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Apical Shear Stress Enhanced Organic Cation Transport in hOCT2/hMATE1 Transfected MDCK Cells Involves Ciliary Sensing

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ABSTRACT

Active transport by renal proximal tubules plays a significant role in drug disposition. During drug development, estimates of renal excretion are essential to dose determination. Kidney bioreactors that reproduce physiological cues in the kidney, such as flow-induced shear stress, may better predict *in vivo* drug behavior than current *in vitro* models. In this study, the role of shear stress on active transport of 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide (ASP+) by MDCK cells exogenously expressing the human organic cation transporters organic cation transporter 2 (OCT2) and multidrug and toxin extrusion protein 1 (MATE1) was investigated. Cells cultured in a parallel plate under continuous media perfusion formed a tight monolayer with high barrier to inulin. In response to increasing levels of shear stress (0.2-2 dynes/cm^2), cells showed a corresponding increase in transport of ASP+, reaching a maximal 4.2-fold increase at 2 dynes/cm^2 when compared to cells cultured under static conditions. This transport was inhibited with imipramine, indicating active transport was present under shear stress conditions. Cells exposed to shear stress of 2 dynes/cm^2 also showed an increase in RNA expression of both transfected human and endogenous OCT2 (3.7- and 2.0-fold, respectively). Removal of cilia by ammonium sulfate eliminated the effects of shear on ASP+ transport at 0.5 dynes/cm^2 with no effect on ASP+ transport under static conditions. These results indicate that shear stress affects active transport of organic cations in renal tubular epithelial cells in a cilia dependent manner.

KEYWORDS: Shear stress, kidney-on-a-chip, organic cation transport, perfusion, deciliation

INTRODUCTION

Despite major advancements in preclinical methods for selection of optimal drug candidates, drug development remains a long and expensive process that has a very low yield of marketed new molecular entities(Watkins, 2011). A major shortfall of preclinical models is the inability to accurately predict clearance and toxicity in humans. Approximately 30% of drugs that are successful in preclinical studies fail in humans due to unanticipated clearance values or toxicities (Kola and Landis, 2004).

The kidney is a particularly important target for *in vitro* cell culture modeling because it is responsible for elimination of over one third of all drugs and a majority of metabolites(Morrissey *et al.*, 2013). The renal proximal tubule is important for drug testing during development because it performs the majority of active transport of drug candidates and is particularly sensitive to toxic injury (Giacomini *et al.*, 2010). The renal tubule is a monolayer of epithelial cells with filtrate flowing across the apical surface. A basement membrane underlies the tubular epithelium and adjacent peritubular capillaries allow for reabsorption of water and solutes. The proximal tubule microenvironment, particularly fluid shear stress and apicobasal oncotic gradients, is incompletely reproduced by conventional cell culture techniques. Epithelial cell bioreactors that capture salient *in vivo* physiology may improve accuracy of clearance and toxicity predictions derived from *in vitro* assays (Pfaller and Gstraunthaler, 1998; Astashkina *et al.*, 2012).

There is growing evidence that proximal tubule cell morphology and functionality can vary with the growth environment such as growth surface porosity, exposure to fluid on both sides and/or fluid shear stress. Previous work from Essig and colleagues as well as from our lab demonstrated that tubular fluid flow induced rearrangement in the apical actin cytoskeleton of proximal tubule cells (Essig and Friedlander, 2003; Ferrell *et al.*, 2010). Subsequently, several studies demonstrated that shear stress affects the expression levels and localization of multiple

uptake and efflux transporters, including NHE3, Na^+/K^+-ATPase, glucose (SGLT2) transporters, ENaC and the endocytosis receptors, megalin and cubulin (Duan *et al.*, 2010; Raghavan *et al.*, 2014; Jansen *et al.*, 2016; Ernandez *et al.*, 2018). Other work has demonstrated that shear flow in a tubular bioreactor system orients and elongates renal tubular cells along the flow path(Venzac *et al.*, 2018). In-vitro evidence indicates that some alterations in cytoskeletal structure and transport function are likely related to the mechanosensory function of cilia (Overgaard *et al.*, 2009; Raghavan *et al.*, 2014). However, few studies have considered the effect of shear stress on drug transporters in renal cells. Furthermore, little is known about the effect of different shear rates on cell functionality, particularly with sustained exposure to shear stress. Most studies have considered a single shear stress rate and have only performed short term experiments (1-6 hours), making it difficult to distinguish between the true effects of shear and the cellular stress response to abrupt changes to the microenvironment (Duan *et al.*, 2010; Jang *et al.*, 2013; Maggiorani *et al.*, 2015).

Here, a microfluidic bioreactor was used to examine the effect of graded levels of shear stress on the renal proximal tubule cell drug transporters OCT2 and MATE1. We demonstrate that increasing apical shear stress leads to increasing organic cation transport and transporter expression and that cilia are involved in this cellular response to shear stress.

MATERIALS AND METHODS:

Device Fabrication and Assembly:

We have previously published our design for a parallel plate bioreactor that provides a fluid flow path of adjustable height across the apical side of the cells and a static reservoir on the basal side (Brakeman *et al.*, 2016). For the work presented here, we adapted our previous design to create a multiplexed device with 4 separate flow paths to allow for testing 4 biologic conditions at once

(Figure 1). Each device is composed of three layers, a base with a 5 mL apical reservoir, a middle plate to hold the Snapwell insert (Costar, Corning) with 1.12 cm^2 cell area and a top plate containing a 1-2 mL basolateral reservoir. The plates were machined from polysulfone to allow for sterilization by autoclaving. Laser-cut silicone gaskets of 500-1000 µm height were sandwiched between each set of plates to seal the fluid compartments and on the apical side to define the channel height. The bioreactor was assembled with screws. The media reservoir and bubble trap were a built-in column. The inlets and outlets were connected using barbed connectors to Masterflex LS-14 silicone tubing with 1.6 mm inner diameter (Cole Parmer). Fluid flow and thus apical shear stress were set and controlled by a peristaltic pump (Cole Parmer).

Cell Culture and Flow:

MDCK cells transfected with either an empty vector or a pair of uptake and efflux transporters (hOCT2/hMATE1) (König *et al.*, 2011) were kindly provided by Dr. Martin Fromm (Erlangen, Germany) and cultured in MEM with Earle's BSS (UCSF Cell Culture Facility) with 10% FBS (Gibco, Thermo Fisher Scientific) and 1% penicillin-streptomycin (UCSF Cell Culture Facility). 500 µg/mL hygromycin (UCSF Cell Culture Facility) and 100 mg/mL geneticin (Sigma-Aldrich) were added to the media for the double transfected cells. For flow experiments, hOCT2/hMATE1 MDCK cells were plated on the underside of Snapwell inserts at a density of 300,000 cells/well (250,000 cells/cm^2), grown under static conditions until confluence and then placed in the bioreactor. Apical media flow was increased over 7 days from 0.1 mL/min to 1-6 ml/min until desired shear stress was achieved and then left stable for 72 hours prior to experimentation. Cells were grown under static conditions for the same amount of time as controls.

Immunofluorescence:

Cells were fixed with 4% paraformaldehyde (Thermo Fisher Scientific), permeabilized with 0.1% Triton-X in PBS (Sigma-Aldrich) and blocked with bovine serum albumin (BSA) (Sigma-

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Aldrich). They were subsequently incubated with 1:50 dilution of Alexa 488-labeled zonula occludens 1 (ZO-1) mouse monoclonal antibody (Life Technologies) or a primary acetylated α-tubulin mouse monoclonal antibody (Life Technologies) for 60 minutes. Cells treated with the α-tubulin antibody were then incubated with a secondary 561 anti-mouse goat antibody (Life Technologies) and phalloidin (Thermo Fisher Scientific) for F-actin staining. Imaging was performed using a Nikon spectral confocal microscope with a 40x oil objective.

Barrier Performance:

Inulin, a 5000 Da polymer, is a well-known marker of glomerular filtration rate and is a marker of

$$\textbf{Barrier Performance} = \frac{\mathbf{C_{apical} - C_{basal}}}{\mathbf{C_{apical}}}$$

Equation 1.

the leakiness of the proximal tubule in vivo (Sohtell *et al.*, 1983). FITC-labeled inulin (Sigma-Aldrich) was added to the apical media and allowed to flow through the devices. Samples were collected from the basal reservoir every 24 hours and analyzed for inulin content using a Genios Pro fluorescence plate reader (Tecan). Barrier function of the cell monolayer was calculated from inulin levels measured in the apical and basal compartments as described by Equation 1. Here, C_{apical} is the concentration of inulin in the apical compartment and C_{basal} is the concentration in the basal compartment. Inulin leak was calculated as concentration in the basal (donor) compartment divided by the cell area after 24 hours.

ASP+ Transport:

Transduced transporter genes were induced with 10 mM Na-butyrate (Sigma-Aldrich) 24 hours prior to a transport experiment, as described previously (König *et al.*, 2011). When appropriate, cells were incubated with an inhibitor (500 μM imipramine or 1 mM cimetidine (Sigma-Aldrich)) on the basal side for 30 minutes, followed by addition of 25 μM 4-(4-dimethylamino)styryl-N-methylpyridinium (ASP+) (Life Technologies) and incubation for 1 hour. Samples were collected

from the apical and basal media. Cells were then rinsed with ice-cold phosphate-buffered saline three times and lysed for measurement of ASP+ accumulation and transport. Transport experiments were simultaneously performed on cells under shear stress and cultured under static conditions. ASP+ concentration was quantified on a Genios Pro fluorescence plate reader (Tecan) at an excitation wavelength of 485 nm and an emission wavelength of 590 nm.

Protein Quantification:

Cells were lysed with 1% SDS-10 M NaOH lysis buffer while shaking overnight. Protein content was measured using a standard Pierce BCA protein assay kit (Thermo Fisher). Where appropriate, protein content was normalized to cell growth area.

RNA Expression:

RNA was extracted from cells using an RNeasy RNA Extraction Kit (Qiagen) and cDNA was generated using an iScript kit (Bio-Rad). cDNA was used for detection of human and dog OCT2 and MATE1 and dog P-gp by qRT-PCR using a Taqman assay and probes (Applied Biosystems) on the Fast Realtime PCR instrument (Applied Biosystems). Ribosomal protein S18 (RS-18) was used as housekeeping control. The effect of shear stress on transporter expression was analyzed using the ΔΔCt method using transporter levels expressed relative to RS-18 (Peters *et al.*, 2007; Schmittgen and Livak, 2008). P-gp was used as a measure of global effects of shear stress on transporter expression.

Deciliation:

Cells were grown to confluence and then incubated in the absence or presence of 30 mM ammonium sulfate (Fluka AG) for 24 hours prior to measurement of ASP+ transport (Overgaard *et al.*, 2009). The presence of cilia was determined by imaging of α-tubulin as described above.

Statistics:

All experiments were performed in triplicate with a minimum of two technical replicates within each experiment. Data are expressed as mean ± standard deviation and graphed as box and whisker plots. Statistical analyses were performed by unpaired one way or two way ANOVA and a p value of <0.05 was considered significant. Data were analyzed using Prism Version 6.0 (Graphpad).

RESULTS:

Cell Morphology and Barrier Function:

Immunofluorescence analysis of cells placed under flow revealed uniform monolayer formation with the tight junction protein, Zonula-occludens-1 (ZO-1), localized to the tight junctions between cells under both static and flow conditions (Fig 2A-C). Quantification of total protein content demonstrated up to a 1.6-fold increase in protein content per cm^2 of monolayer in response to shear stress (Fig 2D). Next, permeability of inulin, a marker of proximal tubule leakiness, across the monolayer was measured. Devices retained a barrier performance rate of 97.9 ± 1.42% over 7 days of culture under up to 2 dynes/cm^2 of shear stress (Fig 2E). This resulted in a final inulin leak rate on day 6 of 0.13-0.69 μg/cm^2/day.

Effect of Shear Stress on Organic Cation Transport:

Transport of ASP+, an auto-fluorescent substrate of OCT2 and MATE1, was measured for one hour at differing levels of shear stress (0.2-2 dynes/cm^2). MDCK cells exogenously expressing hOCT2 and hMATE1 showed a 4.2-fold increase in ASP+ transport in response to shear stress of 2 dynes/cm^2 as reflected in measures of both cellular accumulation (Fig 3A) and transcellular transport (Fig 3B). The effect of shear stress on ASP+ accumulation or transcellular transport was similar under shear stress of 0.5 dynes/cm^2 and 2 dynes/cm^2. To determine if active ASP+ transport was increased, transport of ASP+ by cells exposed to 0.2 dynes/cm^2 of shear stress was measured with or without pretreatment with 500 μM imipramine, an OCT2 and MATE1 specific inhibitor.

ASP+ transport was inhibited by imipramine 60.3 ± 15.8% under shear stress conditions compared to 47.6 ± 19.7% under static conditions (Fig 3C).

To further understand this observation, the effect of shear stress on the expression of human OCT2 (transfected), canine OCT2 (endogenous) and canine P-gp (endogenous) was measured in cells exposed to varying levels of shear for 72 hours after a slow ramping up of shear over 7 days (Fig 4). In comparison to cells cultured under static conditions, MDCK cells exposed to shear showed increased expression of transfected human and endogenous OCT2 (up to 3.7- and 2.0-fold, respectively), with no significant effect on the expression of transfected MATE1 or endogenous P-gp.

Role of Cilia in Response to Shear:

Imaging showed that double transfected MDCK cells express cilia under both static and flow conditions (Fig 5A,E). Exposure of the cells to 30 mM ammonium sulfate for 24 hours caused a complete loss of cilia (Fig 5C,G). Importantly, deciliation had no gross effects on cell membrane junctions, as measured by imaging of F-actin which anchors tight junction proteins at the cell membrane junctions in epithelial cells (Figures 5D and 5H)(Stevenson and Begg, 1994). While there is some thickening of the F-actin staining in these images, this was not a consistent finding, and thus we did not quantitate this effect. Deciliation had no effect on transport by cells cultured under static conditions but completely eliminated the effects of shear stress on ASP+ transport in cells exposed to 0.5 dynes/cm^2 of shear (Fig 6).

DISCUSSION

The kidney plays a central role in the elimination of drugs in the human body. It is important to accurately mimic the complexity of renal physiology for *in vitro* drug testing, particularly with respect to fluid flow. Shear stress from fluid flow has been shown to affect cell morphology and ion transporters *in vitro*, but little is known about the effects on drug transporters

(Duan *et al.*, 2010; Ferrell *et al.*, 2012; Jansen *et al.*, 2016). Additionally, most previous studies have only measured the effect of short-term shear (1-6 hrs), which is unlikely to fully isolate shear stress effects from other cellular stress responses (Duan *et al.*, 2010; Jang *et al.*, 2013; Maggiorani *et al.*, 2015). In these studies we focused on the effects of sustained (7 days) shear stress from fluid flow on drug transport to better mimic the sustained shear stress experienced by renal tubular cells *in vivo*. Understanding how drug transporters respond to sustained shear stress can improve *in vitro* predictions of *in vivo* drug handling by the kidney. Accurately designed *in vitro* models of the kidney may standardize preclinical testing and reduce drug failure rates.

In this work, a parallel plate microfluidic bioreactor was used to determine the effects of sustained shear stress on organic cation transport by OCT2 and MATE1 in a renal tubule cell line. Several cell lines were considered for these studies, including human cell lines such as HPCTs from the Hopfer group(Orosz *et al.*, 2004) and RPTECs (ATCC CRL-4031) (Wieser *et al.*, 2008), but the double transfected hOCT2/hMATE1 MDCK cells were considered the most appropriate choice for this study for several reasons. First, the MDCK cells consistently demonstrate robust attachment to the transwell inserts, which allows them to withstand the initial stress of fluid flow. Second, these cells form tight monolayers, which prevents leakiness and is essential for study of transcellular transport of marker substrates. Finally, exogenous expression of transporters allows for improved detection of active transport and the effect of perturbations.

Cells placed under flow formed a confluent monolayer and localized a tight junction protein to the periphery of the cell. They also retained high barrier function as measured by inulin permeability. The ability of the epithelial cells to form a monolayer that prevents fluid and protein leak is extremely important for proper function of the tubule. Here, the leak through the MDCK cells was minimal at less than 1 $\mu g/cm^2$/day and significantly lower than that through human cells. We have previously shown that human kidney cell monolayers have a 10-20 $\mu g/cm^2$/day inulin

leak (Brakeman *et al.*, 2016), supporting the conclusion that the hOCT2/hMATE1 MDCK cells formed a robust monolayer.

Transport of ASP+ was significantly increased in hOCT2/hMATE1 MDCK cells exposed to varying levels of shear stress for 72 hours when compared to static controls. ASP+ is taken up by OCT2 (*SLC22A2*) and effluxed by MATE1 (*SLC47A1*), two organic cation transporters that work in concert to facilitate the renal secretion of commonly used drugs such as metformin and cisplatin(Biermann *et al.*, 2006; Wittwer *et al.*, 2013). Similar effects of shear stress have been reported for proximal tubule ion transporters. Increases in albumin uptake, ion reabsorption and megalin and cubulin expression and function have been reported in response to increased shear stress (Overgaard *et al.*, 2009; Duan *et al.*, 2010; Ferrell *et al.*, 2012; Raghavan *et al.*, 2014). Exposure of renal proximal tubule cells to shear stress is also associated with reduced apoptosis and faster recovery from acute cisplatin toxicity and enhanced inhibition of organic anion transport (Jang *et al.*, 2013). Since shear stress from fluid flow is constantly present in the proximal tubule, these findings collectively support the use of more physiological *in vitro* model systems to predict renal drug disposition and toxicity. It is interesting to note that there is no significant difference in transporter function between 0.5 dynes/cm^2 and 2.0 dynes/cm^2 of shear stress. Prior studies by Essig et al. had found that a minimum level of shear of 0.17 dynes/cm^2 is required to elicit an alteration in cell morphology and other studies have measured effects on physiology at up to 1 dyne/cm^2 (Essig and Friedlander, 2003; Duan *et al.*, 2010). This is the first study to explore the effects of a range of higher and sustained shear stress levels on functionality of renal drug transporters, and it is possible that the magnitude of biological difference between the 0.5 dynes/cm^2 and 2.0 dynes/cm^2 of shear stress that we evaluated was not large enough to allow for identification of differences between these levels of shear stress in our assays. While further investigation is required, our results shed light on the required conditions for physiologically

relevant models and the effects of increased shear stress levels due to disease on renal drug handling.

One of the limitations of our experimental methods is that we only addressed transport at pH 7.4 using media with pH 7.4 on both apical and basal sides mimicking the experimental methods previously described for the hOCT2/hMATE1 MDCK cells (Konig *et al.*, 2011). An apical to basal pH gradient can effect OCT2 and MATE1 function, and there is typically a pH gradient under physiologic conditions (Muller *et al.*, 2013). Our methods therefore limited our ability to evaluate the interaction between an apical to basal pH gradient and shear stress on OCT2 an MATE1 function. Since we used consistent pH of 7.4 throughout all experimental conditions, this lack of a pH gradient is unlikely to have affected our findings but potentially limits the applicability of our findings to OCT2 and MATE1 transport in the presence of an apical to basal pH gradient.

Surprisingly, the mRNA expression of transfected human and endogenous OCT2 was increased in cells exposed to all levels of shear stress compared to static controls. This upregulation of transporter expression was specific, with no measurable effect on endogenous P-gp expression or transfected MATE1. The upregulation of transporter expression gives insight into the mechanism behind the increased transport and may be a result of enhanced mRNA stability or increased mRNA transcription. It is interesting to note that both the transfected and endogenous transporters were upregulated, which is unexpected since transfected OCT2 was expressed under a CMV promoter and should not be subject to endogenous gene expression regulatory mechanisms. While surprising, this observation is not unprecedented. A similar effect on OAT1 transfected proximal tubule cells exposed to perfusion has been reported but the mechanism for the increased expression remains unclear (Jansen *et al.*, 2016). In another study, Nrf2 signaling was found to play a role in increasing endogenous MATE2-K expression in response to shear stress

(Fukuda *et al.*, 2017). Further study into this is warranted and will be the subject of future investigations.

We also identified that the introduction of apical shear flow increased protein content per cm^2 of cells. This effect was the same magnitude for both 0.5 dynes/cm^2 and 2 dynes/cm^2 of shear similar to the effect seen for mRNA expression and ASP+ transport. There is some evidence that shear stress induces taller renal epithelial cells which would result in a larger volume of cells per cm^2 and thus a likely increase in protein per cm^2; however, this previously published phenomena was only a minor observation in a larger body of data and not quantitated (Long *et al.*, 2017). The increase in total protein in cells grown in the presence of apical shear flow may represent the normal protein content for healthy renal tubular epithelial cells that can be achieved only by the exposure of the cells to apical shear flow. Therefore, the lower levels of protein content in cells grown in static culture may represent an abnormal cellular state as could occur clinically in situations of low apical filtrate flow such as acute kidney injury. This phenomena deserves further study.

Cilia are known to have mechanosensory roles in the proximal tubule (Raghavan and Weisz, 2016). Therefore, to determine whether they play a critical role in the increased OCT2 and MATE1-mediated transport reported here, the effect of cilia removal on transporter function was measured. The complete blockade of shear-dependent increases in ASP+ transport by removal of cilia in our studies is similar to the effect found in studies by Raghavan et. al. who demonstrated a cilia-dependent upregulation in endocytosis in response to fluid flow (Raghavan *et al.*, 2014). Ammonium sulfate likely has other uncharacterized effects on cell behavior and functionality that may indirectly impact solute transport. Despite possible off-target effects, this method of deciliation does test the role of ciliary sensing on transporter-medicated movement of organic solutes. The mechanism of signaling between the mechanosensory proteins in the cilia and the

transporter is currently unknown. One hypothesis is that function of the organic cation transporters is changed due to altered ion transport. Removal of cilia is known to alter solute motility in the proximal tubule cells, specifically by reducing Na^+/K^+-ATPase membrane localization and modifying paracellular transport (Overgaard *et al.*, 2009). It is possible that this results in alterations in MATE1 function, an antiporter dependent on the H^+ gradient. Another hypothesis is that sensing of shear stress affects expression of organic cation transporters. It has been found that shear stress modulates MATE2-K function through Nrf2 signaling (Fukuda *et al.*, 2017). Other organic cation transporters may respond similarly to shear stress, which would be eliminated when the mechanosensory cilia are removed. Much of this is unknown and warrants further study. Interestingly, cilia removal did not have a significant effect on ASP+ transport by cells exposed to 0.2 dynes/cm^2 of shear stress, but a robust effect was observed when shear stress was increased to 0.5 dynes/cm^2. This suggests a threshold shear stress level is sensed by the cilia which in turn signal changes in transporter expression and function before measurable effects on transport are expected. Overall, the dependence of organic cation transport on ciliary sensing of shear stress provides insight into the mechanosensory signaling pathway involved and the minimum level of stress that might be required to trigger a robust response to shear.

In summary, these data demonstrate that shear stress from fluid flow has a significant effect on organic cation transporter function and expression in MDCK cells. Furthermore, upregulation of organic cation transport was dependent on the presence of cilia. We propose that apical shear stress is an important component of any *in vitro* modeling of renal tubular cells and is likely to be an important component of modeling renal secretory clearance and nephrotoxicity of drugs. The specific mechanism by which mechanical stress signals increased transporter activity and expression is still unclear and will require further study.

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Authorship Contributions.

Participated in research design: Jayagopal, Brakeman, Soler, Ferrell, Fissell, Kroetz, Roy.

Conducted experiments: Jayagopal, Brakeman.

Contributed new reagents or analytic tools: Konig, Fromm

Performed data analysis: Jayagopal

Wrote or contributed to the writing of the manuscript: Jayagopal, Brakeman, Soler, Ferrell, Fissell, Kroetz, Roy.

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FOOTNOTES

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Jayagopal A, Soler P, Ferrell N, Brakeman P, Kroetz D, Fissell W, Roy S. "Apical Shear Stress Enhances Organic Cation Transport in hOCT2/hMATE1 Transfected MDCK Cells." American Society of Nephrology, Annual Meeting, 2015.

FIGURE LEGENDS:

Figure 1. Bioreactor design. A) Top down view of the four-channel device. Boxed area indicates one channel with inlet, 5 mL apical reservoir with bubble trap, outlet and a 1-2 mL basal reservoir; **B)** Exploded schematic of the four-channel device set up with 1.12 cm^2 cell area, gasket (500-1000 μm) defined apical fluid flow path and basal reservoir shown.

Figure 2. Effects of shear stress on MDCK cell monolayer formation. A-C) Nucleus (DAPI) and tight junction (ZO-1) stains of cells under **A)** no, **B)** 0.5 dynes/cm^2 and **C)** 2.0 dynes/cm^2 levels of shear stress. Shear stress was slowly ramped up to target shear stress over 7 days and then held stable for 72 hours prior to experimentation. Blue = DAPI for nucleus and Green = ZO-1; **D)** Protein content of cells under varying flow conditions. Shear stress was slowly ramped up to target shear stress over 7 days and then held stable for 72 hours prior to experimentation. Experiments were performed in triplicate and data is shown as box (median with 25^{th} to 75^{th} percentile) and whiskers (min and max); * indicates $p<0.05$; **E)** Barrier performance of cells under flow as measured by inulin permeability across the monolayer every 24 hours for 6 days. Data presented is from a single representative device at each shear stress level.

Figure 3. ASP+ transport in hOCT2/hMATE1 double transfected MDCK cells exposed to shear stress. **A)** Accumulation and **B)** transcellular transport of ASP+ by a monolayer of cells exposed to 0-2 dynes/cm^2 of shear stress for 72 hours followed by basolateral addition of ASP+ for 1 hour. Shear stress was slowly ramped up to target shear stress over 7 days and then held stable for 72 hours prior to experimentation. Data is expressed in percent relative to accumulation or transport by cells cultured under static conditions **C)** ASP+ accumulation into cells exposed to 0-0.2dynes/cm^2 of shear stress for 72 hours followed by basolateral addition of ASP+ for 1 hour with or without an exposure to imipramine, an OCT/MATE1 inhibitor. Experiments were

performed in triplicate and data is shown as box (median with 25th to 75th percentile) and whiskers (min and max); * indicates $p<0.05$.

Figure 4. Effect of shear stress on RNA expression of human (transfected) and endogenous canine OCT2 (organic cation transporter), transfected human MATE1 and canine P-gp in hOCT2/hMATE1 double transfected MDCK cells. Cells were exposed to 0-2 dynes/cm^2 for 72 hours after slowly ramping up to target shear stress over 7 days before mRNA expression was measured. Experiments were performed in triplicate and data are shown as box (median with 25th to 75th percentile) and whiskers (min and max); * indicates $p<0.05$.

Figure 5. Effect of deciliation on morphology of DT-MDCK cells. Staining of nuclei (DAPI) and cilia (α-tubulin) or tight junctions (F-actin) in cells exposed to 0-0.5 dynes/cm^2 for 72 hours after slowly ramping up to target shear stress over 7 days and where labeled, 30 mM ammonium sulfate for 24 hours. **A,C,E,G)** Blue = DAPI, Green = α-tubulin; **B,D,F,H)** Blue = DAPI, Green = F-actin

Figure 6. **Effect of deciliation on ASP+ transport by hOCT2/hMATE1 double transfected MDCK cells. A)** Accumulation and **B)** transcellular transport of ASP+ by a monolayer of cells exposed to 0-0.5 dynes/cm^2 of shear stress for 72 hours with and without treatment with 30 mM ammonium sulfate for 24 hours as indicated, followed by basolateral addition of ASP+ for 1 hour. Shear stress was slowly ramped up to target shear stress over 7 days and then held stable for 72 hours prior to experimentation. Data are expressed in percent relative to accumulation or transport by cells cultured under static conditions. Experiments were performed in triplicate and data are shown as box (median with 25th to 75th percentile) and whiskers (min and max); * indicates $p<0.05$.

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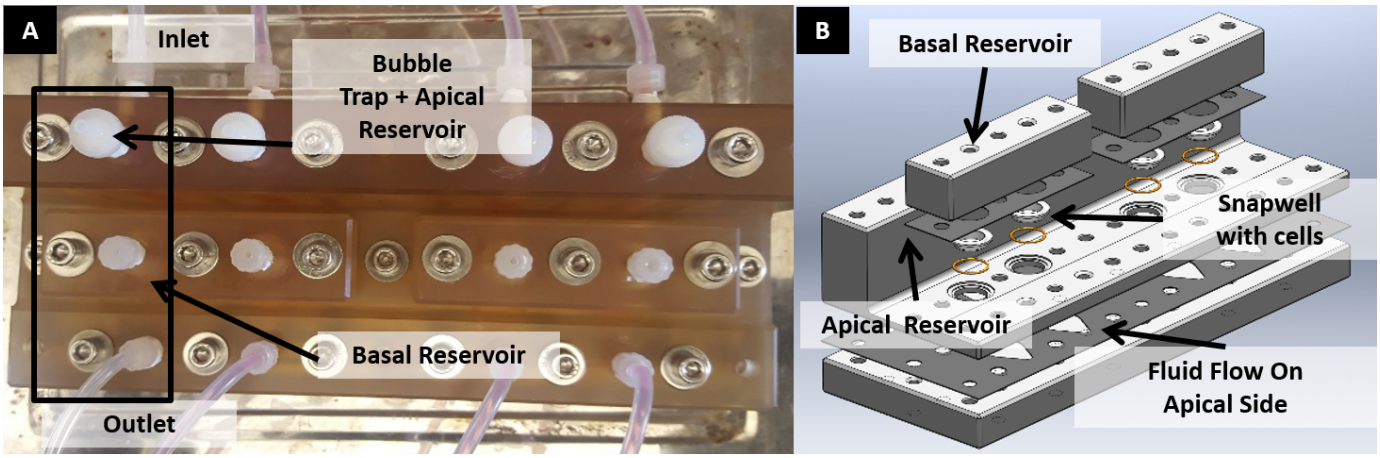
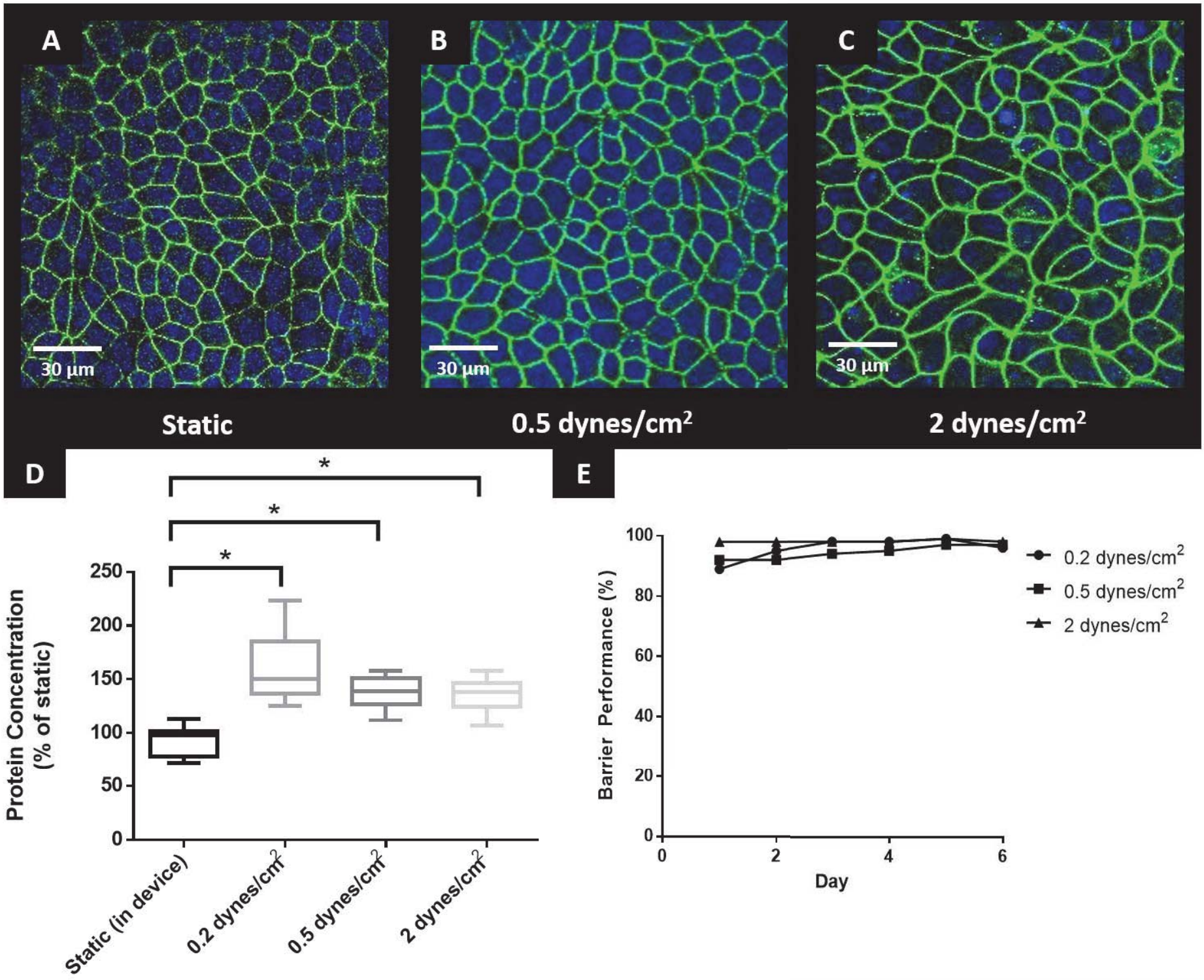


Figure 1



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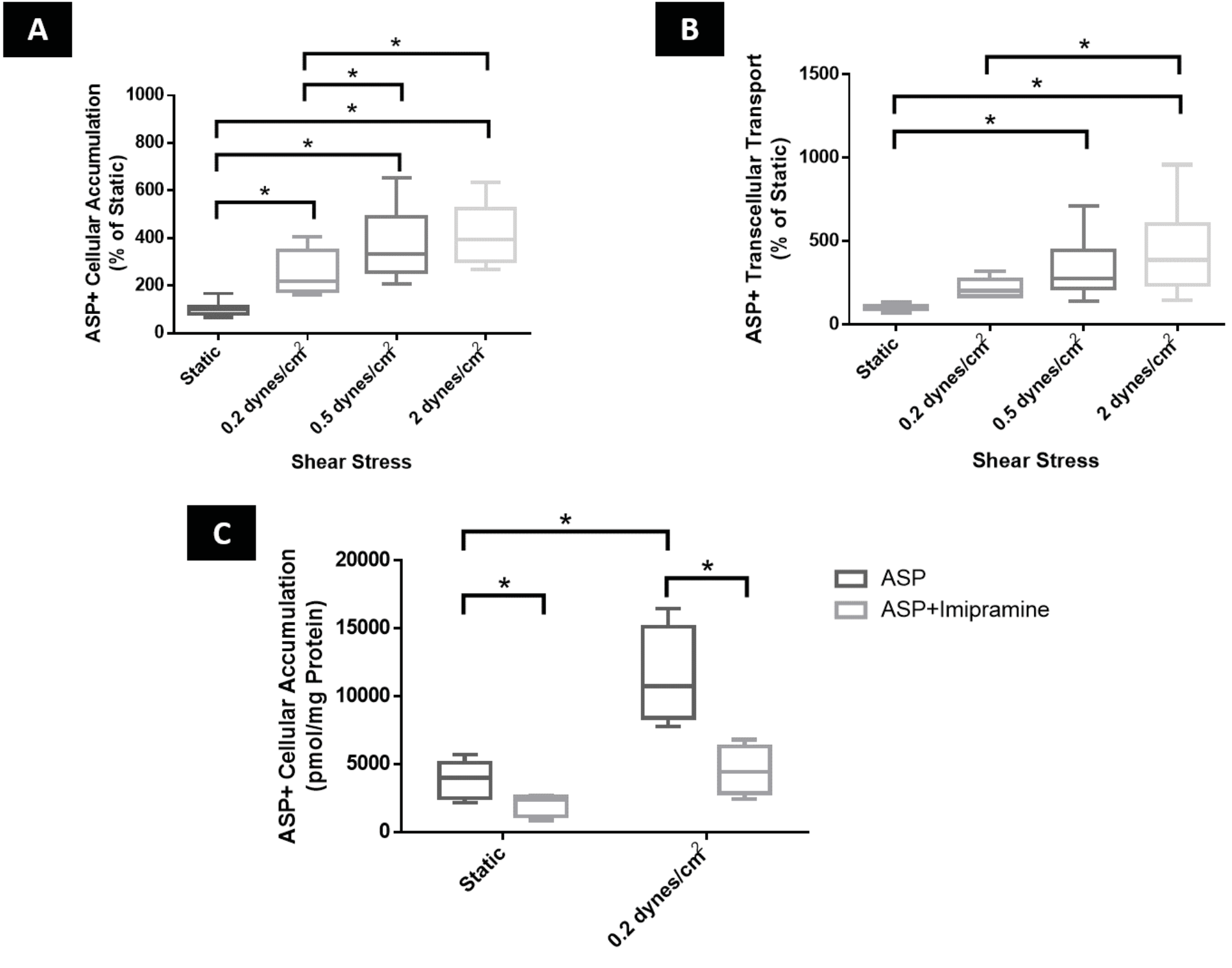

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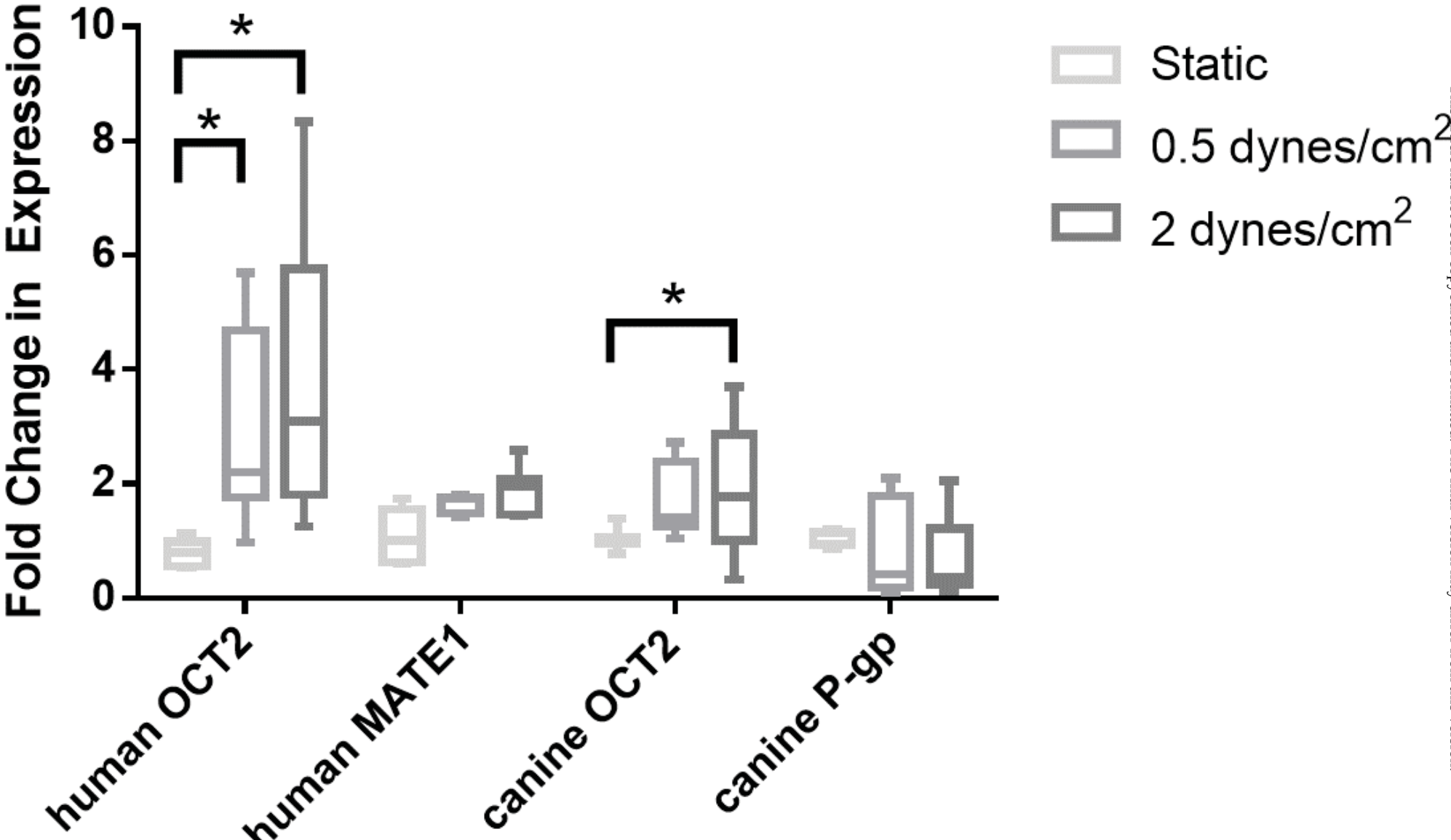

Figure 4

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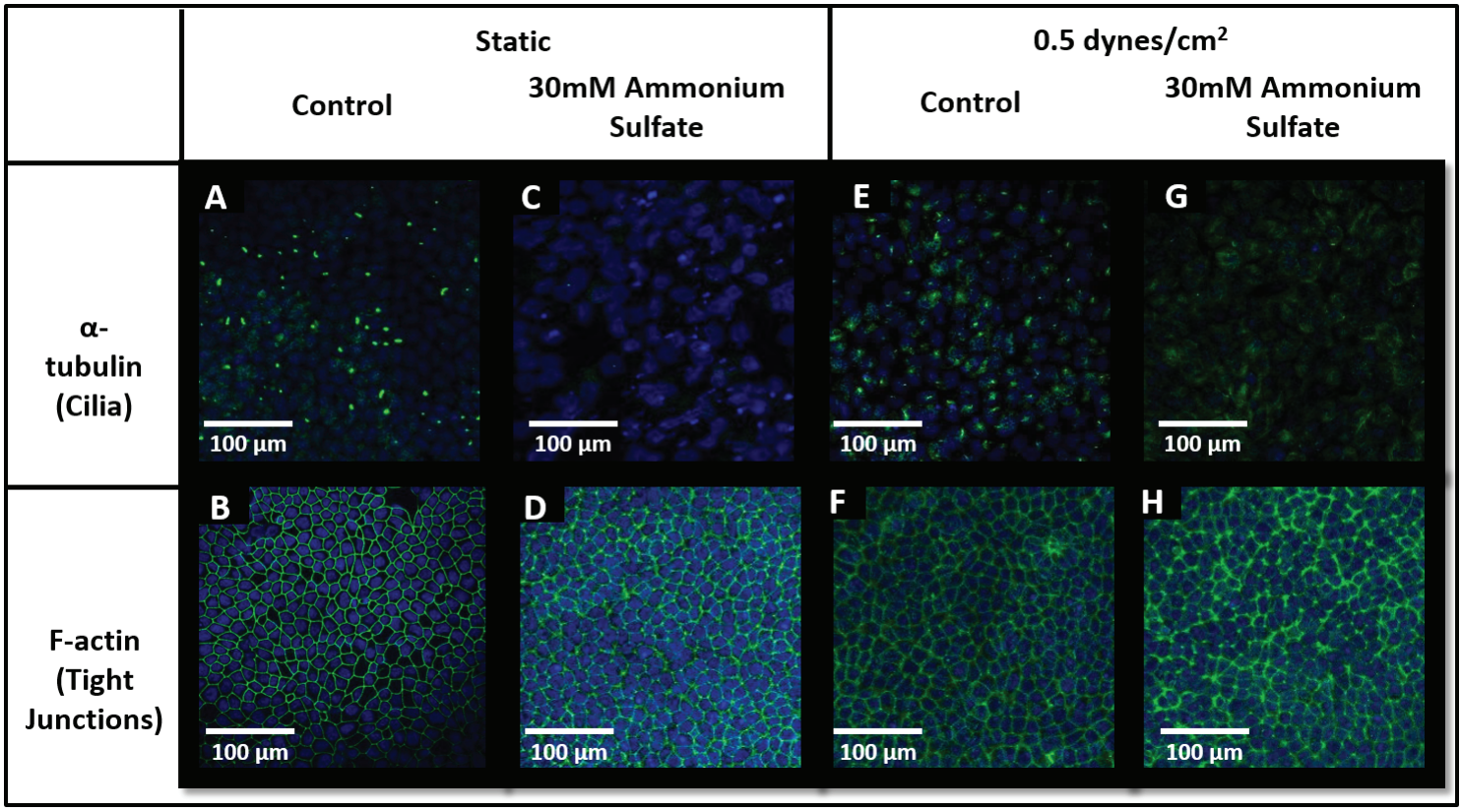

Figure 5

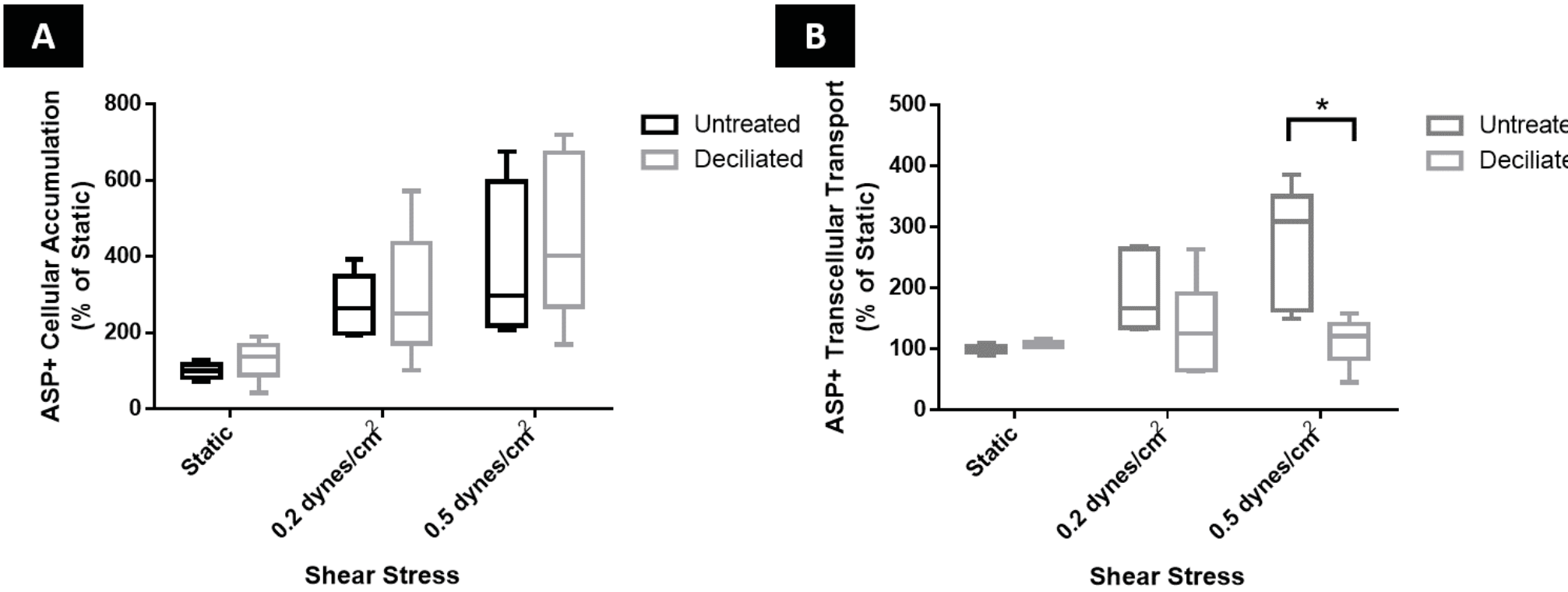

Figure 6