Drug absorption related nephrotoxicity assessment on an intestine-kidney chip

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Drug absorption in the intestine is tightly related to drug-induced nephrotoxicity, which is a relatively common side effect in clinical practice. It highlights a great need to develop predictive models with high accuracy in the early stage during new drug discovery and development. Herein, we presented a novel intestine-kidney chip, which recapitulated drug absorption in the intestine and its resultant drug toxicity on the kidney. This work aims to provide an integrated tool for accurate assessment of drug absorption-related nephrotoxicity *in vitro*. A microfluidic device with multi-interfaces was designed, which facilitated the co-culture of the intestinal and glomerular endothelial cells in compartmentalized micro-chambers. Thus, drug absorption and following nephrotoxicity could be explored in a single assay based on the formation of the intact intestine function on the chip. Specifically, we adopt digoxin (DIG) as a model drug combined with colestyramine (COL) or Verapamil (VER), which significantly influence DIG absorption in the intestine. Different degrees of nephrotoxicity under drug combinations were further observed on the chip, including cell apoptosis, cell viability, and lactate dehydrogenase leakage. These features were consistent with the variance of DIG absorption by the intestinal cells. In agreement with clinical observations, our data demonstrated that DIG-induced nephrotoxicity was enhanced combined with VER but weakened with COL. All of these findings suggest that the established microdevice might provide a useful and cost-effective platform *in vitro* for testing drug absorption and nephrotoxicity in preclinical trials during new drug development. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.4984768]

I. INTRODUCTION

Due to the exceptional ability of the kidney for filtration, secretion, and reabsorption, the kidney is an essential organ maintaining homeostasis of the body and thus is highly susceptible for drug-induced toxicity.[1,2] Drug-induced nephrotoxicity remains a major problem in the clinical setting, which frequently leads to acute or chronic kidney injury. The best strategy to settle these problems observed would be the discovery of new drugs with decreased nephrotoxic potentials. Actually, nephrotoxicity results in 2% and 19% of new drug candidates fail in preclinical trials and phase III clinical trials, respectively.[3] Such problems substantially increase the costs of drug development in the pharmaceutical industry. In addition, drug absorption greatly affects its bioavailability and bioactivity, which is important for accurate assessment of drug nephrotoxicity. The oral route of administration is the most convenient way, for which drugs are absorbed within the gastrointestinal tract. Currently, drug evaluation *in vitro* is mainly

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focused on the drug itself, not involved with the drug absorption in preclinical trials, and nephrotoxicity of many drugs is detected in the late development stage accordingly. Thus, early prediction of the drug nephrotoxicity in humans may contribute to reduce costs and speed the time for drug development. However, the nephrotoxic potential of newly developed medicines is often underestimated, due to the less-appreciated role of drug absorption in nephrotoxicity. For these reasons, it is highly desirable to develop a new model, which could reflect drug absorption, thereby better predicting nephrotoxicity in the early stage.

At present, evaluation of drug nephrotoxicity is mainly based on the animal model and cell-based model.[4,5] As an effective method, the *in vivo* tests come with the own set of drawbacks including high costs, ethical issues, and the impossibility to perform quantitative study or high throughput assay, which limit their use. Therefore, the *in vitro* cell-based tests, utilizing cultured kidney cells, such as renal tubular cells, have been widely used as an alternative to animal testing relied on the simplicity.[6–10] Many kidney models *in vitro* have been exploited over the years to investigate renal cell behaviors and predict the toxicity of various chemicals.[11–15] However, these cell-based *in vitro* models fail to recapitulate the biological responses in the body, such as drug absorption, leading to the extremely poor prediction in drug-induced nephrotoxicity.

The rapid development of organ-on-chip technology may provide a good solution to simulate the complex *in vivo*-like physical environment *in vitro*.[16–25] In recent years, many studies have attempted to build a renal tubular system relied on such micro-devices, named kidney-on-a-chip. These chips display rearranged cytoskeleton and enhanced cell junctions as well as cell functions through creating the physical microenvironment *in vitro*.[26] Significant progress has been made in nephrotoxicity testing by using kidney-on-a-chip. As discussed above, these assays just focus on the role of drug itself on nephrotoxicity, ignoring the potential influence of drug absorption and utilization. It has not been attempted to establish an *in vitro* model for assessment of nephrotoxicity following drug absorption, which remains a challenge in the toxicity test so far. As intestine is of great importance in drug absorption, it is significant to develop an *in vitro* functional intestine-kidney model, which could recapitulate drug absorption and enable the followed assessment of drug nephrotoxicity.

Digoxin (DIG) is an oral medication for tachycardia, atrial fibrillation, and heart failure, which has a narrow therapeutic index and frequently induce nephrotoxicity due to an excessive dose. Therefore, it is of great importance to investigate drug nephrotoxicity under the consideration of drug absorption.[27] Actually, the absorption of digoxin could be influenced by many factors, such as P-gp inhibitors, increasing DIG absorption.[28] In this work, we developed a novel intestine-kidney chip with multi-interfaces and compartmentalized micro-chambers for the assessment of drug absorption related nephrotoxicity. The chip was fabricated with two layers, with human intestinal cell line Caco-2 on the upper layer to simulate drug absorption in intestine and glomerular endothelial cells (GECs) on the bottom layer to observe drug toxicity in the kidney. We adopted DIG as a model drug for subsequent drug absorption related nephrotoxicity testing and further monitored nephrotoxicity by drug combination [e.g., Verapamil (VER) and Colestyramine (COL)]. Specifically, this chip allows us to identify the permeability of GECs, enabling to investigate the toxic effect of drugs on glomerular filtration *in vitro*. The integrated intestine-kidney chip could assess drug absorption related nephrotoxicity effectively and provide a reliable tool for drug testing at the multi-organ level.

II. EXPERIMENTAL

A. Chip fabrication

As shown in Fig. 1, the intestine-kidney chip was fabricated using soft lithography and micromolding as previously used.[29] The microfluidic device was fabricated using soft lithography technology. The masks were designed by AutoCAD (Autodesk) and printed on a plastic film with 4000 dpi resolution. First, to prepare the template, the SU-8 photoresist was spin-coated onto two clean glass wafers and then selectively cured under an ultraviolet light source by using different masks. Afterwards, the mixture of polydimethylsiloxane (PDMS) monomer

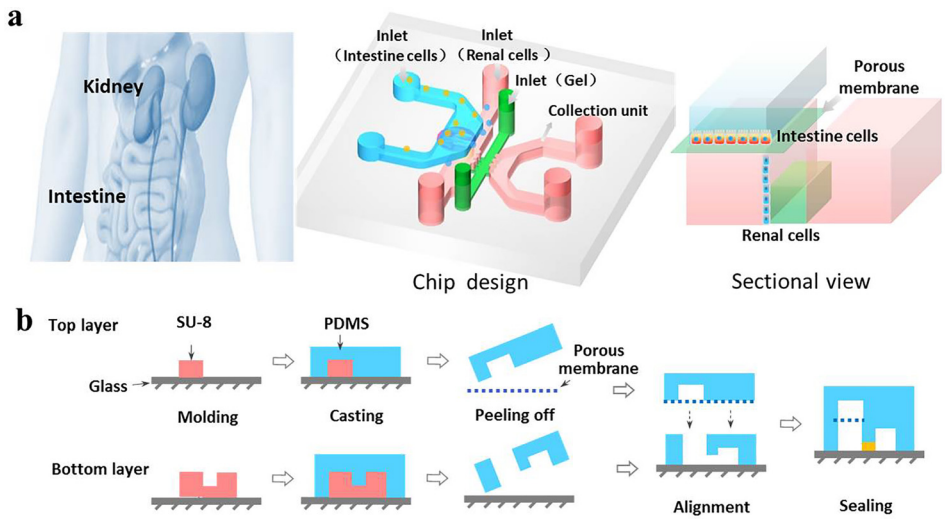

FIG. 1. Design and fabrication of the intestine-kidney chip. (a) Schematic diagram of the design of intestine-kidney chip and sectional view. An explanatory illustration of the chip with multi-interface construction. The top layer, porous membrane, and bottom layer were sealed together. The intestine cells mimicking the intestine were cultured in the chamber on the top layer, and renal cells were seeded in the chamber on the bottom layer. (b) Schematic depiction of the fabrication of the microfluidic device.

(Sylgard 184) and curing agent (Dow Corning Corp.) at the ratio of 6:1 by mass was used to generate two PDMS layers. Finally, the two PDMS replicas were sealed together with the middle polycarbonate porous membrane (pore size: 0.4 μm, Whatman Corp.).[30,31] The sealing was completed using a glue, which is actually the mixture of PDMS monomer and curing agent at the ratio of 20:1 by mass. Specifically, the top layer PDMS and porous membrane were bonded together after oxygen plasma treatment and then assembled with the bottom layer by PDMS glue. The sealed device was cured for 1 h at 80 °C in an oven.

The top layer of the chip had one cell culture chamber which was 300 μm in height, 2 mm in width, and 11 mm in length. The bottom layer consisted of two higher channels separated by a lower channel with gel: the higher channel had one cell culture chamber which was 300 μm in height and the lower channel was 100 μm in height. The porous membrane was sandwiched between the top and bottom layers, allowing the transition of absorbed drugs to the bottom layer. GECs were seeded on the surface of gel-channel between the higher channel and the lower channel, which allowed the transition of constituents from the cell culture chamber to the collecting chamber. The major component of the gel-channel was matrigel (BD), which has been widely used for a variety of biological experiment to mimic the native extracellular matrix.[32] All the chambers are connected with one inlet and one outlet for samples in and out.

B. Culture of intestine and kidney cells on chip

Caco-2 cells (colorectal adenocarcinoma cells), were cultured with Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco) and 100 U/ml penicillin and 100 U/ml streptomycin in the cell incubator with 5% CO_2 at 37 °C. Cells were passaged weekly and the culture medium was changed every 48 h. It is often limited due to the difficulties in obtaining the human derived tissues. Primary glomerular micro-tissues were isolated from rat kidneys according to a previously described protocol.[33] The isolated glomerular micro-tissues were cultured on a collagen I-coated Petri dish in the endothelial cell medium supplemented with 10% FBS, 100 U/ml of penicillin, and 100 U/ml of streptomycin with 5% CO_2 at 37 °C. The cells spread around the glomerular tissues after culturing for 3 days under static conditions. As the glomerular micro-tissues contained podocytes and mesangial cells, we used differential digestion to purify the endothelial cells. As GECs are more easily digested than podocytes and mesangial cells, the endothelial cells were digested using trypsin

for 2 to 3 min after culturing for 5 to 7 days. The digested endothelial cells were transferred to a new Petri dish to expand.

After the fabrication of multilayer PDMS device, matrigel of a high concentration was used to mimic the natural extracellular matrix in the kidney. The matrigel solution with the final concentration of 6 mg/ml was pumped into the matrigel channel in the device at 4 °C and finally gelled at 37 °C for 30 min. The microchambers of the device were treated with a 8 μg/ml matrigel-containing medium for 12 h, which facilitated cell adhesion and culture. The GECs were centrifuged and resuspended in a cell culture medium to the density of 1×10^4 cells/ml. Then, the GECs were injected into the lower cell culture channel from the inlet. The microchip was rotated 90° to culture the cells on the surface of the gel-channel containing matrigel. The GECs was side cultured for 24 h to enable adhering of cells onto the surface of matrigel before the microchip was placed horizontally. After incubation in a humidified incubator at 37 °C for 3 days, GECs on the surface of the gel grew to form an intact cell layer in the chip.

Before loading of GECs for 3d, Caco-2 cells were harvested and resuspended in the medium to the density of 1×10^6 cells/ml. Then, cells were injected into the top chamber. After the cells attached onto the channel, the micro-pump system was connected to the chamber to provide a continuous flow. Cells finally formed an intact monolayer under the fluid shear stress of 0.02 dyne/cm^2.[34]

C. Evaluation of the intestine function

The integrity of the human intestinal epithelial cell monolayer was evaluated by staining the tight junction protein ZO-1 (Abcam) and measuring transepithelial electrical resistance (TEER). The TEER of the Caco-2 monolayer was measured using a voltage-ohm meter. The baseline resistance value measured in the absence of cells was subtracted from the results obtained with the Caco-2 monolayer, and specific TEER values were determined through multiplying the specific resistance times by the total cell culture surface area on the membrane.

The functionality of the human intestinal epithelial cell was measured by quantitating alkaline phosphatase (ALP) activity of the brush border on the apical and basolateral sides using an ALP assay kit(Jiancheng Bioengineering Institute). The high ratio of ALP activity on the two sides indicates the highly differentiated human intestinal Caco-2 cell monolayers in the chip. Specifically, the culture medium was collected from the top and lower chambers of cells after culturing for 5 days. The solution was transferred to a 96 well plate where the ALP activity was quantified in a microplate reader at 405 nm using culture medium as a reference.

D. Immunofluorescence staining

After stimulation, the cells quickly rinsed with Phosphate Buffered Saline (PBS) and fixed in 4% paraformaldehyde for 15 min. The fixed cells were permeabilized with 0.2% Triton-X100 before incubating with the blocking buffer for 1 h at room temperature. Then, cells were incubated with primary antibody ZO-1 (diluted 1:300) overnight at 4 °C, followed by incubation with alexa 488 conjugated goat secondary antibody (diluted 1:100, Beyotime Company) for 1 h, DAPI for 15 min. Samples were finally inspected using a confocal microscope (Leica).

E. Assessment of drug induced toxicity

To assess DIG toxicity, the Caco-2 cells were exposed to a series of concentrations (0, 10, 20, 40, 80, and 160 μM) of DIG for 48 h. CCK-8 assay (cell counting kit-8, Dojindo) was performed to quantify cell vitality. In order to assess drug nephrotoxicity on the intestine-kidney chip, the cell culture medium containing DIG (10 to 160 μM), DIG (80 μM) and COL (200 μg/ml), or DIG (80 μM) and VER (20 μM) was pumped into the chamber with Caco-2 cells, respectively. DIG was absorbed in this cell chamber and then transited into the lower chamber, where GECs were stimulated by drugs absorbed. After drug stimulation for two days, medium from the top layer was gathered to quantify released lactate dehydrogenase (LDH) using a LDH detection Kit (Jiancheng Bioengineering Institute) by a microplate reader. To check apoptosis, cells were stained using a

Live/Dead Kit (BD), which displayed green (live cells) and red (dead cells). Intracellular fluorescence was measured using fluorescence microscopy. CCK-8 assay was performed to quantify cell vitality. 5 μl of CCK-8 reagent with 45 μl of cell culture medium was added into the cells' culture channel. The solution was collected into a 96-well plate, and then, the absorbance at 450 nm was measured using a microplate reader after incubation for 2 h.

F. Permeability testing of the GEC layer

To evaluate the permeability of GECs with drug exposure, we examined the fusion of protein with large molecules through the GEC layer. The permeability was investigated by loading Fluorescein isothiocyanate conjugated IgG (FITC-IgG) (250 μM) and albumin (ALB) (40 mg/ml) to the cell culture channel. FITC-IgG across the GEC layer was measured based on the fluorescence intensity. Time-lapse images of IgG were collected at 0, 15, 30, and 60 min. ALB transported into the collection channel was quantified using an albumin (ALB) assay kit (Jiancheng Bioengineering Institute).

G. MS detection of absorbed drugs

DIG was detected by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Cell medium containing DIG (80 μM) and COL (200 μg/ml) or DIG (80 μM) and VER (20 μM) was added into the top chamber. Then, medium in the bottom chamber was collected 48 h later for the detection of DIG.

To prepare samples for LC-MS/MS, 500 μl of ethyl acetate was added into 50 μl medium containing drugs and then vortexed for 1 min. After centrifuging at 3000 rpm for 8 min, the top layer about 400 μl was transferred into a polythene tube and dried under a gentle stream of nitrogen at 37 °C. The dried residue was then re-dissolved in 50 μl of the mobile phase solution. Finally, a 10 μl aliquot was injected into the LC-MS/MS for analysis. The mobile phase consisted of acetonitrile and water (80:20, v/v). DIG was analyzed by multiple reaction monitoring (MRM) using the precursor to product ion transitions of m/z 779 $\rightarrow$ 649.

H. Image and statistical analysis

In this study, all images were analyzed using Image J software. All data were presented as mean $\pm$ standard error. All experiments were performed at least in triplicates. Comparisons of two groups were performed by using the two-sample t-test. $P < 0.05$ was considered significantly different.

III. RESULTS AND DISCUSSION

A. Creating the intestine-kidney chip

In this work, we designed and fabricated a microfluidic device with multi-interfaces to create an intestine-kidney chip and characterize the DIG absorption process by the intestine as well as its resultant toxicity on GECs simultaneously *in vitro*. The design of the organ-on-a-chip device with multiple interfaces facilitates the co-culture of different cells in compartmentalized micro-chambers and the characterization of drug absorption-related toxicity on GECs in a single assay. The device consisted of a top layer and a bottom layer with compartmentalized micro-chambers. A porous membrane with a pore size of 0.4 μm was sandwiched between the two layers to enable the transportation of drug from the top layer to the bottom layer. The membrane was glued with the two layers by liquid PDMS. The two layers could be separated conveniently, contributing to the following analysis.

The intestinal epithelial barrier, which is created by the Caco-2 cell confluent monolayer on a porous membrane, is often used as the model for drug screening as well as biological studies.[35] Herein, Caco-2 cells were cultured in the chamber of the top layer with a continuous flow, which contributes to cell differentiation and polarization. The Caco-2 cell monolayer finally displayed specific morphology and functionality, resembling human intestine *in vitro*. To

investigate the nephrotoxicity of DIG combining COL or VER on chip, GECs were seeded into the cell culture chamber of the bottom layer, mimicking the glomerular capillary lumen compartment. Drugs absorbed by the intestinal cells could be transported into the bottom layer with GECs after exposure to Caco-2 in the top layer. Then, the nephrotoxicity of different drugs on GECs after Caco-2 absorption was further examined (Fig. 2).

The proposed intestine-kidney co-culture model could characterize drug absorption and drug nephrotoxicity in a single assay. The multi-layered design of this organ-on-chip not only constructs the well-organized system with multi-organ compartments in a single device but also facilitates the assessment of absorption related drug nephrotoxicity in a simple manner. The present data have demonstrated that the intestine-kidney chip might be applied to investigate drug absorption by the intestine and drug toxicity on the kidney to some extent *in vitro* and provide superior flexibility, functionality, and performance.

B. Characterization of drug absorption on intestine-kidney chip

As the structural and functional integrity of intestine barrier in the chip is highly related to the evaluation of drug absorption and nephrotoxicity, we next explored the specific features that contribute to the barrier properties, including the expression of tight junction associated protein ZO-1, ALP activity, and TEER of the Caco-2 cell monolayer. First of all, immunofluorescence staining demonstrated the expression of ZO-1, indicating the integrated tight junction assembly in Caco-2 cells [Fig. 3(a)]. ALP, a brush border enzyme that is expressed exclusively in the apical side of the intestinal barrier, is commonly used as a marker for the differentiation of human intestinal epithelial cells. As shown in Fig. 3(b), the ALP expression of the apical side was around three times that of the basolateral, revealing high differentiation of the intestinal monolayer in our chip.[36] Next, we further measured TEER of the monolayer on the porous membrane, which is a functional parameter to monitor the quality of the barrier *in vitro*. With cells proliferated in the chip, TEER increased and finally stabilized at 1200 Ω cm^2 after 4 days of culture. Caco-2 cells commonly must be grown in the Transwell system for at least 3 weeks to exhibit differentiated intestinal barrier functions. In previous studies, the application of physiological fluid flow and shear stress across the apical surface of the intestinal epithelium accelerates cell differentiation.[34] Our data are consistent with the previous reports using the same cell model. Obviously, these data suggest that the integrated intestinal barrier was formed in our

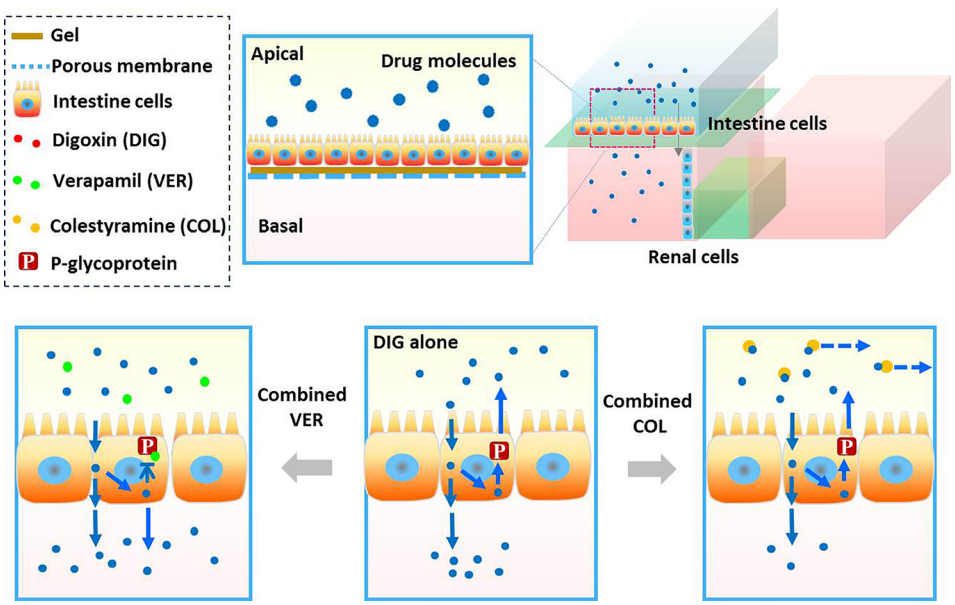

FIG. 2. Schematic diagram of the drug absorption in intestine cells under different drug administration formats. Representative diagram of the process of DIG absorption on the intestine barrier combined with VER or COL.

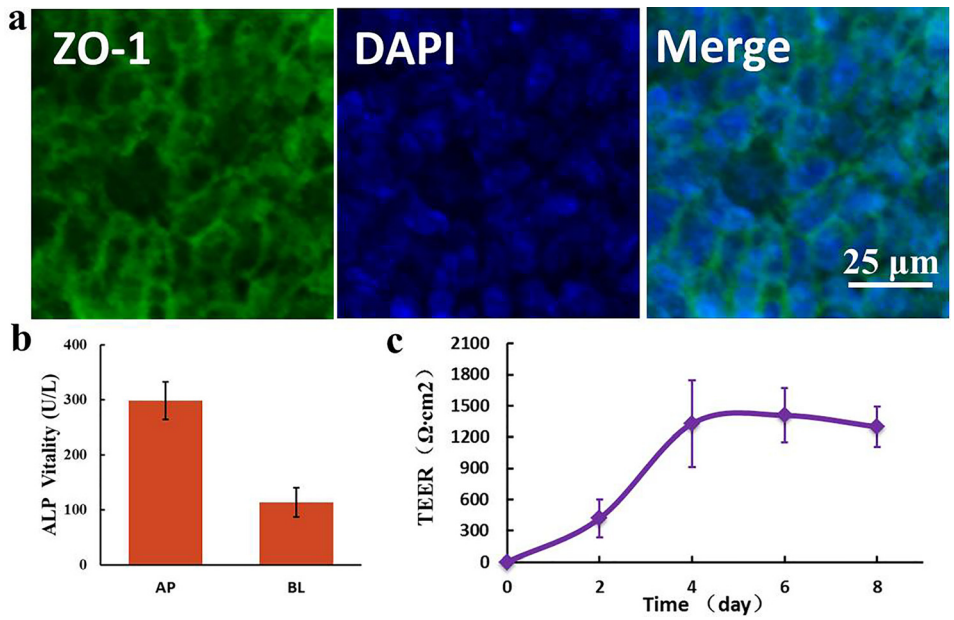

FIG. 3. Evaluation of intestinal barrier formed by Caco-2 cell monolayer in the intestine-kidney chip. (a) Immunofluorescence staining showed the distribution of the tight junction protein, ZO-1(green), in the epithelial monolayers. (b) Computerized quantification of ALP activity in the apical (AP) and basolateral (BL) sides of the barrier. (c) TEER of the Caco-2 monolayer in the microfluidic chip was quantified from day 0 to day 8.

chip with the perfused system, and it could be available for subsequent testing of drug absorption related nephrotoxicity.

Here, we chose DIG as a model with drug combinations (e.g., VER and COL) to evaluate drug absorption by the intestine. DIG has been identified as a substrate of P-glycoprotein (P-gp), which mediates intestinal DIG transport in humans. This drug is mainly excreted in the urine, thereby inducing nephrotoxicity frequently. Colestyramine (COL) as a strong ion exchange resin is generally used to treat hypercholesterolemia, which could remove DIG from the body by forming insoluble complexes in the intestine.[37] Verapamil (VER), a calcium channel antagonist, is clinically useful in hypertension. As a P-gp inhibitor, VER could reduce the efflux of DIG so as to increase drug absorption in the intestine, leading to elevated digoxin concentrations in plasma and its side effects on the body.[38]

More specifically, intestine cells were treated by DIG combined with COL or VER, and then, the medium from the lower channel was collected 48 h later for detection of absorbed DIG. As displayed in Fig. 4(a), DIG was transported to the basolateral side of the intestinal barrier based on the performance of LC-MS/MS. DIG transportation was quantified, which demonstrated higher DIG concentration in the presence of VER compared to that of COL [Fig. 4(b)],

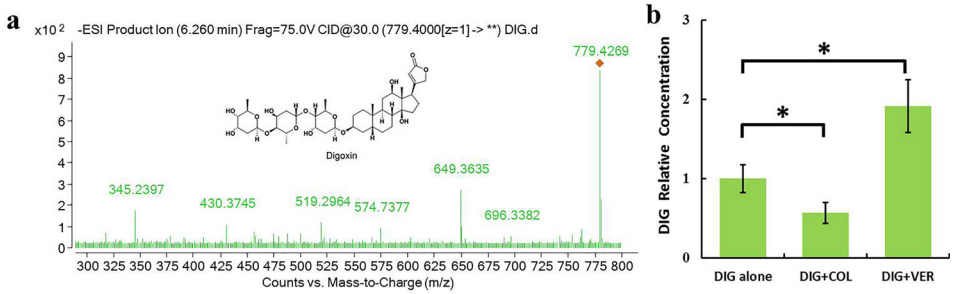

FIG. 4. Characterization of the DIG absorbed by intestinal cells. (a) Mass spectrum detection of DIG from the medium in the bottom channel after exposure for 48 h. (b) Comparisons of DIG concentrations of three groups: DIG alone and DIG combined with COL or VER. The data were shown as mean ± SD (n = 3). *, p-value < 0.05.

consistent with clinical observations. Collectively, the fluctuations of DIG absorption under different drug combinations were well recapitulated in our intestine-kidney chip *in vitro*.

C. Characterization of absorption dependent nephrotoxicity

In the body, the glomerulus is particularly susceptible to toxicity due to continuous exposure to the high concentration of drugs. Therefore, we further assessed DIG nephrotoxicity with different drug combinations (VER and COL) based on the intestine-kidney chip *in vitro*. In our previous experiments, 80 μM DIG resulted in a significant decrease in GECs viability but Caco-2 viability (Figs. S1 and S2, supplementary material). So, it is suitable for the application in this work.

Generally, cell apoptosis and growth is an excellent marker for cell damage in the studies of drug nephrotoxicity. We initially examined cell vitality under drug treatment by performing Live/Dead staining and quantitative CCK-8 assay. The intestinal cells on the top layer were exposed to 80 μM DIG combined with COL (200 μg/ml) or VER (20 μM) for 2 days. According to the above data, the drug absorbed by intestine cells was then transported into the bottom layer with GECs. Live/Dead staining revealed increased cell death by DIG exposure, especially combined with VER [Fig. 5(a)]. CCK-8 assay allows us to quantify the number of viable cells under different conditions. As shown in Fig. 5(b), the viability of renal cells with drug exposure in the decreasing order was DIG + COL, DIG, and DIG + VER, consistent with the concentrations of absorbed DIG by intestine cells. These data indicated that the absorbed drugs by intestine cells induced the toxicity on GECs, including increased cell apoptosis and decreased cell proliferation.

Next, the effects of drug combinations on the structure of GECs were further evaluated. Tight junction protein (ZO-1) was examined by immunostaining after treatment of GECs with drugs for two days, which revealed that exposure of intestine cells to DIG significantly destroyed the expression of ZO-1, especially under the exposure of DIG and VER. In contrast, the existence of COL slightly alleviated the damage to GECs [Fig. 5(c)]. Additionally, lactate dehydrogenase (LDH) release is a highly reliable biomarker for the assessment of renal damage *in vitro*. Hence, LDH leakage by GECs with exposure to drug combinations was examined. In agreement with the immunofluorescence staining, the addition of VER and COL displayed

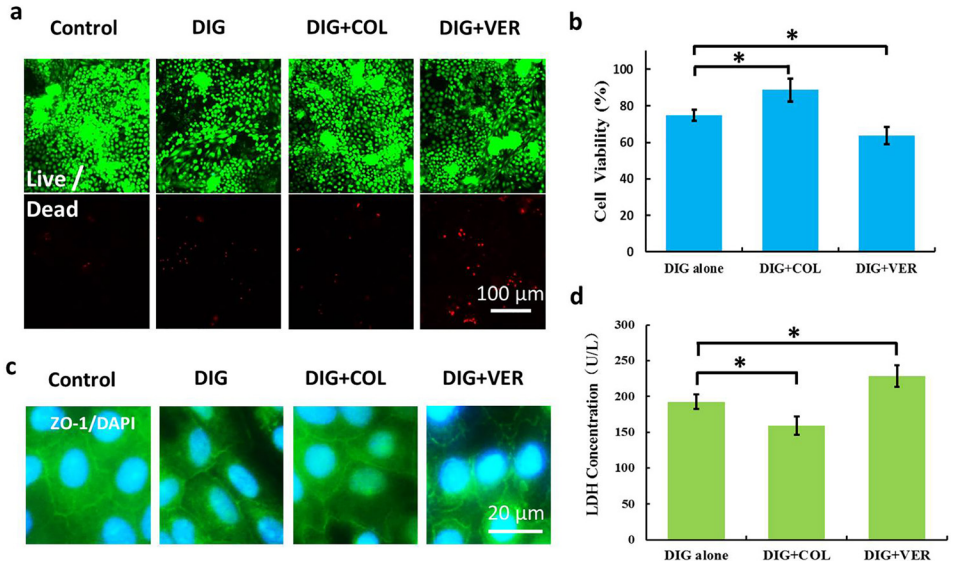

FIG. 5. Assessment of drug-induced cytotoxicity in GECs after exposure to different drugs. (a) Live/dead assay of GECs after treatment with DIG(80 μM), COL(200 μg/ml), and VER(20 μM) for 2 days. Live cells were marked with green, and dead cells were marked with red. (b) CCK-8 assay of GECs after treatment with drug for 2 days. (c) Immunofluorescence staining of the tight junction protein ZO-1 (green) in GECs after drug exposure for 2 days. DAPI is marked with blue. Bar: 20 μm. (d) Leakage of LDH after treatment with drugs for 2 days was quantified. *, p-value < 0.05.

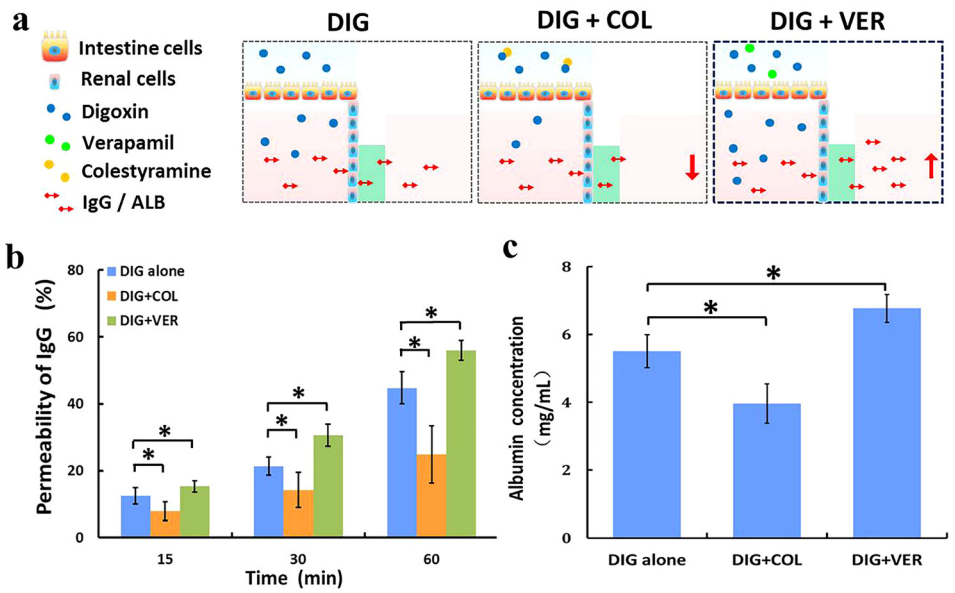

FIG. 6. Assessment of effects of drug on the permeability of the GEC layer. (a) Schematic of the effects of drugs on the permeability of the GEC layer. (b) Quantitative graphs showing the permeability of FITC-IgG (IgG, MW = 150 kD, 250 μM) across the GEC layer after treatment with DIG (80 μM), COL(200 μg/ml), and VER(20 μM) for 2 days. The fluorescence of IgG was quantified by fluorescence quantitative analysis. (c) Quantitative graphs revealed the permeability of albumin across the GEC layer after treatment with different drugs for 2 days. Albumin (MW = 70 kD, 40 mg/ml) across the GECs layer was quantified using an albumin (ALB) assay kit within 60 min.

significant difference [Fig. 5(d)]. These findings suggested that VER enhanced but COL weakened DIG toxicity to the renal cells on the chip and proved that degree of nephrotoxicity was tightly related to drug absorption in the intestine.

D. Drug-induced toxicity on GEC permeability

Glomerular filtration is the key indicator of renal function, which is directly determined by the permeability of the glomerular barrier. The glomerular barrier is a dynamic and highly selective filter and essential to regulate the filtration of large molecules.[39] Here, we explored the permeability of the GEC layer to large molecules with drug combinations by using fluorescein tracer IgG (MW = 150 kD, 250 μM) in our model, representing large molecules.

The diffusion of the fluorescein tracer through the GEC layer in dynamic conditions is shown in Fig. 6(a). After exposure to different drug combinations, the diffusion of IgG was monitored at 15, 30, and 60 min. Quantification of fluorescence demonstrated increased leakage with VER treatment but decreased with COL [Fig. 6(b)], which was in agreement with our above data.

We assessed the permeability of albumin through the GEC layer as well, which could be observed under physiological conditions. In this case, albumin with molecular weight approximately 70 kD was applied to our chip. As a large molecule, albumin is the most abundant plasma protein in blood, and a small fraction of albumin may pass through the slit pores in the body. Herein, albumin permeability under different drug combinations was analyzed based on our chip. More specifically, a similar change was observed in the permeation of albumin [Fig. 6(c)].

Taken together, all of these findings reveal much detail about the difference in nephrotoxicity caused by various drug combinations and offer significant evidence that the intestine-kidney chip could simulate the process of drug absorption and nephrotoxicity *in vitro*.

IV. CONCLUSION

In this work, we developed a novel integrated intestine-kidney chip for the assessment of drug toxicity in consideration of the influence of drug absorption on nephrotoxicity. We chose

DIG combined with COL or VER as the model to explore the effects of drug absorption by the intestine on renal toxicity *in vitro*. Drug absorption in the intestine displayed great variance. Our system demonstrated that DIG combined with VER significantly induced nephrotoxicity in GECs, while DIG and COL exposure markedly decreased drug toxicity on the kidney. Obviously, drug absorption is of great concern in drug toxicity assessment. The intestine-kidney chip recapitulates drug nephrotoxicity following drug absorption in a manner not possible by conventional methods and reveals great advantages in early, preclinical prediction of nephrotoxicity. It may provide a promising and cost-effective alternative *in vitro* method for drug testing and development.

SUPPLEMENTARY MATERIAL

See supplementary material for the drug-induced cytotoxicity in GECs and Caco-2 cells after exposure to different drugs.

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