Role of Shear Stress on Renal Proximal Tubular Cells for Nephrotoxicity Assays

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Drug-induced nephrotoxicity causes huge morbidity and mortality at massive financial cost. The greatest burden of drug-induced acute kidney injury falls on the proximal tubular cells. To maintain their structure and function, renal proximal tubular cells need the shear stress from tubular fluid flow. Diverse techniques to reintroduce shear stress have been studied in a variety of proximal tubular like cell culture models. These studies often have limited replicates because of the huge cost of equipment and do not report all relevant parameters to allow reproduction and comparison of studies between labs. This review codifies the techniques used to reintroduce shear stress, the cell lines utilized, and the biological outcomes reported. Further, we propose a set of interventions to enhance future cell biology understanding of nephrotoxicity using cell culture models.

The inability to accurately identify nephrotoxicity is a major issue for drug development. Nephrotoxicity is the commonest reason to prolong hospital stays in the United States and elsewhere [1]. Acute kidney injury commonly progresses to end-stage renal disease and the need for renal replacement therapy, e.g., dialysis or transplantation, with its substantial costs and morbidity [2–4]. Fourteen drugs were withdrawn from the market between 1990 and 2010 for nephrotoxicity that had not been detected with available screening strategies [5].

There is currently no FDA-approved in vitro test for nephrotoxicity [6]. A major contributing factor is the lack of a readily available cellular target that is an accurate, representative, and physiologically relevant model of cells in the living kidney. Proximal tubule cells (PTC) are a prime candidate for an in vitro assay of nephrotoxicity. PTC are primarily responsible for the uptake and metabolism of many commonly prescribed clinical drugs including aminoglycoside antibiotics, amphotericin B, radiocontrast media, immunoglobulins, and diverse antineoplastic agents [7, 8].

PTC rapidly dedifferentiates under traditional 2D culture conditions, e.g., 96-well tissue culture plates, which severely limits the utility of this format for in vitro toxicity assays [10]. What PTC need is exposure to fluid shear stress. In vivo, PTC are exposed to fluid shear stress as the blood filtrate from the glomerulus flows past them en route to becoming urine. They sense this shear stress and respond with structural and biochemical changes, changes that need to be maintained in the in vitro environment.

Fukuda et al. found that human primary PTC exposed to fluid shear stress for 24 hours increased their expression of several drug transporters, including SLC37A2, SLS33A2, and SLC47A1 (also known at MATE2-K [11]). Xu et al. found that primary rat tubules maintained their express of P450
CYP1A1 for 12 days if exposed to shear fluid stress in a gyrorotatory culture [12]. Mollet et al. found that HK-2 cells exposed to fluid shear stress in a bioreactor, compared to static cultures, maintained their expression of multiple membrane transporter proteins for 21 days, including PEPT1 (SLC15A1), PEPT 2 (SLC15A2), OCT1 (SLC22A1), OAT3 (SLC22A8), gamma glutamyl transferase (gGT), and sodium-glucose cotransporter-2 (SGLT2) [13]. Unfortunately, none of these authors reported the intensity of the fluid shear stress that was applied to the PTC.

The magnitude of the shear stress to which PTC are exposed is dependent on the quantity of filtrate flowing past, the viscosity of the filtrate, and the internal structure of the proximal tubule. The proximal tubule narrows as one moves distally, the length and density of their microvilli changes, and the composition of the fluid changes as the PTC reabsorb water, proteins, and other components. However, the flow in the initial portion of the proximal tubule can be estimated from the single-nephron glomerular filtration rate of 30 and 90 nL/min [14]. This would suggest that PTC in vivo are exposed to approximately 0.05–0.17 dynes/cm² of shear stress [15] which is much lower than the 5 to 100 dynes/cm² that endothelial cells encounter in the vascular system [16]. These low levels of shear stress are challenging to reproduce in vitro in a uniform manner, particularly when they must be implemented in high throughput applications.

In vitro studies with PTC have used a wide range of shear stresses applied for varying durations, making it impossible to compare results between laboratories. What is needed is a standard uniform method of applying shear stress in vitro that is simple and easy to implement. Two issues have limited studies on shear stress. First, the equipment is usually high-priced, which creates significant capital barriers to experimentation [17–19]. Second, few techniques to reintroduce shear have thoroughly defined the parameters for reproduction by other labs [20].

This review seeks to categorize the known literature on reintroduction of shear stress on renal proximal tubule cell and the utility of suspension culture models which reintroduce shear to model renal damage. The current aim is to understand the amount of shear induced by different cell culture methods, the cell types utilized, and the outcomes assayed. These insights allow us to recommend interventions in the field of drug-induced nephrotoxicity to move the field forward. We performed PubMed searches using the terms renal proximal tubular cells, suspension culture, bioreactor, proximal tubule, renal cell shear stress, nephrotoxicity, drug toxicity, acute tubular necrosis, and renal genomics. We identified 25 papers that used PTC (or PTC cell lines) and specified the intensity of the shear stress flow and the duration of the stimulus.

Figure 1 compares the intensity and duration of shear stress applied to PTC in these 25 publications. The marker shape indicates the method utilized to generate the shear stress, most of which are microfluidics and parallel plate studies. Each study is referenced with a number, defined in Table 1. This graph gives a stark account of why the field has not come to a universal model for studies of nephrotoxicity: the miscarriage of cell types, shear levels applied, and duration of exposure defies simple interpretation.

Fluid shear stress induces structural changes in PTC (Table 1). Reorganization of actin fibers and the cytoskeleton was frequently observed, particularly in experiments using higher intensities of fluid shear stress. The studies that applied higher intensities of shear stress are useful as elevated levels of shear stress on PTC have been implicated in the progression of renal disease [41–45]. Increased expression of microvilli in the presence of fluid shear stress was also noted by multiple authors (Table 1). This is critical, as the microvilli are the sensors for tubular flow and shear stress [23, 29, 31, 46, 47].

Of critical importance to the development of an in vitro nephrotoxicity assay, fluid shear stress also increases the quantity and/or activity of transporters that take up proteins and drugs (Table 1). Exposure to fluid shear stress causes PTC to express more megalin and cubilin, transporters that are central part of the proximal tubular uptake of albumin, many other proteins, and drugs [48–50]. Indeed, albumin transport increases when PTC are exposed to fluid shear stress (Table 1). Renal cells employ a variety of organic anion transporters (OATs) and organic cation transporters (OCTs) [51] in the uptake and secretion of drugs. Fluid shear stress has also been observed to upregulate many of these on PTC including MATE (SLC47A1), OCT2 (SLC22A2), P-gp (ABCB1 or MDR1), MAT2K (SLC472K), and MRP2/4 (ABCC2/4). Jang et al. noted that the P-gp efflux by human primary PTC exposed to 0.2 dynes/cm² shear stress in vitro was closer to that observed in vivo compared to PTC in static 2D cultures [27].
Table 1: The study reference, shear stress in dynes/cm², duration in hours, technology utilized, and cell type.

| Reference number | Shear stress (dynes/cm²) | Duration (hours) | Fluid shear stress generated with | Cell | Reorganized actin and cytoskeleton | Increased cubulin/megalin, albumin transport | Increased expression of drug transporters |
|------------------|--------------------------|-----------------|----------------------------------|------|-----------------------------------|---------------------------------------------|--------------------------------------------|
| 1. Bhat 1995 [21]| 0.02–0.27                | 12–15           | Spinner flask with stirrers       | MDCK (canine) | X |                                |                                |
| 2. Cowger 2000 [22]| 0.04–0.12               | 48              | Rotating wall vessel suspension   | Primary PTC (human) | |                                |                                |
| 3. Hammond 1999 & 2000 [20]| 0.04–0.12 | 144          | Rotating wall vessel suspension   | Primary PTC (human and rat) | |                                |                                |
| 4. Raghavan 2014 [23]| 0.1*                    | 0.25–0.5        | Microfluidics                     | LLC-PK1 (pig), OK (possum) | X |                                |                                |
| 5. Miravete 2011 [24]| 0.5–5                   | 1               | Parallel plate                    | HK-2 (human) | |                                |                                |
| 6. Shimony 2008 [25]| 0.1                     | 24–48           | Slow rotation)                    | MDCK (canine), HK-2 (human) | X |                                |                                |
| 7. Xu 2020 [12]   | 0.2                     | 2.5             | Microfluidics                     | HK-2 (human) | |                                | X |
| 8. Jayagopal 2019 [26]| 0.2–2                   | 240             | Parallel plate                    | MDCK (canine) | |                                | X |
| 9. Jang 2013 [27]| 0.2                     | 72              | Microfluidics                     | Primary PTC (human) | X |                                | P-gp |
| 10. Duan 2010 [28]| 0.2                     | 3               | Parallel plate                    | Primary PTC (murine) | X |                                | X |
| 11. Homan 2016 [29]| 0.1 – 0.5               | 1008            | Perfused 3-D construct            | PTC-hTERT1 | X |                                | X |
| 12. Carrizoza- gaytan 2014 [30]| 0.2          | 0.5             | Parallel plate                    | mpkCCD (murine) | X |                                |                                |
| 13. Kaysen 1999 [31]| 0.5–1                   | 240–384         | Rotating wall vessel suspension   | Primary PTC (rat and human) | X |                                | X |
| 14. Brakeman 2016 [32]| 0.5–5                   | 5               | Microfluidics                     | Primary PTC (human) | |                                | X |
| 15. Frohlich 2012 [33]| 0.5                     | 2               | Parallel plate                    | HK-2 (human) | |                                | X |
| 16. Fukuda 2017 [11]| 0.5                     | 24–48           | Parallel plate                    | Primary PTC (human) | X |                                | MAT2K |
| 17. Essig 2001 [15]| 0.04–0.17               | 2–24            | Parallel plate                    | Primary PTC (murine) and LLC-PK1 Immortalized hu PTC | X |                                | X |
| 18. Vriend 2020 [34]| 0.5–2.0                 | 216             | Microfluidics                     | Primary PTC (murine) | X |                                | X |
| 19. Duan 2008 [35]| 1.0                     | 5               | Parallel plate                    | Primary PTC (murine) | X |                                | X |
| 20. Ferrell 2012 [36]| 1.0                     | 3               | Microfluidics                     | Primary PTC (murine) and SV40 transformed PTC (murine) | X |                                | X |
| 21. Kunnen 2017 [37]| 1.9                     | 4–20            | Parallel plate and cone-plate     | MDCK (canine) | |                                | X |
| 22. Cattaneo 2011 [38]| 2.0                     | 6               | Parallel plate                    | SV40 transformed PTC (murine) | X |                                | X |
| 23. Kunnen 2018b [39]| 2.0                     | 4–16            | Parallel plate                    | SV40 transformed PTC (murine) | X |                                | X |
With a specific focus on suspension culture and shear stress effects on renal proximal tubular cells, this review expands and enhances a specific segment of the Good Cell Culture Practice (GCCP) initiative [52] started by the former European Center for the Validation of Alternative Methods (ECVAM) [53]. The GCCP program tries to define standardized protocols to cultivate all relevant human tissues/ organs to test toxicity of newly developed drugs and chemicals.

The diversity of shear stress levels and durations in the studies reviewed here emphasizes the need for systematic reporting of specific criteria in order to produce a knowledge base to support harmonized protocols. Our lab proposed this more than a decade ago, embodied in the Bonn criteria [20]. While there are developments on the way to generate harmonized protocols that should allow for prediction of nephrotoxicity during the preclinical phase of drug development [54–56], each methodology will need the kind of summary review presented here to allow useful progression of the initiatives.

The duration of shear exposure and cell type have striking effects on cellular responses (Table 1). It is a conundrum to compare studies not only because of differing shear stress, duration, and cell types, but the various studies utilized diverse outcome measures. A few studies have examined different shear levels and demonstrated changes dependent on shear levels [21, 29, 31]. There is scant, if any, data on the time course of changes in selected outcomes. Hence, study of changes in outcomes over time is one of our suggestions for future study.

Only with harmonized protocols that define the shear stress applied on renal proximal tubules can many of the questions pivotal to predicting nephrotoxicity be answered: does shear induce certain specific patterns in gene expression? Is there an interdependency between the magnitude of shear stress and the expression of specific genes? How do the effects of cells exposed to shear stress or under microfluidic conditions compare? Can the change in phenotypic function of proximal tubular cells in culture towards an in vivo equivalent state be achieved by shear stress alone?

### 1. Conclusions

We propose three strategies to move the field towards a uniform model to test nephrotoxicity of drugs.

First, in 2010, we proposed a minimum data set to be reported to allow reproduction of suspension culture studies in other labs [20]. As the meeting where the proposal was presented was in Bonn, we termed these the Bonn criteria [20]. They include the vessel diameter, rotation speed, media viscosity, media density, cell/organoid/spheroid diameter, and density. The Bonn criteria remain critical to interpret data between labs and allow accurate experimental reproduction between labs.

Second, if different labs continue to use different techniques and reagents including cell types, some tradeoff or bake-off studies will be indispensable to understand differences between approaches.

Last, the development of a low capital, inexpensive, to-use suspension culture technology would allow far more labs access to the technology and occasion the opportunity for studies to include more replicates and conditions cost-effectively.

The search for a uniform model to study nephrotoxicity is severely limited by the use of a multiplicity of methods and techniques, which cannot be simply compared. Laboratories use different cell types approximating renal proximal tubular cells and apply diverse shear stress methods, and there is no systematic and adequate reporting of culture parameters. The morbidity, mortality, and cost of drug induced acute renal injury should make an integrated cell biology approach to nephrotoxicity an urgent priority.

### Abbreviations

- gGT: Gamma glutamyl transferase
- MATE: Multidrug and toxin extrusion protein
- MDCK: MadinDarby Canine Kidney
- Pgp: P glycoprotein transporter
- PETP: Peptide transporter
- PTC: Proximal tubule cells
- OAT: Organic anion transporters
- OCT: Organic cation transporters
Data Availability
All articles cited are freely available on PubMed and other academic media.

Disclosure
The content does not represent the views of the Department of Veterans Affairs or the United States of America.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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