Jungbin Yoon @Jungbin_Y · Jul 12

It's a great honor to have an opportunity to share my latest research (biofabrication of gut-kidney integrative multiorgan-on-a-chip) with everyone. Please leave the comments or questions if you have any. Thank you :) #Biofab2023 @ISBioFab

2 12 846

You Retweeted

에버랜드 @withEverland · Jul 11

[최초공개] 에버랜드 쌍둥이 아기 판다 탄생 현장.jpg

Establishment of microfluidic *in vitro* multiorgan model using 3D bioprinting technique for understanding the intestine-kidney axis related in secondary hyperoxaluria disease

Author list: Jungbin Yoon[1], Dong-Woo Cho[1], and Jinah Jang[1,2]

Affiliation: [1] Department of Mechanical Engineering, Pohang University of Science and Technology, Pohang, Republic of Korea [2] Department of Convergence IT Engineering, Pohang University of Science and Technology, Pohang, Republic of Korea

Acknowledgements: This work was supported by the Korea Institute for Advancement of Technology (KIAT) and the Ministry of Trade, Industry & Energy (MOTIE) of the Republic of Korea (No. P0021109).

Abstract: Secondary hyperoxaluria is a multifactorial disorder that extends from an inflamed intestine with oxalate malabsorption to kidney stone disease. Studying the intestine-kidney axis has been challenging due to lacking *in vitro* multiorgan model that describes dynamic interactions between the native intestine and the proximal tubule. Here, we used multi-biofabrication techniques to address the challenge and successfully developed *in vitro* multiorgan model with enhanced fluidic connectivity between organ modules. The improved biophysical properties of *in vitro* multiorgan model recapitulated the critical pathophysiology of secondary hyperoxaluria, including intestine damage, calcium oxalate crystallization, and kidney injuries. Further, the efficiency of *in vitro* model was validated by assessing damage recoveries from both organs after perfusing the device with drugs such as trisodium citrate and grape seed extract. Therefore, this optimized *in vitro* multiorgan model could be a promising platform for discovering integrative therapeutics to reverse intestinal inflammation and kidney stone disease in a single assay.

Introduction/Background: **Secondary Hyperoxaluria (SH)** is a multifactorial disease that affects the large intestines and kidneys. The SH spectrum extends from the inflamed intestinal epithelium with oxalate malabsorption to recurrent kidney stone formation, followed by severe kidney infections to chronic kidney disease. SH is diagnosed when excess ingested oxalate is absorbed into the inflamed gastrointestinal (GI) tract and excreted in the urine. Most oxalates in the circulatory system are filtered into the glomerulus of a nephron, and the oversaturated glomerular filtrate with oxalate and calcium ions most likely form calcium oxalate (CaOx) stones inside the nephron. CaOx crystals can be easily precipitated within the proximal tubule (PT) because most oxalates in the glomerular filtrate are metabolized in the PT. The CaOx crystals bind to the tubular epithelial cells and obstruct filtration. Further, approximately 5% of all patients with oxalate malabsorption issues are diagnosed with recurrent kidney stone disease. This has motivated the development of an integrative 3D *in vitro* SH disease model that can precisely mimic oxalate malabsorption-related intestinal epithelium and kidney stone formation.

METHODS

Studying the **intestine-kidney axis** has been challenging due to lacking *in vitro* multiorgan model that describes dynamic interactions between the native intestine and the proximal tubule. Here, we used multi-biofabrication techniques to address the challenge and successfully developed an *in vitro* multiorgan model. In brief, an *in vitro* multiorgan model is designed to separate two functional organ units (intestinal epithelium and vascularized PT) that are spatially separated but physiologically interconnected. First, a housing casket is fabricated to compartmentalize intestinal epithelium and vascularized PT models. The co-axial HK-2 (human PT epithelial) cell bioprinting of 3D-PT (3D-PT) and bioprinted co-axial GE (human glomerular endothelial) cell-bioprinted 3D PV adjacent to 3D-PT are established using multiple biomaterials and 3D bioprinting techniques. Human intestinal epithelial cells (Caco-2s) are seeded on a Transwell insert, and a single insert is placed inside the *in vitro* multiorgan model. Overall, the Transwell insert is expected to recapitulate the intestinal barrier of the native intestinal villus. Finally, the intestinal epithelium and 3D bioprinted kidney units are uniquely assembled in a **single** *in vitro* multiorgan model (**Fig. 1**).

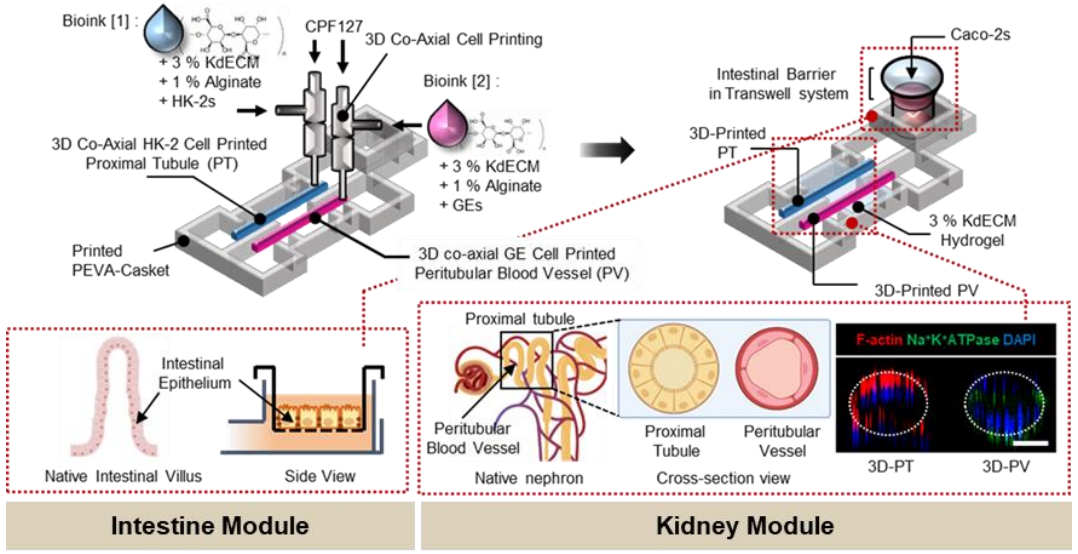

Figure 1.
Schematic diagram of *in vitro* multiorgan model. A vascularized proximal tubule module and intestinal barrier model were fluidically interconnected in single *in vitro* platform *All images are quoted from Yoon et al., Appl. Phys. Rev. (2022)*

RESULTS & DISCUSSIONS

A microfluidic system is added to the ***in vitro* multiorgan model** on the fourth day after biofabrication. The mixed media are perfused into the *in vitro* multiorgan model; the media are circulated to each unit and returned to each media reservoir (**Fig. 2**). The first perfusable circuit is designed to allow the media to pass through the intestinal model, subsequently through the open lumen of the 3D-PT structure, and then to return to the reservoir [1]. In the second independent circuit, the media flowed into the hollow lumen of the 3D peritubular vascular system and circulated back to the reservoir [2] (**Figs. 2A, B**). The maturities of selected intestine-specific and vascularized proximal tubule-specific genes were enhanced upon the microfluidic system in an *in vitro* multiorgan model. Hence **microfluidics** is a promising method for reducing the time required to create a multiorgan-integrated platform with qualified performance. In addition, this *in vitro* multiorgan model enabled the assessment of the absorptive function of the intestinal epithelium and the reabsorptive/excretory part of the PT in a single operation.

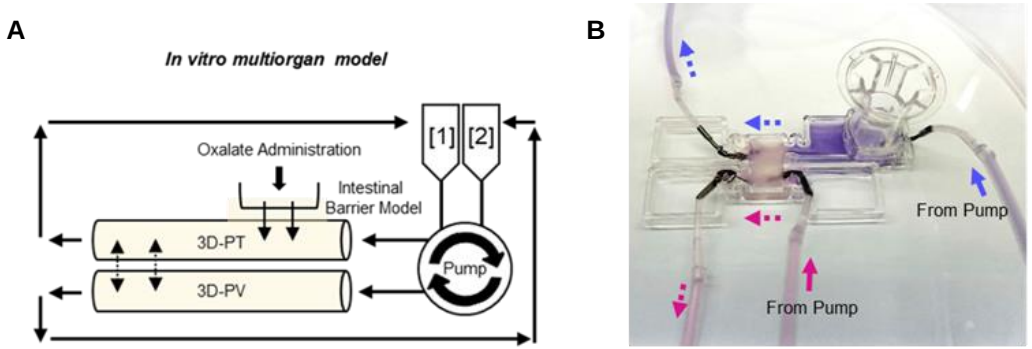

Figure 2.
A. Schematic shows media and drugs flow directions in the peristaltic pump connected to the *in vitro* multiorgan model.
B. Photograph of microfluidic *in vitro* multiorgan model. *All images are quoted from Yoon et al., Appl. Phys. Rev. (2022)*

The improved biophysical properties of *in vitro* multiorgan model recapitulated the critical pathophysiology of secondary hyperoxaluria, including **intestine damage**, **calcium oxalate crystallization**, and **kidney injuries**. In the Transwell insert, oxalate malabsorption is recapitulated by inducing an intestinal barrier disruption (that is shown in IBD patients) by treating it with an inflammation inducer, TNF-α (100 ng/ml), for 24 h on day seven (**Figs. 3A, B**). Furthermore, the excess oxalate ions freely entered the open lumen of the PT. They induced CaOx crystallization after perfusing in vitro multiorgan model with Na_2Ox (0.04 mmol) and $CaCl_2$ (12.0 mmol) from days eight to ten (**Figs. 3C, D,** and **F**). The three-dimensional confocal image on day ten showed that precipitated CaOx crystals increased PT epithelial cell death (**Fig. 3E**). These mimicries successfully resemble intestine leakage and recurrent kidney stone disease-related kidney injuries on ***in vitro* SH model**.

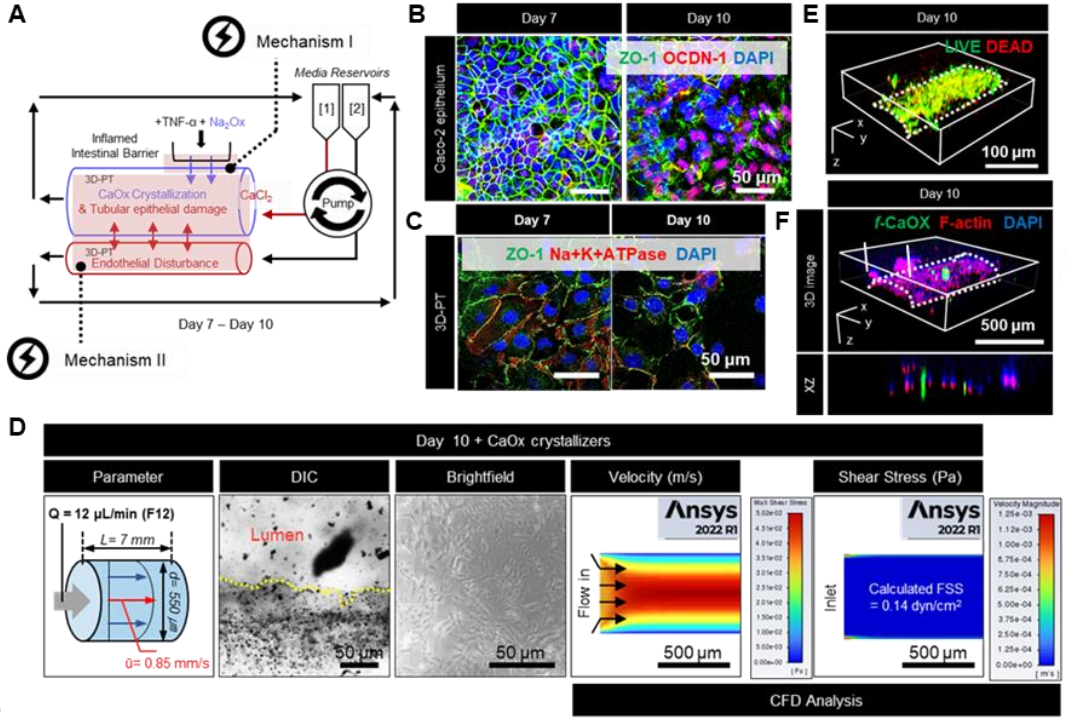

Figure 4.

A. Timeline for establishing complex SH condition on the *in vitro* multiorgan model.
B. Confocal images of Caco-2 epithelium in the Transwell inserts.
C. Confocal images of 3D PT epithelium.
D. Various analyses (imaging, CFD simulation) on a perfusable 3D PT.
E. Live/dead assays for perfusable 3D-PT
F. 3D confocal image of 3D-PT with green-colored CaOx crystals. *All images are quoted from Yoon et al., Appl. Phys. Rev. (2022)*

The novel *in vitro* SH construct can be actively used for **testing potential drugs** or therapeutics to cure integrative SH diseases. Thus far, most kidney stones (0.5 cm or larger) have been removed surgically. Other treatment options for CaOx stone disease remain limited. Currently, there exist no specific pharmacological therapies for SH. Citrate treatment has been widely used in clinical studies to reverse CaOx crystallization. Citrate also interferes with the inflammatory cascade implicated in IBD when citrate is delivered to the site of intestinal inflammation. Here, trisodium citrate (TC; $Na_3C_6H_5O_7$) is perfused at increasing concentrations (0.2 M and 1.6M) in the *in vitro* SH model for three days (days 10–13) to validate the systemic performance of the *in vitro* SH model as a drug testing platform (**Fig. 5A**). Using this *in vitro* SH model, the **recovery** of TC from the CaOx crystal-induced damage is observed (**Figs. 5B, C**). Combined with antioxidant grape seed extract (GSE), the drug is successfully transported to the intestinal barrier unit and then moved to the fluidically linked vascularized PT unit, finally showing intestinal disruption recovery and CaOx crystal dissolution (**Fig. 5D**).

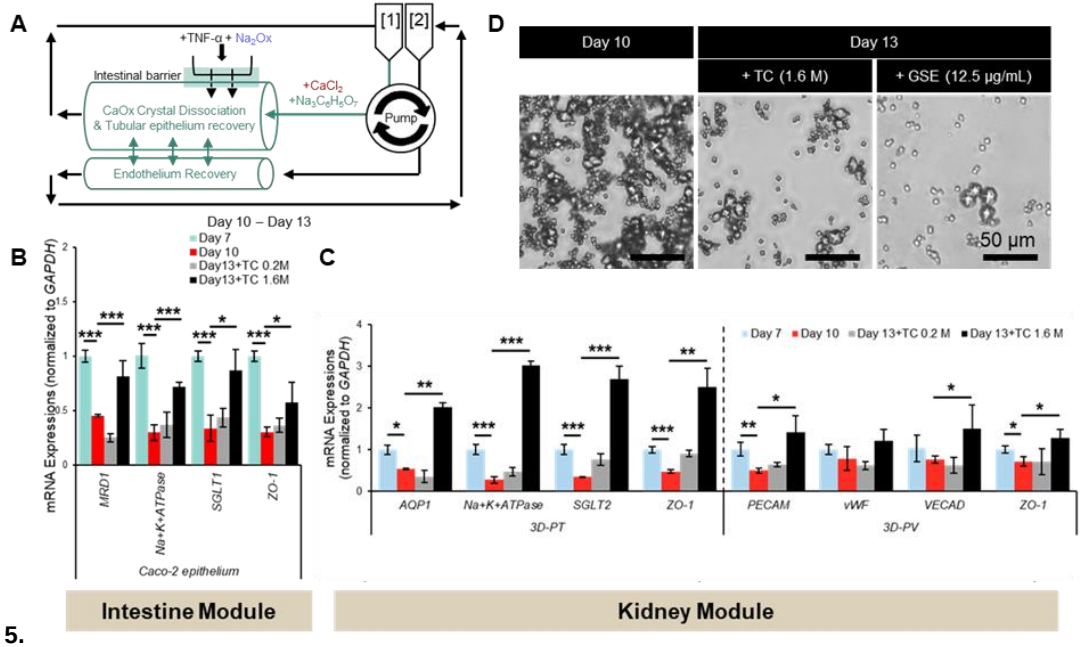

Figure 5.

A. Timeline for reversing the progression of SH by utilizing *in vitro* SH model.
B. Gene expression examination on the intestinal barrier using intestinal barrier integrity-related markers after TC perfusions.
C. Gene expression quantifications on 3D vascularized PT model (3D-PT) after TC perfusions.
D. Microscopic images of the collected CaOx crystals after TC and GSE perfusions. *All images are quoted from Yoon et al., Appl. Phys. Rev. (2022)*

CONCLUSIONS

This study successfully mimicked the key pathophysiological features of SH in a perfusable *in vitro* multiorgan model. In addition, 3D coaxial cell printing technology and microfluidic approaches were actively used to enhance the systemic performance of fluidically interconnected intestinal epithelium and vascularized PTs in an integrative *in vitro* multiorgan model.

Based on these two organ interconnections, the leaky intestinal barrier, CaOx crystallization, and CaOx crystal induced proximal tubular injuries—often diagnosed in SH patients—were reflected in the *in vitro* SH model. Finally, after testing with TC and GSE, the efficacy and systemic responses of the SH platform were validated by observing intestinal epithelium damage recovery and CaOx crystal dissolution.

This integrative *in vitro* multiorgan model highlighted the interconnected communication between the intestine and kidney in a single platform. This phenomenon is like the complexity of human systems *in vivo*; this success allows the creation of a novel *in vitro* SH model. Improved systemic performance of the *in vitro* SH model is achieved by adapting the advantages of 3D bioprinting and microfluidic technologies. The *in vitro* SH model provides expanded opportunities for investigating the efficacy of many potential SH therapies over a brief period.

Yoon et al., Appl. Phys. Rev. (2022)