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Potential Strategies of tissue engineering and regenerative medicine for kidney diseases

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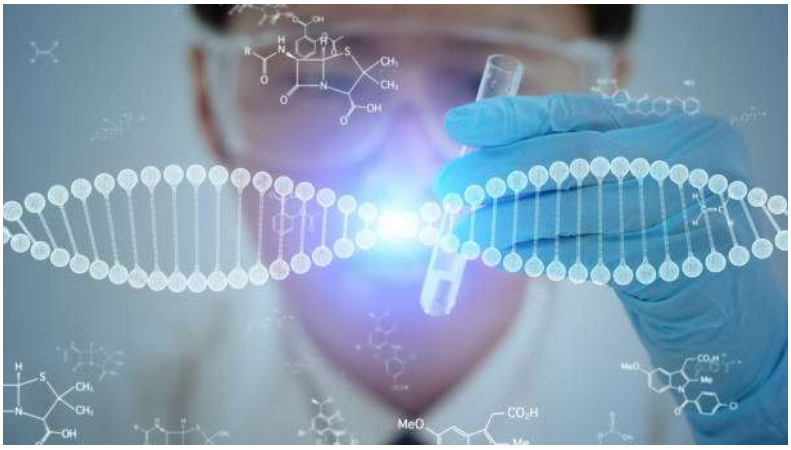

Abstract:

Kidney transplant or kidney dialysis are the current treatment for end stage chronic renal failure (ESRF) and acute renal failure (ARF). Although these therapies have proved the ability to increase life expectancy and improve the quality of life of patients, but there are several limitations of such treatments. Tissue engineering and regenerative medicine can offer alternative modalities that could improve, restore or replace renal function. Cell based therapy showed promising results in kidney regeneration and improving kidney function in an experimental model to treat CKD. However, it is limited by the complexity of the kidney and is not effective for end stage renal failure (ESRF) where complex structure of kidney including its scaffold is deteriorated severely. Consequently, ESRD requires whole kidney regeneration which is another approach to replace damaged kidney.

In this article, we will review different approaches in kidney regeneration, and highlights the obstacles that limit the application of each approach for kidney regeneration in patients with kidney diseases.

Keywords: Tissue engineering, Regenerative medicine, Stem cells, Scaffold, kidney dialysis.

Introduction:

Renal diseases including chronic kidney disease (CKD) and acute renal failure (ARF) are the leading causes of mortality and morbidity in the world and the number of patients is growing gradually. Stage 5 CKD or what is called end stage renal failure (ESRF) and ARF require treatment based on renal replacement therapies, whether dialysis or kidney transplant. Although these therapies have proved the ability to increase life expectancy and improve the quality of life of patients, but there are several limitations of such treatments. Dialysis treatment leads to progression of pathological conditions such as coronary artery disease. In addition, Cumulative Dialysis treatment costs are greater than that for cancer treatment. Kidney transplant also needs lifelong immunosuppressive therapy (1). Moreover, in 2012, the shortage of donor organs puts 116,000 patients on the waiting list for transplantation, let alone the increasing number over years (2). As well as that, 40% of recipients will die or lose graft within 10 years (3). These limitations necessitate the need for alternative approaches in treating renal diseases.

Tissue engineering and regenerative medicine can offer alternative modalities that could improve, restore or replace renal function. Cell based therapy showed promising results in kidney regeneration and improving kidney function in an experimental model to treat CKD. However, it is limited by the complexity of the kidney and is not effective for end stage renal failure (ESRF) where complex structure of kidney including its scaffold is deteriorated severely. Consequently, ESRD requires whole kidney regeneration which is another approach to replace damaged kidney. Whole organ regeneration has revealed successful clinical results to replace simple structure such as bladders, urethras, vessels and upper airways. But kidney is a complex organ that has more than thirty different cell types – glomerular podocytes, endothelial cells, interstitial cells connecting duct cells, mesangial cells and tubular epithelial cells - 2 million glomeruli, numerous arterioles, capillaries and tubules which cooperate together to carry out several functions such as blood filtration and waste excretion (4,3). This complexity in term of anatomical structure and function make the whole kidney regeneration to alleviate patients with ESRF a challenging target.

1. Kidney development:

Regeneration therapy of kidney require full understanding of the molecular basis of kidney development or nephrogenesis. This development proceeds through sequential phases: the pronephros, the mesonephros, and the metanephros. A mature kidney consists of two metanephroi. This mature kidney form by interaction of uretric bud which can give rise to the collecting duct and the ureter and metanephric mesenchyme which form all other elements of nephrons, the interstitium and the vasculature. Signals released from the uretric bud induce the formation of a new nephron by undergoing a mesenchyme to epithelial transition. After forming a permanent kidney, regeneration of new nephron cannot be achieved. Instead, it is able to regenerate some structures in a process called cellular repair in response to injury like other organs. Cellular repair process can help to regenerate the tubules of the medulla after being destructed due to ureter obstruction. Renal stem cells, bone marrow derived stem cells and tubular cells can help kidney to regenerate some tissues but not a new nephrons by trans-differentiation in response to injury. However, irreversible glomerular and tubular damage as a

result of end stage renal failure cannot be repaired by cellular repair process and whole kidney regeneration approach is required at this stage (5).

2. Stem cells for kidney regeneration:

There are several types of stem cells that can be used for both approaches of kidney regeneration, whether cell based therapy or whole kidney regeneration.

2.1.Embryonic stem cells

Embryonic stem cells are derived from the inner cell mass of blastocyst-stage embryos. They have the ability to self-renew in culture and to differentiate into more than 200 types of cells from the three germ layers; ectoderm, mesoderm and endoderm. Several studies showed the ability of mouse ES cells to differentiate into renal progenitors cells and renal cells that form different renal structures such as tubules and glomerular tuffs if cultured with a mixture of suitable growth factors (bFGF, TGF-β 1, activine-A, BMP-4, HGF, EGF, and β-NGF etc.) or injected into mouse kidney (4). On the other hand, several issues related to teratoma formation, ethical issues and possible immune rejection make embryonic stem cells unsuitable for clinical regeneration therapy.

2.2.Induced pluripotent stem cells

Induced pluripotent stem cells are generated by transferring 4 transcription factors (Oct4, Sox2, Klf4 and C-myc) using retrovirus or other techniques to somatic cell. This will reprogram the somatic cell to become pluripotent cell that have similar properties of embryonic stem cells. But this type of cells have no ethical issues and no immune rejection as in embryonic stem cell. Although there are oncogenic risks associated with Klf4 and C-myc. However, recent studies showed that the reprograming can occur without those transcription factors eliminating the risk of cancer formation. Recently, induced pluripotent stem cells are generated from both mesangial and epithelial cells derived from urine. Moreover, podocytes and proximal tubular cells are used also to produce iPSCs (3). Despite encouraging results, some issues have to be considered before using iPSC for clinical applications. The molecular mechanism that transfer cell from pluripotent state to functional kidney cell must be identified. In addition, a defined optimal culture conditions for targeting cells is needed (3).

2.3.Mesenchymal stem cells (MSCs)

Bone marrow consists of different types of adult stem cells such as MSCs and Hematopoietic stem cells. Although bone marrow derived stem cells have shown the ability to regenerate several parts of kidney and treat several injured renal tissues including podocyes, endothelial cells and tubular epithelial cells, but donor BMDC is limited in its ability to transdifferentiating and migrating into the kidney. Several studies showed that MCSs derived from bone marrow, kidney and adipose tissue can result in kidney repair in ARF, CKD and diabetic nephropathy (DN). These cells can produce cytokines and growth factors which are important to prevent kidney fibrosis, reduce inflammation, induces the migration and proliferation of epithelial cells in the kidney and

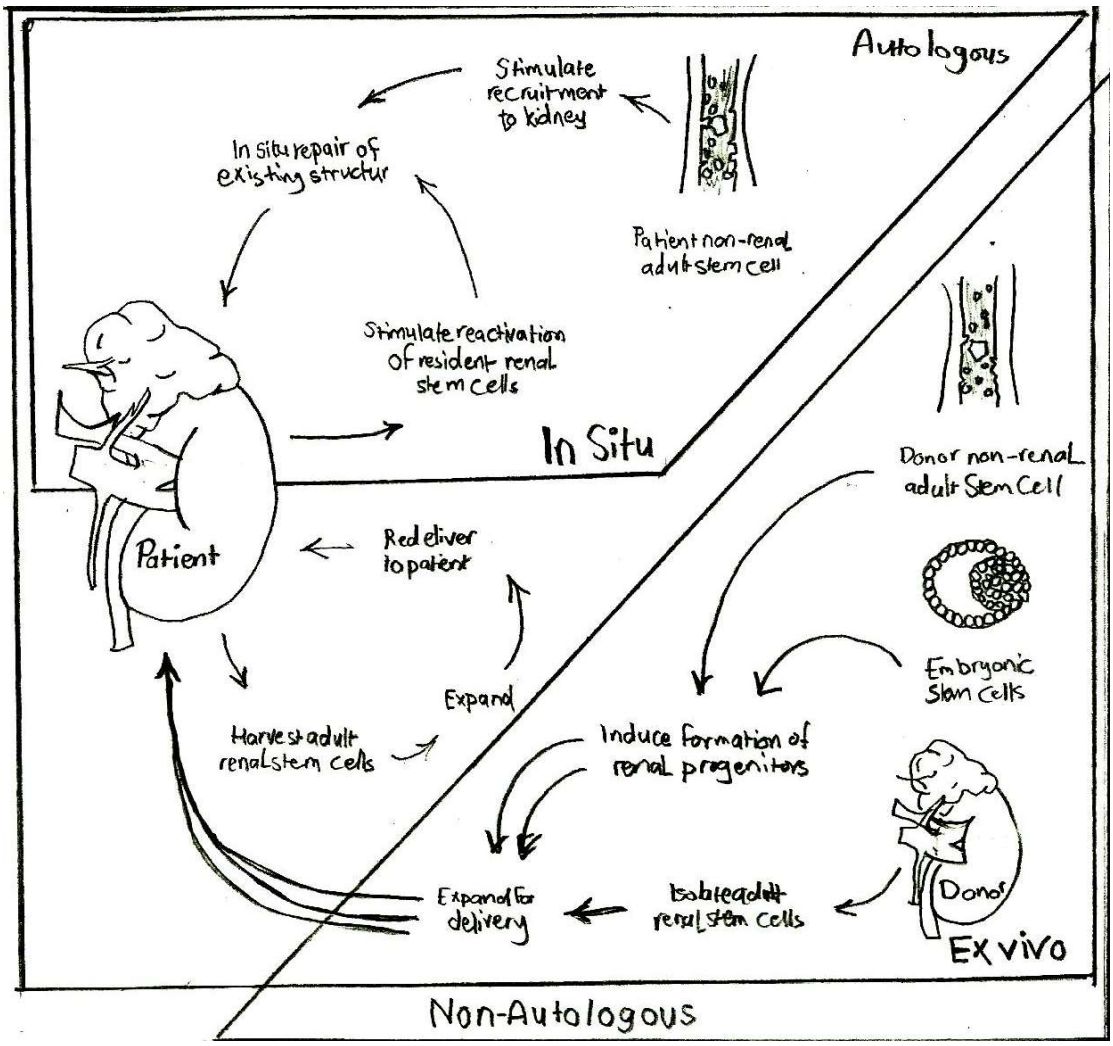

Figure 1: Cellular therapeutic options for the treatment of renal disease (5).

accelerate repairing of damaged kidney tissue in a process which is similar to that shown in figure 1. However, recent reports revealed that MSCs may maldifferentiate into glomerular adipocytes. In addition, MSCs derived from patient with renal failure showed decreased expression of VEGF and SDF-1α which are important for kidney regeneration. This mean that CKD patients cannot benefit from their own MSCs. Further studies are required to consider these issues related to MSCs before using them clinically for kidney regeneration (4).

2.4.Renal stem /progenitor cells

Recent studies revealed the presence of renal stem cells that possess the multi-potency and self-renewal characteristics in different locations in adult kidney including Bowman's capsule, renal papilla and tubular epithelial cells. Different methods have been used to investigate the presence and function of these cells. One approach involves administration of bromodeoxyuridine (BrdU) followed by long chase period. Quiescence Renal stem cells maintain high level of BrdU in their

genomes in comparison to other fully differentiated cells which dilute it as they divide. BrdU labeled cells were found in papilla and tubules and investigating them in vitro shows their ability to give rise to fibroblasts, proximal tubule and collecting duct cells. Another approach depends on the expressing of stem cell surface markers such as CD133 and CD24. Recent research exhibits that the urinary pole of the Bowman's capsule contains cells that express CD133/CD24 surface markers. These cells can give rise to podocytes and tubular cells in vitro and play an important role in regeneration of podocytes and tubular cells after injection into an animal model with acute renal failure (4).

Renal stem/progenitor cells avoid the risks of mal-differentiation or tumorigenicity. In addition, they provide autologous source for cell therapy, avoiding immune rejection associated with allogeneic sources. As well as that, no ethical issues for such type of cells. However, renal stem cells are difficult to isolate from an adult kidney as they constitutes 0.1% of its cells. Moreover, they are difficult to be expanded in culture (4).

2.5.Fetal renal stem cells

Fetal renal stem cells derived from animal embryos have a great potential to be used for nephrogenesis in whole kidney bioengineering. One of their advantages is the availability. Another advantage is the lack of rejection due to absence of donor dendritic cells in fetal renal anlagen. Furthermore, no teratoma formation risk is associated with this type of cells because they develop within a defined window. However, possible transmitting of pathogen from animals is a major concern that need to be considered (6).

2.6.Amniotic fluid-derived stem cells

AFDSCs are pluripotent stem cells which can differentiate to form different types of cells from the three germ layers. One study showed their ability to differentiate along renal cell lines when injected into murine embryonic kidney. Another study revealed its great therapeutic potential when infused in an animal model with acute renal injury. Moreover, AFDSCs used for treating animals with acute renal injury showed better results in term of restoration kidney function and tubular repair than BMDCs, since they are more efficient in paracrine signaling and transdifferentiation. They secrete growth factors and other signaling molecules important for tissue regeneration.

3. Scaffold for whole kidney regeneration:

Kidney is a complex organ in term of cellular heterogeneity, anatomical structure and structural arrangement of ECM components such as growth factors, proteins and glycosaminoglycan. This makes the attempts to produce scaffold that mimic the ECM of human kidney using current level of technology such as electrospining, three dimensional printing and self-assembling a difficult, if not impossible task. However, specially treated decellurized kidney matrices originated from xenogeneic or allogeneic sources can form appropriate scaffolds for whole kidney regeneration (4).

ECM consist of different large and small proteins, polysaccharides and biochemical molecules necessary for organ growth and function. It consists of structural proteins such as collagen and elastin that give the three dimensional structure to tissues and organs. It also secret and store cytokines and growth factors that direct cell proliferation, migration, differentiation, and apoptosis. As well as that, it contain adhesive proteins such as laminin and fibronectin that promote cell adhesion. These characteristics and others such as biocompatibility and existence of intact vasculature make decellurized kidney matrices a perfect scaffold for whole kidney engineering.

Decellularization involves using detergent, enzymes or acid to irrigate tissue or organ repeatedly through its innate vasculature. Successful decellularization mean that all cellular components, DNA, and cell surface antigens are removed while the structural integrity, vascular network and attachment sites of ECM are preserved. Following that, sterilization with ethylene oxide is required to disinfect the scaffold without denaturation of ECM proteins or growth factors. Simple organs such as nerve, valve, respiratory tract, bladder and tendon were successfully decellularized and recellularized. However, this procedure can be challenging for larger, complex organs as it need applying stronger detergents for longer period and at higher perfusion pressure. This consequently may cause undesired damage to ECM proteins and organ vasculature (6).

4. Recellularization of whole kidney scaffolds:

Kidney has several main functions such as filtration, absorption, secretion and drainage of urine that the new construct should maintain in order to replace the damaged kidney. So, cells suspension of epithelial cells, endothelial cells, podocytes, renal fibroblasts and mesengial cells that will form glomeruli, tubules, collecting ducts and other anatomical components of the kidney must be used for successful kidney regeneration. As illustrated in figure (2.a) a special bioreactor is used for cells seeding. The whole organ is placed in a sterile chamber at negative pressure to generate pressure gradient across the organ. Then, cells suspension is perfused through the ureter. This approach allowed for cell distribution throughout the whole scaffold in comparison to positive pressure approach that produced uneven cellular distribution, with cells failing to reach glomeruli. In the subsequent stage, kidney construct is transferred to a perfusion bioreactor (figure 2.b) where an environment that mimic the physiological environment in term of temperature, pressure and pH etc. is maintained. In addition, dynamic culture is typically adopted to provide oxygenation, nutrients supply to the construct which is perfused by a nutrient-rich culture media through renal artery. Meanwhile, the vein and ureter were allowed to drain passively into the reservoir (7).

Recent studies has applied the principle of decellulrization and recellularization techniques in animal experimental models. Ross et al decellulariced rat kidneys in a way that retained the three dimensional architecture glomeruli, vascular network and tubuli and preserved matrix signals such as growth factors that would guide differentiation process. Murine ESCs was then recellularized using physiological perfusion method to reach and attach glomerular, vasculature, and tubular structures and then differentiate into renal cell lines (8). Another study showed that

pretreatment of the Murine ESC with nephrogenic factors such as Activin A, BMP7 and retinoic acid promote differentiation into more kidney specific cells (9)

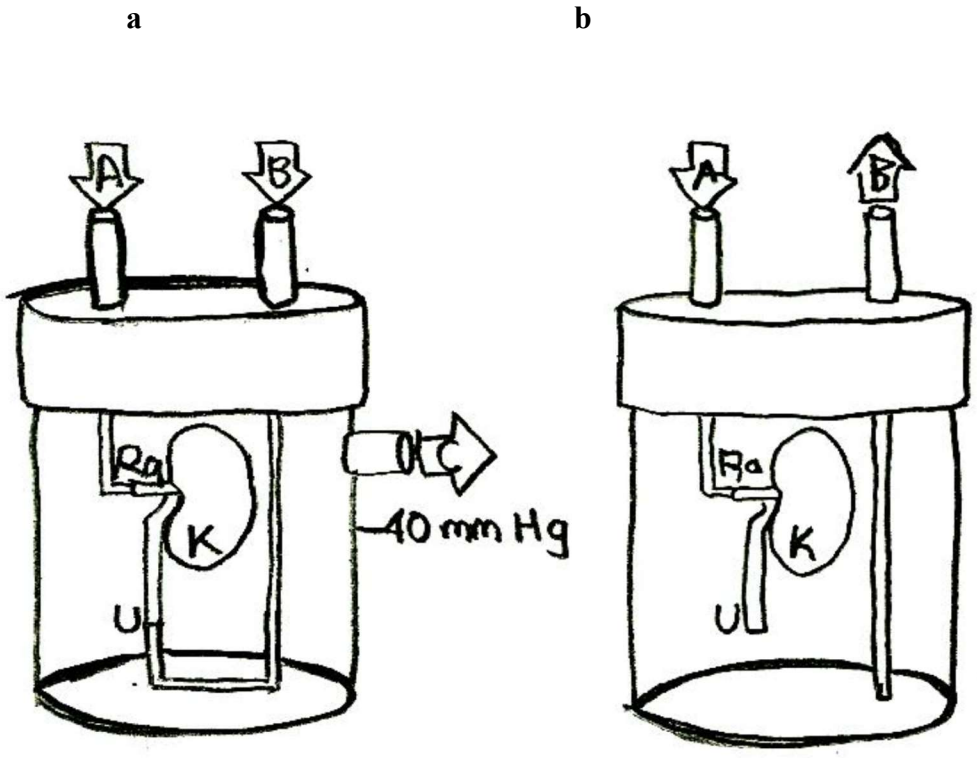

Figure 2: :Bioreactors used for Cell seeding and whole-organ culture of decellularized rat kidneys (a) Schematic of a cell-seeding bioreactor (b) Schematic of a whole-organ culture in a bioreactor (7).

More recently, porcine kidneys have been successfully used to produce ECM scaffold. Following that, several decellularization studies using scanning electron microscopy, histological staining and immunohistochemistry have showed complete cell clearance with preservation of vascular network and scaffolding architecture of the glomeruli, tubules, and vessels. After implanting this decellularized, sterilized scaffold for 2 weeks in vivo, the blood pressure was sustained and no bleeding was observed which mean the vasculature integrity of the new organ. However, thrombus formation and inflammatory infiltrate complications was observed (6).

Complicated structure of human kidney necessitate further work and studies in the field. Effective decellularization protocols in rats and pigs do not mean the same effectiveness for human kidney. Future studies are required for higher mammalian kidneys which are closer to human kidney in term of complexity and cellular heterogeneity. Thrombus formation and

inflammation in the vascular network are essential issues that must be tackled to move forward in kidney regeneration. Other questions related to the effectiveness of regenerated kidney to perform several functions such as homeostasis, resorption, metabolism, endocrine regulation, and immune modulation must be answered in future. In other words, whole human regenerated kidney that accomplish the minimum requirements of appropriate size, precise structure, urine production, performing other functions and biocompatibility is far from being considered as a clinical treatment option. So, it is necessary to enhance the field of kidney regeneration and consider it more thoroughly as it lag behind the dramatic advances achieved in other organs such as skin, cartilage, bone and heart valves.

5. Conclusion:::

We have reviewed different approaches in kidney regeneration and pointed the obstacles that limit the application of each approach for kidney regeneration in patients with kidney diseases.

SC therapies for clinical treatment of kidney disease should firstly overcome several technical, economic and regulatory issues. ES cells are pluripotent, but they are unaccepted ethically because of manipulating embryos. Likewise, IPSC are pluripotent, but teratoma formation and using retrovirus for transduction of transcription factors limit their application clinically. Renal stem cells are limited in their number and difficult to isolate and maintain in culture. On the other hand, MSCs are easy to isolate, and they have a great potential to regenerate damaged tissue in kidney. However, MSCs isolated from patients with CKD are inappropriate and inefficient for kidney regeneration (4).

Recent years have witnessed numerous studies related to using a decellurized whole kidney scaffold seeded with stem cells and cultured in a perfusion bioreactor. However, the transition to safe and effective clinical implementation need collaborative efforts to produce a whole regenerated kidney capable of replacing damaged kidney in end-stage kidney failure patients.

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