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Abstract

Introduction: Bladder Cancer is one of the most expensive cancers to treat due to its high cost of therapy as well as the surveillance expenses incurred to prevent disease recurrence and progression. Thus, there is a strong need to develop safe, efficacious drug formulations with controlled drug release profiles and tumor targeting potential, for improved therapeutic outcomes of bladder cancer patients.

Areas covered: Previously published reviews concentrated on micro- and nano-particulate systems; reviewed biological systems and focused on disease diagnosis. This review aims to provide an overview of drug formulations that have been studied for potential bladder cancer treatment in the last decade; highlight recent trends in bladder cancer treatment; mention ongoing clinical trials on bladder cancer chemotherapy; detail recently FDA-approved drug products for bladder cancer treatment and identify constraints that have prevented the translation of promising drug formulations from the research laboratory to the clinics.

Expert Opinion: This work revealed that surface functionalization of particulate drug delivery systems was required for targeted drug delivery to bladder tumors. Also, incorporating the nanoparticles into in situ gelling systems could facilitate controlled drug release for extended periods, and improve the prognosis of bladder cancer treatment. Future research directions could incorporate multiple drugs into the drug delivery systems to treat advanced stages of the disease. In addition, smart nanomaterials, including photothermal therapies could be exploited to improve the therapeutic outcomes of bladder cancer patients.

Keywords: Bladder cancer, Therapeutic delivery system; preclinical; *in vitro* and *in vivo* evaluation; clinical trials; FDA approval

1. Introduction

The human urinary bladder (Fig. 1a) prevents harmful substances from being retained within the body through its periodic removal of urine from the body. Bladder cancer (BC) is the most common malignant disease affecting the human urinary bladder. There are two main types of bladder cancer; non-muscle-invasive bladder cancer, which is confined to the bladder whereas the muscle-invasive bladder cancer affects neighboring tissues and organs [1]. The various stages of bladder cancer have been highlighted in Table 1. BC patients often present with hematuria, painful urination, and low back pain [1].

Bladder cancer is ranked 4th and 8th, respectively, in terms of disease morbidity and mortality rate amongst men in the United States. The cost of BC therapy over the lifetime of the patients is high, requiring $3.98 billion in the US and €4.9 billion in Europe annually to manage the disease [2], [3].

Table 1: Bladder Cancer staging. Reprinted from [1] with permission from Elsevier

Category	Stage	Description and extent of tumor coverage
Ta, N0, M0	Stage 0a	Non-invasive papillary carcinoma – hollow center of the bladder
Tis, N0, M0	Stage 0is	Flat, non-invasive carcinoma (carcinoma in situ) – inner bladder lining
T1, N0, M0	Stage I	Invasive - connective tissues beyond the urothelial lining
T2a or T2b, N0, M0	Stage II	Invasive – inner half (T2a); outer half (T2b) of the muscular region
T3a, T3b, T4a, N0, M0	Stage III	Invasive – fatty tissue region visible with microscope (T3a); readily visible (T3b); spread to prostate, uterus and/or vagina (T4a)
T4b, N0, M0, N1-3, M1	Stage IV	Invasive/metastatic – pelvic or abdominal wall (T4b), single pelvic lymph node (N1); ≥ 2 lymph nodes (N2); iliac arterial lymph nodes (N3); beyond the bladder to distant sites like bones, liver or lungs

Note: N0 and M0 denote that lymph nodes and distant sites (metastatic tumors) were not affected.

Intravesical drug delivery (IDD), or a direct instillation of drug formulations to the bladder through the urethra, has been widely explored to improve drug availability at the malignant urothelial tissues and avoid systemic side effects. IDD has been used to treat hyperactive bladder syndrome [4], interstitial cystitis [5] bladder infections, urinary obstructions [6], and bladder cancer [7]. However, the efficiency of this localized route of drug delivery remains limited by various factors highlighted in Fig. 1b. For instance, hydrophobic anticancer agents are used to treat bladder cancer to improve their bladder permeability potential. Nevertheless, drugs that do not possess satisfactory physicochemical properties could be incorporated into drug delivery systems to improve their intracellular delivery. Also, the

urothelium is the bladder permeability barrier that prevents drug delivery to malignant urothelial tissues [1], [8]. Various active strategies employed to surmount the urothelium, include electromotive and thermal techniques. Also, drug permeation through the bladder has been enhanced using dimethyl sulfoxide, protamine sulfate, and biomolecules such as chitosan and polycarbophil [8]. In addition, the process of urine formation and removal could reduce drug residence time, necessitating frequent catheterization to maintain the therapeutic level of the drug within the bladder and resulting in bladder irritation and infection as well as poor patient compliance to the dosage regimen [1].

The high cancer recurrence rates in high-risk NMIBC patients might be attributed to the poor efficacy of conventional dosage forms and treatment regimen [9], [10]. Thus, drug delivery researchers have investigated improved dosage forms for the intravesical treatment of bladder cancer. Promising drug formulations should be safe, effective, with sustained drug release profiles to reduce drug dosage and dosing frequency.

Some published reviews have focused on micro- and/or nano-particulate drug delivery systems for bladder cancer treatment [11-19], including chitosan-based nanoparticles [20]. Tran and colleagues reviewed some studies on bladder cancer biology and therapy [21]. Hu and coworkers discussed advanced drug carriers for bladder cancer diagnosis and therapy [22], Bogen et al., focused on biotherapeutics [23], and Banerjee et al highlighted challenges that have prevented the clinical translation of promising drug formulations [24]. However, these articles did not provide a timeline for ongoing clinical trials that pertain to bladder cancer drug formulations. Therefore, this review aims to identify bladder cancer cell membrane proteins that are overexpressed in bladder cancer patients; give a recent update on chemotherapeutic dosage forms that have been investigated for the potential treatment of bladder cancer in the last decade; create a timeline for ongoing clinical trials, and highlight important bladder cancer drug delivery parameters that need to be evaluated to ascertain that BC medicines are safe and effective. The formulations intended for bladder cancer diagnosis are not covered in this review.

2. Potential membrane proteins as bladder cancer therapeutic targets

Membrane proteins on bladder cancer cell surfaces interact with various receptors such as integrins, receptor tyrosine kinases, and G-protein coupled receptors and they alter the normal cell cycle, resulting in bladder cancer cell proliferation, malignant cell survival, apoptosis, invasion, and angiogenesis [25]. The membrane proteins could be released into the patient's biological fluid, promoting the diagnosis of the disease, including its severity.

Bladder cancer recurrence occurs due to overexpression of some membrane proteins; altered cell membrane composition; reduced level of drug transporters, and improved efflux pump, inhibiting the accumulation of therapeutic agents at target malignant urothelial tissues. Potential membrane protein biomarkers associated with bladder cancer (Table 2) have been identified.

Table 2: Potential membrane proteins for bladder cancer diagnosis and prognosis. Reprinted from [25] under a CC BY license.

Protein biomarkers	Types of proteins/receptors	In vitro method of detection	Clinical study		
			Expression in cell lines	Method of detection	Expression in specimens
Pgp-1	Transporter	WB	Highly expressed in 253J and J82 cells	IHC	Highly expressed in 39 of 55 BC specimens (71%), China
Her 2	RTK	WB	Expressed in BC cell lines but 10-fold lower in the breast cancer cell, SKBR3 cells	IHC	Overexpressed more in NMIBC patients (21%), China
TCSTD2	RTK	RT-PCR	Highly expressed in multiple BC cell lines	IHC	Highly expressed in 27.3% of the 99 patients, Japan
VEGFR1	RTK	Immunoblot	Higher expression in TCCSUP	IHC	Increased 2-fold in BC specimens compared with normal patients, USA

VEGFR2	RTK	Immunoblot	Highly expressed in J82 and HT1376 BC cells	IHC	Increased 55 % in BC specimens compared with the normal patients, USA
Integrin β 8	Integrin	Immunofluorescence assay	Overexpressed in Biu87 and T24 BC cells	IHC	Increased two-fold higher in highly malignant BC, China
FGFR3	RTK	WB	Highly expressed in RT4, RT112 and SW780 cells	IHC	Highly expressed (40%) in patients with p71 BC, Korea
CXCR7	GPCR	Q-PCR	Highly expressed (3-10-fold in 5637 and HT1197 cell lines than in other cell lines	IHC	Highly expressed (5-10-fold) in BC tissues than in normal tissues, Florida, USA

Abbreviations: Pgp-1, P-glycoprotein-1; Her 2, Human epidermal growth factor receptor 2; VEGFR1, Vascular endothelial growth factor receptor 1; VEGFR2, Vascular endothelial growth factor receptor 2; CXCR7, Chemokine receptor 7; FGFR3, Fibroblast growth factor receptor 3; IHC, immunohistochemistry; RT-PCR, reverse transcriptase-PCR; RT-qPCR, reverse transcriptase-quantitative PCR; WB, western blot

3. Advanced drug delivery systems

Advanced drug formulations for bladder cancer treatment have been designed based on the knowledge of carrier surface characteristics; surface targeting design; and interactions between targeting molecules and bladder cancer membrane proteins. For example, the surface of drug delivery systems could be decorated with mucoadhesive and intracellular targeting moieties such as boronate groups so that they could be retained at target body sites for prolonged periods, which will facilitate effective therapy. The drug carriers for bladder cancer treatment have been prepared using different techniques (Fig. 2), and

the surfaces of the particulate drug delivery systems have been modified using various chemical methods.

3.1. Liposomes

Liposomes comprise synthetic or natural phospholipids that self-assemble to form bilayered vesicles surrounding an aqueous core. They can encapsulate both hydrophilic and hydrophobic drugs, including DNA plasmids. The loaded drug would be taken up into the target cells via endocytosis [26]. Liposomal systems could be functionalized to improve their physicochemical properties and bioavailability.

3.1.1 Anticancer drug-loaded surface-modified liposomes

Cisplatin therapy has been associated with severe side effects such as nephrotoxicity and neurotoxicity [27]. Polyethylene glycol (PEG) is a hydrophilic, non-ionic, biocompatible, non-immunogenic polymer that is composed of repeating ethylene glycol units [-($CH_2CH_2O)_n$], that is widely used to prepare drug products intended for internal, external and transmucosal routes of administration due to its protein repellent properties that efficiently shields it from the host's immune system and prevent premature elimination of the drug delivery system from systemic circulation [28]. The spherical shape, unilamellar vesicles, and low polydispersity index of cisplatin-loaded PEGylated liposomes and fluorescein sodium-loaded maleimide-functionalized liposomes [29], [30] are beneficial for improved urothelial malignant cellular uptake and cargo delivery to target sites [31]. Liposomes with unilamellar vesicles are preferable for drug delivery because they release drugs to the target sites more readily than their bilayer or multilayer liposomal counterparts [32]. The particle size of cisplatin-loaded PEGylated liposomes ranged from 221 nm to 274 nm [30] while that of fluorescein sodium-loaded maleimide functionalized PEGylated liposomes (FS-Mal-PEG-Lip) was 90±1 nm [29]. Notwithstanding, they have satisfactory particle size for intravesical drug delivery (50-300 nm). The cisplatin liposomes and fluorescein sodium liposomes exhibited variable particle sizes due to differences in the method of preparation of the liposomes as well as the constituents of the liposomes [33]. For instance, cisplatin liposomes were prepared using the reverse-phase evaporation method [30] while fluorescein sodium liposomes were formulated using the thin film hydration method [29]. The respective zeta potential values for blank unmodified liposomes, cisplatin-loaded unmodified liposomes, and cisplatin-loaded PEGylated liposomes were -27.0±1.3 mV, - 20.0±0.9 mV, and – 7.0±10.3 mV, suggesting that positively charged cisplatin and polyethylene glycol (PEG) reduced the negative particle surface charge of the liposomes [30]. Moreover, the relatively positive surface charge of the drug-loaded PEGylated liposomes was beneficial for the improved interaction of the drug formulation with negatively charged urothelial membranes [30]. On the other hand, the maleimide functionalized drug delivery system could interact with urothelial tissues via superior covalent linkage (Michael addition reaction) relative to PEGylated liposomes.

The drug encapsulation efficiencies (EE) of cisplatin-loaded unmodified liposomes and PEGylated liposomes were 34 ± 2 % and 37 ± 2 %, respectively, suggesting that PEGylation of the liposomes improved solubilization of hydrophilic cisplatin [30]. Conversely, the encapsulation efficiencies (EE) of fluorescein sodium decreased with PEGylation and maleimide functionalization of nanoparticles as the EE of unmodified, PEGylated, and maleimide PEGylated liposomes were 53±6 %, 27±2 %, and 25±2 %, respectively [29]. These findings revealed that the model drug (fluorescein sodium) may not be the ideal candidate for evaluating drug encapsulation efficiency and loading capacity of the drug delivery system.

The respective amounts of cisplatin released from drug-loaded unmodified liposomes and drug-loaded PEGylated liposomes after 48 h were 43.0±2.0 % and 39.0±1.9 % [30]. These findings revealed that surface modification of liposomes with PEG may facilitate controlled cisplatin release for an extended period [30]. On the other hand, all the fluorescein sodium (model drug) loaded into conventional liposomes was released in 2 h whereas 95-100 % of fluorescein sodium was released from PEGylated and Mal-PEGylated liposomes within 4 h and 8 h [29], respectively. These studies revealed that PEG and/or maleimide functionalization of liposomes could facilitate sustained release of the loaded anticancer agent or model drug.

Fluorescein Sodium-loaded maleimide functionalized liposomes exhibited superior porcine bladder mucosal retention relative to conventional liposomes (samples retained after 1 h: 32% versus 18 %). Also, PEGylated liposomes and maleimide functionalized PEGylated liposomes exhibited urine wash-out50 (WO_{50}) values of 24 mL and 48 mL, respectively [29]. Interestingly, PEGylated liposomes exhibited improved bladder mucosal penetration tendency in comparison to maleimide functionalized liposomes, which may be due to the mucus penetrating effect of PEG whereas maleimide interacts with the thiol groups within the bladder mucosal tissues via covalent bonding, inhibiting penetration into underlying urothelial membranes [29]. Cisplatin-loaded PEGylated liposomes exhibited superior bladder cancer antitumor activity relative to cisplatin-loaded unmodified liposomes and free cisplatin solution (91% versus 78% versus 59 %) [29]. There was a good correlation between the IC_{50} values of the studied delivery systems and the tumor volume of rats treated with these formulations (Fig. 3).

The drug-loaded liposomal formulations were safer than the free drug, as rats treated with free cisplatin, drug-loaded unmodified liposomes, and cisplatin-loaded PEGylated liposomes exhibited body weight gain of 3, 8, and 11 %, respectively. Also, the serum concentrations of blood urea nitrogen, creatinine, alkaline phosphatase, alanine transaminase, and aspartate aminotransferase in the liver and kidney were significantly decreased after the administration of cisplatin-loaded liposomal formulations, inferring that the new formulation was biocompatible [27][28].

3.2. Anticancer agent-loaded surface-modified polymeric nanoparticles

All the studied polymeric nanoparticles for bladder cancer treatment were spherical and displayed low PDI (< 0.4), indicating that they have a narrow particle size distribution [34]. In addition, they exhibited zeta potential values that ranged from ±10 mV to ±36 mV, depicting good colloidal stability [35]. Though, doxorubicin-loaded PEGylated PAMAM dendritic nanoparticles (PEG-PAMAM-Dox) exhibited ZP of 2.78 mV [9], it had satisfactory colloidal stability in simulant urine over 24 h, which may be due to the self-assembly of the dendritic particles, facilitating their stability in the biological fluid.

Amphiphilic N- [1-(2, 3-dioleoyloxydioleoyloxy) propyl] - N, N, N-trimethylammonium chloride (DOTAP) has mucoadhesive property, and Jin and coworkers utilized it to modify the surface of hybrid nanoparticles formulated using methoxy poly (ethylene glycol)-poly (lactide), (MPEG-PLA) diblock copolymer [36]. Doxorubicin-loaded PEGylated PAMAM-dendritic nanoparticles [9] and doxorubicin-loaded DOTAP-modified polymeric micelles [37] displayed comparable average particle sizes of 13 nm, and 18.7 nm, respectively. The drug-loaded nanoparticles' relatively small size could be due to their self-assembly properties. Nevertheless, these nanoparticles may be beneficial for bladder cancer treatment due to their ability to resist premature clearance from the systemic circulation; accumulate within tumor tissues via the enhanced penetration effect, and possibly improve therapeutic outcomes of bladder cancer patients [37]. These nanoparticulate systems will be suitable for parenteral administration. Conversely, Sahatsapan and coworkers reported that doxorubicin-loaded maleimide-chitosan-catechol-alginate nanoparticles (Dox- Mal-CHI-Cat-ALG NPs) containing 0.05 % of Mal-CHI and 0.05 % Cat-ALG exhibited particle size of 115.8 ± 0.9 nm, and it would be suitable for parenteral and localized bladder cancer drug delivery.

The clinical use of belinostat has been limited by its poor aqueous solubility (0. 14 mg/mL) as well as its potential to induce geno-, hepato-, hematologic, and gastrointestinal toxicity [38]. Thus, belinostat was formulated as polymeric nanoparticles to improve its aqueous solubility, anticancer efficacy, and tolerability [34], [37]. Poly (guanidinium oxanorbornene) (PGON) is a non-toxic, cationic synthetic polymer with cell-penetrating properties. Martin et al. studied PGON-modified PLGA nanoparticles for the intravesical delivery of belinostat. The particle diameter of unmodified (NP-Bel) and modified PLGA (NP-Bel-PGON) nanoparticles was 144±40 and 151±32 nm, respectively [39]. Also, the unmodified chitosan nanoparticles and CHI-PCL NPs studied by Erdogar and coworkers displayed mean diameters of 166 ± 6 nm and 319 ± 5 nm, respectively. These findings revealed that surface modification of polymeric nanoparticles increased particle size. The modified nanoparticles would be suitable for localized bladder cancer treatment because nanoparticles with a diameter greater than 200 nm will trigger the complement system, resulting in their removal from the bloodstream and elimination to the liver and spleen. On the other hand, Garcinia mangostana extract-loaded catechol-modified

nanoparticles [40] that exhibited particle size of 155-186 nm would be valuable for the systemic treatment of advanced stages of bladder cancer.

Cook and coworkers synthesized thiolated microgels by co-polymerizing 2-(acetylthio) ethylacrylate (ATEA) with 2-hydroxyethyl methacrylate (HEMA), and ethylene glycol dimethacrylate served as a cross-linker [41]. Interestingly, the microgels exhibited a particle diameter of 635-977 nm, and the drug carrier may not be suitable for intravesical instillation or systemic therapy based on its size. Nevertheless, the thiolated drug carrier could exploit covalent interaction with mucin glycoproteins, facilitating their cellular uptake into target sites [42]. The surface modification of nanoparticles with mucoadhesive moieties such as thiol, amine, maleimide, catechol, methacrylate, and boronate groups could improve their drug encapsulation efficiency and loading capacity. For instance, the belinostat loading capacity of PGON-modified NPs was greater than that of unmodified nanoparticles (12.9 % versus 3.9%), indicating that surface modification of PLGA nanoparticles with PGON could enhance their drug loading capacity [39]. Also, doxorubicin-loaded polymeric micelles exhibited drug encapsulation efficiency of 90 % and drug loading capacity of 8 %, suggesting that doxorubicin was efficiently solubilized into the copolymeric micellar system [36]. In addition, doxorubicin-loaded PEGylated PAMAM dendritic nanoparticles exhibited an EE of 85.2%. Furthermore, thiolated microgels displayed doxorubicin encapsulation efficiency that ranged from 75 % to 86% [41]. In addition, amine-functionalized polyacrylamide nanogels exhibited docetaxel loading capacity greater than 90% [42], indicating that these microgels and nanogels can solubilize hydrophilic and hydrophobic drugs. The 30 mol% and 80 mol% ATEA/ HEMA-based microgels displayed satisfactory doxorubicin loading capacity of 2.5 mg mL^{-1} and 2.7 mg mL^{-1} doxorubicin, respectively (30), which was greater than the therapeutic doses of doxorubicin (1–2 mg mL^{-1}; 25–100 mL solution) [43], [44].

The type of polymeric nanoparticles; including their surface modification and the pH of the release medium may or may not influence the drug release pattern of polymeric nanoparticles. For example, doxorubicin was favorably released at pH 5.5 (60%) from DOTAP-modified hybrid nanoparticles compared to the amount of drug that was released at pH 6.5 (23%) and 7.4 (37%) [36], suggesting that these drug formulations could be preferentially released into the slightly acidic tumor regions, with a pH value of 5.5 [36]. On the other hand, the amount of mitomycin C released from chitosan NPs and chitosan-coated PCL NPs after 3 h at pH of 5.5 and 7.8 was more than 90 %, indicating that the drug formulation will be suitable for both local and systemic drug delivery. Doxorubicin was released from thiolated microgels into simulant urine in a sustained manner over 5 h [41]. Also, the amount of docetaxel released from amine-functionalized polyacrylamide nanogels over 9 h and 9 days were 30 % and 76 %, respectively [42], revealing that the microgels and nanogels could facilitate controlled drug release. The amount of Garcinia mangostana released from unmodified alginate NPs and catechol-modified NPs over 4 h was 57 % and 90%, respectively [40].

The mucoadhesive properties of nanoparticles could dictate their cellular uptake and intracellular drug delivery. For instance, doxorubicin-loaded microgels containing 80 mol% and 30 mol% of ATEA exhibited the greatest and least extent of thiolation, respectively [41]. The highly thiolated microgels displayed superior resistance to artificial urine wash-out from porcine bladder mucosal tissues relative to formulations with low levels of thiolation. This may be associated with thiol groups from the drug carrier forming covalent disulphide bridges with the cysteine-rich regions of urothelial mucins. Also, fluorescein-loaded unmodified and modified PLGA-PEG nanoparticles exhibited urine wash-out$_{50}$ values of 5 mL and 15 mL, respectively [45]. In addition, Catechol-alginate nanoparticles exhibited superior mucoadhesiveness relative to unmodified alginate nanoparticles, with 45% and 20% of the formulation retained on the porcine bladder tissues after 1 h [40]. Furthermore, the number of nanoparticles retained on porcine bladder mucosal surfaces after 1 h for unmodified, Catechol-modified alginate NPs and Mal-CHI-Cat-ALG NPs was 13 %, 43 %, and 55%, respectively [46]. These findings revealed that the type and amount of mucoadhesive moieties conjugated to the surfaces of nanoparticles could influence their mucoadhesive properties. Moreover, maleimide conjugated to the nanoparticles interacts with cysteine residues on the bladder cell membrane via a Michael-type addition reaction whereas the catechol group of the drug carrier could form irreversible covalent bonds with bladder mucin's thiols and amines, forming o-quinolones, facilitating strong covalent linkage between urothelial tissues and the drug formulation [46]. Some research groups conducted cytotoxicity testing of their novel drug products using murine and human bladder cancer cell lines. For instance, Doxorubicin loaded DOTAP modified polymeric micelles displayed a lower IC_{50} value against MB49 murine cells than doxorubicin loaded unmodified nanoparticles and free doxorubicin solution (0.281 µg/mL versus 0.638 µg/mL versus 0.815 µg/mL) [36]. Also, Amine-modified polyacrylamide nanogels exhibited superior human UMUC3 inhibitory effect relative to T24 cells, and the cytotoxicity increased with an increase in exposure time of the cells to the tested drug formulations [42] (Fig. 4).

In addition, Garcinia mangostana extract-loaded catechol-modified alginate nanoparticulate formulation exhibited improved MB49 murine bladder carcinoma cell inhibitory effect compared to unmodified nanoparticles (IC_{50} of 3 µg/mL versus 7.4 µg/mL) [40]. Furthermore, doxorubicin-loaded Mal-CHI-Cat-ALG nanoparticles and free doxorubicin solution displayed IC_{50} values of 2.62 µg/mL and 3.21 µg/mL, respectively [46]. These studies revealed that surface modification of drug-loaded nanoparticles improved their bladder cancer cytotoxic profile. However, there are concerns about the standardization of herbal extract-loaded nanoparticles, limiting their patient acceptability, ease of drug product development by formulation scientists, and approval by the drug regulatory authorities.

The bladder tumor-inhibitory effect of surface-modified nanoparticles was evaluated as a function of the residual tumor volume or the number of animals that remained alive after treatment. Tumor growth was insignificant after administrating belinostat-loaded surface-modified nanoparticles to xenograft

murine models for 11 days. In contrast, the tumor volume of mice treated with belinostat-loaded unmodified PLGA nanoparticles and drug-free PGON-coated PLGA nanoparticles increased by at least two-fold relative to mice treated with belinostat-loaded PGON-modified nanoparticles [39]. Also, Doxorubicin-loaded DOTAP-modified hybrid NPs exhibited superior tumor inhibition relative to doxorubicin NPs and free doxorubicin (Fig. 5) [36].

In addition, MMC-loaded chitosan-coated PCL NPs exhibited the greatest anti-tumor effect as they recorded the highest number of tumor-bearing rats alive for the longest period. Also, the surface-modified formulation was accumulated and retained within the rat bladder for a prolonged time [47] Furthermore, doxorubicin-loaded PEGylated PAMAM dendritic nanoparticles and free doxorubicin solution exhibited residual tumor volume of 15±5 mm^3 and 75±32 mm^3, respectively [9]. These studies suggested that the therapeutic index of anticancer agents could be improved through their encapsulation into surface-modified nanoparticles.

The safety of the surface-modified nanoparticles has been evaluated in terms of biochemical analysis, staining/microscopy, mucosal irritation, healthy/malignant bladder cancer cytotoxicity testing, or weight gain of formulation-treated animals. The unmodified MPEG-PLA nanoparticles, DOTAP-coated nanoparticles [36] and amine-modified polyacrylamide nanogels [42] did not induce hemolysis, bladder inflammation, or other adverse reactions. Also, there was no tissue damage or edema after intravesical instillation of mitomycin-loaded chitosan nanoparticles and chitosan-coated PCL NPs, and 80 % of the rats treated with MMC-loaded chitosan-coated PCL NPs exhibited weight gain of about 60 % [47]. In addition, slug irritation studies confirmed that maleimide-functionalized PLGA-PEG NPs were non-irritant [45]. Also, blank dual mucoadhesive functionalized Mal-CHI-Cat-ALG nanoparticles and PEGylated PAMAM dendritic NPs were non-toxic to MB49 cells and SV-HUC-1 cells [9], [46]. Furthermore, rat liver and kidney functions were not impaired after intravesical instillation of dendritic nanoparticles, and the integrity of rat bladder tissues was not compromised based on hematoxylin-eosin staining reactions [9]. These results revealed that these surface-modified nanoparticles are biocompatible.

Hyaluronic acid (HA) is an important component of the extracellular matrix that preserves the elastoviscosity of liquid connective tissues such as skin, joints, and eye fluid; supports the structure of tissues; and preserves cell viability [48]. Also, hyaluronic acid-binding receptors such as CD44 and RHAMM have been utilized as therapeutic targets for the treatment of bladder cancer [49], [50]. Mannitol could be used to modulate the drug loading and release behavior of the nano- or microparticles. Recently, Sahiner et al investigated mitomycin C-loaded hyaluronic-, mannitol- and hyaluronic/mannitol-based particles for bladder cancer treatment [48] (Fig. 6).

Figure 6: Schematic diagram revealing the interaction between mitomycin-C and HA/MN particles that facilitated drug delivery to bladder cancer cells, reproduced with permission from [48]

Optimized HA/MN (1:3) particles could be used to treat superficial bladder cancer stages, due to their particle size (835-1101 nm) and low PDI (0.27-0.36) [51]. The zeta potential values of blank and mitomycin C-loaded HA, MN, and HA/MN particles ranged from -29 mV to -36.7 mV. There was a moderate decrease in their negative zeta potential values after the incorporation of mitomycin C into the particles. The HA/MN-based particles could interact with CD44 and RHAMM receptors on malignant bladder tissues, facilitating cellular uptake of mitomycin-C into the cells. The most promising formulation, HA/MN (1:3) particles, displayed mitomycin C loading and encapsulation efficiency of 18.4 mg/g and 15.6 %, respectively. The respective amount of drug released from the particle at pH 4.5, pH 6.0, and pH 7.4 was 31.9%, 35.3 %, and 43.1 % after 25 days, revealing that rapid mitomycin C release would be induced with parenteral drug administration while localized drug delivery to the bladder facilitated controlled drug release [48]. The drug release kinetics for the optimized drug formulation fitted well with the Korsmeyer-Peppas model [48], indicating that polymer degradation and drug diffusion promote drug bioavailability at target sites.

Free mitomycin C solution destroyed 90 % of L929 fibroblast cells at 100 µg/mL. In addition, the studied fibroblast cells remained viable after incubation with 1000 µg/mL of HA/MN particles, indicating that hyaluronic acid/mannitol-based drug carriers are biocompatible. In addition, hemolytic and blood clotting tests confirmed that MN/MN particles were safe. HA/MN-MMC exhibited 50 % HTB-9 human bladder cell inhibition at a concentration of 1000 µg/mL within 24 h and the cytotoxic effect of the drug formulation increased to 92 % after 72 h at a drug concentration of 500 µg/mL [48]. However, the mucoadhesive potential of the formulation was not evaluated. This study is critical to assess their retention in the bladder cavity for a prolonged period.

3.3 Silica-based nanoparticles

Silica nanoparticles have been well-researched as vehicles for transmucosal drug delivery [52] due to their large surface area, tunable pore size, biocompatibility, biodegradability, and renewability [53]. Moreover, they are easily functionalized to obtain advanced drug carriers for bladder cancer therapy [54].

3.3.1 Anticancer drug-loaded thiol-functionalized mesoporous silica nanoparticles

All the silica nanoparticles investigated for bladder cancer treatment, are spherical and they have particle sizes that range from 76-168 nm [54], [55]. Zhang and coworkers reported that the degree of

thiolation for doxorubicin-loaded thiolated mesoporous silica nanoparticles (MSNs) (Fig. 7) was 2.2 ± 0.4 μmol g^{-1} [55].

Doxorubicin-loaded thiolated silica nanoparticles exhibited a smaller particle size than doxorubicin-loaded polydopamine-modified and polydopamine/peptide-modified nanoparticles (76±3 nm versus 168±8 nm versus 170±8 nm) [54], [55]. Also, the diameter of the silica nanoparticles increased with polydopamine and peptide functionalization (125±6 nm versus 168±8 nm versus 170±8 nm) [54]. Nevertheless, all the studied silica nanoparticles exhibited satisfactory particle size required for systemic and localized bladder cancer therapy (50 – 300 nm) [54]. The peptide and/or polydopamine-modified mesoporous silica nanoparticles exhibited negative zeta potential values between -16±2 mV to -23±4 mV, with negative zeta potential values decreasing with surface modification of nanoparticles (Table 3). Nevertheless, negatively charged nanoparticles can interact with positively charged groups on the urothelial mucin, improving drug residence time in the bladder and promoting therapeutic success.

Table 3: Characterization parameters of MSNs, DOX-loaded MSNs, DOX-loaded MSNs@PDA and DOX-loaded MSNs@PDA-PEP. Reprinted from [54] under a CC BY license.

Sample	Particle size[a] (nm)	Zeta potential (mV)	Drug Loading content (%)	BET surface area (m^2/g)	Pore volume[b] (cm^3/g)	Pore size[c] (nm)
1	124.6±7.3	-22.8±3.7	N/A	274.82	0.56	2.58
2	125.1±6.4	-14.7±2.4	16.61	97.51	0.29	1.46
3	168.3±8.1	-17.3±3.1	16.48	42.96	0.14	N/A
4	170.2±7.5	-15.9±1.6	16.25	36.55	0.12	N/A

1: MSNs; 2: DOX-loaded MSNs; 3: DOX-loaded MSNs@PDA; 4: DOX-loaded MSNs@PDA-PEP.
N/A: not applicable.
aNPs size was measured by dynamic light scattering.
bBJH cumulative pore volume for pores between 1.7 and 300 nm in width.
cMost probable pore size.

Also, the zeta potential values of unmodified nanoparticles (MSNs,) amino-modified NPs (MSNs-CD-NH_2) and thiolated nanoparticles (MSNs-CD-(NH_2)-SH) in artificial urine were -20.0±0.9 mV, +33.9±0.5 mV and +33.5±0.6 mV, respectively [55]. These findings revealed that surface modification of silica NPs with positively charged moieties such as amino and polydopamine groups increased their cationic nature and improved their colloidal stability and mucoadhesive potential.

Doxorubicin loading capacity into thiolated silica nanoparticles was 40 % whereas that of polydopamine-modified NPs (MSNs@PDA) and peptide/PDA-modified NPs (MSNs@PDA-PEP) were 16.5% and 16.3%, respectively, indicating that surface modification of nanoparticles decreased their pore size (Table 2) and reduced their doxorubicin loading potential. The amount of doxorubicin released from thiolated nanoparticles into artificial urine (pH 6.1) was greater than that of PBS (pH 7.4) after 48 h (63 % versus 13 %) [55]. Similarly, Dox-loaded MSNs@PDA-PEP displayed pH-dependent drug release kinetics, with a greater amount of doxorubicin released at pH 5.0 than at pH 6.0 and pH 7.4 (70.5% versus 48.9 % versus 22.4 %) over 24 h [54]. These results suggested that doxorubicin would be readily released from the drug formulation within the slightly acidic malignant bladder environment [54], [55].

Based on mucin-particle interaction studies, thiol-functionalized mesoporous silica nanoparticles exhibited superior mucoadhesiveness relative to hydroxyl and amino-functionalized NPs [55]. However, the mucoadhesive properties of MSNs@PDA and MSNs@PDA-PEP were not evaluated. Although doxorubicin-loaded thiolated NPs [Dox- MSNs-CD-(NH_2)-SH] exhibited lower UMUC3 cytotoxic effect than free doxorubicin (IC_{50}: 3.92 ± 1.06 μgmL^{-1} versus 0.45 ± 0.05 μgmL^{-1}) based on MTT assay, the nanoparticles still exerted satisfactory UMUC3 cytotoxic potential [55]. Also, polydopamine and peptide-modified nanoparticles displayed the greatest cytotoxic effect on HT-1376 cells, with IC_{50} of 4.02±0.58 and 0.46±0.05 µg/mL after 24 h and 48 h, respectively [54]. These findings revealed that surface-modified nanoparticles exert improved UMUC3 and HT-1376 cytotoxic effects compared to unmodified nanoparticles and anticancer drug solutions. The extent of Dox-loaded MSNs@PDA-PEP uptake into human HT-1376 cells was doxorubicin concentration-dependent, as formulations containing 1, 5, and 10 µg/mL of doxorubicin resulted in a 1.8-fold, 2.1-fold, and 2.4-fold increase in HT-1376 cellular uptake efficiency, respectively, in comparison to Dox-loaded MSNs@PDA [54]. However, the bladder cancer cellular uptake profile of doxorubicin-loaded thiolated silica nanoparticles was not investigated [55]. The in vitro cellular uptake findings reported for MSNs@PDA-PEP correlated well with *in vivo* studies using orthotopic mice model, as Dox-loaded MSNs@PDA-PEP exhibited the greatest tumor inhibitory effect [54]. The studied drug formulations are presented in the following order of increasing tumor volume 16 days post-treatment: Dox-loaded MSNs@PDA-PEP (100 mm^3) > Dox-loaded MSNs@PDA (500 mm^3) > free doxorubicin (750 mm^3) > saline (1600 mm^3) [54].

Blank thiolated silica nanoparticles were biocompatible and non-toxic to the UMUC3 BC cells up to 250 μgmL^{-1} [55]. Similarly, unmodified and PDA/Peptide-modified silica NPs were safe as they exhibited negligible cytotoxicity against healthy human embryonic HEK-293 cells after 48 h, at concentrations of up to 500 µg/mL [54]. Also, the polydopamine/peptide-based drug carriers did not induce significant morphological changes in the heart, liver, spleen, lung, and kidney, compared with

the saline group. On the other hand, damaged heart and liver were evident in the free doxorubicin-treated mice groups, revealing that incorporation of doxorubicin into peptide/ polydopamine-modified nanoparticulate system could improve its antitumor efficacy and bladder tumor targeting without compromising its biocompatibility [54]..

3.4 *In situ* gelling systems

In situ gelling formulations are dosage forms that typically exist in liquid, syringeable form at room temperature and transform into solid gel at physiological conditions such as pH, temperature, and ionic composition [56], [57]. The in-situ gelling drug delivery systems have been used for mucosal administration to the eyes, ears, nose, buccal cavity, bladder, vagina, and rectum [57]. They are used alone or in combination with surface-modified nanoparticles for controlled drug release and targeted drug delivery.

3.4.1 Anticancer drugs containing in situ gelling systems

Most researchers did not report the pH and syringeability of their studied drug formulations but Kolawole et al., (2019) reported that the pH of three chitosan/beta-glycerophosphate-based formulations (containing 1 % of LCHI/MCHI/HCHI and 12% of beta-glycerophosphate) range from 7.1 to 7.3 [58], which is suitable for intravesical drug administration due to its similarity with physiological pH of 7.4. The formulations presented in order of increasing order of syringeability: HCHIGP (26.0±1.4 N·mm) < MCHIGP (24.6±2.1 N·mm) < LCHIGP (16.3±2.2 N·mm) [58]. These formulations were more syringeable than the poloxamer and chitosan-based gel systems studied by Senyigit and colleagues that exhibited work of compression ranging from 30 to 130 N·mm [58], [59].

Brotherton and coworkers prepared aldehyde-functionalized copolymer-based thermoresponsive hydrogels from 2-hydroxypropyl methacrylate/methacrylic precursor bearing cis-diol pendant groups using the reversible addition-fragmentation chain transfer aqueous dispersion polymerization technique [60]. Small-angle X-ray scattering technique confirmed the worm morphology of the aldehyde-modified copolymer-based gels. In addition, it revealed the transition of the hydrogel system to a spherical structure upon cooling to 5°C [60]. The optimized thermoresponsive and mucoadhesive gel system displayed aldehyde functionalization of 30 %. Also, it exhibited a gelation temperature of 22 °C, whereas CHIGP systems reported by Kolawole et al exhibited gelation temperature and time of 29-30 °C and 5-15 min, respectively [58]. Nevertheless, in situ gelling systems prepared from homopolymers and copolymers exhibited satisfactory ease of gelation. Anticancer drug-loaded copolymer-based worm gels could be prepared and their antitumor and safety profile investigated to their bladder cancer treatment potential.

de Lima et al did not evaluate the gelation tendency of their gemcitabine-containing carboxymethyl cellulose/polyvinyl alcohol (CMC/PVA) and gellan gum-based hydrogels but they reported that the drug formulations exhibited shear-thinning behavior, and papain (proteolytic enzyme) reduced the lag-time required for drug permeation into bladder tissues. Also, the urothelial permeability of gemcitabine was improved by two-fold [61]. The CMC/PVA-based hydrogels exhibited superior stability relative to gellan gum-based samples. Also, the mucolytic and gemcitabine permeation-enhancing effect of native papain was significantly retained in SCMC/PVA-based hydrogel systems in comparison to gellan gum-based formulations after 90 days of product storage at 4 °C (78.1 % versus 54.8 %) [61].

HCHIGP thermosensitive gels prepared using a high molecular weight grade of chitosan exhibited improved resistance to urine wash-out in comparison to MCHIGP and LCHIGP, with WO_{50} values of 9.3 mL, 7.9 mL, and 6.1 mL, respectively [58]. There was a good correlation between the mucoadhesive properties of the drug formulations assessed in a flow of simulated urine using fluorescence microscopy and the data generated using a tensile test. HCHIGP exhibited the greatest mucoadhesive performance with the work of adhesion around 0.13±0.01 N [58]. The mucoadhesiveness (WO_{50} values) of fluorescein-loaded aldehyde-functionalized copolymer-based gel product (30% functionalization) may be superior relative to that of chitosan/glycerophosphate systems (44 mL versus 6-9 mL) [58], [60] There was a good correlation in the mucoadhesive properties of CMC/PVA- and gellan gum-based hydrogels evaluated using gel-mucin interaction studies and artificial urine flow through/fluorescence microscopic examination of porcine bladder mucosal tissues [61]. Also, CMC/PVA-based hydrogels exhibited improved mucoadhesiveness compared to gellan gum-based samples [61].

The cumulative amounts of mitomycin-C released after 6 h from MMC/LCHIGP, MMC/MCHIGP, and MMC/HCHIGP were 63%, 39%, and 37%, respectively [58], suggesting that MMC/HCHIGP was the most promising formulation for the potential intravesical treatment of bladder cancer. All the gemcitabine loaded into CMC/PVA-based systems was released in 24 h and the formulation exhibited the greatest drug flux across porcine bladder mucosa after 7 h (172±57 μg/cm^2) [61]. However, bladder cancer cellular uptake and tumor inhibitory assay were not conducted for aldehyde-functionalized worm gels and CHIGP systems [58], [60]. These tests should be carried out to facilitate the clinical translation of the new drug formulations.

The CMC/PVA and gellan gum-based formulations exhibited pseudoplastic behavior and these hydrogels were non-irritant based on the HET-CAM assay. Nevertheless, V79-4 fibroblast and HUVEC cell viability studies revealed that the CMC/PVA-based systems were more biocompatible than gellan gum-based formulations [61]. Overall, their work revealed that CMC/PVA-based systems were the most promising formulation for the intravesical delivery of gemcitabine in terms of its mucoadhesiveness, stability, and retention of papain activity. However, there are regulatory concerns about the use of papain to formulate topical drug products due to its toxicity and immunogenic potential

[62]. Kolawole et al did not evaluate the biocompatibility of chitosan/beta-glycerophosphate-based thermosensitive systems because the safety of chitosan and beta-glycerophosphate had been previously established [58].

3.5 Composite systems of nanoparticles and stimuli-responsive hydrogels

The composite systems of nanoparticles and hydrogels used for intravesical bladder cancer treatment are: the floatable and non-floatable mucoadhesive drug delivery systems. The floatable formulations resist elimination from the bladder by floating while the latter dosage forms adhere to urothelial mucosal surfaces [1], resulting in improved drug residence in the bladder and possible improvement in the therapeutic outcomes of bladder cancer patients.

3.5.1 Floatable composite system of nanoparticles and hydrogels

The floatable formulations intended for intravesical drug delivery should exhibit a gelation temperature of about 30 ^{o}C; float in the biomimetic environment (bladder cavity) within 2 min; and exhibit prolonged erosion time for at least 12 h to facilitate sustained drug release [1]. There are a few floatable formulations that have been developed for the potential treatment of bladder cancer due to the requirement of urine acidification for the therapeutic activity of the drug formulation to be activated.

3.5.1.1 Composite system of doxorubicin-loaded human serum albumin (HSA) nanoparticles and Poloxamer 407 gel

The composite system of doxorubicin nanoparticles and poloxamer *in situ* gelling, floatable delivery systems was formulated to float in the urine-containing bladder cavity; serve as a drug depot to release doxorubicin gradually, and avoid urinary obstruction associated with highly viscous hydrogel systems. The doxorubicin-loaded nanoparticles were 103 nm in diameter; and the novel composite drug carrier (NP-Dox-Gel) exhibited a gelation temperature (GT) of 10 °C and gelation time (Gt) of 2 min at 37 °C while the composite system of doxorubicin -loaded nanoparticles and non-floating hydrogel displayed a gelation temperature of 12 to 18 °C and gelation time of 2–5 min at 37 °C [63]. Doxorubicin (89.6%) was immediately released from the drug-loaded nanoparticles into the citric acid buffer system (pH 5.0) whereas composite nanoparticles-hydrogel system floated within one minute of introducing the drug carrier into the release medium, and doxorubicin was released at a controlled rate, with 81.9 % of drug released over 10 h [63]. There was no urine collection within 4 h of administering the non-floating gel formulation to rats. In contrast, the volume of urine was collected from untreated rats as well as rats treated with doxorubicin nanoparticles and the composite system of floatable gels and nanoparticles (NP-Dox-Gel) (0.2 to 0.4 mL of urine) within 4 h was comparable, implying that the new formulations

did not disrupt the urine-voiding functions of the bladder [63]. *In vivo* rat studies revealed that doxorubicin nanoparticles exhibited an immediate drug release profile, with urothelial drug concentration reaching its maximum level immediately after administration, followed by a sharp decline in drug concentration to zero after the first urine voiding whereas the amount of drug released from NP-Dox-Gel was 25.2 % after the second urination. These findings demonstrated that NP-Dox-Gel was resistant to elimination from the bladder during urine voiding and it exhibited a sustained drug release profile [63]. However, the anti-tumor efficacy of the floatable formulations was not investigated, thus, the bladder tumor regression capabilities of the new doxorubicin dosage form could not be ascertained.

3.5.2 Non-floatable mucoadhesive composite systems of nanoparticles and hydrogels

The mucoadhesive drug delivery systems for potential bladder cancer treatment are non-floatable, and they adhere to bladder mucosal surfaces for a prolonged period, facilitating drug uptake into malignant bladder tissues, inhibiting bladder tumor, and improving therapeutic outcomes of bladder cancer patients.

Monomethoxyl poly (ethylene glycol)-poly-ε-caprolactone (MPEG-PCL) diblock copolymer is a biodegradable and biocompatible amphiphile. Men *et al.* improved deguelin's aqueous solubility and residence time in the bladder using a composite system of DOTAP-modified MPEG-PCL [DMP] hybrid nanoparticles and thermo-sensitive in situ gelling Poloxamer 407 [64]. Deguelin-loaded hybrid nanoparticles and gemcitabine-loaded thiolated nanoparticles were spherical, and they exhibited narrow particle size distribution (PDI < 0.3) and zeta potential (21-30 mV) [59], [64]. The particle size of DOTAP-modified hybrid MPEG-PCL NPs [64] was smaller than that of thiolated chitosan nanoparticles studied by Şenyiğit and colleagues [59] (35 nm versus 175-190 nm), probably due to the self-assembly properties of the MPEG-PCL copolymeric system. The deguelin encapsulation efficiency (EE) of DOTAP-modified hybrid nanoparticles was 98.2 % while gemcitabine EE into thiolated chitosan nanoparticles was 19.2 % [59], [64]. On the other, the gemcitabine loading capacity of thiolated chitosan NPs was 9.4 % [59] while that of deguelin-loaded hybrid NPs was 4.9% [64]. These findings revealed that the drug loading capacity and the encapsulation efficiency of nanoparticulate drug delivery systems depended on the chemical nature of the therapeutic agents and the type of drug delivery system. Senyigit dispersed thiolated NPs into chitosan gel or poloxamer gel and the nanoparticulate gel formulations are presented in order of increasing syringeability: CHI gel (130.1±2.9 N·mm) < Plx gel (99.8±1.5 N·mm) < CHI-TGA NPs/CHI gel (82.8±0.8 N·mm) < CHI-TGA NPs/Plx gel (30.7±1.4 N·mm) [59]. However, Men and coworkers did not evaluate the syringeability of their deguelin formulation.

Deguelin-loaded nanoparticulate gel system was gradually dissolved into the release medium (PBS, pH 7.4) by surface erosion; and deguelin was released from the delivery system at 37 ^{o}C in a sustained pattern [64]. On the other hand, gemcitabine release from gemcitabine formulations was conducted using a buffer solution with pH 6.5 (44), which may be more representative of the urine. Moreover, all the loaded drug in the gemcitabine solution was released within 2.5h, whereas the respective amount of drug released from CHI-TGA NPs, CHI-TGA NPs-CHI gel, and CHI-TGA NPs-Plx gel within 4 h was 51.0 ± 3.7 %, 33.4 % ± 5.0 % and 19.6 % ±1.6 % [59] There was no significant statistical difference between the amount of gemcitabine released from the thiolated NPs within 4 h and 24 h [59]. The rate of drug release from the CHI-TGA nanoparticles after incorporation into chitosan gel or poloxamer gel was reduced by a magnitude of 1.5 and 2.6-fold, respectively, indicating that poloxamer gel-based formulations exhibited superior sustained drug release profile relative to chitosan gel-based drug carriers [59]. Nevertheless, CHI-TGA NPs/CHI gel was more resistant to simulant urine dilution than the poloxamer gel-based formulation, CHI-TGA NPs/Plx gel (Storage modulus: 15 Pa versus 6 Pa).

Tensile studies revealed that CHI-TGA NPs/CHI gel displayed improved bioadhesive properties compared to poloxamer gel-based carriers (1.70 ± 0.04 N mm versus 1.10 ± 0.08 N mm). In addition, combination of the chitosan and poloxamer gel-based drug carriers with simulant urine resulted in a 51% and 80% reduction in their bioadhesive properties, respectively [59]. The studied formulations are presented in order of increasing urothelial mucosal drug permeation over 4 h: composite poloxamer gel/NP-based carrier (18.78 ± 1.97 %) < Gemcitabine solution (21.96±1.20 %) < composite thiolated chitosan NP/CHI gel-based carrier (33.16 ± 5.11 %) < thiolated chitosan nanoparticles (37.32 ±3.48 %), suggesting that thiolated chitosan-based delivery systems may be preferred for improved drug delivery to underlying bladder cancerous tissues. Moreover, histopathological evaluation of bovine bladder mucosa treated with the studied composite gemcitabine nanoparticle/hydrogel formulations confirmed their safety as they did not damage healthy bladder mucosa [59].

Coumarin 6-loaded DOTAP-modified hybrid nanoparticles exhibited improved T24 bladder cancer cellular uptake compared with the unmodified MPEG-PCL nanoparticles. In addition, fluorescent composite particulate gel system was observed in the mice's bladder within 10 min of intravesical administration and the residence of D/DMP-F gel in the bladder was sustained for 2 h [64]. Furthermore, *in vivo* biocompatibility studies revealed that mice treated with the new formulation containing 2 mg/kg of the deguelin were alive whereas the rats injected with a drug solution (2 mg/kg) died. This finding suggested that the D/DMP-F system is a safe formulation [64]. However, future studies could evaluate the bladder tumor inhibitory properties of advanced deguelin and gemcitabine formulations to confirm their bladder cancer treatment potential.

3.5.3 Non-floatable mucoadhesive composite systems of liposomes and hydrogels

The blank and drug-loaded folate-modified liposomes were spherical, with an average diameter of 150 – 160 nm, and polydispersity indices of 0.14 to 0.18. There were no remarkable differences in the particle size of the liposomes after rapamycin encapsulation and folate surface modification. However, the negative values of zeta potential for the drug-loaded folate-modified liposomes (R-FL) were increased by about 2-fold in comparison to the drug-loaded unmodified liposomes (R-CL: -8.0±0.3 mV versus R-FL: -17.3±0.3 mV) [65]. These findings may be due to negatively charged folate groups anchored on the liposomal bilayer, improving the repulsive forces between liposomal particles, and promoting their colloidal stability. R-CL and R-FL displayed moderately low encapsulation efficiency (≈ 42 %) and drug loading (≈ 6 %), which might be attributed to the hydrophobicity of rapamycin, facilitating its localization within the phospholipid bilayer [65].

The colloidal dispersions of R-CL and F-CL were stable at 4 ^{o}C over 4 weeks, as there were negligible changes in their particle size, polydispersity index (PDI), zeta potential, and drug loading capacity, possibly due to their cholesterol content (10 % molar composition of the liposomes), increasing the phospholipid molecular packing and improving the liposomal vesicle's resistance to aggregation [65]. At 25 ^{o}C, there was no visible aggregation of the liposomes but the particle size of the liposomes increased and PDI was greater than 0.3. In addition, there were no significant statistical differences in their zeta potential values [65]. The test cells are presented in order of increasing folate-modified liposomal internalization: murine MBT2 cells < human grade III HT 1376 cells < human grade II 5637 cells, suggesting that folate-modified liposomes will be more readily taken up into human bladder tumors than murine urothelial cancerous tissues [65]. Interestingly, the most invasive form of bladder cancer cells, HT 1376 cells, exhibited the greatest sensitivity to all rapamycin-loaded formulations, as 60 % of the bladder cancer cells were viable after treatment with 1 µg/mL of rapamycin loaded folate modified liposomes for 48 h, whereas 95 % of the cells were viable after treatment with rapamycin solution, inferring that the drug loaded folate-modified liposomes improved the antitumor activity of rapamycin [65].

Poloxamer (P407), R-CL/P407, and R-FL/P407 systems displayed similar gelation temperature of 21 ^{o}C, gelation time of 29 s, and gelation duration of 12 h, suggesting that the incorporation of unmodified or folate-modified liposomes into the poloxamer solution did not compromise its gel-forming capacity [65]. The unmodified and modified liposomal gel formulations (R-CL/P407 and R-FL/P407) exhibited zero order drug release kinetics, and 100 % of rapamycin was released from the rapamycin-loaded unmodified liposomes over 12 h whereas less than 5 % of rapamycin was released from the modified rapamycin liposomes within similar time [65]. Gel erosion facilitated the release of liposomal vesicles

from the gel matrix and the liposomes interacted favorably with the urothelial membranes, improving intracellular drug delivery [65].

R-FL/P407 exhibited superior bladder tumor regression ability relative to R-CL, R-FL, and rapamycin solution. There were statistically significant differences between the extent of tumor regression evident on day 11 (P=0.0273) and day 14 (P=0.0088) for orthotopic BC mice models (MBT2/Luc cells) treated with R-CL/P407 and R-FL/P407. *In vitro* cytotoxic profile of the new liposomal formulation was consistent with the *in vivo* Western blot analysis of harvested, formulation-treated bladder tumors, as R-FL/P407 treatment downregulated the mTOR signaling pathway and increased PARP cleavage (an apoptotic marker), inducing autophagy and cell death. These *in vivo* results suggested that R-FL/P407 significantly retarded bladder tumor growth by inducing apoptosis through inhibition of mTOR phosphorylation. These findings showed that R-FL/ P407 was a potential rapamycin delivery system that could destroy folate-receptor-expressing bladder cancer cells [65].

4 Characterization of bladder cancer drug formulations

The physicochemical and biological properties of bladder cancer drug delivery systems are evaluated to ascertain that bladder cancer medicines are safe and effective. The parameters investigated for particulate drug delivery systems include particle size and size distribution, surface charge, drug encapsulation efficiency, drug loading capacity, and stability. In addition, the specific surface area and pore diameter of mesoporous silica nanoparticles were evaluated using the Brunauer-Emmett-Teller and Barrett-Joyner-Halenda analysis, respectively. Moreover, these parameters dictate the drug encapsulation potential of silica NPs [54], [55]. Parameters that pertain to gel formulations include syringeability, gel strength, gelation duration, gelation temperature, and gelation time. In addition, the properties that are usually investigated for particulate and/or hydrogel systems include drug release profile, mucoadhesiveness, malignant cellular uptake, *in vivo* penetration, cytotoxicity, antitumor inhibitory effect, biocompatibility, and biodistribution of drug formulations. An overview of the techniques used to evaluate desirable bladder cancer drug delivery systems is detailed in Table 4.

Table 4: Overview of techniques used to characterize bladder cancer drug delivery systems

Parameters	**Techniques**
Particle morphology	Transmission electron Microscopy, Scanning Electron Microscopy
Particle size/size distribution/surface charge	Dynamic Light Scattering; Transmission electron Microscopy, Scanning Electron Microscopy
Particle surface modification	Fourier Transform Infrared Spectroscopy; Ellman assay, ^{1}H NMR spectroscopy

Drug Encapsulation efficiency/Drug Loading Capacity	HPLC, UV-visible spectroscopy; Fluorescence spectroscopy
Viscosity/gelation potential	Viscometry; rheometry
Syringeability	Tensile analysis (Texture analyzer)
Drug release profile	HPLC, UV-visible spectroscopy; Fluorescence spectroscopy
Mucoadhesiveness	Fluorescence microscopy/simulant urine flow-through technique; tensile test
Bladder cancer cellular uptake	Flow cytometry
Bladder cancer cytotoxicity testing	MTT assay; WST-1 assay
Bladder tumor inhibitory potential	Tumor volume evaluation; anti-angiogenic potential using a transgenic zebrafish model
Biocompatibility studies	Mice weight evaluation; bladder tissue staining/microscopy; cytotoxicity test against healthy fibroblast cells; HET-CAM assay; hemolytic test; blood clotting index;
Biodistribution studies	Animal Organ Harvesting/HPLC analysis
Stability profile	Visual and drug content evaluation after storage at 4 °C and 37 °C for 3 months

HET-CAM: hen's egg-chorioallantoic membrane; HPLC: High-Performance Liquid Chromatography

5 Recent trends in the treatment of bladder cancer

In recent times, researchers explored photodynamic techniques to promote bladder cancer treatment. Examples of such delivery systems have been detailed in Table 5. These drug delivery systems offer a new and effective strategy to combat bladder cancer progression and improve the prognosis of the disease.

Table 5: Photo/Chemodynamic and photothermal-based delivery systems

Delivery system	**Mechanism of action**	**Ref**
Iron oxide/chlorophyII clustered nanoparticles	Iron induces malignant cell apoptosis	[66]
Organic dye-loaded polymeric nanosponges	Organic dye induces malignant cell apoptosis	[67]
Gold@Iron/chlorophyII nanorods	Iron induces malignant cell apoptosis	[68]

Glucose oxidase/iron/bovine serum albumin/polypyrrole/manganese oxide nanoparticles	Glucose/Glutathione co-triggered tumor hypoxia	[69]
Reactive oxygen species-activated self-amplifying pro-drug nanoagent	Copper-chelate chemotherapy and cascaded photodynamic therapy	[70]
Iron oxide-loaded porphyrin-grafted Lipid Nanoparticles	Iron induces malignant cell apoptosis	[71]

6 Clinical trials: bladder cancer therapeutic drug formulation

Lipid and polymer-based nanoparticles and/or in situ gelling systems are currently being investigated in clinical trials alone or in combination with conventional therapies [72]. The duration of the clinical studies is detailed in Figure 9.

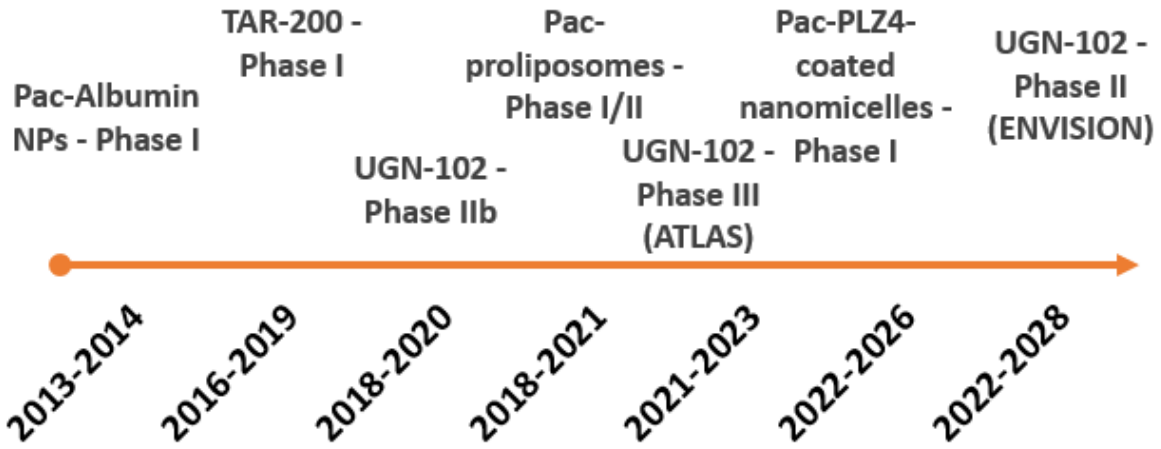

Figure 9: Clinical trials Timeline for bladder cancer treatment (2013-2028)

Phase II clinical trial [73] revealed that 10 out of 28 patients who have been non-responsive to BCG therapy and treated using paclitaxel-loaded albumin-bound nanoparticles displayed complete response which was maintained for up to a year [73] At 21 months follow-up, 67.8% of patients retained their bladder, and disease progression or distant metastases were not recorded. Another Phase 1, open-label trial (NCT02722538) [74], [75] revealed that radical cystectomy should be carried out on muscle-invasive bladder cancer patients before administering gemcitabine intravesical drug delivery system (TAR-200) to improve the therapeutic outcomes of bladder cancer patients. A single-arm phase 1/2 study revealed that paclitaxel-loaded liposomal formulation (Lipax/TSD-001)[76] induced a bladder cancer recurrence-free survival rate (RFS) in 83 % of patients with low-grade, non-invasive papillary

Ta bladder cancer that have undergone TURBT whereas patients receiving conventional standard-of-care therapies experienced cancer recurrence-free survival of 49% [77]. The paclitaxel formulation was well tolerated at 2 years-follow up as the urinary well-being of the patients was not compromised and the patients did not experience systemic paclitaxel toxicity [77].

A Phase 2b, open-label, single-arm trial (NCT03558503) using mitomycin containing thermoreversible gel formulation (UGN-102) to treat low-grade intermediate-risk NMIBC patients revealed that 65 % of patients had a complete response (CR) in 3 months; 73 % CR in 12 months whereas 32 % of participants reported disease recurrence [78], [79]. A Phase III randomized ATLAS study (NCT04688931) was carried out on bladder cancer patients, with UGN-102 administered with or without transurethral resection of bladder tumor (TURBT) in comparison with TURBT alone [80]. Results from the study revealed that UGN-102 intravesical therapy facilitated a 55% reduction in the risk of disease recurrence, progression, or mortality. Also, the complete response (CR) rate after 3 months was 64.8% for those administered with UGN-102 while patients managed with TURBT had a CR rate of 63.6 % [81]. Another Phase III trial (ENVISION) on UGN-102 (NCT05243550) [82] showed that the low-grade intermediate-risk NMIBC patients managed using UGN-102 displayed a CR rate of 79.2% at three-month follow-up [81]. Both trials demonstrated that UGN-102 was safe and effective and achieved the primary endpoint of disease-free survival [81]. The secondary endpoint for the duration of therapeutic response is currently being studied in the ENVISION trial, and a new drug application for UGN-102 could be submitted to the FDA in 2024 if positive research findings are reported from the ongoing ENVISION trial. Nevertheless, the clinical studies are expected to be completed by 2028 [81].

Phase I clinical study of PLZ4-coated paclitaxel loaded nanomicellar dosage form (NCT05519241) [83] is the first-in-human trial using the paclitaxel formulation. It was given once weekly for six weeks at three different paclitaxel doses (25 mg, 50 mg, and 75 mg) to evaluate the therapeutic dose that will be used for Phase 2 trials; assess systemic absorption after intravesical instillation of drug formulations; and effectiveness of treatment [83]. The ongoing study is expected to be completed in August 2026 [83].

7 FDA approvals: Drug formulations for bladder cancer treatment

The majority of nanomedicines approved by the U. S. Food and Drug Administration and the European Medicines Agency for bladder cancer treatment are lipidic and biological [84]. Since the focus of our review is on chemotherapeutic agents, thus, only drug formulations that have been approved for bladder cancer treatment in the last decade will be detailed (Table 6). Interestingly, the Indian national regulatory authority has approved Bevetex™, a paclitaxel-loaded polymeric-lipidic nanoparticulate

formulation for the treatment of bladder cancer [85] Also, mitomycin-C containing thermosensitive gel formulation (Jelmyto®) has recently been approved to treat low-grade bladder cancer [86].

Table 6: FDA-approved drug formulations for bladder cancer treatment (2013-2023)

Brand name/manufacturer	Active ingredient	BC stage	Dosage regimen	Reference
Jelmyto® (UroGen Pharma, USA), approved April, 2020	Mitomycin-containing thermosensitive gel	Low grade non-muscle-invasive	2 x 40 mg MMC reconstituted with 20 mL hydrogel; 4 mg/mL via a urethral catheter or nephrostomy tube, once weekly for 6 weeks	[86]
Lipodox® (Sun Pharma, India), approved February 2013	Liposomal doxorubicin coated with PEG	NMIBC	2mg/mL; < 90 mg dose in 250 mL and >90 mg in 500 mL dextrose saline; intravenous at 1 mg/min	[87], [88]
Bevetex™ (Sun Pharma, India), approved in India	Paclitaxel-loaded polymeric-lipidic nanodispersion	Metastatic urothelial adenocarcinoma	100 mg injection concentrate; administered based on doctor's prescription	[89]

8 Conclusion

This review offers a comprehensive overview of drug formulations studied for potential bladder cancer treatment over the last decade. Also, it highlights various techniques for characterizing novel bladder cancer medicines. Additionally, it seeks to shed light on ongoing clinical trials related to bladder cancer chemotherapy, outline recently FDA-approved drug products for treating bladder cancer, and pinpoint the challenges that have hindered the translation of promising drug formulations from research laboratories to clinical application. Over the last two decades, researchers have investigated different advanced formulations containing chemotherapeutic agents such as cisplatin, belinostat, doxorubicin, mitomycin-C, docetaxel, deguelin, gemcitabine, and rapamycin, to treat various stages of bladder cancer. The recently developed drug-loaded particulate and/or in situ gelling systems would be beneficial to reduce the frequency of drug dosing, and improve the therapeutic outcomes of bladder cancer patients. However, there is a need to deduce drug dosage that will be effective in humans to speed up the clinical translation of promising drug formulations for the treatment of bladder cancer.

9. Expert Opinion

Various *in vitro* and *in vivo* models were used to study the preclinical performance of promising intravesical drug formulations, and satisfactory safety and antitumor efficacy data were generated. However, some authors provided limited or no information regarding certain experimental conditions, making it challenging to compare various preclinical data across studies. Thus, the harmonization of acceptable and realistic study protocols for carrying out *in vitro* and *in vivo* studies of bladder cancer drug formulations would be valuable to translate promising drug delivery systems from the research laboratory to the clinics.

Few promising drug formulations have made it to the market due to various reasons. For instance, the poor understanding of new molecular and cellular targets involved in bladder cancer progression and metastasis could undermine new drug product development. Also, the toxic effects of some nanoparticles prepared using organic solvents could limit patient acceptance and compliance to dosage regimen, resulting in therapeutic failure. In addition, the cost of raw materials, including manufacturing a new drug formulation is expensive. For example, the cost of producing conventional paclitaxel and doxorubicin powder for injection is cheaper than that of Abraxane™ (paclitaxel loaded albumin-bound nanoparticles) and Doxil™ (doxorubicin-loaded PEGylated liposomes), respectively [90]. Furthermore, the entire timeline for commercializing a new drug formulation could take 10-15 years, requiring approximately $1 billion [91]. Thus, the clinical benefits of a new nano pharmaceutical should be significant to rationalize patients paying higher prices than that of a conventional drug formulation [92].

Some factors have hindered national regulatory authorities from approving the sale and commercialization of promising drug formulations to treat bladder cancer. For instance, nanomedicines need to be characterized on a batch-to-batch basis using multiple techniques to ascertain that the processing conditions such as sterilization [93] and sonication does not alter the properties of drug formulations such as particle size, morphology, drug content, encapsulation, release, safety, toxicity, stability and *in vivo* therapeutic activity [94], [95]. Also, the storage conditions of the drug products as well as the state at which these products are stored could influence their physicochemical properties [84].

The execution of clinical trials for a new drug product is expensive, which prevents the clinical translation of safe, quality, and effective bladder cancer nanomedicines. Thus, there is a strong need for collaborations between different pharmaceutical industries so that they can afford the cost of new drug product development. Also, the constitution of international consortia made up of academics, clinicians, pharmaceutical formulation scientists, and regulatory authorities would be beneficial to assess the benefits and risks of new drug products within a short time, thereby reducing the timeline for clinical translation of new drug formulations from the laboratory to the clinics. In addition, the use of multiple drugs or biologics-loaded delivery systems for the treatment of bladder cancer is currently being

investigated [96]. However, their clinical application could be associated with adverse events and immunologic reactions. Future direction for bladder cancer treatment will explore intravenous cell-based therapies such as modified interleukin which could destroy cancerous cells [97].

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Figure 1: a) Anatomy of the human urinary bladder [1]; b) barriers to efficient intravesical drug delivery

Figure 2: Major techniques for preparing drug formulations intended for bladder cancer treatment; RAFT: reverse addition-fragmentation chain-transfer

Figure 3: (a) Cytotoxicity (IC_{50} values) of Cispt (cisplatin), LCispt (cisplatin loaded unmodified liposomes), and PLCispt (cisplatin loaded PEGylated liposomes) after 24 h and 48 h incubation; (b) The tumor volume (mm^3) in bladder cancer-bearing rats that received different formulations, including phosphate buffered saline (PBS), Cispt, LCispt and PLCispt, reproduced with permission from [30].

Figure 4: In vitro viability of T24 cells (a and b) and high-grade human urothelial carcinoma cell lines (UMUC3 cells) (c and d) after exposure to docetaxel loaded unmodified nanogels (PAm-DTX), PAm-NH2-DTX (docetaxel loaded amine-modified nanogels) or free docetaxel solution (DTX) at different

concentrations for 4 h or 72 h. The cells after 4 h treatment was further cultured for 72 h in fresh growth medium. Control experiments were carried out without nanogels and DTX. Cell viability is expressed as % of control. Data shown is the average of at least 3 independent experiments, reproduced with permission from [42].

Figure 5: Anticancer effects in vivo; **Notes:** (**A**) Tumor growth curve in subcutaneous tumor model. (**B**) Tumor weight in subcutaneous tumor model. (**C**) Image of tumors in each treatment group in orthotopic bladder tumor model. (**D**) Weight of tumor-bearing bladders in each orthotopic bladder tumor model treatment group. The normal group is the bladder not bearing tumor. For all graphs, error bars indicate mean ± SEM; n=3 independent experiments. **P*,0.05 and ***P*,0.01 (Student's *t*-test).

Abbreviations: SEM, standard error of the mean; NS, normal saline; Dox, doxorubicin; Dox/PP, Dox loaded methoxypoly (ethyleneglycol); Dox/DPP, Dox-loaded DPP; DPP, 1,2-dioleoyl-3-trimethylammonium propane/methoxypoly (ethyleneglycol); Originally published by and used with permission from Dove Medical Press Ltd [36].

Figure 6: Schematic diagram revealing the interaction between mitomycin-C and HA/MN particles that facilitated drug delivery to bladder cancer cells, reproduced with permission from [48]

Figure 7: Synthesis of amino-modified; hydroxyl-functionalized and thiolated mesoporous silica nanoparticles; reprinted with permission from American Chemistry Society [55]