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A Physiology Based Three-Layer Diffusion Reaction Model For *In Vitro* To *In Vivo* Translation Of Intravesical Mitomycin C Exposure In The Human Urinary Bladder Wall

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Abstract

Intravesical mitomycin C reduces recurrence after resection of non-muscle-invasive bladder cancer, but response varies widely between patients, and dose, instillation volume, and dwell time are still chosen empirically. The concentration reached within the wall should govern those choices, but it is neither measured in routine care nor resolved by compartmental models that treat the wall as one or two well-mixed regions. Layer-resolved concentrations exist for human tissue *in vitro* and in patients, but no model has connected them. We asked whether a physiology-based model parameterized only from human *in vitro* permeation data could predict clinical tissue concentrations. The wall was represented as three layers: urothelium (0-200 μm), lamina propria (200-1500 μm), and muscularis (1500-4000 μm), each governed by Fick's second law with a layer-specific diffusion coefficient (D_i) and first-order loss constant (k_i), with harmonic-mean flux continuity at the interfaces. Three of six transport parameters were estimated from published human *in vitro* data; three were fixed after an identifiability assessment. For the clinical simulation only, the luminal boundary was replaced, by a dynamic urine-dilution function, with no tissue parameter re-estimated. Urothelial diffusion coefficient was 84-fold below lamina propria. Predicted urothelial and lamina propria concentrations at 120 min fell within 1.25-fold of biopsy means from seven patients, inside the pre-specified 2-fold criterion; the muscularis prediction exceeded the observed mean by 2.42-fold. Parameters measured in human tissue *in vitro* therefore predicted clinical exposure in the layers that define the non-muscle-invasive target, giving a basis for setting regimens against tissue rather than urinary exposure.

Keywords: non-muscle-invasive bladder cancer. intravesical chemotherapy, *in vitro in vivo* translation, diffusion-reaction model, partial differential equation, mechanistic modelling

Introduction

Bladder cancer is the ninth most commonly diagnosed cancer worldwide and the sixth in the United States[1, 2]. It is broadly classified into two major types: non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). Approximately 75% of newly diagnosed cases present as NMIBC, in which the tumor is confined to the outer and inner layers of the bladder wall (the urothelium and lamina propria) without invasion of the detrusor muscle[3, 4]. NMIBC carries favorable survival but recurs 50 to 70% of high-risk tumors return within five years, and 10 to 30% progress to muscle-invasive disease[3]. That cycle of recurrence, monitoring, and repeated intervention makes bladder cancer among the most expensive malignancies to manage over a patient's lifetime. After transurethral resection of bladder tumour (TURBT), intravesical chemotherapy is standard adjuvant treatment. Mitomycin C (MMC) is the most widely used chemotherapeutic agent, alongside Bacillus Calmette-Guérin, gemcitabine, and epirubicin. A single immediate post-TURBT instillation reduces the relative risk of recurrence by about 39% compared with resection alone[5, 6]. Despite this benefit, response to intravesical MMC varies widely from one patient to the next. Much of that variability traces back to how the dose is chosen. Current clinical regimens rely on empirically derived dose, volume, and dwell-time regimens that do not account for differences in bladder physiology or what concentration reaches the different tissue layers for targeted action of chemotherapeutic agents[7].

Intravesical administration delivers drug directly into the bladder lumen via catheter, where it is retained during a certain dwell period before emptying of bladder. This route has a pharmacokinetic advantage of having a local drug concentration at the urothelial surface can exceed systemic concentrations by three to four orders of magnitude, maximizing target-site exposure for bladder cancer while limiting systemic toxicity[8, 9]. However, therapeutic efficacy depends not on urinary drug concentration, but on concentrations achieved within the bladder wall tissue particularly in the lamina propria, where residual malignant cells, tumor re-implantation, and microinvasive disease may persist following TURBT[10]. Although intravesical therapy has major pharmacokinetic advantage there are two process that limit the exposure of drug in the bladder walls: (i) urine is produced continuously during the dwell period of administration which dilutes the instillate, so luminal concentration falls with time (ii) The urothelium, with its umbrella cells, tight junctions, and apical uroplakin plaques, imposes a substantial permeability barrier for the permeability of drug into the bladder walls[10, 11]. As a result, drug concentrations decline sharply with increasing tissue depth, creating a pharmacokinetic gradient that cannot be adequately captured by conventional compartmental models. The lack of well-characterized data on enzyme expression and active transport mechanisms within the bladder wall limits the development of a fully mechanistic PBPK model to predict tissue distribution of drug in the bladder walls.

Modelling work in bladder cancer has largely addressed other questions. A recent scoping review identified 97 simulation studies, most concerned with disease progression, screening, surveillance, treatment outcomes, or cost-effectiveness rather than with drug concentration in tissue[12]. There were two pharmacokinetic frameworks that were relevant (i) Grabnar *et al.* (2006) connected urine, bladder-wall regions, and systemic absorption through transfer rate constants and used the resulting model to explore permeability enhancement[13] and, (ii) Wientjes *et al.* (1993) combined urine pharmacokinetics with a simplified penetration relationship and pharmacodynamic data to rank modifiable treatment factors, concluding that dose, residual urine, urine production, and instillation volume mattered more than extending dwell time from 2 to 4 h[14]. Both studies established that quantitative modelling could inform intravesical therapy, but neither resolves concentration in relation to depth and time across the physiological and anatomical layers of the bladder wall.

The bladder wall is not a homogeneous medium, and its three layers differ in exactly the properties that govern drug transport. The urothelium is a stratified epithelium of three to seven cell layers, capped by superficial umbrella cells whose apical membrane is covered by uroplakin plaques and sealed by tight junctions, with an overlying glycosaminoglycan layer[11, 15]. Together these make it one of the least permeable epithelia in the body, evolved to hold a concentrated and often cytotoxic urine away from the tissue beneath. Immediately below it, the lamina propria is loose connective tissue rich in collagen and elastin and carries a dense subepithelial capillary plexus, so it offers far

less resistance to diffusion while providing, for the first time, a route by which drug can leave the tissue into the systemic circulation. It is also where the clinically decisive events occur residual disease after resection, tumour re-implantation, and the invasive front of T1 tumours all sit within this layer[15, 16]. Deeper still, the muscularis propria consists of thick, densely packed smooth muscle bundles and makes up most of the wall's thickness and it is the target only when disease is already muscle invasive. A drug crossing this sequence therefore meets a steep barrier, then a permeable but vascularised layer that both transmits and removes it, then bulk muscle, a series arrangement in which resistance and loss are distributed unevenly with depth[16]. Human measurements confirm that this structure produces a steep concentration field rather than a uniform tissue concentration. Di Stasi et al. measured MMC concentration-depth profiles in full-thickness human bladder wall after passive diffusion[17]. In patients, Wientjes et al. found that concentrations fell semi-logarithmically with depth from the urothelium to deep muscle, reaching a plateau near 2000 μm [14]. Both datasets describe the same thing: the bladder wall is a multilayer system in which each layer contributes its own resistance to transport, so that concentration at any depth depends on everything the drug has crossed to reach it. Capturing this physiological phenomenon requires a model in which each layer carries its own transport parameters.

The knowledge gap is therefore narrow and specific. Layer-resolved MMC concentrations exist for human tissue *in vitro* and for human tissue *in vivo*, but no published analysis has estimated layer-specific transport parameters from human bladder tissue *in vitro* and carried them, unchanged, into prediction of independently measured human tissue concentrations. The question this work addresses is whether a mechanistic, physiology-based model can translate *in vitro* bladder permeation data into quantitative predictions of *in vivo* tissue pharmacokinetics, and whether it can do so across the full thickness of the wall, so that its predictions are meaningful for both NMIBC and MIBC. That requires a model which predicts concentration as a function of depth and time, transfers from a controlled experiment to the patient, and remains physically interpretable at every layer. We address this with a physiology-based partial differential equation model[18] that applies Fick's second law of diffusion[19] to describe MMC concentration as a continuous function of tissue depth and time across three anatomically defined layers after intravesical administration. It helps us resolve sub-millimetre gradients across the urothelium, lamina propria, and muscularis simultaneously is what makes the *in vitro* to *in vivo* transfer possible: parameters estimated from a controlled diffusion-cell experiment are carried directly into prediction of clinical tissue-biopsy concentrations without re-fitting. The work has three aims: (i) to estimate layer-specific transport parameters from published human *in vitro* passive-diffusion data; (ii) to identify which parameters govern exposure at clinically relevant depths; and (iii) to evaluate the model against independent human tissue concentrations using a dynamic urine-dilution boundary, with no re-estimation of tissue parameters.

For our work we use Mitomycin C as an example for this translation work. MMC itself is well suited and widely used to this treatment[20]. It is a bioreductive alkylating agent from *Streptomyces caespitosus* ($\log P \approx -1.4$) that is activated intracellularly to a reactive quinone, forms interstrand DNA cross-links at guanine residues, and triggers apoptosis[21]. Clinical regimens use 20-40 mg in 20-50 mL of diluent with a 60-120 min dwell. Interest in the delivery problem has sharpened since the June 2025 FDA approval of mitomycin intravesical solution (Zusduri, UroGen Pharma) for recurrent low-grade intermediate-risk NMIBC[22].

Methods

Overview

The workflow had four stages. *In vitro* concentration-depth-time data were first examined to establish the qualitative transport behaviour and justify the governing equation (Section 2.3). A three-layer PDE model was then specified and implemented numerically (Sections 2.4-2.5). Transport parameters were estimated from the *in vitro* data in two stages, the second constrained by an identifiability assessment of the first (Section 2.6). Finally, the estimated parameters were transferred without modification into a simulation of the clinical instillation, in which only the luminal boundary condition was changed, and the resulting predictions were compared with independent clinical biopsy data (Section

2.7). Sensitivity analysis was performed on the *in vivo* model (Section 2.8). All symbols and units are defined in Table 1.

Table 1 Nomenclature used in the manuscript

Symbol	Definition	Units
$C(x,t)$	MMC concentration in tissue at depth x and time t	$\mu\text{g/g}$
x	Depth from the luminal surface	cm
L	Total bladder-wall thickness (0.40 cm)	cm
t	Time from start of instillation	s
D_i	Effective diffusion coefficient in layer i ($i = \text{uro, lam, mus}$)	cm^2/s
k_i	First-order local loss constant in layer i	s^{-1}
D_{eff}	Harmonic-mean diffusion coefficient at a layer interface	cm^2/s
$F_{i\pm 1/2}$	Diffusive flux at the midpoint between adjacent nodes	$\mu\text{g cm}^{-2} \text{s}^{-1}$
Δx	Spatial grid spacing (4×10^{-4} cm)	cm
N	Number of spatial nodes, 1000	
C_0	Initial luminal MMC concentration	$\mu\text{g/mL}$ or $\mu\text{g/g}$
$C_{\text{lumen}(t)}$	Luminal MMC concentration during the dwell period	$\mu\text{g/mL}$ or $\mu\text{g/g}$
V_0	Bladder lumen volume at the start of the dwell period	mL
q	Urine formation rate	mL/min
$S_{C,p}$	Normalised sensitivity coefficient of concentration C to parameter p	
uro, lam, mus	Urothelium, lamina propria, muscularis	

Data sources

In vitro permeation data

The *in vitro* dataset used for model parameterization was obtained from a study by Di Stasi *et al.* in 1999[17]. In this study, full-thickness bladder wall specimens were harvested from patients undergoing radical cystectomy for bladder cancer. Samples were carefully selected from cancer-free regions of the bladder wall to ensure histologically normal tissue architecture. A passive diffusion setup was used in which the luminal surface of each bladder specimen was exposed to an MMC solution (40 mg in 100 mL sterile saline; approximately 400 $\mu\text{g/mL}$). Tissue samples were collected at 5, 15, 30, 45, and 60 minutes following initiation of MMC exposure. Following collection, tissue was dissected into anatomically defined layers (urothelium, lamina propria, and muscularis), individually homogenized, centrifuged, and the resulting supernatant was analyzed for MMC content by high-performance liquid chromatography (HPLC). MMC concentrations were normalized to wet tissue weight and reported in units of $\mu\text{g/g}$ tissue. This dataset provides a high-resolution, three-layer, time-resolved concentration profile that captures the spatial dynamics of MMC diffusion across the bladder wall - a dataset uniquely suited for informing the PDE based diffusion model.

In vivo clinical data

Wientjes et al. in 1993 conducted a comprehensive pharmacokinetic study in 24 patients receiving intravesical MMC (20 mg/40 mL) prior to radical cystectomy[10]. The study measured urine MMC concentrations throughout the dwell period, plasma MMC concentrations reflecting systemic absorption, and serial tissue biopsy concentrations at defined depths parallel to the urothelium, lamina propria and muscularis, following bladder removal. Of the 24 patients enrolled, 17 patients were excluded from the tissue penetration analysis either because the final urine MMC concentration was below 66 µg/mL- indicating inadequate luminal drug exposure- or because more than 30 minutes had elapsed between draining the MMC solution and ligation of the bladder vasculature, which would allow systemic clearance to reduce tissue concentrations prior to sampling and compromise measurement accuracy. Only the remaining 7 patients, in whom luminal exposure was confirmed adequate and vascular ligation was performed promptly, provided the tissue biopsy data used in this work. Of all measurements collected, the plasma concentrations and urinary concentration-time profiles, while pharmacokinetically informative but were not useful for the purposes of the present diffusion model. The only measurements directly relevant to parameterizing and validating a spatially resolved diffusion model are depth-specific tissue concentrations, that described how much drug reached each anatomical layer at a defined time point. It is precisely this information that the seven-patient biopsy dataset provides, with tissue MMC concentrations measured at three depths- concentration in urothelium around 140 µm, concentration at depth 700 µm (representative in lamina propria layer), and concentration at depth 2000 µm (representative in muscularis layer) at 120 minutes following instillation. These point-in-space, point-in-time measurements serve as the direct experimental counterpart to the model output $C(x, t)$ evaluated at those specific coordinates, making them uniquely suited as a validation reference for the physiology-based diffusion framework developed in this work.

Exploratory data analysis of *in vitro* data

Before moving to model development, it is important to perform exploratory data analysis on *in vitro* data MMC concentration-depth-time profile data to first understand nature of drug transport across bladder wall in various layers. Layer-specific concentration distributions were visualized using box plots to characterize the spatial gradient and within-layer variability (Figure 1a). Mean concentration time profiles were plotted for each anatomical layer (Figure 1b).

Model structure

Spatial domain and layer definitions

A one-dimensional diffusion-reaction model was developed to describe MMC transport across the bladder wall. The spatial domain extended from the luminal surface ($x = 0$ cm) to the serosal boundary ($x = L = 0.40$ cm), representing the total physiological thickness of the human bladder wall. The domain was divided into three anatomically defined layers: urothelium (0-0.02 cm), lamina propria (0.02-0.15 cm), and muscularis (0.15-0.40 cm). Each layer was assigned a layer-specific diffusion coefficient (D_i) and first-order elimination rate constant (k_i). Figure 2 shows how the anatomy of the bladder wall was converted into the model domain.

Governing equation

Within each layer, MMC concentration changes through two processes: diffusion down the local concentration gradient, and first-order loss from the tissue. Diffusion is described by Fick's second law with a layer-specific diffusion coefficient and loss/elimination of drug is described by a layer-specific rate constant that lumps local degradation, non-specific binding, and *in vivo* uptake into the cell. Combining the two gives, for layer i

$$\frac{dC(x, t)}{dt} = D_i \cdot \frac{d^2C}{dx^2} - k_i \cdot C \quad (1)$$

where $C(x, t)$ is drug concentration (µg/g) as a function of depth x (cm) and time t (s). The elimination term accounts for drug loss via local degradation, non-specific tissue binding, and systemic absorption through bladder capillary perfusion.

At the interface between two layers of differing diffusivity, flux continuity was done using the harmonic-mean effective diffusion coefficient[23],

$$D_{eff} = \frac{2 \cdot D_1 \cdot D_2}{D_1 + D_2} \quad (2)$$

which is the standard finite-difference treatment for multilayer diffusion and is equivalent to placing the two layers' resistances in series across the interface.

Boundary and initial conditions

Fick's second law describes how concentration redistributes within tissue but not what drives it but for every differential equation to be solved we require a condition at each boundary is before the solution is unique.

Luminal boundary (Dirichlet): A Dirichlet/luminal boundary is a boundary condition that fixes the value of a solution at the domain edge. Concentration at $x = 0$ is fixed. In the *in vitro* configuration the donor compartment is amount of drug entering the tissue, so concentration is treated as constant. The luminal boundary was fixed at $C_0 = 400 \mu\text{g/mL}$ throughout the experiment, reflecting the constant donor compartment concentration in the Franz diffusion cell.

$$C(0, t) = C_0 = 400 \mu\text{g/mL} \quad (3)$$

In the *in vivo* configuration, urine produced during the dwell period dilutes the instillate. Assuming complete mixing in the lumen and no removal of drug from the lumen other than by dilution,

$$C_{lumen}(t) = \frac{C_0 \cdot V_0}{V_0 + q(t)} \quad (4)$$

with $C(0, t) = C_{lumen}(t)$. Equations 3 and 4 differ only in whether the value is constant or dynamic, which is what allows the same tissue model to serve both settings: the transition from diffusion cell to patient is a substitution at a single boundary, with no structural change to the solver.

Serosal boundary (Neumann): A no-flux condition was applied at $x = L$, ensuring drug cannot leak beyond the outermost bladder wall boundary. This is physiologically justified because MMC concentrations decay to negligible levels well before reaching the last depth in a standard dwell condition.

$$\left. \frac{\partial C}{\partial x} \right|_{x=L} = 0 \quad (5)$$

Initial condition: The tissue is drug-free at the start of the instillation, $C(x, 0) = 0$, for all x .

Assumptions

The model rests on the following assumptions:

A1: Transport is passive Fickian diffusion, with no convection, active uptake, or carrier-mediated transport.

A2: Bladder is as a planar, uniform structure and drug spreads radially the same everywhere.

A3: Tissue density is 1 g/mL , the standard soft-tissue approximation, so $\mu\text{g/mL}$ and $\mu\text{g/g}$ are interchangeable.

Numerical implementation

The PDE was discretized on a uniform grid of $N = 1000$ nodes ($\Delta x = 4 \times 10^{-4} \text{ cm}$) using a flux-based finite-difference scheme. Diffusive fluxes were evaluated at the midpoints between adjacent nodes following Fick's first law,

$$F_{i+1/2} = D_{i+1/2} \cdot \frac{C_{i+1} - C_i}{dx}, \quad F_{i-1/2} = D_{i-1/2} \cdot \frac{C_i - C_{i-1}}{dx} \quad (7)$$

where $D_{i+1/2}$ is the harmonic mean of the diffusion coefficients of the two nodes. Each interior node ($i = 2$ to $N - 1$) was advanced from the difference between the fluxes on its two faces, less first-order local loss,

$$\frac{dC_i}{dt} = \frac{F_{i+1/2} - F_{i-1/2}}{dx} - k_i \cdot C_i \quad (8)$$

The interior-node equations were evaluated as a single vectorised operation rather than node by node. Because a fine grid combines fast diffusive modes with slow first-order loss, the resulting system of ordinary differential equations is stiff, so it was integrated with the implicit VODE (Variable-coefficient Ordinary Differential Equation) solver. An implicit solver suits a stiff system because it stays stable without needing a tiny time step. VODE also sets its own step size from the local error estimate: small steps while concentrations rise quickly during early urothelial accumulation, and larger steps once the profile levels off. Since the solver is stable for this kind of diffusion-reaction problem.

Parameter estimation

Six parameters were estimated from the *in vitro* dataset: D_{uro} , D_{lam} , D_{mus} , k_{uro} , k_{lam} , and k_{mus} . Layer-mean concentrations at each of five sampling times (3 layers $\times$ 5 time points = 15 observations) formed the estimation dataset. All parameters were reparametrized in log space ($\theta = \log p$) to enforce positivity. The objective function was the sum of squared residuals with unit weights. Model predictions at each observed time-depth coordinate were obtained by taking the spatial node nearest the experimental depth and interpolating linearly in time between solver output steps. Optimization used L-BFGS-B (optim, R; maximum 500 iterations; factr = 1×10^{12}). Relative standard errors were obtained from the numerical Hessian at the optimum.

Stage 1 estimated all six parameters simultaneously, starting from $D_{uro} = 1 \times 10^{-7}$, $D_{lam} = D_{mus} = 1 \times 10^{-6}$ cm²/s, $k_{uro} = 1 \times 10^{-3}$, and $k_{lam} = k_{mus} = 1 \times 10^{-5}$ s⁻¹. Initial values were taken from published diffusion coefficients for small molecules in soft tissue and from plausible ranges for first-order loss constants. Parameter k_{uro} was seeded an order of magnitude above the deeper-layer values to reflect the expectation that most local loss occurs at or immediately beneath the urothelium. The parameter estimation process gave estimates for all six parameters, however, four parameters: D_{lam} , D_{mus} , k_{lam} , and k_{mus} , showed high relative standard errors (RSE%), indicating poor conditioning of the full six-parameter problem with the available data. The poor identifiability had two distinct, interconnected causes. The parameters were correlated and mutually compensating. In the lamina propria, D_{lam} (driving drug forward/inward) and k_{lam} (removing drug) act in opposing directions. Increasing D_{lam} and k_{lam} proportionally produces near-identical concentration profiles the optimizer cannot distinguish between the many such combinations that fit equally well. The drug reaching the muscularis must first pass through the lamina propria, ambiguity in D_{lam} propagated forward, hence D_{mus} and k_{mus} additionally uncertain as a downstream process. Hence, the parameters were collectively entangled across layers. Secondly, the muscularis concentrations were near-zero across all five time points with minimal temporal variation almost any positive combination of D_{mus} and k_{mus} produced equally flat, and an almost zero predictions. The current estimates were: D_{uro} (1.29×10^{-7} cm²/s) and k_{uro} (1.528×10^{-3} s⁻¹) were reasonably well identified. The remaining four parameters were not: D_{lam} (7.143×10^{-6} cm²/s), k_{mus} (9.99×10^{-6} s⁻¹) and D_{mus} and k_{lam} were both (1.002×10^{-3} cm²/s and 4.021×10^{-5} s⁻¹, respectively).

This Stage 1 was used as an identifiability diagnostic rather than as a final estimate. To resolve both identifiability problems simultaneously, three parameters were fixed as per their sensitivity to the *in vivo* model at their previously estimated values. In the estimation process the and only D_{uro} , D_{lam} , and k_{uro} were estimated in Stage 2. Fixing k_{lam} and k_{mus} broke the inter-parameter correlation chain allowing D_{lam} to be estimated against a fixed elimination term. Fixing D_{mus} removed the non-identifiable muscularis parameter from the search space. The resulting three-parameter problem was tractable given the available data and produced well-identified estimates with RSE% values confirming genuine identifiability.

***In vivo* translation and model evaluation**

The parameter set obtained in Stage 2 was transferred to the clinical simulation without modification. The only change was the luminal boundary condition, which was replaced by the dynamic dilution function of Equation 4 with $C_0 = 500 \mu\text{g/mL}$ (20 mg in 40 mL)[10], $V_0 = 40 \text{ mL}$, and $q = 1.5 \text{ mL/min}$, taken from average literature values[24, 25]. Simulations were run for 120 min as per the *in vivo* dwell time. Predicted concentrations were extracted at 140, 700, and 2000 μm and compared with the corresponding observed means from the seven-patient dataset. The evaluation criterion was pre-specified as a predicted-to-observed ratio within 2-fold at each depth, the convention used for physiologically based model evaluation and ratios within 1.25-fold were taken to indicate close agreement.

Local sensitivity analysis

A local sensitivity analysis was performed on the *in vivo* model to quantify the influence of nine parameters on predicted MMC concentrations at three midpoint tissue depths: 140 μm (urothelium), 700 μm (lamina propria), and 3000 μm (muscularis). Beyond the six transport and elimination parameters, three clinically controllable parameters were included: initial luminal concentration (C_0), initial bladder lumen volume (V_0), and urine formation rate (q). A one-at-a-time (OAT) central finite difference approach was used, each parameter was perturbed by $\pm 10\%$ while all others were held constant, and the full model, including spatial field vector reconstruction was re-executed for each perturbation. The central finite difference derivative was computed as:

$$\frac{dC}{dp} = \frac{C_{high} - C_{low}}{2\Delta p} \quad (9)$$

This was normalized to produce a dimensionless proportional sensitivity coefficient:

$$S(C, p) = \frac{p_0}{C_{base}} \cdot \frac{dC}{dp} \quad (10)$$

where C_{base} is the baseline predicted concentration and p_0 is the nominal parameter value. $S = 1.0$ means a 1% change in the parameter causes a 1% change in the predicted concentration; $S = -0.5$ means a 1% parameter increase causes a 0.5% concentration decrease. Results were visualized using a faceted bar plot showing signed sensitivity coefficients at each tissue depth, ordered by mean absolute sensitivity across all depths.

Software

All modelling, estimation, and analysis were performed in RStudios 2026.07.0 Build 139 using *deSolve* for integration and base *optim* for optimization.

Results

Exploratory data analysis

The *in vitro* data show the two features that distinguish diffusion-limited from reaction-limited transport (Figure 1). Mean MMC concentration declined steeply from urothelium through lamina propria to muscularis (Figure 1a), the depth gradient expected of diffusion through a layered resistive medium. Variability was largest in the urothelium, consistent with sensitivity of the most superficial layer to the surface boundary condition and to variation in dissection depth between specimens. Urothelial concentrations rose rapidly over the first 15 min and then plateaued through 60 min (Figure 1b), which is the signature of transport-limited accumulation approaching quasi-steady-state flux and in deeper layers accumulated more slowly, consistent with a diffusion front propagating inward. These patterns support Fick's second law with first-order loss as the governing description and support treating the three layers as separate transport domains rather than one homogeneous medium.

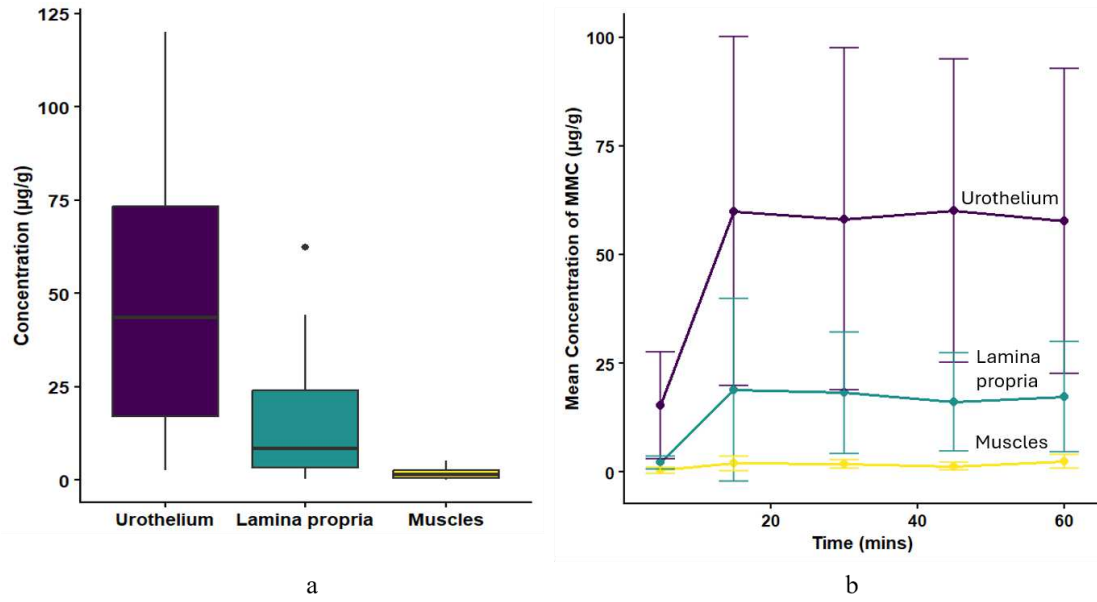


Fig. 1 Exploratory analysis of the in vitro MMC penetration data.(a) Box plot of MMC concentration (µg/g) distributions across three bladder-wall layers: urothelium, lamina propria, and muscularis. (b) Mean concentration-time profile of MMC across the three bladder wall layers: urothelium, lamina propria, and muscularis

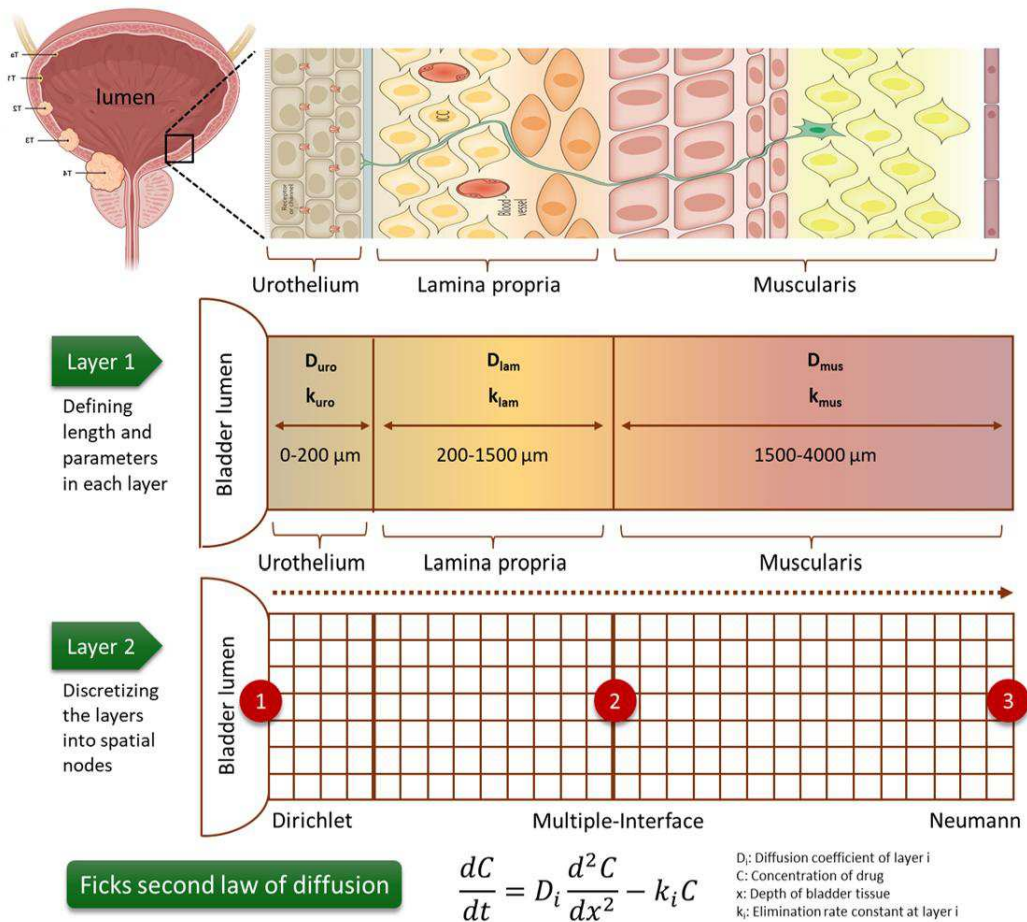


Fig. 2 Structure of the three-layer bladder-wall model. The urothelium (0-200 μm), lamina propria (200-1500 μm), and muscularis (1500-4000 μm) are each assigned a diffusion coefficient D_i and a first-order loss constant k_i . Drug enters through a prescribed-concentration (Dirichlet) boundary at the luminal surface (1) and cannot leave through the no-flux (Neumann) boundary at the serosal surface (3); the domain is discretized over 1000 nodes with inter-layer boundary interface (2).

Parameter estimation

The final parameter estimated were reduced three-parameter model achieved $R^2 = 0.95$ across all three tissue layers and all five experimental time points. Estimated parameters are presented in Table 2.

Table 2 Stage 1 and Stage 2 parameter estimates.

Parameter	Units	Stage 1 estimate	Stage 1 status	Stage 2 value	RSE (%)	Stage 2 status
D_{uro}	cm^2/s	1.29×10^{-7}	Estimated	1.164×10^{-7}	11.6	Estimated
D_{lam}	cm^2/s	7.143×10^{-6}	Estimated, imprecise	9.804×10^{-6}	136.7	Estimated
D_{mus}	cm^2/s	1.002×10^{-3}	At upper bound	1.002×10^{-6}	-	Fixed
k_{uro}	s^{-1}	1.528×10^{-3}	Estimated	1.673×10^{-3}	17.0	Estimated
k_{lam}	s^{-1}	4.021×10^{-5}	Estimated, imprecise	4.021×10^{-5}	-	Fixed
k_{mus}	s^{-1}	9.99×10^{-6}	Estimated, imprecise	9.996×10^{-6}	-	Fixed

D_{uro} ($1.164 \times 10^{-7} \text{ cm}^2/\text{s}$) was approximately 84-fold lower than D_{lam} ($9.804 \times 10^{-6} \text{ cm}^2/\text{s}$), quantifying the dominant permeability-barrier function of the urothelium. The high first-order urothelial elimination constant ($k_{uro} = 1.673 \times 10^{-3} \text{ s}^{-1}$; biological half-life ≈ 6.9 minutes) reflects the combined pharmacokinetic of local MMC degradation, non-specific tissue binding and incorporating into the dense subepithelial vasculature. The lamina propria behaves in the opposite way. Its loose connective tissue matrix of collagen and elastin offers little resistance to diffusion ($9.804 \times 10^{-6} \text{ cm}^2/\text{s}$). Local loss is correspondingly slower ($k_{lam} = 4.021 \times 10^{-5} \text{ s}^{-1}$, roughly 40-fold below), so once drug has crossed the urothelium it is both transmitted and retained more readily. Figure 3 shows fitting results of model-predicted versus observed data with different time point how concentration is fitted to differing depth.

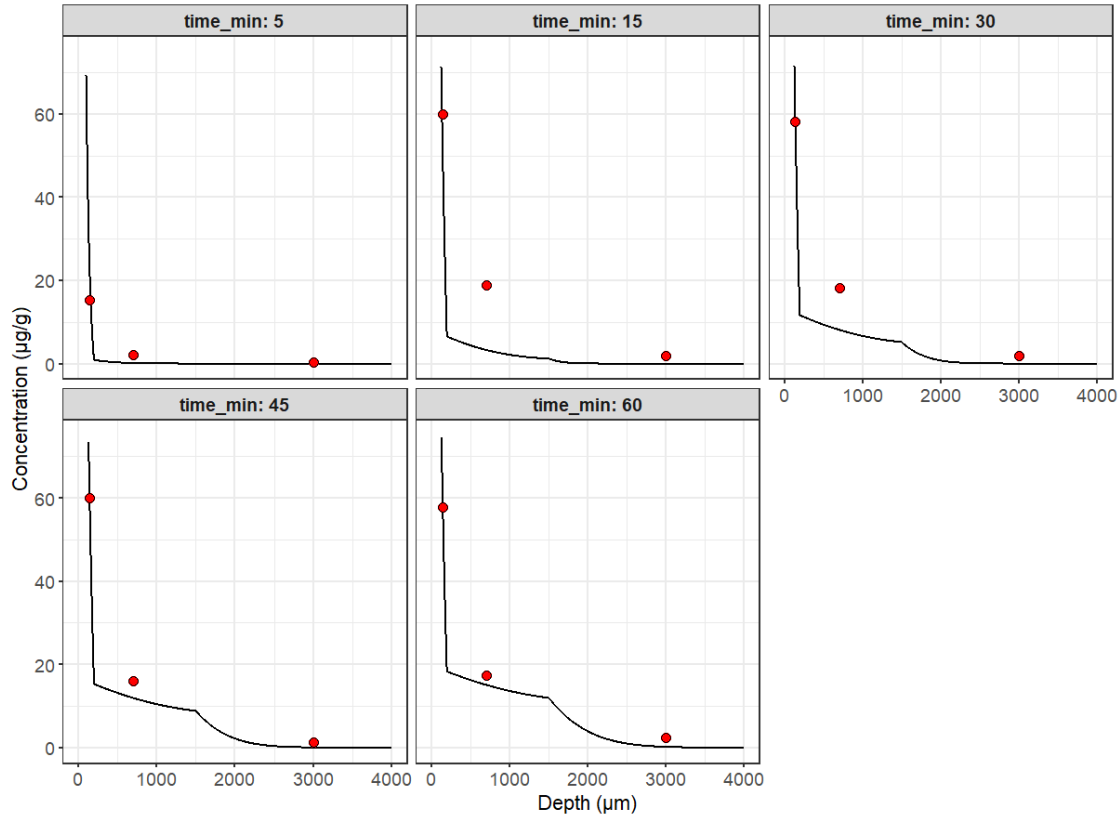


Fig. 3 *In vitro* model fit. Predicted (lines) and observed mean (red dot) MMC concentration-depth profiles in the urothelium, lamina propria, and muscularis over 60 min

***In vivo* translation**

The Stage 2 parameter set was transferred to the clinical simulation unchanged, and the constant luminal boundary was replaced by the dynamic dilution function of Equation 4. Table 3 gives the complete *in vivo* parameter set.

Table 3 Parameter set for the *in vivo* simulation

Parameter	Units	Value	Source
D_{uro}	cm^2/s	1.164×10^{-7}	Estimated from <i>in vitro</i> data
D_{lam}	cm^2/s	9.804×10^{-6}	Estimated from <i>in vitro</i> data
D_{mus}	cm^2/s	1.002×10^{-6}	Fixed (Section 2.6)
k_{uro}	s^{-1}	1.673×10^{-3}	Estimated from <i>in vitro</i> data
k_{lam}	s^{-1}	4.021×10^{-5}	Fixed (Section 2.6)
k_{mus}	s^{-1}	9.996×10^{-6}	Fixed (Section 2.6)
C_0	$\mu\text{g}/\text{mL}$	500	Clinical dose, 20 mg in 40 mL[10]
V_0	mL	40	Residual volume[26]
q	mL/min	1.5	Rate of urine formation[25]

Predicted concentrations at 120 min are compared with the clinical biopsy data in Table 4 and Figure 4. Predictions in the urothelium and lamina propria fell within the pre-specified 2-fold criterion, and within 1.25-fold. The muscularis prediction exceeded the observed mean by 2.42-fold.

Table 4 Predicted and observed MMC concentrations at 120 min.

Layer	Depth	Predicted ($\mu\text{g/g}$)	Observed mean ($\mu\text{g/g}$) ($\pm\text{SD}$)	CV% on observed data	Predicted/observed
Urothelium	140 μm	14.4	11.65 (10.02)	86	1.24
Lamina propria	700 μm	7.05	5.63 (5.69)	101.1	1.25
Muscularis	2000 μm	3.77	1.56 (1.48)	94.7	2.42

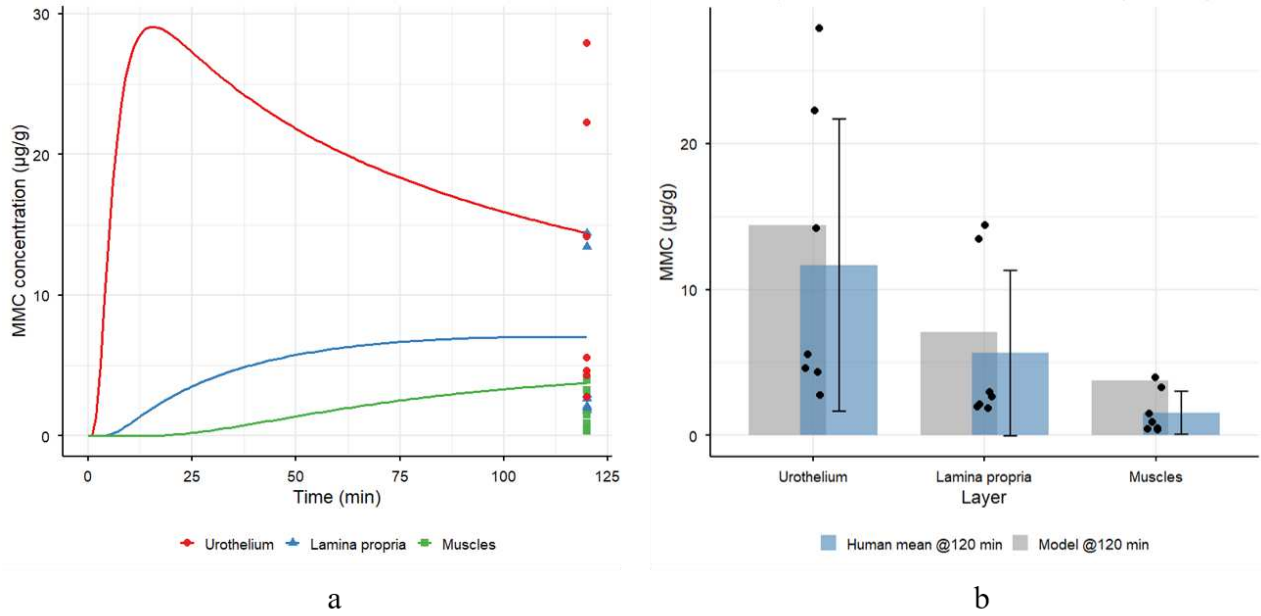


Fig. 4 In vivo predictions and clinical evaluation data. (a) Predicted MMC concentration–time profiles in the urothelium, lamina propria, and muscularis over a 120 min dwell under the dynamic urine-dilution boundary. (a) Predicted (grey) and observed mean (blue) concentrations at 120 min; individual patient observations are shown as points and error bars represent $\pm$ SD.

Sensitivity analysis

Sensitivity coefficients differed systematically with depth (Figure 5). In the urothelium, D_{uro} and C_0 were the most influential parameters, both with positive coefficients: urothelial exposure is set by the diffusion resistance of the epithelial barrier and by the concentration presented at the luminal surface. In the lamina propria, both D_{uro} and D_{lam} were influential, reflecting transport in series through the two layers. In the muscularis, D_{lam} dominated, identifying lamina propria transport as the rate-limiting step for deep penetration. Urine formation rate q had moderate influence at all depths, acting through the dilution boundary. The three parameters fixed in Stage 2: D_{mus} , k_{lam} , and k_{mus} showed low sensitivity at all depths under the nominal parameter set.

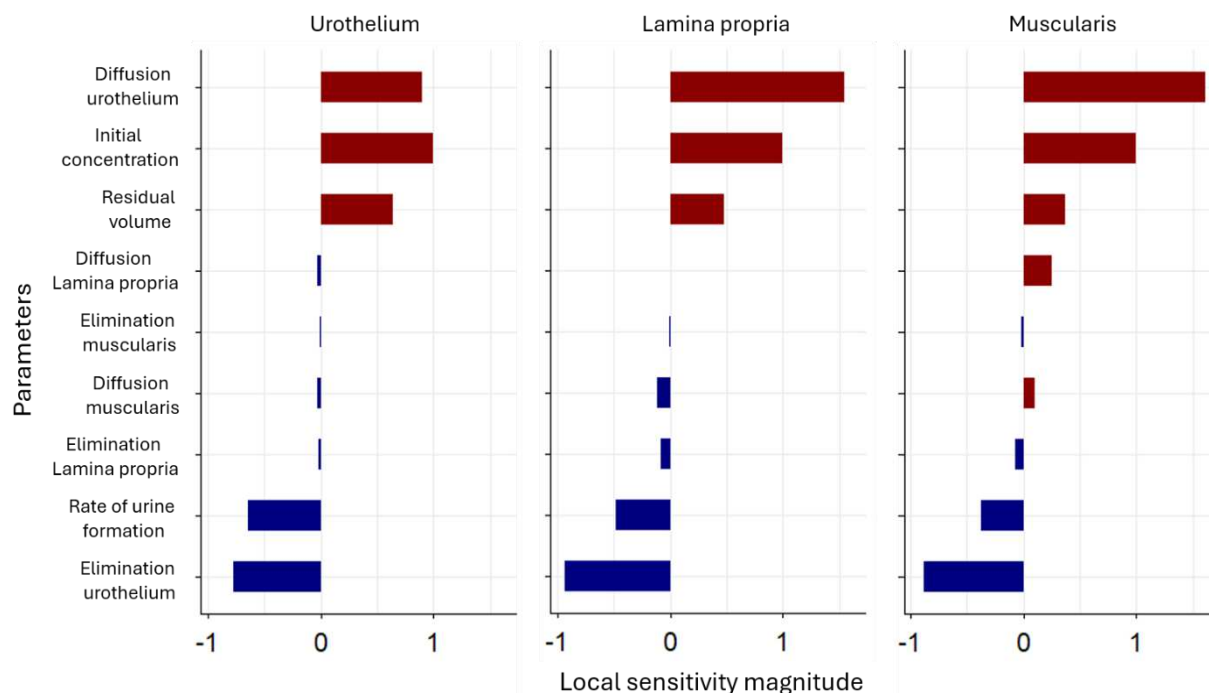


Fig. 5 Local sensitivity analysis. Normalised sensitivity coefficients (S) for nine model parameters with respect to predicted MMC concentration at three tissue depths after a 120 min dwell. Positive coefficients (red) indicate that increasing the parameter raises predicted concentration; negative coefficients (navy) indicate the opposite.

Discussion

This study was designed to test whether *in vitro* data can be used to build a mechanistic model, and whether that model can then simulate the *in vivo* condition and predict tissue concentration in the bladder as a function of both time and depth within the wall. Drug transport parameters estimated from a controlled *in vitro* diffusion experiment in human bladder tissue predicted measured clinical biopsy concentrations without re-fitting. Only the luminal boundary changed, from a fixed donor concentration to a function describing progressive urine dilution. Under that single substitution the model reproduced urothelial and lamina propria concentrations within approximately 1.25-fold of the observed means (predicted/observed 1.24 and 1.25). This is the premise on which physiologically based modelling and IVIVE rest: parameters with physical meaning, placed in an anatomically faithful structure, should predict *in vivo* behaviour without empirical adjustment. Two qualifications apply to that claim here. Three of the six transport parameters were fixed rather than estimated, and the choice of which to fix drew on the *in vivo* configuration (Section 2.6), so the transfer is not fully independent of the target of prediction. The muscularis prediction did not meet the 2-fold criteria, and the manuscript's translational claim is restricted accordingly: the model is evaluated for the urothelium and lamina propria, which together define the NMIBC target, and is not yet evaluated at muscle depth.

The two urothelial parameters describe different things and separating them is what a diffusion model is about. A low D_{uro} limits how fast drug crosses the epithelium. A high k_{uro} determines how much of what crosses is subsequently lost rather than retained. A compartmental model may combine both into one transfer constant and cannot distinguish a tissue that admits little drug from a tissue that admits drug and then clears it quickly. There are two different concepts that need to be highlighted here. The first is addressed by permeability of drug across bladder wall. Lipophilic agents such as thiotepa partition into the urothelium almost completely but are cleared into the capillary bed within a short distance, whereas MMC partitions less favorably at the surface but is retained over a longer distance and therefore reaches deeper[27]. High surface partitioning without retention is not sufficient for deep exposure, the present estimates place MMC toward the retained end of that spectrum. The magnitude of the urothelial barrier is specific to

drug and tissue rather than a fixed property of the epithelium. Grabnar et al., fitting pipemidic acid diffusion in porcine bladder to a two-region model[13], reported a urothelial diffusion coefficient of $2.94 \times 10^{-7} \text{ cm}^2/\text{s}$ against $6.32 \times 10^{-7} \text{ cm}^2/\text{s}$ in the underlying tissue, roughly a 2-fold difference, an order of magnitude smaller than the 84-fold difference estimated here for MMC in human tissue. Species, compound, and model structure all differ between the two analysis, so the comparison bounds the plausible range rather than validating either estimate[13]. Human bladder transport data are sparse, so the parameters estimated here are hard to check against anything independent. Moch et al. shows the one useful comparison where they measured mitomycin C penetration and permeation through porcine bladder urothelium in four different setups: a Franz cell, two QuixSep microdialysers, and the Vitropharma device and fitted each profile to Fick's second law for a single homogeneous membrane, D/L^2 rather than D directly[28]. With a specimen thickness (L) of about 200 μm , their D/L^2 values translate to diffusion coefficients near 3.4×10^{-7} to $9.1 \times 10^{-7} \text{ cm}^2/\text{s}$. Porcine bladder is a widely used surrogate for the human organ in pharmacological work, on the basis that it resembles it closely in anatomy and physiology[29, 30], and Moch et al. adopted it for that reason. Our estimate for Duro ($1.164 \times 10^{-7} \text{ cm}^2/\text{s}$) is of the same order as these values.

The over-prediction in 2000 μm has a mechanistic explanation that also tells the model's main structural gap. The loss constants were estimated in *in vitro* tissue, where the only routes of loss are local degradation and non-specific binding: there is no perfusion, so nothing is removed from the tissue by absorption. *In vivo* the lamina propria is densely vascularised, and drug arriving there is continuously drawn into the capillary bed and carried into the systemic circulation. That mechanism cannot operate in a diffusion cell and cannot be contained in a k_{lam} fitted to one. Carrying the *in vitro* value into the *in vivo* simulation unchanged leaves drug in the lamina propria that a perfused bladder would be removing/absorbing, and whatever passes through an under-cleared layer arrives at the muscularis in excess.

Grabnar et al. connected urine, tissue regions, and systemic absorption through transfer rate constants, capturing the features that matter is urinary concentration varying with urine formation and voiding, biphasic tissue distribution[13], and absorption from the perfused deep layers. But the deep-layer transport was summarised by a single empirical half-width. Wientjes et al. combined urine pharmacokinetics with a simplified penetration relationship to rank modifiable treatment factors for trial design[14], again without resolving the gradient inside the wall. The present model keeps what both established the dynamic urinary dilution and layer-dependent transport that replaces the compartmental abstraction with a concentration field solved at 4 μm resolution. Since concentration falls by more than an order of magnitude across the first 200 μm , a coarser representation cannot locate the therapeutic front where dosing decisions need it. Therefore, the novelty claim is purposely narrow. Spatially resolved transport models of layered tissue are well established, in the bladder wall and elsewhere. What has not been done, as far as we are aware, is to estimate layer-specific parameters from human bladder tissue *in vitro* and carry them unchanged into prediction of independently measured human tissue concentrations. Human-to-human extrapolation is the contribution the manuscript is trying to achieve. That generality also means the framework should transfer to other intravesical agents and delivery systems through a change of parameters rather than a change of form.

The model is deliberately simple, and several of its simplifications are bound to some limitation that model still possesses. D_{lam} was estimated with uncertainty (RSE 137%) because of its correlation with the fixed k_{lam} . The sensitivity analysis indicates that the fixation had little effect on predictions under the nominal parameter set, but the deep-layer parameters remain the least certain quantities in the model. Fixing three of six parameters is a pragmatic response to an identifiability problem, not an independent measurement, and those fixed values carry whatever bias the Stage 1 fit carried. Because the *in vitro* preparation is unperfused, k_{lam} and k_{mus} are expected to underestimate *in vivo* loss. The evaluation set comprises seven patients with inter-patient variability above 80% CV, which limits how strongly the translational claim can be stated. Model predictions are end-of-dwell values and observed concentrations are post-drainage values. After drainage the luminal source is removed and drug already in the wall continues to redistribute inward, so post-drainage sampling would if anything raises deep-layer concentrations relative to the end-of-dwell prediction; hence the washout therefore does not account for the muscularis over-prediction, although it may contribute at the urothelial surface. Sampling depth is also approximate: the model reports a point concentration while

a biopsy although given specific depth concentration may also contribute to variability of human bladder or the concentration might be integrated over a section.

Three extensions could follow directly, in order of how much they would strengthen the translational claim. The first is a vascular loss term where the muscularis over-prediction to absent perfusion-driven clearance in the lamina propria, and that hypothesis is testable here by raising k_{lam} to a value representing capillary uptake. The plasma data from the same clinical study, not used here, would show how much drug was absorbed and so put a bound on that term. The second is a probabilistic treatment of anatomy. Variability above 80% CV at every depth is consistent with real heterogeneity in wall thickness, layer boundaries, capillary density, and urothelial permeability, and no single-profile model can represent it. Sampling correlated anatomical parameter sets and running the PDE model once per sampled anatomy would convert a deterministic profile into a distribution, without altering the transport model, and would let it answer the question a clinician actually asks what fraction of patients reaches a target lamina propria concentration under 20 mg in 40 mL, and how does that fraction shift at 40 mg or a 90 min dwell. The third is regimen optimization. Once the model predicts reliably at all depths, dose, volume, and dwell time can be searched against an objective defined on predicted tissue concentration: maximum exposure at a target depth, subject to a ceiling on urothelial exposure. Combined with the virtual population, that search could run per anatomical subgroup rather than against an average patient. Both extensions rest on the same prerequisite confidence that transport generalizes beyond the depths at which the model has been evaluated.

Conclusion

We developed and evaluated a physiology-based, spatially resolved model of intravesical mitomycin C transport through the human bladder wall. Exploratory analysis of human *in vitro* permeation data showed a decline in concentration with depth and a urothelial plateau within 15 min, the signature of transport-limited rather than reaction-limited kinetics, which justified a diffusion reaction description over a compartmental one. Three of six transport parameters were estimated from those data and three were fixed after an identifiability assessment. Carried unchanged into a clinical simulation in which only the luminal boundary was changed, the model predicted biopsy concentrations from seven patients within approximately 1.25-fold at 140 and 700 μm , and over-predicted by 2.42-fold at 2000 μm . Within those limits and recognizing that the lamina propria diffusion coefficient remains imprecisely determined, the model provides a mechanistically interpretable basis for evaluating dose, instillation volume, and dwell time against depth-resolved tissue exposure in NMIBC, where the lamina propria is the therapeutic target. Extending the same confidence to the muscularis, and therefore to muscle-invasive disease, will require a vascular loss term informed by *in vivo* absorption data and evaluation in a larger clinical dataset, together with a probabilistic treatment of inter-patient anatomical variability.

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Declarations

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval

This study did not involve new experiments on human participants or on animals. It is a secondary analysis of previously published, de-identified, aggregate data, and institutional review board approval was therefore not required.

Consent to participate

Not applicable. The analysis used previously published aggregate data.

Data availability

No new experimental data were generated. All data supporting the findings of this study were extracted from the published sources cited as references literature, and the extracted values used for parameter estimation and model evaluation are reported in full in the tables and figures of this article.

Code availability

The R code implementing the three-layer diffusion-reaction model, the two-stage parameter estimation, and the sensitivity analysis is available from the corresponding author on reasonable request.

Author contributions

Conceptualization: Rodrigo Cristofolletti, Kajal Gupta; Methodology: Kajal Gupta, Rodrigo Cristofolletti; Software: Kajal Gupta; Formal analysis and investigation: Kajal Gupta; Writing - original draft preparation: Kajal Gupta; Writing - review and editing: Kajal Gupta, Rodrigo Cristofolletti; Supervision: Rodrigo Cristofolletti. All authors read and approved the final manuscript.

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