Bioprinting in Renal Regenerative Medicine

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Contributor: Chrysanthos D. Christou , Stella Vasileiadou , Georgios Sotiroudis , Georgios Tsoulfas

In this new era of technological advancement, three-dimensional (3D) printing has emerged in medicine, promising to revolutionize surgical practices. Three-dimensional printing could be defined as “translating” a digital image into a 3D solid object by printing consecutive thin layers of materials. The fusion of tissue engineering and 3D printing has given rise to bioprinting. This technique employs biocompatible printers and "bio-ink" to create intricate tissue structures, while the complete fabrication of functional organs remains a research objective. 3D bioprinting has already shown promising results, especially in the field of microfluidic devices with the development of tissues demonstrating proximal tubules, glomerulus, and tubuloinerstitium functions. Such models could be applied in renal disease modeling and during drug development for nephrotoxicity investigation.

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1. Introduction

Renal transplantation constitutes the most commonly performed solid organ transplantation. Specifically, the Global Observatory on Donation and Transplantation estimated there were 80,926 renal transplantations (32% from living donors) conducted in 2020, accounting for 62.4% of global transplantation activity [1]. For patients with end-stage kidney disease (ESKD), renal transplantation with a living or deceased donor transplant remains the treatment of choice when compared with peritoneal dialysis or hemodialysis since it provides substantially greater quality of life and is associated with lower long-term morbidity and mortality [2]. Nevertheless, renal transplantation is still associated with various postoperative complications, including urological complications (urine leak and urinary obstruction), peritransplant fluid collections (hematomas, lymphoceles, urinomas, and abscesses), vascular complications (renal artery stenosis, renal artery thrombosis, arteriovenous fistulas and pseudoaneurysms, renal vein thrombosis), calculous disease, neoplasms, gastrointestinal complications, and herniation complications [3]. The introduction of novel technologies and the improvements in medical imaging and surgical techniques have significantly lowered the prevalence of these complications, ameliorating their negative impact on the surgical outcome.

In this new era of technological advancement, three-dimensional (3D) printing has emerged in medicine, promising to revolutionize surgical practices. Three-dimensional printing could be defined as “translating” a digital image into a 3D solid object by printing consecutive thin layers of materials [4]. Originally, 3D printing materialized in non-medical disciplines to serve the pressing demands of rapid engineering of prototypes. However, it has since expanded to other disciplines, including surgery, where 3D printing has been used for educational purposes to

facilitate the comprehension of complex anatomy, for preoperative planning, and particularly for operations involving complex vasculature, for crafting customized surgical tools, and for patient counseling [5][6].

Despite the expansion of selection criteria, including “marginal” renal grafts from substandard donors, renal transplantation is limited by the shortage of transplants [7]. Specifically, in the US, only 25% of the waitlisted patients receive a transplant within five years, with patients being removed from the list due to deterioration of health or premature death [7]. Thus, the lack of donors worsens the already vast healthcare burden associated with ESKD patients on dialysis. Therefore, justifiably, kidney regeneration has been a long-standing challenge for tissue engineering. The fusion of tissue engineering and 3D printing has given rise to bioprinting [8]. This technique employs biocompatible printers and “bio-ink” to create intricate tissue structures, while the complete fabrication of functional organs remains a research objective. Bioprinting achieves the fabrication of structures of precise internal and external architecture that provide high cell viability and imitation of natural tissue features (biomimicry) [9][10].

2. 3D Bioprinting Is Employed in Renal Regenerative Medicine

Renal transplantation, despite being the gold standard, intriguingly it is also a halfway measure since it does not address the underlying disease while, at the same time, it does not cure the patient rather than transforming and lessening the morbidity from that of chronic dialysis to the morbidity of long-term immunosuppression therapies. Except for leaving patients vulnerable to opportunistic infections and predisposing to malignancy development, immunosuppression therapy is a main alloantigen-independent factor in renal chronic allograft nephropathy [11][12]. Up to 50% of kidney transplanted patients lose the graft due to chronic allograft nephropathy within ten years from transplantation [13]. Kidneys are particularly complex organs with more than 20 different cell types [14]. Bioprinting a kidney whose cell lines retain their viability and functionality long-term is a herculean task. The 3D bioprinting approach holds potential due to its ability to achieve detailed structures, which may lead to better biomimicry [15]. For organ 3D bioprinting, two different strategies have emerged: scaffold-based and scaffold-free [16]. While revolutionary, 3D bioprinting is still in its foundational stages, especially concerning the production of complex structures. Some of the primary challenges include ensuring vascularization, creating a functional nephron unit, and addressing the intricate balance of cellular interactions. Additionally, the issue of scalability and reproducibility across different bioprinting platforms poses significant hurdles. Currently, the field is seeing advancements primarily in microfluidic device development that demonstrate renal function, which represents a more immediate and tangible step towards replicating kidney function. In this section, the related literature where 3D bioprinting is employed in developing renal structures has been identified and presented.

Table 1 summarizes the identified studies where 3D bioprinting was employed in the development of renal cultures/tissues. The first study utilizing 3D bioprinting to develop convoluted renal proximal tubules in vitro was published in 2016 [17]. Homan KA Et al. developed perfusable microfluidic-based chips that housed renal proximal tubules that were fully embedded in an extracellular matrix [17]. The proximal tubules were characterized by an open-lumen architecture, which was circumscribed by proximal tubule epithelial cells that maintained cell viability and functionality for over two months [17]. During printing, a fugitive ink (containing a triblock copolymer of

polyethylene-polypropylene-polyethylene and thrombin) was used that was then removed before cell seeding. Gene expression analysis of 33 key proximal tubule epithelial cells genes revealed cells that these cells were transcriptionally similar to primary renal proximal tubule epithelial cells [17]. Finally, the researchers demonstrated how their model could be used to investigate drug-induced tubule damage mechanisms by successfully inducing dose-dependent tubular damage using cyclosporine A [17]. Notably, their model lacked vasculature, limiting its application in renal reabsorption studies. In 2019, researchers from the same department published a study aiming to develop a 3D bioprinted a microfluidic-based vascularized proximal tubules model, embedded in extracellular matrix, to investigate the reabsorption of solutes via tubular-vascular exchange [18]. Notably, the markers observed confirmed the presence of endothelial tissue and the perfused model demonstrated active reabsorption of albumin and glucose [18]. Additionally, the researchers explored the role of the model in disease modeling by inducing hyperglycemic conditions and monitoring endothelial cell dysfunction [18].

Table 1. Studies developing 3D-bioprinted renal models.

	First Author	Cell Lines-Subjects	Printer Type/Bioink	Printing Strategy	Aim	Results
1.	Homan KA. [17]	PTEC	AGB 10000, (©Aerotech Inc., Pittsburgh, PA, USA)/gelatin-fibrin hydrogel, fugitive ink, silicone elastomer	Scaffold based	Develop 3D convoluted renal proximal tubules within microfluidic chips	The microfluidic-based model showed high cell viability, gene expression pattern close to primary renal PTEC, and superior functional albumin uptake compared with 2D controls
2.	Lin NYC. [18]	PTEC, vascular endothelial cells	3D-Bioplotter (©EnvisionTEC)/gelatin-fibrin-based ECM, fugitive ink	Scaffold based	Vascularized proximal tubules (microfluidic platform) demonstrating reabsorption of solutes (tubular-vascular exchange)	The model demonstrated active albumin and glucose reabsorption.
3.	King MS [19]	HUVEC, adult, renal, fibroblast, and renal PTEC	NovoGen Bioprinter Instrument (©Organovo Inc., San Diego, CA, USA)/NovoGel Bio-Ink	Scaffold based	Develop a renal proximal tubule model in vitro supported by renal fibroblast and endothelial cells.	The model demonstrated functions of the native proximal tubule, drug-induced

	First Author	Cell Lines-Subjects	Printer Type/Bioink	Printing Strategy	Aim	Results
						nephrotoxicity, and renal fibrosis.
4.	Ali M. [20]	Porcine kidneys/human primary kidney cells	ITOP system/KdECMMA-based	Scaffold based	Investigate the role of KdECMMA-based bio-ink in supporting 3D bioprinted renal constructs from human primary kidney cells	The constructs demonstrated high cell viability, and significantly higher sodium reabsorption and hydrolase activity compared to the control group.
5.	Addario G. [21]	pmTEC, HUVEC fibroblasts	Microfluidic bioprinter (©RX1 Aspect Biosystems, Canada)/alginate, gelatin, pectin	Scaffold based	Development of a microfluidic-based tubulointerstitium model for in-vitro studies	The authors achieved to develop multiple models of different cell-line/bio-ink formulations comparing the cell viability and metabolic activity of the various constructs
6.	Lawlor KT. [22]	hPSCs	NovoGen MMX extrusion-based 3D cellular bioprinter (©Organovo Inc., San Diego, CA, USA)/Cellular Bio-Ink.	Scaffold free	Develop renal organoids of highly reproducible cell number and viability by extrusion-based 3D cellular bioprinting.	Achieved the formation of renal organoids demonstrating a high resemblance to nephron histology, high reproducibility/cell viability, and drug-induced nephrotoxicity
7.	Jo H. [23]	Autologous omentum tissue/UUO Rats	Dr. INVIVO (©ROKIT Healthcare, Inc., Seoul, Korea)/fibrinogen, thrombin	Scaffold free	Transplantation of an autologous omentum patch in the renal subcapsular space for immune regulation and tissue regeneration	Reduced tubular injury and downregulation of fibrosis-inducing mechanisms were observed in the omentum patch group.

	First Author	Cell Lines-Subjects	Printer Type/Bioink	Printing Strategy	Aim	Results
8.	Singh NK. [24]	Porcine kidneys, hBMMSC, renal PTEC, and HUVEC, UUO mice	In-house developed 3D cell-printing system/decellularized ECMs, alginate, pluronic	Scaffold based	Develop a 3D microfluidic vascularized renal tubular tissue-on-a-chip. Transplant grafts in UUO mice	Perfusable tubular constructs were developed with the ability to switch between monolayer and bilayer. Markers of tissue maturation were observed regarding renal tubular tissue and vascular tissue. UUO

Abbreviations: ECMs: extracellular matrices, hBMMSC: human bone marrow-derived mesenchymal stem cells, hPSCs: human pluripotent stem cells, HUVEC: human umbilical vein endothelial cells, ITOP: integrated tissue-organ printing, KdECMMA: photo-crosslinkable kidney extracellular matrix, pmTEC: primary murine tubular epithelial cells, PTEC: proximal tubule epithelial cells, UUO: unilateral ureteral obstruction.

Proximal tubules constitute the main site of nephrotoxicity, and thus, 3D bioprinted renal proximal tubule constructs could be applied during drug screening. In a study by King SM et al., the role of 3D bioprinting in producing constructs regarding drug-induced tubular damage was further investigated by developing fully cellular human in vitro proximal tubule interstitial interface that consisted of primary human renal proximal tubule epithelial cells supported by interstitial cell types including fibroblast and endothelial cells aiming to provide a microenvironment that supports the health and function of the polarized epithelia [19]. Notably, following 30 days of culture, the tissues demonstrated sufficient metabolic activity with stable levels of expression of many important renal transporters and a viable intrarenal renin-angiotensin system [19]. In addition, the 3D printed tubules demonstrated cisplatin-dependent nephrotoxicity and a TGFβ induced fibrotic response [19]. Such models could be employed in the early stages of the drug development pipeline to reduce the occurrence of costly failures at the late stages of development. In addition, it is highlighted that the choice of the bio-ink that will encapsulate the 3D bioprinted cells is crucial in their long-term viability and functionality [25].

In a study Addario G et al. aimed to develop microfluidic-based renal tubulointerstitium models for in-vitro studies employing primary murine tubular cells, endothelial cells, and fibroblasts using a microfluidic 3D bioprinter [21]. The effect of different materials of the bio-ink was investigated, with a recorded cell viability on day 7 of >91% and >82%, for alginate-based and pectin-based bio-ink, respectively [21]. Limited growth and gradual death of endothelial cells was observed when cultured in a medium lacking the vascular endothelial growth factor, highlighting the essential role of the bio-ink in the support and maturation of the cell lines used. In a different study by Ali M et al., the role of porcine kidney extracellular matrix-derived bio-ink in facilitating renal tissue formation and maturation was investigated [20]. Initially, the porcine kidneys were decellularized while the extracellular matrix was

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presented that the matrices were through solubilization and methcrylation to derive photo-crosslinkable hydrogels [20]. The derived hydrogels were tested using a Quantibody Growth Factor Array, which revealed that despite the processing, the hydrogels maintained a plethora of cytokines and growth factors [20]. The hydrogels were used to formulate a bio-ink, which was then tested for its ability to support the cell viability, proliferation, and adhesion of human primary kidney cells [20]. The bio-ink allowed for a high proliferation with an increase in the number of cells on days 1, 3, and 5 of cell cultures, whereas in the control group (gelatin methacrylate was used), a gradual decrease in the number of cells was observed. Additionally, the cell viability was higher than 95% [20]. Finally, 3D-bioprinted renal constructs were developed, mixing the human primary kidney cells with the derived bio-ink. Notably, a ~90% cell viability at day 14 was observed, while at the same time, the bioprinted construct demonstrated at day 14 a significant amount of sodium uptake and significantly higher hydrolase activity when compared to control constructs [20].

3D bioprinted renal organoids can be fabricated for renal disease models, during drug development and screening, and in renal regenerating medicine. In a study by Lawlor TK et al., the role of cellular extrusion bioprinting was explored in providing rapid and high throughput generation of kidney organoids with high cell viability [22]. Employing human pluripotent stem cells, they manage to produce 3D-printed organoids that, within 20 days of culture, formed nephrons with the presence of podocytes, proximal tubules, distal tubules, loop of Henle thick ascending limb, connecting segments, and additional cellular components including endothelial cells and renal stroma [22]. The researchers investigated how changing various bioprinting parameters, including well format, the speed of tip movement for a given rate of cell extrusion, and the organoid conformation, affect the properties of the resulting organoids in terms of tissue thickness, coefficient of differentiation, and nephron number [22]. Notably, changing to a 3D bioprinted tubular conformation demonstrated elevated nephron number [22]. The researchers evaluated cell viability following ramipril exposure, which significantly decreased in 2D-induced nephrotoxicity [22]. Renal organoids have been proven more effective in predicting drug-induced nephrotoxicity compared to 2D cultures of renal proximal tubule epithelial cells due to their rapid differentiation and loss of key transporters and metabolic enzymes [26][27]. Finally, the researchers managed to generate a kidney patch that contained 4×10^6 cells across a total field of approximately 4.8 × 6 mm [22]. Studies have reported the vascularization and maturation of such organoids following transplantation under the renal capsules of mice [28].

Therefore, except for bioprinting transplantable kidneys, 3D bioprinting could be used in the management of ESKD in regenerative medicine by partially restoring renal function. Intriguingly, restoring as little as ten percent of the renal function could allow patients with ESKD to disengage from dialysis, significantly improving their quality of life [29]. In a recent study, Jo H. et al. developed an autologous omentum patch to investigate its role in the treatment of ESKD [23]. Specifically, the researchers investigated the effect of transplanting the omentum patch in the renal subcapsular space of rats suffering from unilateral ureter obstruction-induced kidney injury [23]. Initially, the researchers utilized autologous omentum tissue, fibrinogen, and thrombin to fabricate two bio-inks [23]. An artificial intelligence tool generated the omentum patches design, printed using a bioprinter [23]. Two weeks after transplantation, renal tubular damage, and fibrosis-related gene expression were measured [23]. In the omentum patch group, decreased tubular damage and under-regulation of fibrotic mechanisms were observed compared to a group of rats transplanted with the fibrin patch group [23]. In a different study by Singh KN et al., the therapeutic

role of transplanted vascularized tubular tissue in a chronic renal disease model was investigated [24]. Initially, porcine kidneys were decellularized and lyophilized to prepare a solution of extracellular matrices, then mixed with sodium alginate to produce a hybrid bio-ink [24]. Along with the bio-ink, human bone marrow-derived mesenchymal stem cells, renal proximal tubular epithelial cells, and human umbilical vein endothelial cells were used in 3D coaxial bioprinting of monolayer and bilayer complex hollow structures [24]. Following four weeks of culture using vascularized renal proximal tubule-on-a-chip conditions, the grafts were transplanted into the renal subcapsular part of unilateral ureter obstruction-modeled immunodeficient mice [24]. Two weeks following transplantation, the unilateral ureter obstruction transplanted models demonstrated decreased expression of alpha smooth-muscle actin and elevated expression of aquaporin 1 compared with the non-transplanted models and also expression of markers indicating neovascularization [24].

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3. Alternative promising approaches for the management of ESKD

ESKD approaches pandemic proportions, which will deteriorate the disequilibrium between available grafts and the demand for transplantable organs. The application of regenerative medicine and bioengineering, including 3D bioprinting, could lead to a new era in renal transplantation. 3D bioprinting has already shown promising results, especially in the field of microfluidic devices with the development of tissues demonstrating proximal tubules, glomerulus, and tubuloinerstitium functions. Such models could be applied in renal disease modeling and during drug development for nephrotoxicity investigation. Finally, focusing on transplantation, studies employing 3D bioprintable tissues for the management of ESKD have demonstrated promising results in animal models restoring part of the renal function.

Alternative promising approaches for the management of ESKD are the use of wearable and implantable artificial kidney devices and xenotransplantation. Wearable hemodialysis devices have achieved proof-of-concept in human clinical trials, while implantable hemodialysis devices have not yet reached human trials. Wearable hemodialysis devices aim to provide continuous renal replacement therapy, achieving higher solute clearance than standard hemodialysis. While wearable and implantable artificial kidney devices demonstrate promising results and, in terms of scalability, could be the most practical approach for ESKD management, they still face several challenges, including the engineering challenge of miniaturizing the devices, optimizing sorbent materials, patient suitability and accessibility, preventive anticoagulation for long-term patency, microbiological contamination, and long-term effectiveness.

Renal xenotransplantation of genetically engineered pigs for human xenotransplantation has, on the other hand, already reached pre-clinical phases and is closer to addressing the graft shortage compared to 3D bioprinting, where the research is still at a founding stage. Specifically, in a recent study, Porrett et al. performed bilateral native nephrectomies in a human brain-dead decedent and then transplanted two bioengineered renal grafts. Notably, the decedent remained hemodynamically stable through reperfusion; no hyperacute rejection or porcine virus transmission was observed, while the kidneys retained viability until termination 74 hours later. In a different study by Montgomery et al., genetically engineered pig kidneys were transplanted into two brain-deaded human

recipients, demonstrating urine and creatinine output following reperfusion without signs of hyperacute rejection. Nevertheless, many challenges are still associated with renal xenotransplantation, including long-term viability and functionality, immunological barriers, the risk of zoonotic diseases, ethical and moral concerns, public acceptance, cost, and accessibility.