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Development of kidney tissue in a laboratory-scale bioreactor

Prasenjit Sarkar

Research Snippets

While the making of kidneys in a laboratory may be termed science fiction, with the advent of embryonic stem cell research and bioreactor technology, it is poised to become a reality. The need for making kidney tissue in a bioreactor is evidenced by the increasing incidence of end-stage kidney disease worldwide as well as in India. While end-stage kidney disease can be treated with dialysis, the average lifespan of patients on dialysis is significantly lower than those with kidney transplantations. However, the number of kidneys available for transplantation is much lower than the demand for kidney transplantations. Due to the shortage of kidneys available for transplantation, recent research has focused on making kidneys in the laboratory from pluripotent stem cells with the hope that these kidneys may be transplanted into patients with end-stage kidney disease. Also, kidney tissue produced in this manner may be engrafted into patients with kidney injury to improve kidney function. Since this endeavor lies in the interface of biology and engineering, a brief description of both aspects will be provided here.

Cells are the building blocks of an organismâ€™s body. Various types of cells are required for various types of functions in the body of an organism. For example, podocytes cells participate in forming the filtration barrier in the kidney. Some cells of the body known as stem cells possess certain unique abilities. Firstly, they can be grown indefinitely in laboratory conditions. Secondly, they can give rise to other types of cells through a process known as differentiation, when given specific signals from the environment. For example, embryonic stem cells are cells that are isolated from the â€¯inner cell massâ€™ tissue of the embryo and can be grown indefinitely in laboratory conditions. Additionally, they can differentiate into any type of cell of the adult body, such as cells of the kidney, brain, stomach, intestines, liver, pancreas, etc. The ability of embryonic stem cells to form any tissue of the adult body can be utilized for engineering purposes to make organs in the laboratory. Current efforts to produce the kidney from embryonic stem cells will be discussed herein.

The kidney is a complex organ that filters the blood and removes toxins. The kidney comprises filtering units known as nephrons. On average, there are about a million nephrons in the adult human kidney. Nephrons themselves are made of various tissues including podocytes, proximal tubules, loops of Henle, and distal tubules. Nephrons connect to the collecting ducts, which collect urine from all the nephrons. Additionally, nephrons are interspaced by the stromal tissue. To engineer a kidney in the laboratory, all of these tissues need to be present together. Recent efforts in the direction of making kidney tissue from embryonic stem cells have achieved success in making podocytes, proximal tubules, loops of Henle, distal tubules, and stromal cells inside a kidney â€œorganoidâ€. Other studies have shown the formation of collecting ducts from embryonic stem cells. Further studies have sought to combine these tissues together to engineer a kidney organoid that resembles the in vivo kidney. However, major challenges remain in making a kidney organoid that faithfully reproduces the functions of the kidney. Currently, a big challenge is that the kidney organoids produced in laboratory conditions are not suitable for engraftment into the body, as shown by engraftment experiments inside mice. When engrafted into mice, these kidney organoids undergo aberrant expansion, with the formation of unintended tissues such as cartilage. Due to such aberrant expansion of the stromal tissue in the engrafted kidney organoid, long-term engraftment of kidney organoids (>4 weeks) has not been demonstrated so far. Yet another challenge is that the engrafted kidney organoid requires requisite blood flow for the survival of the constituent tissues. While current research has demonstrated the formation of blood vessels inside the in vivo engrafted kidney organoid, the quantity of these blood vessels is inadequate. Further research needs to be conducted to increase the formation of blood vessels into the engrafted kidney organoid.

An engineering challenge in the use of kidney organoids derived from currently published methods is that the kidney organoid formed in the laboratory is 5 orders of magnitude smaller in volume than the actual human kidney. Therefore, the process of forming kidney organoids needs to be scaled up in a bioreactor. While the production of kidney organoids has been attempted in a bioreactor, these organoids were shown to be unsuitable for engraftment due to aberrant expansion of the stromal cells. Therefore, further research is required in terms of process development to make kidney organoids that are stable and do not display aberrant expansion of the stromal tissue. Additionally, further research is also required for the scale-up of kidney organoids in a bioreactor such that they may be used for the purpose of transplantation into patients. To address these engineering challenges, a molecular-level understanding of the kidney formation process is required.

At the molecular level, the process of conversion of embryonic stem cells to any other tissue is governed by environmental signals, which in most cases take the form of specific proteins present in the environment of the stem cell. Different protein signals from the environment may guide the stem cell to become different tissues. Therefore, differentiation of embryonic stem cells involves the engineering of specific protein signals that can allow the formation of the product tissue of interest. To design the differentiation process, some guidance can often be taken from the literature on the developmental biology of the tissue of interest. The differentiation process for forming kidney tissue from embryonic stem cells has been identified recently, with many research labs proposing their protocols. Common to all these protocols is the activation of the Wnt signaling pathway followed by the FGF9 signaling pathway. The Wnt signaling pathway is a set of biochemical reactions that occur when a cell is exposed to the Wnt protein. The information that the Wnt protein is present in the environment of the cell, is conveyed through this set of biochemical reactions into the cell. This process causes the cell to respond in various ways, depending on the cell type. For example, embryonic stem cells respond to the Wnt signal in the environment by differentiating, i.e. the identity of the cell changes. Embryonic stem cells can be guided through the Wnt signaling pathway and the FGF9 signaling pathway towards differentiation to the precursor of nephrons, known as nephron progenitor cells. Thereafter, activation of the Wnt signaling pathway and Notch signaling pathway is needed for induction and proper patterning of nephrons, respectively. Molecular-level studies on the Wnt and Notch signaling pathways are required to better understand the process of formation of nephrons, and to engineer stable kidney organoids in the laboratory.

The biomolecular engineering research group at IIT Jodhpur aims to conduct molecular-level studies on the Wnt and Notch signaling pathways with the goal of forming stable kidney organoids which may be used for long-term engraftment. To address the problem of long-term engraftment, a bottom-up approach will be adopted instead of the top-down approach reported in the literature. Kidney organoids will be formed that comprise of nephrons sans the stromal cells, aided by molecular-level studies on the Wnt and Notch signaling pathways. New hypotheses will be formulated and tested in order to obtain

nephrons that are induced and patterned through the Wnt and Notch signaling pathways, respectively. These organoids will be engrafted into mice to study their long-term stability. Thereafter, more complexity will be added to the organoids by adding the stromal tissue and the collecting ducts. Apart from kidney organoid engineering, research will also focus on process engineering for the production of kidney tissue in a larger scale in bioreactors. Current methods for making kidney organoids are carried out in cell culture dishes which is not a scalable process. Therefore, process development will be carried out to adapt these methods to a bioreactor setting. Process parameters such as the Reynolds number and the Power number will be optimized to ensure that mass transfer limitations are minimized, localized protein concentration gradients are minimized, but cells do not die due to excessively high shear force. Kidney organoids produced in the bioreactor will be tested for long-term stability by engraftment experiments in mice.

To complete the discussion about embryonic stem cell research, ethical aspects of such research need to be addressed. Since embryonic stem cells are derived from the embryo and harvesting the inner cell mass tissue invariably leads to the destruction of the embryo, embryonic stem cell research raises ethical concerns. Moreover, kidney organoids made from embryonic stem cells cannot be engrafted into patients since they will be rejected by the immune system of the body as foreign tissue. The solution to these problems came with the discovery of induced pluripotent stem cells (iPSCs). This technology allows scientists to derive iPSCs, which are identical to embryonic stem cells in terms of their ability to be maintained indefinitely in cell culture, and their ability to form any tissue of the body. Moreover, iPSCs can be derived from other sources, such as skin cells, and therefore do not raise ethical concerns. Thus, patient-specific iPSCs can be derived, which can then be turned into kidney tissue. Such tissue will not evoke an immune response since the cells are derived from the patientâ€™s own body. Together with this technology, it is envisioned that personalized medicine will soon become a reality.

References:-

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About the Author

Dr. Prasenjit Sarkar,
Assistant Professor,
Department of Chemical Engineering
(psarkar@iitj.ac.in)