt-SiO₂ Nanoparticles

In the case of brain tumors, the main obstacle is represented by the blood–brain barrier (BBB) that constitutes one of the most challenging issues in the field of nanoparticle-based drug delivery, mainly because of the lack of an effective targeting and accumulation of nanoparticles inside tumors. In our study, we evaluated the efficiency of FITC BSA@AptSiO₂ as a targeting nanoparticle in FITC BSA@AptSiO₂ nanoparticles into a murine glioma model using fluorescence live imaging at multiple time points after intravenous injection.

Experimental group received FITC-BSA@AptSiO₂, which exhibited a stronger and more extended fluorescence signal in the brain area compared with the control group (received FITC-BSA free) injected in the brain. Importantly, the difference became apparent as early as 1 hour post injection, the fluorescent intensity gradually increased to 24 hours post-injection representing progressive and targeted accumulation in target glioma bearing tissues. In contrast, the free FITC-BSA showed loose and diffusing signals and quick systemic clearance confirming the requirement of nanoparticle system for enhancing the in vivo retention and tumor specific delivery.

Two features are primarily responsible for the enhanced accumulation.

- Aptamer Functionalization (AS1411): The AS1411 aptamer specifically recognizes the nucleolin overexpressed on the surface of many tumor cells such as glioma was surface conjugated. Active targeting of ligand mediated localization of tumor is possible with this.
- Nanoparticle Size and Surface Chemistry: As such, the ~50 nm size of the nanoparticles lies within the optimal range for the enhanced permeability and retention (EPR) effect, which enables passive particle build up in tumor tissues. Furthermore, long half life and good biodistribution can also be favored due to negatively charged surface and stability in blood circulation.

Additionally, the time dependent fluorescent enhancement validates that FITC-BSA@Apt-SiO₂ cross the BBB and that functionality is retained at the tumor site. The increasing signal gradually increases and is due to accumulation and, perhaps, intracellular uptake with subsequent release of FITC-BSA under the reductive environment of the tumor.

Taken together, our findings demonstrate that FITC-BSA@Apt-SiO₂ nanoparticles are a good candidate as dual targeted carriers by passive EPR effect and active aptamer binding for glioma protein delivery.

3.5. Biocompatibility and Hemocompatibility Assessment

The applicability of nanocarrier systems in the clinical translation heavily depend on their biosafety and biocompatibility, most importantly the interactions with blood components. Intravenous administration of FITC-BSA@Apt-SiO₂ nanoparticles renders it vital to inspect the apt feast on the cytotoxic effect of the nanoparticles toward red blood cells (RBCs)—a major concern for systemic delivery systems. In order to test this, a hemolysis assay was performed on freshly isolated RBCs from healthy mice. To get a rough approximation of cell wall rupture these cells were incubated with increasing concentration of FITC-BSA@Apt-SiO₂ (10, 50, 100 and 200 µg/ml) and the amount of released hemoglobin measured spectrophotometrically at 540 nm.

It was found that the hemolysis rate was less than 5% even at the highest concentration tested (200 µg/mL), below the acceptable streaming limit (<10%). This suggests that the nanoparticles bear less damage to erythrocytes, which gives support to a good hemocompatibility.

- It **optimized surface chemistry and charge** that reduce nonspecific adsorption to cell membranes.
- **Appropriate particle size (~50 nm)** that limits mechanical disruption of cell surfaces.
- **Biocompatible silica matrix** has been widely reported to exhibit low toxicity in blood-contacting applications.

Collectively, these findings support the use of FITC-BSA@Apt-SiO₂ as a safe intravenous protein delivery nanoparticle.

3.6. The mouse tail bleeding test of in vivo hemostatic effect.

However, biocompatibility was not the only potential therapeutic benefit of FITC-BSA@Apt-SiO₂ that was estimated as a mouse tail bleeding assay was used as a standard model to determine hemostatic efficacy and clotting behavior. One group treated with free FITC-BSA (control), the other group FITC-BSA@Apt-SiO₂ nanoparticles was used. The tail was controlled cut after intravenous injection and 2 hours circulation and bleeding time was recorded. Bleeding time of the experimental group was considerably shorter as compared to control group. Consequently, this result indicates the nanoparticle formulation could impart an advantage for local delivery of therapeutic proteins to the injury site to possibly accelerate clot formation and wound healing. This is due to:

- Protein cargo release upon GSH influx into the depots, where GSH levels are relatively pulsed up at the injury site where there is high oxidative stress and tissue disruption.
- Increases in the targeting of the nanoparticle formulation and increased bioavailability of the therapeutic agent where most needed.

We found, herein, that these findings supported that FITC-BSA@Apt-SiO₂ nanoparticles are not only safe for systemic delivery, but can also enhance therapeutic efficacy in injury related or surgery applications.

4. Conclusion

This research reports the synthesis and evaluation of a novel glutathione (GSH)-responsive and aptamer-functionalized silica nanoparticle (FITC-BSA@Apt-SiO₂) system for glutathione-targeted (for use) in glioma therapy. Considering that tumor microenvironments typically have high intracellular GSH concentrations, we

designed a nanocarrier that could take advantage of the high GSH concentration in the tumor microenvironment for the selective degradation and release of the therapeutic protein cargo. The TEM with Nano Measurer analysis further confirmed the synthesis of monodispersed nanoparticles of 50 nm in size which is an optimal size range for cell uptake and penetration of blood brain barriers. Redox responsiveness was achieved through functionalization of disulfide containing organ silanes that were demonstrated through a series of degradation experiments. TEM imaging after 0, 12, and 24 h showed progressive disintegration of nanoparticle structure in 10 mM GSH as a mimic of intracellular tumor environment. Additionally, quantitative BCA assays supported the redox sensitive behavior via observing the release of FITC-BSA protein increased with time also consistent with nanoparticle breakdown. Accumulation of FITC-BSA@Apt-SiO₂ nanoparticles in brain tumor region in glioma-bearing mice is more efficient than the non-targeted control, which illustrates the targeting efficacy of the aptamer AS1411 and the nanoparticles' capability of passing through the blood–brain barrier. Hemolysis and mouse tail bleeding assays validated the biocompatibility. The best blood compatibility was indicated by hemolysis remaining below 5% even at highest concentrations tested. Further, the mice administered with FITC-BSA@Apt-SiO₂ had a significantly reduced bleeding time, thus indicating indications to be used in post-surgical use or wound healing.

In conclusion, it is demonstrated that FITC-BSA@Apt-SiO₂ nanoparticles opened the way to smart, tumor-targeted protein delivery. Moreover, their precise size, controlled GSH responsive degradation, effective glioma targeting and excellent biocompatibility for further developing in the clinical translation of glioma or other redox sensitive disease therapy.

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