Cell-derived Secretome for the Treatment of Renal Disease

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Kidney disease is a major global health issue. Hemodialysis and kidney transplantation have been used in the clinic to treat renal failure. However, the dialysis is not an effective long-term option, as it is unable to replace complete renal functions. Kidney transplantation is the only permanent treatment for end-stage renal disease (ESRD), but a shortage of implantable kidney tissues limits the therapeutic availability. As such, there is a dire need to come up with a solution that provides renal functions as an alternative to the current standards. Recent advances in cell-based therapy have offered new therapeutic options for the treatment of damaged kidney tissues. Particularly, cell secretome therapy utilizing bioactive compounds released from therapeutic cells holds significant beneficial effects on the kidneys. This review will describe the reno-therapeutic effects of secretome components derived from various types of cells and discuss the development of efficient delivery methods to improve the therapeutic outcomes.

Key words: Renal disease, Regenerative Medicine, Secretome therapy, Secretome delivery

Introduction

Recent developments in regenerative medicine have provided attractive solutions for the treatment of renal diseases. Several technologies developed in regenerative medicine have been applied to treat renal failure¹⁻³⁾. Among the technologies, cell-based therapy has been primarily considered as a therapeutic intervention to provide renal functions¹⁻³⁾. A commonly used approach in cell-based therapy is cell transplantation, where therapeutic cells are exogenously administered to achieve the repair. The cell transplantation approach using various cell types have resulted in reno-therapeutic outcomes in many pre-clinical¹⁻³⁾ and clinical studies⁴⁾. For example, primary renal cells isolated from kidney tissues were introduced to improve renal functions from kidney diseases in a pre-clinical⁵⁾ and clinical study⁴⁾. Similarly, various stem cells, including adult, fetal, embryonic, and induced pluripotent stem cells also have been extensively studied as therapeutic cell sources and demonstrated the beneficial therapeutic effects in renal failure⁶⁾. While the cell transplantation approach has demonstrated promising outcomes, in terms of improvement of renal function, several safety issues still remain to be addressed prior to applying clinically. These include immune rejection, pulmonary

embolism, and teratoma formation by uncontrolled proliferation of pluripotent stem cells^{7,8}. While cellular therapy has become a potential therapeutic modality for selected populations, its mode of action remains controversial. Many investigators believe that the effects of cell therapy may be primarily due to trophic factors secreted by the administered cells (secretomes). Consequently, research using cell-derived secretomes has been actively pursued in various areas, including the kidneys.

Secretome is generally referred to as the trophic factors present in conditioned medium of cell culture (CM)⁹, and is comprised of soluble and insoluble molecules enriched with bioactivity that is suitable for tissue regeneration. Several studies reported that exogenous delivery of secretomes resulted in therapeutic efficacy on the improvement of renal functions in pre-clinical and clinical studies¹⁰⁻¹³. The therapeutic outcome by the secretome treatment is largely attributed to various secretome-derived bioactive compounds such as cytokines, chemokines and growth factors, lipids, free nucleic acids, and extracellular vesicles (EV)¹⁴⁻¹⁶. The important roles of the secretome compounds localized within the tissue injury site are believed to be angiogenesis, modulation of inflammatory, and inhibition of apoptosis of renal cells¹¹ (Fig. 1). In this article, we will review these secretome components and applications using various types of cell-derived secretomes for treatment of renal diseases (Table 1). Engineering platforms for efficient delivery of the secretomes will also be discussed (Table 1).

Secretomes used for kidney applications

1. Mesenchymal stem cell (MSC)-derived secretome

MSC is a stem cell type that is widely used in cell transplantation for tissue regeneration due to their availability in many tissue types and their ability to regenerate tissues for repair^{12,13}. The primary role of MSC underlying the regenerative mechanism following transplantation is known to enhance angiogenesis, induce anti-apoptosis, and modulate inflammation within the injured tissue¹¹. Several studies reported that the regenerative effects are mediated by paracrine actions via trophic factors secreted from the localized MSC into the injury site. For example, MSC infusion into the damaged kidney tissue facilitated the

improvement of renal functions that are attributed to the pro-angiogenic capability. Pro-angiogenic soluble factors^{11,17,18}, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), placental growth factor (PGF) and monocyte chemoattraction protein 1 (MCP-1) were reported to improve vasculogenesis and angiogenesis of injured kidney^{19,20}. In one study, Rota C et al. reported that amniotic fluid-derived MSCs reduced cisplatin-induced renal injury and the reno-protective effect was significantly improved by the angiogenic capability of VEGF, stromal cell-derived factor-1 (SDF-1) and insulin-like growth factor-1 (IGF-1)²¹. Moreover, another study reported by Takahashi et al. using an ischemic tissue model, VEGF and IGF-1 were found to exert anti-apoptotic effects on the improvement of tissue functions²², indicating a possible therapeutic mechanism of MSC secretome-derived anti-apoptosis for the renal repair.

In addition to the angiogenic and anti-apoptotic effects of MSC-derived soluble factors, the anti-inflammatory function also contributes to the improvement of renal functions. Other soluble factors such as transforming growth factor-beta 1 (TGF- β 1), prostaglandin E₂ (PGE₂), nitric oxide (NO), interleukin-6 (IL-6) and indoleamine 2,3-dioxygenase (IDO) present in the MSC-derived secretome have demonstrated the immunomodulatory effects within the damaged tissue. These factors are believed to play a critical role in transforming of the macrophage phenotype to anti-inflammatory subsets and regulating the balance of T helper 1 cell and TH17 cell (pro-inflammatory)

Fig. 1. Secretome therapy for treatment of renal disease. Cell-secreting molecules such as soluble factors and extracellular vesicles (EV) in the secretome contain therapeutic ability to recover the renal functions from the kidney injury. The possible mechanism underlying the beneficial effects can be explained by anti-apoptotic, anti-inflammatory, anti-scarring, immunomodulatory and pro-angiogenic property of the treated secretome into the damaged kidney.

to T_H2 cell and regulatory T cell (anti-inflammatory)²³⁻²⁵. These findings have suggested that the immune-modulatory action through the factors support the improvement of renal function²⁶.

Based on the positive paracrine effects of the trophic factors released from MSC, several studies utilized MSC-derived secretome to examine the therapeutic effects on kidney recovery. Abedi et al. administered human MSC-secretome via intraperitoneal injection to the gentamicin-induced kidney injury and observed partial tissue regeneration²⁷. In another study, da Silva et al. demonstrated the therapeutic outcomes of MSC-secretome treatment using a CKD model²⁸. Secretome derived from rat MSCs was administered into a unilateral ureteral obstruction (UUO)

model through the abdominal vena cava after total ligation of the left ureter. In this study, administration of MSC-derived secretome improved fibrosis progression and factors involved in apoptosis, inflammation, cell proliferation, and epithelial-mesenchymal transition in rats subjected to UUO²⁸. More recently, Liu et al. reported a possible tool of human MSC (hMSC) secretome for the treatment of a UUO kidney model²⁹. They collected human MSC secretome from human umbilical cord MSC culture (hMSC-secretome) and introduced through an intravenous injection, which resulted in improvement of renal functions. They believe that the therapeutic efficacy was likely mediated by reduced anti-apoptosis, inflammatory markers, and fibrosis progression as well as improvement of renal

Table 1. Cell Secretomes used for Kidney Applications

Cells	Origin	Component	Delivery	Therapeutic effects (species; disease; functions)	References
MSC	Human bone marrow	Whole secretome	I.P.	Rats; Gentamicin-induced AKI; injection time-dependent function improvement	Abedi et al. ²⁷
MSC	Rat bone marrow	Whole secretome	I.V.	Rats; UUO; reduced fibrosis progression and renal cell apoptosis	da Silva et al. ²⁸
MSC	Human umbilical cord	Whole secretome	I.V.	Rats; UUO; reduction in fibrosis progression, inflammation and renal cell apoptosis, proliferation of renal cells	Liu et al. ²⁹
MSC	Human cord blood	EV from secretome	I.V.	Clinical trials; CKD with 15–60 mg/ml/min in GFR; amelioration of inflammatory immune reaction and overall function improvement	Nassar et al. ⁸⁰
MSC	Human bone marrow	MV from secretome	I.V.	Rats; IRI; inhibition of apoptosis and stimulation of tubular cells	Gatti et al. ⁴⁰
MSC	Human bone marrow	MV from secretome	I.V.	SCID mice; rhabdomyolysis-induced AKI by glycerol intramuscular injection; tubular cell proliferation through horizontal transfer of mRNA	Bruno et al. ³⁸
MSC	Human bone marrow	miRNA-let7c-EV	I.V.	C57BL mice; UUO; anti-fibrotic function of miRNA let7c-expression	Wang et al. ⁴⁹
Renal cells	Rat kidney	EV from tubular cells	I.V.	Rats; ischemia; improved renal functions, reduced tubular damages and fibrosis	Dominguez et al. ⁵³
MSC	Human ESC	Whole secretome	I.V.	Rats; 5/6 nephrectomy; inhibition of CKD progression, improved tubular and glomerula injury, no therapeutic effects using MSC-EV only.	van Koppen et al. ⁵⁵
iPSC	Murine iPSC	Whole secretome	I.P.	Rats; IRI; down-regulation of oxidative stress-related pathway	Tarng et al. ⁵⁸
EPC	Human peripheral blood	MV	I.V.	Rats; IRI; reprogramming of host renal cells by miRNA delivery in MV	Cantaluppi et al. ⁶¹
EPC	Human EPC	EV	I.V.	Rats; experimental anti-Thy1.1 glomerulonephritis; inhibition of antibody- and complement-mediated injury of mesangial cells	Cantaluppi et al. ⁶²
ESC	Mouse ESC	Whole secretome recovered by self-assembled nanofiber	I.P. delivery of nanofiber hydrogel	C57BL mice; LPS-induced AKI; reduced apoptosis of renal cells	Wang et al. ⁷³
PSC	Human	Whole secretome combined with PRP	Intrarenal delivery of PRP and secretome	Rats; IRI; reduced apoptosis and improved proliferation of renal cells	Yim et al. ⁷⁷

Intraperitoneal injection (I.P.), intravenous injection (I.V.), Unilateral ureteral obstruction (UUO), chronic kidney disease (CKD), glomerular filtration rate (GFR), microvesicle (MV), ischemic-reperfusion injury (IRI), severe combined immunodeficiency (SCID), messenger RNA (mRNA), extracellular vesicle (EV), embryonic stem cell (ESC), placental stem cell (PSC), platelet rich plasma (PRP)

cells proliferation.

While the whole secretome, which contains all factors secreted by cells, has been successfully used as a therapeutic cue for renal treatment, a number of studies attempted to use a specific compound derived from the secretome. Use of well-defined secretome factors would be more practical for clinical use, in terms of safety and minimizing regulatory concerns⁷. Extracellular vesicle (EV), as a compound of cell secretome, has been studied for therapeutic efficacy on renal treatment. EV is a broad term that describes membrane vesicles formed by various cell types that are released to the extracellular environment³⁰ and plays a crucial role in cell-cell communication^{31,32}. EV includes microvesicles (MVs), exosome, and apoptotic bodies^{13,33}. MVs (100–1,000 nm) is originated from the outward membrane budding and released directly from the plasma membrane, whereas the exosomes (30–100 nm) originate from the inward budding of multivesicular bodies (MVB) that are formed as intraluminal vesicles (ILVs) and are released by fusion of MVB and the plasma membrane^{13,33-36}. The apoptotic bodies (500–4,000 nm) are usually generated by cells undergoing cell death³⁷.

A number of early studies have shown therapeutic outcomes of MSC-derived EV from the improvement of renal failure³⁸⁻⁴⁴, where the regenerative effects are mediated by various molecules such as protein, messenger RNA (mRNA), and micro RNA (miRNA)¹³. The introduction of insulin growth factor 1 receptor (IGF1R) mRNA enriched MSC-derived EVs showed improvement in cell proliferation, when co-incubated with damaged proximal renal tubular epithelial cells in an *in vitro* cisplatin-induced AKI model, suggesting of the renoprotective effects⁴³. In an ischemia-reperfusion injury (IRI) model using rats, Gatti et al. demonstrated the therapeutic outcomes of MVs from human BM-MSC on the improvement of renal functions, where the beneficial effects are believed to be attributed to the transfer of RNA for renal cell activation⁴⁰. Another study by Bruno et al. emphasized the transfer of mRNA and showed that the administration of MSC-derived MVs improved the survival rate of mice with the glycerol-induced AKI³⁸. The therapeutic mechanism is believed to be due to the proliferation of tubular cells by the administrated MVs, of which MV RNA was internalized into the targeting cells. However, abolishing of MVs with RNase abrogated the

protective results, suggesting RNA dependent biological effects. This study suggests that the mRNA transfer through MV internalization to the targeting cells could be considered as a new therapeutic platform for the treatment of renal failure³⁸. Interestingly, MSC-EVs derived from different cell origin have heterogeneous mRNA contents¹³. In human bone marrow MSC-derived EVs, mRNAs for transcription (e.g. TCFP2, RAX2, IRF6), cell cycle regulation (e.g. SENP2, RBL1, CDC14B), immune regulation (e.g. IL1RN, MT1X, CRLF1), extracellular matrix remodeling (e.g. COL4A2, IBSP), cytoskeleton (e.g. DDN, MSN, CTNNA1), cell differentiation (e.g. RAX2, EPX, SCNN1G) and hematopoietin (e.g., HK3, EPX) were identified^{13,38,39}. On the other hand, adipose tissue-derived MSCs-EVs contained mRNAs for transcription (e.g., MDFIC, POU3F1), angiogenesis (e.g., HES1, TCF4), adipogenesis (e.g., CEBPA, KLF7), Golgi apparatus gene (ARRB1, GOLGA4), and TGF- β signaling (e.g., TGFB1, TGFB3)^{39,44}. These findings suggest that appropriate selection of the secretome-derived EVs is needed for targeting treatment of specific conditions.

In addition to the beneficial effects of mRNA from EVs, several studies have confirmed the potential regenerative effects of miRNA in the MSC-derived EVs⁴⁵⁻⁴⁷. miRNAs are known to regulate 30–70% of mRNA expressions at the post-transcriptional level^{13,45,48} and considered as a key contributor to the overall biological functions and therapeutic features of EV (exosome)⁴³. In an *in vitro* model of IRI induced by ATP depletion, miR-410, miR-495, miR-548c-5p, and let-7a improved the renal recovery by down-regulation of mRNA coding associated with apoptosis, cytoskeleton reorganization and hypoxia, such as CASP3 and 7, SHC1 and SMAD4^{39,46}. The up-regulation of miR-375, miR-548c-5p, and miR-561 down-regulates SHC1, impeding the pro-survival EGFR-ras-ERK pathway^{44,47}. In a unilateral ureteral obstruction (UUO) model, Wang et al. miRNA-let-7c in MSC derived EVs improved renal injury and decreased fibrotic markers, such as collagen IV IVa1, TGF- β 1, and TGF β R1[49]. In addition, miR-29b, which contributes in epithelial-mesenchymal transition (EMT), downregulates angiotensin II-induced EMT via PI3k/AKT signaling pathway to target renal interstitial fibrosis⁵⁰ and also miR-21 was reported to protect the epithelial cell from IRI by preventing cell apoptosis and dendritic cell maturation⁵¹. Overexpression of the therapeutic

miRNA such as miR-29b and miR-21 in the EVs could be a potential therapeutic for improving renal function^{50,51}.

2. Secretome derived from other cell types

While most of the secretome studies have focused on MSC as a source of secretome, several studies have reported that different types of therapeutic cells could be used as secretome sources. As noted above, primary renal cells demonstrated therapeutic effects on the improvement of renal functions^{4,5,52}. Dominguez et al. showed the therapeutic effects of primary renal cell-derived secretome on kidney improvement⁵³. They collected EV compartments from rat renal tubular cells and intravenously injected the EV into a renal ischemia model in rats. The injection of EV significantly improved renal functions and reduced tubular damages and fibrosis. They suggested that the therapeutic effects by the EV therapy are likely due to a reduction of the large renal transcriptome drift observed after ischemia⁵³.

Secretomes derived from embryonic stem cells (ESC), and induced pluripotent stem cell (iPSC) have also been studied. Several reports using ESC-derived secretome demonstrated immunosuppression and proliferation ability, and have suggested that this system may be used as a therapeutic option for renal treatment^{54,55}. One study performed by van Koppen et al. used a rat CKD model to examine the therapeutic outcomes of ESC-derived secretome⁵⁵. Intravenous injection into the CKD rats showed that human embryonic MSC-derived secretome decreased the progression of CKD and improved tubular and glomerular injury. They suggested that the therapeutic effect is likely due to enhanced endothelial cell regeneration through active DNA damage repair⁵⁵. Interestingly, they also mentioned that the exosome only treatment from the MSC-derived secretome was not effective in the improvement of renal function. This finding suggests that incorporation of soluble factors and EV is essential to facilitate maximized therapeutic outcomes from the CKD⁵⁵.

Likewise, iPSC-derived secretome has shown to have antioxidant, anti-inflammatory, and anti-apoptotic effects^{56,56}. A study by Li et al. showed anti-inflammatory effects of the iPSC-derived secretome. They showed the suppression of macrophage inflammatory protein-2, malondialdehyde, and increased total glutathione and inhibition of PI3K/Akt pathway⁵⁷. Furthermore, the iPSC-derived sec-

retome significantly increased the expression of interferon gamma-induced protein-10 (IP-10) and mRNA, mitigating the tissue damage. These results suggest a protective effect of iPSC-derived secretome through a paracrine regulatory mechanism. In accordance with the previous studies, Tarnag et al. have discovered that iPSC-derived secretome facilitated the significant improvement of the renal condition and reduced apoptosis through intraperitoneal (IP) administration⁵⁸. A decrease in reactive oxygen species (ROS) production and inflammatory cytokines expression was observed in the iPSC secretome-treated group. They suggested that improvement of renal function is due to down-regulation of the oxidative stress-related pathway in the renal ischemia⁵⁸.

Based on the results that angiogenic effects of the MSC, ESC, and iPSC-derived secretomes are able to improve renal function, endothelial progenitor cells (EPC) appear to be a promising secretome source, since EPC was found to improve angiogenesis and neovasculogenesis⁵⁹⁻⁶². Recent studies showed that EPC-derived secretome contains pro-angiogenic, anti-apoptotic, and antioxidant features. Urbich et al. examined the expression profile of angiogenic growth factors such as VEGF-A, VEGF-B, SDF-1, and IGF-1 in different endothelial cell types, and found that EPC showed higher levels of the angiogenic ability than that of mature endothelial cells, such as human umbilical vein endothelial cells (HUVEC) or human microvascular endothelial cells (HMVEC) as well as monocyte⁵⁹. The release of soluble factors was significantly higher in EPC as compared with other cells. Furthermore, EPC-derived secretome increased the migration of endothelial cells. This study concludes that the release of various factors from EPC enhances the survival and functions of cells in a paracrine manner, expediting angiogenesis and regeneration of tissues. Another study by Yang et al. examined the effect of antioxidant of EPC features by treating HUVEC with EPC secretome under a hypoxic condition⁶⁰. The EPC-secretome treated HUVEC showed a significant reduction in apoptosis and improvement in tubular formation, which exerts the anti-oxidative effect of the EPC-secretome. Cantaluppi et al. tested the therapeutic effects of EPC-secretome components on the improvement of renal functions in an AKI model using rats⁶¹. They intravenously injected miRNA-enriched microvesicles in the AKI rats

and found that the injected microvesicles were localized within the peritubular capillary and tubular cells. Furthermore, they demonstrated that the delivered microvesicles induced tubular cell proliferation, reduced apoptosis, and amelioration of fibrosis. In a miRNA depletion assay, they confirmed that the renoprotective effect is due to the bioactive miRNA in the microvesicle that contributes to the reprogramming of host renal cells for the renal functions. In a subsequent study, Cantaluppi et al. also demonstrated that EVs derived from EPCs exert a protective effect in Thy 1.1 glomerulonephritis by inhibiting complement-mediated renal injury⁶².

Secretome delivery for renal treatment

While cell secretome has shown to improve renal function in multiple studies, the delivery method has been a limitation to achieve maximum therapeutic effects of the secretome. For instance, Xing et al. reported that MSC-secretome was not effective in improving renal function in an IRI kidney injury model in mice, which may be due to inefficient delivery of the secretome in a low concentration state⁶³. Similarly, Abedi et al. observed partial tissue regeneration when MSC-derived secretome was intraperitoneally injected into animals with kidney failure²⁷. In either study, the injected trophic factors in the secretome may have lost their biological activity within a short time, likely due to a high diffusion rate and short half-life following injection⁶⁴⁻⁶⁶. To address this issue, the controlled release of secretome factors into the injured site is necessary to achieve improved therapeutic effects.

Drug delivery system has been developed as a promising option to facilitate a controlled and sustained release of bioactive factors to exert maximized outcomes in tissue regeneration⁶⁷⁻⁷⁰. Delivery vehicles, including natural and synthetic materials, have been designed to physically encapsulate target factors or immobilize the factors on the vehicles, thereby protecting them from the effect of enzymatic environment and prolonging therapeutic effects *in vivo*⁶⁷⁻⁷⁰. As a delivery vehicle, hydrogel systems have been widely used, and a combination of hydrogel with the secretome has been proposed as a possible platform due to its versatility required for biological use⁷¹. In several studies,

secretome encapsulated in the hydrogel was used by directly adding hydrogel-initiating materials into the cell culture. In a study, Bakota et al. developed an injectable multidomain peptide (MDP) nanofiber hydrogel system for delivering secretome⁷². The MDF hydrogel is designed to soak secretome factors when placed into the ESC cell culture. The self-assembled MDP hydrogel loaded with stem cell-derived secretome demonstrated stability over time, sheer thinning tolerance, allowing for targeting release condition that can be easily delivered through needle injection. In a release kinetic study in mice, the administered hydrogels with secretome through an intraperitoneal injection remained localized for 24 hours, indicating the effectiveness of secretome delivery via MDP hydrogel system. In a further study, Wang et al. examined whether the MDP nanofiber hydrogel system was able to exert reno-protective effects using lipopolysaccharide (LPS) induced both an *in vitro* and an *in vivo* model⁷³. LPS exposure resulted in a significant increase in activation of caspase-3, which mediates apoptosis⁷⁴, and RhoA/Rho kinase, which involves in cell permeability and apoptosis⁷⁵, but the presence of preconditioned nanofibers reduced cell apoptosis in an *in vitro* by preventing these activations. The introduction of preconditioned nanofibers, which were developed by combining stem cell with the nanofibers, improved LPS-induced kidney in both an *in vitro* and an *in vivo* study. In an *in vivo* study using LPS-induced kidney injury, a rise in serum blood urea nitrogen (BUN) and creatinine levels in the LPS injected mice was significantly reduced when treated with nanofibers hydrogel with secretome. They suggested that the nanofiber hydrogel system could be used as a secretome delivery platform for the treatment of renal failure. More recently, Waters et al. developed a nanocomposite hydrogel system to facilitate secretome-based a dual-action therapy⁷⁶. They used 2D synthetic nanoclay material LAPONITE® nanosilicates to form a complex with growth factors in the secretome⁷⁶. By UV crosslinking of mixtures of nanoclay-secretome and gelatin methacrylate (GelMA), they were able to load the crosslinked complex of nanoclay-growth factors within a nanocomposite hydrogel that potentially deliver secretome released from human MSC. The nanoclay hydrogel showed a controlled release of growth factors such as VEGF and FGF2, by slowing secretome release overtime in an *in vitro* study.

Comparing with nanoclay hydrogel without secretome, the angiogenesis potential of the hydrogel with secretome was confirmed by a significant increase in endothelial cell proliferation and migration observed in a microchannel migration assay and the inhibition of apoptosis was observed in a TdT-mediated dUTP nick end labeling (TUNEL) assay. They suggest that the nanoclay hydrogel system containing pro-angiogenesis and tissue-protective ability has the potential to provide a valuable therapeutic platform for tissue regeneration.

Recently, our group developed a clinically relevant hydrogel-based delivery system to efficiently deliver human placental stem cells (hPSCs)-derived secretome to treat kidney ischemia in rats⁷⁷. Unlike the previous three studies where hydrogel formation with cell secretome occurs on the cell culture *in situ* through a one-step process^{72,73,76}, our approach consists of two steps; 1) collection of cell secretome from cell culture; 2) incorporation of the collected secretome into a hydrogel system. Thus, our strategy enables for an “off-the-shelf” therapy and efficient quality control of the secretome by utilizing cryopreserved secretome. As the clinically relevant hydrogel source, platelet-rich plasma (PRP) hydrogel was used since PRP is biocompatible and safe for clinical applications^{78,79}. Furthermore, autologous PRP obtained from the patient is therapeutically beneficial to minimize immune response as compared with that of an allogeneic or xenogeneic source. In our study, we demonstrated that hPSC-derived secretome contained the pro-proliferative and anti-apoptotic ability for endothelial and primary human tubular cells. A release kinetics study showed that incorporation of secretome within the PRP gel system facilitated controlled release of secretome factors in an *in vitro* and *in vivo* study. When the PRP encapsulated secretome was tested in an IRI injury model in rats, the secretome+PRP treated group showed significantly lower serum creatinine (Cr) and neutrophil gelatinase-associated lipocalin (NGAL) levels than that of other groups, demonstrating improvement in renal function compared with other controls. We confirmed that the therapeutic outcomes by the delivery of secretome through PRP gel are due to minimized renal tissue damages through anti-apoptotic and pro-angiogenic capability. Our results suggest that secretome encapsulation with PRP gel could be an effective approach for the controlled delivery of secretomes for re-

covery of damaged kidneys.

Perspective

While the cell-derived secretome approach demonstrates promising reno-therapeutic outcomes in pre-clinical and clinical studies⁸⁰, a number of challenges need to be addressed before using this modality for clinical translation. A major issue in the use of secretome is attributed to the unidentified characteristics of the cell-secreted factors⁷. Further studies are needed to characterize better and define the secreting factors, which allows for improved control and regulation for clinical translation. Furthermore, clinical efficacy and indication need to be assessed in well-designed and controlled clinical trials⁸¹.

In the manufacturing process for commercialization, production, and concentration of secreted molecules in quantities sufficient for clinical administration are also challenging, in terms of efficient therapeutic effects. A recent study involving the EV production on a large scale³⁶ would enable efficient delivery of sufficient quantities of EV containing miRNAs could be adapted for clinical translation. Also, it is important to note that differential expression of specific molecules in the secretomes might result in different level of beneficial outcomes. For example, Pires et al. demonstrated different profiles of secretomes from BM-MSC, adipose derived cells (ASC), human umbilical cord perivascular cells (HUCPVCs) using a proteomic analysis⁸². Interestingly, secretion profiles of the specific secretomes from BM-MSC allowed more anti-oxidative stress than that of ASC and HUCPVCs. Interestingly, BM-MSC and HUCPVCs showed better anti-apoptotic properties than ASCs, while HUCPVCs is not appropriate as anti-scarring agent. Therefore, appropriate selection of the secretome is needed to maximize therapeutic outcomes for efficient renal treatment.

In addition, the engineering strategy of secretome components has a significant potential to develop a treatment for kidney damage efficiently. For example, engineering of exosome as a therapeutic cue may be a viable approach to improve kidney function and promote regeneration, where exosome could be modified to load therapeutic drugs passively or the exosome surface can be anchored with the

targeting moieties to efficiently home to the repair site⁸³⁾. Such engineering strategies would be beneficial to enhance therapeutic outcomes compared with the naïve type.

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