Chapter 3
Stem Cells: Use in Nephrology

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3.1 Introduction: Stem Cells and Kidney Diseases

Kidney diseases continuously raise serious concerns for human health and pose a challenging and costly public health problem at a global level. In recent years, a greater number of cases of kidney diseases worldwide have been reported, associated with aging and demographic transition processes, resulting from the increase in the population's life expectancy [1]. Hypertension, diabetes, and stress are multiplying factors, as well as socioeconomic, racial, and gender disparities, considered determinant factors for kidney diseases [2, 3].

The therapy for kidney diseases is intimately connected to its physiopathology [4]. The therapeutic maneuvers used to prevent the progression of kidney disease are not completely effective, whereas the treatment framework and therapeutic options, in addition to the limitation of certain drugs in acting on certain mechanisms, remain major obstacles. This scenario leads patients with kidney diseases to undergo replacement therapies such as dialysis, a temporary solution coexisting with an inferior quality of life for patients, or kidney transplantation [5]. Thus, there is a call to find new, more viable and effective strategies to prevent or stop progression, or even reverse kidney disease and, thus, to improve quality of life and patient survival.

Some steps have been taken in the past; however, the next necessary one seems to be toward stem cells and regenerative medicine. The use of stem cells and the ability to manufacture functional human tissue prove to be advantageous due to the possibility of their use in applications such as disease modeling and drug tracking

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and, ultimately, in tissue and organ repair and regeneration. However, human tissues are inherently complex and often organized into architectures composed of multiple cell types, extracellular matrix, and vasculature. These procedures increase with the complexity of structural organs [6]. Kidney is an intricate organ, made up of more than 26 different specialized cell types [7, 8], with a complex extracellular matrix of proteins and glycosaminoglycans, and organized into approximately 1 million microarchitectures, namely the nephrons. These structures are responsible for the regulation of volume, composition of body fluid compartments, maintenance of acid–base balance, excretion of metabolic waste, and production of hormones that control blood pressure and erythropoiesis [9, 10].

In recent decades, there have been numerous studies on the potential use of stem cells from different sources for the treatment of kidney diseases [11–13]. Stem cells can act by secreting bioactive paracrine factors and extracellular vesicles with immunomodulating and repairing properties of injured renal tissues [14, 15]. The evidence is complemented by the establishment of induced pluripotent stem cells (IPSC) and the targeted ability to differentiate into a renal lineage of these cells to self-organize and generate organoids for disease modeling, drug screening, and even for renal replacement [16].

This chapter summarizes an overview of the characteristics of different types of stem cells, organoids, mechanisms of action, clinical studies, and ethical issues on cell-based therapy for kidney diseases.

3.2 Sources of Stem Cells for Renal Therapy

There are a diversity of cells that can be employed as cellular therapy for kidney and can be helpful also for kidney physiopathological studies:

a. Embryonic stem cells (ESC) and induced pluripotent stem cells (IPSC)
b. Mesenchymal stem/stromal cells
c. Renal stem cells/progenitors cells

Some products from those cells elicit a better response than the proper cells. In this scenario, stem cell extracellular-derived vesicles (EV) are showing great results mainly on an experimental basis, and we will exploit it here.

3.2.1 Embryonic Stem Cells (ESC) and Induced Pluripotent Stem Cell (iPSC)

Embryonic stem cells (ESC) are obtained from the inner cell mass of blastocysts under certain culture conditions. From Thompson's pivotal experiment to nowadays, a lot of improvements have been made in the culture of these cells: use of defined medium, avoidance of animal-based products in culture, phenotypic

markers, genetic studies, and so on [17]. ESCs have two main characteristics: (1) self-renew and (2) huge potency to differentiate in other cells from body tissues, under specific factors and stimuli. This last characteristic defines them as pluripotent cells. Moreover, this high pluripotency is the appeal for regenerative medicine: you can generate the cell you need! [18].

Renal organogenesis is a highly complex process [19]. It results from the interaction of several cells and from different origins, stimulated by distinguished factors, elegantly reviewed by several authors [20–22]. More recently, with advances in global gene analyses, even single-cell transcriptomes, we now can understand the role of each cell and the multiple factors involved in this process [23]. From this knowledge, researchers have been working with ESC to recreate a kidney or, at least, to generate some cells [18, 24], although several multistep processes are still laboring and challenging. Time-dependent factors, dose-dependent stimulus, and cell-dependent interactions are necessary to generate one cell from the kidney [18]. And after generating this one, you should mix all cells together to create a kidney. In 2D culture conditions, it is quite impossible. Organoids or 3D cultured cells are simplifying those steps, and we are close to recreate a kidney in a petri dish. Organoids will be more detailed below. Then, it is possible to generate a precursor of a renal cell. Nephron progenitor cells (NPCs) and ureteric buds (UB) have been generated by differentiation-based protocols from ESC [25].

During renal organogenesis in mammals, Osr1+ cells give rise to the metanephric mesenchyme (MM), which condenses to form the cap mesenchyme (CM) [26]. MM gives rise to the nephrons and interstitium, while UB differentiates to elaborate the lower urinary tract from the collecting ducts to a part of the urinary bladder.

Protocols for differentiation of ESC to NPC or UB are different from each group. However some molecules and markers can be summarized (see Fig. 3.1) [27, 29–34]:

Due to ethical issues, tumorigenesis/teratomas formation, and all rejection problems, the use of ESC needs attention when translating to clinical therapeutics. Despite this, all knowledge that ESC can give us is invaluable [35].

In 2006, Takahashi and Yamanaka published an article that changed the research worldwide. They recreated a pluripotent cell from an adult cell [36]. Induced pluripotent stem cells (iPSC) are adult cells that go back to the past. Usually working with blood cells or skin fibroblast, some genes are integrated at the cell (OCT4, Nanog, SOX2 mainly) and then cells go back to its pluripotent stage, like an ESC stage. Due to its pluripotency and self-renew capability, iPSC once obtained work as an ESC cell and may generate as many cells as possible when stimulated. Groups have worked hard to obtain kidney cells from IPSC, using a lot of strategies (Table 3.1).

Cell replacement therapy (isolating cells from the same patient) and drug screening are the main attractive issues from IPSC research regarding kidney cell therapy. However, some issues still need investigation. There are few studies regarding epigenetics in kidney-derived IPSC. Since the cell is obtained from a human adult cell, a lot of genetic modifications have already happened [45, 46]. Moreover, genetic

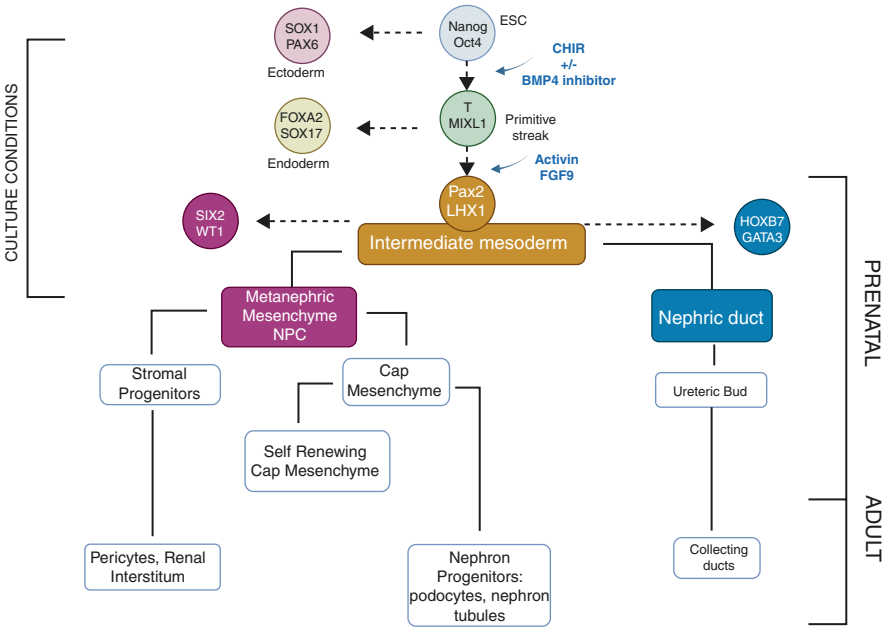

Fig. 3.1 Differentiation steps to generate nephron progenitor cells (NPC) and ureteric buds (UB) from embryonic stem cells (ESC) in culture conditions and following the lineage tree of renal cell types in human development. Stage proteins used as parameters for differentiation in culture conditions are identified in the circles. In blue, main molecules that must be present/absent at medium for differentiation in culture. Abbreviations: BMP, bone morphogenetic protein; FGF9, fibroblast growth factor 9; LHX1, LIM homeobox 1; LPM, lateral plate mesoderm; NPC, nephron progenitor cell; OCT4, POU class 5 homeobox 1; PAX2, paired box 2; ESC, embryonic pluripotent stem cell; SIX2, SIX homeobox 2; SOX2, SRY-box 2; T, brachyury; WT1, Wilms tumor 1. Image adapted from Takasato et al. [27] and Schumacher et al. [28]. Created with BioRender.com

abnormalities and tumorigenic concerns are still a problem since it is a culture cell and due to its pluripotency [47]. One positive aspect is that the ongoing studies have demonstrated that those kidney-derived cells are functional cells as seen in Table 3.1. Since organoids and those differentiated cells are not able to get vascularized, some researchers go further and repopulate decellularized kidneys with kidney derived-IPSC cells or organoids, and they have been showing better results than single-cell administration and a more feasible and closer clinical translation [48]. In the field of generation kidney-derived IPS, standardization of culture protocols urges attention. It is a difficult goal. There are a diversity of techniques to induce kidney cell generation and poor reproducibility that, nowadays, restrict scalability.

Despite clinical therapeutics for kidney-derived IPSC cells still needing to fight strong battles, IPSC easily creates an incredible tool: once IPSC is obtained from patients, we can exploit and better understand several genetic diseases [49–53].

Table 3.1 IPSC induction to generate kidney cells and its functional assays

Origin cell	Derived cells	Functional assay	References
IPSC	Podocytes	Cytoplasmic contractile response to angiotensin II, functional evidence of albumin uptake in the cytoplasm of iPS podocytes comparable to human podocytes, integration capacity of iPS-derived podocyte progenitors in an in vitro nephrogenesis reaggregation assay	Song et al. [37]
IPSC	Podocytes	transplantation method using spacers that release the tension of host kidney capsules, allowing the effective formation of glomeruli from human iPS cell–derived nephron progenitors	Sharmin et al. [38]
IPSC	Renal progenitors, i.e., nephrogenic intermediate mesoderm and metanephric mesenchyme	Intravenously infused iPSC-derived RPCs in a cisplatin mouse model	Imberti et al. [39]
IPSC	Kidney organoids	Dextran uptake by proximal tubules, upon implantation of the kidney organoids at renal capsule of immunocompromised mice they observed an increase in the number of vessels and glomeruli gradually acquired a much more mature architecture and the size-selective dextran handling	Low et al. [40]
IPSC	Ureteric epithelium and the metanephric mesenchyme in monolayer culture, following organoid culture conditions and fully kidney formation	Dextran uptake assay showing endocytic ability, cisplatin model at kidney organoid leading to cell apoptosis	Takasato et al. [41]
IPSC	Kidney organoids contain nephrons associated with a collecting duct network surrounded by renal interstitium and endothelial cells. Within these organoids, individual nephrons segment into distal and proximal tubules, early loops of Henle, and glomeruli containing podocytes elaborating foot processes and undergoing vascularization	Proximal tubules endocytose dextran and differentially apoptose in response to cisplatin	Takasato et al. [41]
IPSC	Metanephric mesenchyme	Glomeruli vascularized upon transplantation	Taguchi et al. [42]
IPSC	Kidney organoids containing podocytes, proximal and distal tubular cells, stromal cells and endothelial cells	Express renin	Shankar et al. [43]
IPSC	Nephron progenitor cells (NPC)	In vitro tubule-like structures in three dimensional culture systems	Kang and Han [44]

Chronic kidney disease experimental model (5/6 nephrotomy) was conducted to analyze the effects of IPS locally administered. Amelioration of CKD parameters was observed; however, tumor-like formations in 5 out of 8 were observed in the remnant kidneys [54].

We can summarize some of the potential studies using ESC and IPSC in kidney area in five approaches (see Figs. 3.2 and 3.3):

1. Generation of specific cells from kidney to understand it and to develop basic research.
2. Generation of cells to seed at scaffold or a decellularized kidney. Since vascularization is a problem at organoids, some researchers understand that replacement therapies must be throughout a real scaffold/decellularized kidney and repopulate these scaffolds with kidney cells derived from IPSC cells [55, 56].
3. Generation of organoids of the kidney to generate more realistic experimental models and drug tests. This subject will be explored below [57, 58].
4. Directly injected into humans. This strategy is being conducted for other diseases, such as Parkinson [59], macular degeneration [60], etc.
5. Modeling diseases and drug discovery: harvesting cells from genetic disease patients is possible to better understand several diseases [51].

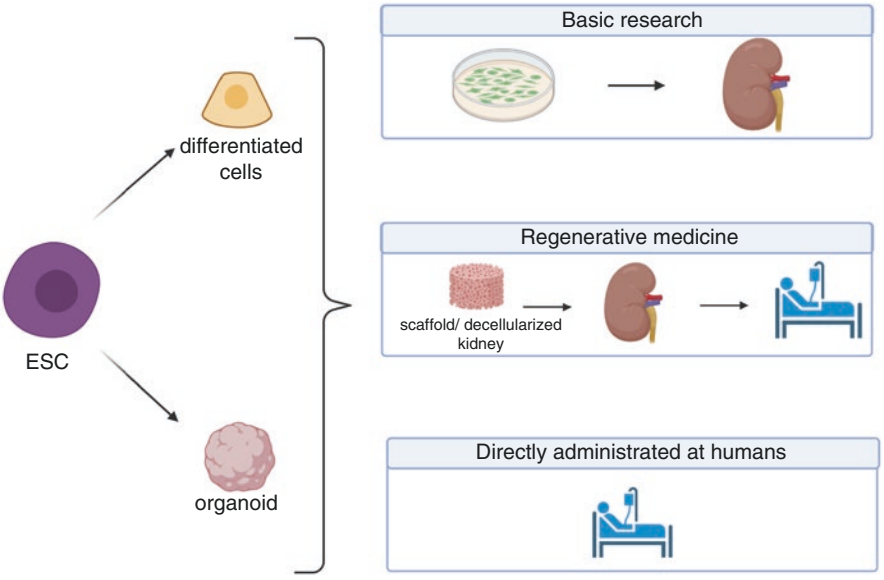

Fig. 3.2 Potential areas of ESC in kidney diseases. Embryonic stem cells (ESC) may be differentiated to the needed cell or to a complex of cells in 3D structure called organoid, under specific stimulus. At this point, they can be studied for basic research or injected in patients, throughout scaffolds or not. Direct administration of ESC at kidney disease in humans has not been tested. Created with BioRender

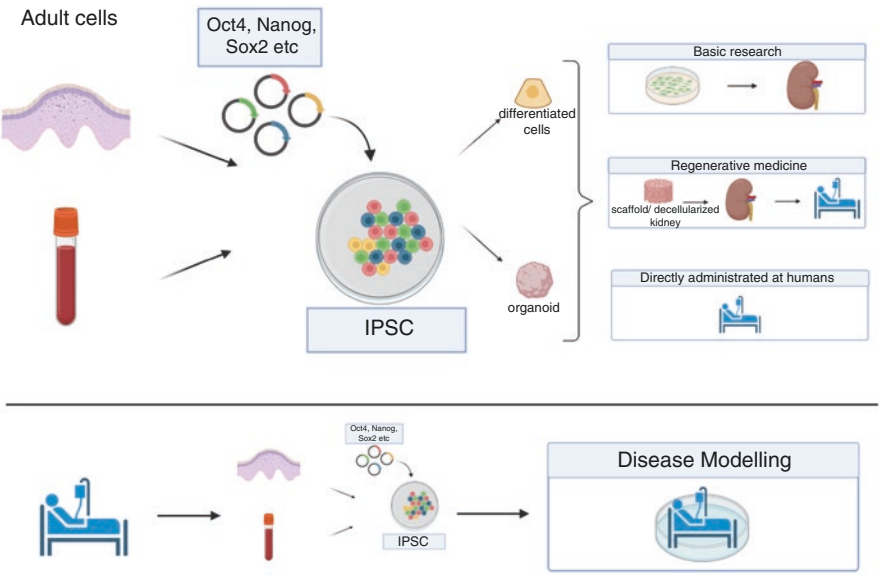

Fig. 3.3 Potential areas of IPS in kidney diseases. Since induced pluripotent stem cells (IPSC) have almost the same potential as ESC, the therapies and areas of studies of IPSC are quite the same as ESC. The advantage of using IPSC is that it is possible to take the cells from the patient, overcoming rejection in transplantation. Moreover, modeling disease is a huge tool for studying several genetics and metabolic diseases. Created with BioRender

3.2.2 Mesenchymal/Stromal Stem Cells

Mesenchymal stem cells, also known as multipotent mesenchymal stromal cells (MSCs), are the most studied cells in cellular therapy for kidney diseases—not only for kidney diseases but also for several illnesses.

These cells are collected from human body sites and then put at culture conditions to be expanded and to be administered into the patient (see Fig. 3.4). Mesenchymal stem cells are found in umbilical cord blood, adipose tissue, Wharton's jelly, bone marrow, dental pulp, and so on [61]. A recent consensus has standardized its abbreviation of MSCs from bone marrow as BM-MSCs, from adipose tissue as AD-MSCs and from umbilical cord as UC-MSCs [62]. Adult stem cells reside in areas where they are protected, namely niches [63, 64]. Some researchers point out that, since MSC can be found in virtually all sites of the body, perivascular areas may be the niche for part of MSC [65]. Other parts of MSC may have originated from nonperivascular sites [61, 66, 67].

How do you know if you collected the right cell and if they are the ones you need? Some consensus has been published and established some patterns. Dominici et al. [68], from International Society of Cell and Gene Therapy (ISCT), have described a minimum criteria to define MSC from bone marrow. Briefly, MSC should be plastic adherent, with positive extracellular proteins, mainly CD90,

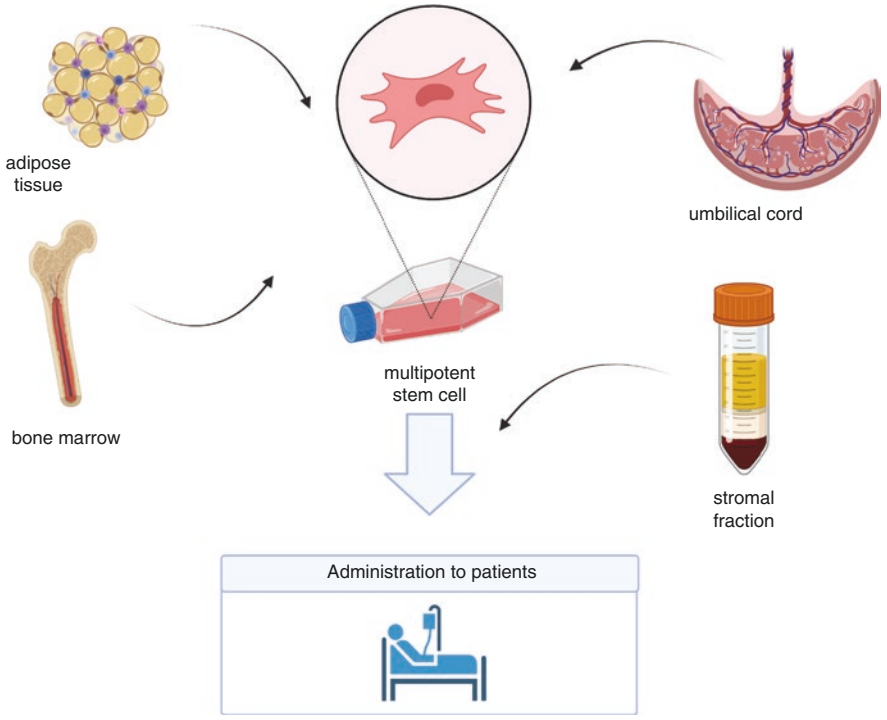

Fig. 3.4 Main sources of mesenchymal stem cell for clinical therapy. MSCs are cells expanded in vitro and then administered to patients. Stromal fraction of bone marrow or from adipose tissue may be used for clinical therapy with successful preclinical trials. Created with BioRender

CD73, and CD105, negative expression of CD11b, CD14, CD19, CD34, CD45, CD79a, and HLA-DR, and perform assays to characterize the multipotency properties (differentiation to adipocytes, chondroblast and osteoblast cells) [68]. In 2019, ISCT updated the consensus [69]; three new criteria have been added:

1. The origin of the MSC should be described: there are tissue-specific regenerative properties depending on the origin of the cell. For instance, CD34 expression is negative at BM-MSC and positive at AD-MSC [70].
2. Evidences of stemness in vitro or in vivo.
3. Functional assays.

However, this consensus does not define specific culture conditions, and it leads to a lot of differences and problems in quality, safety, and reproducibility to clinicals trials. In 2018, the marketing for MSC therapy has been authorized in Europe [71]. Nowadays, more than 250 trials are enrolled at Clinicals Trials.gov (studies found for: stem cell | kidney, also searched for Renal, Progenitor Cell, Process, and more, search date: October 6, 2021).

Since it has become a therapy, more attention should be paid to GMP manufacturing, quality control and safety, and efficiency tests. Regarding this issue, several articles have described some guidelines to achieve those goals [72–75].

The MSC's culture is heterogeneous. It is composed of fibroblasts, myofibroblasts, progenitors, among other possible cells. In this way, ISCT claims to analyze the functional properties of MSC that involves mainly its mechanism of action: analyze the secretion of trophic factors, the immunomodulation role, and its angiogenic pattern [76].

For kidney therapies, MSC treatment consists of administration of MSC expanded in vitro in patients. Since MSC is not fully recognized by the immune system, allogeneic transplantation is not a problem [77]. Administration of allogeneic MSC in patients does not elicit rejection, even with multiple infusions [77, 78]. On the other hand, the amount of cells, the MSC donor characteristics, the route of administration, periodicity or not of infusion, autologous or allogeneic MSC administration are not a well-standardized protocol and vary from study to study [79, 80]. How MSCs exert their therapeutic effect will be exploited below.

Among MSCs, adipose stem cells (AD-MSC) have several attractives to substitute BM-MSC: easy to collect and cultivate and quite the same properties of BM-MSC. Some differences have been observed, mainly in their response to injury. The source of MSC elicits differences in therapeutics responses [80]. Stromal fraction of bone marrow and from adipose tissue may be a noncultured option for cellular therapies. Indeed, these stromal fractions include a lot of other cells; they should be autologous administered, but they do not have the problems of a cultured cell: senescence and tumorigenesis [81, 82] (Fig. 3.4).

3.2.3 Renal Stem Cells

As mentioned above, virtually all tissues may harbor stem cells, some from a perivascular niche, other cells called progenitors from other niches that are unknown. The search for a tissue-specific adult stem cell is a huge task [63, 65, 83].

Is there a kidney renal progenitor? The fast regeneration of the kidney after an insult supports the idea of ready cells to repair. Highly turnover tissues harbor resident stem cells. Organs and tissue with low turnover may rely on progenitors cells [84]. At the kidney, progenitor cells may be the one closer to the lesion leading to this fast regeneration.

Several groups have been studying this population of cells. BrdU+-labeled retaining of cells [85], isolation of a highly proliferative cell from a nephron (named rKS56) [86], isolation of a CD133+ cell from kidney that expresses Pax2 (an embryonic marker) capable of generated tubules with epithelial markers [87], a Sca1+Lin- cell isolated from kidney that contribute to tubule repair [88] show that a kidney progenitor cell exists [89].

The parietal cells from Bowman's capsule, specially at the urinary pole, have been suggested as kidney progenitors. These cells were CD133+CD24+ (residual markers from embryonic kidney), and they differentiate in podocytes or tubule cells under culture conditions [90, 91].

Humphreys et al. elegantly described that the tubular epithelial regeneration after an injury is due to surviving epithelial cells, thus other cells/progenitors did not

contribute to its repair [92]. So, if the MSC or progenitor cells did not replace/differentiate into epithelial cells, the better outcomes obtained after their administration may be through its paracrine effects (see below at "Mechanism of Action").

Lindgren et al. described a possible tubular progenitor cell in humans. Sorting kidney cells ALDH^{high+} out, they found CD133+CD24+CD106- cells with regenerative properties. They can be located at the proximal tubule and at distal convoluted tubule [89, 93].

Renal papillae has also been described as a niche for kidney progenitor cells. Oliver et al. injected BrdU in mouse and rat pups and followed them for 2 months. After this period, they found BrdU retaining cells at the kidney papillae in numerous amounts [94].

Recently, a cell with stemness properties has been isolated from urine. Derived from renal papillae or from tubule, this cell is very attractive for cell therapy since it is obtained easily in a noninvasive way with similar properties of MSC [95].

The niche of kidney progenitor cells in adult kidney needs further studies [96]: proximal tubules, distal convoluted tubules, Bowman's parietal cells, papillae; should we consider multiple niches for kidney progenitors cells? (see Fig. 3.5).

In all those articles, the kidney cells show proliferative ratio, specific markers of embryonic cells, such as CD146, CD133, and CD24, form spheres at culture and when administered in animals models of kidney diseases, they lead to improvement of the disease and differentiation to tubules or podocytes. Epigenetics have a huge role in these cells. The final outcome/fate of a progenitor cell depends on its

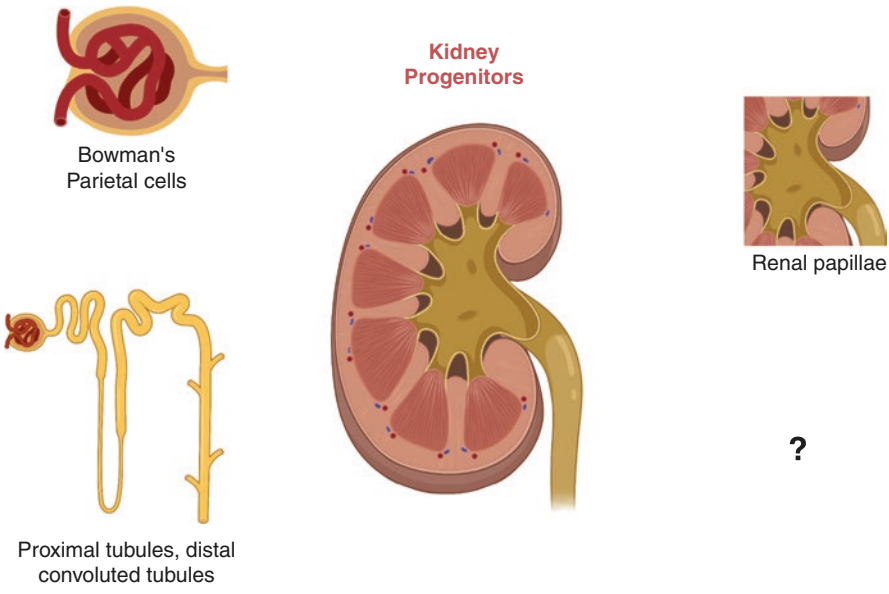

Fig. 3.5 Possible niches for kidney progenitors in adult kidneys. Those three sites may be potential areas for niches for kidney progenitors. Are there multiple niches? Created with BioRender.com

stimulus received. Injury is the main stimulus to a progenitor cell to differentiate: it is the basis of regeneration in cellular therapy [97, 98]. How injury leads to progenitor cell activation or its exhaustion is an area that still needs a lot of work.

3.2.4 Cell-Free Therapy: Extracellular Vesicles from IPS, MSC, and Kidney Progenitor Cells

Extracellular vesicles (EV) are an area of research that is increasing exponentially in the late years. EVs are vesicles composed of lipid bilayer with transmembrane contents and bear proteins, lipids, DNA, RNA, miRNA, etc., leading them to participate in several processes of cell-cell communication [99, 100]. Extracellular vesicles can be categorized by size and release from cell:

- Exosomes: 40–100 μm/budding from plasma membrane
- Microvesicles: 150–1000 μm/fusion of internal multivesicular compartments with plasma membrane
- Apoptotic body: 1000–5000 μm/cell fragmentation/blebbing

EVs are released by several cells (MSCs, cancer cells, immune cells, epithelial cells, etc.) and in several body fluids (blood, urine, milk, amniotic fluid) [100–103]. A detailed guideline summarizing the criteria to isolate and characterize EVs is defined by the International Society of EVs [104].

The main function of EVs is to regulate cell-cell communication [105]. In the first works of EVs, it was thought that EVs were only for cell clearance [102]. It is known that both resting cells and stressed cells release EVs. EVs released from cells in a disease environment may participate in the progression of the disease. In this sense, block EV release may be a therapeutic action to halt its progression. When EVs are released by donor cells, molecules from cytoplasm and/or membrane can come together. From this idea, the EVs can be used as disease biomarkers in therapies and studies of cell-cell communication.

Therapy with EV arises from the idea that those vesicles can carry "good" molecules if they are from "good" cells. The opposite is also right: "bad" cells generate vesicles with "bad" molecules, as described above. In this sense, those EVs can be used for disease biomarkers [101] and the understanding of disease progression. Nowadays, it is possible to engineer the donor cell to generate the EV you want. The EV donor cell can be upregulated or downregulated to specific molecules to modulate the recipient cell [101].

EVs cargo varies immensely, and it is related to donor cell microenvironment and to the stimulus of donor cell [102, 106]. EVs cargo are proteins, DNA, mitochondrial DNA (mtDNA), messenger RNA (mRNA), microRNA (miRNA), long noncoding RNA (lncRNA), circular RNA (circRNA), lipids, minerals, and also foreign molecules from infectious diseases [107, 108]. There are databases that summarize its cargo constantly: Vesiclepedia, ExoCarta, and EVpedia [109–111]. All these cargo can be absorbed by the recipient cell and can modify/reprogram its response [112].

Table 3.2 Extracellular vesicle therapies for kidney models, selected articles

Extracellular vesicles (EVs)	Kidney disease	Reference
Microvesicles from BM-MSC	Mouse AKI	Bruno et al. [114]
Microvesicles from BM-MSC	Human tubular cell/in vitro ATP depletion	Lindoso et al. [115]
Microvesicles from Wharton-Jelly MSC	Rat AKI	Zou et al. [116]
Extracellular vesicles from ESC	Mouse AKI and fibrosis after AKI insult	Yu et al. [117]
Extracellular vesicles from UC-MSC and UC-MSC overexpressing Oct4	Mouse AKI	Zhang et al. [118]
Exosomes from AD-MSC	Rat cisplatin AKI model	Lee et al. [119]
Exosomes from BM-MSC and from melatonin preconditioned BM-MSC	Rat AKI model	Alzahrani [120]
Extracellular vesicles from endothelial progenitor cells	Culture human glomerular endothelial cells and podocytes	Medica et al. [121]
Exosomes from UC-MSC	Rat unilateral ureteral obstruction (UUO)/fibrosis model	Liu et al. [11, 12]
Extracellular vesicles from BM-MSC	Diabetic model/streptozotocin	Grange et al. [122]
Exosomes from urine-derived stem cells	Diabetic model/streptozotocin	Jiang et al. [123]

EVs from several cells have been studied for kidney diseases from diagnosis to therapy [102, 113]. Table 3.2 summarizes some studies in EV therapy in the kidney models.

EV therapy is at its initial steps. Standardization of protocols, cargo definition, and routes of administrations may need to be analyzed cautiously.

3.3 Mechanism of Action of Stem Cells on Cellular Therapy

In literature, the use of stem cells in therapy in experimental models is always correlated with good outcomes, improvement of tissue, and amelioration of cell function. There is a lot of evidence that stem cell treatments are indeed a good option for several diseases/models. How does it work? How can a cell modulate the injury? We will review these questions below.

3.3.1 ESC and iPSC

Since ESC/IPSC therapy was established, the more attractive of this therapy is related to kidney replacement using scaffolds or a decellularized kidney filled with IPSC-derived renal cells, as better described below.

Injection of renal progenitors from IPS can also elicit a good response. Toyohara et al. have administered at the renal subcapsular the IPS-derived renal progenitors in

experimental models of AKI, showing improvement of renal disease. The mechanism of action is related to iPSC-derived renal progenitors secretion of growth factors such as HGF, VEGFa, and ANG-1 that are renoprotective and lead to tissue amelioration [124]. Another group injected IPS-derived renal progenitors intravenously in AKI model, leading to amelioration of renal impairment symptoms. They suggested that this could occur due to engraftment of IPS-derived renal progenitors to damaged tubule, proliferation, and acquisition of tubular epithelial phenotype [39].

3.3.2 MSC

In the field of MSC the mechanism of action is a more studied subject. Paracrine effects are the main mechanism of action of MSC that leads to modulation of inflammation and regenerative properties (anti apoptotic and angiogenic effects).

Nevertheless, it must be said that when we think about MSC therapy, it is the administration of MSC at a recipient patient, i.e., there is administration of exogenous MSC. We must bear in mind that the MSC mechanisms of action are what this cell should do in its niche/tissue. In a physiological state, MSC is quiescent. After an injury, the inflammatory response plays a role in regenerating the tissue, releasing a storm of cytokines and inflammatory factors. These molecules by its turn activate MSCs that come to downregulate the inflammation response and finish the repair process. If there is a balance between inflammatory response and MSC, tissue returns to its integrity and homeostasis. If this balance is not achieved, there is a disruption in the healing process, and diseases are established. Since endogenous MSC can not work properly due to pathologic state, exogenous MSC are administrated to the patient to heal and generated the homeostasis to the damaged area. And it mainly happens throught immunomodulation of inflammatory response [125–127].

Why do exogenous MSCs need to be administered to patients? Why are resident MSCs unable to exert their own function? Because resident MSCs are exhausted and/or depleted. Exhaustion of MSC occurs in metabolic alterations, such as obesity and aging, as well as in a pathogenic scenario. Obesity interferes with stem cell response. Mice fed with a high fat diet (HFD) have hair follicle stem cells depleted. HFD promotes a lipid-induced stress, which in turn activates IL-1R and inhibits SHH signaling [128]. AT-MSC from aging, diabetes, and obese donor patients leads to a different response in therapy, once these pathological states decrease the regenerative potential of the AT-MSC [129]. Neural stem cell also is impaired with aging [130].

Isolated BM-MSC from rat with CKD model showed signs of premature senescence: spontaneous adipogenesis, reduced proliferation capacity, active senescence-associated β-galactosidase, accumulation of actin, and a modulated secretion profile. So CKD inputs some modifications at resident MSC that impairs its functionality [131]. The same occurs with AT-MSC in a long-term exposure to uremic toxin leading to disruption of regenerative properties [132]. These data show that resident MSC can be modulated by metabolic and pathogenic stimulus and more importantly show the need to carefully assess the patient's suitability for autologous MSC transplantation.

Some authors suggested a way to stimulate resident MSC by modulating the niche and manipulating MSC in vivo [133]. This is an interesting area but still needs a lot of work. So if resident MSC is not working properly, exogenous MSC can help to cope with the injury. Once administered, the MSC biodistribution in the body is still controversial. There is evidence that MSC can be engrafted in the lung, liver, and kidney. MSC engraftment depends on the site of MSC administration. Most experimental models injected MSC intravenously; however, this decision depends on the mechanism of action needed [134, 135].

MSC can reach the injured site, in few numbers, despite the route of cell administration. Locally administration of MSC seems to be more effective than other routes. Exougenous MSC administered to patients may home to damaged areas throughout several chemokines and receptors, for instance CXCL12-CXCR4, CCL27-CCR10, and CCL21-CCR7 [136], and then exit the bloodstream. Ullah et al. greatly described strategies to improve this homing and migration of MSC for clinicals therapy [137].

Now, at damaged areas, MSC modulates inflammatory response in several ways. MSCs secrete IDO2,3, TGFb, PGE2, TSG6, and sHLA-G5 that act in immune cells, leading to an immunomodulation, mainly through increased population of regulatory T cells (Treg cells), suppressed proliferation, and activation of T cells, promoting regulatory DCs and M2 macrophages and then stimulating anti-inflammatory response [138–141]. More detailed mechanisms of MSC in adaptive and innate immune responses are recapitulated in well-written reviews [127, 138].

Some authors, on the other hand, suggested that the trapped MSC in the lung is the way MSC elicits its response. Monocytes and macrophages at lung phagocyte MSC and then change themselves to a regulatory pattern that are systematically distributed [142]. Since most clinical trials injected MSC intravenously, and these cells are trapped in the lung, it generated a controversy of how MSC exerts its function. Recent works suggest that the living MSC is not needed at the injury site. Some fragments of MSC cell or even MSC inactivated can elicit modulation of inflammation [143, 144]. It can change the previous works in the area regarding MSC mechanism of action and homing [145].

Besides modulation of inflammation, MSC has anti-apoptotic and angiogenic properties. Several works describe that MSC can secrete angiogenic factors, such as VEGF, FGF, HGF, placental growth factor (PGF), monocyte chemotactic protein 1 (MCP-1), stromal cell-derived factor 1 (SDF-1), and angiopoietin-1 (Ang-1). Regarding anti-apoptotic effects, MSC secretes survivin, VEGF, HGF, insulin-like growth factor-I (IGF-I), stanniocalcin-1 (STC1), TGF-β, FGF, and granulocyte–macrophage colony-stimulating factor (GM-CSF). MSC can also regulate reactive oxygen species (ROS) since it can produce HO-1 [139]. Modulation of ROS should also occur due to mitochondrial transfer via *tunneling nanotubes* (TNT) or via exosome transfer [146].

At kidney models, MSCs promote immunoregulation, anti-apoptotic effects, and angiogenic profile (Table 3.3).

Table 3.3 Exogenously MSC treatment at kidney models

	Model	Amount of cells and route of administration	Outcomes	Reference
Acute kidney disease (AKI)	40-min bilateral renal pedicle clamping	Intracarotid administration of MSC (approximately 10(6)/animal) either immediately or 24 h after renal ischemia	Improved renal function, higher proliferative, and lower apoptotic indexes, as well as lower renal injury and unchanged leukocyte infiltration scores	Tögel et al. [147]
	Sepsis-associated AKI/mouse cecal ligation and puncture operation	(1 × 10 cells intravenously) 3 h after surgery	Alleviate sepsis-associated AKI and improve survival, inhibition of IL-17 secretion and balance of the proinflammatory and anti-inflammatory states	Luo et al. [148]
	Polymicrobial sepsis induced by cecal ligation and puncture (CLP) in mice	5.0 × 105 BM-MSC cells from HO-1+/+ or HO-1−/− mice injected via the tail vein 2 h post-CL. Additional tail vein i.v. injections of 2.5×10^5 cells in 200 μl of PBS were given 24 and 48 h post-CLP. Lung-derived fibroblasts, at a dose of 5.0×10^5 cells as control were administered too	Amelioration of sepsis outcome, increased survival. After onset of CLP-induced sepsis, enhanced phagocytosis of bacteria by neutrophils and increased bacterial clearance	Hall et al. [149]
	Mouse cisplatin model	Human BM-MSC (5×10^5 cells) intravenously (i.v.) injected into tail vein	Decreased proximal tubular epithelial cell injury and ameliorated the deficit in renal function, resulting in reduced recipient mortality	Morigi et al. [150]
	Glycerol-induced mouse model	1×10^6 mice BM-MSC i.v. injected	Morphological and functional recovery	Herrera et al. [151]
	60 min bilaterally clamping of renal pedicles	Six hours after injury, MSC (2×10^5 cells) were administered intravenously	Morphological and functional recovery, reduced renal inflammation	Semedo et al. [152, 153]

(continued)

Table 3.3 (continued)

	Model	Amount of cells and route of administration	Outcomes	Reference
AKI > CKD	Folic acid model followed for 4 weeks	1×10^6 cells AT-MSC IP 24 h after folic acid	Reduced kidney fibrosis and chronic inflammation	Burgos-Silva et al. [154]
	Unilateral severe ischemia by clamping the left renal pedicle for 1h	6 h of reperfusion, 1×10^6 cells. Bone marrow mononuclear cells (BMMCs) were administered intraperitoneally	Reduced tissue inflammation, decreased fibrosis	Semedo et al. [155]
	Unilateral hypoxia followed for 6 weeks	2×10^5 AT-MSCs IP after 4h of injury or 2×10^5 IP AT-MSC at 6 weeks followed until 10th weeks	Reduced tissue inflammation, decreased fibrosis	Donizetti-Oliveira et al. [156]
Chronic kidney disease	5/6 nephrectomy	2×10^5 cell BM-MSC IV multiple doses	Reduced inflammation systemically and locally, reduce fibrosis progression	Semedo et al. [152, 153]
	UUO (unilateral ureteral obstruction)	10^6 human Wharton's Jelly-derived MSCs were injected into the aorta inferior to the renal artery after surgery in rats	Decreased fibrosis	Kheradmand et al. [157]
	UUO (unilateral ureteral obstruction)	Human MSCs (1×10^6/rat) immediately before operation	Exogenously administered MSCs significantly reduced these indicators of renal fibrosis, MSCs protect against obstruction-induced renal fibrosis, in part, by decreasing STAT3 activation and STAT3-dependent MMP-9 production	Matsui et al. [158]
	Chronic kidney disease in collagen4A3-deficient mice (Alport model)	At 6 weeks of age, COL4A3-deficient mice were divided into two groups that received tail vein injections of either 1×10^6 MSC in 200 µl isotonic saline or saline only at weekly until death	Renal parameters without changes, prevented the loss of peritubular capillaries, and reduced markers of *renal fibrosis*, that is, interstitial volume, numbers of smooth muscle actin-positive *interstitial cells*, and interstitial collagen deposits as compared to saline-injected COL4A3-deficient mice	Ninichuk et al. [159]

Table 3.3 (continued)

	Model	Amount of cells and route of administration	Outcomes	Reference
	Streptozotocin-induced diabetic nephropathy (STZ-DN)	2×10^6 Human UC-MSCs via the tail vein at week 6. Analyses after 2 weeks	Ameliorated functional parameters, such as 24-h urinary protein, creatinine clearance rate, serum creatinine, urea nitrogen, and renal hypertrophy index. In the kidney tissue, this improve of renal function were correlated with significant reductions in renal vacuole degeneration, lower inflammatory cell infiltration and less renal interstitial fibrosis	Xiang et al. [160]
	2K1C/Renal arterial stenosis	2×10^5 cells BM-MSC IV/weekly	Prevented the progressive increase of arterial pressure, reduced fibrosis, proteinuria and inflammatory cytokines, reduced fibrosis proteinuria and inflammatory cytokines and suppressed the intrarenal RAS	Oliveira-Sales et al. [161]

3.3.3 Extracellular Vesicles

Extracellular vesicles (EVs) have three main potential functions in kidney diseases: (1) diagnostic biomarkers, (2) progression of the disease, and (3) therapy. Jin et al. described the role of exosomes as diagnostic parameters for kidney diseases [101]. Karpman et al. have recently reviewed the role of EVs in pathophysiological [162, 163].

Several steps must be considered to generate exogenous EVs to therapy. Usually, a "good" cell is the one used. MSC is the most used cell to generate EVs. However, this EV area of study is calling attention and has been flourising. Nowdays it is possible to obtain therapeutic EVs from renal epithelia cell as well as from IPSC [163]. EVs release for donor cells can be increased during cellular activation and/or cell stress. After that, EVs are isolated and then ready for therapy [102].

Once intravenously administered, exogenous EVs can be found in the liver, spleen, and gastrointestinal tract [164]. As well as MSC, EV biodistribution depends on the route of administration.

How can EV cargo enter the cell? First, EVs must be near the recipient cell. Several receptors expressed at EVs may participate in this process, depending on the EV-cell origin. Moreover, the expression of these receptors can be modulated by bioengineering. To delivery EV's cargo, EVs must be internalized and the EV cargo delivery at the

recipient cell. EVs can be internalized by recipient cells by a variety of endocytic pathways, including clathrin-dependent endocytosis, and clathrin-independent pathways such as caveolin-mediated uptake, macropinocytosis, phagocytosis, and lipid raft–mediated internalization [165]. EV cargo enters the endosomal system in early endosomes (EEs); however, they are not degraded. This mechanism is still unknown [164].

EV cargo can act directly in the recipient cell. mRNA, microRNA, some proteins, and lipids can modulate the recipient cell [166]. Hade et al. have recently reviewed the molecules that are described to be EV-derived from MSC [167]. All the molecules, specially miRNA, as extensively described by several authors, can reprogramme the recipient cell, leading to amelioration of the kidney disease model [168, 169].

Several mechanisms of action of EVs are still unknown and need further studies: how do EVs reach the damaged cell? Is this amelioration obtained after administration of a transient process, since the amount of EVs cargo is limited?

3.4 Kidney Organoids

For decades, in vitro 2D culture and animal models have been widely used as an important research platform to address a range of scientific issues, from basic science to development, disease modeling, and evaluation of new drugs and therapeutics. Two-dimensional culture models are simpler systems, with relatively low cost and reproducible using primary or immortalized cells; however, they have limitations due to their simplicity. Primary renal cell cultures are defined as cells that were recently isolated from renal tissue. Recently renal epithelial cells were also obtained from human urine [170]. Their application is indicated because they mimic the physiological state of cells in vivo with more accuracy; however, they have limited growth capacity, due to the rapid process of dedifferentiation and with a predetermined number of cell divisions before entering senescence, with loss of their phenotype over time, which would make them unfeasible for long in vitro studies long term [171]. Despite these limitations, the use of primary renal cells still remains a reliable choice in studies of nephrotoxicity and basic renal cell functions [7].

Several immortalized cell lines of renal origin have been established due to their unlimited growth capacity and a more stable phenotype, which provides more reproducible results than primary cultures. Some immortalized human cell lines such as HK2, ciPTEC, RPTEC, caki-2, and other animal-derived cell lines as MDCK, LLC-PK1, NRK-52, and OK have been used because they maintain indefinite proliferation and sufficient phenotypic parameters for specific in vitro studies [171]. The RPTEC cells immortalized in two-dimensional cultures have been used for nephrotoxicity assays; however, they lack anion and cation transporters that are essential for drug excretion, making them unsuitable for predictive nephrotoxicity assays [172]. Disadvantages of these cells include the immortalization process is usually elicited by transfection and/or injection of Simian virus (SV40), papillomavirus (16E6/E7) genes, and human telomerase reverse transcriptase (hTERT) which can result in important changes in their characteristics and functions over time [173, 174].

In this context, two-dimensional cultures do not support growth in the vertical dimension resulting in abnormal polarity for specific renal cells [169]. Thus, the

lack of tissue-specific architecture and the absence of cell–cell interactions and cell–matrix interactions lead to loss of cell function and cannot accurately simulate the necessary microenvironmental factors and fail to modeling crucial elements of renal physiology that are highly influential in the study of the disease and the effectiveness of the drug for the treatment [175].

The use of experimental animal models are important tools for these studies, although they preserve the inherent complexity of interconnected tissues, they have shown little predictive and translational power for the human response due to discrepancy between species, which exhibit genetic and physiological differences in relation to basal metabolism, immune system function, and lifetime (de [176]). Most experimental studies use different species of animals such as mice, rats, hamsters, rabbits, zebrafish, guinea pig, xenopus toads, primates, dogs, and cats. In the field of therapeutics for kidney disease, most studies use rodents as a preclinical experimental model; however, these tested drugs fail in human clinical trials [177]. Each model has its own unique advantages and limitations [178, 179]. In vivo studies are time-consuming, of low yield and high cost as they require specific installations, adequate equipment and special training [180].

However, currently, many complex legal and ethical issues are raised about the pertinence of animal use leading to important restrictions on in vivo testing in the United States and Europe. The United States Environmental Protection Agency has prioritized the reduction of in vivo studies until the year 2035 and the Food and Drug Administration has established the use of the Principle 3 Rs (replacement, refinement, and reduction) in studies involving animal models [181]. This 3R strategy by Russell and Burch [182] suggests some ways to make animal experiments more humane, with minimal use of animals, that is, "reduction" in the total number of animals used in the experiment. This use must be carefully planned and "refined," and, if possible, higher animals must be "replaced" by alternative methodologies [178]. In 1995, the 4th R [183] was introduced which implies the addition of "responsibility" for the original three R's of Russell and Burch, based on the integrity and honesty of the results and the scientific correctness of proper and reasonable use of animal models necessary for research [184].

Thus, the existence of failures in replicating experiments in humans, the high costs spent, and the ethical and legal issues involved in the use of animals for disease models, tests of drugs, and therapy are under scrutiny. There is, therefore, a need for an intermediate path that can generate reliable forecasts and meet unresolved needs.

There is, therefore, a need for an intermediate path that can generate reliable predictions and meet needs not addressed by 2D models and animal models. With the improvement in 3D culture techniques, particularly the development of three-dimensional systems called organoids can help to overcome some of these limitations and concerns, making a "bridge" between studies based between in vitro and in vivo with great potential for applicability for disease modeling, personalized therapy, cancer research, and regenerative medicine.

Organoids constitute a complex three-dimensional multicellular collection, typically of human origin, derived from pluripotent stem cells, neonatal or progenitor cells, in which the cells spontaneously self-organize into differentiated functional cell types and present a structural and functional behavior similar to organ in vivo

[3, 185, 186]. Self-assembly and differentiation are essential characteristics of organoids resulting from the signaling pathways that regulate these processes provided by the extracellular matrix, growth factors in the medium and the constituent cell types [41, 187]. It is important to point out that the extracellular matrix is essential for the mechanical support of organoids in cell growth, migration, differentiation, and cell survival [188].

Organoids can be obtained from various sources such as pluripotent stem cells (PSCs), including embryonic stem cells (ESCs), and induced pluripotency stem cells (iPSCs) and tissue-specific adult stem cells [189, 190]. These distinct organoids have unique and complementary characteristics, as organoids derived from pluripotent stem cells mimic organogenesis during embryonic development and generally resemble fetal stage tissues, while organoids derived from adult stem cells recapitulate adult tissue [191]. WnT signaling has been identified as a key factor that allows the generation of organoids derived from adult stem cells [192]. PSC-derived organoids are generated through directed differentiation, mimicking specific combinations of growth factors that drive the induction of germ layers during development [177]. To date, several organoids derived from PSCs have been established that resemble various tissues, including functional organs such as brain, pancreas, intestine, liver, kidney [32], and heart, the last organ to be generated as an organoid [193] (Fig. 3.6).

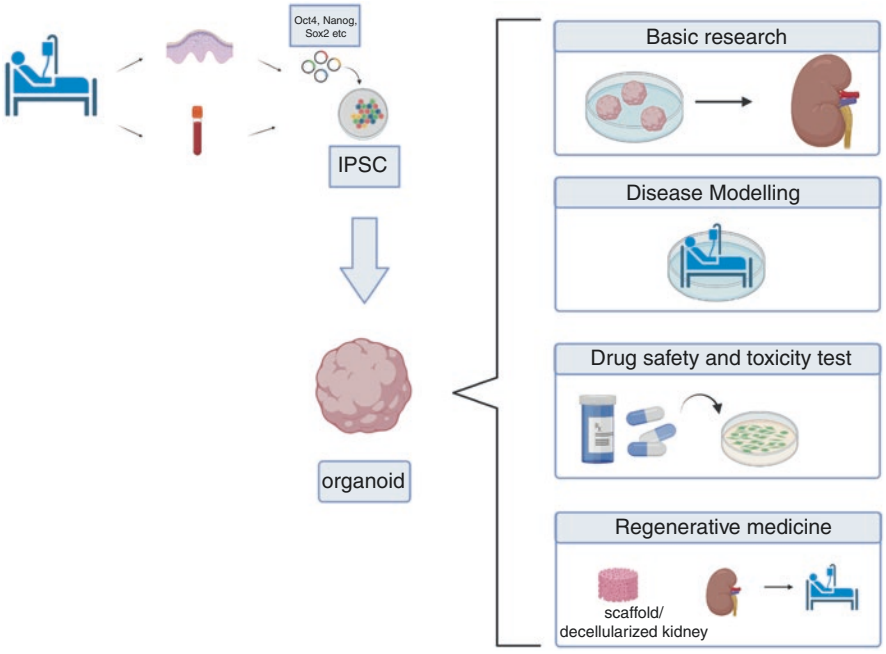

Fig. 3.6 Translational applicability of organoids. Organoids can be used for (1) studies of kidney development, aiming to understand human development and organogenesis processes; (2) disease modeling, to unveil the mechanisms that regulate and drive disease progression of various human pathologies; (3) drug efficacy and toxicity screening; (4) regenerative nephrology. Created with Biorender

3.5 Stem Cell/Renal Stem Cells Applied to Renal Tissue Engineering

Despite the great attractiveness of stem cell therapy, there are several obstacles to be overcome, such as the short duration of cell survival and function, the need for immunosuppression, in addition to the mandatory safety and fate studies of long-term implanted cells that limit its application [194]. Tissue engineering, as a therapeutic strategy, is based on the idea that bioactive substances facilitate the targeting, differentiation, and proliferation of stem cells seeded in three-dimensional scaffolds, leading to better cell engraftment [195]. Thus, this technology represents a new interdisciplinary field of knowledge, which aims to develop biological substitutes that mimic native tissue and can be used for the repair and regeneration of compromised tissues and organs [195, 196].

Complex organs such as kidney require an intact vascular network that can be reconnected to circulation after transplantation in the recipient in order to deliver nutrients and oxygen to the entire organ for clinical applications that must be processed in a three-dimensional (3D) fashion. Furthermore, given its highly organized multicellular structural complexity and the need for execution of vital essential functions, it would not be possible to reproduce these functions by traditional tissue engineering techniques. Only strategies such as decellularization and bioprinting are capable of generating functional and transplantable three-dimensional organs for future clinical application. Even in this context of difficulties in relation to renal complexity and its regeneration, tissue engineering proposes to help to simultaneously overcome the challenges inherent to dialysis, the need for organs for transplantation, and the prevention of patient exposure to immunosuppressive drugs.

Decellularization is a very attractive option to overcome these challenges, and it is anticipated that this new approach will be cost-effective in the long run, compared to the lifetime costs of dialysis or immunosuppressive drugs required for transplantation. The process of decellularization of xenogenic or allogeneic donor kidneys is the generation of whole organ scaffolds from which new kidneys are prepared, using the three-dimensional geometry, the vasculature, and components of the intact extracellular matrix, originating from the kidney tissue itself [197]. Through this strategy, vascularized acellular scaffolds are obtained intact, with total preservation of the vascular tree in order to facilitate the in vitro perfusion and reconnection to the bloodstream, which will provide nutrients and oxygen, in addition to the removal of metabolites after transplantation [198].

Furthermore, it has the essential advantage of providing structural integrity of the tissue, where synthetic and natural polymers used cannot replicate the precise spatial organization of cell architecture complex, as found in native renal tissue [199].

Several renal decellularization procedures have been described in the last years, in studies based on prolonged perfusion in the vasculature kidney through detergents or enzymes and successive washings [200]. These and other cell lysis solutions solubilize cell structures preserving intact the structural components necessary to maintain the mechanical and biological properties of the extracellular matrix (ECM) [201]. The vast majority of decellularization protocols depend on the use of

these detergents such as non-ionic Triton X-100 or the anionic dodecyl sulfate of sodium (SDS), but other existing techniques to assist this process include the use of alternating freeze-thaw cycles, shock osmotic and deoxyribonuclease to degrade nuclear material. In the face of exposure, an ideal protocol for decellularization will be one that efficiently removes all cellular material with less damage to the composition, to the biological activity and mechanical integrity of the extracellular matrix [202].

The recellularization of the resulting decellularized three-dimensional matrix of the kidney is the most complex and challenging phase due to the numerous differentiated cells that form the kidney [203]. Choosing cell sources to repopulate the resulting three-dimensional structure is important in obtaining a functional organ. The cells used should preferably be patient-derived to eliminate immunological rejection after implantation with ease of characterization and expansion, in addition to being functional in their new environment [197]. However, several cell types have been used for renal recellularization, including renal epithelial and endothelial cells, embryonic stem cells (ESCs), and IPSC.

Epithelial cells are an autologous source; however, they do not provide all cell types needed for kidney recellularization. Furthermore, cell expansion of these cells is not achieved due to the limited number of passages, making its clinical application unfeasible.

The use of ESCs in the recellularization process is limited by ethical issues due to the destruction of embryos, as well as because of in vivo teratoma formation [204]. Human IPSCs reprogrammed to be pluripotent cells avoid the ethical dilemma of embryonic cells, however IPSCs have already been shown to be tumorigenic, which limits their translation potential [56].

The recellularization methodologies used for decellularized renal matrices are critical to achieving success in repopulation whether it be cell distribution and differentiation [205]. In an attempt to circumvent these obstacles, different delivery routes were tested, when the cells were injected through the renal artery, they only reached the glomerular capillaries, while via the renal route they reached only the peritubular capillary [206].

Despite the promising results, the repopulation of decellularized renal matrices requires further optimization with the improvement of functional parameters, since the functionality of these organs is not yet accepted for transplants.

Bioprinting or organ printing**,** an extension of tissue engineering, recently defined as computer-aided additive biomanufacturing of cell tissue, applies additive manufacturing technology to structure living and nonliving materials with an organization bi- or three-dimensional pre-established, in order to directly produce biological tissue structures [207], substitutes that restore, maintain, or improve the function of a tissue or an entire organ. These emerging technologies are important tools to promote tissue regeneration with great potential for the bioengineering of living organs [208, 209]. Bioprinting systems use adaptations of additive manufacturing technologies and thus, based on their operating principles, can be classified as direct laser-induced engraving, inkjet printing (continuous or drop-by-drop), extrusion deposition, lithographic printing (stereolithography [SLA] and digital light

projection [DLP]), and electrostatic wiring [210]. Although bioprinting is a promising technology as an interface between engineering and tissues, each technique has its limitations.

Considering the prolonged time to print tissues and organs on a larger scale, a relevant disadvantage of encapsulating live cells in biomaterials is the need for the biomaterial cell suspension to be stored considerably in advance in the reservoir, which compromises cell viability and limits its bioactivity [207]. Mechanical resistance, structural integrity, and processability of biofabricated structures are also a common disadvantage among bioprinting techniques, as they use hydrogels with a high water content to favor biocompatibility [211].

Overall, cell encapsulation in biomaterials allows for cell patterning with potential for organ impression; however, the subsequent formation of extracellular matrix (ECM), digestion and degradation of the matrix of biomaterials, and the proliferation and colonization of encapsulated cells are not trivial. In order to overcome these problems, a new concept was introduced by Mironov et al. [212]. The proposal consists of tissue spheroids as building blocks that direct self-assembly for organ fabrication, demonstrating developmental morphogenetic principles such as cell organization and tissue fusion, based on the recognition that "nature is wise" [213, 214].

After bioprinting an organ, the produced structure needs to be transferred to a perfusion bioreactor, used to provide an ideal environment for the maturation process, by transporting nutrients, growth factors, and oxygen to the cells and extracting waste metabolic, so that the cells can grow and fuse, forming the organ [207].

Considering the path to transplantation in a human, this includes (1) modeling a three-dimensional model of an organ with its vascular architecture; (2) generation of a design for bioprinting, (3) isolation of stem cells, (4) differentiation of stem cells into specific target organ cells, (5) preparation and loading of organ-specific cells, vessel cells, blood samples, as well as the support medium, (6) bioprinting process followed by (7) organogenesis in a bioreactor and (8) transplantation. When building larger scale organs, the mechanical integrity of the bioprinted structures and their vascularization are the main challenges for the success of the approach in the search for the replacement or restitution of tissues and organs [215].

3.6 Clinical Translation of Stem Cells in Kidney Diseases

By searching for "Mesenchymal Stem Cell" at ClinicalTrials (www.clinicaltrials.gov), it retrieves 1270 studies between completed, recruiting, and not patients (other term ["Mesenchymal Stem Cell"], search date October 7, 2021). Adding the word "kidney" at the search, it results in 58 studies (conditions or diseases [kidney] + other term ["Mesenchymal Stem Cell"], search date October 7, 2021).

No trials with administration of exogenous exosomes/microvesicles or extracellular vesicles were found at ClinicalTrials for kidney disease. However, there is one work using patients and extracellular vesicles in kidney diseases. Forty stage III and

IV CKD patients were enrolled, and 20 patients received two doses of umbilical MSC-derived extracellular vesicles, showing amelioration of inflammatory response and improvement of kidney function [216, 217].

Hickson et al. summarize the progress of regenerative therapies into clinical translation in the four areas of nephrology: renovascular disease, sepsis-associated AKI, diabetic kidney disease, and kidney transplantation. The trials in diabetic kidney diseases for regenerative cellular therapies are evidently more prevalent since there is an exponential increase of diabetes in the world [218]. Completed trials using the exogenous MSC have been demonstrating amelioration of kidney diseases and no adverse effects in humans [217, 219]. However, it is difficult to compare all trials. These results can have interference of several parameters: lack of standardization on the production of the cells, lower quality and safety analysis, biases at donor MSC patients, route of administration and biodistribution of MSC in recipients, and mainly its mechanism of action in humans [220].

From the 58 clinicals trials, eight studies are completed (Table 3.4). All these studies showed safety and tolerability of MSC infusion in patients.

Table 3.4 Clinical trials with MSCs

w	Status	Study title	Conditions	Interventions	Locations	Publication
1	Completed	Mesenchymal stem cells transplantation in patients with chronic renal failure due to polycystic kidney disease	Chronic renal failure Polycystic kidney disease	Biological: intravenous injection autologous mesenchymal stem cells	Royan Institute Tehran, Iran, Islamic Republic of	Makhlough et al. [221]
2	Completed	Autologous bone marrow derived mesenchymal stromal cells (BM-MSCs) in patients with chronic kidney disease (CKD)	Chronic kidney disease	Biological: intravenous injection	Royan Institute Tehran, Iran, Islamic Republic of	
3	Completed	Induction therapy with autologous mesenchymal stem cells for kidney allografts	Renal transplant rejection	Procedure: kidney transplantation with MSCs infusion	Stem cell therapy center, Fuzhou General Hospital	
				Procedure: kidney transplantation without MSC infusion	Fuzhou, Fujian, China	

Table 3.4 (continued)

w	Status	Study title	Conditions	Interventions	Locations	Publication
4	Completed	MSC for occlusive disease of the kidney	Atherosclerotic renal artery stenosis	Drug: arterial infusion of autologous mesenchymal stem cells	Mayo Clinic in Rochester	Camilleri et al. [222]
			Ischemic nephropathy		Rochester, Minnesota, United States	
			Renovascular hypertension			
5	Completed	Hypoxia and inflammatory injury in human renovascular hypertension	Renal artery stenosis	Drug: mesenchymal stem cell	University of Alabama	Lerman [223]
			Ischemic nephropathy	Procedure: mesenchymal stem cell delivery with stent placement	Birmingham, Alabama, United States	
			Renovascular disease		Mayo Clinic	
			Chronic kidney disease		Rochester, Minnesota, United States	
					University of Mississippi	
					Jackson, Mississippi, United States	
6	Completed	Mesenchymal stem cells and subclinical rejection	Organ transplantation	Procedure: mesenchymal stem cell infusion	Leiden Universitary Medical Center	Reinders et al. [224]
					Leiden, Netherlands	
7	Completed	Allogeneic amniotic mesenchymal stem cell therapy for lupus nephritis	Lupus nephritis	Drug: human amniotic mesenchymal stem cell		
			Mesenchymal stem cells			
8	Completed	Evaluate the safety of CS20AT04 inj. in subjects with lupus nephritis	Lupus nephritis	Biological: allogeneic bone marrow derived mesenchymal stem cells	Hanyang university hospital	Jang et al. [225]
					Seoul, Korea, Republic of	

There are a lot of points to carefully look at and that need attention when translating stem cell therapy to humans. Moreover, transparency is needed [226]. The following questions reflect some concerns of procedures to culture the MSC that are quite open, and no standardization were determined.

1. Source of stem cell: from bone marrow, from adipose tissue, from IPS, etc., how these cells were isolated, etc.
2. MSC donor patient criteria: age, comorbidities related, pathogens exclusion, obesity, etc.
3. Cell culture conditions: use of defined mediums, the presence of fetal bovine serum, time of expansion, how passages were performed, how many passages, etc.
4. Preconditioning culture: how it was performed, etc.
5. Safety and quality control of cells before injection: viability test, karyotype tests, mycoplasma tests, pathogens tests, immunophenotype assays, secretion and function assays, etc.
6. Amount of cells injected at recipient patient and its periodicity
7. Route of administration: locally or systemically; whether systemic, arterial, or venous
8. Biodistribution: cells got trapped at lung, at liver, etc.?
9. Analysis of mechanism of action: systemic cytokine analysis before and after treatment, profile of immune cells before and after treatment, functional parameters of kidney before and after treatment, etc.
10. Recipient patient with inclusion and exclusion criteria well defined
11. Long-term follow-up after treatment: analysis of the recipient patients parameters after 1, 6, 12, 24, 72 months, for instance, to evaluate changes and tumorigenic potentiality, etc.

Regarding replacement therapies with organoids or scaffolds/decellularized kidneys with stem cells, they are in basic research yet due to kidney complexity [197].

Bringing EVs to clinical trials requires a lot of standardization protocols. Due to its infinitude of possible sources and internal cargo, a myriad of therapies can be generated. Drug delivery through EVs creates more parameters to increase variables for standardization. There are several EV isolation protocols, several characterization of EV protocols, EV half-life, storage conditions, biodistribution, dosage control, and route of administration that requires more studies before using them in humans [227, 228].

3.7 Ethics and Legal Regulation

With the large number of patients suffering from chronic and incurable diseases such as kidney diseases, interest in stem cells grows and generates great expectations in terms of possible benefits related to therapeutic applications. However, regardless of the potential and hope that stem cells will improve and save lives, there are many ethical, religious, legal, and security challenges and controversies to overcome.

hESCs are derived from the cell mass of embryos and have high levels of telomerase activity and normal karyotype. They are able to differentiate into cell types of the three germ layers under in vitro and in vivo conditions. However, human embryonic stem cell research presents a fundamental dilemma related to the moral status of the embryo from which embryonic stem cells are derived: is it morally acceptable to seek new therapies to cure disease at the expense of destroying a human embryo? Opponents of use argue that the embryo is capable of developing into a human being, and its destruction would be immoral and unethical. Proponents deny any moral status of the embryo whose potential benefits justify embryonic research [229]. This ethical dilemma is portrayed in different laws that regulate embryonic research around the world.

Safety issues related to hESC-based therapy are of primary concern for its clinical use. The pluripotency of hESCs allows these cells to differentiate into several different cell types, which would make it difficult to control after transplantation in vivo. When these cells are transplanted, tumors that contain the three germ layers can be formed and are called teratomas. Currently, it is believed that differentiating hESCs into the desired cell type before transplantation is the only way to prevent the formation of teratomas.

Thus, the use of stem cells contributes to disease modeling, drug discovery and testing, biobanks, organoids, and therapeutics, and it is important to always take ethical and legal considerations to each specific field of application.

3.8 Conclusion

Stem cell therapies and their subproducts, mainly EVs, are a promising therapy for kidney disease, with higher expectations as well as higher challenges. Basic research is still needed to understand the mechanisms and biological properties of stem cells. Moreover, standardization protocols, higher quality control parameters, therapeutic efficacy, and safety of using stem cells in humans are required for the flourishing of regenerative medicine using stem cells.

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