The Regeneration of Renal Tissue

by Hannah Thompson

Abstract: As of today, there are approximately 100,791 individuals waiting for kidney transplants, with a new patient added every fourteen minutes.[33] Treatments for renal failure and kidney disease are limited to dialysis, renal replacement therapy, or transplantation. However, advancements in renal regeneration diminish the need for these transplants. Several techniques allow for kidney recovery after development of renal disease, including bioengineering mechanisms, stem cell therapies, drug therapeutics, and renal regenerative techniques. In this review, the role of induced regeneration will be discussed in relation to renal damage. The mechanisms of embryonic kidney development have been elucidated, allowing researchers to recreate self-organizing renal tissue and nephrons by using human pluripotent stem cells. Although kidneys have a limited regenerative ability, understanding the mechanisms of developmental biology and kidney morphology have allowed researchers to identify the process of self-renewing damaged renal tissue. The role of regenerative medicine in repairing damaged kidneys serve as a model of hope for the hundreds of thousands affected by kidney illnesses.

Introduction and Objectives

Renal disease and illness are worldwide public health issues due to the shortage of organ donors and treatments available.[1] These illnesses and diseases arise when renal structures, such as nephrons, are unable to perform their function.

Kidney disease is one of the most common illnesses, with an astonishing one in three American adults at risk for developing it.[5] This illness can lead to end-stage renal disease (ESRD), commonly known as kidney failure, resulting in a complete loss of renal function. Other common illnesses include malignant hypertension, diabetes, glomerular disease, tubular diseases, and interstitial diseases.

The renal system, composed of several organs, such as the kidneys, ureters, bladder, and urethra, works to maintain homeostasis of the human body.[2] The kidneys, specifically, are responsible for the filtration of bodily fluids and excretion of waste in the form of urine.[3] The kidneys also serve to maintain metabolic (chemical), hemodynamic (cardiovascular), immunologic (immunity), and endocrinologic (hormone) levels of the body.

Each kidney consists of approximately one million functional units referred to as nephrons, which are responsible for filtering blood through two structures, the glomerulus and tubule. Glomeruli filter proteins, blood cells, and other large molecules from fluid that passes through them; this fluid then proceeds to the proximal tubule where urine is produced, waste is removed, and minerals are returned back to the bloodstream.[4] Any illness involving these structures (Figure 1) is serious and potentially life threatening. Traditionally, the treatment for kidney diseases, such as ESRD, is either dialysis or a kidney transplant. However, an imbalance exists between patients requiring treatment and available treatments. The lack of kidneys available for transplant and deficiencies in dialysis result in over 89,000 deaths each year. The possibility of the regeneration of renal tissue brings to light a new possibility in this dreadful reality.

Renal regeneration allows for the repair and regrowth of renal structures, resulting in the restoration of function.[6, 7] Nephrons form during embryonic development, and thus regeneration as an adult is impossible. However, animal models provide a better understanding of neoneprogenesis, a mechanism that results in the formation of new nephrons. Identifying these animal models and understanding kidney morphogenesis has allowed

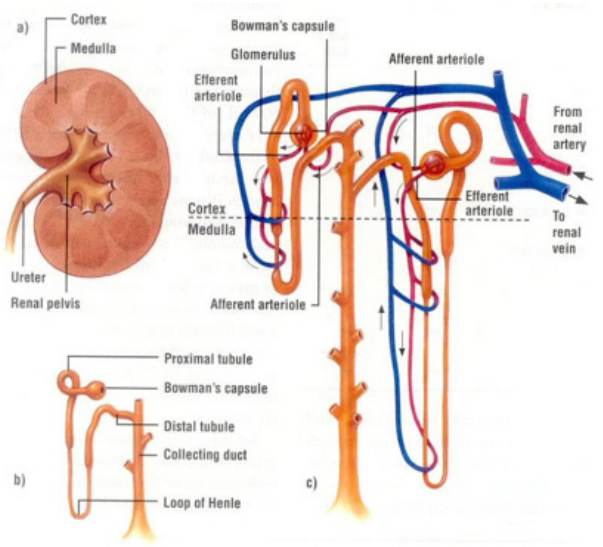

Figure 1. Depicted in this diagram is the filtering units of the kidney, specifically the glomerulus and proximal tubule.[29]

for advancements in the growth of kidney cells through human pluripotent stem cells.[1, 8, 9, 10] Stem cells are currently being explored in both human and animal models. In the future, stem cell therapies may be used to halt the progression of disease and illness in patients who are diagnosed with other renal diseases but have not yet developed ESRD.

A common symptom of kidney diseases includes the inability of the vasculature to function properly; without this efficient blood flow, the renal system is unable to maintain homeostasis. Angiogenesis, the formation of new blood vessels from preexisting ones, allows for the restoration of this crucial function of the renal system. Bioengineering uses tissue decellularization processes to manipulate genetic material and create artificial limbs, tissues, or organs. Three dimensional bioprinting, an aspect of bioengineering, involves the growth and development of bodily structures using biological material and engineered systems.

This review will discuss renal regeneration in three objectives. Objective 1 will discuss the methods of embryonic renal development and its application to the use of stem cells in regenerating nephrons and renal structures. Objective 2 will discuss angiogenesis and other techniques used to prevent complete loss of renal function. Objective 3 will describe the future of renal regeneration, including drug therapeutics and bioengineering. These objectives all relate to a common goal of renal regeneration, combating against renal disease and illness.

Discussion

Objective 1: Embryonic Renal Development Applied to Stem Cell Use

Within the past decade, there has been great success with the use of stem cell treatments. Varying types of stem cells, such as induced pluripotent stem cell (iPSC), bone marrow-derived cells (BMDC), organ-specific stem cells, and adipose derived stem cells (ADMSC), are capable of differentiating into kidney cells.[7,11] These stem cells have the ability to mimic renal embryonic development through laboratory conditions similar to the environment of an embryo.[12] By identifying the processes of embryonic renal development and its physical and chemical environment, scientists are able to use stem cells to form nephrons.

Renal development begins with the primitive streak, a structure that gives rise to the gastrula and forms the mesoderm, which in turn arranges the metanephric kidney or final functioning kidney. This mesoderm forms several mesonephros, such as the ureteric bud, metanephric mesenchyme, and mesonephric duct (Figure 2).[8]

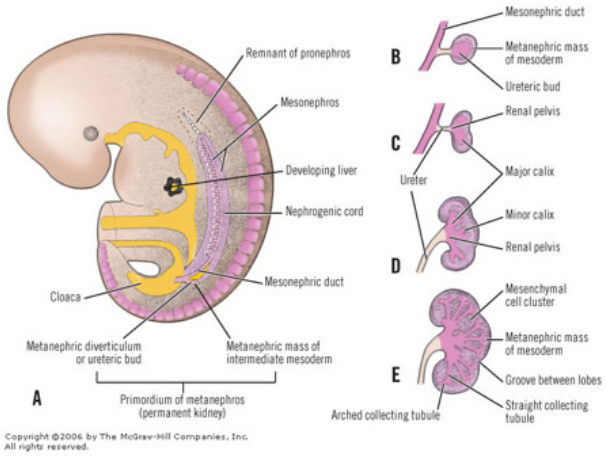

Figure 2. Depicted in this diagram are the mesonephros and the process these structures use to transform into the metanephric kidney.[28] Also depicted is the progression in which these mesonephros form the metanephric kidney.

The uretic bud is responsible for forming the collecting ducts, renal pelvis, ureter, and parts of the bladder. The metanephric mesenchyme develops into the glomeruli, proximal and distal tubules, and loops of Henle. The mesonephric duct forms the trigone of the bladder. It also releases the mesonephros into the cloacal, where one wall of the bladder is assembled. As previously mentioned, these structures all compose the renal system and contribute to its function in the human body.[7,4]

Signal transduction transfers information from the exterior of a cell to its interior using signal pathways and growth factors. The differentiation of cells then uses this mechanism by expressing particular genes. For instance, Wnt signaling pathways are responsible for gastrulation (the process by which a blastula forms into a gastrula), body axis formation, cellular differentiation, and cell migration. To function properly, Wnt signaling pathways must be activated by a Wnt protein. In addition to signal pathways, growth factors in the form of a protein or hormone allow for cellular differentiation. Bone morphogenetic proteins (BMP) are a type of transforming growth factor protein that specialize in the formation of bone, the heart, and central nervous system. Unlike the name suggests, this protein is crucial to the growth, regulation, and maintenance of several different cells.[7, 13] The expression of BMP7, for example, is crucial for the development of the kidney and other renal structures. This protein is found in high concentrations in cells found in the Bowman's capsule, the distal tubules and collecting ducts of the kidney. This protein may decrease in concentration or even disappear when disease strikes one's renal structures.[32] Nodal is another transforming growth factor protein that conducts a signal transfer using receptors that regulate gene expression. The binding of these receptors transfer information to the primitive streak and mesoderm.[7,14]

Using combinations of BMP, nodal protein, and signal transduction pathways, scientists have successfully used human pluripotent stem cells to develop the primitive streak.[30] Animal studies have suggested varying combinations of the growth factors and signal transduction pathways to develop this structure.[12] For instance, rats injected with mesenchymal stem cells showed a decrease in further renal damage as well as decreased symptoms

Processes Used for Renal Cell Replacement in Mice

Biological Subject Used	Damage to the Nephron	Process of Stem Cell Induction	Biological Mechanism	Targeted Cell Possible
Mouse adult HSCs	Acute Tubular Necrosis (the death of tubular epithelial cells)	Intravenous Transplantation	Cell Fusion, & Transdifferentiation	Proximal Tubular Cells
Mouse adult BMDCs	Acute/Subacute Glomerular Injury	Intravenous Transplantation	Bone Marrow Hemangioblast	Glomerular Endothelial
Mouse adult MSCs	Acute Tubular Necrosis	Intrarenal & Intravenous Transplantation	Plasticity	Glomerular Mesangial Cells

Figure 3. A variety of media conditions and processes can be used for renal cell replacement in mice.[7]

of hyperglycemia (excess of glucose in the body) and glycosuria (excess of sugar in waste).[15,31] Different stem cells, such as hematopoietic stem cells (which result in blood cells), bone marrow derived stem cells, and mesenchymal stem cells (stromal cells), can be used to prompt the response of a biological mechanism, which in turn can lead to the success of renal cell replacement (Figure 3).

Stem cells can be induced using either intravenous transplantation or intrarenal transplantation. Intravenous transplantation introduces the stem cells into veins, whereas intrarenal transplantation introduces the stem cells into renal structures. The damage to the nephron is caused by illnesses and diseases that lead to the loss of renal function. Although the specifics of media conditions vary, they all recognize that they must mimic the processes of forming the primitive streak in embryonic development in order to form it using stem cells. Through the process of stem cell induction and biological mechanisms, renal function can be restored.[7,14,15,31]

The structures and signal transduction methods mentioned are all components of the early development of an embryo. However, some components of the fully developed kidney are not expressed until later development. For the metanephric kidney to form, the final interactions between the nephric duct and metanephric mesenchyme must occur (Figure 2). These interactions allow the formation of the ureters through the migration of the nephric duct to the bladder. This is the final stage in embryonic renal development; when stem cells are used, this will also be the concluding step.[16]

Humans are unable to perform neonephrogenesis, the process by which organisms create new nephrons from their own cells after renal injury or damage has

occurred. However, animal models, such as zebrafish, can. The genomes of humans and zebrafish are quite similar. Over seventy percent of human genes have a similar gene sequence to that of zebrafish.[17] These genes are referred to as Orthologs and recognize that genes from different species evolve from common ancestors, but differentiate through speciation. Due to these similarities, zebrafish are a fitting model for studying renal regeneration in application to humans. This model has led to advancements in the research of the growth and production of human nephrons.

Zebrafish perform neonephrogenesis in two stages. The first stage involves the expeditious regeneration within existing nephrons that in turn replace the damaged nephrons. In the second stage, entirely new nephrons are created using renal progenitors.[18] Zebrafish are capable of regenerating nephrons that have been damaged by diseases, such as acute kidney injury (AKI).[18,19] To duplicate the conditions of AKI in humans, scientists administered nephrotoxins to the embryos of several zebrafish. This drug exposure created symptoms similar to AKI, including the loss of function in the proximal tubule.[17] As a result, zebrafish created entirely new nephrons to replace those damaged by the nephrotoxins. Researchers identified that 4-(phenlythio)-butanoic acid was produced when stimulated by the nephrotoxins. This acid increased the abundancy of renal progenitors that initiate the development of new nephrons. These renal progenitors were transplanted to secondary and tertiary recipients to confirm that progenitors have the ability for self-renewal. From this study, further research is being performed to determine if the induced treatment of 4-(phenlythio)-butanoic acid in human pluripotent stem cells serves any function in regeneration.[9, 17, 19] Scientists also identified that when a zebrafish experiences renal injury, mesenchymal stem cells (MSCs) emerge from renal progenitors. These MSCs restore the structure and function of damaged renal cells. When these cells divide, the resulting cells differentiate into nephrons and have function as well (Figure 4).

Scientists have been able to identify the pro-regenerative agents and chemical elements that initiate regeneration. These discoveries make it possible for the conditions of nephron renewal

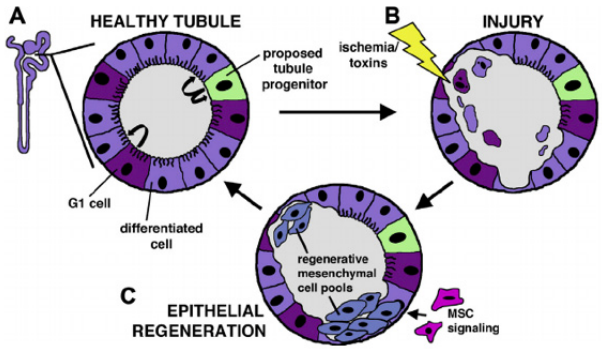

Figure 4. Renal progenitors release MSCs upon renal injury of a zebrafish. These cells repair the structure of the renal cell, as shown above, once signaled by the injury.[9]

in zebrafish to be replicated in humans.[9, 17, 18, 19] Further research of embryonic renal development, neonephrogenesis in animals, and the use of human pluripotent stem cells will be required to successfully regenerate nephrons.

Objective 2: Regenerative Techniques Used to Prevent Complete Loss of Function in Early-Stage Patients

Regenerative medicine can be used to halt the progression of illness in patients who have not developed ESRD, but have been diagnosed with more temperate diseases. Chronic kidney disease (CKD) is one of these diseases, in which patients progressively lose kidney function over several months or years. By using stem cell therapies, scientists can regenerate nephrons. Scientists have identified three different types of optimal cells capable of renal cellular repair. These ideal target cells include vessels, stroma, and nephron epithelia.[10,20]

Once these target cells were determined, scientists determined the type of stem cell they would use to promote regeneration.[10,20] Through a variety of experiments, scientists found that tissue-specific stem cells were the best source for cellular regeneration in early-stage patients.[10,20] Tissue-specific stem cells can generate a large supply of cells without the risks of affecting other cells of the body. These stem cells can be obtained and isolated from embryos or adult organisms.[10,20]

Two processes can be used to reprogram the renal progenitors that will produce the new nephrons.[10] One process involves reprogramming adult cells back into a pluripotent condition and then

differentiating them again into renal progenitors. This indirect process is much more time consuming and difficult in comparison to a more direct process. Alternatively, another process automatically reprograms the ideal target cells, such as the stroma and nephron epithelia, into induced renal progenitor cells. Unlike the indirect process, this more direct process avoids reprogramming to the earliest state and instead reverts to the particular cellular state required to instigate renal regeneration.[10] These processes can be used to replace the damaged cells in patients with chronic kidney disease. While current studies are using these processes in kidney tissue, they have yet to be performed in human patients.

Many patients diagnosed with kidney diseases experience the inability of their vasculature to function properly. Proper blood flow is necessary for the renal system to maintain hemodynamic levels of the body and, in turn, homeostasis. The restoration of blood flow is possible with angiogenesis, the formation of new blood vessels from ones that already exist in the body. This induced vascular growth can reduce the symptoms of patients diagnosed with kidney diseases.

There are several different types of angiogenesis that will occur, depending on the severity of vascular damage. These processes are possible due to the formation of angioblasts, the tissue that produces blood vessels. Sprouting angiogenesis results in the addition of blood vessels to tissues that lack properly functioning blood vessels. This method occurs when there is a complete loss of function. Suggested by its name, sprouting angiogenesis is categorized by the sprouting mechanism through which endothelial cells grow towards a stimulus. This stimulus is a reaction site where the formation of new blood vessels occurs.[21] In comparison, intussusceptive growth is a splitting process where an existing blood vessel is cleaved and split into two blood vessels. This is used in cases where the existing blood vessels have maintained some function but require a larger supply of vascular tissue. Intussusceptive growth increases the number of blood vessels and capillaries; however, it does not increase the number of endothelial cells as sprouting angiogenesis does.[22] It is important to distinguish the difference between vasculogenesis and angiogenesis. While angiogenesis forms blood

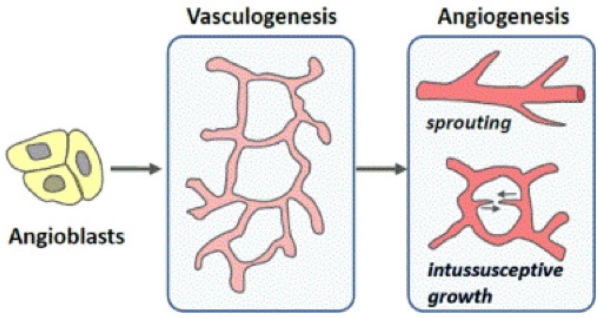

Figure 5. The basic types of vascular growth are illustrated in this diagram. Angioblasts develop into blood vessels. New blood vessels can be formed in two processes: Vasculogenesis and Angiogenesis. The mechanisms of these processes are illustrated above.[21]

vessels from preexisting ones, vasculogenesis is the process of creating entirely new blood vessels (Figure 5). This can occur embryonically or can be replicated through the use of stem cells.

Endothelial cells are affected when blood flow is restricted. Some endothelial cell progenitors, such as the CD133+, CD34+, and KDR+ cells, have the ability to differentiate into cells that promote endothelial cellular repair. Growth factors, such as the vascular endothelial growth factor, are induced to promote the process of replication and its speed and accuracy.[20]

Using stem cells and regenerative processes, scientists have discovered mechanisms that successfully result in the production of healthy nephrons to replace diseased ones found in early-stage patients. Angiogenesis has resulted in the ability to restore blood flow and the hemodynamic levels of the natural body. All of these processes can be used to restore homeostasis in patients with early-stage kidney diseases.[21, 22, 23]

Objective 3: The Future of Renal Regeneration

Advancements made in renal regeneration within the past ten years have mainly concerned the use of stem cells. However, that is not the only option for renal regeneration. Scientists have recently discovered drug therapeutics and developed bioengineering mechanisms that can be applied to regenerative renal construction. These discoveries have the potential to lead to a revolution in organ regeneration.

Specific drug delivery systems have been shown to foster the growth of several renal tubules when grown between layers of polyester fleece. For instance, in a recent study, Minuth, Denk, and Glashauser isolated and cultured renal cells in a serum-containing medium.[24] Cells were then coated with extracellular matrix proteins that promote the growth of tubules. However, the study found that this coating leads to substantially low levels of respiratory gas exchange. Researchers determined that for the development of tubules to occur, they must first replicate the environment required by stem cells. To do this, they created an artificial interstitium, the space between a biological structure, out of polyester fleece. They sandwiched the cells and isolated embryonic material between layers of the fleece. This material fostered the exchange of respiratory gas and nutrition (Figure 6). Next, they placed this sample in a perfusion culture container maintained at 37°C for thirteen days. Within this period, the tubules matured and were capable of function. These scientists are currently studying the possibility of implanting this biomaterial. The growth of these tubules was dependent on the serum-containing material and polyester fleece and its growth factors.[24] Upon the success of this experiment, one may expect to see drug therapeutics used more often to create artificial biological structures with the goal of eventual implantation.[24]

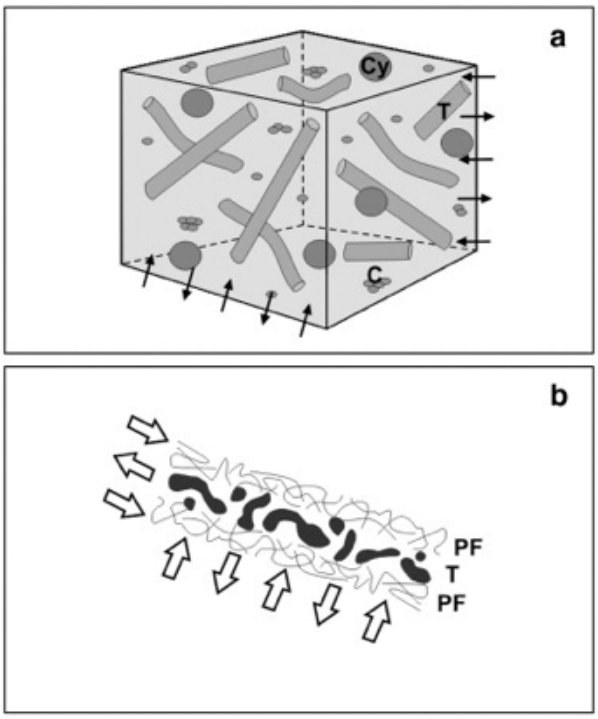

Figure 6. This figure illustrates the coating of cells using extracellular matrix proteins (a) in comparison to using the polyester fleece interstitium (b).[24] The cells coated with extracellular matrix proteins are unable to exchange respiratory gas and lack the space required to fully develop. The small arrows on part a represent the low gas exchange levels, whereas the large arrows on part b represent the spatial development and exchange of gas.[24]

Bioengineering involves the process of decellularization where the extracellular matrix is separated from its cells. By manipulating genetic material, artificial limbs, tissues, and organs can be created.[25] Three dimensional bioprinting uses stem cells or cells isolated from a patient. These cells are placed in a growth medium where they replicate in laboratory settings. Once replication has occurred, the cells are placed in a cartridge. This resulting product has been coined bioink. The bioprinter ejects the bioink into specific layers that are immersed with hydrogel, a substance that acts as a temporary mold around the cells. Once the printing is complete, the tissue continues to mature until the hydrogel is removed. After these processes have occurred, the tissue has complete function.[26]

These processes are relatively new, and as a result, more findings are becoming available each day. Recently, Homan et. al described the ability to bioprint 3D renal proximal tubules using perfusable chips.[27] Although it may be several years until the regeneration of renal tissue through drug therapeutics and bioengineering is optimized, it seems to be within the future grasp of the scientific community.

Conclusion

An imbalance exists between treatments available and patients requiring treatment for kidney illnesses and diseases. By understanding the processes of embryonic renal developments, scientists have been able to apply this knowledge to renal regeneration using human pluripotent stem cells. The model of neonephrogenesis has also been used to promote the use of stem cells. While some patients require newly constructed renal structures or nephrons, others require revival and replacement of damaged

nephrons. Using angiogenesis, blood function can be restored in patients suffering from kidney conditions. To prevent complete loss of function in early-stage patients, scientists must be able to distinguish the mechanism needed to restore function and structure. The future of renal regeneration seems promising with the use of drug therapeutics and other mechanisms of bioengineering. Perhaps one day, those diagnosed with kidney illnesses will undergo renal regenerative therapies, restoring a vigorous life.

References

[1] Lin YQ, Wang LR, Pan LL, Wang H, Zhu GQ, Liu WY, Wang JT, Braddock M, Zheng MH. Kidney bioengineering in regenerative medicine: An emerging therapy for kidney disease. Cytotherapy. 2016;18(2):186-97.

[2] Robson JS, Mitchell GAG, Le Vay D. Renal system [Internet]. Encyclopedia Britannica Online; 2016 Nov 28. Available from https://www.britannica.com/science/human-renal-system

[3] von Schulthess GK, Margulis A, Fuchs WA. Morphology and function in MRI: Cardiovascular and renal systems. Berlin: Springer; 1989. p. 111-130.

[4] Your kidneys and how they work [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2014. Available from https://www.niddk.nih.gov/health-information/kidney-disease/kidneys-how-they-work

[5] Kidney disease: The basics [Internet]. NY (NY): The National Kidney Foundation; 2016. Available from https://www.kidney.org/news/newsroom/factsheets/KidneyDiseaseBasics

[6] Chou YH, Pan SY, Yang CH, Lin SL. Stem cells and kidney regeneration. Journal of the Formosan Medical Association. 2014;113(4):201-09.

[7] Battler A, Leor, J. Stem cell and gene-based therapy: Frontiers in regenerative medicine. London: Springer; 2006. 208-231.

[8] Little MH, Combes AN, Takasato M. Understanding kidney morphogenesis to guide renal tissue regeneration. Nature Reviews Nephrology. 2016;12(10):624-35.

[9] Mccampbell KK, Wingert, RA. New tides: Using zebrafish to study renal regeneration. Translational Research. 2014;163(2):109-22.

[10] Harari-Steinberg O, Pleniceanu O, and Dekel B. Selecting the optimal cell for kidney regeneration. Organogenesis. 2011;7(2):123-34.

[11] Stem cell key terms [Internet]. California's Institute for Regenerative Medicine; 2016. Available from https://www.cirm.ca.gov/patients/stem-cell-key-terms

[12] Gouon-Evans V. The race for regeneration: Pluripotent-stem-cell-derived 3D kidney structures. Cell Stem Cell. 2014;14(1):5-6.

[13] Sykaras N, Opperman LA. Bone morphogenetic proteins (BMPs): How do they function and what can they offer the clinician? Journal of Oral Science. 2003;45(2):57-73.

[14] NODAL Nodal growth differentiation factor [Homo sapiens (humans)] [Internet]. National Center for Biotechnology Information; 2016. Available from https://www.ncbi.nlm.nih.gov/

[15] Eirin A, Lerman, LO. Mesenchymal stem cell treatment for chronic renal failure. Stem Cell Research & Therapy. 2014;5(4):83.

[16] Davidson, AJ. Mouse kidney development. StemBook [Internet]. Cambridge (MA): Harvard Stem Cell Institute; 2008.

[17] Poureetezadi SJ, Wingert, RA. Little fish, big catch: Zebrafish as a model for kidney disease. Kidney International. 2016;89(6):1204-210.

[18] Mccampbell KK, Springer KN, Wingert, RA. Analysis of nephron composition and function in the adult zebrafish kidney. Journal of Visualized Experiments. 2014;90.

[19] Diep CQ, Ma D, Deo RC, Holm T M, Naylor RW, Arora N, Wingert RA, Bollig F, Djordjevic G, Lichman B, Zhu H, Ikenaga T, Ono F, Englert C, Cowan CA, Hukriede NA, Handin RI, Davidson AJ. Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. Nature. 2011;470(7332):95-100.

[20] Reinders MEJ, Rabelink TJ, Briscoe, DM. Angiogenesis and endothelial cell repair in renal diseases and allograft rejection. Journal of the American Society of Nephrology. 2006;14(4):932-42.

[21] Adair TH, Montani JP. Angiogenesis. San Rafael (CA): Morgan & Claypool Life Sciences; 2010.

[22] Eichmann A, Bouvrée K, Pardanaud L. Vasculogenesis and angiogenesis in development. Cancer Treatment Res. 2004;117:3-32.

[23] Maeshima Y, Makino H. Angiogenesis and chronic kidney disease. Fibrogenesis & Tissue Repair Fibrogenesis Tissue Repair. 2010;3(1):13.

[24] Minuth WW, Denk L, Glashauser A. Cell and drug delivery therapeutics for controlled renal parenchyma regeneration. Advanced Drug Delivery Reviews. 2010;62(7-8):841-54.

[25] Jansen J, Fedecostante M, Wilmer MJ, Peters JG, Kreuser UM, van den Broek PH, Mensink RA, Boltje TJ, Stamatialis D, Wetzels JF, van den Heuvel LP, Hoenderop JG, Masereeuw R. Bioengineered kidney tubules efficiently excrete uremic toxins. Scientific Reports. 2016;6(26715).

[26] Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nature Biotechnology. 2014;32(8):773-85.

[27] Homan KA, Kolesky DB, Skylar-Scott MA, Herrmann J, Obuobi H, Moisan A, Lewis JA. Bioprinting of 3D convoluted renal proximal tubules on perfusable chips. Scientific Reports. 2016; 6(34845).

[28] Sonya M. Embryology of the kidney [Internet]. Interactive Biology; n.d. Available from http://www.interactive-biology.com/3695/embryology-of-the-kidney/

[29] Physiology of kidneys. Role of kidneys in keeping homeostasis [Internet]. N.p.; n.d.

[30] Takasato M, Er PX, Chiu HS, Little MH. Generating kidney organoids from human pluripotent stem cells. Nature Protocols. 2016;11(9):1681–1692.

[31] Klinkhammer BM, Kramann R, Mallau M, Makowska A, Roeyen CRV, Rong S, Buecher EB, Boor P, Kovacova K, Zok S, et al. Mesenchymal stem cells from rats with chronic kidney disease exhibit premature senescence and loss of regenerative potential. PLoS ONE. 2014;9(3).

[32] Mitu G, Hirschberg R. Bone morphogenetic protein-7 (BMP7) in chronic kidney disease. Frontiers in Bioscience. 2008;13:4726–4739.

[33] Organ Donation and Transplantation Statistics [Internet]. NY (NY): The National Kidney Foundation; 2017. Available from https://www.kidney.org/news/newsroom/factsheets/Organ-Donation-and-Transplantation-Stats