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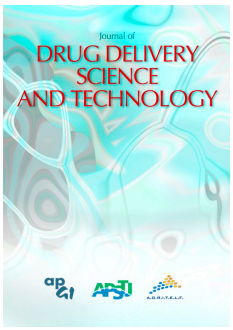

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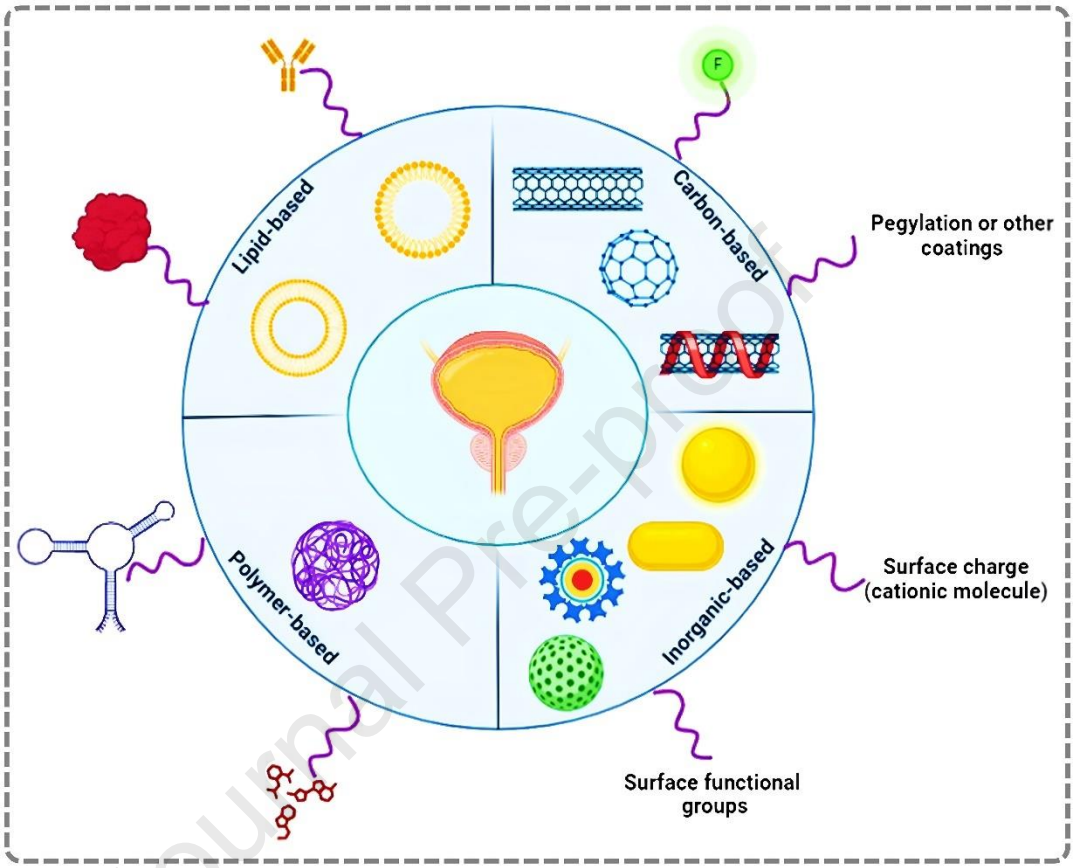
Graphical Abstract



Cell-targeting Nanomedicine for Bladder Cancer: A Cellular Bioengineering Approach for Precise Drug Delivery

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Abstract

Bladder cancer poses considerable therapeutic difficulties owing to its elevated rates of recurrence and the constraints of existing treatment methods. This study examines the possibility of cell-targeting nanomedicine as a viable strategy to improve the accuracy of bladder cancer treatment. The article explores the use of carbon-based nanostructures, metallic nanoparticles, and new methods in designing tailored drug delivery systems, drawing on knowledge from cellular bioengineering. This study focuses on the interaction between nanoparticles and the urothelium, with a specific emphasis on the potential of silver nanoparticles (AgNPs) in treating non-muscle invasive bladder cancer (NMIBC). The study highlights how AgNPs may induce apoptosis and stop the cell cycle, offering promising prospects for NMIBC therapy. Furthermore, the use of carbon nanotubes (CNTs) in precise and targeted treatment using photo-thermal ablation is examined. This study highlights the revolutionary capabilities of nanotechnologies, indicating a fundamental change towards improved and individualized therapies for bladder cancer. Therefore, this paper is an essential reference for researchers, physicians, and academics who are committed to progressing cancer therapies.

Keywords: *Bladder Cancer; Nanomedicine; Drug Delivery; Urothelium; Targeted Therapy.*

1. Introduction

Bladder cancer (BC) is the most common urological malignancy worldwide. BC has several histological subtypes that have typical features [1]. Transitional cell carcinoma (TCC), also known as urothelial carcinoma (UC), accounts for more than 90% of cases. UC originates from the urothelium, which lines the bladder. It can be classified as either invasive or non-invasive, depending on its penetration of the bladder wall [2]. Squamous cell carcinoma (SCC) is the second most common type of bladder cancer, accounting for approximately 1-5% of all diagnoses. This subtype originates from the squamous epithelial cells that line the bladder's surface [3]. However, SCC is frequently detected at a late stage, which renders treatment less effective than that of UC. Additionally, the fact that both sexes are equally affected by SCC may be attributed to the equivalent rates of chronic inflammation and infections in males and females [4, 5]. Small cell carcinoma, which originates from neuroendocrine cells (< 1%) and adenocarcinoma, which arises in mucus-secreting glandular cells (1%) are other uncommon forms of BC [6]. Consequently, in order to facilitate more accurate diagnosis, distinct subtypes necessitate distinct treatments.

The development of BC is influenced by a multifaceted interplay of factors. Smoking continues to be the primary risk factor, accounting for more than thirty percent of cases. Some additional factors may be relevant, such as chronic exposure to chemical pollutants at work, prior radiotherapy for neoplasia, and parasitic infections such as schistosomiasis. Additionally, genetic predisposition contributes to the likelihood of developing BC, as family history amplifies the risk. Additionally, individuals who have experienced persistent urinary tract infections, particularly those affecting the bladder, or specific metabolic disorders may be at a higher risk [7]. The current diagnostic instruments for BC have limitations that can impede the early detection and optimal treatment planning. The sensitivity of urine cytology is notably low, especially in the detection of early malignancies, as it examines exfoliated cells from the bladder [8]. Biopsy is regarded as the gold standard for diagnosis, as it involves the removal and microscopical examination of tissue samples, despite the fact that it is invasive and may not always provide a comprehensive view of a tumour [9]. Cystoscopy, which employs an illuminated scope to observe the bladder's interior, can identify visible tumours; however, it may overlook microscopic lesions. These drawbacks frequently result in delayed diagnoses and occasionally a worse prognosis, among other factors [10].

The standard treatment for breast cancer is contingent upon its stage and aggressiveness.

Intravesical instillation therapy is a non-invasive treatment for UC that entails the direct administration of medication into the bladder to either prevent or eliminate the growth of cancer cells. This therapy commonly uses medications such as Mitomycin C, Gemcitabine, and Bacillus Calmette-Guérin (BCG). Mitomycin C and Gemcitabine are chemotherapy agents that inhibit DNA synthesis in cancer cells, while BCG is an immunotherapy that stimulates the immune system to attack cancer cells [11]. Nevertheless, the apical membrane of the urothelium, which is impermeable, can impede the penetration of drugs into the deeper cell layers. Additionally, the frequency of urination will facilitate the elimination of medications from the body, which will reduce their effectiveness [12]. More extensive measures, such as surgery (cystectomy) and radiation, may be necessary in muscle-invasive or advanced BC. Unfortunately, these have a negative impact on the quality of life of the patient. In certain instances, systemic chemotherapy is employed; however, it frequently induces off-target effects, including injury to normal tissues in addition to tumours, which can result in adverse effects such as fatigue, hair loss, and vertigo. The objective is to create anticancer compounds that are capable of selectively eliminating cancer cells while causing minimal damage to benign tissues [13].

Nanotechnology has the potential to resolve the current issue of BC treatment and diagnosis. However, nanoparticles are capable of crossing the urothelial barrier and delivering medications directly to cancer cells as a result of their diminutive size. This also enhances the efficacy of drug delivery and mitigates systemic adverse effects through the passive or active targeting of these carriers on individual cells. In addition, it is feasible for them to serve as diagnostic agents by being laden with nanoparticles for imaging purposes, which would facilitate the visualisation of tumours during treatment [14]. Biological bioengineering demonstrates significant potential for cancer therapy by permitting targeted modifications to the genetic makeup of malignant cells. These modifications can include gene editing techniques such as CRISPR-Cas9 to correct mutations, gene silencing to inhibit the expression of oncogenes, and gene addition to introduce tumor suppressor genes [15]. This could entail the transfer of specific nucleotides that would alter the characteristics of malignant cells, thereby increasing their susceptibility to pharmaceutical treatments. Additionally, scientists have employed CRISPR-Cas9 gene editing technology to inactivate and wipe out genes that are involved in the growth and survival of tumours [16].

Ligand-based targeting is where the molecules used are antibodies, peptides, nucleic acids and small molecule that can bind specifically to receptors or antigens over-expressed on cancerous

cells. Such ligands function as homing devices that guide therapeutic payloads loaded nanoparticles to target sites. This method of drug administration increases drug carrying capacity while reducing off-target exposure in normal tissues [17]. On the other hand, environmental-based targeting strategies make use of the peculiar microenvironment of tumors; such as different pH, temperature or redox potential between tumor and normal cells. In this regard, specific nanoparticles are engineered to sense such changes in the surroundings allowing them to release their cargo only upon reaching the tumor site. Taking advantage of natural body processes enables cell-based targeting approaches for drug delivery into specific locations within the body [18]. An alternative method involves using immune cells like neutrophils and leukocytes which naturally migrate towards sites of infection and inflammation [19]. This is because researchers are able to alter nanoparticles so that they crosstalk with these cells and end up in the tumor microenvironment. An alternative method includes the use of stem cells or bladder-resident cell types to deliver drugs directly into cancer cells. Different ways exist of targeting cells in terms of which there are few benefits as compared to other conventional treatment methods. This can greatly enhance drug delivery specificity whilst limiting off target effects leading to possible minimization of side effects and improved patient prognosis. Moreover, cellular targeting may be useful for personalized medicine where therapeutic approaches reflect specific characteristics of a cancer patient [20, 21].

The treatment of BC is presently being investigated using a variety of nanocarriers. Polymeric nanoparticles, liposomes, micelles, and dendrimers appear to be the most promising. Polymeric nanoparticles are distinguished by their distinctive characteristics. They can be engineered to contain a variety of therapeutic agents and exhibit a wide range of properties to facilitate targeted delivery [13]. Other biocompatible carriers, such as liposomes, replicate the structures of cell membranes. Consequently, they have the ability to merge with malignancy cells and release their contents. Micelles, on the other hand, are self-assembling structures that have the ability to entrap hydrophobic substances within themselves [22]. The enhanced permeability and retention (EPR) effect enables them to readily accumulate into tumours [23]. Lastly, dendrimers, which are extremely branched, offer a substantial surface area for the attachment of therapeutic compounds or targeting ligands [24]. The ongoing development of these most recent nanocarriers has resulted in enhanced biocompatibility, targeted efficacy, and controlled drug release, propelling BC therapy into a more promising future.

The present review will delve into the physiological aspects of cells associated with bladder cancer, focusing on potential targets for effective treatment. The study will also examine the various types of nanoparticles and their role in cellular targeting and precise drug delivery against bladder cancer. Cellular targeting refers to the ability of nanoparticles to specifically identify and bind to cancer cells, thereby enhancing the accuracy of treatment. Precise drug delivery involves the use of these nanoparticles to transport therapeutic agents directly to the cancer cells, minimizing damage to healthy tissues and improving treatment efficacy. The novelty of this review lies in its focus on cellular targeting strategies using nanoparticles for bladder cancer treatment. While the review covers the established knowledge about BC subtypes, risk factors, diagnosis, and current treatments, the in-depth exploration of ligand-based, environmental-based, and cell-based targeting methods with nanoparticles is the unique contribution.

2. Cell Types Associated with Bladder and Their Relation to Bladder Cancer Pathology

Bladder cancer presents a complex pathology characterized by its heterogeneous nature and the involvement of various cell types within the bladder. Understanding the specific roles of these cells in both normal physiology and disease states is crucial for developing targeted therapies.

2.1. Urothelium

The urothelium, a special type of epithelium lining the inner surface of the urinary tract and bladder, is divided into over eight types, depending on the number of cell layers and its morphology [25]. It is made up of three chief cell components: superficial (umbrella), intermediate and basal cell layers (see **Figure 1**). Each layer has unique morphology and protein markers such as cytokeratins -7, -8, -18, and -19 which are similar across all the layers. This layered arrangement prevents urine permeability to some extent, allows for signal transmission and provides defense against infections in body's immune response system [26].

2.1.1. Superficial or Umbrella Cell Layer

The superficial or umbrella cell layer is the outermost layer, consisting of a single layer of massive, polyhedral cells that are highly differentiated. These cells are termed umbrella cells. Due to polyploidy, these cells manifest a large nucleus, multi-nuclei, and apical-basolateral polarity. Their specialized features include a perforated apical membrane and microplicae-covered surfaces, and

they are in direct contact with urine. The shape of these cells undergoes a significant transformation, transitioning from an approximately cuboidal shape in a voided bladder to a highly elongated shape when the bladder is filled [27]. **Table 1** contains a list of the specific protein markers that are associated with these cells. These highly differentiated cells form the outermost barrier and are crucial for protecting underlying tissues from toxins in urine. Disruption of their barrier function can lead to increased susceptibility to carcinogens and the initiation of bladder cancer [28]. In the context of cancer, umbrella cells are often the first line of defense that fails, leading to exposure of deeper cell layers to harmful agents [29].

2.1.2. Intermediate Cell Layer

The intermediate cell layer is situated beneath the umbrella cells. The species and micturition cycle determine the number of cells in this stratum, which can range from one to several. Desmosomes and gap junctions connect these pear-shaped cells to umbrella cells, which express proteins such as DFV and uroplakins. They are partially differentiated and possess the capacity to differentiate further in order to replace depleted umbrella cells [30]. These cells serve as a transitional layer, providing structural support and the ability to regenerate into umbrella cells. Their partial differentiation state makes them susceptible to genetic alterations that can lead to cancerous transformation [31]. Intermediate cells are critical in maintaining urothelial integrity, and their malfunction or transformation can contribute to cancer progression, particularly in the form of non-muscle invasive bladder cancer (NMIBC) [32].

2.1.3. Basal Cell Layer

The innermost layer, situated beneath the intermediate layer, is the basal cell layer. It is composed of a single layer of cells that are in close proximity to the capillary bed. Hemidesmosomes facilitate the adhesion of these cells to the basement membrane through β4-integrin. This stratum offers an optimal environment for urothelium stem cells, which are capable of regenerating all three categories of urothelial cells [33]. These cells are the proliferative base of the urothelium and contain stem cells responsible for regenerating the urothelial layers. Mutations in basal cells are often linked to the development of more aggressive, muscle-invasive bladder cancer (MIBC) [26]. The proximity of basal cells to the bladder's blood supply increases the risk of metastasis if these cells undergo malignant transformation [34].

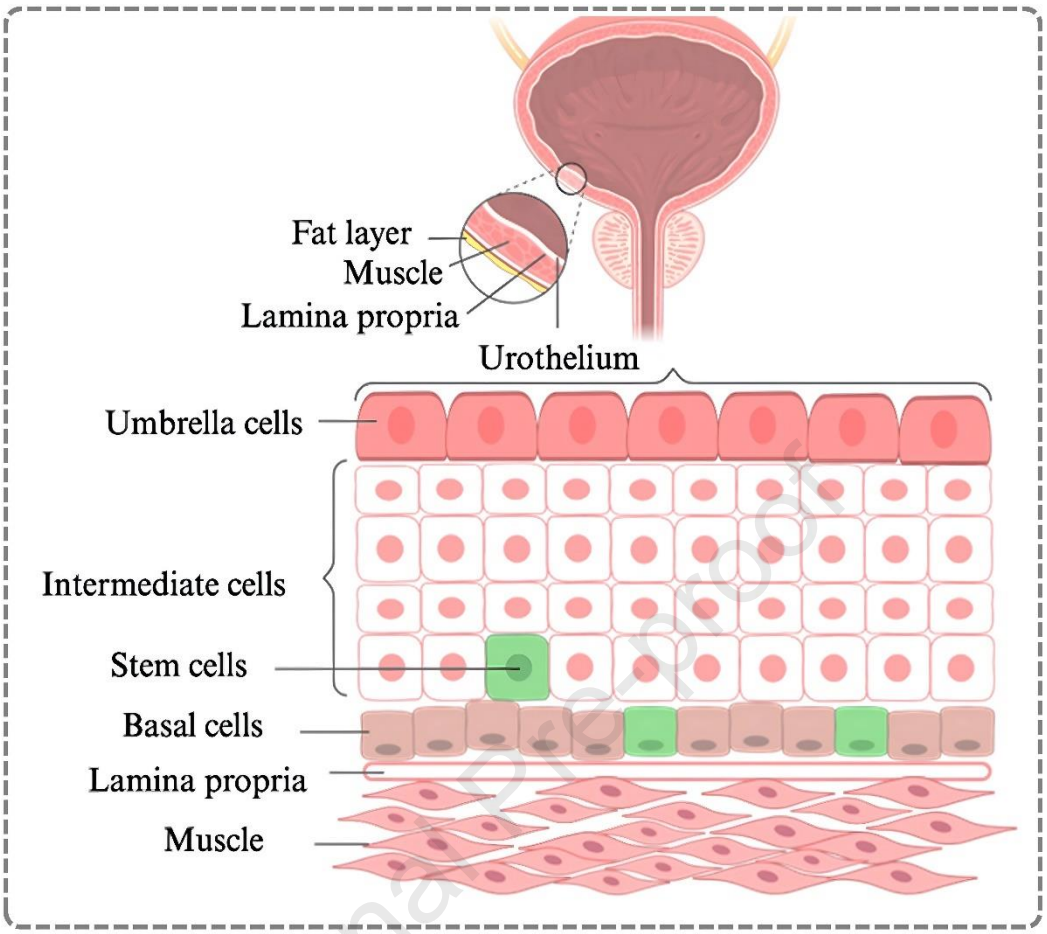

Figure 1. Basal cells, intermediate cells, and superficial or umbrella cells are the three cell types of urothelium. Under infection basal and intermediate cells may serve as stem cells and differentiate to umbrella cells. Figure is prepared in BioRender.com.

2.2.Urothelium Stem Cells

The urothelium stem cells of the bladder are essential for the preservation of epithelial integrity. They have a turnover rate of 3-6 months, which increases in response to injury or infection. Following an injury, such as an E. coli infection, these basal cells, which are identified by markers such as KRT5, SHH, and KRT14, can regenerate into umbrella cells [35]. Regenerative potential is also demonstrated by intermediate cells that express uroplakin. Genetic and epigenetic modifications can induce the transformation of normal urothelium stem cells into cancer stem cells [36]. For example, non-muscle invasive BC can result from mutations in the Ha-ras or FGFR3 genes in stem cells, whereas muscle-invasive BCs are associated with mutations in the P53, Rb, and PTEN genes. These cancer stem cells, which are identified by markers such as CD44+, CD47,

EMA-, 67LR, and BCMab1+, are characterized by self-renewal and proliferation abilities. They contribute to the resistance of cancer to conventional treatments [37]. Urothelial stem cells are crucial for the regeneration and repair of the bladder lining. However, genetic mutations within these cells, such as those affecting the FGFR3, P53, or PTEN genes, can lead to the formation of cancer stem cells, which are resistant to conventional therapies [38]. The presence of cancer stem cells in bladder cancer is associated with high recurrence rates and resistance to treatment, making them a significant challenge in managing the disease [39].

2.3.Smooth Muscle Tissue

The urothelium is enveloped by many layers of smooth muscle tissue, mostly the detrusor muscle, which contracts to release urine. Smooth muscle cells are elongated and spindle-shaped, usually measuring 300-400 μm in size. They are mostly comprised of actin and myosin. The Sonic Hedgehog (Shh) growth factor, produced by the urothelium, is crucial for the transformation of smooth muscle cells from embryonic mesenchyme. Smooth muscle differentiation is characterized by the presence of distinct protein markers at different stages. In the early stages, alpha actin (SMAA) is seen, whereas calponin is present during intermediate stages, and SM22a is shown at advanced differentiation stages [40, 41]. The bladder wall contains two types of smooth muscle tissue: muscularis propria (MP) and muscularis mucosae (MM). MP is characterized by large, thick, dense bundles of muscle fibers, while MM comprises smaller, slender bundles located in the lamina propria. Superficial bladder cancers often invade the MM without deeply involving the MP, depending on the size of the muscle bundles involved [42]. Smooth muscle tissue, particularly the muscularis propria (MP), plays a critical role in bladder function. Its involvement in bladder cancer often signifies a more advanced stage of the disease, with invasion into these layers indicating a poor prognosis [43]. The infiltration of bladder cancer into the muscularis propria is a key factor in the progression to muscle-invasive disease, which is associated with a higher risk of metastasis and mortality [44].

2.4.Cancer Associated Fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs) are a diverse and abundant population of cells seen inside the cancer microenvironment. They may arise from several sources, including tissue-resident cells, progenitor cells derived from bone marrow, pericytes, endothelial cells, and even epithelial cells.

CAF detection has utilised multiple prognostic markers such as alpha-smooth muscle actin (ASMA), Fibroblast-Specific Protein 1 (FSP1 or S100A4), vimentin, fibroblast activation protein (FAP), desmin, and platelet-derived growth factor receptors (PDGFRs α/β) due to the diverse origins of CAFs. Nevertheless, it is crucial to acknowledge that none of these characteristics are exclusive to fibroblast cells [45]. CAFs play a crucial role in the tumour microenvironment by actively facilitating cancer development, invasion, and metastasis via several mechanisms. The process of tumour formation and the construction of new blood vessels (angiogenesis) are promoted by the release of growth factors, cytokines, and components of the extracellular matrix. CAFs has the capacity to modulate the immune response inside the cancer, suppressing the body's innate defensive mechanisms against the tumour and inducing a state of diminished immunological activity [46]. CAFs contribute to the tumor microenvironment by promoting cancer cell growth, invasion, and immune evasion. Their diverse origins and functions make them a complex and challenging component of bladder cancer pathology [47]. Targeting CAFs in therapy is complicated by their heterogeneity and the lack of specific markers, which hampers the development of effective treatments aimed at modifying the tumor microenvironment [48]. Overall, CAFs provide a significant challenge for cancer therapies due to their diverse origins and inherent unpredictability. Table 1 shows Urothelium cell layers characteristics.

Tabel 1. Urothelium cell layers characteristics.

Cell type	Number of Nuclear	Where	Expressed protein markers	Size (µm)	Reference
Basal cells	mononuclear	Between urothelial cells and lamina propria	Keratin (KRT) 5, KRT 14, Tumor Protein p63 (TP63), Sonic Hedgehog (SHH), CD44, Cd49f, β1, and β4 Integrin, laminin receptor, specific "basal" cytokeratins (CK-5/14, CK-17)	5-10	[49-54]
Intermediate cells	Mononucleate or binucleated	between basal and superficial cells	KRT7, KRT5, TP63, SHH, or Cd49f or Uroplakins (UPKs)	~20	[49, 53-58]
Superficial cells	mono- or multinucleate (depending on species), polyhedral (typically 5- or 6-sided)	face the urinary space	KRT20, UPKs, and cytokeratin-20	80-120	[49, 50, 53, 58, 59]

3. Pathways to Differentiation of Bladder Cells

3.1.Stem Cells

The urothelium is comprised of stratified epithelium, housing three distinct cell populations, basal, intermediate, and superficial or umbrella cells based on the level of their differentiation, each distinguished by their location and marker expression [60]. It is widely accepted that the basal cellular layer, positioned above the lamina propria, harbors undifferentiated progenitor cells that transition into intermediate cells before maturing into superficial cells [61]. These progenitor cells play a crucial role in the regeneration and repair of the urothelium. Studies have shown that these cells express markers such as CK5 and CK14, which are indicative of their undifferentiated state. As they differentiate, they lose these markers and gain others, such as CK20, which are characteristic of mature superficial cells [62-64]. Nevertheless, some studies have proposed the existence of two separate cell lineages for basal/intermediate cells and superficial cells [51, 65].

Under normal conditions, urothelial cells have a low turnover rate with minimal cell division. However, in response to stimuli like chemical exposure, mechanical stress, surgery, or infection, stem cells residing in the basal layer shift from a quiescent state to an active phase to proliferate rapidly and regenerate functional urothelium within days to weeks [66]. Epithelial proliferation in urinary bladder is regulated through various signaling pathways and growth factors such as bone morphogenetic protein 4 (BMP4) signaling, Sonic Hedgehog, Wnt signaling, Notch-Delta, ELF3, retinoids, and TP63, exchanged between the basal layer of urothelium and the stromal cells within the lamina propria [52, 55, 56, 66-68].

During embryonic stage, mesenchyme-derived signals play the key role in urothelial differentiation. Fibroblast growth factor (FGF) signaling is one of these signals which promotes the stratification in bladder epithelium expressing FGFR2, specifically the formation of intermediate cell layer [69]. In addition, mesenchymal-derived retinoic acid (RA) signaling is another key regulator of differentiation of embryonic P cells and postnatal intermediate cells, [49, 52] increased in response to injury. RA signals through its nuclear receptors on urothelial cells, which binds to RA response elements (RAREs), near target genes to regulate their transcription.

Some studies have indicated the role of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARG) in differentiation of urothelial cells [70, 71]. According to Liu et al.' findings, PPARG is crucial for mitochondrial biogenesis and regulates the specification and

differentiation of I and S cells during development and homeostasis. Additionally, their RNA-Seq analysis demonstrated that the absence of PPARG led to the upregulation of squamous markers in the urothelium, indicating PRAG role in preventing squamous differentiation in basal cells[71].

Bone morphogenetic protein 4 (BMP4) is predominantly expressed in the mesenchymal layer beneath the urothelium and plays a crucial role in development and terminal differentiation of superficial cells. However, in response to bacterial injury of the bladder, basal cells within the urothelial layer upregulate Sonic hedgehog (SHH) expression which activates FOXF1 expression in mesenchyme. This leads to the secretion of BMP4/BMP5 from stromal cells, initiating the proliferation and differentiation of KRT5+ basal cells [55, 67, 72]. Its regulatory effects on urothelial proliferation and differentiation are mediated through signaling receptors Bmpr1a and Bmpr1b, with downstream canonical Bmp signaling modulated via Smad proteins [67].

The SHH and Wingless-related integration site (WNT) signaling pathways are synergistically involved in cell proliferation, differentiation, and tissue development and repair during embryonic days and in adult tissues, with the activation of one pathway modulating the expression or activity of components in the other. The WNT/β-catenin pathway is pivotal in maintaining the proliferation of progenitor cells within the bladder. WNT signaling leads to the accumulation of β-catenin in the cytoplasm which in turn translocates to the nucleus to interact with transcription factors and activate target genes expression. Among its pathways, the canonical WNT or β-catenin-dependent pathway is essential for basal urothelium regeneration post-injury. Administration of SHH-neutralising antibodies significantly or systemic administration of a WNT inhibitor reduced urothelial proliferation after bacterial infection [55].

The involvement of NOTCH signaling pathway in urothelium differentiation and integrity has been studied. Through RNA-seq analysis, Santos et al. showed that inactivation of NOTCH signaling in D-organoids prevents lumen formation and reduces the expression of luminal markers, shifting them towards a P-organoid phenotype [54]. Additionally, Paraskevopoulou et al. revealed that conditional inactivation of NOTCH signaling in basal or superficial urothelial cells reduced integrin expression and led to hyperplasia, superficial cell detachment, barrier dysfunction, and downregulation of cell-cell and cell-extracellular matrix interaction genes, recovered by exogenous activation of NOTCH [73].

3.2.Supporting Cells

The detrusor muscle of bladder consists of smooth muscle fibers with autonomic control. The differentiation of smooth muscle from bladder mesenchyme is induced by signals from epithelial cells whereas the pattern of smooth muscle is determined by mesenchyme [74, 75]. However, smooth muscle-inducing signals are inhibited by the local environment in the adult bladder, limiting the plasticity of adult bladder for repair [76]. The differentiation of smooth muscle is directed by sonic hedgehog (shh) growth factor which is expressed by urothelial cells. Shh activates the gene expression of Wnt family and the bone morphogenetic proteins.

4. Bladder Gene Transfection

Traditional treatments for bladder cancer including surgery, chemotherapy, and radiation therapy have shown efficacy to varying degrees, yet they often come with significant side effects and limited long-term success. Consequently, in recent years we have witnessed growing interest in innovative therapeutic approaches to combat bladder cancer. Among these approaches, bladder gene therapy emerges as a promising strategy for targeted and personalized treatment. Bladder gene transfection involves the delivery of therapeutic genes directly into the cancerous bladder cells with mutated genes to restore normal cell growth as well as to modulate underlying molecular mechanisms driving tumorigenesis, tumor progression, and metastasis. This can be achieved through viral and non-viral vectors.

4.1.Viral Gene delivery

Viral vectors serve as carriers for genes that have the potential to impede tumor growth, trigger cell death, or activate the immune system to target cancer cells. The success of viral gene delivery relies on efficient viral entry into target cells. To enhance viral transduction efficiency, researchers have explored various strategies, including the use of chemical compounds known as permeation or penetration enhancers. These enhancers facilitate viral entry by promoting cellular uptake and endosomal escape, which improve gene delivery efficacy [77]. An example of this is the association of adenovirus with a polyamide surfactant, Syn3, to improve the efficiency of intravesical adenovirus [78]. The choice of viral vector plays a crucial role in determining the success of gene delivery. While adenovirus is known for its high transduction efficiency and broad

host cell range, lentivirus offers the advantage of stable and long-term gene expression due to its ability to integrate into the host cell genome [79, 80]. Adeno-associated virus (AAV) is another viral vector choice due to its low immunogenicity and ability to maintain persistent gene expression without genomic integration [81]. Furthermore, oncolytic viruses, such as herpes simplex virus (HSV), have gained interest for their dual ability to selectively replicate in and destroy cancer cells while sparing normal tissues. Using these vectors, researchers studied the potential of gene therapy such as tumor suppressor genes like p53 and PTEN have been investigated to restore normal cell growth regulation and induce apoptosis in cancer cells [82-84]. Suicide genes such as HSV-thymidine kinase (HSV-TK) and cytosine deaminase have been utilized to sensitize cancer cells to prodrugs, leading to selective cytotoxicity within the tumor [85, 86]. Additionally, immunomodulatory genes like interleukins and interferons have been explored to enhance the anti-tumor immune response [87, 88]. **Table 2** provides a concise overview of the many viral vectors used in cancer gene therapy. It highlights their distinct characteristics, modes of transportation, and their vital functions in delivering therapeutic genes to cancer cells.

4.2.Non-Viral Delivery

Non-viral vectors offer distinct advantages over traditional delivery systems like viral and bacterial vectors. One key benefit is their ability to be engineered with minimum intrinsic immunogenic and cytotoxic properties. For instance, adenovirus as a common viral vector, can provoke inflammatory responses [89]. In such cases, immunosuppressive regimen is administered to mitigate immunogenic side-effects of viral vectors [90]. Additionally, viral and bacterial vectors carry the risk of integrating their DNA into the host genome, potentially leading to unpredictable transformations within cancerous cells. Viral vectors also carry the risk of migrating to other unintended organs, potentially inducing off-target side-effects [91].

Non-viral vectors not only mitigate these risks, but also exhibit several advantages such as lower pathogenicity, reducing the likelihood of adverse immune reactions or cytotoxic effects, localized gene expression, and easier and cheaper manufacturing compared to their viral and bacterial counterparts. These characteristics make non-viral vectors an attractive option for gene delivery in cancer therapy, offering a safer and more accessible alternative for therapeutic interventions [92]. Non-viral vectors encapsulating nucleic acids, DNA, mRNA, siRNA, within drug delivery systems such as exosome, surfactant, and lipid-, polymer-, metal-based nanoparticles. For example, small

interfering RNA (siRNA) can be delivered to target cells via liposomes to silence genes at the mRNA level. Polo-like kinase-1 (PLK-1), which regulates mitotic progression in mammalian cells, is often upregulated in human tumors, and its depletion can inhibit cell growth and lead to apoptosis [93-96]. In fact, PLK-1 is considered as a prognostic marker for non-muscle invasive bladder cancer [97, 98]. In a study, intravesical therapy of a complex of PLK-1 siRNA coated with cationic liposomes in murine bladder cancer cells provided a localized, high concentration of siRNA. Using siRNA/cationic liposomes the expression of PLK-1 was inhibited effectively in a time- and dose-dependent manner, preventing cancer cell proliferation [99].

Exosomes are naturally occurring vesicles within cells with minimized immunogenic potential, considered as another drug delivery system that can carry RNA payloads to trigger translational changes in cancerous cells [100]. Accordingly, Greco et al. used isolated exosomes from HEK293 cells and mesenchymal stem cells as a vector to carry PLK-1 siRNA into bladder cancerous cells. They electroporated UMUC3 cell line, derived from a urinary bladder transitional cell carcinoma as a bladder tumor model, to introduce siRNA-loaded exosomes into the cancer cells, resulting in decreased expression of PLK-1 in cancer cells [101].

Table 2: Summary of Viral Vectors Used in Cancer Therapy.

Gene	Effect	Vector, Route	Cancer	Reference
interferon α	antiproliferative activity, cytotoxicity, anti-angiogenic effect	Adenovirus, Intravesical	superficial bladder tumors	[102]
interferon α2b		Adenovirus, Intravesical	BCG-non-responsive nonmuscle invasive bladder cancer	[78, 103]
-	-	Poxvirus, Intravesical	muscle invasive carcinoma of the bladder	[104]
interferon α		Lentivirus, Intravesical	BCG-unresponsive bladder cancer	[84]
tumor suppressor p53	Inhibit tumor growth, antiproliferative	Poxvirus, Intravesical	Orthotopic invasive tumor model, MB-49	[105]

5. Drug delivery systems

The transurethral resection of non-muscle-invasive tumours is the standard treatment for bladder cancer, which is followed by intravesical chemotherapy. However, the specificity of conventional intravesical chemotherapy is substantially restricted, primarily as a result of drug dilution and rapid elimination through urine excretion. This restriction necessitates the administration of multiple dosages, which may result in localized irritation and other adverse effects. Nanoparticle-based therapies are emerging as a promising solution to these challenges. These systems provide significant benefits, including the capacity of nanomedicines to adhere to bladder tumour surfaces for extended periods, thereby increasing the retention time of therapeutic agents on the targeted tissues. Additionally, nanoparticles have the ability to release drugs directly to bladder cancer cells, thereby minimizing non-specific drug activity and ensuring more precise targeting. Numerous nanoparticle-based platforms have been created to facilitate the targeted delivery of drugs to bladder cancer tissues (**Figure 2**). Currently, clinical studies are being conducted to investigate the effectiveness and safety of these advanced formulations that use nanoparticles (**Table 3**). Albumin-bound paclitaxel nanoparticles (nab-paclitaxel) are a kind of nano-formulation that have shown significant potential in bladder cancer therapy. They are known for their improved drug delivery and lower toxicity, as seen by several studies investigating their efficacy. The ABI-009 formulation, which was studied in non-muscle invasive bladder cancer (NMIBC), has successfully completed Phase 1/2 studies, indicating its potential for clinical success. On the other hand, the discontinuation of certain studies, like the one examining nab-paclitaxel in urothelial cancer, underscores the difficulties and intricacies involved in the practical use of these treatments. These varied results highlight the need of ongoing research to enhance nanoparticle formulations that achieve a balance between effectiveness and safety, thereby enabling future clinical uses. The preliminary results are encouraging, suggesting that the drug's efficacy has been improved and that the number of adverse effects has been reduced [106]. This section explores the diverse nanoparticle-based approaches to bladder tumour treatment. It evaluates their therapeutic potential, mechanistic principles, and the most recent developments in clinical research.

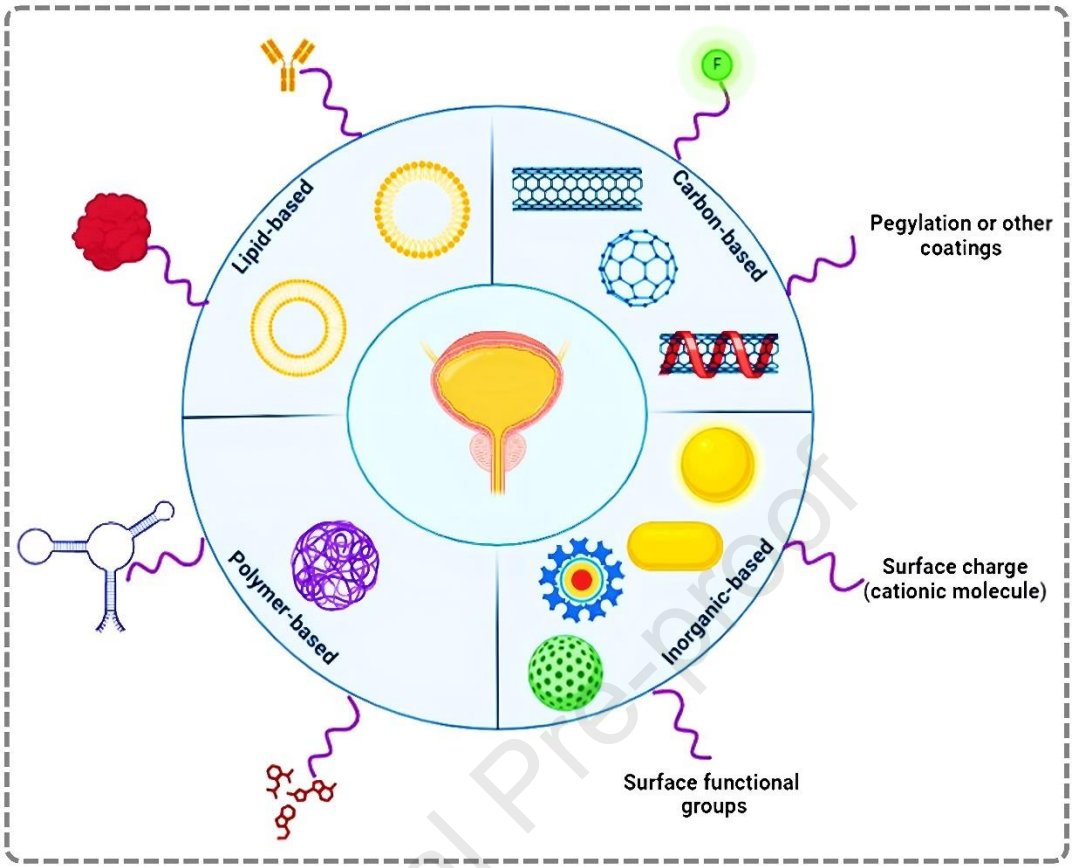

Figure 2. The figure presents a comprehensive overview of nanoparticle-based strategies for active targeting in bladder cancer therapy. It categorizes nanoparticles into four main classes: lipid-based, polymer-based, carbon-based, and inorganic-based. Each type is engineered with specific surface modifications to improve targeting efficacy. Lipid-based nanoparticles, characterized by lipid bilayers, can be functionalized with antibodies or ligands for targeted delivery to cancer cells. Polymer-based nanoparticles, composed of synthetic polymers, can be modified with surface functional groups to improve binding to specific cancer cell receptors. Carbon-based nanoparticles, including carbon nanotubes and graphene, are tailored with surface coatings to enhance biocompatibility and reduce immune clearance. Inorganic-based nanoparticles, like gold nanoparticles and quantum dots, can be functionalized with cationic molecules to facilitate cellular uptake.. Figure is prepared in BioRender.com.

Table 3. Clinical trials of nanoparticle-based therapeutic platforms for bladder cancer

Intervention	Condition	Supporter/ Colleagues	Status	NCT Number
Paclitaxel, nanoparticle albumin-bound	Bladder Cancer	Columbia University/ Celgene Corporation	Unknown (Phase I & II)	NCT00583349
Drug: Paclitaxel Albumin-Stabilized Nanoparticle Formulation	Bladder Cancer	Mayo Clinic/ National Cancer Institute (NCI)	Early Phase 1	NCT02646319
Drug: Ferumoxtran-10 (USPIO)* **Procedure: MR lymphangiography**	Bladder Cancer Genitourinary Cancer Prostate Cancer	M.D. Anderson cancer Center/ M.D. Anderson Cancer Center	NA	NCT00147238
Drug: ABI-009 **Drug: Gemcitabine**	Non-muscle Invasive Bladder Cancer (NMIBC)	Aadi Bioscience, Inc./ National Cancer Institute (NCI)	Completed (Phase 1/2)	NCT02009332
Drug: nab-paclitaxel **Drug: Gemcitabine**	Urothelial Carcinoma Bladder Cancer Transitional Cell Carcinoma	SCRI Development Innovations, LLC/ Celgene	Terminated	NCT02887248
Drug: NC-6004 **Drug: Gemcitabine**	Solid tumors	NanoCarrier Co., Ltd.	Completed	NCT02240238

* Ferumoxtran-10: A Magnetic Resonance Imaging contrast agent, NA: Not Applicable

5.1.Cell Targeting Excipients in Polymer Nanoparticles

The favorable attributes of polymers, including ease of synthesis, adaptable characteristics, scalability in terms of size, and surface properties, position them as highly desirable carriers for delivering anti-cancer drugs [107]. Polymer-based nanoparticles are created by natural or synthetic polymers. These polymers should be biodegradable, non-toxic, and able to transport drugs or genes to specific locations for targeted drug delivery [108]. Administering drugs directly to the bladder urothelium (the lining) poses challenges due to the bladder permeability barrier (BPB). To overcome this, scientists developed nanoparticles (NPs) that effectively adhere to the bladder urothelium and penetrate urothelial and bladder cancer cells. Martin et al. (2013) developed Poly(lactide-co-glycolide) (PLGA) nanoparticles loaded with the histone deacetylase inhibitor belinostat. Furthermore, the nanoparticles were modified using a polymer called poly(guanidinium oxanorbornene) (PGON) that has the ability to penetrate cells. The NP-C6-PGON formulation, which contained fluorophore coumarin (C6), demonstrated a remarkable 10-fold improvement in tissue penetration within the bladder of mouse model and ex vivo human ureter. Notably, tumors treated with PGON formulation exhibited a substantial 70% alleviation in volume and increased intratumoral acetylation of histone H4. Overall, results showed this PLGA – based platform with PGON surface modification hold promise for enhancing drug delivery and chemotherapy efficacy in bladder cancer (**Figure 3**) [109].

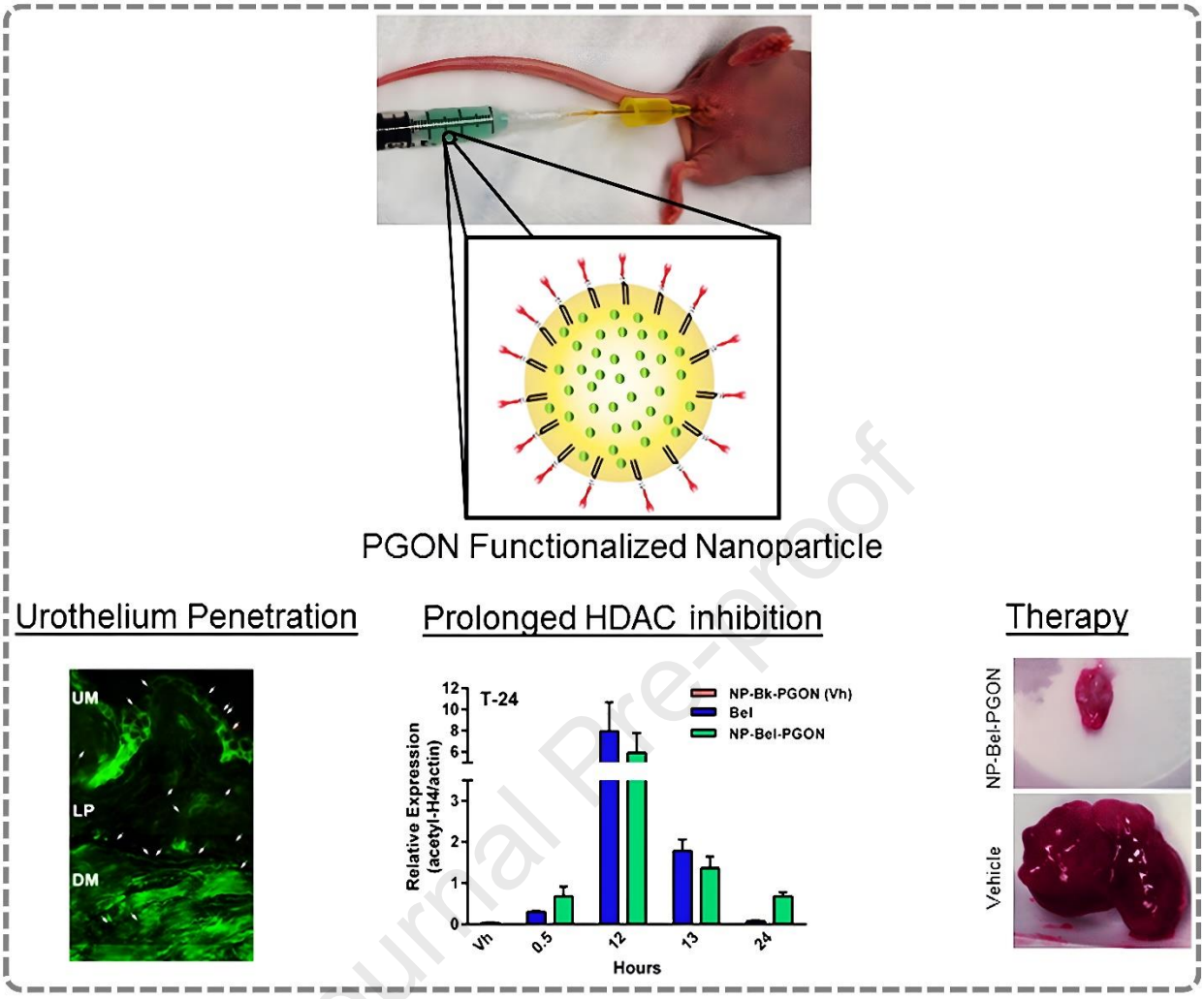

Figure 3. Illustration of belinostat's enhanced efficacy in PGON-modified PLGA nanoparticles, demonstrating increased transurothelial migration, tumor cell uptake, cytotoxicity, prolonged HDAC inhibition, and tumor regression in a mouse bladder cancer model. Reprinted with permission of [109].

Survivin is a protein that plays a crucial role in preventing cell death (apoptosis) and allowing tumor cells to evade the effects of therapy-induced senility. Elevated levels of survivin expression are linked to more aggressive bladder cancer and an increased risk of recurrence. Martin's group employed a PLGA-based platform for delivering survivin siRNA directly to the bladder urothelium. They modified PLGA nanoparticles by incorporating a positively charged mucoadhesive polysaccharide called chitosan onto the NP surface. These chitosan-modified NPs exhibited significantly enhanced binding to and uptake in mouse bladders and human ureters compared to unmodified NPs. Importantly, the survivin siRNA encapsulated within the chitosan-modified NPs remained bioactive for up to 9 days in vitro. Furthermore, in xenograft tumor

models, treatment with chitosan-modified NPs containing survivin siRNA led to a substantial 65% reduction in tumor volume and decreased survivin expression. Overall, Martin et al. demonstrated that this localized drug delivery approach, utilizing low molecular weight chitosan, effectively facilitates siRNA transport across the urothelium and improves therapeutic outcomes in bladder cancer [110]. In numerous research papers, albumin has been employed as a surface modification to enhance the targeted delivery of nanoparticles to tumors. However, the impact of different surface modification methods on albumin conformation and NP performance remains uncertain. In Hyun et.al. investigation, they employed three surface modification techniques: physisorption, interfacial embedding, and dopamine polymerization, to modify PLGA nanoparticles with albumin (**Figure 4**). The results suggest that dopamine polymerization maintains the integrity of the albumin structure, creating a layer on the surface that enhances the transport of nanoparticles (NPs) and facilitates drug delivery to tumors by interacting with albumin-binding proteins. Conversely, the interfacial embedding method produces NPs with altered albumin, which does not significantly contribute to interactions with cancer cells but promotes uptake by the mononuclear phagocyte system (MPS) through scavenger receptor A [111]. The research underscores the importance of controlling surface-bound albumin interactions to achieve favorable cancer therapeutic outcomes.

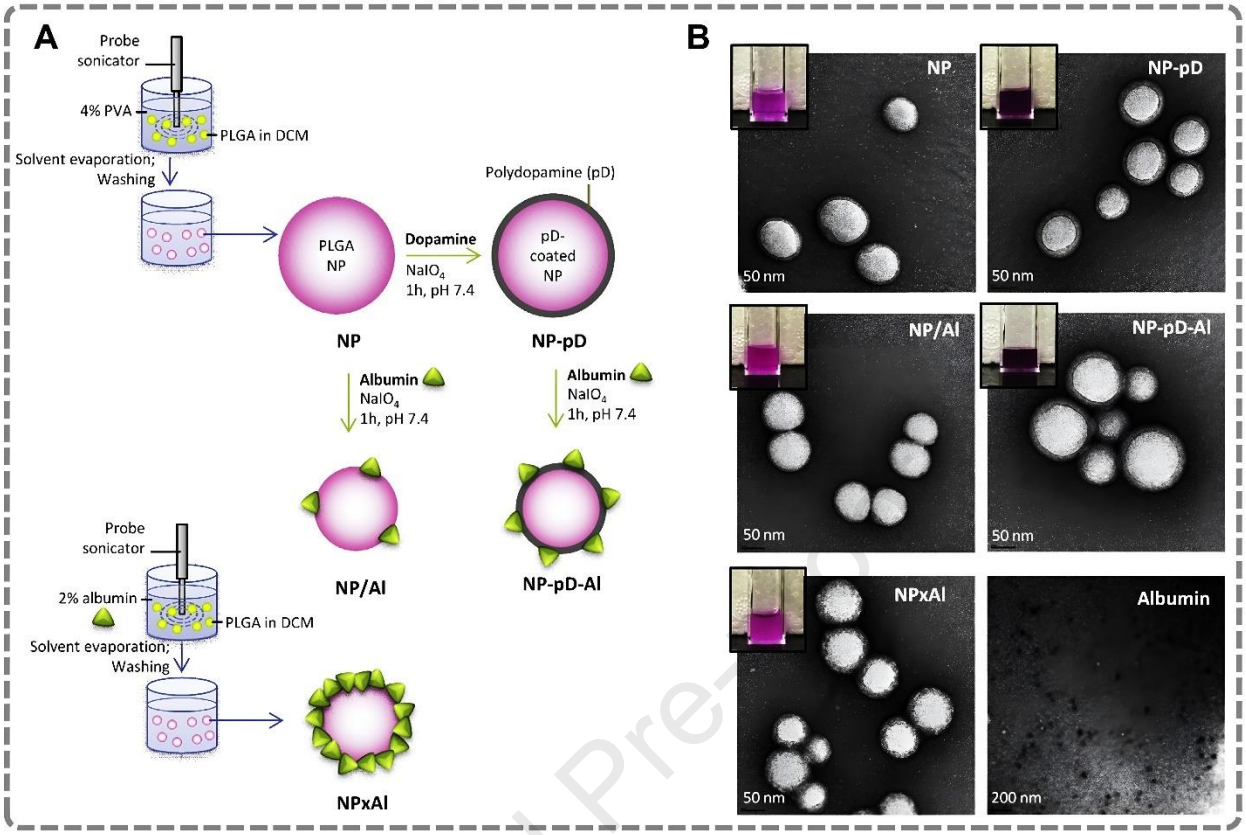

Figure 4. (A) Illustration of diverse techniques employed for the conjugation of albumin to PLGA nanoparticles. (B) Transmission electron microscopy (TEM) imagery showcasing rhodamine-labeled nanoparticles, NP-pD, NP/Al, NPxAl, NP-pD-Al, and albumin, all negatively stained with 2% uranyl acetate. The dark purple hue of NP-pD and NP-pD-Al suspensions (visible in the inset) signifies the existence of polymerized dopamine (pD). Reprinted with permission of [111].

Zhu et al. (2020) developed dual-sensitive nanoparticles (DOX&IR780@PEG-PCL-SS NPs) with remarkable stability due to internal cross-linking. These advanced nanoparticles exhibited dual responsiveness: first, they were sensitive to elevated glutathione levels in bladder cancer cells, and second, they responded to near-infrared laser irradiation. By combining chemotherapy (using doxorubicin) with photothermal therapy (via IR780), these NPs effectively combated bladder cancer. Bladder cancer tissue is characterized by a high concentration of reduced glutathione (GSH) in the tumor microenvironment. Glutathione triggers structural changes in the nanoparticles, leading to the preliminary release of drugs. Additionally, near-infrared laser irradiation facilitates complete drug release from the nanoparticles, inducing a photothermal effect that destroys tumor cells. DOX&IR780@PEG-PCL-SS NPs significantly enhanced the concentration of anti-cancer drugs within tumor cells. In experimental mice, treatment with these

NPs resulted in a substantial depletion in volume of the tumor [112]. In Wang et.al (2020) study, researchers focused on modifying mesoporous silica nanoparticles (MSNPs) with poly(amidoamine) (PAMAM) dendrimers. The modification process involved sequential attachment of PAMAM onto the surface of MSNPs. The mucoadhesive properties of PAMAM-functionalized nanoparticles were assessed on pig bladder walls, along with their controlled drug release pattern. The adhesive characteristics increased with the number of PAMAM amino groups. Maximum particle adhesion occurred after two generations of PAMAM were attached to the nanoparticle surface. The study emphasizes that the adhesion strength and drug release properties of MSNPs can be carefully regulated by adjusting the PAMAM dendrimer layers on the nanoparticle surface (**Figure 5**). Moreover, the scientists encapsulated the antineoplastic drug doxorubicin within the mesopores of PAMAM-functionalized MSNPs. These drug-loaded nanoparticles demonstrated a sustained drug release mechanism, triggered by acidic pH. Importantly, the research emphasizes that the adhesive properties and drug release behavior of MSNPs can be fine-tuned by adjusting the number of PAMAM dendrimer layers on the nanoparticle surface. This finding has significant implications for the development of mucoadhesive drug delivery systems, particularly for bladder cancer therapy [113].

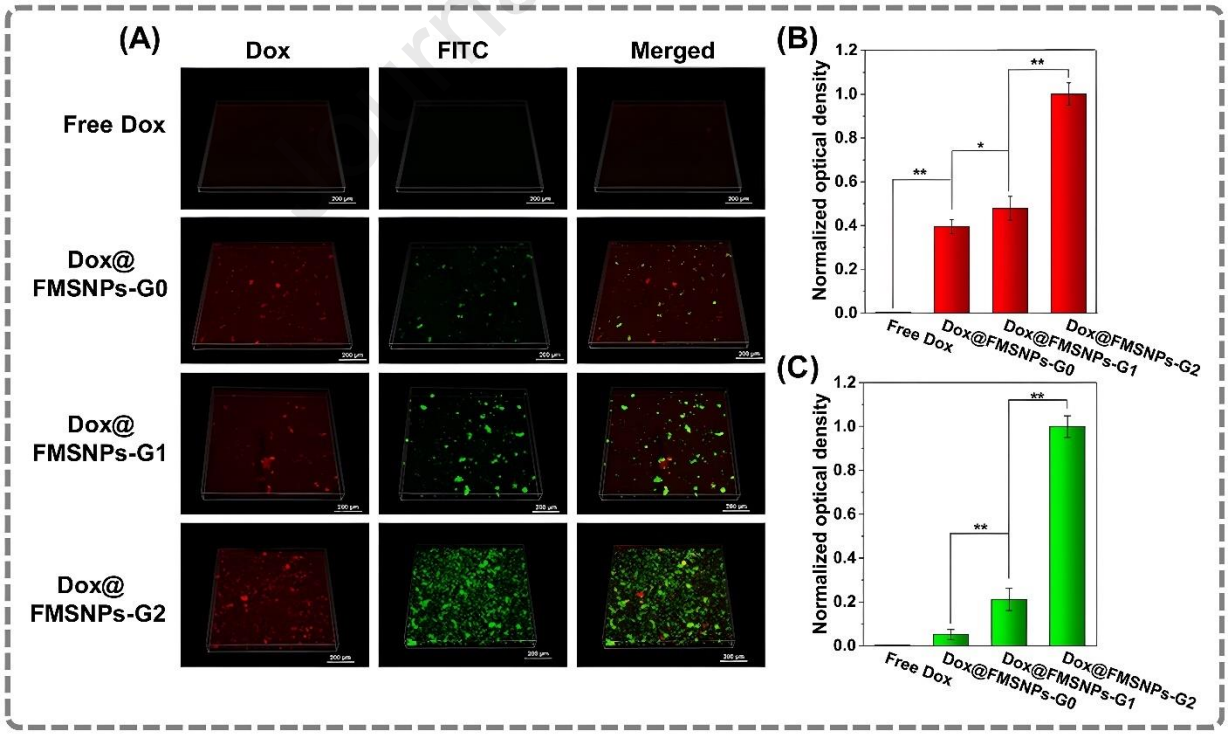

Figure 5. (A) Doxorubicin (Dox)-loaded nanoparticles' mucoadhesive capabilities on the porcine bladder wall is analyzed by Confocal Laser Scanning Microscopy (CLSM). The fluorescence intensities show the retention of free Dox, Dox@FMSNPs-G0, G1, and G2 on the porcine urinary bladder mucosa after three artificial urine washes and a 3-hour incubation. The green fluorescence comes from FITC-labeled MSNPs on the bladder wall, whereas the red comes from released Dox and nanoparticle Dox. (B) and (C) show statistical analysis of Dox and FITC fluorescence intensity optical density. Data are shown as mean ± SD (n = 3; *p < 0.05, **p < 0.01). Reprinted with permission of [113].

5.2.Cell Targeting Excipients in lipid-based Nanoparticles

Lipid-based nanoparticles, such as liposomes, are characterized by their large surface area due to their bilayer structure. This characteristic facilitates efficient drug loading and interaction with biological systems. The surfaces of liposomes can be easily modified by researchers by attaching ligands, antibodies, or other functional molecules. These modifications enhance the targeting specificity of liposomes, making them ideal for tumor localization. Liposomes are versatile and can be tailored for specific drug payloads. They can encapsulate drugs and release them gradually over time, reducing the need for frequent dosing and improving patient compliance [114]. Liposomal BCG, a primary treatment for bladder cancer, has been shown to effectively minimize BCG-related side effects such as fever, achiness, chills, fatigue, and hematuria, while preserving the therapeutic activity of BCG. Notably, BCG-liposomes have demonstrated significant tumor reduction when compared to unencapsulated BCG [115]. A novel approach known as RNA activation (RNAa) has been introduced by Kang et al [116]. This method utilizes double-stranded RNA (dsRNA) to enhance the activity of specific genes. Unlike RNA interference (RNAi), which silences genes, RNAa activates them. This can be thought of as a specialized type of RNA acting like a cancer-fighting drug, a tiny messenger instructing cells on how to behave. When this RNA drug is encapsulated within lipid nanoparticles (LNP) and directly delivered into the bladder, remarkable effects are observed. The bladder cells readily absorb these nanoparticles, and by activating the p21 gene, a tumor suppressor, they fight bladder cancer. In mice with bladder cancer, this treatment has been shown to prolong their lifespan, and some tumors even shrink or completely vanish. This study presents an exciting avenue for treating bladder cancer using precisely targeted RNA particles

Cui et.al (2015) investigated whether siRNA (small interfering RNA) could reduce survivin expression and enhance the effectiveness of mitomycin C, a chemotherapy drug, in treating human RT4 bladder transitional cell tumors. They used two newly developed carriers for delivering siRNA: PCat and PPCat. These carriers are pegylated cationic liposomes, with PPCat containing an additional component, paclitaxel, to improve in vivo delivery and transfection of survivin siRNA. The results showed that treatment with mitomycin C at a 50% cytotoxic concentration increased survivin mRNA and protein levels. Also, adding PPCat or PCat containing survivin siRNA reversed survivin induction and enhanced mitomycin C activity ($p < 0.05$). In mice with subcutaneous tumors, single-agent mitomycin C delayed tumor growth but significantly increased survivin protein levels in residual tumors. Adding PPCat-survivin siRNA, which alone caused a minor survivin decrease (<10%), completely reversed mitomycin C-induced survivin expression and further enhanced mitomycin C activity ($p < 0.05$). The results suggest effective in vivo survivin silencing. There appears to be synergism between mitomycin C and PPCat-survivin siRNA. In this study, a critical approach involved the utilization of cationic liposomes. These liposomes not only enhance the efficiency of nucleic acid transfection but also reinforce the inhibitory impact on tumor cells by facilitating endosomal escape (**Figure 6**) [117].

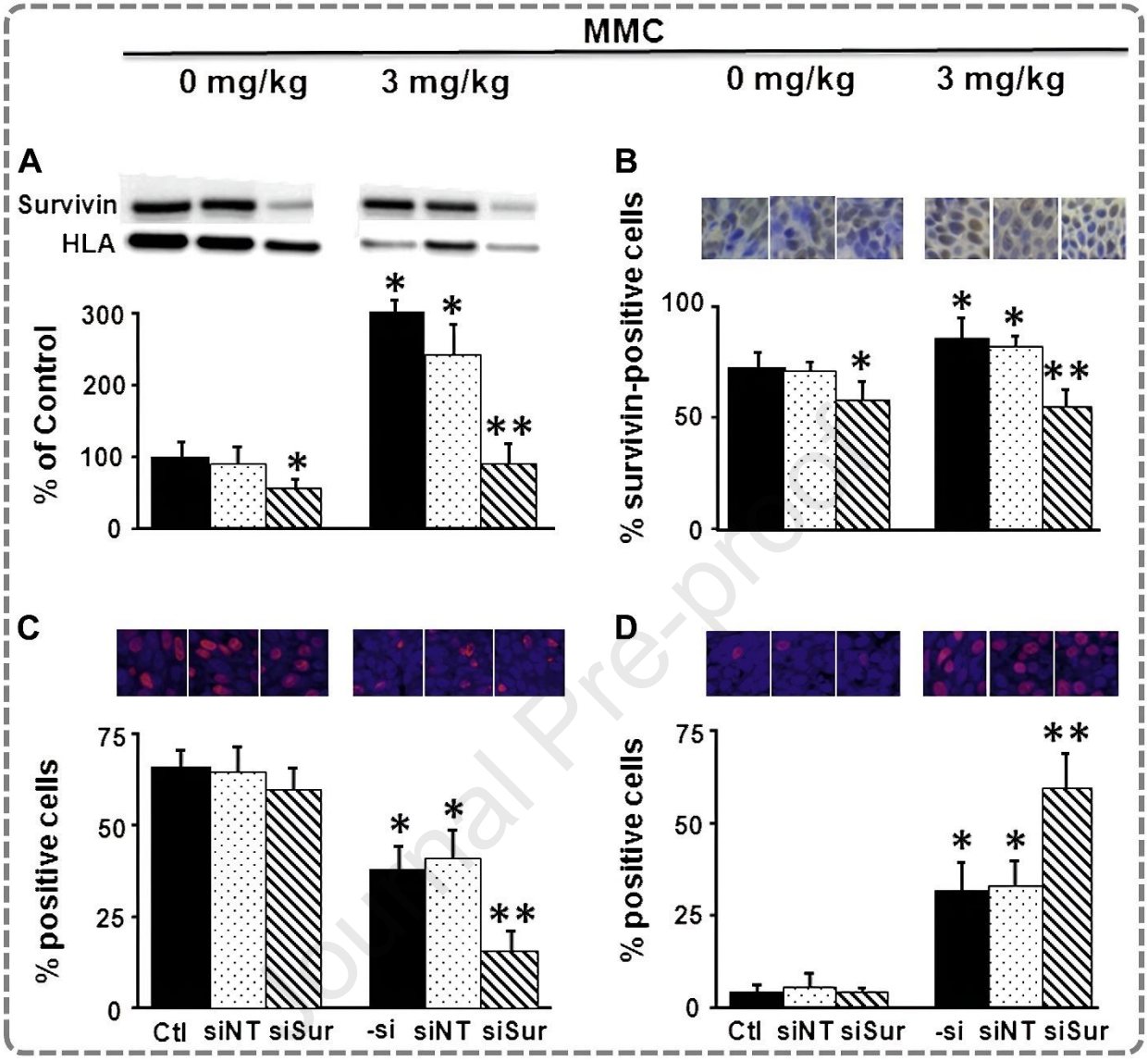

Figure 6. Illustration of the amplified antitumor activity of MMC in vivo, achieved through the silencing of survivin. Post two days of treatment, the mice were anesthetized and their tumors were subjected to Western blot, immunohistochemical staining, and quantitative imaging analysis. The findings reveal notable disparities in tumor expression, levels of survivin protein, and the expression of apoptotic cells when compared to controls and MMC plus PPCat-siNT. The data, depicted as mean + 1 SD of 3 to 5 tumors, underscore these significant differences [117]. The figure is licensed under CC BY.

In contrast to neutral lipids, cationic liposomes exhibit toxic effects. They have the potential to trigger reactive oxygen species (ROS) production and elicit inflammatory reactions. As a solution, researchers developed pegylated cationic liposomes. Mikhail et al. (2017) investigated the efficacy of lyso-thermosensitive liposomal doxorubicin (LTLD, ThermoDox®) combined with loco-

regional mild hyperthermia (HT) for precise drug delivery to the bladder wall, particularly for bladder cancer treatment. These responsive liposomes minimized off-target effects and facilitated higher doxorubicin accumulation and distribution within the bladder wall compared to free intravenous doxorubicin [118]. Guha Sarkar et al. (2017) developed a novel approach known as the 'liposome-in-gel' (LP-Gel) system to overcome limitations in intravesical drug delivery. The LP-Gel incorporates fluidizing nanoliposomes within a urine-triggered hydrogel. This hydrogel not only co-delivers the suspended nanocarriers but also enhances their adhesion to the mucin layer on the urothelium. By mimicking both the lipid membranes and mucosal layer of the urothelial barrier, the LP-Gel system achieves prolonged drug localization within the bladder. In experiments with rat and human bladder cancer cells, LP-Gel exhibited significant cytotoxicity. Upon instillation into rat bladders, it adhered more effectively to the urothelium and penetrated deeper into the bladder wall. Remarkably, paclitaxel-loaded LP-Gel retained the drug for at least 7 days, a substantial improvement over free drug administration, which has only a few hours of effectiveness and minimal systemic exposure. The LP-Gel platform holds promise for targeted drug delivery in bladder-related conditions [119]. Vila-Caballer et al. (2016) created a novel system known as pH-responsive stealth liposomes loaded with bovine serum albumin (BSA) designed to transport therapeutic proteins to the bladder epithelium. When administered to mice via bladder instillation at pH 6.5, the pH-responsive liposomes effectively delivered the loaded FITC-BSA to the bladder epithelium. The utilization of pH-sensitive liposomes offers an advantage for bladder cancer treatment, capitalizing on the naturally acidic nature of urine to enable targeted drug delivery to bladder cancer cells [120].

5.3.Cell Targeting Excipients in Carbon-based nanostructures

Carbon-based nanostructures (CBNSs), including carbon nanotubes, fullerenes, nanodiamonds, graphene, and carbon quantum dots, demonstrate efficient cellular uptake. This property facilitates effective drug delivery to cancer cells. Their high drug loading capacity, potential for thermal ablation, and promising chemical, thermal, physical, optical, mechanical, and electrical properties make CBNSs a valuable tool in cancer therapy, offering unique features and targeted drug delivery capabilities [121]. Incomplete removal of cancer during transurethral resection (TUR) can lead to recurrence. Researchers propose a novel treatment for superficial bladder tumors involving heat. The approach aims to reduce recurrence by using single-walled carbon nanotubes (SWCNTs)

specifically targeted to the tumor. SWCNTs are precisely directed toward tumor cells through the interaction of annexin V (AV) and phosphatidylserine. While phosphatidylserine is typically internalized in healthy tissue, it becomes externalized in tumors and tumor vasculature. By conjugating SWCNTs with AV, they selectively bind to bladder cancer cells. These nanotubes are delivered into the bladder at a very low dose. After 24 hours, a brief treatment with near-infrared light (NIR) heats the bound nanotubes. The heat selectively targets the tumor while preserving the healthy bladder wall. In mice studies, this innovative therapy resulted in no visible tumors on the bladder wall after NIR light treatment and no bladder damage. Additionally, a separate survival study demonstrated a 50% cure rate. This promising approach holds potential for treating bladder cancer and minimizing recurrence [122]. Rieger et al. (2015) investigated multi-walled carbon nanotubes (MW-CNTs) with varying aspect ratios to find the most effective CNT type for multifunctional drug transport. They synthesized four CNT variants (CNT-1, CNT-2, CNT-3, and CNT-4) with different lengths and diameters. Among these, the shorter CNTs (CNT-2 and CNT-4) exhibited more defects and lower aspect ratios compared to the pristine CNT-1 and CNT-3. The researchers assessed how well these CNTs adhere to bladder tissue. When exposed to mouse bladders, all four types of CNTs adhered to the bladder lining (urothelium) and covered approximately 5–10% of the area. Furthermore, in vitro studies using bladder cancer cells (UM-UC-3 and EJ28) revealed that CNT-2 and CNT-4 had stronger inhibitory effects than CNT-1 and CNT-3. Overall, CNT-1 and CNT-3 appear most promising for further developing a multifunctional drug transporter to treat bladder cancer (**Figure 7**) [123].

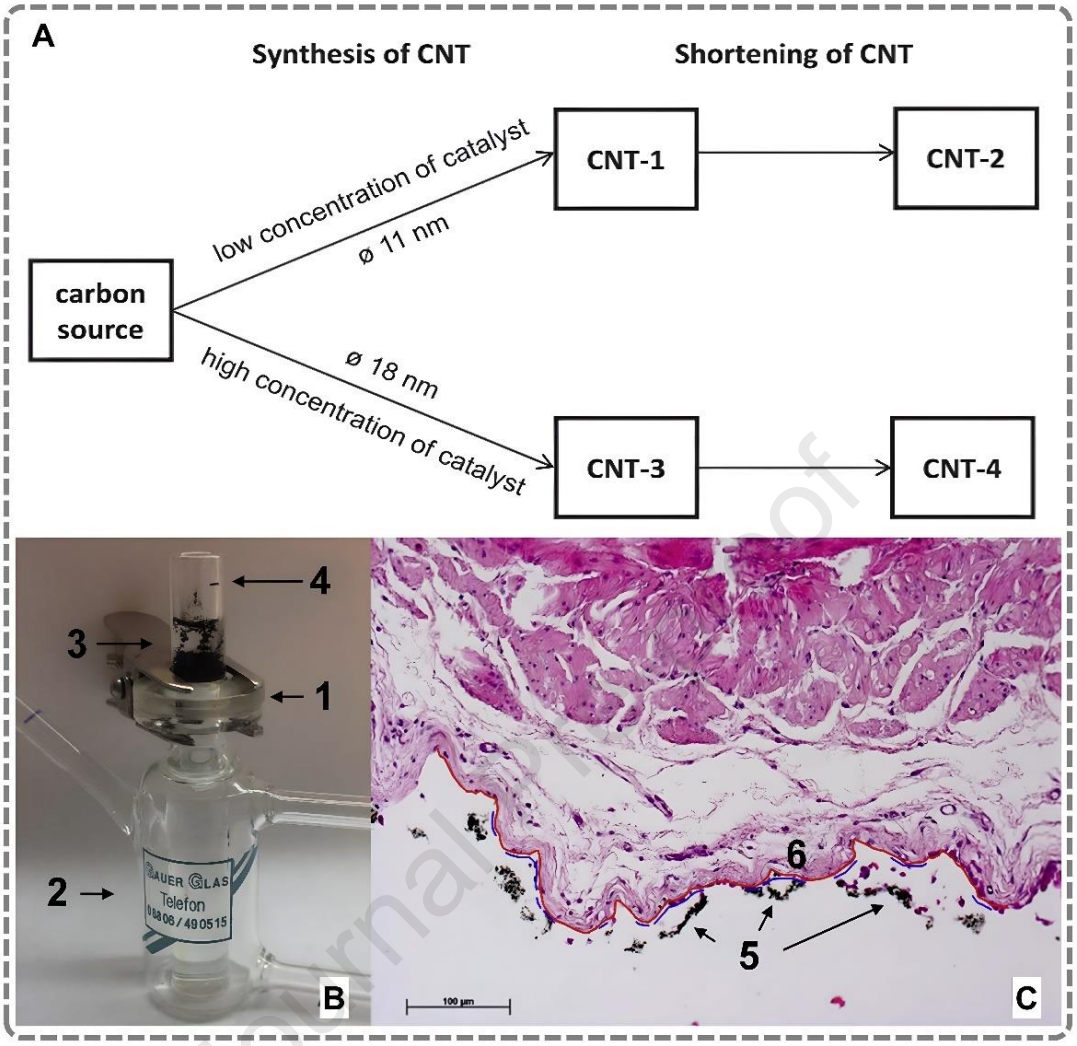

Figure 7. (A) Illustrates the process flow of the synthesis and shortening of various types of Carbon Nanotubes (CNTs), along with the experimental setup for mucoadhesion studies on explanted urinary bladders from mice. (B) Depicts the mounting of mouse bladder tissue (1) onto the receptor chamber of the Franz diffusion cell (2), followed by the placement of CNT suspensions (3) into the donor chamber (4) for an incubation period of 1 hour at room temperature. (C) Showcases the histological analysis of the bladder tissue section with CNTs attached to the urothelium, performed microscopically. The total urothelial surface and the urothelial surface covered by the CNTs are represented by red and blue lines, respectively. Reprinted with permission of [123].

5.4.Cell Targeting Excipients in

5.4.1. Metallic nanoparticles

Inorganic nanoparticles display a wide range of properties and can be categorized based on their composition and characteristics. The primary groups of inorganic nanoparticles include metallic nanoparticles and non-metallic nanoparticles [124].

MNPs improve the stability and extend the half-life of drug carriers in circulation, ensuring prolonged drug effectiveness and better treatment outcomes. MNPs can be tailored for passive or active targeting. Active targeting involves attaching ligands (such as antibodies or peptides) to MNPs, enabling specific binding to cancer cells. This precise targeting minimizes harm to healthy tissues. MNPs efficiently distribute to the desired target site due to their small size and surface properties. They can navigate biological barriers and reach tumor regions effectively. MNPs' large surface-area-to-volume ratio makes them ideal for surface modifications [125]. These alterations enhance drug loading, improve biocompatibility, and enable controlled release. exhibit high biocompatibility and stability, rendering them suitable for medical applications. Metal NPs possess anti-cancer effects, including apoptosis induction, cell cycle arrest, inhibition of tumor angiogenesis, metastasis, and inflammation. These actions collectively contribute to halting cancer proliferation [126]. Ferreira et al. (2019) investigated the use of silver nanoparticles (AgNPs) as an anticancer treatment for non-muscle invasive bladder cancer (NMIBC). They induced bladder cancer in female mice using N-methyl-N-nitrosourea (MNU) and administered different concentrations of biogenic AgNPs via the bladder (intravesical route). While the 0.5 mg/mL AgNP treatment did not effectively reduce cancer lesions, the 0.2 mg/mL concentration showed some tumor regression. Notably, the lowest concentration (0.05 mg/mL) led to significant tumor regression, with some animals exhibiting normal tissue. Their findings also revealed that AgNPs induce cell death through apoptosis and inhibit cell migration and proliferation in human bladder cancer cells (5637 cells) [127]. Polikarpov et al. (2019) aimed to create specialized nanocomplexes for detecting bladder cancer cells. These nanocomplexes consist of Upconversion Nanoparticles (UCNP), which can convert low-energy light (such as near-infrared) into higher-energy visible light. The UCNP were coated with silica. When combined with the Anti-Glypican-1 Antibody MIL38, these nanoconjugates selectively bind to urothelial carcinoma cells (bladder cancer cells). The antibody specifically targets Glypican-1, a protein found in bladder cancer cells. Upon

exposure to near-infrared light, the nanocomplexes emit visible light (photoluminescence), making the cancer cells visible. This technology holds promise for improving bladder cancer detection during surgery using light-based techniques (**Figure 8**) [128].

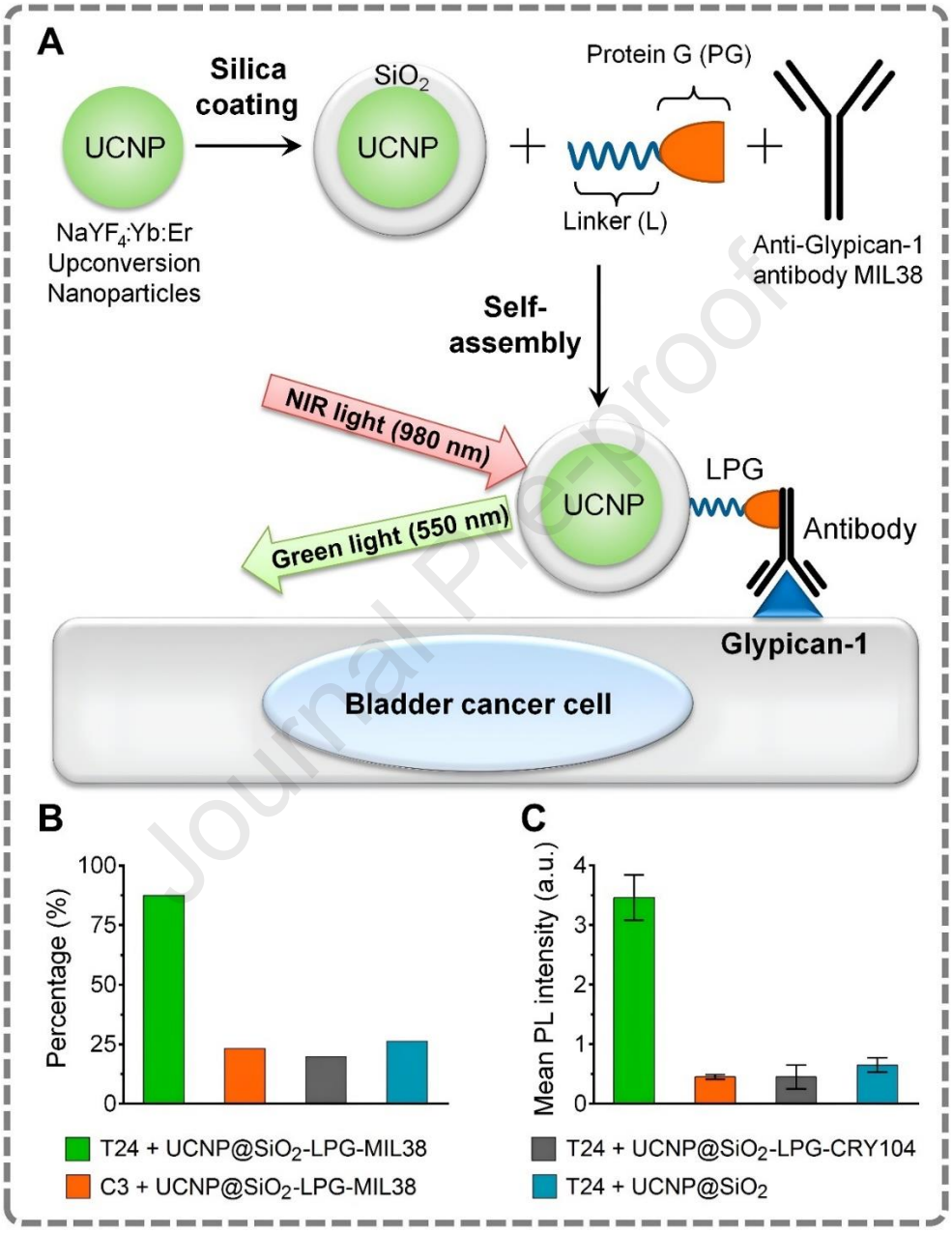

Figure 8. (A) Depicts the synthesis and utilisation of targeted upconversion nanoconjugates, UCNP@SiO2-LPG-MIL-38, to mark bladder cancer cells and interact with urothelial carcinoma cells. (B) Shows the proportion of Glypican-1 high T24 and low C3 urothelial carcinoma cells labelled by targeted and non-targeted nanoconjugates. (C) Shows T24 and C3 cell average PL intensity labelled by targeted and non-targeted nanoconjugates. The error bars show the mean 95% confidence interval [128]. The figure is licensed under CC BY.

5.4.2. Non-Metallic nanoparticles

Yuan et al. (2018) aimed to create specialized fluorescent probes for detecting bladder cancer cells. These probes combined Quantum Dots (QDs) with a Prostate Stem Cell Antigen (PSCA) antibody. Specifically, they linked QDs (with a wavelength of 605 nm) to the PSCA antibody. When these probes were used, they specifically recognized the PSCA protein expressed in bladder cancer cells. Importantly, the fluorescence from the probes remained stable and had a long duration. These fluorescent probes hold promise for targeted imaging of bladder cancer cells, aiding in non-invasive tumor detection and early diagnosis during medical imaging [129]. Wang et al. (2020) developed a system that adheres to the bladder lining and gradually releases drugs. They achieved this by modifying mesoporous silica nanoparticles (MSNPs) with poly(amidoamine) (PAMAM) dendrimers on their surface. Additionally, they loaded an anticancer drug (doxorubicin) into the MSNPs' pores. These drug-loaded nanoparticles released the drug gradually, triggered by acidic pH. The more PAMAM amino groups they added, the better the MSNPs adhered to the bladder. The maximum adhesivity occurred with two-generation PAMAM. In summary, adjusting the PAMAM layer on MSNPs allows control over their mucoadhesive and drug release properties [113]. Wei et al. (2017) developed a system where they coated mesoporous silica nanoparticles (MSNs) with Polydopamine (PDA). This modified system, called DOX-loaded MSNs@PDA-PEP, includes a peptide called CSNRDARRC that specifically targets bladder cancer cells. These nanoparticles gradually release doxorubicin (DOX) and are sensitive to changes in pH. In lab tests, they were efficiently taken up by bladder cancer cells due to the peptide's recognition. Both in vitro and in vivo experiments demonstrated that DOX-loaded MSNs@PDA-PEP performed better than free DOX and other MSN formulations. These targeted nanocarriers hold promise for improving bladder cancer [130].

6. Concluding Remarks, Obstacles, and Anticipated Developments

Research on bladder cancer has made substantial progress, revealing novel therapy strategies and sophisticated diagnostic techniques that have the potential to greatly enhance patient outcomes. This work highlights the crucial significance of metallic nanoparticles (MNPs) and carbon-based nanostructures (CBNSs) in improving medication delivery and accuracy in treatment, which has the potential to completely transform bladder cancer therapy. The use of conjugating single-walled

carbon nanotubes (SWCNTs) with annexin V for targeted thermal ablation, as well as the utilization of silver nanoparticles (AgNPs) to induce apoptosis, showcase the strong anticancer properties of these nanomaterials. Furthermore, the effective containment of survivin siRNA inside chitosan-modified nanoparticles with mucoadhesive characteristics demonstrates the promise of targeted drug delivery methods in diminishing tumor size. Together, these progressions provide the basis for efficient therapies that might reduce the recurrence of bladder cancer and enhance the prognosis of patients.

Although the potential of nanomedicine in treating bladder cancer is significant, there are numerous crucial obstacles that need to be overcome in order to effectively use these technologies in clinical settings. An important problem is the need for comprehensive research on biocompatibility and toxicity in order to comprehend the long-term consequences of nanoparticle buildup in tissues. For instance, although AgNPs have shown the ability to decrease the growth of tumors, research has also indicated the possibility of harmful consequences at larger dosages. This highlights the need of optimizing the dosage and conducting safety assessments. Furthermore, the technological difficulties associated with ensuring uniformity in the quality and effectiveness of nanoparticles at a large-scale industrial level might impede their broad use in therapeutic settings. The problem of reproducibility continues to be a significant obstacle, since even little discrepancies in nanoparticle production may result in significant disparities in therapeutic results.

Furthermore, nanoparticle-based medicines must successfully negotiate the intricate immunological responses of the body, which might result in unexpected outcomes such as fast elimination or off-target effects. The stringent regulatory framework that governs the approval of innovative medical technologies adds significant complexity to the translation of these promising cures into clinical practice. This is seen in the long and intricate approval procedures that nanoparticle-based drug delivery systems must undergo.

In order to address these obstacles, it is imperative that future studies in the field of bladder cancer treatment prioritize the development of collaborative efforts involving several disciplines. Important research inquiries that must be answered are:

- *How can we further improve the specificity and effectiveness of medicines based on nanoparticles?* Subsequent inquiries should focus on the advancement of superior ligands and

targeting moieties that can accurately guide nanoparticles to cancer cells while reducing unintended side effects.

- *What are the enduring consequences of the buildup of nanoparticles in the human body?* Thorough investigations on the distribution, breakdown, and elimination of nanoparticles are crucial to guarantee their safety for extended clinical use.

- *How can nanoparticle-based medicines be combined with current treatment methods?* Further investigation is necessary to explore the possible synergistic effects of nanoparticles and other treatments, like as radiation or immunotherapy, in order to enhance the overall effectiveness of treatment.

- *Is it possible to create individualized medicine with nanoparticle-based therapies?* Customizing these medicines based on the exact tumor features and genetic profiles of individual patients should lead to more accurate and efficient treatments.

In order to enhance the process of translating preclinical research into clinical applications, it will be essential to have strong clinical trials, regulatory assistance, and collaborations with the industry. Ongoing investment in these fields is crucial for conquering existing challenges and guaranteeing the advancement of inventive, secure, and efficient therapies for bladder cancer. An impartial and comprehensive assessment of the advantages and difficulties of nanomedicine is crucial in order to fully exploit its promise in cancer treatment.

CRediT authors statement

Conceptualization, **Abbas Rahdar**; Writing-original draft preparation, **Zakiyeh Sadat Hoseini , Somayeh Zeinalilathori , Safieh Zeinali , Bazla Siddiqui, Zelal Kharaba**; Writing-review and editing, **Sonia Fathi-karkan**, **Abbas Rahdar**, **Sadanand Pandey**; Supervision, **Abbas Rahdar, Sadanand Pandey, Sonia Fathi-karkan**. All authors have read and agreed to the published version of the manuscript.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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