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Modeling Catheter-Associated Bladder Mucosal Adhesion and Microtrauma Using a Human Urothelial Microtissue Model

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ABSTRACT

Animal models have been used to investigate urinary tract catheters, but small animals do not accurately recapitulate human bladder physiology nor support different types of catheters. Recent advances in three-dimensional models mimicking tissue microenvironments have improved our understanding of human tissue development and allowed us to model many diseases. Here, our objective was to characterise bladder urothelial mucoadhesion and microtrauma associated with intermittent catheterisation. We employed our three-dimensional Urine-tolerant Human Urothelial model (3D-UHU) to investigate two different intermittent catheter types, a polyvinylpyrrolidone (PVP)-coated catheter (PVP-CC) and a coating-free integrated amphiphilic surfactant (IAS) catheter. We showed that pressing catheters onto the models for 2 min caused compression of the 3D-UHU model in contact regions, with disruption of urothelial umbrella cells detected by immunofluorescence staining of surface markers uroplakin III, cytokeratin 20 and chondroitin sulphate, alongside occasional effacement of apical surfaces exposing intermediate layers. PVP-CC had a significantly higher number of urothelial cells adhered to its surface after catheter removal compared with IAS. Moreover, application and removal of PVP-CC caused a decrease in transepithelial electrical resistance, suggesting barrier disruption, whereas IAS did not cause a statistically different effect. However, no change in paracellular permeability rates assessed by FITC-dextran were observed in models after application of catheter pieces. Finally, compression of models induced trauma-triggered inflammatory cytokine responses. Specifically, we observed increased secretion of the pro-inflammatory cytokine IL-1 β as well as the cell adhesion molecule CEACAM1 after exposure to PVP-CC compared with IAS. These data demonstrate that the 3D-UHU model holds promise as an alternative to animal models for investigating the effects of urinary catheters on the urothelium.

1 | Introduction

Urinary catheters are among the most commonly used medical devices in healthcare, primarily employed to assist patients with impaired ability to control micturition [1]. Urethral catheters, which are inserted transurethraly, include both indwelling and intermittent catheter (IC) types. Indwelling catheters are the most frequently used and may be inserted into the urethra for short (less than 30 days) or long (more than 30 days) durations [2]. In

contrast, ICs are single-use, and catheterisation is performed an average of four to six times daily, up to 5 min each time, to ensure sufficient voiding of the bladder [3, 4]. It is estimated that 90,000 adults have a urinary catheter in the UK, with approximately 50 000 of these using ICs, up to five times daily [5].

Repeated intermittent catheterisation poses the risk of urethral trauma and bleeding (urethrorrhagia), and persistent urethral bleeding occurs in 10.7%–28% of IC users [6, 7]. Furthermore,

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repositioning of the IC to completely empty the bladder can cause additional trauma. This mucosal trauma can compromise the urothelial barrier, with or without the presence of haematuria, thus increasing the risk of urinary tract infections (UTIs). The trauma caused by catheterisation can range from minor urethral microtrauma to serious complications, such as false passage [8–10].

To reduce the trauma associated with ICs, hydrophilic-coated catheters were introduced in the late 1980s. Water binds to the hydrophilic coating on the catheter, leading to increased lubricity when compared with hydrophobic uncoated catheters [11, 12]. Uncoated catheters require the use of a lubricant to aid insertion, while hydrophilic catheters coated with polymers such as poly(ethylene oxide)s or polyvinylpyrrolidones (PVPs) aim to improve and ease insertion, reducing the risk of urethral trauma and UTI [13, 14]. Several catheters are used in clinical settings with different types of coating, such as hydrogel-coated, silver-coated, polytetrafluoroethylene (PTFE)-coated and pre-lubricated catheters [2].

Currently, the pathobiological effects associated with ICs are less clear due to a lack of appropriate or tractable models. Small animals such as rodents and rabbits do not recapitulate human bladder accurately; larger animals, such as the pig, are more expensive, therefore limiting the sample size; and comprehensive investigations are not feasible in humans based on ethical grounds. Therefore, there is a significant gap in the understanding of urethral trauma associated with ICs. Current advancement in cell culture techniques has led to the development of three-dimensional (3D) in vitro models with the potential to mimic the complex structure of human primary tissues. These models hold promise for many applications including modeling a wide range of diseases [15]. Recently, we developed a 3D Transwell-based model of the human bladder urothelium [16]. The 3D Urine-tolerant Human Urothelial model (3D-UHU) differentiates and stratifies to human urothelial thickness of seven to eight layers, forms a tight barrier and exhibits the key human bladder biomarkers, including uroplakin proteins and a glycosaminoglycan layer, and innate immune responses. Importantly, its apical surface is fully tolerant to 100% human urine, allowing experiments to be performed in the correct physiological milieu. Here, we used the 3D-UHU model to investigate the bladder mucosal adhesion and corresponding microtrauma caused by catheterisation using two different catheters currently in clinical use, a hydrophilic-coated and a coating-free catheter.

2 | Method

2.1 | Human Bladder Epithelial Cells and 3D-UHU Culture

The growth condition of human bladder cells and establishment of 3D-UHU has been described previously [16]. Briefly, HBLAK human bladder progenitor cells (CELLnTEC, Switzerland) were cultured in CnT-Prime medium (CnT-PR, CELLnTEC) and passaged when they reached 70% to 90% confluency. Cells were detached using Accutase and seeded at a density of 4000 cells/cm². To establish 3D human urothelial models, HBLAK cells within passages 8–12 were seeded onto 12 mm Transwell with 0.4 µm pore polycarbonate membrane (VWR, United Kingdom) at the

density of 2×10^5 cells per insert. The apical and basolateral chambers were filled with 0.5 and 1.5 mL of CnT-PR medium, respectively, so that the medium levels were equal. After 48 h of incubation at 37°C and 5% CO₂, CnT-PR was replaced with 3D Barrier Medium (CnT-PR-3D, CELLnTEC) and incubated overnight. To initiate 3D cultures, the inserts were transferred into a ThinCert cell culture plate, and filter-sterilised human urine (pooled gender) (BioIVT, UK) was added to the apical chamber while fresh CnT-PR-3D medium was added to the basolateral chamber. Urine and CnT-PR-3D medium were changed twice per week until days 18–20 when 3D models were fully stratified and differentiated.

2.2 | Catheters

Two different types of catheters were utilised in this study: SpeediCath Flex (Coloplast, UK) and GentleCath Glide (Convatec, UK). The SpeediCath has a hydrophilic PVP coating and is a soft catheter with a dry sleeve which was removed prior to use. The GentleCath Glide is comprised of a coating-free alternative to PVPs, fabricated with integrated amphiphilic surfactant (IAS) technology. Both catheters were sliced into 8–10 mm segments to fit inside the Transwell inserts (membrane diameter 12 mm) and maintained lubricated in water provided by the manufacturer.

To examine catheter dwelling effects, urine and CnT-PR-3D were aspirated from apical and basolateral chambers of the 3D-UHU models, respectively, to resemble events that happen in catheter users. As urine drains through the catheter lumen, it reduces the rinsing of the urethral walls that normally occurs during bladder voiding. This leads to catheter sticking to the urethra. The 3D-UHU model resembles the bladder neck, internal sphincter and the proximal urethra where the cells are the same as the bladder cells. The 3D models were then placed in the incubator at 37°C and 5% CO₂ to dehydrate. After 15 min, the cultures remained visibly moist, comparable with wetness of the ex vivo pig model published by Burns et al. [17]. Cultures were removed from the incubator, and one piece of catheter was placed centrally on a 3D-UHU model using tweezers. Catheters were pressed down gently onto the models for 2 min manually to mimic the in vivo dwell time needed to drain urine [17, 18]. The applied pressure was equivalent to 3 N force in accordance with previous studies [4]. We established this by comparing the imprints of catheter pieces into agar pads made with a Lloyd LXR PLUS Tensile Machine.

2.3 | Immunostaining and Imaging

3D-UHU cultures were washed with PBS and then fixed with 4% methanol-free formaldehyde (Thermo Fisher Scientific, UK) overnight at 4°C. Membranes were washed with PBS, excised and permeabilised in 0.2% Triton-x100 (Sigma-Aldrich, UK) in PBS for 20 min at room temperature (RT) and then blocked with 5% normal goat serum (Thermo Fisher Scientific) at RT for 1 h. Membranes were incubated at 4°C overnight with the following primary antibodies in 1% bovine serum albumin (BSA)/PBS: mouse anti-uroplakin-III (UPKIII) monoclonal antibody (1:50 dilution, sc-166808, Santa Cruz); mouse anti-cytokeratin 8 (CK8) monoclonal antibody (1:50 dilution, MA1-06318, Thermo Fisher Scientific);

rabbit anti-cytokeratin 20 (CK20) polyclonal antibody (1:100 dilution, PA5-22125, Thermo Fisher Scientific); and mouse anti-chondroitin sulphate antibody (1:100 dilution, ab11570, Abcam).

Post-incubation, membranes were washed with 1% BSA/PBS and incubated with secondary antibodies (1:500 dilution, goat anti-mouse or goat anti-rabbit) conjugated with Alexa Fluor-488 (Thermo Fisher Scientific) at RT for 1.5 h. To label filamentous actin, membranes were washed before adding Alexa Fluor-633 phalloidin (1:500 dilution, Thermo Fisher Scientific) at RT for 30 min, followed by DAPI nucleic acid stain (DAPI dihydrochloride, 300 nM in PBS, Invitrogen, UK) at RT for 5 min.

To image the 3D-UHU cultures, membranes were washed, then mounted with ProLong Glass antifade mountant (Invitrogen) and imaged on a Leica SP8 microscope (Leica Microsystems Ltd, UK). Images were acquired using 63× magnification with 0.3 µm Z step size giving the 50% image overlap required for 3D image reconstruction (the laser power was set to 0.1%–2% with gain adjustment for HyD detectors ranging below 100–500 to avoid pixel saturation). Images were processed using LAS X software version 3.5.7.

To examine the dwelling effects of the catheters, the 3D-UHU models were first stained with 5 mM DRAQ5 Fluorescent Probe, a far-red live DNA stain (1 µL, 62252, Thermo Fisher Scientific), for 4 min at 37°C, and then dried and treated with catheters. Subsequently, the membranes were stained either with Alexa Fluor-488/555 phalloidin (1:500 dilution, Thermo Fisher Scientific) to assess the F-actin as described above, or wheat germ agglutinin (WGA) to examine the cell membrane.

To stain with WGA, the membranes were fixed with formaldehyde solution and then washed with Hanks' Balanced Salt solution (HBSS), (Thermo Fisher Scientific). Next, the membranes were stained with 5 µg/mL of WGA, Alexa Fluor-555 conjugate (Thermo Fisher Scientific) for 1 h at RT. The membranes were mounted and subsequently imaged as described above.

Concomitantly, the catheters were dried for 2 h at RT, then placed on a cell culture dish (µ-Dish 35 mm, Ibidi, Germany) with Vectashield PLUS antifade mounting media (Vector Laboratories, USA) and imaged on a Leica SP8 microscope using 20× magnification. Images were acquired from four different areas, including both ends, and two mid-sections from four biologically independent experiments. Urothelial cell nuclei count from different areas was averaged and presented as the number of cells adhered to catheter pieces (8–10 mm).

2.4 | Measuring Transepithelial Electrical Resistance (TEER) and Paracellular Permeability

The EVOM3 with a STX2-Plus electrode (World Precision Instruments, UK) was used to measure the integrity of the 3D-UHU barrier pre- and post-catheter contact. TEER measurements were conducted according to the manufacturer's instructions. Briefly, the equipment was set up with a 1000 Ω test resistor; then, blank handling mode was selected to allow subtraction of the blank control from the current resistance measurement of cultures. Plates were allowed to equilibrate to a consistent

temperature prior to measurement. Each measurement was recorded and stored on the device for further analysis.

The paracellular permeability of the 3D-UHU models was assessed using fluorescein isothiocyanate (FITC)-dextran (MW 4000, Sigma-Aldrich, UK). FITC-dextran solution (1 mg/mL) was prepared in urine, filtered and added to the apical chamber. Aliquots of medium from the basolateral side were collected at 0, 8 and 24 h post-catheter contact, and fluorescence was measured by a Tecan Spark microplate reader (Tecan, Switzerland) at an excitation of 485 nm and an emission of 538 nm. The FITC-conjugated dextran concentration was presented as relative fluorescence units (RFU).

2.5 | Measuring Immune Response Using Luminex Multiplex Assay

To measure inflammatory cytokines/chemokines in response to catheter dwelling, 3D-UHU models were re-incubated with fresh urine (apical chamber) and CnT-PR-3D (basolateral chamber) post-catheter treatment. At 12 h post-incubation, the apical supernatants were collected and stored at –80°C.

A customised human Luminex high-performance assay panel (Bio-Techne, UK) was used to determine selected biomarker concentrations in the supernatants according to the manufacturer's instructions. The following analytes were analysed using a Luminex 200 instrument (Bio-Techne Ltd, UK): CCL2, CXCL1, CEACAM-1, IL-1β, IL-6, IL-18, TNF-α, IL-1α, IL-1Ra, IL-8, CD44 and Pentraxin 3.

2.6 | Statistical Analysis

Data were analysed using GraphPad Prism 10.3 for Windows (GraphPad Software, USA). At least three independent biological replicates in technical replicates of two were performed for statistical analysis. Differences in measurements of urothelial cells adhered to the catheters were analysed by *t*-test with Welch's correction. Two-way ANOVA was used to calculate statistical significance in TEER and paracellular permeability measurements followed by Tukey's multiple comparisons test to compare the variables, and Dunnett's multiple comparisons test to compare the variables with the control, respectively. Tukey's multiple comparisons test was employed to compare the expression of the cytokine/chemokine between different groups.

3 | Results

3.1 | Media Removal Effect on 3D-UHU Models

To mimic an *in vivo* catheterisation setting, it was necessary to remove apical and basolateral media from 3D-UHU models to create a semi-dehydrated environment. The models were stained with DRAQ5 first; then, the media was removed; and models were incubated at 37°C for 5, 10 and 15 min. Next, they were stained with phalloidin or WGA (not shown) to examine the effect of dehydration on 3D-UHU DNA, F-actin and cell membrane, respectively. In the non-dehydrated control condition, models showed a typical 3D-UHU structure comprised of six to seven

cell layers with distinctive umbrella cells on the apical side (Figure 1A). The dehydrated models retained the 3D-UHU structure, although, at 15 min post-media removal, membrane ruffling and occasional DNA fragmentation were observed (Figure 1B). As the membranes showed sufficient dehydration at 15 min post-media removal and the observed impairment was minimal, we employed the 15 min dehydrating condition throughout the study.

3.2 | Dwelling Effect of Catheters on 3D-UHU

To examine the impact of catheter application on urethral mucosal adhesion and microtrauma, the 3D-UHU models were fluorescently probed with DRAQ5 prior to catheter application. The stained models were then dehydrated, and PVP-CC or IAS catheter pieces were pressed onto models manually for 2 min followed by staining for cell membrane (Figure 2A–C) or F-actin (Figure 2D–F) with WGA or phalloidin, respectively, to examine the 3D-UHU morphology.

The cell membrane staining indicated that cell layers that were in contact with the PVP-CC or IAS catheters shown in the demarcated areas were compacted and flattened (PVP, Figure 2B; and IAS, Figure 2C) compared with the un-treated but dehydrated 3D-UHU models (Figure 2A). Similarly, pressing pieces of PVP-CC and IAS onto models caused cell flattening and compression in the contacted area (PVP, Figure 2E; and IAS, Figure 2F) compared with the control models stained with F-actin (Figure 2D). Overall, the 3D-UHU models showed a similar structural alteration in response to application of PVP-CC and IAS catheter pieces, but they appeared to tolerate the catheter application relatively well.

3.3 | Host Cell Adhesion to Catheter Surface

Catheter pieces in contact with 3D-UHU models were dried at RT, and cell nuclei were imaged to investigate cell adhesion to the catheters (Figure 3). Images were captured from four different areas on each catheter including both ends and two mid-sections (Figure 3A), and the adhered nuclei quantified (Figure 3B). PVP-CC pieces exhibited a scalelike surface, with adhered cells frequently seen (representative images in Figure 3C–F). Contrarily,

IAS catheters had a smoother surface, with fewer cell nuclei observed on the catheter exterior (representative images in Figure 3G–J); in fact, many areas on the IAS pieces showed no adhered nuclei at all. The quantification (Figure 3B) showed that the number of urothelial cells adhered to PVP-CC was significantly higher than to IAS catheters ($p < 0.001$). These results suggest that the PVP-CC material is more adherent than the IAS.

3.4 | Bladder Mucosal Microtrauma and Injury

We have shown previously that 3D-UHU models express key biomarkers including uroplakin IA, II and III; cytokeratin (CK) 8, 13 and 20; Toll-like receptor 2, 4 and 5; E-cadherin; zonula occluden-1; Claudin-1 and Claudin-3; and chondroitin sulphate [16]. We assessed the microtrauma and injury induced by the catheter application via confocal microscopy after removal, focusing on uroplakin (UP)-III (Figure 4); cytokeratin CK8 and CK20; and chondroitin sulphate, a glycosaminoglycan (GAG) layer component (Figure 5).

UPIII was expressed in the umbrella cells of the dehydrated but un-treated control models (Figure 4A), while the cultures contacted with either PVP (Figure 4B) or IAS (Figure 4D) showed disruption or effacement of the UPIII displayed in the demarcated area. As a control, we imaged regions well away from the contact area of both catheters (PVP, Figure 4C; and IAS, Figure 4E) which seemed unaffected and exhibited a typical expression of UPIII.

Next, we examined the CK8 which, as expected [16], was expressed throughout the basal, intermediate and umbrella cell layers (Figure 5A). Application of PVP-CC (Figure 5B) and IAS (Figure 5C) caused comparable disturbance of CK8-presenting cells in the contacted area of the top-most layers. Although the underlying layers were flattened, no disruption in CK8 biomarker was detected. Furthermore, CK20, a terminal differentiation marker expressed specifically by umbrella cells, was detected at the apical surface of the control 3D-UHU model (Figure 5D), but its presence was disrupted in the cultures contacted by PVP-CC (Figure 5E) and IAS (Figure 5F). Similarly,

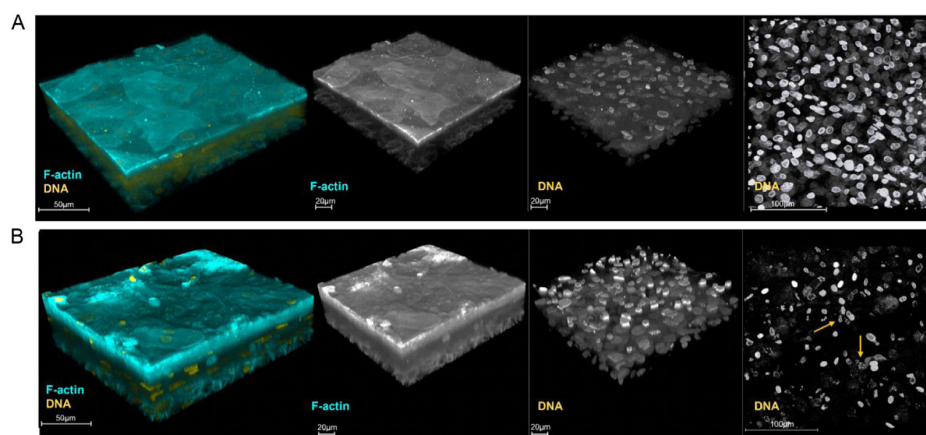


FIGURE 1 | 3D-UHU model tolerates media removal. (A) 3D confocal image of the 3D-UHU model in normal culture conditions; (B) 3D confocal image of cultures fixed and stained 15 min post-media removal showing slight membrane ruffling and DNA fragmentation (yellow arrows). Phalloidin-stained F-actin is presented in cyan and DRAQ5-stained DNA is presented in yellow (far right images are top-down); scale bars are as shown. Images are representative of at least three biologically independent experiments.

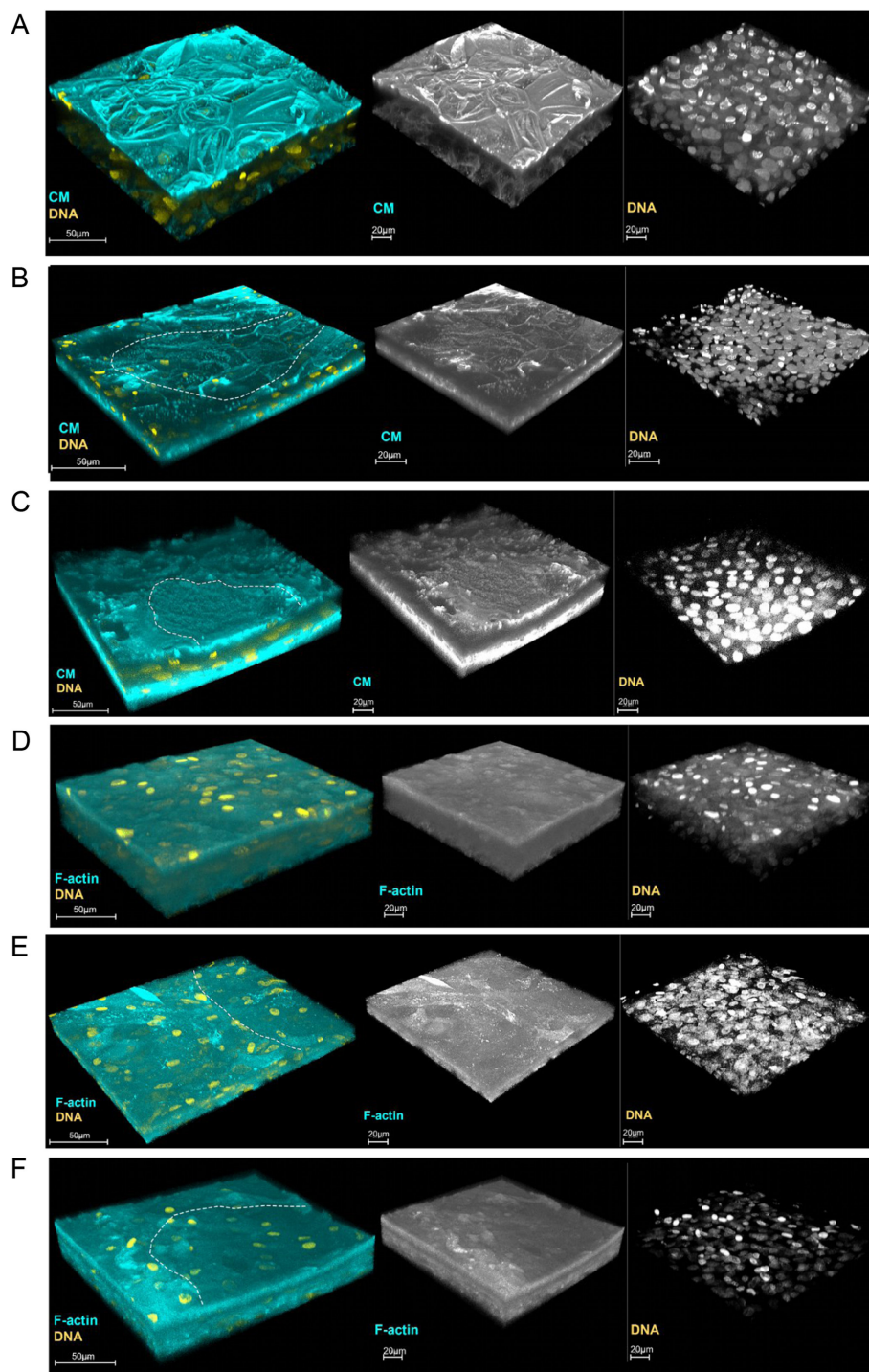


FIGURE 2 | Catheter application onto models caused 3D-UHU cell layer compression. 3D confocal images of (A) dehydrated 3D-UHU before treating with catheters; (B) demarcated area indicating the dwelling effect of the PVP-CC and (C) IAS; (D) 3D-UHU before catheter application; (E) the white lines indicate the indented impression formed by application of the PVP-CC and (F) IAS catheters. (A–C) show one set using WGA to stain the cell membrane (CM, cyan), while (D–F) show another set, using phalloidin to stain F-actin (cyan); DRAQ5 was used in both sets to stain DNA (yellow); scale bars are as shown. Images are representative of at least three biologically independent experiments.

the GAG layer expressed at the apical surface of the 3D-UHU (Figure 5G) was disturbed by PVP-CC (Figure 5H) and IAS (Figure 5I). We also noted in some cases that application of both catheters affected the intermediate layers, causing significant microtrauma (Figure 5E,F,I). Variation in deeper damage may be the result of slight variations in catheter pressure during the manual press.

3.5 | Effect of Catheters on Barrier Integrity in 3D-UHU Models

Next, we examined the effect of catheter contact on barrier integrity by measuring the TEER before and after application of catheter pieces (Figure 6A). The removal and dehydration condition did not cause a significant TEER change in the 3D-UHU

models; however, a significant reduction was observed in the 3D-UHU models treated with PVP-CC ($p < 0.01$), while IAS caused no statistical difference. Consistent with these data, the barrier disruption caused by PVP-CC was statistically significantly higher compared with IAS ($p < 0.05$).

To assess the functional consequences of catheter application on barrier integrity, a FITC-dextran (4 kDa) marker was used to measure the paracellular permeability (Figure 6B). Neither catheter showed a significant change at 8 h or 24 h post-catheter contact compared with the control and dry control.

3.6 | Secretion of IL-1 β and CEACAM1 Is More Pronounced With PVP-CC Application Than IAS

We next sought to determine whether urothelial damage could initiate the innate immune response. We measured analytes known to be involved in inflammation and damage response in the urothelium, namely, pro-inflammatory CXCL1, CCL2, IL-8, IL-6, IL-1 α , IL-1 β , IL-1Ra, IL-18, TNF- α cytokines and chemokines, CEACAM1, Pentraxin and CD44 in the collected supernatants following catheter contact.

No statistically significant differences were observed between the non-dehydrated and dehydrated controls for any measured analytes, except for Pentraxin (control mean 291.2 vs. dry control mean 448.6) and CD44 (control mean 1192.7 vs. dry control mean 2259.3). PVP-CC catheter elicited responses that were statistically significantly higher compared with non-dehydrated control in the case of IL-1 α ($p < 0.0001$) (Figure 7E), IL-18 ($p < 0.01$)

(Figure 7F), IL-1 β ($p < 0.001$) (Figure 7G), IL-1Ra ($p < 0.01$) (Figure 7H), CEACAM1 ($p < 0.001$) (Figure 7I), Pentraxin 3 ($p < 0.0001$) (Figure 7K) and CD44 ($p < 0.0001$) (Figure 7L). The IAS catheter caused responses that were also statistically significantly higher compared with control in the case of IL-1 α ($p < 0.0001$) (Figure 7E), IL-18 ($p < 0.05$) (Figure 7F), IL-1 β ($p < 0.05$) (Figure 7G), IL-1Ra ($p < 0.001$) (Figure 7H), CEACAM1 ($p < 0.01$) (Figure 7I), Pentraxin 3 ($p < 0.001$) (Figure 7K) and CD44 ($p < 0.01$) (Figure 7L). Application of catheters stimulated comparable IL-1 α , IL-18, IL-1 β , IL-1Ra, Pentraxin and CD44 innate immune responses with the following exceptions: PVP-CC caused a significant increase in IL-1 β ($p < 0.05$) (PVP-CC mean 3290.1 vs. IAS mean 2154.89 pg/mL) and CEACAM1 ($p < 0.01$) (PVP-CC mean 23 368 vs. IAS mean 20 024.4 pg/mL) compared with IAS.

4 | Discussion

Urinary catheters are one of the most frequently used medical devices for bladder management in patients with urinary retention and/or incontinence [1]. Intermittent catheterisation is the gold standard method and preferred over indwelling catheters, which is considered a risk factor for developing UTIs [19, 20]. Therefore, it is important to understand how urinary IC insertion may adversely affect an individuals' urinary tract, such as causing adhesion and damage, which could in turn cause pain and impact quality of life. Although UTI is a commonly considered outcome for indwelling catheters users, UTI also occurs with IC, and induced trauma during IC has been described as a risk factor

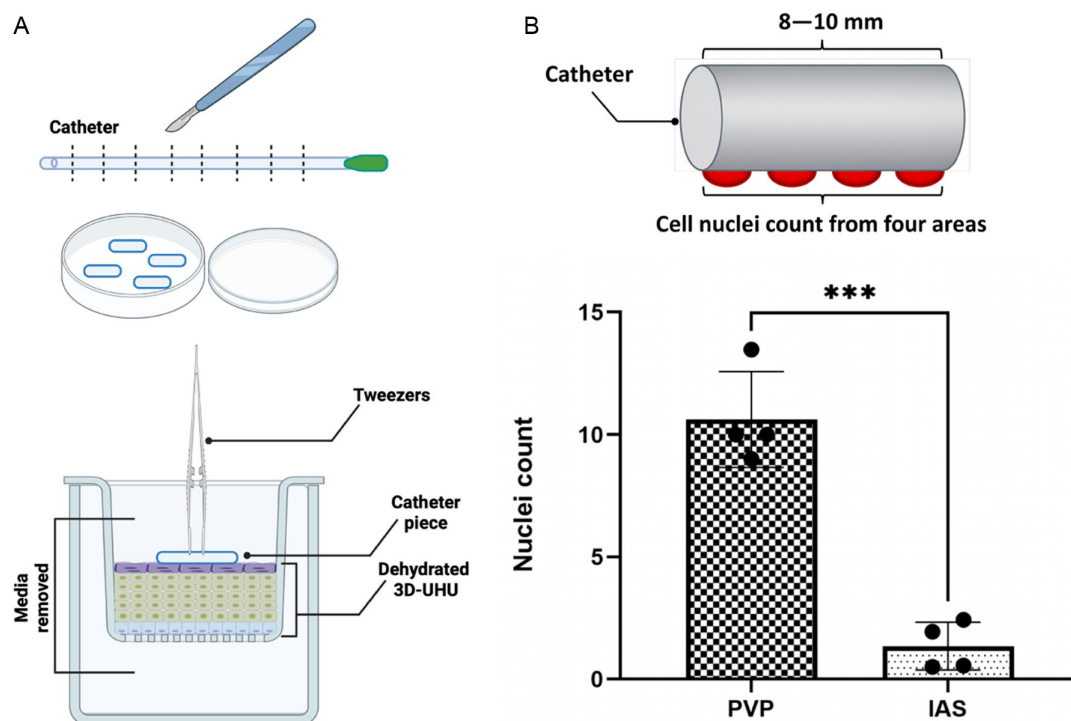


FIGURE 3 | Catheters show variation in host cell adhesion. (A) A graphical representation of preparation and application of catheter pieces. Created with BioRender.com, <https://BioRender.com/3aaekce>; (B) a schematic representing four areas imaged on the catheters post-contact with 3D-UHU cultures, and number of urothelial cell nuclei adhered to the catheters; (C–F) images of four different areas across the length of PVP-CC; (G–J) IAS following 3D-UHU model catheter contact. Unpaired *t*-test with Welch's correction was used to calculate *** $p < 0.001$; data represent mean $\pm$ SD, $n = 4$ biologically independent experiments.

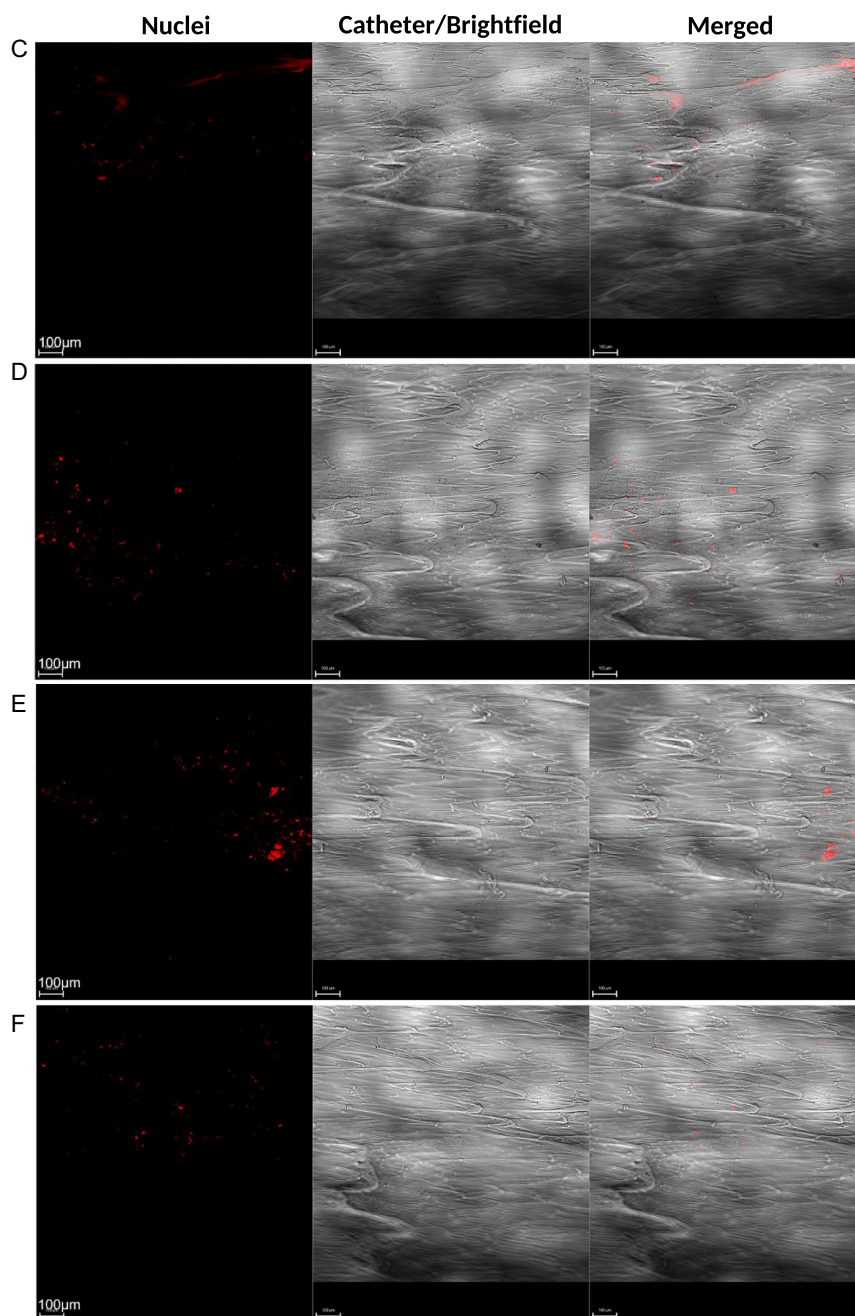


FIGURE 3 | Continued.

[10, 21]. In fact, in one study, there was no difference in UTI frequency in indwelling vs. IC cohorts [20], albeit the indwelling catheter is likely to facilitate infection via biofilm formation on the catheter surface. If IC-mediated damage can also predispose the individual to subsequent infection, developing consistent and reliable in vitro models is therefore crucial to assess the tissue effects of urinary catheters, including catheter materials and lubricants, and to guide subsequent animal and clinical studies.

Several animal models have been used to investigate urinary catheters, such as mice, rats, rabbits and dogs [21–25]. However, small animals do not accurately recapitulate the human bladder. The rodent urothelium in particular differs significantly from that of humans [26]. Although porcine models are preferred due to their anatomical and physiological relevance to humans [27–32],

they are more expensive compared with smaller animal models, which limits the sample size, and as large animals, such research also involves complex infrastructure. Additionally, ex vivo porcine bladder models have been developed and tested; however, their use has also been limited [33–35].

Traditionally, two-dimensional (2D) cell culture methods have been the pre-animal mainstay, but lately, the use of new and improved methods has offered valuable insights, with 3D cell culture, organoids and microphysiological systems permitting experiments in a more complex and relevant microenvironment [36, 37]. These more advanced cultures have been shown to mimic the in vivo microenvironment in key ways, offering more relevant cell–cell interactions, nutrient access and cellular mechanics compared with 2D cultures [38]. As a result, they

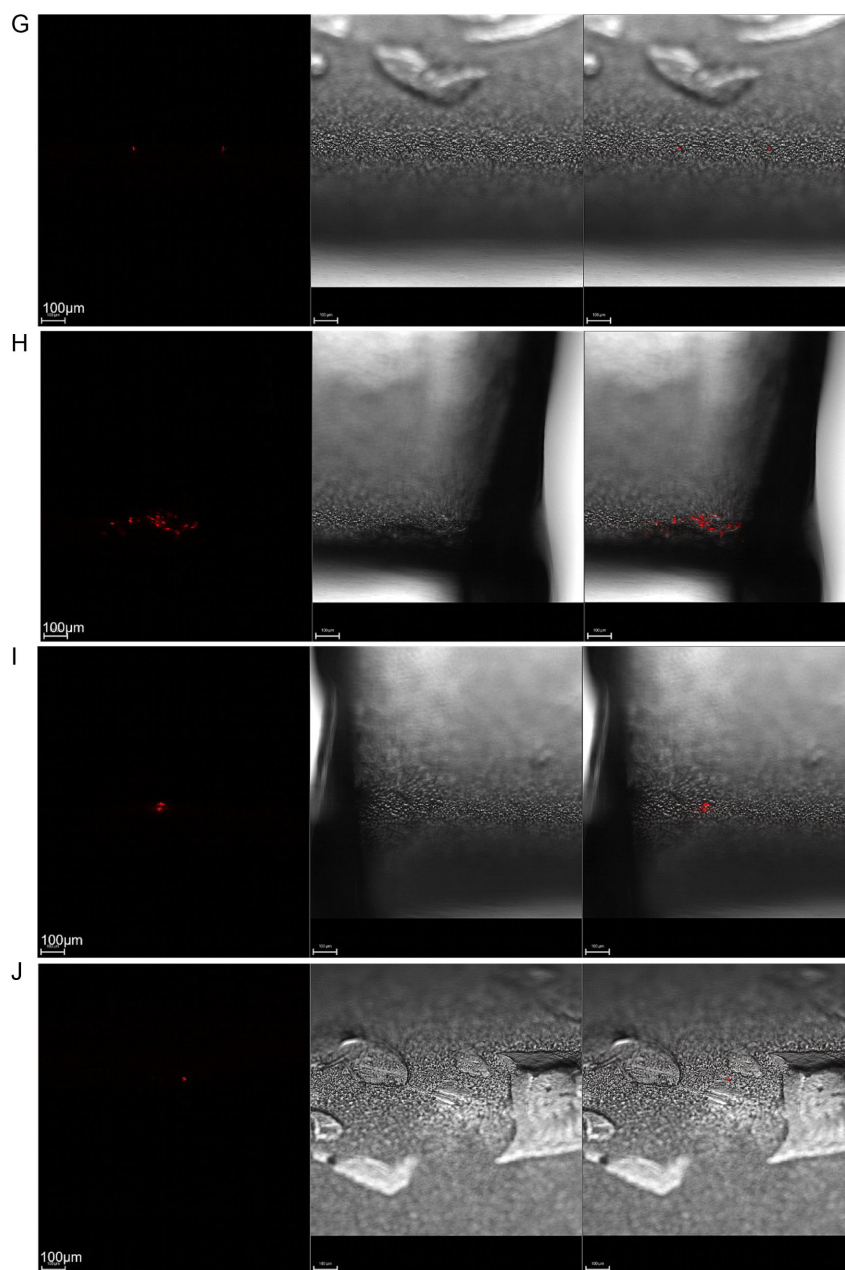


FIGURE 3 | Continued.

can often bridge the gap between 2D cell culture and animal models, and as such have been employed in many applications such as cancer research, stem cell research, metabolic profiling, drug discovery and modeling various types of diseases [37, 39–43]. These models not only allow researchers to reduce animals use, allowing faster and more economical experimentation alongside increased adherence to the ‘3Rs principles,’ but can offer a more human-specific view of biological processes.

In vitro models of the human urothelium that closely recapitulate physiological characteristics are increasingly being developed. These systems have enhanced understanding of urothelial biology and provide a valuable complement to animal models. Several studies have utilised established cell lines to assess their capacity to form organoid structures, including the generation of urothelial organoids under microgravity conditions and the development of bladder-on-a-chip platforms. In addition, normal human

urothelial (NHU) cells have been employed to create biomimetic tissue models, including complex three-layered bladder assembloids. However, the 3D-UHU model remains the only system to date to stratify into approximately seven to eight cell layers while maintaining barrier functionality and full urine tolerance [16].

To our knowledge, this is the first report of an in vitro, fully stratified and differentiated human cell-based 3D urothelial microtissue being used to study application of catheter pieces. The urothelium is a stratified epithelium that lines the bladder and serves an important barrier function. It consists of three cell types: basal cells, intermediate cells and umbrella cells located on the apical surface. The fully differentiated umbrella cells are primarily responsible for maintaining the impermeability and barrier function of the urothelium [44]. Here, we investigated the immediate trauma and inflammation following the use of two commercially available ICs with different surface compositions, PVP and IAS.

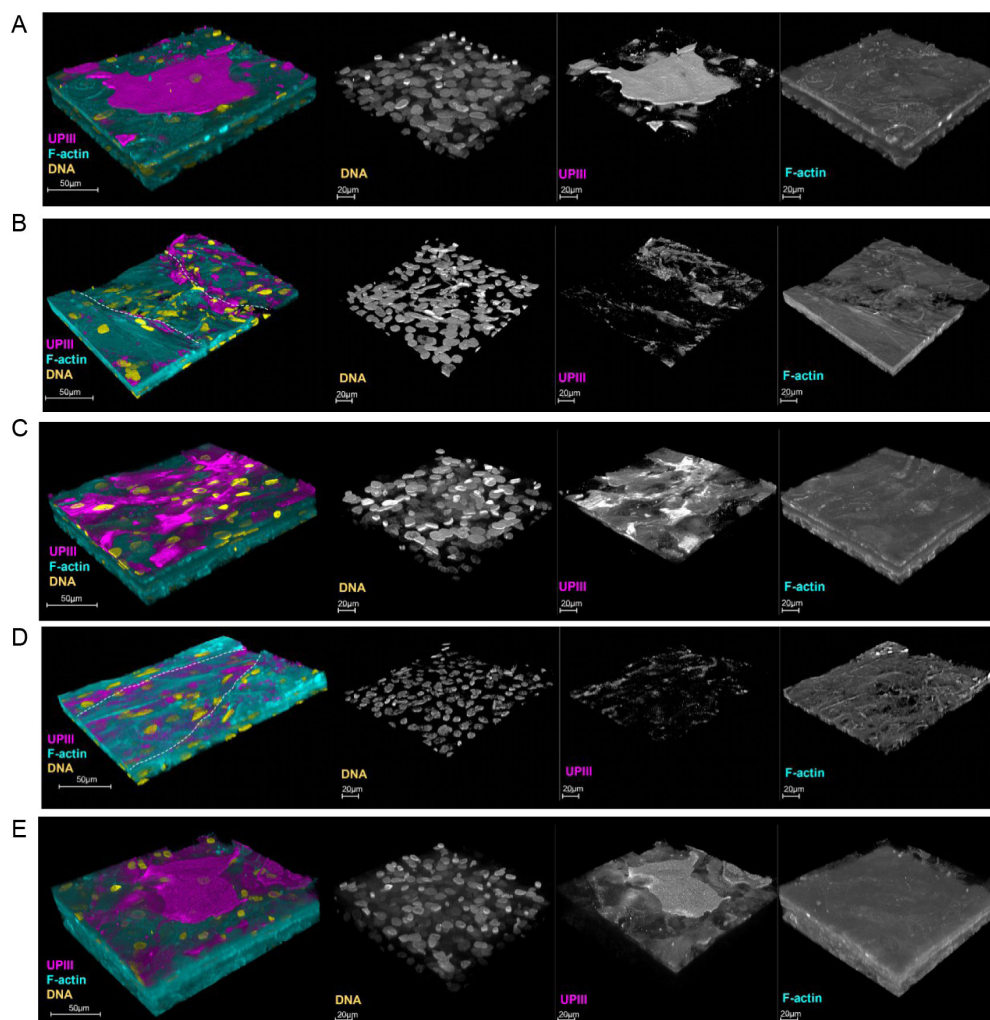


FIGURE 4 | Catheter contact leads to urothelial microtrauma. 3D confocal image of UPK-III (magenta): (A) 3D-UHU untreated model; (B) PVP-CC contact zone; (C) area outside the PVP-CC contact zone; (D) IAS contact zone; (E) area outside the IAS contact zone. White lines indicate the contact regions. Phalloidin-stained F-actin is presented in cyan and DAPI-stained DNA is presented in yellow; scale bars are as shown. Images are representative of at least three biologically independent experiments. The outer area images are acquired from the same models as the contact area images.

The polymer properties of the catheter surface represent the key differentiating factor between PVP-CC and IAS catheters. In PVP hydrophilic coatings, the polymer absorbs the wetting fluid, swells and becomes highly lubricious, facilitating catheter insertion. However, a key limitation arises during the indwelling period, as the catheter progressively dries out. When hydration levels fall below approximately 75%, the coating becomes adhesive and may adhere to the urethral wall. Upon removal, this adhesion may result in increased tissue damage.

In contrast, the IAS catheter utilises an amphiphilic molecule composed of a hydrophilic head and a hydrophobic tail. The hydrophobic tail remains embedded within the base polymer, while the hydrophilic head migrates to the surface, generating a hydrophilic interface that becomes highly lubricious upon exposure to water. As the catheter dries, the transient hydrogen bonds dissipate, allowing the material to revert to its original state, thereby minimising adhesive interactions and reducing surface stickiness [18, 45].

We have previously shown that the 3D-UHU model shares key structural and physiological features with human bladder

urothelium including stratification to over seven layers, expression of key biomarkers and urine tolerance [16], providing a suitable in vitro human urothelial model to study a range of human bladder diseases [16, 46, 47]. To assess the effect of the catheter pieces on the urothelium, culture media was removed from apical and basolateral compartments and 3D models were allowed to semi-dehydrate, representing voiding by IC. Although cell cultures can tolerate brief media removal during culture medium exchange, restoring the autocrine and paracrine communications through the cell secretome [48], the long-term effect of media removal on cell cultures is unclear. The 3D-UHU model was unaffected at 5- and 10-min post-media removal, but, at 15 min, showed minimal membrane ruffling and DNA fragmentation. Nevertheless, morphological structure and spatial arrangements were not affected by the dehydration conditions.

Next, we wanted to examine the effect of two different catheters on 3D-UHU model. The PVP version is a hydrophilic-coated catheter while the IAS version is a coating-free catheter with IAS technology, allowing the amphiphilic surfactant to orientate the surface hydrophilic headgroups, creating a hydrophilic exterior

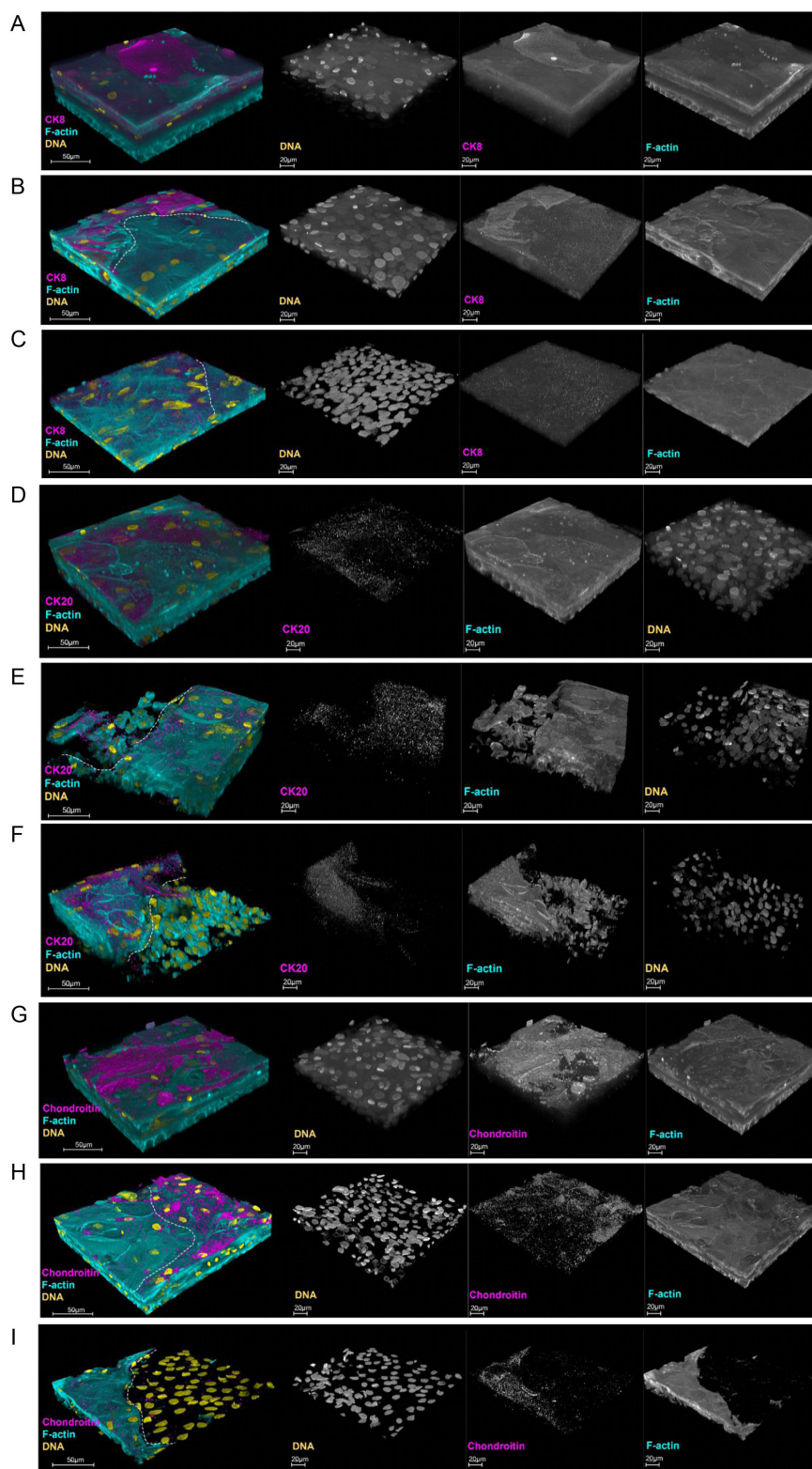


FIGURE 5 | Catheter application onto models induces urothelial injury. 3D confocal images of CK8 (magenta) expressed throughout the 3D-UHU layers (A) untreated and treated with (B) PVP-CC and (C) IAS. CK20 (magenta) expressed at the terminally differentiated umbrella cells (D) untreated and treated with (E) PVP-CC and (F) IAS. Chondroitin sulphate (magenta) expressed at the apical surface of the 3D-UHU models (G) untreated and treated with (H) PVP-CC and (I) IAS. Phalloidin-stained F-actin is presented in cyan and DAPI-stained DNA is presented in yellow; scale bars are as shown. Images are representative of at least three biologically independent experiments.

[4]. Following 2 min catheter pressure on 3D-UHU models, both catheters caused similar structural alteration indicated by flattened and compacted cells in the contact area.

Studies have suggested that the number of the epithelial cells on catheters following withdrawal is indicative of urothelial friction and trauma [18, 49, 50]. To assess the microtrauma caused by

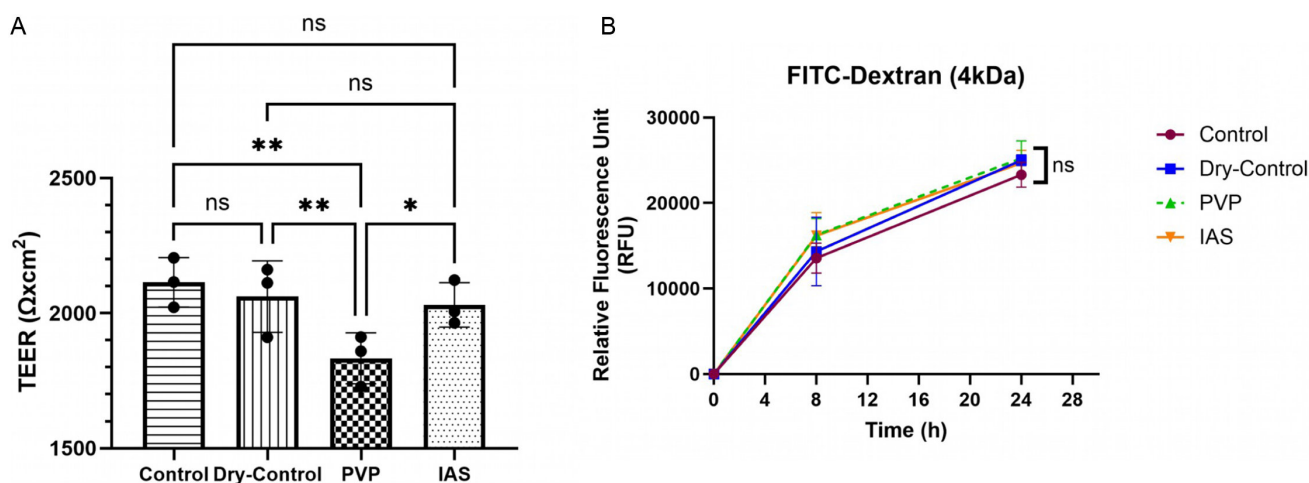


FIGURE 6 | 3D-UHU models retain barrier integrity following catheter application. (A) TEER measurements were recorded pre- and post-dehydration and pre- and post-catheter (2 min) exposure; (B) the barrier integrity was measured by assessing the FITC-dextran influx into the basolateral compartment at 8 and 24 h post-treatment. ** $p < 0.01$; * $p < 0.05$; ns ≥ 0.05 in TEER measurements were calculated by two-way ANOVA followed by Tukey's multiple comparisons test. Two-way ANOVA followed by Dunnett's multiple comparisons test were used to calculate ns ≥ 0.05 in analysing FITC-dextran levels; data represent mean $\pm$ SD, $n = 3$ biologically independent experiments.

the catheters after removal, catheters were examined for the presence of cell nuclei in four different regions spanning each catheter piece. Although catheter application caused comparable structural alteration in the contact area of the microtissue, the adhesion of urothelial cells to the surface of the PVP-CC was significantly higher than that seen in IAS.

We further explored catheter-induced microtrauma by examining urothelial biomarkers. Many studies have investigated bladder or urine biomarkers for bladder cancer or UTI [51–53], yet to our knowledge, no studies have used them to assess urothelial trauma and injury. There have been a few studies that investigated catheter-associated bladder mucosal trauma using other criteria in either in vivo or ex vivo porcine models [31, 35, 54]. These researchers visually examined the porcine bladder using cystoscopy, while Willumsen et al. assessed the patho-morphology of the tissue sections using haematoxylin and eosin staining to identify tissue injury and mucosal inflammation. Stærk et al. additionally observed the infiltration of neutrophils across the mucosa as another proxy for trauma. Here, we show that both PVP-CC and IAS catheter application caused the disorganisation of the key biomarkers normally evident in the umbrella cells that line the mucosal surface of the urinary tract, including UPIII, CK8, CK20 and chondroitin sulphate (a key constituent of the glycosaminoglycan layer). This disruption occurred within the contacted areas, but not outside, suggesting it was contact-dependent. It is difficult to know whether the markers disappeared because they were sheared from the umbrella cell surface, or whether the umbrella cells that expressed them were themselves removed by the catheter. Furthermore, a subset of 3D-UHU models exhibited significant microtrauma induced by application of both catheters, indicating that injury can lead to loss of multiple layers of bladder epithelia in this model. The variability of this outcome could be because we used manual pressure to apply the catheters, which would have been subject to inevitable human variation.

To define whether urothelial damage may have affected the barrier integrity and paracellular permeability of the urothelium,

we measured the TEER and evaluated the influx of a fluorescent FITC-dextran probe from the apical to the basolateral chamber, respectively. A significant loss of barrier integrity was observed in 3D-UHU models after application of PVP-CC but not IAS when compared with their own normal and dehydrated but un-treated controls. PVP-CC also caused a significant barrier disruption compared with IAS. This is consistent with the observation of a greater number of cells adhered to the surface of PVP-CC, whose loss may have led to barrier disruption. A dissimilar result occurred with paracellular permeability, which is defined and controlled by tight junctions. Despite the injury, 3D-UHU models exhibited minimal impairment with either catheter type, indicating that the model can maintain the barrier function 24 h post-trauma. It is important to recognise that a rapid reduction in TEER with minimal FITC-dextran permeability change suggests early functional tight-junction remodeling rather than substantial epithelial disruption, because TEER is highly sensitive to ionic conductance across tight junctions, whereas FITC-dextran assays primarily detect larger paracellular defects and macromolecular leak [55]. Current evidence suggests that catheter-related urothelial microtrauma does not simply heal; instead, it creates a transient pro-adhesive, inflammatory environment that promotes epithelial repair but may simultaneously increase susceptibility to bacterial colonisation, persistence and recurrent UTI [56, 57]. Catheterisation can cause urothelial microtrauma through mechanical shear stress, epithelial disruption and inflammation, which may increase susceptibility to catheter-associated urinary tract infection (CAUTI). Experimental and clinical studies show that injury to the bladder mucosa promotes fibrinogen deposition and bacterial adhesion, facilitating biofilm formation on catheter surfaces [58, 59].

Subsequently, we assessed innate immune response induced by catheter application at 24 h post-catheter contact. Studies using CAUTI models have described that the presence of a urinary catheter alone triggers pro-inflammatory responses in the bladder [60–62]. Rousseau et al. showed that catheterisation of female mice caused significant gene expression changes and migration

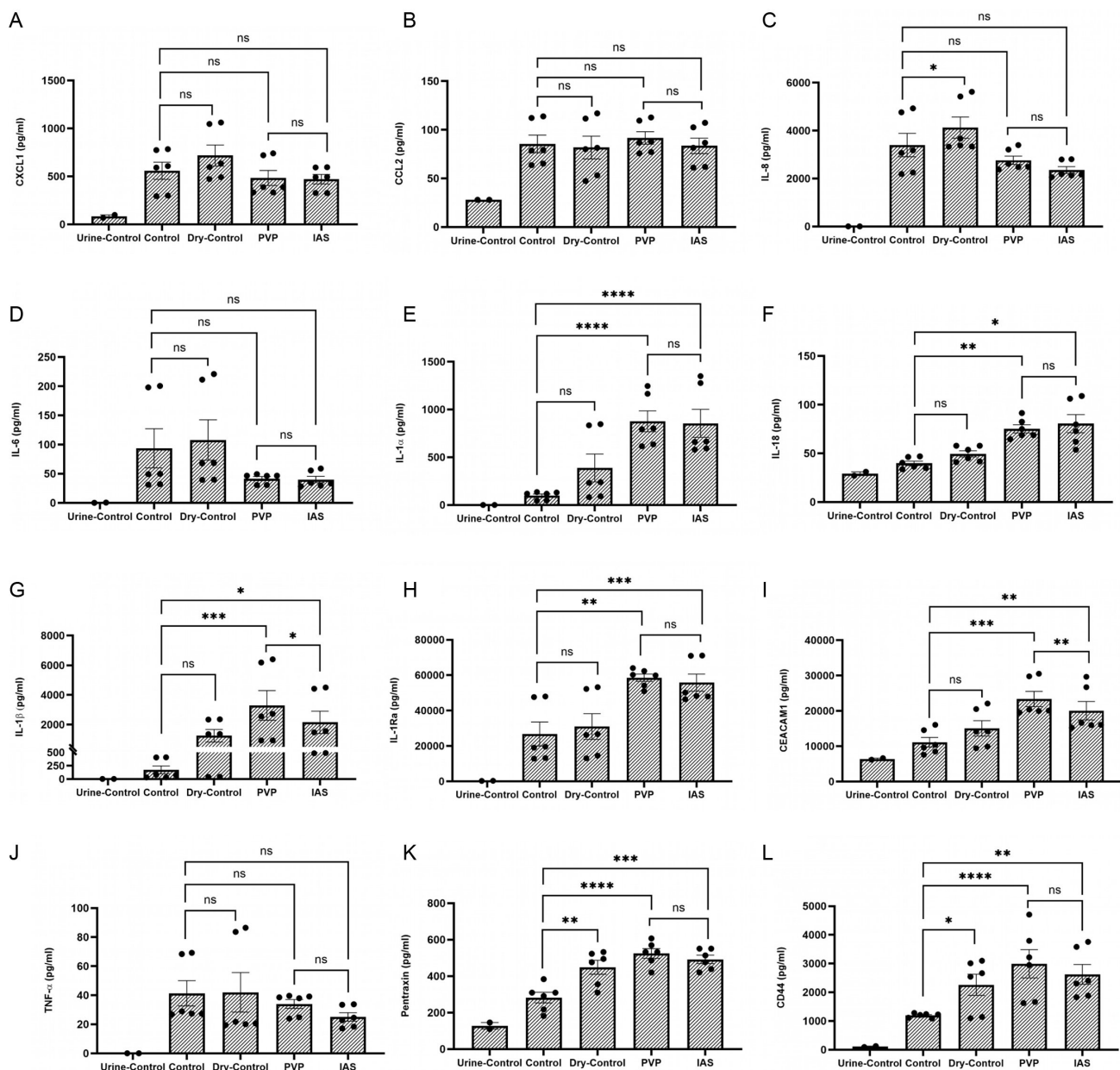


FIGURE 7 | Catheter application triggers inflammatory immune responses. (A) CXCL1; (B) CCL2; (C) IL-8; (D) IL-6; (E) IL-1α; (F) IL-18; (G) IL-1β; (H) IL-1Ra; (I) CEACAM1; (J) TNF-α; (K) Pentraxin 3; (L) CD44 release was measured by Luminex in response to catheter contact with PVP-CC and IAS at 24 h post-treatment. Data represent mean ± SD, $n = 6$ biologically independent experiments. **** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns ≥ 0.05 calculated by two-way ANOVA followed by Tukey's multiple comparisons test.

of innate immune cells into the bladder. We observed that application of both catheters induced similar cytokine and chemokine responses, with the exception of IL-1β and CEACAM1, which were significantly increased for PVP-CC compared with IAS.

Although the expression of CXCL1, CCL2 and IL-8 chemokines was not changed compared with the un-treated controls, a significant inflammatory response was measured following application of both catheters including IL-1α, IL-1β, IL-1Ra and IL-18. The IL-1 family is a key regulator of innate and adaptive immunity and consists of two agonists, IL-1α and IL-1β, and a specific receptor antagonist, IL-1Ra; the balance between IL-1 and IL-1Ra plays an important role in the susceptibility and severity of several diseases [63, 64]. Cytokines and chemokines produced in response to

UTIs are predominantly generated within the bladder urothelial cell layers and subsequently released into the urine. IL-1β is a potent pro-inflammatory cytokine that has been associated with dysregulated inflammation and increased severity of UTIs [65, 66]. Previous studies have demonstrated that IL-1β can upregulate the expression of virulence genes, including *fimH* and *papC*, in uropathogenic *Escherichia coli*, thereby enhancing bacterial adhesion to and invasion of urothelial cells [67].

The expression of Pentraxin 3, a soluble pattern recognition molecule which can recognise pathogen- and danger-associated molecular patterns and has been associated with tissue remodeling [68], was elevated in response to tissue damage caused by application of both catheters. Interestingly, Pentraxin 3 was elevated without

catheter application in response to dehydration. Furthermore, catheters augmented the secretion of the adhesion molecules CD44 and CEACAM1. CD44 is constitutively expressed on the urothelium, and studies have shown that it is upregulated in renal injury [69]. More importantly, CD44 has been shown to play a role in UTI [70]; here, like Pentraxin 3, it was also secreted in response to dehydration in the absence of catheter application, perhaps as a prelude to potential downstream tissue damage. Similar to CD44, CEACAM1 has been reported as an important target for bacterial pathogens that utilise CEACAM1 as host receptor on epithelial cells to invade the host and evade the immune system [71, 72], but its function in response to injury and trauma remains undetermined. Studies have shown that UPEC and other Gram-negative uropathogens expressing CEACAM-binding adhesins can suppress urothelial exfoliation in response to infection and thereby promote bladder colonisation [73].

This study, designed to explore the initial feasibility of 3D-UHU for testing catheter-induced microtrauma, did have some limitations. First, as noted above, reproducibility would be improved by deploying a system that could deliver standardised and relevant forces to all cultures in an experimental plate simultaneously in a sterile environment; we are currently developing such a system for a follow-up study. Second, while we asserted pressure using a gentle downward force, a real catheterisation event would involve a transverse sliding force. For the future, it should be feasible to develop a system whereby such forces could be assessed in the Transwell environment. Finally, while the human urethra is lined in large part by urothelium in women, only the first third proximal to the bladder (the prostatic region) contains urothelium in men; the middle area of the male urethra (the membranous and part of the spongy urethral zone) is lined with pseudostratified columnar epithelium; and in the most distal region of both the male and female urethra, squamous epithelia [74]. Thus, 3D-UHU can only model damage to the more proximal urethra and, in the case of the catheter tip, to the bladder neck or trigone regions.

5 | Conclusion

Our study is the first to use a 3D human bladder microtissue model to examine the effect of catheters on the urothelium. By applying two different IC catheters on the 3D-UHU model, we demonstrate the development of a novel in vitro platform for studying catheter-induced trauma. Together, these findings support a model of catheter-induced urothelial microtrauma in which catheter contact exerts compressive and shear forces on the superficial urothelium, resulting in disruption and partial detachment of umbrella cells and loss of key apical differentiation markers, including UPIII, CK20 and chondroitin sulphate. Increased urothelial cell adhesion to the catheter surface further suggests that catheter withdrawal contributes to mechanical exfoliation or injury of superficial cells, consistent with the observed disruption of umbrella cell-associated biomarkers. This structural injury is accompanied by a reduction in TEER, indicative of early tight-junction dysfunction and impaired ionic barrier integrity, despite the absence of substantial FITC-dextran flux, suggesting a subtle yet functionally significant barrier defect rather than overt epithelial breakdown. Concurrent increases in IL-1 β and CEACAM1 secretion further support activation of innate inflammatory and epithelial stress responses secondary to catheter-induced injury,

with the magnitude of the inflammatory response correlating with the degree of cell adhesion and barrier disruption. This system forms the basis for future development towards a tractable, early catheter tested to be employed as a comparative system alongside animal models and clinical studies.

Author Contributions

Nazila V. Jafari: data curation (lead), formal analysis (lead), investigation (lead), methodology (lead), project administration (equal), software (lead), validation (lead), visualisation (lead), writing – original draft (lead). **Jennifer L. Rohn:** conceptualisation (lead), funding acquisition (lead), project administration (equal), resources (lead), writing – review and editing (lead).

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Conflicts of Interest

JLR has share options in the University College London spinout company AtoCap Ltd, to whose work the manuscript is not related.

Data Availability Statement

All data generated and analysed during the current study are included in this published article.

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