Journal of Controlled Release 285 (2018) 46–55

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

# A gel system for single instillation of non-muscle-invasive bladder Cancer: A "divide-and-rule" strategy

Pengyu Guo[a,b,c,d,1], Wenting Shang[a,b,c,d,1], Li Peng[a,b,c,d,1], Lu Wang[a,d], Hongzhi Wang[a,d], Ziyu Han[b,c], Hongmei Jiang[b,c], Jie Tian[b,c,**,2], Kun Wang[b,c,**,2], Wanhai Xu[a,d,*,2]

[a] Urology Surgery Department, The Fourth Hospital of Harbin Medical University, Harbin, Heilongjiang 150001, PR China
[b] CAS Key Laboratory of Molecular Imaging, The State Key Laboratory of Management and Control for Complex Systems, Institute of Automation, Chinese Academy of Sciences, Beijing 100190, PR China
[c] University of Chinese Academy of Sciences, Beijing 100080, PR China
[d] Heilongjiang Key Laboratory of Scientific Research in Urology, Harbin, Heilongjiang 150001, PR China

ARTICLE INFO

*Keywords:*
Bladder cancer
Single instillation
Gel
Mouse model

ABSTRACT

Single instillation (SI) reduces recurrence of non-muscle-invasive bladder cancer by chemoresecting floating tumor cells and residual tumor lesions (RTLs) after transurethral resection of the bladder, but with limited efficacy. Current studies improved this by prolonging retention time and increasing penetration to bladder wall, ignoring that the two separate factors should be treated in different ways. Here, we introduced a smart gel system-based SI to prevent re-implantation of tumor cells (RTCs) and ablate RTLs in a "divide-and-rule" approach. The gel system was synthesized by PEG-PAMAM and dextran aldehyde and composed of gold nanorods and gemcitabine for photothermal therapy and chemotherapy, respectively. It was developed to provide dextran aldehyde-selective adhesion with tissue amines. Since tumor surface expressed high levels of collagen, the exposed amines could act as adhesion points for the gel system. Thus, the gel presented more affinity to tumor tissues. When being instilled, it could form a protective layer on the inner face of the entire bladder wall immediately, preventing RTCs in early time. And it persisted long at the tumor site, ablating RTLs. Our data proved the gel system improved intravesical treatment efficacy in a "divide-and-rule" approach and might be a promising treatment strategy for SI.

## 1. Introduction

Bladder cancer (BCa) is the fourth most common malignancy in man worldwide [1]. In the United States, approximately 81,190 new cases will be diagnosed in 2018 and 17,240 will die from it [2]. Approximately 75% of first diagnosed BCa cases are non-muscle-invasive bladder cancer (NMIBC). Transurethral resection of the bladder (TURB) is the standard treatment for NMIBC. Owing to its high recurrence rate (5-year recurrence up to 70%) [3] and necessity for relative management after the first therapeutic session, its treatment is one of the most expensive treatments for solid tumors [4]. To reduce its recurrence, immediate single instillation (SI) is recommended by the American Urological Association and European Association of Urology after TURB for low and intermediate risk NMIBC. It acts by ablating floating tumor cells and chemoresecting residual tumor lesions [5, 6]. However, current therapeutic agents may not completely eradicate the malignancy. Their efficiency is limited by drug dilution of urine during retention, relative short drug retention time and low drug penetration at the tumor site [7]. Although under regular chemotherapy regimen, its recurrence rate is still relative high (5-year recurrence ranges from 44.8% to 55.8%) [8]. Thus, there is a desirable need for new therapeutic strategies to improve the present situation.

Currently, multiple studies have improved intravesical drug delivery system using nanoparticles or mucoadhesive biomaterials and show improved efficacy compared with simple drug instillation [9–11]. Most of these biomaterials simply prolonged drug retention time and enhanced drug penetration of the entire bladder wall rather than actively targeted to malignance tissues [12–14]. However, if the entire

* Correspondence to: Wanhai Xu, Yiyuan Street #37, Nangang District, Harbin, Heilongjiang 150001, PR China.
** Corresponding authors at: Zhongguancun East Road #95, Haidian Dist., Beijing 100190, PR China.
*E-mail addresses:* jie.tian@ia.ac.cn (J. Tian), Kun.Wang@ia.ac.cn (K. Wang), xuwanhai@163.com (W. Xu).
[1] The parallel first authors
[2] The parallel corresponding authors

https://doi.org/10.1016/j.jconrel.2018.06.036
Received 20 March 2018; Received in revised form 6 June 2018; Accepted 29 June 2018
Available online 02 July 2018

bladder were continuously exposed to cytotoxic drugs, it will cause severe side effects, such as urinary irritation, cystitis and hematuresis. Therefore, simple improving drug retention time and penetration to the bladder wall was not an ideal strategy for SI.

In fact, re-implantation of tumor cells (RTCs) and residual tumor lesions (RTLs) were two major factors responsible for the high recurrence [5]. An ideal intravesical instillation agent should treat the two separate factors in different ways. Preventing RTCs should be performed within the first several hours after TURB, after that they would seed into the bladder wall firmly and were covered by extracellular matrix. Thus the efficacy of flowing drugs inside the bladder might compromise owing to indirect contact to tumor cells [15]. For RTLs, an accurately and rapidly drug delivery system was needed to prolong retention time and enhance drug penetration at the tumor site rather than the entire bladder wall to minimize side effect. Thus a smart intravesical instillation agent should act on the whole bladder wall in the first few hours and only attach to the tumor site in long term [12]. Here we hypothesised that use of a "divide-and-rule" approach might be better to maximum ablation effect of malignance and minimize side effect of the entire bladder. Specifically, early isolation of tumor cells from the bladder wall might be an effective method of preventing their re-implantation, and longer retention time and improved drug penetration at the tumor site (for RTLs) might induce significant tumor regression with reduced injure to normal tissue.

On the basis of the above assumption, we introduced a smart gel system for SI with a combination of photothermal therapy and chemotherapy. The gel platform was synthesized using PEGylated PAMAM (PEG-PAMAM) and dextran aldehyde. An aldehyde-amine cross-linking occurred between the two components to form a gel platform and provide a dextran aldehyde-selective adhesion with tissue amines [16]. Tumor surfaces presented more collagen than normal tissues, exposing amines as adhesion points for the gel system [17]. According to the above histological basis, the gel immediately formed an extensive protective layer on the inner face of the bladder wall while being instilled, which hindered RTCs. Moreover, owing to its higher affinity for tumors, the gel identified and attached to tumors surface, which prolonged retention time and continuously released drugs, resulting in enhanced treatment efficacy. Gold nanorods (AuNRs) and gemcitabine (GEM) were incorporated within the gel to conduct photothermal therapy [18] and chemotherapy, respectively. The gel system could induce RTLs regression through photothermal ablation and enhance chemotherapy by prolonging acting time and improving drug penetration with hyperthermia [19]. Two emerging models, a mouse cell implantation (CI) model and an air-pouch bladder cancer (APBCa) model were developed to simulate RTCs and RTLs. We assessed the safety and efficacy of the gel system in these two models as well as in an orthotopic BCa model. We show that the smart gel system provided a "divide-and-rule" approach for preventing RTCs and ablating RTLs, which might be a promising SI strategy for NMIBC.

## 2. Materials and methods

### 2.1. Animal models and ethical statement

Two emerging models, a mouse cell implantation (CI) model and an air-pouch bladder cancer (APBCa) model were developed to simulate RTCs and RTLs. An intravesical photothermal therapy was performed in the CI model to prevent implantation of tumor cells and ablate tumor lesions. An air pouch was developed in the APBCa model and tumor cells were seeded on the inner face of the pouch wall to develop an APBCa model, subsequently. All the animal experimental protocols, including experimentation and surveillance, were approved by the Institutional Animal Care and Ethics Committee of the Fourth Hospital of Harbin Medical University.

### 2.2. Establishment of the mouse air-pouch BCa (APBCa) model and instillation treatment

BALB/c nude female mice (5-week-old) were purchased from the Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Mice were anesthetized using isoflurane, and then 3 ml filtered sterile air was injected subcutaneously into their backs to create a 2.5 × 2 cm air pouch, followed by injection of 1 ml sterile air on alternate days to preserve the air pouch size. After five days, RT112 cells ($1 \times 10^6$) in 50 μl Matrigel were seeded on the inner face of the pouch wall. Before measurement of tumor size, air in the pouch was released. Tumor size was measured using vernier calipers, and calculated using formula: (length × width$^2$)/2. Mice with tumor volume of ~100 mm$^3$ were selected for further experiments. For treatment experiment, different agents (500 μl, ≥5 mice for each group) were injected into the pouch on day 0 and incubated for 60 min to simulate SI treatment on RTLs. After saline irrigation, five-min near-infrared ray (NIR) irradiation was conducted for photothermal therapy [20]. Then, the pouch was emptied for better measurement of tumor size.

### 2.3. Orthotopic experiment and single instillation treatment

#### 2.3.1. Orthotopic BCa model establishment and SI treatment

Mice were anesthetized using sodium pentobarbital and catheterized with a 24-G catheter. Then, a wire was inserted into the bladder through the catheter to attach to the bladder wall. The external part of the wire was attached to an electrotome. Monopolar high frequency coagulation was conducted twice at 2.5 W for 1 s, mimicking a TURB injury [21]. Subsequently, 5637 that stably expressed luciferase (5637-luc) were infused (100 μl, $1 \times 10^6$) into the bladder and incubated for 2 h. Tumor growth was monitored by IVIS (LB983 NC100, Berthold, Germany). Fifteen minutes before imaging, the mice were intraperitoneally injected with 150 μl D-luciferin (30 mg/ml, Perkin Elmer) in phosphate buffered saline (PBS). Treating agents (gel system, GEM or saline. 100 μl, ≥5 mice for each group) were instilled into the bladder and incubated for 60 min at day 0 when luciferase signal researched > $1 \times 10^5$. After saline irrigation, a micro-optical fiber was inserted through the catheter for a 5-min NIR irradiation.

#### 2.3.2. CI model establishment and treatment for preventing cell implantation

A CI model was introduced to evaluate the efficacy of SI in preventing tumor cell implantation. Briefly, the bladder was irrigated with saline to simulate clinical routine bladder irrigation after 2 h of 5637-luc retention in the bladder, as described above. Treating agents (gel system, GEM or saline, $n = 20$ for each group) were instilled into the bladder immediately and incubated for 60 min. After saline irrigation, a micro-optical fiber was inserted for 5-min NIR irradiation (readers recommended to see the Supplementary Video S1 for experimental details). Tumor growth was assessed at day 15 by fluorescence imaging using IVIS and pathological section.

### 2.4. Masson staining and preparation of the gel platform

Masson staining was performed on tumor tissues and normal epithelium of the APBCa model, orthotopic BCa model and human samples. The gel platform was synthesized as described with minor modifications [20]. Briefly, 0.65 g HO-PEG-COOH (2000 Da, Beijing Chemgen Pharma Co Ltd., China) was dissolved in 1 ml 10% PAMAM-$NH_2$ (Weihai CY Dendrimer Technology Co Ltd., China) over night to seal parts of amines. The viscosity of the gel increased with decrease in the proportion of HO-PEG-COOH. For instillation, the optimal mixing weight ratio between PAMAM and HO-PEG-COOH was 1–6.5. To conduct different function, the solution was doped with PEGylated gold nanorods (PEG-AuNRs, AuNRs contents: 50 μg/ml) and gemcitabine (GEM 10 mg/ml) (Shanghai Mackin Biochemical Co Ltd. China 10 mg/