Advantageous uses of nanotechnology as a method for removing cancerous cells from the brain

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Incidences of brain cancers across the globe as well as the associated costs continue to drastically climb, contributing to over $80 billion per year of medical costs for cancer alone. With few options for treatment, the economic and societal impact of those affected desperately desire a safer, more efficient, and more cost-effective option for high-rate remission with minimal side effects. As targeted therapy treatment options have become a better option for cancer treatment, we believe that utilizing these current methods in combination with graphene, a highly diverse biomaterial, programmed nanoparticles could potentially play a large role in cancer research and treatment in the near future that may minimize the negative impact cancer has on society. Through injection into the cerebrospinal fluid, roughly one-hundred nanobots would be contained within a simple saline solution to be given a direct line of access into the brain. These nanoparticles comprised of graphene would ideally carry out similar functions to that of a killer T-Cell, as seen in the human body, as a method to target specific identified and marked cancerous cells. Once these nanobots have reached the identified cell, the nanobots will release an incredibly small amount of chemotherapy into said cell to maximize the efficiency in which chemotherapy is delivered to invoke minimal side effects and consequences on the human body. In this analysis of the newly rising cancer treatment method in the field of targeted therapy, our research attempts to shed light on this additional option for individuals suffering from brain cancers.

Background

Although being diagnosed with a form of brain cancer is not always an immediate death sentence, a cancer diagnosis can greatly impact the emotional health of patients, families, and caregivers (American Cancer Society, 2020). Anxiety, distress, and depression are all common feelings that individuals experienced when they or a loved one has been diagnosed, which can ultimately impact their roles at home, school, or work (American Cancer Society, 2020). It has been seen that among individuals with no previous psychiatric history, a diagnosis of cancer is associated with heightened risk of these common mental disorders, which may adversely affect cancer treatment and recovery, as well as quality of life and survival (Niedzwiedz et al., 2019). When compared to melanoma, for example, the estimated five-year survival rate of early-diagnosed individuals is roughly 99 percent as early-stage melanoma has become fairly routine to treat (Skin Cancer Foundation, 2020), and interestingly enough, in a study assessing the mental health of advanced-stage melanoma patients, only about 28 percent of patients felt the need to utilize the offered mental health services provided over a 12-month period during treatment (Hanna et al., 2017). In contrast, a similar study was conducted with individuals diagnosed with brain tumors, an often much more rigorous and aggressive treatment path, and it was found that over the span of their treatment, up to 74 percent of patients experienced mental health distress with up to 47 percent of patients reporting significant levels (Randazzo & Peters, 2016).

With this as well as many issues associated with economic impact, brain cancer treatment has seen a huge surge in the development of novel strategies for management of disease as a more efficient and cost-effective treatment option is needed (Ray et al., 2014). According to the Agency for Healthcare Research and Quality, the estimated direct medical costs of all cancers in the United States in 2015 were $80.2 billion (American Cancer Society, 2020), averaging roughly $32,000,000 each year for brain cancer treatment (Ostrom et al., 2013), scientists have seen a drastic need to develop a cost-effective mode for patients suffering from this illness. Although there are many relatively efficient methods of removal, with a surprisingly high success rate, the current options for removal and/or interventions could be considered drastically risky and cost-inefficient (Ray et al., 2014). Right now, brain cancer is known for being one of the most expensive cancers to treat, with a high success rate if caught early enough (Dudley et al., 2008). The cost of primary malignant tumor treatment in 2007 was observed to be roughly $8,478 per month (Raizer et al., 2015) with an associated total median cost of around $138,787 if patients received two main forms of treatment, and

roughly $79,099 if patients had received neither primary treatment option (Ray et al., 2014). Many studies have unsurprisingly shown that the costs associated with malignant brain tumors increase after patients undergo surgery as a method of removal, which after only one year following these surgeries, patients who also received other methods of treatment that was not as effective totaled roughly $184,107 in healthcare expenditures (Ray et al., 2014). For chemotherapy treatment, temozolomide (TMZ), an oral chemotherapeutic agent, has now become part of standard care for patients with these types of brain cancers, but has been noted to have shown a total cost of treatment with this drug to be $17,847 (Raizer et al., 2015). From the completion of the TMZ treatment to relapse, progression, or death, the average cost was viewed at $4,389 per month (Raizer et al., 2015). Temozolomide, specifically, is one of the primary cost-inefficient methods that plague patients that suffer from this disease, forcing them to pay more each year as the drug price continues to climb (Ray et al., 2014). Though TMZ is noted as a primary treatment option, the use of TMZ is known to potentially indicate greater disease severity, making it a less efficient method for eradicating the existence of cancer cells in a patient (Ray et al., 2014). Further cost-effectiveness as well as a cost-benefit analysis will serve a more in-depth description on this issue as it compares to the treatment option as this paper suggests.

With roughly 24,000 new incidents of brain cancer each year in the United States alone (Park et al., 2017), scientists have recently begun to look at a biomolecular method as a method of cancer remediation, rather than the current and common chemical or surgical approaches. As it has been continuously researched, the incidents of malignant brain tumors have been rising significantly over the years, showing no signs of slowing down (Park et al., 2017). Patients who have been diagnosed with specific types of brain cancers such as metastatic melanoma have a median survival rate of 8 months, and a 2-year survival rate as low as 10-15 percent (Dudley et al., 2008). In the United States, the 5-year relative survival rate after the diagnosis of a brain tumor that is malignant or non-malignant was observed to be 34.7 percent (Park et al., 2017). The average observed mortality rate in the United States between the years of 2009 and 2013 was 4.32 per 100,000, with roughly 73,450 deaths annually attributed to primary malignant brain and other central nervous system tumors (Park et al., 2017). These numbers have been seeing an upward trend over recent years with 22.36 cases per 100,000 and growing, of individuals who have been diagnosed with these types of tumors in the United States (Park et al., 2017). These numbers further suggest the need for effective treatment methods as these numbers rise parallel with

the need for prevention, care, and treatment of co-morbid depression and anxiety among diagnosed individuals (Niedzwiedz et al., 2019). After examining the climbing incidence and mortality rates for these types of tumors, one could deduce that the current options in place for individuals with brain cancer does not adequately address the growing problem facing society. In recognizing that this multi-pronged problem may not have an easy fix, this research group proposes a way in which an ideal scenario can be reached by combining multiple methods into one comprehensive and effective method that could potentially ease the overall negative societal impact that various forms of brain cancer causes. This group has focused on finding solutions to this multi-faceted problem by suggesting the potential to see a drastic decrease in the cost associated with curing brain cancer. This could potentially be done at a low-risk rate, while still maintaining high success percentages, therein easing much of the stress and worry often associated with diagnosis.

It is seldom seen that a patient treated for brain cancer of any kind will not experience side effects from the treatment itself (Ray et al., 2014). Many of the available treatment options given to patients currently are highly individualized by an experienced multidisciplinary team to target the best treatment for the patient (Perkins & Liu, 2016). The often-preferred treatment for primary brain tumors is surgical removal and is considered to be the safest option for many patients; however, as with any surgery, especially in brain surgery, there is always a large risk factor associated with the procedure (Perkins & Liu, 2016). As previously mentioned, this option is often passed aside at first by many as it is easily the most expensive treatment option (Raizer et al., 2015). After a procedure such as this, common complications that may follow include deep venous thrombosis, pulmonary embolisms, wound infection, seizures, depression, intracranial bleeding, systematic infections, adverse drug reactions, and worsening neurological status (Perkins & Liu, 2016). It is also said that of patients who undergo resection of brain tumors, roughly thirty percent will likely develop seizures along with cognitive deficits such as attention, memory, depression and other mood problems, and executive functioning (Perkins & Liu, 2016). Radiotherapy is another slightly more effective option for patients with high-risk, low-grade gliomas, however, common side effects those who receive this treatment may experience include, but are not limited to: skin reactions, swelling, hair loss, tiredness and fatigue, etc. (Perkins & Liu, 2016). Chemotherapy is another popular option that many patients opt for as when in combination with radiation therapy, this treatment has been shown to improve the survival in many cases (Ostrom et al., 2013). Regardless of how effective

chemotherapy has proven to be, there are also many known side effects that accompany treatment as chemotherapy drugs kill fast-growing cells such as cancerous cells, however, fast-growing healthy cells such as blood-forming cells within bone marrow, hair follicles, and cells in the mouth, reproductive system, and digestive tract are all likely to get damaged in the process (Bagnyukova et al., 2010). Given the staggering number of patients who experience such high accounts of adverse side effects from these life-saving treatments, other methods must be developed to minimize these reactions, and by altering methods of chemotherapy treatment to be highly targeted, we believe that the use of nanotechnology will help us accomplish this goal.

Carbon materials are known to be one of the world's most abundant groups of materials and can be seen in applicable uses that can range from small electronics to surgical technologies to diamonds (Tadyszack et al., 2018). Graphene is another such instance, appearing as a two-dimensional nanomaterial that is comprised of sp^2 bonded carbon atoms that possess an astonishingly high number of optical, thermal, mechanical, and electronic properties (Shen et al., 2012). With graphene's high potential to be an outstanding applicator for the world of nanoparticle usage, the seminal report on the use of graphene oxide (GO) expresses its ability to be an efficient nanocarrier for drug and gene delivery in 2008 (Shen et al., 2012). Additionally, graphene has shown potential in other biomedical applications such as forming antibacterial materials, biological sensing and imaging, and biocompatible scaffolds for cell cultures (Shen et al., 2012). This single layer of carbon atoms, covalently bonded to three neighbors, naturally forms a honeycomb-like structure of densely packed benzene rings (Tadyszack et al., 2018). Pure forms of graphene can be obtained by a typical top-down approach from graphite, consisting of layers of graphene stacked parallel to one another in a three-dimensional, crystalline, long-range order (Tadyszack et al., 2018). Each individual layer of graphene has a fascinatingly high specific surface area at roughly 2630 m^2/g as well as exceptional electronic conductivity of a measured 200,000 $cm^2V^{-1}s^{-1}$ mobility of charge carriers (Shen et al., 2012). Additionally, graphene has been observed to have high thermal conductivity of roughly 5,000 W/m/K and mechanical strength as well as intrinsic biocompatibility, scalable production, facile biological and chemical functionalization of GO, all at low costs (Shen et al., 2012). As mentioned, graphene is an ideal nanocarrier for efficient drug delivery, having GO typically be one to three layers thick with size ranging from a few nanometers to several hundred (Shen et al., 2012). By utilizing this potential of graphene and graphene oxide, this material is able to add valued properties

such as target specificity, high loadings, and controlled or sustained release kinase kinetics to the field of cancer research and treatment (Tiwari et al., 2019). Many similar nanocarriers are currently being used for the delivery of numerous different therapeutic molecules, allowing graphene to show high potential as a possible successful candidate as well (Tiwari et al., 2019).

Nanobots programmed and designed to fight cancers on a molecular level is developing as an alternative to the many invasive and destructive cancer treatments seen today (Besser et al., 2013). The nanobots and associated nanoparticles are used to deliver immunostimulatory treatments to an individual's system (Cheng et al., 2013), (National Cancer Institute, 2017) as well as actively eradicate both benign and malignant manifestations of cancer in a patient's body (Mi et al., 2016). The nanobots are programmed with synthetic receptors capable of attaching to tumor-specific proteins and lysing them upon extended contact (Cheng et al., 2013), (National Cancer Institute, 2017). The process will be a more systematic implementation of the CAR T cell method (Cheng et al., 2013), (National Cancer Institute, 2017), allowing the nanobots to function as a "living drug" within the user's system, capable of operating and reproducing autonomously (National Cancer Institute, 2017). These nanobots are also seen as a method for cancer radiotherapy by improving radioisotope (radionuclide) delivery through nanomedicine (Mi et al., 2016). There are two ways in which nanotechnology can facilitate the chemoradiotherapy: by delivering the chemotherapeutics by nanoparticles combined with external irradiation for combination therapy due to the radio-sensitizing effect of some chemotherapeutic drugs; the other is to co-deliver both chemotherapeutics and radio-sensitizers/radioisotopes in the same nanoparticle, which achieves the simultaneous delivery of agents at lesion as well as concise radio control (Mi et al., 2016). Both methods of applying nanotechnology to cancer treatment benefits from decreased toxicity in normal tissues and preferential accumulation in tumors (Mi et al., 2016). The review of Mi, et al showed one instance of a study where nanotechnology was successfully used as a method for changing the physiological properties of tumors using the second aforementioned approach (Mi et al., 2016). This study expressed that the difference in effectiveness of these nanoparticles in combination with the drug was sizably more effective in reducing tumor volume in comparison to administering each drug alone (Mi et al., 2016). As the field of medicine and hospitals may quickly become split in advocating or condemning the use of this engineered technology, there maintains an undeniable truth to the benefits that these

nanobots could potentially provide to patients with this disease (Dudley et al., 2008).

Methods

Lumbar Puncture

The lumbar puncture is a procedure in which every physician should be adequately trained to perform (Pardridge, 2011), therefore little to no additional training would be needed for the physicians administering the treatment, ultimately lowering the cost and time associated with this aspect of treatment, while increasing chances of success. Also known as a spinal tap, this minimally invasive procedure is performed in a patient's lower back, lumbar region, where a needle is inserted between two lumbar vertebrae to remove a sample of or inject into the cerebral spinal fluid (CSF), which is fluid that surrounds the brain and spinal cord to protect them from injuries (Pardridge, 2011). Although lumbar punctures are typically used to help diagnose certain infections or disorders of the central nervous system (CNS), including brain and spinal cord cancers (Pardridge, 2011), physicians can also use this procedure to inject certain medications or chemotherapy drugs into the CSF (Pardridge, 2011). Since this method is a minimally invasive, routine procedure for patients with brain cancers, it is ideal for the execution of the suggested treatment.

Additionally, injection of saline solution directly into the CSF by lumbar puncture would prove to be ideal in administering the nanobots, as saline is known for its harmless, often therapeutic effects in the CSF (Pardridge, 2011). It is common practice for hospitals to administer patients with a 0.9% saline solution for fluid intake. Here, it is implied that the nanoparticles can be inserted into the saline solution to be injected directly into the CSF of patients.

As it is known, the pathway of CSF flow within the human body moves through arterial blood into the lateral, third, and fourth ventricle's choroid plexuses before the CSF flows into the lateral, third, and fourth ventricles in addition to the subarachnoid space of the brain (Pardridge, 2011). As the CSF offers a direct route for the nanoparticles to the brain, it is ideal for a lumbar puncture into the CSF to be the primary method for this treatment. By utilizing this method, the nanoparticles would be capable of acting more efficiently by having this direct path to the brain, where the cancerous cells need to be located.

The injection of the nanoparticles into the saline solution would be a relatively easy process as nanoparticles are often utilized in liquid substances (Mi et al., 2016), allowing for ease of treatment administration. Roughly 100 of these microscopic

nanobots within the saline solution will be able to directly be injected into the CSF at once, minimizing the need for patient discomfort while maximizing the effectiveness of the treatment.

The Nanobots and associated nanoparticles will be used to deliver immunostimulatory treatments to the individual's system as well as actively eradicate both benign and malignant manifestations of cancer in a patient's body (National Cancer Institute, 2017). In a process similar to those seen within lytic viruses, the nanobots will seek out tumor cells and cause them to go through a form of induced apoptosis followed by the digestion of residual portions by the body's lymphocytes (Cheng et al., 2013), (National Cancer Institute, 2017). This will be accomplished by modifying and programming the engineering particles with synthetic receptors capable of attaching to tumor specific proteins and lysing them upon extended contact/interaction (National Cancer Institute, 2017), (National Cancer Institute, 2017). This area is prone to variation given that the bots can either release targeted chemotherapy directly into the cells they interact with, or by directly implanting a marker within the cells that indicates their need for removal, generally a more systematic implementation of the CAR T cell method (National Cancer Institute, 2017). The latter option allows for a training of the body's immune system and ultimately limits the volume of bots needed for initial implantation/introduction (Besser et al., 2013). However, this method requires additional bioengineering and may pose issues in time or cost-sensitive cases (Besser et al., 2013).

For this reason, as is seen with current chemotherapy methods, the deployment of bots outfitted with chemotherapeutic medication would be useful in systemic cases, but more costly given that only a finite amount of therapy can be housed within each engineered particulate (Besser et al., 2013). In addition to providing targeted relief to the patient's system, the goal is to have the nanobots function as a "living drug" capable of operating and reproducing autonomously (Singleton et al., 2017). Autonomous replication can be enabled via supplementary capsules that house provisions/additional therapy doses that allow for the same bots to remain within the system continually "patrolling" different zones within the body, or replication can by facilitated via the usage of tumor cells (Suthakorn et al., 2003). In the case of tumor cell reproduction, the bots would be outfitted with RNA strands that code for specific, similarly tumor targeting proteins that operate like lytic viruses (Suthakorn et al., 2003). In this way, the body's tumor population continues to decrease while the population of engineered proteins rises exponentially (Suthakorn et al., 2003). However, in order to implement a cost-effective model of the nanobots/particles, computational improvements regarding the

proliferation limits and genotoxicity will be required (Besser et al., 2013). These will enable the bots to be greatly less expensive than current adoptive cell transfer methods (Besser et al., 2013).

T-Cell Isolation

In the current scientific environment, advancements in bioengineering and gene coding have facilitated the research of practical applications to novel ideas (de Witte et al., 2006). In the case of cancer, new forms of radiation and tumor targeting are making considerable strides in providing additional avenues for treatment outside of traditional chemotherapy and hormonal treatments (de Witte et al., 2006). One such methodology that has shown incredible progress is the isolation and reprogramming of the body's own T lymphocytes to manage and potentially eradicate the presence of desired cancer variants within an individual's system (de Witte et al., 2006). A three-fold process is required to properly functionalize these cells to target and breakdown the masses: isolation, programming, and application (Krummel et al., 2016). When utilized properly, there exists the possibility to incorporate similar mechanisms in nanobots capable of quickly and efficiently treating segments of the body that reprogrammed lymphocytes would take considerably longer to produce results for (Lefort & Kim, 2010).

The lymphocyte mechanism that facilitates the targeting of specific antigens is that of T-cell migration (Lefort & Kim, 2010). T-cell migration is essential for allowing the detection of antigens within congregate areas (Krummel et al., 2016). The process of isolating lymphocytes in vivo is a straightforward, but delicate one (Lefort & Kim, 2010). The peripheral blood mononuclear cells (PBMCs) need to be isolated from the patient's blood and cultured to confluence (Lefort & Kim, 2010). These newly cultured T-cells have the potential, via ex vivo modification to treat viral infections and target specific tumor antigens within the body (Krummel et al., 2016). This can either be accomplished via the extraction and proliferation of tumor infiltrating lymphocytes (TILs) from tumor biopsies or from the PBMCs located in the surrounding blood (Sharpe & Mount, 2015). Alternatively, through genetic modification, T-cells can be modified to target specific antigens expressed by tumors (Sharpe & Mount, 2015). The modification can result in the formation of specific receptors with enhanced antigen specificity that can effectively target tumors without the need for formal activation (Krummel et al., 2016).

T-Cell receptor therapies alter T-cell specificity by mediating the antigen recognition capabilities of cells via TCR alpha and beta chains (Sharpe & Mount, 2015). By isolating target sequences from tumor-reactive cells, the isolation and cloning of

specific vectors enables the production of tumor-antigen-specific T-cells (Sharpe & Mount, 2015). The two primary methods for doing so within the current scientific climate involve either rat immunization or TCR gene transfer (Sharpe & Mount, 2015). Rat immunization follows a very similar format to the process of producing antibodies for venomous exposures (Sharpe & Mount, 2015). By exposing transgenic mice that express human leukocyte antigens to tumor proteins, an accelerated production of antigen specific T-cells occurs which can be isolated and utilized as needed within patients (Stanislawski et al., 2001). Alternatively, TCR gene transfer isolates tumor-specific T-cells from a patient currently going through remission, hoping that the TCRs present on the cells are capable of replicating their effectiveness within a new host (Stanislawski et al., 2001).

However, the limitation of gene transfer lies in the specificity of the transferal process given that the recipient of the cells must share the same disease and be unresponsive (de Witte et al., 2006). This method differentiates itself from similar treatments utilizing Chimeric Antigen Receptors (CARs) due to the fact that CARs are capable of not only recognizing proteins, but also glycolipid and carbohydrate structures expressed in tumors (Driessens et al., 2009). The gap that exists between this method and traditional T-cell therapies lies in the need for co-stimulation (Heslop, 2010). Without the ability to produce tumor necrosis stimulators via receptor binding, CARs have shown to be unresponsive upon insertion, however, if the target sequences for stimulatory sequences can be programmed to the CAR T-cells beforehand, enhanced tumor regression effects can be seen (Driessens et al., 2009).

Programming and Creating Nanobots

The nanobot is meant to be programmed and designed to fight cancers on a molecular level as an alternative to the many invasive and destructive cancer treatments seen today (Bagnyukova et al., 2010). The successes seen by TCR therapies provides immense utility in future clinical assertions that the method is capable of providing widespread utility following the isolation and proliferation of tumor-specific T-cells for patients with only a need for small amounts of initial TCRs to complete the process (Schmitt et al., 2009). However, for the nanobot application discussed within the current analysis, the variability of CAR T-cells poses a much more viable avenue forward. The specificity for cellular pairing provides a unique pairing with the availability of nanocomposites to deliver drugs to a site (Goenka et al., 2014). Traditionally, graphene oxide (GO) constructs consist of single atom thick layers of graphene sheets whose peripheral carboxylate

groups provide colloidal stability and pH-dependent negative surface charges (Park et al., 2009). While the material has a high affinity for biological interaction, where its size, shape and thickness directly contribute to the toxicity and uptake availability of the composites by cells, the structures are not biodegradable which poses a few issues in terms of application and the oxidative stress that could occur as a result of the utilized components being left within an individual's system following deployment (Goenka et al., 2014).

In addition to GO's ability to reliably interact with DNA, RNA and cell membranes, the high specific surface area and hydrophobic interactions of GO have shown the potential to be exploited to achieve highly efficient loading of poorly soluble drugs without having to sacrifice their efficiency or potency (Liu et al., 2008). This is enabled through the ability of GO constructs to enter cells via endocytosis (Liu et al., 2008). For peak efficiency, it is vital that the drug carrier is not hampered by endosomal compartmentalization and is able to release its load in the cytosolic compartments of the cell (Goenka et al., 2014).

Apoptosis

Cell mediated death, commonly referred to as cell suicide is known as the process of apoptosis. Apoptosis is the method used to help regulate the number of animal cells (Alberts et al., 2010). This cell death can be triggered by intracellular signals, or externally as well. The process of apoptosis is triggered billions of times every hour, and is used to help form structures, prevent cancerous growths and maintain organ size (Alberts et al., 2010). Cell death can take place in several forms, including: cell necrosis and apoptosis (Fink & Cookson, 2005). Apoptosis is the cell death method that is further explored within this paper and is different from cell necrosis fundamentally. Cell necrosis is characterized as accidental and passive cell death that unlike apoptosis is not self-contained and can spill cellular contents into surrounding tissue and damage other cells (Fink & Cookson, 2005).

This method of cell death is programmed, different than cell necrosis, because the contents are not released when death occurs in apoptosis by neatly containing contents and preventing damage to neighboring cells (Alberts et al., 2010).

This process of cell death is a universal fundamental component in the development and homeostasis of tissue (Favaloro et al., 2012). This action of homeostasis is involved in the functions relation to balancing mitosis within the body (Favaloro et al., 2012). Through the research of many diseases, caspase dependent apoptosis has been identified to play a role in different cancers, as well as; neurological, cardiovascular and autoimmune

diseases (Favaloro et al., 2012). However, in this proposed method of action for the treatment of cancerous cells in the brain, the universal action of apoptosis found in virtually all people will be manipulated for the betterment of society and medicine. By working with this fundamental biological function, the foundation of this treatment does not need to be implemented as it can be considered a naturally occurring resource within the body, which can impact the overall work and monetary requirements for treatment with this advantageous nanotechnology.

The intracellular method of triggering apoptosis is done through an intracellular proteolytic cascade (Alberts et al., 2010). This cascade is made up of caspases that are of the protease family and are found in animal cells (Alberts et al., 2010). Caspases are produced by the inactive precursor procaspase, which produce the caspase when intracellular signals for apoptosis are released (Alberts et al., 2010). Two active caspases are formed that work together to disassemble a cell, known as the initiator and executioner caspases (Alberts et al., 2010). The executioner caspase triggers a cascade of more executioners being produce; self-amplifying the proteolytic cascade (Alberts et al., 2010). Key proteins are dismembered within the cell, weakening structures like the nuclear lamina which then allows nucleases to enter and break down the contained DNA (Alberts et al., 2010). When all parts of the cell have been dismantled, the remains are then taken up by another cell, leaving behind no damage to surrounding cells (Alberts et al., 2010). Once the apoptosis cascade is triggered, it is carried out in an “all-or-nothing” fashion, making this cascade irreversible, and because of this, the apoptosis processes and signals are tightly controlled and monitored (Alberts et al., 2010).

Once apoptosis is triggered, irregular bulges will form on the outside surface of the cell (Alberts et al., 2010). These bulges are also known as blebs, and will shrink and condense as the cytoskeleton collapses, the nuclear envelop disassembles, and DNA breaks up (Alberts et al., 2010). The cell that will then engulf the carcass of the dead cell is a phagocytic cell, which are drawn to the dead cell by the altered cell surface (Alberts et al., 2010). These phagocytic sells have an increased response rate for apoptotic cells that engulf them before the cell can release its contents, avoiding any damage to surrounding cells (Alberts et al., 2010).

Extracellular signals produced by neighbor cells can also trigger apoptosis. This method can take two forms; activation of the cell death program, discussed later, or the activation of cell death through a cell surface receptor (Alberts et al., 2010). There are many different types of cell surface death receptors, however for the of this paper, the Fas receptor will be discussed due to the

extensive research on this receptor. The Fas receptor is found on the membrane of most mammalian cells and is activated by the membrane bound protein Fas ligand found on killer lymphocytes (Alberts et al., 2010). These immune cells have the specific job of regulate immune responses that induce apoptosis, when the ligand interacts with the Fas receptor on the soon to be dead cell, a death-inducing signaling complex that will lead to cell death (Alberts et al., 2010).

Both methods of triggering apoptosis involve the Bcl2 family of intracellular proteins. These proteins are key in the communication that leads to apoptosis (Alberts et al., 2010). A part of this family are proteins dedicated to promoting caspase activation and cell death, while another part is dedicated to inhibiting this process and promoting cellular survival (Alberts et al., 2010). Well-known death promoting members of Bcl2 are Bax and Bak proteins, which initiate apoptosis by signaling the release of Cytochrome C from mitochondria into the cytosol which will activate initiator caspases and induce cell death (Alberts et al., 2010). This class of proteins is vital to signaling apoptosis and will play a key role in nanotechnology triggering this form of cell suicide (Alberts et al., 2010).

The proposed method of apoptosis triggered by nanotechnology involved the extracellular signal method discussed above and the use of a nanobot. The nanobot will serve as the immune cell that projects an artificial death receptor ligand. This ligand will interact with the specified death receptor, such as the Fas receptor, to trigger the signaling of apoptosis in the targeted cell. Once apoptosis is triggered, due to it's all or nothing nature, the process will continue as any normal apoptosis would. Once the cells surface changes and attracts the phagocytes the cell will be engulfed. The specific job of the proposed nanobot would be to mimic a leukocyte to trigger apoptosis on specific cancerous cells by incorporating target DNA of these cancerous cells into the bot. The bot will also need to have an artificial glycocalyx that will allow it the mobility and camouflage of a leukocyte. Further research would be required in how to model the nanobot to imitate a leukocyte, and another possible route would be to investigate viral proteins that have also been found to trigger apoptosis (Fink & Cookson, 2005).

The Process

Once the specific cell(s) have been identified and marked, the graphene nanobots could then be built with a simple 3D printer and programmed to locate those specific markers/indicators (Yang et al., 2015). The nanobot capsule would then be filled with a very minute amount of chemotherapy or the specific gene to trigger

apoptosis, enclosed, and the batch would be inserted into a 0.9% sodium chloride solution. After mixing, the solution would be distributed into the patient's cerebrospinal fluid (CSF) by lumbar puncture between either the L3/L4, L4/L5, or L5/S1 locations (Pardridge, 2011). The patient would then be on bedrest with a regular intake of fluids, continuously being monitored, and periodically tested to find any traces of the markers/indicators previously searched for (Cheng et al., 2013), (National Cancer Institute, 2017). By method of programming these nanobots, they would be able to search for and approach the marked cells (Li et al., 2018). As the nanobots are composed of the graphene material, the nanobots that would not be able to complete the job would become free floating nanobots that would safely dissolve into the body (Li et al., 2018). Similarly, once the nanobots complete their job, the empty carbon capsule will float along the patient's CSF, break down, passed through the body, and excreted as bodily waste (Yang et al., 2015).

Future Directions

For the experiment itself, a lot of background tests would have to be first conducted on the patient in order to mark the exact location(s) of the cancerous cell(s) within the patient's brain by use of either apoptosis indicator or by gene marker regulators (Hassan et al., 2014). In the long-run, the nanobots would most likely be administered by lumbar puncture as it is the most efficient method of transporting fluids directly to the brain (Pardridge, 2011). The only additional training that may be required may be the information on the nanobots themselves and how they will work within the patient's body. In terms of the T-cell isolation, this may prove to be the most extensive training necessary due to the complex and time-consuming nature of this process (Besser et al., 2013). If this process were to be conducted in the field, within the hospitals themselves, the hospital's researchers would need to familiarize themselves with the procedures necessary to construct adoptive cell transfers, or simply outsourcing to a third-party manufacturer (Dudley et al., 2008). This is all similar to the process of manufacturing graphene and programming the nanobots themselves. Since it's highly uncharacteristic for a hospital to have its own nanoparticle lab, third-party sources would enable them to keep costs low and patient accessibility high (Yang et al., 2015).

The field of medicine and hospitals as individual entities may quickly become split in advocating or condemning the use of this engineered technology, potentially creating a rift in quality of care (Dudley et al., 2008). This new technology, however, has the potential to open avenues of exploration for biologists, engineers, microbiologists/immunologists, etc. via the multitude of disciplines

necessary for the refinement and implementation of such novel innovation in the field of microbiological engineering (National Cancer Institute, 2017). The ability to program the receptibility of biological material based on its environment and necessary triggers not only creates opportunities for graduate education but spurs the creation of practical tech jobs necessary to the maintenance of highly specific machinery vital to the sustainability of labs (Von Andrian & Mackay, 2000). A bridging of the divide that currently exists between conventional engineering and biological sciences enhances the possibilities for collaboration between the leading authorities of both fields. Engineers developing diversified uses for graphene can expect an upward shift in workforce and avenues for research expansion following the initial implementation of nanoparticles (Yang et al., 2015). Given that graphene is not an inherently biocompatible material, having biologists in the lab working to increase the receptibility of the particles into a patient's system by allowing them to cross the blood-brain barrier uninhibited and enabling the bots to reproduce in a method similar to biological systems would not only create an influx of enthusiasm for the goal they're endeavoring to reach, but an increase in the funds available to finance the research (Pinto et al., 2013).

In addition to potentially jump-starting the coalescence of two fields, microbiological engineering stands to create waves in the general social sphere given its potential for controversy and misconception. In the months that have followed the first instance of engineered babies completely resistant to the AIDS virus on the Asian peninsula by a Chinese doctor, individuals from all sides of the aisle have come forward to either condemn or promote the use of bioengineering to facilitate the growing need for disease attenuation in the developed world (Dudley et al., 2008). The nanoparticles presently discussed will undoubtably create similar controversy, but also be a beacon for a future of individuals who do not need to go through trials of uncertainty and depression in response to a brain cancer diagnosis. The path forward is typically one of struggle, and doctors and engineers will be required to stand behind innovative medicines that may sound foreboding to the layperson but have the potential to save countless lives (Yoda, 2015). Advancing will take extensive testing, validation and social coverage, but with the support of the sciences and fields with the potential to disseminate truth in the face of hyperbole, a new future can be realized. The first step is to utilize this concept to bring researchers together for the common goal of eradicating cancer with a methodology not previously tested. While it will undoubtedly take time to materialize, the future is bright and the minds that will bring it there are even brighter.

Additional Info

This review serves solely as an analysis of the current developing topic in medicine and biomedical engineering as its potential to positively impact society. The information provided was researched from various other articles and benchwork that had been conducted in recent years. Although the paper had not included any of the group's data, the information provided, and conclusions had been put together and evaluated by the group themselves. As the group has not currently done any testing, they are always looking for new ways to bring to light what this new technology is capable of and how it can make a difference in medicine. The group always invites talks of potential testing of their own accord as this is a project the group is highly passionate about. With assistance and support from the Grand Canyon University's College of Science, Engineering, and Technology department's faculty members, the group would like to acknowledge and thank the CSET college for the support.

Additionally, the core focus of this review was primarily on the treatment of various forms of brain cancers and tumors as well as the impact these treatment options have on society. With the diverse applicability that targeted therapy and this new form of nanotechnology offers, the group understands that this method could potentially be applied to other forms of cancerous cells throughout the body. If this method were to be adopted for other forms of cancer in the body, the collective view of the group could potentially see this as continuing to perform as an efficient model of treatment of cancer as a new standard for patients. To reiterate, although this method may be applicable to other forms of cancer, this possibility had not been explored by the group in this review as brain cancer, specifically, was the focus for this paper.

Conclusions

In looking at brain cancer treatment from both sides of the spectrum – patient and provider – treating and eradicating cases of brain cancer is incredibly costly, resource-inefficient, and risky. For those individuals who suffer from this type of cancer, if treatment is successful, life expectancy and quality of life improves drastically, however, the financial burden often hangs a dark, lingering cloud overhead. For providers that help treat this form of cancer, the few available treatment options cost a great deal and utilize many resources from many different departments, creating an inefficient process that holds few alternatives. This research proposes a way in which brain cancer treatment costs, risks, and resource-usage can be immensely reduced, while simultaneously increasing the success rate of treatment at the benefit of both the patient and the provider, positively impacting both aspects of

society. This can be accomplished by utilizing nanotechnology to program a nanoparticle to locate and eradicate cancer cells in the brain by saline injection into the cerebrospinal fluid through a lumbar puncture.

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