Review Article

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Chitosan-based drug delivery systems for brain cancer therapy

Ryan Harris

Department of Materials Science and Engineering, University of Washington, Seattle, WA 98195, USA

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Abstract

Chitosan is a material known for its biocompatibility, biodegradability and ease of modification. The focus of this review is to illustrate the large variability in the uses of chitosan in the field of treating brain cancers such as Glioblastoma Multiforme (GBM). This paper will first introduce and discuss chitosan, its applicability to the medical field and the material properties that make it a viable solution for treating brain cancer tumors. Constraints such as the Blood Brain Barrier (BBB) and biocompatibility will be mentioned, and how chitosan is a viable solution for bypassing many limitations. Entrance points to the body will then be discussed, the different mucosal entrance points as well as oral and ocular drug delivery pathways, and how chitosan can be equipped and modified to fit the parameters of these pathways. Different drug delivery methods all use chitosan to improve their effectiveness in reaching and delivering the predetermined drug to the GBM or respective indicator.

Corresponding author: Ryan Harris (harrisrc@uw.edu)

1. Introduction

Cancer is a leading cause of death worldwide and techniques continue to be improved in order to combat its effects. A recent progression in the cancer therapy field is the use of chitosan (CS), a derivation of chitin. This paper will analyze the processing, performance, and alteration of properties of CS with respect to the treatment of brain cancer.

Chitin is the second most plentiful naturally occurring polymer. It is a primary component of exoskeletons in crustaceans thus its biocompatible applications are vast; it is commonly used for skincare products, tissue repair, and drug delivery. Chitosan is created via the deacetylation of Chitin and two main derivations occur-N,N,N-trimethyl-CS and N,O carboxymethyl-CS, both of which can be applied medically[1]. CS by nature is cathodic and hydrophilic which can be beneficial, though also a hinderance depending on the desired drug delivery method, location, and therapy used.

Brain cancer therapies often struggle to get across the blood brain barrier: a barrier comprised of blood vessels that tightly regulate the flow of ions to the brain[2]. Drug delivery methods using chitosan's property of biocompatibility, such as hydrogels, micelles, emulsions, lipid layers, and nano capsules all are able to effectively pass this barrier and deliver the therapy in a designed fashion. There are both benefits and issues with all methods listed, some of which include fast degradation, burst release, and more.

One of the most aggressive forms of brain cancer contains tumors commonly known as Glioblastoma Multiforme (GBM). Specific targeting efforts towards these tumors are often the ones researched[3]. Methods may include indicators such as pH, specific R group indicators and swelling[1, 4].

Chitosan-based drug delivery systems continue to be researched for the treatment of tumors. Utilizing many different indicators and treatment methods creates a wholistic approach towards GBM and cancer research.

2. Overview of Chitosan Properties

Chitosan (CS) is increasing in popularity within fields such as agriculture, cosmetics and drug delivery. Its malleability in

terms of physical properties continue to allow for innovation and increasing usability. CS has been found in mushrooms as well as a multitude of other organisms. This vast quantity makes chitosan a promising material for drug delivery due to its immediate availability and low cost [5].

Chitosan is made by deacetylation of Chitin, a process that occurs by refluxing Chitin in an NaOH solution for 2 hours, then rinsing with deionized distilled water until neutralized. Pure Chitosan is then extracted by drying the material for 24 hours [5]. Kata et al. analyzed CS derived from six different invertebrate species, considering their crystalline index, chitosan productivity levels, dry weights, and thermal stability. This research will be used as a starting point for determining the effectivity of different chitosan derivations. However, specific forms defined in the following section are best for use in the medical industry. It was determined that the dry weight of a select few species was higher than the others, thus deemed the most efficient form of chitin source in terms of obtaining the greatest desired yield of chitosan from chitin. Species with the highest dry weights include Agabus bipustulatus and Anax imperator, a beetle and a dragonfly respectively [5].

Kaya et al.'s research determined that chitin and chitosan may be classified as having a porous and microfibrillar structure, non-porous or microfibrillar structure, or only microfibrillar structure, each morphology benefitting different applications [5]. Specific to drug delivery, porosity is often closely analyzed as different pore sizes allow for drastically different drug diffusion rates [5]. Consequently, this constitutes the requirement of comprehending the porosity of the chitosan sample in use with respect to the desired method of delivery.

Chitosan based drug delivery relies on the modification of the material's physicochemical properties. At a molecular level, modification of the -NH2 and -OH groups are what allow for properties such as the mucoadhesion, stability, and solubility to be altered for specific treatment techniques. Common modification techniques include blending, copolymerization and curing techniques often used in combination with processing methods such as electrochemical radiation[4]. Physical alteration consists of the addition of one or more polymers to the chitosan microstructure where crosslinking occurs between the two polymers. This procedure adjusts the structure of the material, directly affecting the degradation properties. For example, increasing tensile strength by the addition of polymers such as PVA (poly vinyl alcohol), the rate of drug delivery is decreases over a given amount of time. This reduction is due to the reduction of water permeability through the membrane structure[4].

Chemical alteration is often used to adjust the mucoadhesion, stability, and permeability properties of chitosan based nanocarriers. The ability to adhere to a mucous membrane allows for the increased uptake of a drug in the host and can directly affect the drug effectivity. Chemical alteration often occurs by interacting with the chitosan by way of radiation, photochemical, chemical, plasma induced and enzymatic grafting methods[4]. For example, chemical alteration through chemically linking D,L lactic acid with the -NH2 groups on the chitosan material allows for a more pH sensitive chitosan drug delivery method[4]. It is important to note that these examples are often specific to one form of drug delivery and cannot be directly transferred to other drug delivery pathways.

3. Constraints (BBB & Tumor Analysis)

The blood brain barrier (BBB) remains one of the largest constraints for brain cancer therapy. The BBB allows for tighter regulation regarding "movement of molecules, ions, and cells between the blood and the CNS," making it difficult for large drug molecules to pass through[2]. This is where CS is brought into use, as its biocompatibility allows for the bypass of 'gatekeeper' endothelial cells within the BBB. Although still being actively researched, it has been experimentally verified that chitosan assists nanoparticles with passing the vascular central nervous system cells. CS's ability to cross this barrier is due to the material's extremely high biocompatibility levels[6]. This is due to the molecular construction of CS, as it is being comprised of only natural elements oxygen, hydroxides, nitrogen, and hydrogen, due to its origin of a primary building block for multiple organisms.

Glioblastoma, known as GBM, is one of the most aggressive types of tumorous cancer growths in the brain, as well as one of the most lethal. This makes it a critical target for drug delivery. Epidermal growth factor, also known as EGFR, was the first molecule to be linked to the oncogenesis in GBM. This means that it is a common target in targeted brain cancer therapy[7]. Processes specific to releasing toxins when directly connected to the target EGFR are still being actively researched. However, much promise was shown when the process was deemed safe during phase 1 of clinical trials. Pathways relating to the r-groups of this target can be exploited with the use of a chitosan based nanocarrier. Specific dipole-dipole bonds and hydrogen bonding that is necessary to attach can be easily achieved with the use of Chitosan. Westphal et al surmised that the redundancy of cellular pathways associated with EGFR and GBM cells was the main limiting constraint to toxic drug delivery methods, as only one pathway can be designed at a time.

Tumors and malignant growth often exhibit a low local pH value. This trait can be exploited for drug delivery. CS has been historically known to degrade at a much faster rate during exposure to a low pH value or low kpα value[8]. This allows for drugs loaded within a CS nanocarrier to be released at a much faster rate than when near low pH value tumors [8].

4. Entrance Points

Drug delivery requires a point of entry and depending on the type of nanocarrier these locations can be drastically different. Hydrogels in particular have a wide range of sizes and depending on the drug, can come out of the nanoscale and into the centimeter range[9]. Larger hydrogels are often injected directly to the desired location, which can be intrusive and disruptive.

A more preferable method for non-invasive drug delivery is through a mucous membrane such as pulmonary, oral, nasal,

or vaginal pathways. These routes all can be accessed without injection or surgery[4]. CS is found to be effective at both paracellular and transcellular transport via its physical and chemical properties. CS has a pKα of 6.5, which is similar to the mucous membranes and epithelial cells within the body ensuring strong biocompatibility, as well as likelihood of adhesion and delivery through the membranes and cells. Thiolated Chitosan, a special modified derivation of chitin, was found to have strong mucoadhesion properties due to special bonds and permeability that occurs[10]. Forming disulphide bonds directly with the glycoproteins on the mucosal regions allows thiolated chitosan to effectively permeate external boundaries of the body. [10]

Osuna et al. performed an experiment that established the idea of chitosan being an effective median between mucous membranes and nano-carriers.[10] Their research found higher CS concentrations allowed for the highest levels of adhesion in the ex vivo experiment with a higher nanocarrier concentration. With nanoparticles coated in varying levels of chitosan or thiolated chitosan, Osuna's group found increasing levels of chitosan directly increased the adhesion ability of the particle independent of the particle size[10]. The exponential growth of adhesion ability with increasing chitosan coating levels illustrates the increased effectivity of having higher levels of chitosan integrated within the nanoparticles being studied.

5. Drug Delivery Applications of CS

5.1 Hydrogels

Hydrogels are one of the primary forms of drug delivery technology that utilize chitosan's unique properties. There are two general forms of hydrogels, physically or chemically induced gels. Both forms are able to be constructed with a chitosan scaffold. Most physically modified hydrogels are known to have faster degradation, which can be either advantageous or disadvantageous depending on the circumstance. Faster degradation may allow for faster drug release, however if the chosen entrance point is far from the targeted release site then a physically modified hydrogel may make it difficult for the drug to release at the proper time and location[8].

In order to work around this potential issue, the modification of chemical properties is often used to accurately release drugs. Hydrogels are known to be hydrophilic and cathodic, making them optimal for water soluble anodic drugs and therapies. This does pose to be an issue when attempting to insert cathodic or hydrophobic drugs. Hydrophobic drugs generally have a low therapeutic index which requires a large dosage in order to be deemed effective[8]. Hydrophobicity must also be considered in conjunction with the porosity of the chitosan structure. A smaller drug molecule is known to release faster through smaller pore sizes, whereas the larger molecule releases slowly, or can be absorbed via endocytosis[9]. Considering both hydrophobicity and porosity is essential when measuring the effectivity of a drug delivery system.

Hydrogels are easily modifiable to meet design parameters as illustrated with Majumder's research on creating a self-assembling hydrogel by the name of Sequogel. The design of the hydrogel's self-assembling nature is a construct of the pH balance in the dissolved solution. When the polymer-based chitosan material is suspended in a buffer, it remains an unfolded monomer. However, as NaCl solution is added to increase the concentration of ions in the solution the peptide material begins to crosslink, creating beta-rich planar networks that are biocompatible and pH sensitive when CS is introduced[11].

There are many other characteristics that are specific to chitosan, such as the efficiency of mucosal adhesion and ability to bypass the blood brain barrier with higher success rates than conventional direct drug pathways[1].

5.2 Nanoparticles

Quantum dots (QD) provide much promise in the drug delivery field as they are multi-functional. QDs can simultaneously perform "diagnosis, targeting, biolabeling, drug delivery, and killing of cancer cells," rendering them to be effective on multiple fronts of cancer research[3]. Their photoluminescence band can be tuned between UV and IR on the electromagnetic spectrum during creation for these many actions listed previously.

The ability to perform all of these tasks simultaneously requires a two-part quantum dot. A fluorescent inorganic core and an organic shell seek out and interact with cancerous GBM cells. An inorganic core is often paired with an outer layer of CS in order to act as a stabilizing agent or as a capping ligand known as the semiconductor-chitosan conjugation[3]. The QD is first created by nucleating a composition of gold, indium, and sulfur into the followed by the deposition of zinc-sulfide, resulting in a core nanoparticle known as ZAIS. Chitosan can then be applied as the shell, creating the conjugation ZAIS/Chitosan. This specific bioconjugation was researched by Santana et al., for combating GBM.

In Santana et al.'s research on "bi-functional quantum dot-polysaccharide-antibody immunoconjugates for bioimaging and killing brain cancer cells in vitro," the group was able to illustrate the effectiveness of using ZAIS-CS QDs in conjunction with antibodies against the GBM tumor cells U87 vs the reference cells HEK 293T. Although some cytotoxicity was observed at high concentration levels, HEK 293T cell viability was preserved in instances where only the QD is presented, and when the antibody is introduced the GBM cells are effectively targeted and killed. Cell viability was observed highest in control with no alterations. However, when comparing HEK 293T cells to GBM cells subjected to the ZAIS-Chitosan-antibody quantum dot, cell viability decreased by about 40% in the cancerous cells comparatively, exemplifying the strong potential for this ZAIS-CS based QD method[3].

There are two main methods for drug incorporation into the nanoparticle. Incorporation during the composition of the QD, or absorption of the drug post QD production are the two methods that are commonly used. There are both nano-capsules and nano-spheres, where capsules retain content inside of the nanoparticle, and nanospheres incorporate the drug into a polymer network[1], [12].

Nanospheres are a very popular form for drug delivery across the BBB, as the use of peptides and proteins are easily able to adhere to nanospheres and create a coating, usually on the interior but also on the exterior of the nanoparticle with the assistance of chitosan being integrated within the particle structure. In Aktas et al.'s research, they were able to utilize a common method involving the chitosan shell attached to PEG dendrimers that interact with a natural bodily compound biotin[13]. This biotin then directly reacts with antibodies, in this case OX26, in order to bypass the BBB effectively. This integration with chitosan allows for negative charges of the brain endothelium cells to interact with the positively charged chitosan, as well as allowing for the OX26 receptor that is bound to the nanosphere to trigger receptor-mediated transport intravenously through the BBB as desired[13].

CS remains to be a prevalent topic in the nanoparticle world, as a stabilization method, organic medium for adhering to drugs and/or r groups, as well as its ability to pass through the blood brain barrier[1], [3], [6].

5.3 Micelles

Micelles are a form of aggregate molecules with hydrophilic heads and hydrophobic lipid tails formed into a spherical shape, with lipid tails congregating in the center. This allows for lipophilic or hydrophobic drugs to easily be encapsulated within a micelles[1].

Chitosan has the valuable property of being able to pass the blood brain barrier with more ease than other nanocarriers, encouraging its use in many drug delivery systems, including micelles. Agrawal et al. have completed research and analysis on synergizing both chitosan and TfR, a transferrin receptor known for its high expression on malignant cells with a biocompatible micelle. In their study, they used DTX, an anticancer model drug [14].

Both spectrometry and spectrophotometry were used to analyze the different combinations of micelles, with particular interest in how the addition of chitosan affected the "particle size and size distribution, surface morphology, drug encapsulation efficiency, drug loading, and drug release profile"[14]. It was noted that the both the size and shape of the CS micelles were "more or less uniform," with the surface being relatively smooth[14]. The encapsulation efficiency was significantly more effective with the addition of chitosan to the micelles, and the conjecture was made that the chitosan on the surface enhanced efficiency due to the adhesive nature of the material. This adds to the effectivity and illustrates the high-performance levels that this material can achieve. The drug release profile was calculated at hour long intervals for DTX, a bioadhesive CS micelles, as well as a bioadhesive CS micelles with a transferring receptor (TfR) used for targeting cancerous tumors. The pure anti-cancer drug DTX was the quickest to be released, as there are no inhibitors directing or slowing the release as the micelles does in the other two cases. The CS micelles without the additional TfR was the next fastest to release, with the TfR CS micelles being the slowest. As the TfR is designed to target malignant cells, and CS nanocarriers tend to release at low pH, this synergized micelles was determined to be the most effective and targeted micelles analyzed [1], [14]. It is important to note that Agrawal et al.'s research does not depict the whole concept of targeted drug delivery, only the concentration levels of measured DTX in plasma.

Cytotoxicity of these micelles was also analyzed, and it was determined that TfR and chitosan are able to directly increase the micelles cellular uptake, implying that there could be a high ratio of DTX to brain cancer cells compared to the use of DTX without a chitosan based nanocarrier[14].

6. Conclusions

The material of Chitosan is highly regarded in the medical field for its applicability for use in drug delivery. Illustrated in this paper are the vast ways that the material can be modified, either during the processing stage or at the structural level with the addition of r-groups like -OH and -NH2 or indicators like Tfr, so that the effectivity and performance can be improved.

Chitosan based nanocarriers have been proven to be especially effective in the treatment of brain cancers such as glioblastoma multiforme. Testing CS parameters such as; targeting, cytotoxicity, solubility, adhesion to mucoadhesion sites, etc. allowed for nanocarrier drug delivery systems such as hydrogels, micelles, and quantum dots to be proven more effective than traditional drug delivery methods. In each of the delivery methods listed, the use of chitosan was able to increase their effectiveness in one or more of the described parameters. Chitosan has been known to reduce the burst effect of nanocarriers and increase biocompatibility of historically bio-incompatible methods such as quantum dots. Chitosan can also be a building block for drug delivery methods such as hydrogel scaffolds or micelles, due to its rigidity and affinity to bonding. All research in this review article may use different technologies and have different entrance and target points, however in all cases the use of chitosan was able to create a more effective drug delivery system and solution.

As additional testing is to be continued on this promising material, more methods and applications are continually identified. This ensures the future of drug delivery is very likely to have chitosan at its core. Chitosan's abundance in nature, its extreme biocompatibility features, its ability to be an indicator as well as the method of delivery are all exemplary reasons for why this material will prove to be capable and at the forefront of drug delivery technology for years to come.

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Conflict of Interest

The author declares no conflict of interest.

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