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Advancements in Nanorobots: Innovating Bladder Cancer Treatments, Diagnosis and Patient Outcomes

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ABSTRACT
Nanorobots are an emerging technology that can deliver cancer treatment with increased precision, potentially decreasing unintended side effects commonly seen with surgery, chemotherapy, and radiation therapy. Recent advancements in in vivo trials have demonstrated significant tumour reduction in mice with bladder cancer, showing the potential of nanorobots to not only treat but also improve diagnostic capabilities in cancer therapy. This review aims to highlight the precision of nanorobots, the current knowledge on their use and their potential in clinical applications. The use of nanorobots could lead to better patient outcomes by providing targeted treatment and reducing side effects. Key components of modern nanorobot technology, including self-driving capabilities, biocompatibility, and biosafety, are analysed. Chemically-driven micro/nanorobots (MNRs) are primarily categorized by the biocompatibility of the materials used and the cytotoxicity of their waste products. Common materials are considered, focusing on size, shape, surface charge, and surface area. While both hydrogen peroxide and urease-driven nanorobots are considered potential fuels for MNRs, urease is regarded as a more biocompatible solution, making it a promising option for cancer treatment. This report emphasizes the potential of nanorobots to revolutionize the treatment of bladder cancer by improving both therapeutic precision and patient quality of life.
Keywords: Nanorobots, biocompatibility, bladder cancer, tumour penetration

1 INTRODUCTION
Bladder cancer remains a formidable challenge in oncology, particularly due to its high recurrence rates and limited effectiveness of current treatment methods. Recurrence rates can be as high as 78% within five years, underscoring the inadequacy of existing interventions such as surgery, chemotherapy, and radiation therapy (Healthline, 2023). Despite substantial research efforts, bladder cancer continues to rank among the top five in incidence and mortality globally (World Health Organization 2023). This calls for a deeper evaluation of targeted therapies and innovative approaches, such as the use of nanorobots to address these critical gaps in treatment.

The several challenges faced in the treatment of bladder cancer are due to its diverse types, including urothelial carcinoma, squamous cell carcinoma, and adenocarcinoma, each requiring different treatment approaches. There is a lack of targeted therapies for bladder cancer, making it difficult to detect at the early stage (Tramfimovich, 2023). Further, additional challenges arise because of the impermeable barrier formed by its lining (Boschi & Malatesta, 2023). Additionally, as the cancer progresses, it can become muscle-invasive bladder cancer, making tumour removal more challenging during surgery). Current treatment methods, including surgery, chemotherapy, and radiation, often result in significant morbidity, including damage to healthy tissues and reduced quality of life in patients (Tramfimovich, 2023). Although these approaches will remain largely applicable in oncology even as MNRs grow more advanced, their side effects call for more research into alternative tumour treatment options, especially concerning harder to reach areas in the body. More critically, these methods lack the precision necessary to avoid collateral damage, highlighting a pressing need for innovative solutions like nanorobots, which offer more targeted therapy with fewer adverse effects.

Diagnosis typically involves cystoscopy, where a tube is inserted into the urethra to examine the bladder, and biopsy to collect tissue samples. Additionally, urine cytology and imaging tests like CT urogram and retrograde pyelogram further assess the presence and extent of cancerous cells or tumours (Mayo Clinic, 2024). Evolutions in the field of cancer research are critical, as tumour response to chemotherapy and radiation can be low, resulting in

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treatment challenges (Tramfimovich, 2023). However, in comparison to current treatments, nanorobots have proven to be safer and more effective in tumour removal (Kong et al., 2023). However, they remain an emerging technology, of which awareness and knowledge is still limited. This report aims to compile major findings in the field with a focus on treatment of bladder cancer.

Nanotechnology is the umbrella term for various emerging technologies that operate at the nanoscale. It includes topics regarding nanorobots, nanoparticles, and nanomaterials. While all these technologies aim to improve cancer treatments, nanorobots offer significant advancements in medicine, specifically in the field of oncology. Nanorobots can perform a variety of medical tasks that can access hard-to-reach parts of the body (Kong et al., 2023). They comprise intricate components and are propelled by various driving force mechanisms. These mechanisms include external magnetic-driven, external ultrasound-driven, biological/chemical-driven, hybrid-driven, enzyme reaction-driven, and light-driven. Their capacity to interact with cells and penetrate organ barriers ensures efficient drug delivery, cancer detection and precise tumour targeting (Kong et al., 2023).

Nanorobots are equipped with key components such as power sources, payloads, sensors, actuators and communication systems (Kong et al., 2023). Firstly, they feature a biocompatible outer casting which is commonly made of materials such as silicon, carbon and diamond. This shell is crucial for ensuring safety and performance. Additionally, the size, shape and surface texture can be customized based on the specific function of the nanorobots. For instance, smooth surfaces decrease the risk of tissue damage, while rough textures help target cancer cells better. With regards to the power source, energy is provided through the means of batteries, hydrogen fuel cells, or enzymatic processes driven by the body's metabolism (Kong et al., 2023). Payloads dictate functions such as drug delivery and tissue repair while the sensors enable their ability to detect internal changes. Finally, actuators enable cell interaction or movement within the body, allowing communication between both them and external devices (Kong et al., 2023).

While nanoparticles also serve as carriers for drug delivery, they lack the driving-force functionalities of nanorobots (Kong et al., 2023). The active propulsion, cell/tumour targeting, and communication systems offer an emerging innovation that has the potential to revolutionize the field of oncology. Should the current hurdles of nanorobotics be addressed, there can be cautious optimism in the increased efficacy of cancer treatments. One of the major, modern challenges is the precise navigation of nanorobots in complex and dynamic environments such as the human body. There, factors like blood flow, immune responses, and the heterogeneous nature of tumours complicate the duty of nanorobots' drug delivery. Additionally, the issue of biosafety, particularly concerning the biodegradability and long-term toxicity of nanorobot materials, has not been fully resolved (Mao & Wan, 2023). It remains difficult to tell to what extent nanorobots will be able to replace standard cancer treatment, however, the incentive of biosafety and precise treatment provides hope that they have yet untapped potential. There is evidence to indicate that the use of nanorobots can minimize side effects by aiding in minimally invasive surgery and overall improving patient outcomes during cancer treatment and therapy (Tramfimovich, 2023).

Therefore, the aim of this report is to investigate the efficacy of urease-powered nanorobots and DNA origami nanorobots in the treatment of bladder cancer. This is accomplished through comparing the mechanisms of movement, power sources, materials, and biocompatibility with a focus on these two nanorobot types specifically. It is to be noted that the internal components of nanorobots include a power source, actuator, and sensors. However, the structure of nanorobots varies depending on their specific use and application.

2 BIOSAFETY, BIODEGRADABILITY AND FATE OF NANOROBOTS *IN VIVO*

During early studies, the biocompatibility and biodegradability of MNRs took a backseat to mobility tests, solely focusing on self-driving capabilities. Undegradable materials were used to process early nanorobots, such as carbon-based materials, platinum, metal oxides and overall non-biodegradable polymers (Wan et al., 2021). MNRs are now designed with materials that can degrade in special environments; therefore, the presence of specific pH levels and enzymes must also be considered. Potential substrates including degradable polymers, active metals such as magnesium or zinc and porous silica were studied for potential biodegradable MNR design (Wan et al., 2021). As the field progressed and the function and applicability of MNRs were further expanded, this new technology's biocompatibility and biosafety aspects were given more consideration alongside the fate of the nanorobots within the patient's body. Therefore, the "survival" of MNRs should be prioritized as it benefits their ability to deliver the therapeutic agents to the desired cells and tissues (Mao & Wan, 2023). After the drugs are delivered, it is often in the patient's best interest for them to be excreted or degraded as soon as their job in the body is accomplished. For nanorobots made of metal nanoparticles in particular, they may become toxic if they are not removed in a timely manner. The interactions between MNRs and the biological environment can affect their fate in the body and impact the patient.

Throughout the lifespan of MNRs *in vivo*, they will travel through and interact with body cells, tissues, organs and organic molecules. The reactions during this process, or lack thereof, are of great note to researchers seeking to advance the practicality and effectiveness of micro/nanorobots as treatment modalities. It is relevant to note that the surface composition, size, morphology and overall structure of nanorobots may change over time, and therefore the biosafety considerations must change accordingly, following along with the dynamic journey of the MNRs as they accomplish their desired goals within the biological environment. Smaller nanorobots and their byproducts can be excreted naturally by the body. Nanorobots under 10 nm can be excreted through urine while microrobots and larger

nanorobots can instead be excreted through feces. These pathways can inform the method of MNR administration depending on the robot design. However, the effectiveness of the particular pathways to fully remove the MNRs from the system still requires dedicated study (Mao & Wan, 2023). There remain many situations where the MNRs can undergo biodegradation *in vivo*, allowing the constituent parts to be reabsorbed by the body or excreted. It is ideal for the MNR material to degrade into smaller portions which are not cytotoxic or overall harmful to the human body. To this end, researchers have started making use of *in vitro* tests to review the cytotoxic properties and hemolysis rate (rate of red blood cell destruction) of MNRs (Wan et al., 2021).

In general, it is important to note that the biological toxicity of MNRs is shown to be closely tied to surface modification, size, shape, methods of preparation, concentration and the time required for the given MNR to complete its goal. Material properties can change drastically from the macro to the nanoscale, developing unique properties at their small size (Gupta & Xie, 2018). This phenomenon can be both a blessing and a curse, with these new properties both providing increased tools, yet also affecting the toxicity of the material at the nanoscale. The most potent example of this is gold. On the macroscopic level, gold is an exclusively face-centred structure (FCC), however, on the microscopic level, it can develop exotic packing structures including body-centred (BCC) and hexagonal close-packed (HCP) structures. Many materials experience notable changes in single electron magnetism, luminescence, excitonic light absorption, catalytic activity, redox properties and various other traits when reduced to ultrasmall sizes (Jin & Higaki, 2021).

The review of the biosafety properties of MNRs is a complex field of study, considering numerous factors. In the book *Biomedical Micro- and Nanorobots in Disease Treatment*, Chun Mao and Mimi Wan review four basic considerations necessary for the understanding of biosafety in micro/nanorobots. These considerations are the following: there is no complete bioinert material, biosafety occurs because of the effect of MNRs on the biological environment, autonomous motion of MNRs must be considered as part of biosafety and finally, biosafety is a dynamic process. With this baseline context established, a more in-depth study of micro/nanorobots' biological safety and security can be conducted.

2.1 Protein Corona Phenomenon

In the field of nanoparticles and the subgroup of nanorobotics, their interaction with their environment *in vivo* is complex and ever shifting. As the MNRs travel throughout the bloodstream, proteins may be adsorbed to the surface of the micro/nanorobots. This accumulation of proteins onto the nanorobot is termed the *protein corona phenomenon.* The protein corona can affect nanoparticles' size, stability, shape, and surface properties, therefore also risking altering their functions. This can impact a nanorobots' ultimate fate within the body, its capacity to deliver therapies, and its toxicity levels (Zanganeh et al., 2016). The size and shape may also cause other issues in the body such as blockages, especially blood vessels.

The protein corona is split into two distinct portions based on the nature of the bound proteins, both the hard and soft corona. The hard corona is formed by strongly adsorbed high-affinity proteins that bind readily to the nanoparticle in question, while the soft corona consists of low-affinity proteins that have weaker bonds with the nanoparticle. Soft corona is more dynamic compared to the hard corona, developing a shorter lifespan and readily changing (Kopac, 2021). The primary causes of protein corona formation are due to the non-covalent bonds between the protein sulfhydryl group and any metal atoms in the substrate of the nanorobot (Mao & Wan, 2023) as well as hydrophobic interactions which form the inner corona and the shielding of electrostatic adsorption which are responsible for the formation of the outer corona (Kopac, 2021).

While it seems completely avoiding the formation of a protein corona is impossible, researchers are instead focused on controlling its formation and mitigating the undesirable effects of the protein corona. Instead, pre-adsorbing certain proteins and controlling the composition of the corona before injection *in vivo* has been shown to improve the circulation of nanorobots (Kopac, 2021). The precise effects of this corona can vary drastically depending on the adsorbed proteins that latch onto the nanoparticle, with effects ranging from disastrous to potentially beneficial.

If nanoparticles are bound to extracellular opsonin proteins, it will trigger opsonization and result in the tagging and removal of a large number of nanorobots by phagocytic cells in the immune system before they're able to complete treatment (Mao & Wan, 2023). A protein corona rich in dysopsonins or apolipoproteins can provide the opposite effect. Dysoposonins such as albumin or allowing the nanoparticles to highly reduce the chance of being detected by the immune system and ingested by phagocytes, giving them a form of "camouflage" (Kopac, 2021). However, this can present its own set of problems. It can eliminate the targeting ability of these nanorobots and severely hamper their ability to deliver therapies to target tissues and organs. It has been discovered that these negative effects can be mitigated while preserving the camouflage ability of the nanorobots by using certain plasma proteins before introduction *in vivo* (Mao & Wan, 2023).

Zwitterionic polymer shells have also been looked at as a possible solution in combatting the effects of the protein corona. They can mimic the phospholipid bilayer of cells; they have hydrophilic properties and have a neutral charge. Therefore, there is an increase in both stability and solubility while blocking the nonspecific adsorption of proteins, and platelets and potentially reducing immunogenicity (Mao & Wan, 2023). While considering these studies, a clear link emerges between the nanomaterials used for MNR design and its safety and function *in vivo*.

2.2 Material Considerations

The material considerations of MNRs, nanorobots especially, is a very complex and high-stakes process. Due to the small size of nanorobots, the materials used in their creation can have unique properties which differ greatly from their macro-scale counterparts (Gupta & Xie, 2018). Furthermore, nanoparticles can differ notably in surface composition, surface structure and core/ligand interface structures due to nanoparticles not being completely identical (Jin & Higaki, 2021). When determining MNR materials, size, shape, surface charge, chemical functional groups on the surface, overall chemical composition, and surface area are relevant factors to consider in MNR design decision-making for biosafety and purposes of biodegradability and ease of removal or reabsorption by the system. The chemistry of the biomaterial is the most significant in determining the overall safety and cytotoxic properties of the MNR in question (Mao & Wan, 2023).

2.2.1 *Safety of Biological Material*

A notable trend in the design of micro and nanorobots is the use of biological materials to increase biocompatibility and safety within the system. To avoid an immune response while allowing for good biosafety, studies have designed MNRs using DNA, exosomes, cells and microorganisms. Researchers have previously made functional microrobots using sperm cells, motile bacteria, neutrophils, cardiomyocytes and macrophages (Wan et al., 2021). Biological surface modifications have been made to prolong the blood circulation cycle and enhance the nanorobots' ability to reach tumour sites. These include albumin, liposomes, red and white blood cell membranes and platelet membranes. Biological materials have benefits beyond their biocompatibility, as natural cells already have better targeting and movement capabilities. Furthermore, aggregates of organic active ingredients are used for their low toxicity and immunogenicity and the relative ease with which they can cross biological barriers such as the blood-brain barrier. Sperm cells can move through more viscous fluids, without needing culturing and are relatively easy to handle. At the same time, neutrophils are already attracted to sites of inflammation, which makes them ideal carriers for therapies (Mao & Wan, 2023).

Despite these benefits, the choice of substrates for purely biological micro and nanorobots is highly limited compared to chemical or physical designs. While they have great risk in biosafety, chemical and physical MNRs provide significantly more control in materials, size, chemical and mechanical properties and just have a general designability which makes them useful for purposeful design. Other biomaterials also must have their immunogenicity and oncogenicity considered. A good middle ground has been found by incorporating biological and synthetic materials. The practice helps ensure that metals become enzyme-resistant and generally increases their stability in the body (Graczyk et al., 2020). The alteration of MNR biomaterial through chemical or physical means has also proven fruitful by imparting novel abilities. Certain highly biocompatible but immobile cells such as red blood cells and platelets have been previously modified with synthetic materials such as enzymes, iron oxide and metal magnesium to move through magnetic fields (Wan et al., 2021).

2.2.2 *Synthetic Material and Metals*

Despite the biocompatibility and safety of synthetic materials often being lower than biological materials, these materials have greater diversity and designability for micro and nanotechnology. There is an abundant choice of materials when it comes to physical and chemical MNRs such as hydrogels, zwitterionic polymers, magnesium-based microspheres, gold, silver, porous silica and certain composite materials. By designing synthetic materials such as organic polymers with similar mechanical properties compared to cells and tissues, further biocompatibility can be achieved. Certain zwitterionic polymers can mimic the phospholipid bilayer of cells, thereby increasing their biocompatibility. These polymers have hydrophilic properties and maintain a neutral charge, not only increasing the solubility and stability of drug delivery but also blocking the non-specific adsorption of organic particles. By doing so, this permits nanoparticles to mitigate the protein corona effect which can lower immunogenicity (Mao & Wan, 2023).

Metallic nanoparticles have been distinctly beneficial due to how versatile they can be. The technology of spherical nucleic acids (SNA) has been founded on the use of these materials, pairing a metal core alongside nucleic acids bound to the surface. The most studied metals in nanomedicine consist largely of gold, platinum and silver. Gold nanoparticles (AuNP) have been one of the most widely used and researched synthetic materials in the field. On the nanoscale, gold is low in toxicity and is quite resistant to chemical and enzyme exposure. Gold can reflect infrared radiation very well and is useful for staining and radiation therapy applications in cancer treatment. It remains non-reactive with most acids and bases, which keeps it largely stable during transport within the body. Furthermore, gold is very malleable, which makes it easy to work with and improves its designability (Graczyk et al., 2020).

However, despite the benefits of gold, there have been studies to suggest that DNA can become unstable in the presence of gold nanoparticles. When a gold nanoparticle approaches the DNA strands, entropy increases, and stability decreases. This can cause damage to the DNA, preventing replication (Izanloo, 2017).

2.2.3 *Surface Modifications of Nanorobots*

Alongside the selection of materials, biosafety considerations should be made for the surface modifications of the MNRs. Surface modifications contribute to the toxicity of the micro/nanorobots as well as the functionalities and in vivo interactions. Certain chemical and physical surface modifications can be used to lower the toxicity of micro- and

nanorobots while maintaining beneficial characteristics. One of the more notable modifications is polyethylene glycol (PEG). PEG is the most studied material for surface modifications of MNRs. The method of application is for the activated PEG to be covalently bonded to the surface of the MNRs. It can couple to the surface of nanorobots to increase stability and water solubility, therefore prolonging circulation time. Furthermore, PEG surface modifications can reduce immunogenicity and antigenicity (Mao & Wan, 2023).

Despite this, PEG itself is not very stable and oxidizes when in the body. PEG also has a rapid clearance rate from within the body, meaning that high PEG within the body can lead to high amounts of anti-PEG immunoglobulins, causing phagocytes to clear out PEG and severely shorten its lifespan in the body. However, there are several solutions to this problem, one of them being PEG-breaking links which break the bonds with polyethylene glycol in special environments. This allows the PEG to keep the MNRs stable throughout the blood circulation and, upon reaching the tumour tissue, the PEG layer breaks off and can be rapidly cleared out by the body while the nanorobot does its job. In other situations, polyvinyl pyrrolidone and betaine as a replacement for the PEG.

Furthermore, there are polypeptide surface modifications which can improve the biostability and binding activity of the MNRs. Polypeptide surface modification also improves the capacity of the therapy to interact with target tissues preferentially as well as their targeting ability. For example, the targeting ability of some MNRs which utilize the RGD (arg-gly-asp) polypeptide aids in targeting tumours. However, the proteolytic enzymes within the gastrointestinal tract can easily destroy them, and the surface treatment causes low solubility, and high antigenicity and can be easily removed by antibodies (Mao & Wan, 2023).

3 RESULTS

3.1 Role of Nanorobots in the Body

Natural nanoscale organisms play an important role in the intricate environment of the human body, showing extraordinary complexities and functionality. Cells contain a variety of organelles such as mitochondria and ribosomes. These organelles carry out vital functions such as protein synthesis and energy production. Additionally, enzymes function as molecular machines that efficiently catalyze chemical reactions with great precision and specificity. Motor proteins such as dynein and kinesin, transport cargo along microtubules (Abraham et al., 2018). These natural nanorobots contribute to the general health and functionality of the body by performing crucial functions in metabolic, cell signalling, DNA replication and cellular transport activities. Natural nanorobots play crucial roles in the body by preserving homeostasis, controlling cellular functions, and coordinating intricate biological processes. Understanding their characteristics and roles has immense potential to solve human biological problems and progress biomedical sciences and technology. When it comes to innovating the field of nanorobotics in healthcare, using these “biological nanorobots” as a template for therapeutic agents will help researchers in creating increasingly potent treatment methods that can easily congregate near target areas and display biocompatible properties.

However, while these biological systems have evolved to function optimally in specific conditions, the use of synthetic nanorobots introduces novel complexities. Unlike naturally occurring nanomachines, synthetic nanorobots must overcome the body's immune defences and biophysical barriers, a challenge that is rapidly becoming the focus of modern studies in the field. Studies have indicated that while nanorobots can mimic certain functions of natural proteins, their long-term integration into complex biological environments remains problematic due to potential immune responses, toxicity and unwanted interactions with the human body (Kopac, 2021). Thus, the creation of synthetic nanorobots that can safely and effectively operate within human systems is still in its developmental stage. Despite this, there is promising research to indicate that this technology is an avenue worth exploring for therapeutic applications such as cancer treatment.

3.2 Biological Nanorobots

Biological nanorobots are microscopic devices made from biological materials such as DNA, lipid membranes and protein. They take building blocks from the human body and use them as materials in their composition, relying on their biosafety, interactions with the human body and pre existing properties. These nanorobots are designed to execute specialized tasks at the nanoscale, taking advantage of the inherent complexity and functionality of biological systems. Unlike typical nanorobots, which are frequently synthetic constructs, biological nanorobots have distinct advantages such as biocompatibility, programmability, and self-assembly. For instance, ribosomes and various intracellular vesicles could be considered basic biological nanorobot forms. Drug delivery targeting is one of the most promising uses for biological nanorobots. To minimize side effects and maximize treatment efficacy, researchers can deliver therapeutic payloads with previously unknown precision by designing nanoscale vehicles that can identify and selectively bind to diseased cells or tissues (Nehru et al., 2022). Biological nanorobots can also be engineered to react to particular stimuli, such as pH changes or the presence of particular biomolecules. This allows for the drug to be released at the appropriate location. Additionally, biological nanorobots can be used in the application of diagnostics and imaging. These nanorobots can be designed to detect and identify particular molecular targets within the body by adding magnetic nanoparticles to their structure (Yan et al., 2023). This can provide important insights into the pathophysiology of diseases and the effectiveness of treatments. Moreover, biological nanorobots can be fitted with sensors or actuators to continuously monitor physiological parameters, opening up new possibilities for healthcare monitoring and personalized medicine.

In the context of bladder cancer treatment, biological nanorobots have prospects for cutting-edge therapeutic modalities, including nanorobots driven by urease. These nanorobots produce a localized increase in the pH within the tumour microenvironment by using the enzyme urease to catalyze the breakdown of urea into ammonia and carbon dioxide (Niu et al., 2023). The pH shift may counteract the acidic environment typical of malignant tissues, which may improve the effectiveness of concomitant antibodies or chemotherapy treatment.

3.2.1 *Cellular Nanorobots*

Cellular nanorobots carry out diagnostic tasks on a microscale by utilizing the inherent capabilities of natural cells. In this context, the word "nanorobots" refers to the reused host cell or enclosed system of nanoscale machinery that is controlled by the cellular environment. A cell is a specialized type of nanorobot that can synthesize and assemble other nanorobots (Rajendran et al., 2021). Cellular nanorobots are a viable method for targeting therapy and diagnosis in the treatment of bladder cancer. These nanorobots can be made to target bladder cancer cells directly while causing the least amount of harm to healthy organs. They can carry therapeutic payloads directly to the tumour site, increasing treatment efficacy and minimizing negative effects. Some examples include chemotherapeutic medicines and molecules that alter DNA. Furthermore, cellular nanorobots can be integrated with sensors or imaging agents to detect tumour growth or identify cancer biomarkers, enabling early diagnosis and therapy response monitoring.

3.2.2 *Molecular Nanorobots*

Compared to other nanorobots in the body, molecular nanorobots are slow but highly efficient for various reasons. Molecular nanorobots are widely used in biological systems for intra- and intercellular tasks. These nanorobots are composed of nucleic acids and protein molecules. A significant advantage of molecular nanorobots is their small size, enabling precise construction at the molecular level. This allows the creation of highly intricate machines comparable in size to large biological molecules, making them well-suited for tasks within the body. Molecular nanorobots have the potential to revolutionize medical procedures with these capabilities, including the meticulous rebuilding of a patient's bladder tumour (Kong et al., 2023). For example, a molecular "smart pebble" nanorobot with chemical change detection capabilities might be created to track bladder health and in the event of an illness, alert other nanorobots to the issue. When molecular nanorobots are provided with biosensors or imaging technologies, they can track a tumour's reaction to treatment in real time, offering valuable information about how well a treatment is working and how the disease is developing (Kong et al., 2023). Clinicians can use nanorobots to detect changes in tumour size, metabolic activity, or molecular signalling pathways and modify treatments to improve patient outcomes and lower the risk of disease recurrence.

Synthetic nanorobots can be created by applying the mechanisms of action observed in natural nanorobots. By making alterations in general functionality, modes of propulsion, and material selection, nanorobots can be fabricated to provide therapeutic effects with improved accuracy and precision. Specifically, chemical and DNA origami nanorobots are two main configurations that are discussed and compared below.

3.3 Chemical Nanorobots

While chemically powered nanorobots demonstrate significant potential through their use of catalytic reactions to drive propulsion, their clinical application remains faced with certain challenges. The use of hydrogen peroxide as a fuel, for example, has been shown to produce cytotoxic byproducts in MNR studies, causing concerns about its biocompatibility (Feng et al. 2022). Although hydrogen peroxide-driven nanorobots have demonstrated effective potential for movement within patients, their cytotoxic nature limits their potential in vivo and can come with its own range of side effects, particularly concerning long-term cancer treatments. Recent studies have instead turned to using biocompatible chemical nanorobots that use molecules such as urease, which offers a safer alternative by breaking down urea in the bladder—into ammonia and carbon dioxide (Feng et al. 2022). The initial research on urea-driven nanorobots is promising even in its infancy, however, the sparse knowledge concerning this treatment should be remedied before serious discussions of clinical applications can be considered.

3.3.1 *General Functionality*

The general structure of the chemical-driven nanorobot encompasses two primary components: its nanoscale frame and surface modification methods. Crucial to its functionality, the nanoscale frame of these robots is based on a fundamental material known as carbon nanotubes. This remarkable material is promising for its function as a framework due to its highly versatile nature and unique properties (Iijima, 1991). Carbon nanotubes have nanometric diameters and lengths ranging from micrometers to millimeters thus providing them with a strong foundation, high mechanical strength and increased electrical conductivity (Anzar et al., 2020). Furthermore, their high surface area to volume ratio gives room for opportunities for surface modification thus increasing the robots' versatility (Anzar et al., 2020). The multiwall arrangement of these structures is also vital to the many diverse properties it provides, such as an increase in motion and flexibility thus portraying it as a favourable component within nanorobots (Kong et al., 2023). Alongside this, carbon nanotubes display great electronic and mechanical properties, thus resulting in the material Young's modulus being five times the magnitude of the material steel (Anzar et al., 2020). The composition of the nanotube wall itself, based on chiral indices, also portrays metallic or semiconductive properties. Other

properties of this material include chemical inertness, low toxicity levels and high mechanical robustness making it an ideal material for multiple biomedical applications such as nanorobots (Kong et al., 2023).

Additionally, surface modifications play an important role in customizing the properties and operations of nanorobots. These modifications are applied through various techniques such as molecular self-assembly, coating deposition and chemical functionalization. However, the two methods that stand out the most within the structure of chemically driven nanorobots are self-assembly and physical vapour deposition, also known as PVD (Feng et al., 2022). This method uses a solid source as a means of material vaporization to form a vapour phase. The vapour then condenses to a thin coating layer which is applied on top of substrates to facilitate surface properties within the nanorobot itself (Feng et al., 2022). A common application for this technique is the creation of islands or hemispherical cap structures through the means of deposition of metal with a catalyst on top of surface silica particles (Feng et al., 2022). This process not only expands the catalytic behaviour of the altered structure but also increases its surface area, promoting more interaction with reactants. On the other hand, self-assembly is also an important technique in which protein molecules and colloidal nanoparticles get arranged into organized structural formats without external intervention (Yao et al., 2021). This is done through the presence of various bonding techniques, specifically hydrogen bonding interactions, Brownian forces, and dipole interactions. Self-assembly provides various advantageous properties within the structure of chemically driven nanorobots such as simplicity, precision, versatility and compatibility with biological systems making it suitable for applications such as drug delivery (Yao et al., 2021).

3.3.2 *Movement and Power Sources*

Chemically driven nanorobots function based on reactions with chemicals in the surrounding environment. They accomplish this by converting the energy stored in biomolecules into mechanical energy used for propulsion. Many of these nanorobots incorporate the use of catalytic mechanisms since enzymes serve as an engine by providing an avenue for energy conversion. They do this by breaking down their corresponding substrates, which release energy used for kinetic motion (Feng et al., 2022). This method for achieving movement is inspired by natural biomotors, such as myosin, which induces intracellular propulsion through decomposing adenosine triphosphate (ATP) (Zhang et al., 2022). Some common enzymes suitable for use in biological applications with nanorobotics include hydrogen peroxide, urea, glucose, and water. Both bimetallic and tubular nanorobot structures are commonly employed to achieve mechanical movement through propulsion mechanisms. Tubular nanomotors include a confined hollow interior that provides space for chemical reactions to be carried out. As a result, tubular configurations can be easily propelled by a strong thrust force produced by bubbling (Zha et al., 2018). They can achieve locomotion in high-strength ionic environments, making them well-suited for use in biological fluids. Additionally, the use of a bimetallic coating introduces an asymmetric distribution of a metal capable of decomposing a specific compound fuel source. These structures have versatile bidirectional movement capabilities based on the placement of the catalytic metal (Wang et al., 2023).

3.3.2.1 Hydrogen Peroxide Driven Nanorobots

Hydrogen peroxide decomposition is commonly employed to produce locomotion in chemically driven nanorobots. This is the case as the bonds within hydrogen peroxide are unstable, which allows for molecular breakdown into water and oxygen due to the action of a catalyst. Platinum is most commonly used in this process, as this noble metal functions as an efficient catalyst. The deposition of platinum helps to promote propulsion on a nano-scale through mechanisms of either bubble ejection or self-phoresis (Fernández-Medina, 2020). In this context, bubble-propelled nanorobots use the recoil force of the oxygen gas released from the breakdown of hydrogen peroxide. This in turn allows for forward movement (Sánchez, S & Pumera, 2009). Conversely, in self-phoresis, spontaneous particle motion is initiated by imposing a quantity gradient related to solute concentration, electric potential, or temperature (Uros et al., 2023). This mode of movement generation is electrokinetic and can be achieved for nanorobots which are bimetallic. An example of this is demonstrated in Pt/Au nanorobots, which are composed of individual segments containing gold and platinum. An anode reaction occurs at the platinum electrode, where hydrogen peroxide is oxidized. Furthermore, the cathode reaction takes place at the gold segment, whereby the hydrogen peroxide becomes reduced. This electron transfer attracts the movement of protons to the surrounding environment of the nanorobot, which creates propulsion at the platinum end (Sánchez, S & Pumera, 2009). However, the use of hydrogen peroxide to achieve such movement has since declined due to cytotoxicity in the body (Feng et al., 2022). Consequently, researchers are now experimenting with new fuel sources to enhance biocompatibility.

3.3.2.2 Urease Driven Nanorobots

Researchers have worked on developing alternative fuel sources derived from biological fluids. Therefore, to allow for more refined applications in living organisms, recent investigations have been made into the use of enzymes such as urease as a viable power source. Incorporating urease into the surface design of the nanorobot allows for the breakdown of urea, which is present in large concentrations within the urinary bladder (Feng et al., 2022). As urea decomposes, it releases carbon dioxide and ammonia gas, producing a product gradient. This allows for mechanical propulsion via self-phoresis, (Uros et al., 2023). Researchers have also studied the use of tubular nanomotors that are fuelled by urea. These nanomotors achieve propulsion by employing enzyme-substrate reactions involving urease

and urea. The products of this reaction accumulate inside the nanotubes, creating an internal flow of gas that propels into the external environment via a small tubular opening.

3.3.3 *Biosafety of Chemical-Driven Micro/Nanorobots*

The biosafety considerations of chemical-driven micro/nanorobots are driven primarily by the biocompatibility of the materials used and the cytotoxic behaviour of any waste products created by the MNR propulsion system. Similarly to physical motors, chemical motors provide numerous material options for MNR design, giving researchers flexibility in choosing suitable components. However, both chemical and physical micro/nanorobots raise significant concerns regarding their overall biosafety. When compared to biologically designed MNRs, their *in vivo* use could be possibly restricted by their harmful effects. Certain waste products created by the reactions that allow the MNR to move through its select medium may not be biodegradable or easily removed from the body (Mao & Wan, 2023). Two major chemical fuels used for MNRs with a high selection of research are hydrogen peroxide fuel and urease.

Some of the earliest micro/nanorobots were chemically driven, using hydrogen peroxide as their fuel source. In 2002, a platinum-catalytic hydrogen peroxide self-driving nanorobot was studied (Wan et al., 2021). It soon came to light that the high hydrogen peroxide concentration (5%-20%) posed a high risk of toxicity. Furthermore, the waste products created by reacting the hydrogen peroxide were also harmful to the human body. The waste products of hydrogen, carbon dioxide, ammonia, magnesium hydroxide and platinum could cause severe harm to the patient's body, with some of the residual materials being non-degradable (Mao & Wan, 2023).

A tested solution to address this fuelling issue was to control the environmental temperature, reducing the necessary concentration of hydrogen peroxide to just 0.1%. Despite this significant decrease in hydrogen peroxide concentration for the fuel, it remains in high enough quantities to be cytotoxic as the average concentration of hydrogen peroxide in the human body is less than 0.002% (Mao & Wan, 2023). In contrast to the hydrogen peroxide option are urease-powered MNRs, which are designed to only function in a high urea environment to move, giving them great potential in treating disease within the bladder. Urease-powered MNRs use the natural urease enzyme which catalyzes the hydrolysis of urea into ammonia and carbon dioxide. Urease is incredibly biocompatible with the human body and therefore does not have as large concerns as using hydrogen peroxide for driving.

3.4 DNA Origami Nanorobot

DNA origami is a new technology in the field of nanorobotics. It is commonly used in the development of DNA nanorobots and plays an important role in achieving targeted drug delivery. By using the principles of DNA base-pairing, the desired shape can be achieved to optimize functionality and use. Due to their highly versatile structure, DNA origami allows for the incorporation of various components into their surface morphology. This includes the integration of biomolecules and functional ligands to enhance performance and targeting ability. Although these nanorobots are typically incapable of autonomous movement, they are well renowned for their precise treatment capabilities (Hu et al., 2020).

3.4.1 *General Functionality*

The general structure of the DNA origami nanorobot is centred around the use of DNA molecules as programmable building blocks, thus allowing for its motion and drug delivery operation. This foundational structure of the nanorobot is accomplished through utilizing the DNA origami technique along with bottom-up fabrication. DNA origami, a technique discovered by Rothemund, involves the principle of complementary base pairing to repeatedly fold a single-stranded DNA molecule. The DNA is then fixed by oligonucleotides (which are short sequences of nucleotides) to achieve its desired structure or shape (Kong et al., 2023). After this hundreds of DNA strands, known as staple strands, are put into various sequences to create a long single-stranded scaffold that can be conformed into the required shape through a process known as one-pot thermal annealing (Bush et al., 2020). This process allows for the construction of 2D or 3D nanoscale objects with high precision. Furthermore, the foundational nanostructure is built through bottom-up fabrication, an approach based on the concept of starting with individual atoms or molecules and assembling them into larger, more complex structures (He et al., 2023). By combining these two approaches and the development of complex shapes through controlled bends and twists, this nanoscale robot is created. However, to incorporate additional reversible motion within these structures a switchable flap that can respond to external stimuli is also developed (Torelli et al., 2014). This flap is controlled by the release of nucleic acid, housed within a tube found inside the robot itself. When the nanorobot is activated, it manipulates the tube, causing the nucleic acid to be released and the flap to transition from a disarmed to an armed configuration allowing for the delivery of drugs within various areas of the body (Torelli et al., 2014).

When considering DNA as a base component of these structures, it is important to consider its material properties. DNA can repair itself when put into stressful environments because of its pliable and robust nature. This structure is also a good application when constructing movable parts due to its malleable and spring-like properties (Nummelin, 2020).

Furthermore, its torsional rigidity, high stiffness and flexibility are ideal when considering the making of detailed origami structures. Lastly, due to its Young's modulus (0.3-1.0 GPa) being close to that of hard plastic and its exceptional flexibility, DNA makes an ideal material for nanoscale structures (Peters et al., 2013).

3.4.2 *Movement and Power Sources*

Scaffolded DNA origami structures can be designed to incorporate stiff double-stranded DNA (dsDNA) and flexible single-stranded DNA (ssDNA) components (Marras et al., 2015). This allows for the creation of nanorobotic devices that can achieve motion with a high degree of precision. Some designs include features capable of linear, rotational, and angular motion. They utilize mechanisms of mechanics such as hinges and sliders to mimic the function of macroscopic machinery. In the context of DNA origami, various forms of motion have been fabricated, including walking, sliding, rotating, and rolling. Many of these mechanisms are inspired by natural biological motors, such as kinesin and dynein. These are motor proteins that function in the body by transporting intracellular payloads through movement along microtubules. Energy for this action is acquired through the breakdown of ATP in a process known as ATP hydrolysis (Zhan et al., 2023). Using the fundamental principles that govern this motion, different DNA structures have been synthesized to provide similar movement and therapeutic effects.

3.4.2.1 DNA Walker System

The DNA walker system uses a track to facilitate movement. This track is composed of single-stranded DNA molecules, as well as anchor strands to form a template. The walker can achieve spontaneous motion through the creation of a free energy gradient. This causes the walker to translocate toward a configuration with a lower energy gradient (Li et al., 2016). In particular, this movement is prompted by the addition of DNA strands called fuel strands, or by the addition of molecules capable of cutting or altering DNA. This can include the addition of DNAzymes or restriction enzymes (Li et al., 2016). Energy changes disturb the initial state of equilibrium, causing the walker DNA to progress along the track (Mao et al., 2022). Advancements in DNA nanotechnology have enabled the development of customizable tracks. For example, as shown in Figure 1, researchers have achieved programmed directional movement of the DNA walker system. In this development, the walking strand is anchored to the track through the implementation of three DNA feet. Each of these feet moves independently along the track in a carefully coordinated manner to prevent the strand from being released. The repeated recycled binding and cleavage of the DNA legs allow for locomotion along the track's length (Mao et al., 2022).

Similar to how these nanorobots react to particular stimuli to accomplish targeted movement, nanorobots intended for clinical use in bladder cancer treatment may be engineered to interact with the complex genetic mutations, epigenetic changes, and dysregulated signalling pathways typical of the illness.

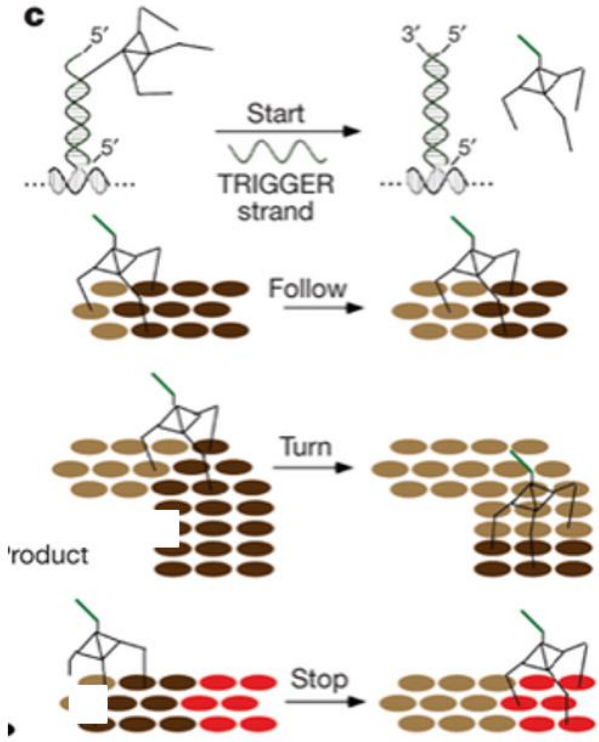

Figure 1: DNA Origami Walker System to Achieve Directional Movement

Note. The implementation of the DNA walking strand with three feet allows for enhanced anchoring ability and more precise movement along the track.

3.4.3 *Biosafety of DNA Origami*

Recent studies of DNA origami nanotechnology safety have been rather promising compared to other forms of treatment. The biosafety of wireframe nucleic acid nanoparticles (wireframe NANPs) has been evaluated by Wamhoff et al. in mouse models with a therapeutically significant dose. The study concluded that DNA-based nucleic acid nanoparticles are not acutely toxic after a single dose for tumour treatment. It did not negatively impact albumin-globulin ratios nor overall levels of globulin nor was monocytosis discovered. There was no kidney or liver damage found despite the main site of accumulation of the nanoparticles being in the liver (Wamhoff et al., 2023).

A significant concern in DNA-based treatments, however, is by causing autoimmune problems by harming tolerance to DNA and triggering the formation of antibodies. During the tests of wireframe NANPs by Wamhoff et al., the researchers found that the treatment did not induce the SLE phenotype, or Systemic Lupus Erythematosus, which is an autoimmune disorder that triggers the creation of autoantibodies. However, higher doses were not tested in this study, and therefore it is important to consider the health hazards of constant DNA origami treatment. The nanoparticles naturally accumulate in the liver, yet seemingly leave it unharmed. Upon accumulation within the liver, the nanoparticles were rapidly cleared out thanks to the high biodegradability of the nanomaterials. Furthermore, the NANPs can have their biodegradability tuned and can be further modified to control their interactions with the immune system (Wamhoff et al., 2023).

4 DISCUSSIONS

4.1 Mechanisms of Bladder Cancer

Bladder cancer is driven by an interaction of genetic mutations, epigenetic modifications and changes in gene expression brought on by exposure to external factors. The unusual proliferation of cells lining the bladder wall is the root cause of bladder cancer, which has many underlying causes. One of the key mechanisms of bladder cancer is genetic mutations, causing abnormal cells to develop out of control in the bladder lining, particularly in tumour suppressor genes such as TP53 and oncogenes such as HRAS (Lokeshwar et al., 2022). The tumour suppressor gene known as the guardian of the genome (TP53) has a vital role in preserving genomic stability and halting the development of malignant cells. Mutations in TP53 limit the protein's capacity to control the process of the cell cycle and initiate DNA repair, which can increase genetic mistakes and uncontrolled proliferation of cancerous cells. In addition to TP53, oncogene mutations such as HRAS have a major role in the formation and advancement of bladder cancer. Oncogenes are genetic factors that, when altered or excessively expressed, promote the development and endurance of cells (Lee & Muller, 2010). Transcriptional mechanisms affecting development and differentiation are carried out by a protein encoded by the proto-oncogene HRAS (Lee & Muller, 2010). When HRAS is constitutively activated due to mutations, tumour development and dysregulated cell growth might follow.

Additionally, environmental factors such as tobacco smoking and industrial chemicals can cause DNA damage in bladder cells, which can accelerate the development of cancer. Aromatic amines like 4-aminobiphenyl (ABP), found in cigarette smoke and industrial chemicals are impacted in BC development due to their ability to form DNA adducts, potentially leading to mutations (Bellamri et al., 2019). DNA adduct formation is influenced by liver enzymes such as glutathione S-transferase M1 (GSTM1), cytochrome P450 1A2 (CYP1A2), and N-acetyltransferase 2 (NAT2) (Halaseh et al., 2022). While NAT2 decreases adduct formation, CYP1A2 enhances adduct formation. Studies highlight the interaction between genetic variables, enzyme activity, and smokers' vulnerability to BC by indicating that people with slow NAT2/rapid CYP1A2 phenotypes and those deficient in the detoxifying enzyme GSTM1 are more likely to develop BC (Halaseh et al., 2022).

4.2 Connection to Nanorobots

For bladder cancer treatments to be developed effectively, it is essential to comprehend these pathways. Nanorobots powered by urease employ the activity of the urease enzyme to directly pinpoint cancer cells, capitalizing on their increased presence in tumours. These nanorobots deliver targeted benefits by efficiently transporting urea-based therapies to cancerous cells. Additionally, DNA origami nanorobots offer a flexible platform for controlled release and accurate delivery of therapeutic agents. They are intricately designed with specific configurations and adeptly navigate the complexities of bladder cancer by targeting the genetic or epigenetic changes that accelerate the progression of the disease. Through molecular sensing, both types of nanorobots make it possible to monitor therapy in real time, thus aiding in the customization of treatments. Given the complex molecular characteristics of bladder cancer, the integration of these cutting-edge nanotechnologies has the potential to improve patient outcomes.

4.2.1 *Man-made and Natural Nanorobots*

Transitioning from the role nanorobots play in bladder cancer, it is crucial to understand the comparison between the roles of man-made and natural nanorobots in regards to their composition, power and function. Chemical/man-made nanorobots are composed of synthetic materials which include carbon, nanotubes, metal nanoparticles, polymers and more (Kong et al., 2023). On the other hand, natural nanorobots are made up of natural biological molecules such as proteins, nucleic acids, enzymes, lipids and carbohydrates (Kong et al., 2023). In terms of their mode of transportation, they can be compared based on their propulsion methods. For instance, the main difference is that natural nanorobots use biological metabolic pathways to circulate in the body to perform their functions. In contrast, man-made/chemical nanorobots use external sources such as light, temperature, and pH that might cause

them to differ in their structure. Natural nanorobots are less controllable by researchers than synthetic ones and react to biological signals and environmental factors. This is because they have been carefully tuned by evolution. The methods of power and their composition allow for the execution of varying functions. Although man-made and natural nanorobots serve the same purpose of benefiting the human body, they differ in their main functions.

Man-made nanorobots aim to target defected sites/tumours and can do so while minimizing the damage to healthy tissues/cells, further reducing side effects. They can also aid in the diagnosis and detection of disease. In the application of bladder cancer, they can precisely penetrate tumours and the impermeable linings of the bladder, allowing for increased tumour targeting. On the other hand, natural nanorobots contribute to the daily functions that occur within the human body. For example, some of the many tasks include protein synthesis, cellular transport as well as DNA replication. Overall, even though chemical/man-made and natural nanorobots share the similar goal of benefiting the human body, their differences in composition, power, and function emphasize their unique capabilities and applications.

4.2.2 *Comparison of DNA Origami and Chemical MNRs*

DNA origami and chemically driven nanorobots demonstrate versatility in terms of their structure and function within a biological setting. When comparing the motion of DNA origami and chemically driven nanorobots, it is important to consider the precision of movement, as well as the mechanisms used for propulsion. Chemically powered nanorobots utilize enzymatic reactions to promote drive, thereby allowing them to adapt to changing conditions within the body environment. Based on their material structure, these nanorobots can be designed to interact with different biological fuel sources and enzymes depending on their specific application. Therefore, this allows for autonomous motion, detection, and treatment of cancer cells, which is advantageous (Saadeh & Vyas, 2014). On the other hand, DNA origami typically employs the use of external mechanisms for propulsion, as previously discussed. Therefore, these nanorobots are not capable of autonomous movement (Hu et al., 2020).

With regards to material comparisons within these two nanorobot types, chemically driven nanorobots have a higher Young's modulus due to the increased strength of their base material, unlike that of DNA origami molecules. This higher modulus of elasticity and high flexibility enables chemically driven nanorobots to have exceptionally high mechanical strength and durability, thus allowing them to have the needed properties to endure the increased levels of mechanical stresses and the rough environment encountered within the body. As a result, chemical nanorobots are expected to be able to maintain their functionality and structural integrity over large periods, making them a more reliable method of curing cancer.

Lastly, chemical nanorobots are designed to respond dynamically to changing environments (such as changes in pH, variations in substrate concentration within tumour microenvironments, etc.) whereas DNA origami nanorobots are mainly designed to react to external stimuli (ex: temperature changes) (Kong et al., 2023). This specific property of the chemical nanorobots serves as an advantageous attribute when considering applications such as the treatment of cancer as their drug delivery response to tumour-specific cues such as pH levels or substrate concentrations are very specific and targeted. Therefore, minimizing the side effects of drugs while maximizing treatment efficacy (Kong et al., 2023). Summary of chemical and DNA origami is presented in Table 1.

Table 1: Summary of the chemical and DNA origami nanorobots

Nanorobot	Material	Power Source	Material Properties	Advantages	Applications
Chemical	**Framework:** Carbon nanotubes	Endogenous power sources are driven by catalytic reactions involving hydrogen peroxide and urea (Hu et al., 2020).	High mechanical strength/stability, high electrical conductivity, high surface area to volume ratio, versatility, flexibility, chemically inert, low density	Autonomous movement, targeted treatment, responsive to stimuli such as pH and temperature changes	Drug delivery, cancer treatment of specified areas (based on substrate/fuel concentration), transportation of nucleic acids and microorganisms, diagnostics, anti-inflammatory and anti-bacterial effects (Sun et al., 2024).
DNA Origami	**Framework:** DNA Strand	Exogenous power sources are driven by external mechanisms such as magnetic fields and light energy (Hu et al., 2020).	Not brittle, pliable, deforms elastically, subtle bending stiffness, high tensile stiffness, strong resistance to bending and twisting, torsional rigidity, and malleability (Peters et al., 2013).	Lower cytotoxicity, enhanced stability and adaptability	Improved efficacy of chemotherapy, reduced side effects of cancer treatment, and reduced drug resistance (Udomprasert & Kangsamaksin, 2017).

4.2.3 *Comparison of Urease and Hydrogen Peroxide*

Whilst considering innovative treatments for bladder cancer one of the most important factors to consider with regards to the chemically driven nanorobot is the fuel source being used at hand. By comparing two of the most widely used fuel sources, hydrogen peroxide and urease, their impact on curing bladder cancer can be revealed. Hydrogen peroxide is an effective fuel source due to the vast array of metals that can be used to decompose it catalytically. However, the use of hydrogen peroxide has limited applications in vivo, due to toxicity in the body. Specifically, the decomposition of hydrogen peroxide leads to the release of reactive oxygen species (ROS), which can lead to necrosis or carcinogenic effects (Shields et al., 2021). It is also important to note that the presence of some specific catalyst may result in a non-homeostatic response within the body. Therefore, the compounds released pose significant risks in biomedical applications, as they can initiate damage within the cells by causing oxidative stress. At the same time with this specific substrate, there is a potential risk of hydrogen peroxide diffusion beyond the targeted tumour area thus causing unintended impacts on healthy tissue around the bladder tumour. This in turn makes hydrogen peroxide well-suited for use in other applications outside of the body. On the other hand, urea is a metabolic product found naturally within the body. As a result, the use of this compound is considered safe and feasible for in vivo applications. Additionally, the release of ammonia upon decomposing urea increases the pH of the surrounding environment (Panja & Adams, 2021). This, in turn, has anti-tumour effects as it helps to neutralize tumour acidity (Pilon-Thomas et al., 2016). Overall, urea demonstrates greater efficacy due to its increased biocompatibility, and its beneficial role in tumour suppression.

4.2.4 *Clinical Applications and Limitations*

The clinical applications of nanorobots for bladder cancer treatment, diagnosis, and therapy demonstrate a huge step in biomedical innovation, despite their many challenges. Although nanorobots have been predominantly tested in animal trials, the integration of micro/nanorobots into clinical settings faces many obstacles such as safety, technical feasibility, regulatory compliance, and market acceptance (Zhang et al., 2022). Although nanorobots have been proven effective in laboratory settings, it is crucial to ensure that their functionality is maintained in human physiological environments (Zhang et al., 2022). In particular, many challenges arise in the navigation of nanorobots in vivo due to the high speed and dense viscosities of bodily fluids (Zhang et al., 2022). Additionally, the optimization of drug delivery efficiency remains a key concern that researchers have explored using high-load capacity carriers or multiple nanorobots to target disease sites effectively (Zhang et al., 2022). Further, another challenge is the safe removal of nanorobots post-treatment. Once the nanorobots have completed their job, for them to be useful for in vivo applications, they must have the ability to break down and decompose into non-toxic compounds without external aid (Zhang et al., 2022).

Nevertheless, recent research has demonstrated the development of urea-powered nanorobots (Institute for Bioengineering of Catalonia, 2024). This propulsion method has the potential to significantly reduce tumours and their effects with minimal invasiveness (Institute for Bioengineering of Catalonia, 2024). These nanorobots are composed of a porous silica sphere (Simó et al., 2024a). On their surfaces, there are various components, each serving specific functions (Simó et al., 2024a). The precise accumulation in tumour tissues is useful in applications regarding medical imaging modalities like medical positron emission tomography (PET) and advanced microscopy (Institute for Bioengineering of Catalonia, 2024). A recent study conducted on mice shows promising progress in bladder cancer treatment using nanorobots. The nanorobots successfully reduced bladder tumours in mice by 90% with a single dose (Simó et al., 2024a). By utilizing the urea-powered nanorobots, they effectively penetrate tumours, offering a targeted approach to therapy (Institute for Bioengineering of Catalonia, 2024). These applications highlight the potential clinical use for targeted therapy, reducing lengths of hospitalization, lowering costs and patient comfortability (Simó et al., 2024a). The advancements in nanorobotics offer a more effective and targeted approach to bladder cancer therapy.

4.3 Treatment Applicability and Commercial Viability

Nanotechnology has been applied in various sectors and fields such as materials engineering and photonics due to the unique nature and versatility of materials at a small scale. The global nanotechnology market was projected to surpass $125 million US dollars by 2024 (Rambaran & Schirhagl, 2022). This is driven by several advancements in technology that permit the manipulation and manufacturing of materials at the microscopic and nanoscopic levels. However, when it comes to the biomedical and pharmaceutical fields, the science is still too new and untested for clinical applications.

One of the largest concerns with MNR use *in vivo* is the biosafety and risk mitigation of the treatment types. Many materials and methods can cause adverse effects in patients, and the safer options such as purely biological nanorobots lack the customizability of potentially more harmful designs such as physical and chemical MNRs (Wan et al., 2021). Nanorobots have the potential to revolutionize drug delivery and provide more targeted therapeutic solutions for various diseases. In the medical domain, micro/nanorobots enhance the precision and refinement of medical treatments and discussions are emerging about conducting clinical trials on humans with nanorobots for medical applications (Cozzi, 2024).

Regulatory bodies such as the FDA remain critical of this novel technology for treatment, with less than 30 FDA-approved nanotherapeutics approved by 2016 (Zanganeh et al., 2016). This restricts the sudden influx of micro and

nanorobots available for clinical use, as the field is still well in its infancy. Many products, such as the Urease-Powered Nanorobot Radionuclide Therapy, are novel and have great potential for the treatment of illnesses like bladder cancer. They inspire and excite at the thought of their potential treatment options; however, they still require rigorous testing before they're made available for therapeutic applications. This is largely why the medical applications of MNRs are lagging behind the applications of micro/nanotechnology in other fields, as the standards to which they are held are significantly higher. When it comes to defining drug delivery nanorobots, there can be a certain ambiguity and there also remains uncertainty about which business models suit these treatments best (Morigi et al., 2012).

5 FUTURE RECOMMENDATIONS

1. It is imperative to initiate a wider public discussion on the risks and advantages associated with nanorobots, as well as explore effective regulatory approaches to maximize the benefits of nanorobots while minimizing potential risks (Rickard & Foss Hansen, 2020).
2. Further research should concentrate on assessing the environmental and human health risks associated with various types of nanorobots before their widespread implementation. This calls for a robust risk assessment and the establishment of regulatory legislation and classification ahead of the potential adoption of medical MNRs (Rickard & Hansen, 2020).
3. Bridging the gap between academia and industry is critical to fostering innovation and ensuring that the use of nanorobots is scientifically validated and commercially feasible (Rickard & Hansen, 2020). The limited understanding of their safety and efficacy in the human body remains a significant hurdle in the validation of medical MNRs. However, with adequate research and investment, nanorobots may have the potential to revolutionize the landscape of cancer therapy.
4. The study conducted by Simó et al. (2024) on tumour-bearing mice seems to indicate that urease-powered nanorobots have the potential as an effective treatment for bladder cancer. Despite proving that MNRs have incredible potential in targeting and eliminating bladder tumours, it remains to be seen if such treatment is feasible in a clinical setting for human models. Further research in biosafety and interactions between MNRs and the human body are crucial to slowly bringing this technology to humans.

6 CONCLUSIONS

1. Nanorobots have shown significant promise in experimental drug delivery and surgical interventions for cancer, although their clinical application remains speculative. Recent evidence from mouse studies suggests that nanorobots may one day transform cancer treatment, but their biocompatibility and safety must be more rigorously studied before human trials can be considered. As of now, it remains inadvisable to jump into human trials without more evidence concerning the validity and safety of MNRs when facing.
2. While nanorobots remain in the early stages of development and awaiting clinical approval, they have demonstrated a high demand in various industries such as healthcare and engineering. Specific applications include imaging technologies, drug delivery, tumour detection, and therapy.
3. DNA origami nanorobots have a precise structural design which is guided by DNA base pairing, indicating greater biocompatibility. On the other hand, chemical nanorobots rely on synthetic materials for their construction and function.
4. Chemical MNRs rely on chemical reactions within their environment to facilitate their movement. This is often achieved through the presence of various catalytic mechanisms involving enzymes and substrates. As a result, they may create waste products, some of which are linked to harmful, cytotoxic effects. Using chemicals with non-cytotoxic byproducts is a crucial step in increasing the safety and validity of chemical MNRs in clinical applications.
5. The use of hydrogen peroxide for driving nanorobot motion has diminished due to its cytotoxic effects on the body. This idea has prompted researchers to explore alternative fuel sources to improve biocompatibility. An important development in the field of nanorobots is the use of enzymes such as urease, which is a natural molecule found within the bladder.
6. Chemically fueled MNRs allow for greater customization of surface properties, interactions and behavior when compared to DNA origami.
7. DNA origami MNRs display greater biosafety than chemical MNRs, emphasizing the need to further study the hybridization of these two concepts. The use of biological materials or compounds naturally occurring within the human body in the construction of MNRs remains the best way to ensure biocompatibility and proper interaction with the target tissue.
8. With one of the first few tests to be conducted in-vivo, urease-powered nanorobots demonstrated an ability to reduce bladder tumour count in mice by 90%, demonstrating a proof-of-concept that could potentially initiate further research in the field.

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