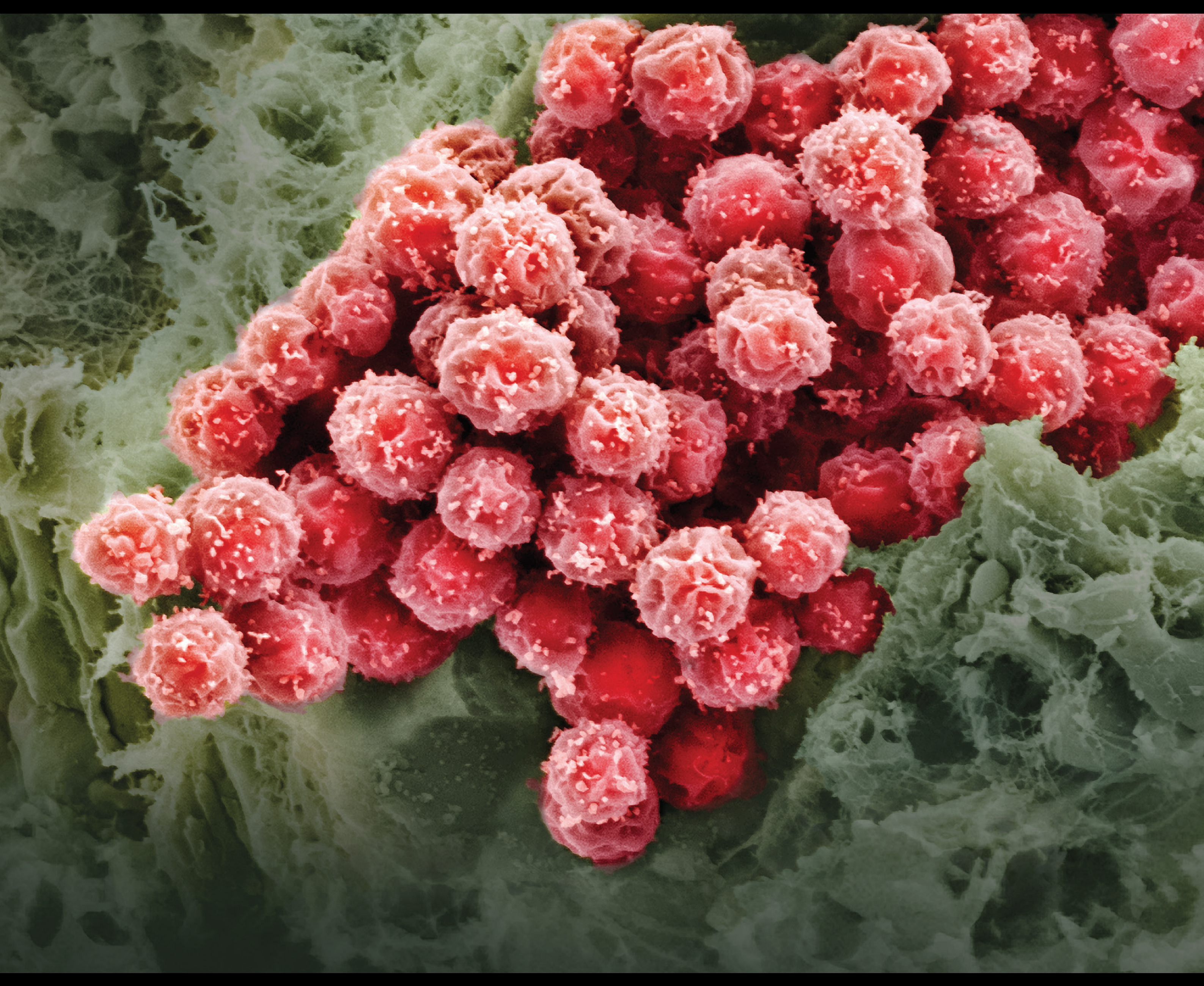
Stem Cells International

Stem Cell-Derived Conditioned Media as a Potential Therapeutic Tool in Regenerative Medicine

Lead Guest Editor: George Kolios
Guest Editors: Vasilis Paspaliaris and Eirini Filidou



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Research Article

Endometrial Regenerative Cell-Derived Conditioned Medium Alleviates Experimental Colitis

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Background. Traditional interventions can play a certain role in attenuating ulcerative colitis (UC), known as one type of inflammatory bowel diseases, but sometimes are not effective. Endometrial regenerative cells (ERCs) have been shown to exert immunosuppressive effects in different models of inflammation, and stem cell-derived conditioned media (CM) have advantages over cell therapy in terms of easy access and direct action. However, whether ERC-CM could alleviate colitis remains unclear and will be explored in this study. *Methods*. Menstrual blood was collected from healthy female volunteers to obtain ERCs and ERC-CM. Acute colitis was induced by 3% dextran sodium sulfate (DSS), and ERC-CM was injected on days 4, 6, and 8, respectively, after induction. The disease activity index was calculated through the record of weight change, bleeding, and fecal viscosity during the treatment process. Histological features, macrophage and $CD4^+$ T cell in the spleen and colon, and cytokine profiles in the sera and colon were measured. In addition, an *in vitro* lymphocyte proliferation assay was measured by using a CCK-8 kit in this study. *Results*. ERC-CM treatment significantly improved the symptoms and histological changes in colitis mice. ERC-CM increased the percentage of Tregs in the spleen and colon but decreased the percentages of M1 macrophages and Th1 and Th17 cells in the spleen and decreased the population of Th17 cells in the colon. In addition, ERC-CM treatment decreased the local expression of TNF-α, IL-6, and iNOS in the colon. Furthermore, ERC-CM increased the levels of anti-inflammatory cytokines IL-10 and IL-27 but decreased proinflammatory cytokines IL-6 and IL-17 in the sera. In addition, ERC-CM significantly inhibited ConA-induced mouse lymphocyte proliferation *in vitro*. *Conclusion*. The results suggest that ERC-CM can exert similar therapeutic effects as ERCs and could be explored for future application of cell-free therapy in the treatment of colitis.

1. Introduction

Since ulcerative colitis (UC), one type of the inflammatory bowel disease (IBD, another type is Crohn's disease) with unknown pathogenesis, was formally established in 1875, medical research on UC has gradually intensified [1]. The pathogenesis of UC is very complex involving genetic factors [2], intestinal microbial factors [3], and intestinal epithelial barrier dysfunction [4]. Innate and adaptive immunity is thought to play a major role in the pathogenesis of UC [5]. Although there are many therapeutic strategies available for UC, such as traditional drugs (mesalamine, glucocorticoids, and thiopurines) [6], biological therapy [7, 8], and diet therapy [9, 10], however, long-term use of immunosuppressive and anti-inflammatory drugs increases the risk of infection and certain malignancies [11]. Unfortunately, many patients with moderate to severe ulcerative colitis inevitably end up needing surgery. In recent years, the treatment of IBD has switched from simply controlling symptoms to normalization of blood markers, complete mucosal healing, and

disappearance of symptoms [12]. However, many existing treatment methods cannot meet the needs of all patients. Thus, a novel treatment for UC is urgently needed.

Endometrial regenerative cells (ERCs) from menstrual blood played as a new source of adult stem cells and not only have the potential of self-renewal [13], mesenchymal stem cell- (MSC-) like phenotype [14], and immune modulation [15] but also have the unique advantages of noninvasiveness, relatively unlimited source, strong proliferation ability, and avoiding ethical problems [16, 17]. Our and others' previous studies have demonstrated that ERCs could modulate immune homeostasis in acute hepatitis and other models, such as heterotopic heart transplantation and kidney ischemia reperfusion injury in mice *via* different pathways [18–22]. In ulcerative colitis, ERCs can not only regulate colitis but also be modified for better therapeutic effects [21, 23].

Despite the growing interest in MSCs, some limitations of an MSC-based strategy still remain; for example, most injected cells are arrested by the complex lung network which leads to weak efficiency [24]. More serious is the possibility of thrombosis with cellular administration and other risks such as arrhythmias, ossification, and calcification [25, 26]. In addition, some cryopreservation protocols may make stem cells less functional and viable [27]. All these effects and concerns can be avoided by cell-free preparations. A growing number of studies demonstrated that the therapeutic effects of MSCs are not only due to the cell-to-cell direct contact but also to their secreted cytokines, chemokines, growth factors, extracellular vesicles, and so on. Therefore, due to the characteristics of MSCs and the existence of various limitations, the cell-free treatment strategy is on the rise and has several advantages over cell therapy, as they can be obtained more easily and more economically and can be manufactured, packaged, and transported straightforwardly [28], and the most important thing is that they would not be rejected. All of secreted proteins, EVs and free mitochondria from MSCs, can be found in the conditioned medium (CM) which may exert similar therapeutic function as cell-based treatment. Furthermore, CM-based therapy has been identified to be functional in treating many inflammatory disorders such as hepatic failure and acute lung injury [29, 30]. However, whether ERC-CM could exert the therapeutic effects on experimental colitis remains unclear. Thus, in the present study, the effects of ERC-CM on alleviating colitis were systematically explored in mice.

2. Materials and Methods

2.1. Isolation and Identification of ERCs. Menstrual blood was collected from healthy female volunteers of childbearing age. All operations towards human being were approved by the Ethics Committee of Tianjin Medical University General Hospital (IRB2021-WZ-116). The protocol for extracting the ERCs was conducted as described previously [19]. Briefly, the menstrual cups were inserted into the vagina for about 4 hours and then removed, and the collected menstrual blood was used for cloudy layer cell collection by the standard Ficoll method and then suspended in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (HyClone) and 1% penicillin/streptomycin and cultured in Petri dishes in an incubator with 37°C and 5% CO_2 condition. When ERCs grow to the passage 3 (P3), morphology and surface markers (CD90, CD105, CD45, CD79a, and HLA-DR) of ERCs were identified by microscopy and flow cytometry, respectively [22, 23, 31].

2.2. Preparation of ERC-CM. ERCs in regular shape and good status of P4-P5 were selected, and the serum-free medium (Yokon, NC0103, China) was replaced until 80% confluency and then collected after 48 h. Next, the cell culture medium, which had been purged of impurities such as cell debris *via* centrifugation (3000 r/min, 20 min), was concentrated 20-fold using an ultrafiltration centrifuge filter unit (Millipore, Billerica, MA, USA) with a molecular weight cut-off value of 3 kDa and then sterilized by filtration through a 0.22 μm centrifugal filter (Millipore, Billerica, MA, USA). The concentrated CM was stored at -80°C for further treatment *in vivo*.

2.3. Animals and Experimental Groups. Male mice weighing 22-25 g in this experiment were purchased from National Institutes for Food and Drug Control of China and were placed in standard breeding environment provided with standard diet and water in Animal Care Facility of Tianjin General Surgery Institute. All animal experimental operations were approved by the Institute of Animal Care and Use Committee at Tianjin Medical University General Hospital and performed in accordance with the Guide for the Care and Use of Laboratory Animals (IRB2021-WZ-116).

Acute colitis was induced in BALB/C mice (Figure 1(a)) that received 3% dextran sulfate sodium (DSS, YEASEN, China) in drinking water for 7 days and then consumed normal water for 3 days. Twenty-four mice were randomly divided into four groups ($n = 6$): (I) naive mice drinking normal distilled water for 10 days (normal group), (II) DSS-induced colitis in mice without any treatment (untreated group), (III) DSS-induced colitis mice injected intraperitoneally with 300 μl of normal serum-free medium (days 4, 6, and 8) as the vehicle control (vehicle group), and (IV) DSS-induced colitis mice injected intraperitoneally with 300 μl of ERC-CM (days 4, 6, and 8) (ERC-CM group). Mice were monitored and recorded daily for weight change, stool consistency, and blood in stool for the disease activity index (DAI) calculation based on standard criteria [32]. The mice were sacrificed, and samples (spleen, colon, and sera) were collected at day 10 from the beginning for further analysis.

2.4. Pathological Examination. Colon tissue was fixed in 10% formalin for 48 hours and then dehydrated and paraffin-embedded and cut into 5 μm sections for hematoxylin and eosin (H&E) staining. Histopathology scores were evaluated and calculated as in previous studies [33] documented based on the following criteria: (a) inflammation severity: 0 (none), 1 (slight), 2 (moderate), and 3 (severe); (b) depth of injury: 0 (none), 1 (mucosal), 2 (mucosal and submucosal), and 3 (transmural); (c) crypt damage: 0 (none), 1 (basal 1/3

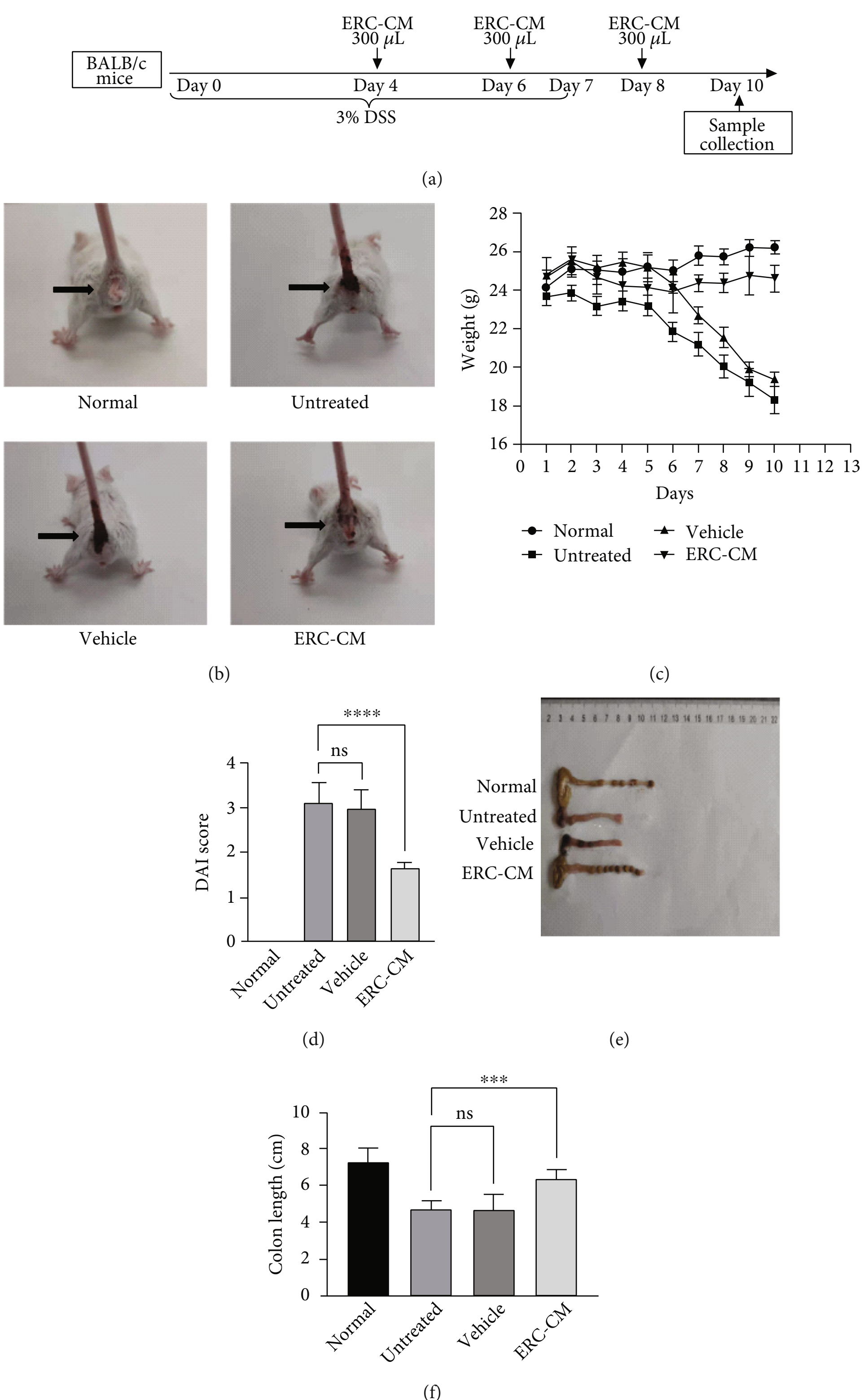

Figure 1: ERC-CM attenuated symptoms in DSS-induced experimental colitis. (a) Study design for the whole process of DSS-induced colitis and interventions. (b) Representative pictures showing bloody stool were taken on the 10th day after DSS induction. The mice in the ERC-CM group were in the best condition than those in other groups. Body weight changes (c) and DAI score (d) of each group of mice were recorded daily. In the ERC-CM group, the weight loss and DAI score were shown lesser than those in other groups. The length of the colon (e, f) in each group was measured and analyzed on day 10 ($n = 6$). Data shown were representative, and the p value was determined by one-way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

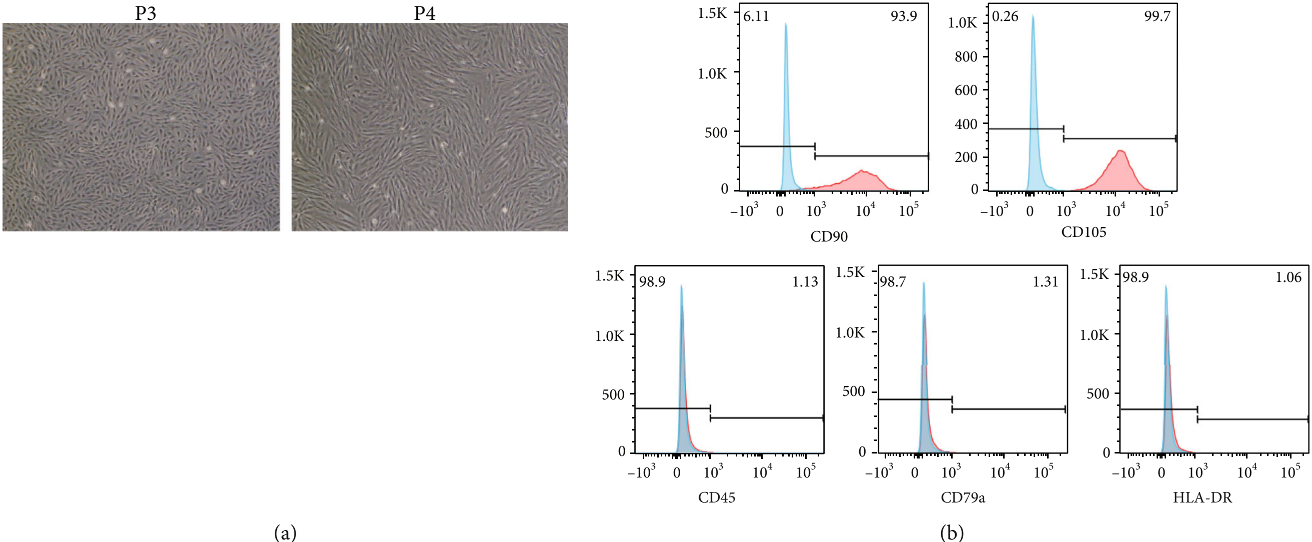

Figure 2: Characterization of ERCs. (a) Cell morphology of ERCs at P3 and P4. (b) Flow cytometry analysis of ERCs. Surface markers CD90, CD105, CD45, CD79a, and HLA-DR were detected.

damage), 2 (basal 2/3 damage), 3 (crypt lost, only surface epithelium intact), and 4 (entire crypt and epithelium lost); and (d) percent involvement: 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%).

2.5. Immunohistochemistry Staining. Immunohistochemistry staining was used to analyze the expression of TNF-α, IL-6, and iNOS in the inflammatory intestine. Firstly, after the slices were hydrated and washed, the slices were submerged in an appropriate EDTA solution and immersed in a 100°C water bath for 15 min to realize antigen retrieval. Secondly, after endogenous peroxidase was eliminated with 3% hydrogen peroxide for 30 min, the nonspecific antibody adsorption was blocked with 10% goat serum for another 30 min, and then, cells were incubated with primary antibodies against TNF-α (1 : 400, Abcam, ab92486, UK), IL-6 (1 : 800, Abcam, ab6672, UK), and iNOS (1 : 1000, Abcam, ab283655, UK), respectively, at 4°C overnight. Next, the sections were washed three times with PBS, and a reaction enhancer was added dropwise and incubated for 20 min at room temperature and then incubated with an anti-rabbit IgG antibody (Zhongshan Jinqiao, PV-9000, China) for another 20 min at room temperature. Finally, the sections were incubated with freshly prepared DAB solution (Zhongshan Jinqiao, ZLI-9018, China) after washing, and hematoxylin was added dropwise for nuclear staining and then scanned for image analysis. ImageJ software was used to analyze the image.

2.6. Preparation of Lamina Propria Mononuclear Cells and Splenocyte Suspension. Isolation of murine lamina propria mononuclear cells from colonic tissue was conducted under the guideline as described [34, 35]. Briefly, mucus and epithelial cells were removed from fresh colon specimens in successive steps with dithiothreitol (DTT, Solarbio, D8220, China) and ethylene diamine tetraacetic acid (EDTA, Solarbio, E8040, China), followed by digestion with collagenase IV (400 U/ml, Solarbio, C8160, China) and deoxyribonuclease I (0.15 mg/ml, Solarbio, D8071, China) for 90 min at 37°C. The cell precipitate was resuspended with 4 ml 40% Percoll (Cytiva, US), and then, 2.5 mL 80% Percoll was slowly added to the bottom of the centrifuge tube. LPMCs were obtained by centrifugation at 1000 g density gradient for 20 min and then washed with PBS.

Mouse spleens were ground separately, and splenocyte suspension was obtained after lysis of red blood cells. After washing twice, the cell concentration was adjusted to 1×10^7/ml with PBS solution.

2.7. Flow Cytometry Analysis. Different immune cells are identified using flow cytometry. All flow cytometry monoclonal antibodies and reagents used in this experiment were purchased from either BioLegend or eBioscience company, mainly including Zombie NIR™ Dye (Dead/Live reagent), anti-mouse CD4 (FITC-labelled), IFN-γ (PE-labelled), IL-17 (Percp-labelled), CD25 (PE-labelled), Foxp3 (APC-labelled), CD11b (FITC-labelled), F4/80 (APC-labelled), and CD86 (Percp-labelled) to detect Th1 cells ($CD4^+IFN\text{-}\gamma^+$), Th17 cells ($CD4^+IL\text{-}17a^+$), Tregs ($CD4^+CD25^+Foxp3^+$), and M1 macrophages ($CD11b^+F4/80^+CD86^+$) in the spleen and/or intestine. Extra- and intracellular staining protocols were carried out as previously described [21]. Furthermore, to accurately identify Th1 and Th17, splenocytes and colonic LPMCs were firstly coincubated with stimulators for 6 hours, respectively, followed by fluorescent antibody staining.

2.8. Enzyme-Linked Immunosorbent Assay (ELISA). After the mice were sacrificed on day 10 after DSS induction, the blood was collected to obtain the sera. The levels of IL-10, IL-27, IL-17, and IL-6 in the sera were used to assess the inflammatory status and were measured using corresponding ELISA kits (DAKEWE, Beijing, China), and details were performed according to the manufacturer's instructions.

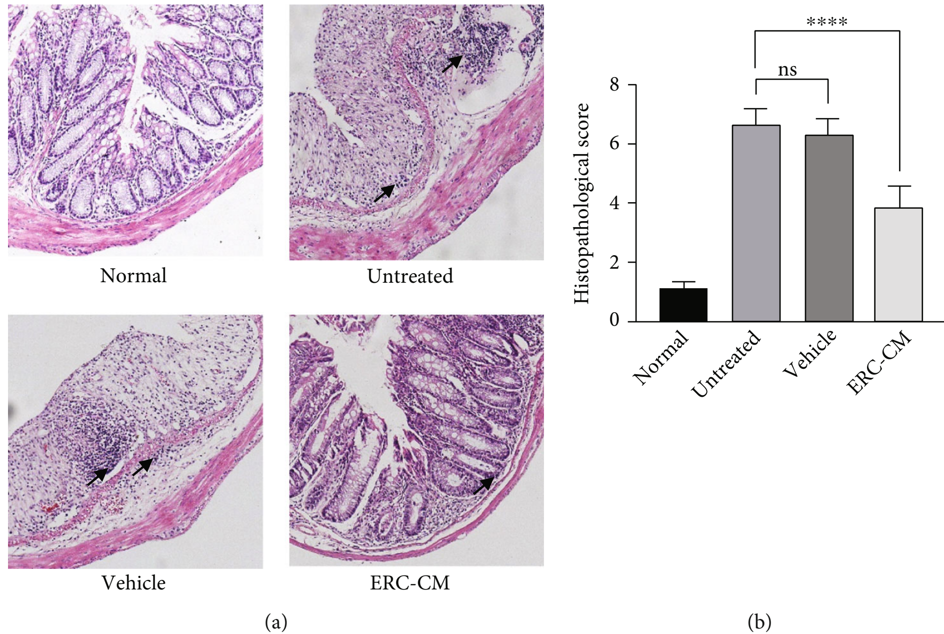

Figure 3: ERC-CM significantly alleviated colon damage in colitis. (a) Representative photomicrographs (100x, hematoxylin and eosin staining) of histological sections of the colon from each group. (b) Histopathology scores were evaluated and calculated as in previous studies [33]. The p value was determined by one-way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

2.9. In Vitro Proliferation Assay. The proliferation of lymphocytes in response to ConA was measured by using the CCK-8 kit. Briefly, BALB/c splenocyte suspension (3×10^5 cells/well) was cultured with ConA (20 μg/ml) in the presence of ERC-CM at indicated concentrations. The cultures were incubated for 72 h, 10 μl of CCK-8 was then added to each well before the end of culture, and $OD_{450\,nm}$ was recorded.

2.10. Statistics. Data shown in this study were expressed as mean ± SD, and the differences among multiple groups were analyzed using one-way analysis of variance (ANOVA); GraphPad Prism 8 software was applied. Throughout the text, figures, and legends, the following terminologies are used to denote statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.001$.

3. Results

3.1. Characterization of ERCs. At P3, ERCs exhibited spindle-shaped, fibroblast-like morphology and colony-forming abilities (Figure 2(a)). ERCs measured by flow cytometry showed high expression of CD90 and CD105 and low expression of CD45, CD79a, and HLA-DR, which are consistent with MSCs' phenotype (Figure 2(b)).

3.2. ERC-CM Attenuated Symptoms in DSS-Induced Experimental Colitis. BALB/C mice suffered from severe colitis induced by DSS (Figure 1(a)) characterized by weight loss, bloody diarrhea, and increased general DAI by drinking distilled water containing 3% DSS (Figures 1(b)–1(d)). The colons collected from different groups exhibited different lengths, and the colon in the ERC-CM group seems much longer than that in untreated and/or vehicle groups (ERC-CM group *vs.* untreated group, $p < 0.001$; ERC-CM group *vs.* vehicle group, $p < 0.001$). This result implied that ERC-CM could significantly attenuate the colon's shortening (Figures 1(e)–1(f)).

3.3. ERC-CM Significantly Alleviated Colon Damage in Colitis. Histopathological changes were assessed by hematoxylin/eosin staining of colonic sections. Mucosal structural disorders, inflammatory cell infiltration, epithelial cells, and structural disruption of the crypt were very intense in the untreated group and vehicle group, whereas these changes were significantly attenuated in the ERC-CM treated group. Histopathological scores were used to assess the damage, and scores in the ERC-CM group were significantly lower than those in the untreated and/or vehicle group (ERC-CM group *vs.* untreated group, $p < 0.0001$; ERC-CM group *vs.* vehicle group, $p < 0.0001$) (Figures 3(a) and 3(b)).

3.4. ERC-CM Decreased the Percentages of Th1 Cells, Th17 Cells, and M1 Macrophages but Increased the Percentage of Tregs in the Spleen of DSS-Induced Colitis in Mice. To determine the immunomodulatory effects of ERC-CM, splenocytes were prepared and stained for analysis. As shown in Figures 4(a) and 4(c), percentages of M1 macrophages, Th1 cells, and Th17 cells were increased in the untreated group and significantly decreased after ERC-CM treatment (Figure 4(d), M1: ERC-CM group *vs.* untreated group, $p < 0.05$; ERC-CM group *vs.* vehicle group, $p < 0.05$; Figure 4(g), Th1: ERC-CM group *vs.* group, $p < 0.001$; ERC-CM *vs.* vehicle, $p < 0.001$; Figure 4(f), Th17: ERC-CM *vs.* untreated, $p < 0.001$; ERC-CM *vs.* vehicle, $p < 0.001$). Furthermore, Treg populations showed a decreasing trend

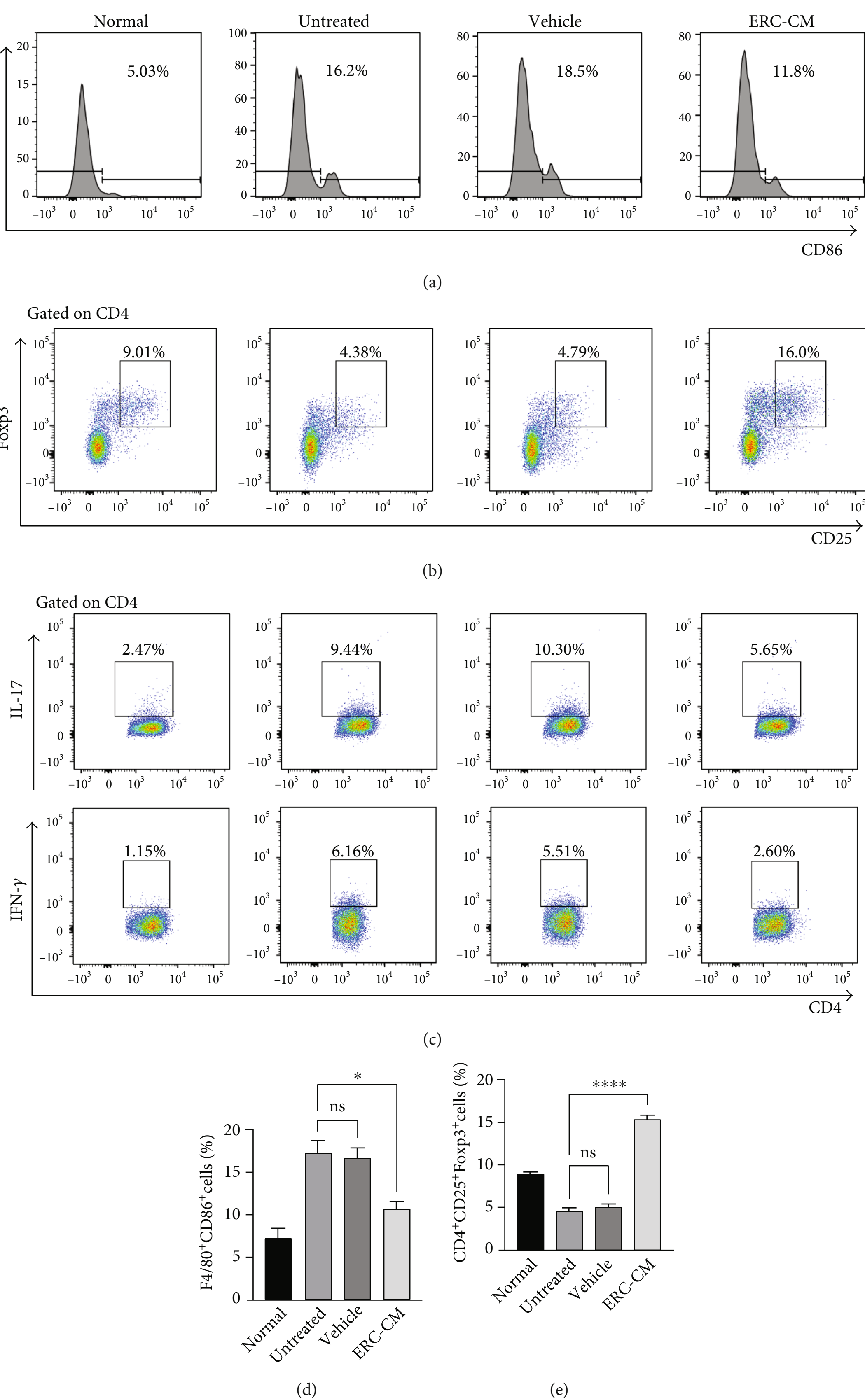

Figure 4: Continued.

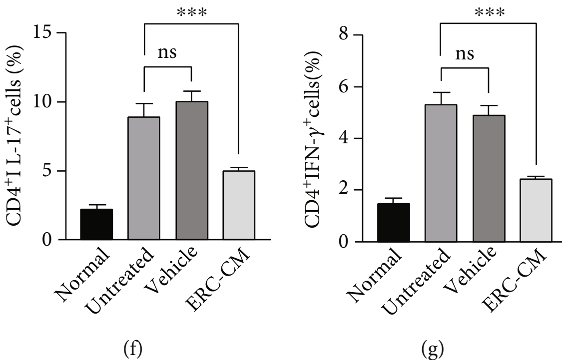

Figure 4: ERC-CM decreased the percentages of Th1 cells, Th17 cells, and M1 macrophages but increased the percentage of Tregs in the spleen of colitis mice. Splenocytes were collected on day 10 after DSS induction. To accurately identify the subpopulation of Th1 and Th17 cells, splenocytes were firstly incubated with a cell stimulation cocktail for 6 hours before being stained with fluorescent antibodies. Percentages of F4/80$^+$CD86$^+$ M1 macrophages (a), representative dot plots of CD4$^+$CD25$^+$Foxp3$^+$ Tregs (b), CD4$^+$IL-17$^+$Th17 cells (c), and CD4$^+$IFN-γ^+ Th1 cells (d) were shown. Data shown were representative, and the p value was determined by one-way ANOVA. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

in the untreated and/or vehicle groups but an obvious increase after ERC-CM treatment (ERC-CM group *vs.* untreated group, $p < 0.0001$; ERC-CM group *vs.* vehicle group, $p < 0.0001$) (Figures 4(b) and 4(e)). This indicated that ERC-CM successfully performs an immunomodulatory function.

3.5. ERC-CM Decreased the Percentage of Th17 but Increased the Percentage of Tregs in the Colon. UC has been shown to be mediated by excessive activation of Th17 cells and deficiency of Tregs [32]. To this end, the expressions of Th17 and Tregs in CD4$^+$T cells were analyzed by flow cytometry. As expected, the accumulation of Th17 cells in the lamina propria was significant in the untreated group of mice compared to the normal group of mice and relatively less in the treated group (ERC-CM group *vs.* untreated group, $p < 0.01$; ERC-CM group *vs.* vehicle group, $p < 0.01$) (Figures 5(b) and 5(d)). In contrast, the number of Tregs in the lamina propria was abnormally downregulated in the untreated group compared to the normal group of mice and significantly increased in the treated group (ERC-CM group *vs.* untreated group, $p < 0.001$; ERC-CM group *vs.* vehicle, $p < 0.001$) (Figures 5(a) and 5(c)). Based on this observation, the regulation of Th17 cells and Tregs in the lamina propria by ERC-CM may be associated with the improvement of colitis.

3.6. ERC-CM Decreased Proinflammatory Cytokine Levels and Macrophage Infiltration in the Colon. To detect the regulatory effect of ERC-CM on proinflammatory cytokines and inflammatory cells in the colon, we detected the expression of TNF-α, IL-6, and M1 macrophages by immunohistochemistry. As shown in Figures 6(a) and 6(b), TNF-α and IL-6 were significantly increased in the untreated group but were decreased after ERC-CM treatment (Figure 6(d), TNF-α: ERC-CM group *vs.* untreated group, $p < 0.0001$; ERC-CM group *vs.* vehicle group, $p < 0.0001$; Figure 6(e), IL-6: ERC-CM group *vs.* untreated group, $p < 0.0001$; ERC-CM group *vs.* vehicle group, $p < 0.0001$). Similarly, as shown in Figures 6(c) and 6(f), M1 macrophages (iNOS$^+$ cells) showed a significant infiltration in the untreated group and were decreased after ERC-CM treatment (ERC-CM group *vs.* untreated group, $p < 0.0001$; ERC-CM group *vs.* vehicle group, $p < 0.0001$).

3.7. ERC-CM Modulated Cytokine Level in the Sera. To further assess the cytokine profile in mice, we performed separate assays from sera. Notably, the proinflammatory cytokines IL-6 and IL-17 were increased, and the anti-inflammatory cytokines IL-10 and IL-27 were decreased in the untreated group in the serum (Figures 7(a)–7(d)). After ERC-CM treatment, the above situation was reversed (IL-6: ERC-CM group *vs.* untreated group, $p < 0.05$; ERC-CM group *vs.* vehicle group, $p < 0.05$; IL-17: ERC-CM group *vs.* untreated group, $p < 0.0001$; ERC-CM group *vs.* vehicle group, $p < 0.0001$; IL-10: ERC-CM group *vs.* untreated group, $p < 0.0001$; ERC-CM group *vs.* vehicle group, $p < 0.0001$; and IL-27: ERC-CM group *vs.* untreated group, $p < 0.0001$; ERC-CM group *vs.* vehicle group, $p < 0.0001$). This suggests that therapeutic improvement of ERC-CM is associated with changes in cytokine levels in inflammatory mice.

3.8. ERC-CM Inhibited the Proliferation of Lymphocytes In Vitro. To assess the immunosuppressive effects of ERC-CM *in vitro*, the splenocyte proliferation assay was performed. As shown in Figure 8, ERC-CM can inhibit the lymphocyte proliferation *in vitro* compared with the vehicle control (ERC-CM group *vs.* vehicle group, $p < 0.01$). ERC-CM significantly inhibits ConA-induced murine splenocyte proliferation.

4. Discussion

UC is a chronic inflammatory disease that plagues the world, and currently effective therapeutic strategies remain to be explored. In this study, acute colitis was successfully induced by drinking 3% DSS in BALB/c mice and identified with

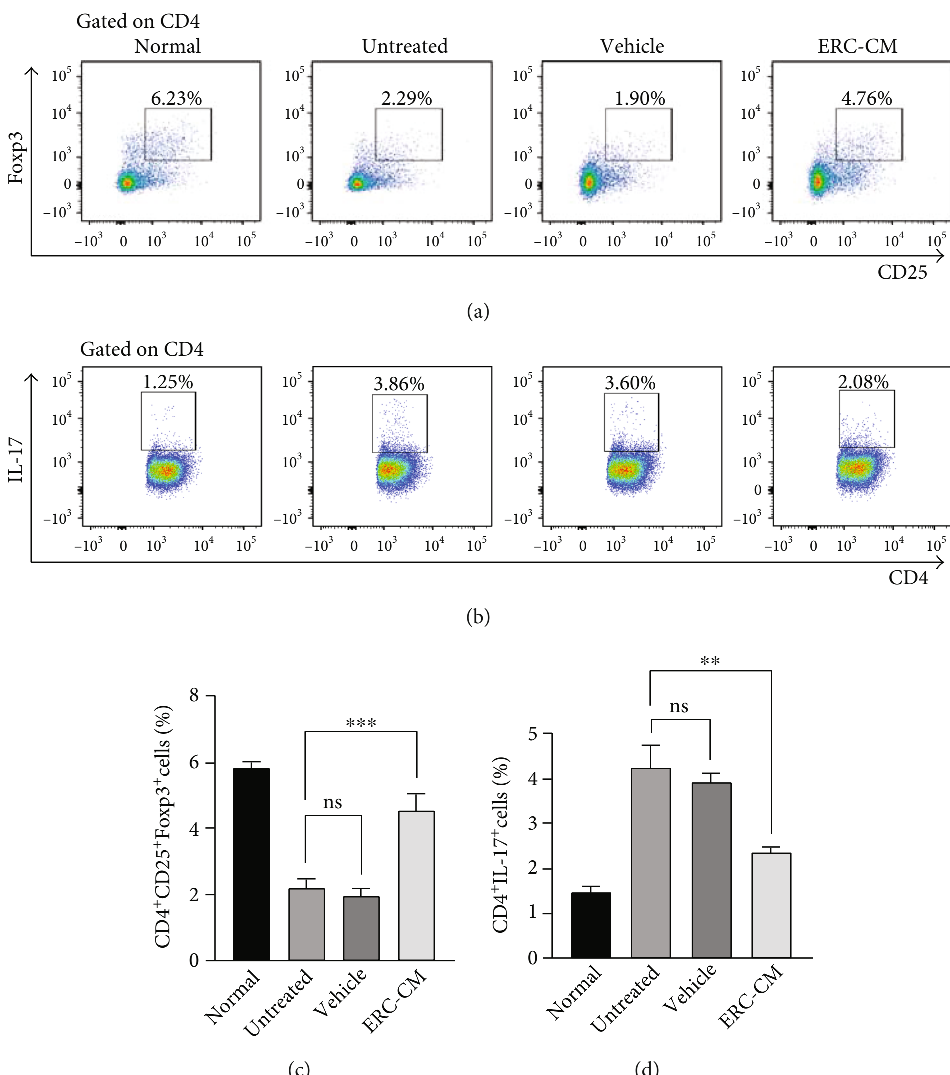

Figure 5: ERC-CM decreased the percentage of Th17 cells but increased the percentage of Tregs in the colon. Representative dot plots of $CD4^+CD25^+Foxp3^+$ Tregs (a), $CD4^+IL\text{-}17^+$ Th17 cells (b) in the lamina propria. Percentage of $CD4^+CD25^+Foxp3^+$ Tregs (c) and $CD4^+IL\text{-}17^+$ Th17 cells (d). Data shown were representative, and the p value was determined by one-way ANOVA. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

clinical symptoms. After receiving different treatments, some changes differentially exhibited in different groups; symptoms of colitis were significantly attenuated in the ERC-CM group. In addition, pathological changes, local inflammatory cell infiltration, and proinflammatory protein expression in the colon, as well as proinflammatory cytokine levels in sera, were significantly ameliorated in the ERC-CM-treated group when compared with those of vehicle and/or untreated groups, which indicated that ERC-CM can effectively exert therapeutic effects in the treatment of colitis. In addition, *in vitro* assays showed that ERC-CM effectively inhibited lymphocyte proliferation.

A growing number of studies suggest that MSCs exert their therapeutic effects depending not only on the way in which they come into contact with cells but also on their paracrine activity, mitochondrial transfer, etc. Paracrine function, on the other hand, is inevitably associated with the secretion of microvesicles and cytokines by MSCs [36, 37]. Various cytokines secreted by MSCs, such as insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), IL-10, and TGF-β, are involved in tissue repair and the regulation of immune cells [38–40]. These extracellular vesicles and cytokines are contained in the CM *in vitro*. Compared with cell therapy, the use of CM can control the dose more accurately, and cell lines can be used for mass production without invasive extraction procedures for patients, which saves time and cost [41]. The CM acts in a more direct manner, unlike cells that may be captured by various host defense mechanisms. In addition, cytokines alone are expensive and may exaggerate the inflammatory response. Therefore, a combination of many different factors is the best way to treat [42]. Conditioned media can meet just such conditions. Innate and adaptive immunity is thought to play a major role in the pathogenesis of UC. Among them, T cells and macrophages are the main players [5], and it has been well documented that cells such as Th1 cells, Th17 cells, and M1 macrophages and the

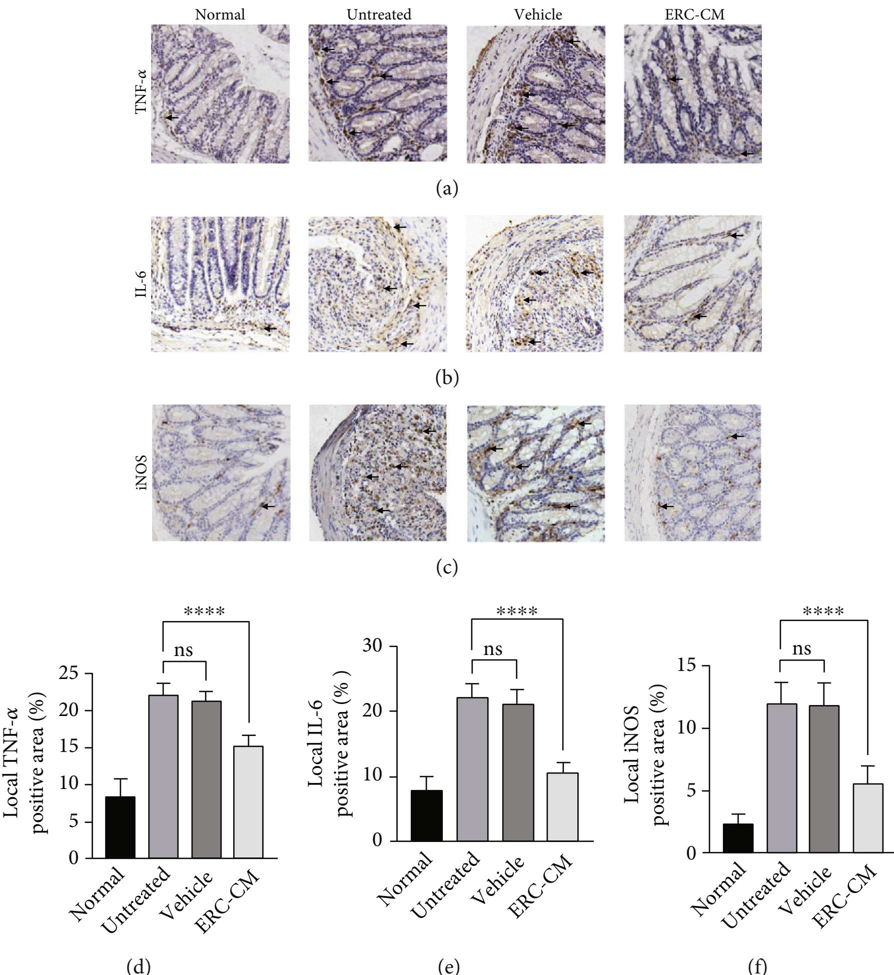

Figure 6: ERC-CM decreased the level of proinflammatory cytokines and macrophage infiltration in the colon. Inflammatory factor levels and macrophage infiltration in the colon were assessed by immunohistochemical staining. Specifically, TNF-α, IL-6, and M1 macrophage infiltration were detected by iNOS staining. Images of IHC staining (200x) for the mouse colon (a–c) and quantitative data of cell counts for each group (d–f) are shown. The p value was determined by one-way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

cytokines they secrete, such as IFN-α, IL-6, IL-17, and TNF-α, are involved in the pathogenesis of UC [43, 44]. It is well known that $CD4^+$ T cells (Th1 cells, Th17 cells, and Tregs) are required for UC and their balance is essential for maintaining intestinal homeostasis. In the inflammatory environment, Th0 cells are converted to inflammatory Th1 and Th17 cells, contributing to driving the initial stage of colitis. The proinflammatory cytokines they release not only recruit neutrophils to the damaged areas but also promote more proinflammatory cytokine production through a negative feedback regulation mechanism. Tregs work as key effective suppressors in autoimmune diseases, not only suppressing the proliferation of Th0 cells *in vitro* and *in vivo* [45] but also exerting their function by producing the immunomodulatory cytokines, such as IL-10 and TGF-β [46]. In addition, previous evidence showed that purified Tregs prevented naive $CD4^+$ T cell transfer-induced colitis in SCID mice [47]. As mentioned above, disruption of the dynamic balance between $CD4^+$T cells is involved in the pathogenesis of colitis, and it is noteworthy that in patients with UC, Th17 cells are enriched and Tregs are scarce, and it has been well documented that the balance between Th17 cells and Tregs can be a potential regulatory target for improvement in colitis models [48, 49]. Macrophages make up a large proportion of intestinal immune cells, and they can initiate and coordinate the immune response against foreign bodies that have breached the epithelial barrier [50]. In inflammatory conditions, blood-derived monocytes/macrophages transform into a proinflammatory phenotype and produce large amounts of inflammatory cytokines such as IL-6 and IL-12, which are often described as classically activated macrophages (e.g., M1 macrophage) [51].

In the present study, percentages of Th1 cells, Th17 cells, and M1 macrophages are significantly increased in both the spleen and the colon in DSS-induced colitis when compared with normal mice, which are consistent with the results that Th1 cells, Th17 cells, and M1 macrophages may be involved in the pathogenesis of UC. In addition, percentages of Tregs in the spleen and the colon are increased in the ERC-CM-treated group when compared with untreated and/or vehicle groups, which implied the therapeutic effects of ERC-CM. *In*

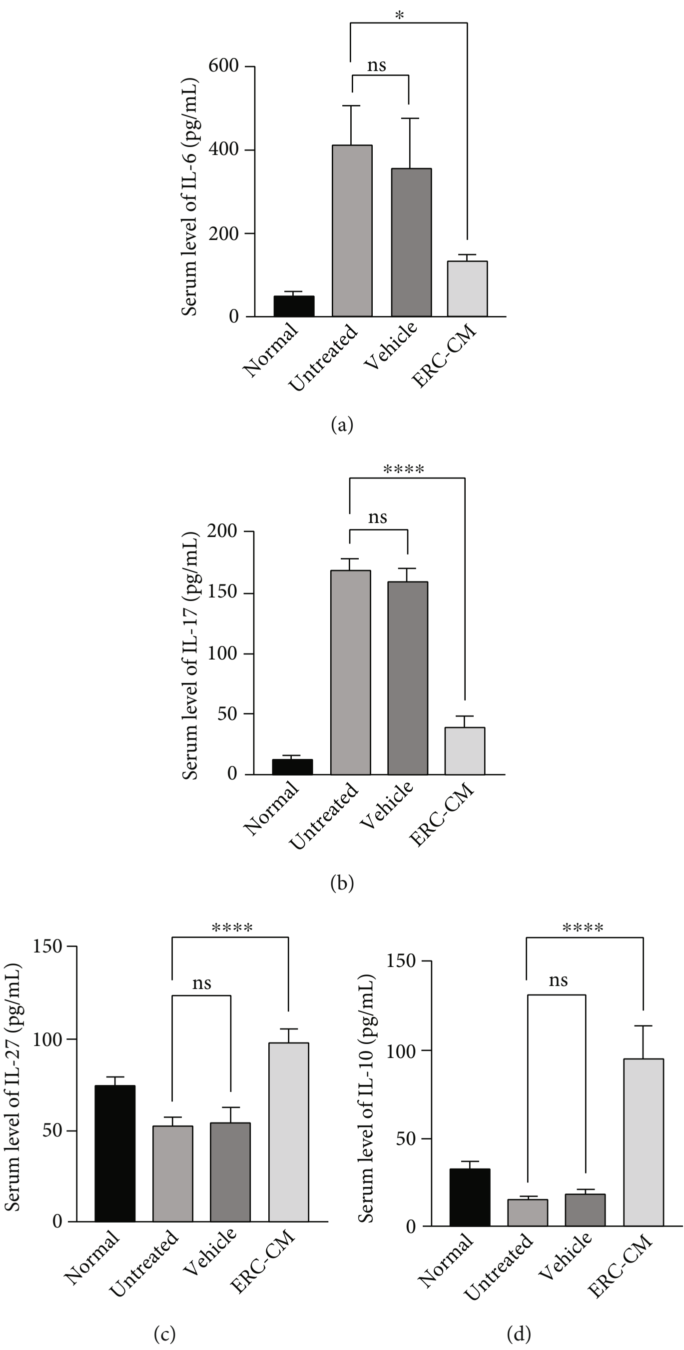

Figure 7: ERC-CM modulated the cytokine level in sera. Measurement of serum levels of IL-10, IL-27, IL-17, and IL-6 using the appropriate ELISA kits. IL-6 (a), IL-17 (b), IL-27 (c), and IL-10 (d) were shown, respectively. The p value was determined by one-way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

vitro experiments have also demonstrated that ERC-CM exerts a therapeutic effect by affecting lymphocyte populations. The mesenteric lymph nodes are an important tissue in response to local inflammation in the intestine and will be further analyzed in our future studies. The various pathogenic pathways of inflammatory bowel disease can interact with each other, and cytokines can be associated with any of them [52]. For example, IL-6 induces activation of the STAT3 signaling pathway, which regulates various genes involved in cell survival, cell migration, and apoptosis [53]. To determine whether ERC-CM ameliorates colitis by regulating cytokine levels, several cytokine levels were assayed. Our study showed that the proinflammatory cytokines IL-6, TNF-α, and IL-17 were decreased and the anti-inflammatory cytokines IL-27 and IL-10 were increased after ERC-CM treatment. It is worth noting that IL-27 was initially thought to be a proinflammatory cytokine, but the anti-inflammatory effects of IL-27 signaling have since been illustrated in many recent studies [54, 55]. It has been shown that IL-27 has a wide range of effects on Th1, Th2, and Th17

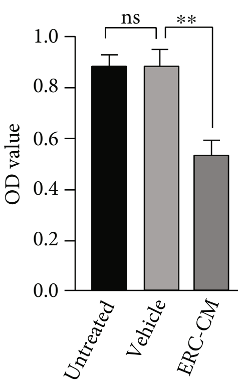

Figure 8: ERC-CM inhibits the proliferation of splenocytes *in vitro*. The proliferation of splenocytes in response to ConA was measured by using the CCK-8 kit. The p value was determined by one-way ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

cells as well as Tregs [56]. This is also verified by the change in the ratio of various cells mentioned above. We therefore infer that the various changes occurring in colitis mice are associated with various cytokines.

We and others have previously demonstrated that ERCs play a therapeutic role in various diseases, including UC [18, 23]; however, whether ERC-CM as a cell-free therapy has a potent effect in attenuation of colitis remains unknown. Based on the previous work and to our knowledge, we are the first to explore the therapeutic effects of ERC-CM in colitis. The results showed very promising therapeutic prospects for ERC-CM. However, there are still some issues including the potential therapeutic mechanisms, and the strategy to improve the therapeutic effects of CM needs further investigation.

5. Conclusion

The results in this study have demonstrated that ERC-CM significantly attenuated colitis in mice, suggesting that ERC-CM could be used as a novel cell-free strategy for potential application in the treatment of inflammatory bowel diseases.

Abbreviations

ERCs: Endometrial regenerative cells
iNOS: Inducible nitric oxide synthase
TGF-β: Transforming growth factor-β
IGF-1: Insulin-like growth factor-1
DSS: Dextran sodium sulfate
DTT: Dithiothreitol
EDTA: Ethylene diamine tetraacetic acid
MSC: Mesenchymal stromal cells
IL-6: Interleukin-6
TNF-α: Tumor necrosis factor-α.

Data Availability

All data included in this manuscript can be available.

Conflicts of Interest

The authors declare that they have no conflict of interests.

Authors' Contributions

Chenglu Sun, Jingpeng Hao, and Hong Qin designed and carried out the research, analyzed the data, and drafted the manuscript. Yanglin Zhu, Xiang Li, Baoren Zhang, Yafei Qin, Hongda Wang, and Guangming Li performed the research. Hao Wang conceived and designed the research, provided administrative and financial support, and helped in revising the manuscript. All authors have read and approved the final manuscript. Chenglu Sun, Jingpeng Hao, and Hong Qin are co-first authors on this paper.

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References

[1] G. G. Kaplan, "The global burden of IBD: from 2015 to 2025," *Nature Reviews Gastroenterology & Hepatology*, vol. 12, no. 12, pp. 720–727, 2015.

[2] M. U. Mirkov, B. Verstockt, and I. Cleynen, "Genetics of inflammatory bowel disease: beyond NOD2," *The Lancet Gastroenterology & Hepatology*, vol. 2, no. 3, pp. 224–234, 2017.

[3] T. Zuo and S. C. Ng, "The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease," *Frontiers in Microbiology*, vol. 9, p. 2247, 2018.

[4] J. L. Pope, A. A. Bhat, A. Sharma et al., "Claudin-1 regulates intestinal epithelial homeostasis through the modulation of Notch-signalling," *Gut*, vol. 63, no. 4, pp. 622–634, 2014.

[5] A. Geremia, P. Biancheri, P. Allan, G. R. Corazza, and A. di Sabatino, "Innate and adaptive immunity in inflammatory bowel disease," *Autoimmunity Reviews*, vol. 13, no. 1, pp. 3–10, 2014.

[6] H. P. Sham, M. Bazett, M. Bosiljcic et al., "Immune stimulation using a gut microbe-based immunotherapy reduces disease pathology and improves barrier function in ulcerative colitis," *Frontiers in Immunology*, vol. 9, p. 2211, 2018.

[7] T. P. Chapman, C. F. Gomes, E. Louis, J.-F. Colombel, and J. Satsangi, "De-escalation of immunomodulator and biological therapy in inflammatory bowel disease," *The Lancet Gastroenterology & Hepatology.*, vol. 5, no. 1, pp. 63–79, 2020.

[8] D. Pugliese, C. Felice, A. Papa et al., "Anti TNF-α therapy for ulcerative colitis: current status and prospects for the future," *Expert Review of Clinical Immunology*, vol. 13, no. 3, pp. 223–233, 2017.

[9] F. Durchschein, W. Petritsch, and H. F. Hammer, "Diet therapy for inflammatory bowel diseases: the established and the

new," *World Journal of Gastroenterology*, vol. 22, no. 7, pp. 2179–2194, 2016.

[10] A. Wędrychowicz, A. Zając, and P. Tomasik, "Advances in nutritional therapy in inflammatory bowel diseases: Review," *World Journal of Gastroenterology*, vol. 22, no. 3, pp. 1045–1066, 2016.

[11] B. G. Feagan, P. Rutgeerts, B. E. Sands et al., "Vedolizumab as induction and maintenance therapy for ulcerative colitis," *The New England Journal of Medicine*, vol. 369, no. 8, pp. 699–710, 2013.

[12] D. Y. Jeong, S. Kim, M. J. Son et al., "Induction and maintenance treatment of inflammatory bowel disease: a comprehensive review," *Autoimmunity Reviews*, vol. 18, no. 5, pp. 439–454, 2019.

[13] J. Verdi, A. Tan, A. Shoae-Hassani, and A. M. Seifalian, "Endometrial stem cells in regenerative medicine," *Journal of Biological Engineering*, vol. 8, no. 1, p. 20, 2014.

[14] C. E. Gargett, H. P. T. Nguyen, and L. Ye, "Endometrial regeneration and endometrial stem/progenitor cells," *Reviews in Endocrine & Metabolic Disorders*, vol. 13, no. 4, pp. 235–251, 2012.

[15] C. E. Gargett, K. E. Schwab, and J. A. Deane, "Endometrial stem/progenitor cells: the first 10 years," *Human Reproduction Update*, vol. 22, no. 2, pp. 137–163, 2016.

[16] X. Meng, T. E. Ichim, J. Zhong et al., "Endometrial regenerative cells: a novel stem cell population," *Journal of Translational Medicine*, vol. 5, no. 1, p. 57, 2007.

[17] M. Bozorgmehr, S. Gurung, S. Darzi et al., "Endometrial and menstrual blood mesenchymal stem/stromal cells: biological properties and clinical application," *Frontiers in Cell and Development Biology*, vol. 8, p. 497, 2020.

[18] Y. Zhao, X. Lan, Y. Wang et al., "Human endometrial regenerative cells attenuate bleomycin-induced pulmonary fibrosis in mice," *Stem Cells International*, vol. 2018, Article ID 3475137, 13 pages, 2018.

[19] X. Lan, G. Wang, X. Xu et al., "Stromal cell-derived factor-1 mediates cardiac allograft tolerance induced by human endometrial regenerative cell-based therapy," *Stem Cells Translational Medicine*, vol. 6, no. 11, pp. 1997–2008, 2017.

[20] P. Sun, J. Liu, W. Li et al., "Human endometrial regenerative cells attenuate renal ischemia reperfusion injury in mice," *Journal of Translational Medicine*, vol. 14, no. 1, p. 28, 2016.

[21] D. Yu, Y. Zhao, H. Wang et al., "IL-1β pre-stimulation enhances the therapeutic effects of endometrial regenerative cells on experimental colitis," *Stem Cell Research & Therapy*, vol. 12, no. 1, p. 324, 2021.

[22] G. Li, D. Kong, Y. Qin et al., "IL-37 overexpression enhances the therapeutic effect of endometrial regenerative cells in concanavalin A-induced hepatitis," *Cytotherapy*, vol. 23, no. 7, pp. 617–626, 2021.

[23] X. Xu, Y. Wang, B. Zhang et al., "Treatment of experimental colitis by endometrial regenerative cells through regulation of B lymphocytes in mice," *Stem Cell Research & Therapy*, vol. 9, no. 1, p. 146, 2018.

[24] X. Li, J. Liao, X. Su et al., "Human urine-derived stem cells protect against renal ischemia/reperfusion injury in a rat model via exosomalmiR-146a-5pwhich targetsIRAK1," *Theranostics*, vol. 10, no. 21, pp. 9561–9578, 2020.

[25] M. G. Chang, L. Tung, R. B. Sekar et al., "Proarrhythmic potential of mesenchymal stem cell transplantation revealed in an in vitro coculture model," *Circulation*, vol. 113, no. 15, pp. 1832–1841, 2006.

[26] M. Breitbach, T. Bostani, W. Roell et al., "Potential risks of bone marrow cell transplantation into infarcted hearts," *Blood*, vol. 110, no. 4, pp. 1362–1369, 2007.

[27] G. Moll, J. J. Alm, L. C. Davies et al., "Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties?," *Stem Cells*, vol. 32, no. 9, pp. 2430–2442, 2014.

[28] F. J. Vizoso, N. Eiro, S. Cid, J. Schneider, and R. Perez-Fernandez, "Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine," *International Journal of Molecular Sciences*, vol. 18, no. 9, p. 1852, 2017.

[29] D. S. Zagoura, M. G. Roubelakis, V. Bitsika et al., "Therapeutic potential of a distinct population of human amniotic fluid mesenchymal stem cells and their secreted molecules in mice with acute hepatic failure," *Gut*, vol. 61, no. 6, pp. 894–906, 2012.

[30] V. Y. Su, C. S. Lin, S. C. Hung, and K. Y. Yang, "Mesenchymal stem cell-conditioned medium induces neutrophil apoptosis associated with inhibition of the NF-κB pathway in endotoxin-induced acute lung injury," *International Journal of Molecular Sciences*, vol. 20, no. 9, p. 2208, 2019.

[31] M. Dominici, K. Le Blanc, I. Mueller et al., "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement," *Cytotherapy*, vol. 8, no. 4, pp. 315–317, 2006.

[32] L. Zhang, Y. Zhang, W. Zhong, C. di, X. Lin, and Z. Xia, "Heme Oxygenase-1 Ameliorates Dextran Sulfate Sodium-induced Acute Murine Colitis by Regulating Th17/Treg Cell Balance," *Journal of Biological Chemistry*, vol. 289, no. 39, pp. 26847–26858, 2014.

[33] B. Zhang, Y. Liu, X. Lan et al., "Oral Escherichia coli expressing IL-35 meliorates experimental colitis in mice," *Journal of Translational Medicine*, vol. 16, no. 1, p. 71, 2018.

[34] B. Weigmann, I. Tubbe, D. Seidel, A. Nicolaev, C. Becker, and M. F. Neurath, "Isolation and subsequent analysis of murine lamina propria mononuclear cells from colonic tissue," *Nature Protocols*, vol. 2, no. 10, pp. 2307–2311, 2007.

[35] V. Dinallo, I. Marafini, D. di Fusco et al., "Neutrophil extracellular traps sustain inflammatory signals in ulcerative colitis," *Journal of Crohn's and Colitis*, vol. 13, no. 6, pp. 772–784, 2019.

[36] L. Biancone, S. Bruno, M. C. Deregibus, C. Tetta, and G. Camussi, "Therapeutic potential of mesenchymal stem cell-derived microvesicles," *Nephrology, Dialysis, Transplantation*, vol. 27, no. 8, pp. 3037–3042, 2012.

[37] S. S. Lin, B. Zhu, Z. K. Guo et al., "Bone marrow mesenchymal stem cell-derived microvesicles protect rat pheochromocytoma PC12 cells from glutamate-induced injury via a PI3K/Akt dependent pathway," *Neurochemical Research*, vol. 39, no. 5, pp. 922–931, 2014.

[38] B. Imberti, M. Morigi, S. Tomasoni et al., "Insulin-like growth factor-1 sustains stem cell mediated renal repair," *Journal of the American Society of Nephrology*, vol. 18, no. 11, pp. 2921–2928, 2007.

[39] F. Tögel, P. Zhang, Z. Hu, and C. Westenfelder, "VEGF is a mediator of the renoprotective effects of multipotent marrow stromal cells in acute kidney injury," *Journal of Cellular and Molecular Medicine*, vol. 13, no. 8B, pp. 2109–2114, 2009.

[40] Z. Selmani, A. Naji, I. Zidi et al., "Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required

to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells," *Stem Cells*, vol. 26, no. 1, pp. 212–222, 2008.

[41] S. el Moshy, I. A. Radwan, D. Rady et al., "Dental stem cell-derived secretome/conditioned medium: the future for regenerative therapeutic applications," *Stem Cells International*, vol. 2020, Article ID 7593402, 29 pages, 2020.

[42] M. Osugi, W. Katagiri, R. Yoshimi, T. Inukai, H. Hibi, and M. Ueda, "Conditioned media from mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects," *Tissue Engineering. Part A*, vol. 18, no. 13-14, pp. 1479–1489, 2012.

[43] H. Cui, Y. Cai, L. Wang et al., "Berberine regulates Treg/Th17 balance to treat ulcerative colitis through modulating the gut microbiota in the colon," *Frontiers in Pharmacology*, vol. 9, p. 571, 2018.

[44] X. Bing, L. Xuelei, D. Wanwei, L. Linlang, and C. Keyan, "EGCG Maintains Th1/Th2 Balance and Mitigates Ulcerative Colitis Induced by Dextran Sulfate Sodium through TLR4/MyD88/NF-κB Signaling Pathway in Rats," *Canadian Journal of Gastroenterology & Hepatology*, vol. 2017, article 3057268, 9 pages, 2017.

[45] T. B. Bertolini, M. Biswas, C. Terhorst, H. Daniell, R. W. Herzog, and A. R. Piñeros, "Role of orally induced regulatory T cells in immunotherapy and tolerance," *Cellular Immunology*, vol. 359, article 104251, 2021.

[46] X. Valencia, G. Stephens, R. Goldbach-Mansky, M. Wilson, E. M. Shevach, and P. E. Lipsky, "TNF downmodulates the function of human CD4+CD25hi T-regulatory cells," *Blood*, vol. 108, no. 1, pp. 253–261, 2006.

[47] C. Mottet, H. H. Uhlig, and F. Powrie, "Cutting edge: cure of colitis by $CD4^+CD25^+$ regulatory T cells," *The Journal of Immunology*, vol. 170, no. 8, pp. 3939–3943, 2003.

[48] L. Zhu, L. Z. Xu, S. Zhao, Z. F. Shen, H. Shen, and L. B. Zhan, "Protective effect of baicalin on the regulation of Treg/Th17 balance, gut microbiota and short-chain fatty acids in rats with ulcerative colitis," *Applied Microbiology and Biotechnology*, vol. 104, no. 12, pp. 5449–5460, 2020.

[49] S. Luo, R. Wen, Q. Wang et al., "Rhubarb Peony decoction ameliorates ulcerative colitis in mice by regulating gut microbiota to restoring Th17/Treg balance," *Journal of Ethnopharmacology*, vol. 231, no. 231, pp. 39–49, 2019.

[50] S. de Schepper, S. Verheijden, J. Aguilera-Lizarraga et al., "Self-maintaining gut macrophages are essential for intestinal homeostasis," *Cell*, vol. 175, no. 2, pp. 400–415.e13, 2018.

[51] C. C. Bain, C. L. Scott, H. Uronen-Hansson et al., "Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same $Ly6C^{hi}$ monocyte precursors," *Mucosal Immunology*, vol. 6, no. 3, pp. 498–510, 2013.

[52] M. L. Chen and M. S. Sundrud, "Cytokine networks and T-cell subsets in inflammatory bowel diseases," *Inflammatory Bowel Diseases*, vol. 22, no. 5, pp. 1157–1167, 2016.

[53] Y. Wang, X. Duan, X. Liu et al., "Rho kinase blockade ameliorates DSS-induced ulcerative colitis in mice through dual inhibition of the NF-κB and IL-6/STAT3 pathways," *Inflammation*, vol. 43, no. 3, pp. 857–867, 2020.

[54] X. Chen, R. Deng, W. Chi et al., "IL-27 signaling deficiency develops Th17-enhanced Th2-dominant inflammation in murine allergic conjunctivitis model," *Allergy*, vol. 74, no. 5, pp. 910–921, 2019.

[55] J. Qi, Z. Zhang, X. Tang, W. Li, W. Chen, and G. Yao, "IL-27 regulated $CD4^+IL\text{-}10^+$ T cells in experimental Sjögren syndrome," *Frontiers in Immunology*, vol. 11, p. 1699, 2020.

[56] H. Yoshida and C. A. Hunter, "The immunobiology of interleukin-27," *Annual Review of Immunology*, vol. 33, pp. 417–443, 2015.

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Research Article

Towards Secretome Standardization: Identifying Key Ingredients of MSC-Derived Therapeutic Cocktail

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The therapeutic potential of the conditioned medium (CM) derived from MSCs (mesenchymal stem/stromal cells) in disparate medical fields, from immunology to orthopedics, has been widely suggested by *in vitro* and *in vivo* evidences. Prior to MSC-CM use in clinical applications, appropriate quality controls are needed in order to assess its reproducibility. Here, we evaluated different CM characteristics, including general features and precise protein and lipid concentrations, in 3 representative samples from adipose-derived MSCs (ASCs). In details, we first investigated the size and distribution of the contained extracellular vesicles (EVs), lipid bilayer-delimited particles whose pivotal role in intercellular communication has been extensively demonstrated. Then, we acquired Raman signatures, providing an overlook of ASC-CM composition in terms of proteins, lipids, and nucleic acids. At last, we analyzed a panel of 200 molecules including chemokines, cytokines, receptors, and inflammatory and growth factors and searched for 32 lipids involved in cell signalling and inflammation. All ASC-CM contained a homogeneous and relevant number of EVs ($1.0 \times 10^9 \pm 1.1 \times 10^8$ particles per million donor ASCs) with a mean size of 190 ± 5.2 nm, suggesting the appropriateness of the method for EV retaining and concentration. Furthermore, also Raman spectra confirmed a high homogeneity among samples, allowing the visualization of specific peaks for nucleic acids, proteins, and lipids. An in depth investigation that focused on 200 proteins involved in relevant biological pathways revealed the presence in all specimens of 104 factors. Of these, 26 analytes presented a high degree of uniformity, suggesting that the samples were particularly homogenous for a quarter of the quantified molecules. At last, lipidomic analysis allowed the quantification of 7 lipids and indicated prostaglandin-E2 and N-stearoylethanolamide as the most homogenous factors. In this study, we assessed that ASC-CM samples obtained with a standardized protocol present stable features spanning from Raman fingerprint to specific marker concentrations. In conclusion, we identified key ingredients that may be involved in ASC-CM therapeutic action and whose consistent levels may represent a promising quality control in the pipeline of its preparation for clinical applications.

1. Introduction

Over the years, the transplantation of autologous or allogeneic stem cells, either naïve, differently primed, or genetically manipulated, has paved the way to the successful clinical management of several diseases whose pharmacological need was previously unmet. In particular, mesenchymal stem/stromal cells (MSCs), thanks to their regenerative and immunomodulatory potential [1, 2], have gained popularity as cell therapy in disparate clinical scenarios, from immunological diseases [3] to orthopedic conditions [4] and central nervous system injuries and disorders (e.g., traumatic brain injury, Parkinson's disease, and ischemic stroke) [5]. Besides the overall promising results, MSC transplantation (as well as cell-based therapy in general) entails evident drawbacks, such as ethical controversies, concerns linked to ex vivo

expansion and high manufacturing costs. Starting from 2006 with the work of Gnecchi et al. [6], a growing body of evidence identifies paracrine signalling as the main effector of MSC therapeutic action, overturning the initial hypothesis that acknowledged cell engraftment, differentiation, and replacement as the main actors. Consequently, in 2010, Professor Caplan, the father of MSCs who firstly characterized and named them, proposed the new terminology of medicine signalling cells to highlight their secretory nature [7]. The term secretome, coined at the beginning of 2000's by Tjalsma and colleagues [8], defines the plethora of factors of different natures (lipids, nucleic acids, and proteins) secreted by a cell, both freely dissolved and conveyed into extracellular vesicles (EVs). The paradigm shift on MSC mode of action promoted cell secretome, intended both as an entire formula and as selected fractions (i.e., soluble and vesicular subcomponents), to a novel class of biological therapeutics. Indeed, the last few years witnessed the entrance of MSC secretome to several clinical trials, mostly in the regenerative medicine field, retracing the path of the clinical application of donor cells [9]. A critical search through ClinicalTrials.gov database, performed at the end of April 2021 using alternatively the keywords "secretome," "conditioned medium," or "extracellular vesicles" and the filter "interventional" as study type, lists a total of 14 studies based on MSC-secretome administration. Interestingly, most of these protocols relied on the use of CM ($n = 11$ versus $n = 3$ studies using EVs) derived from allogeneic MSCs ($n = 8$ versus $n = 1$ study specifically following an autologous setting). Thus far, only 3 of these studies are completed (NCT04315025, NCT03676400, and NCT04134676), but unluckily, there are no available results yet. Nevertheless, this picture allows us extrapolating some general considerations on the state of the art of MSC-based cell-free therapies:

(i) To date, the clinical use of complete secretome, accounting for both soluble and vesicular fractions, seems more easily applicable than isolated EVs. However, at the moment, EV potential in diagnostics remains pivotal, as confirmed by the high number of clinical trials relying on their use in this field

(ii) Allogeneic settings are widely implemented, confirming the lack of immunogenicity and allowing to minimize interdonor variability and to avoid the need of performing additional procedures on patients for cell harvesting, thus excluding also donor site morbidity

(iii) Donor MSCs are harvested from both neonatal (mostly umbilical cord) and adult (e.g., adipose tissue and bone marrow) tissue sources

(iv) As for MSC-based cell therapy, the nature of the targeted pathologies is extremely various (among others, COVID-19 pneumonia, chronic wounds, alopecia, and osteoarthritis)

It is worth noting that up to now, the regulatory framework for the clinical use of cell secretome, or its subproducts, has not been clearly stated by any national nor international agency such as the FDA or the EMA. In the light of a successful translation to the clinics, there are still many technical issues to be addressed, mainly concerning the mode of action, scalability, standardization, and characterization.

In recent years, our research focused on the investigation of the conditioned medium (CM) from adipose tissue-derived MSCs (ASCs) in terms of biochemical composition [10–13] and therapeutic action, both *in vitro* [14, 15] and *in vivo* [16, 17]. Most of these studies provided the comparison between ASC-CM, consisting of both soluble factors and vesicle-conveyed ones, and ultracentrifuge-isolated EVs. Here, we decided to focus selectively on complete secretome. The present work takes a step forward in the perspective of ASC-CM characterization by quantifying a wide panel of molecules (cytokines, chemokines, receptors, growth and inflammatory factors, and bioactive lipids) in 3 different samples, in order to define some quality control criteria in the light of its future translation into clinics as an innovative cell-free therapeutic.

2. Materials and Methods

2.1. Cell Cultures. ASCs were isolated from the subcutaneous adipose tissue of 3 nonobese (BMI < 30) donors (1 male and 2 females, 54.7 ± 2.3 years old) who underwent total hip replacement surgery ($n = 2$) or liposuction ($n = 1$). All tissues were collected at IRCCS Istituto Ortopedico Galeazzi upon Institutional Review Board approval. Every donor provided a written informed consent. Adipose tissue samples were shredded with a sterile scalpel, digested for 30 min with 0.75 mg/ml type I collagenase (Worthington Biochemical Corporation, Lakewood, NJ, USA), and filtered with a 100 μm cell strainer (Corning Incorporated, Corning, NY, USA). ASCs were grown in a culture medium composed by high-glucose DMEM (Sigma-Aldrich, St. Louis, MO, USA), 10% fetal bovine serum (FBS, Euroclone, Pero, Italy), 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C, 5% CO_2.

2.2. CM Production. ASCs from V to VII passage at 90% of confluence were incubated in starving conditions (without FBS) for 72 h. No signals of cell suffering were ever recorded during the period. The medium was then collected and centrifuged at 2500g for 15 min at 4°C with the purpose of eliminating cell debris, dead cells, and large apoptotic bodies. The supernatants were then concentrated about 60 times by centrifuging at 4000g for 90 min at 4°C in Amicon Ultra-15 Centrifugal Filter Devices with 3 kDa molecular weight cut-off (Merck Millipore, Burlington, MA, USA). This procedure allows the retention of the vesicular component of cell secretome, as previously demonstrated in [12, 13, 15]. The safety and efficacy of the final product obtained through this procedure have been already tested both *in vitro* [14, 15] and *in vivo* [16, 17].

2.3. Nanoparticle Tracking Analysis (NTA). ASC-CM samples were appropriately diluted in 0.22 μm triple-filtered PBS and analyzed by NanoSight NS3000 (Malvern

Panalytical, Salisbury, UK). Three videos, each one lasting 1 min, have been recorded for every sample. All measurements respected the quality criteria of 20-120 particles/frame, concentration of $10^6 - 4 \times 10^9$ particles/ml, and number of valid tracks > 20%. After capture, the videos have been analyzed by the in-build NanoSight Software NTA.

2.4. Protein Array. Undiluted ASC-CM samples were analyzed by RayBiotech facility (RayBiotech, Norcross, GA, USA). The concentration (pg/ml) of 200 analytes of different natures (cytokines, chemokines, receptors, and inflammatory and growth factors) was investigated using the Quantibody® Human Cytokine Array 4000 Kit, a combination of Human Cytokine Array Q4, Human Chemokine Array Q1, Human Receptor Array Q1, Human Inflammation Array Q3, and Human Growth Factor Array Q1 (https://www.raybiotech.com/quantibody-human-cytokine-array-4000/). Obtained values were normalized on donor cell number (pg/10^6 ASCs).

2.5. Raman Spectroscopy. ASC-CM samples were diluted in sterile saline solution and analyzed with the Raman microspectroscope (LabRAM Aramis, Horiba Jobin Yvon S.A.S., Lille, France) equipped with a 532 nm laser following a previously reported protocol [10, 13]. CM samples were deposited on a calcium fluoride slide and air-dried, and then, measurements were performed in the spectral ranges 600-1800 and 2600-3200 cm^{-1}. At least 15 spectra per sample were acquired and processed (baseline correction, unit vector normalization, and postacquisition calibration) taking advantage of the integrated software LabSpec 6 (Horiba Jobin Yvon S.A.S., Lille, France).

2.6. Targeted UHPLC-MS/MS-Based Lipidomics. Polyunsaturated fatty acids, eicosanoids, endocannabinoids, and N-acylethanolamides were quantified on a QTRAP 5500 triple quadrupole linear ion trap mass spectrometer (Sciex, Darmstadt, Germany) coupled with an Agilent 1200 Infinity pump ultrahigh-pressure liquid chromatography (UHPLC) system (Agilent Technologies, Palo Alto, CA, USA) using the UHPLC-MS/MS methods previously reported [18]. Briefly, undiluted ASC-CM samples (approximately 200 μl/sample) were spiked with deuterated internal standards and 1 ml of cold acetonitrile was added for protein precipitation. After centrifugation, the supernatants were extracted with a 4 ml of dichloromethane/isopropanol (8 : 2; *v*/*v*) and centrifuged again. The organic layer was separated, dried, and reconstituted in 60 μl methanol. 3 μl aliquot was analyzed for endocannabinoids and N-acylethanolamides. The remaining solution was added with 500 μl hydrochloride acid (0.125 N) and 4 ml ethyl acetate/n-hexane (9 : 1; *v*/*v*). The organic phase was dried, and the residue was reconstituted in 60 μl acetonitrile. 30 μl aliquot of methanol obtained from the neutral extraction and 30 μl aliquot from acid extraction were merged, and 10 μl was analyzed for polyunsaturated fatty acids and eicosanoid determination. Data acquisition and processing were performed using Analyst®1.6.2 and MultiQuant®2.1.1 software (Sciex, Darmstadt, Germany), respectively.

2.7. Validation of Selected Proteins and Lipids. The validation of selected proteins was performed on 5 additional ASC-CM samples (deriving from cells harvested from 2 female and 3 male donors, mean age = 54.6 ± 22.3 years old). The Human Magnetic Luminex Screening Assay Rk4yTGNI (R&D Systems, Minneapolis, MN, USA) was customized to contain 5 molecules: MCP-4, PDGF-AA, TNF RI, DKK-1, and RAGE. Duplicates of each ASC-CM (50 μl/sample) were tested, undiluted, and read through Bio-Plex Multiplex System (Bio-Rad, Milan, Italy) following standard procedures. Data analysis was performed with MAGPIX xPONENT 4.2 software (Luminex Corporation, Austin, TX, USA). The validation of SEA and PGE2 levels was performed through the UHPLC-MS/MS methods previously described on the CM derived from 5 additional ASC populations (all female donors, mean age = 49.0 ± 11.1 years old).

2.8. Data Analysis and Statistics. Statistics was performed with GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA) and Excel. p values < 0.05 were considered statistically significant. NTA data were analyzed by the Kruskal-Wallis test to evaluate interdonor variability. For the Raman spectra, descriptive and multivariate statistical analyses were performed with Origin2021 (OriginLab, Northampton, MA, USA). Principal component analysis (PCA) was performed to reduce the dimensionality of Raman spectral datasets and to highlight differences between the spectra, with the resulting principal components (PC) representing these spectral differences with increasing percentage of variance. For protein array data, D'Agostino and Pearson omnibus normality test was used to determine whether the samples come from a Gaussian distribution. None of the datasets passed the normality test and correlation (Spearman r), and linear regression analyses were then performed accordingly. Coefficient of variation (CV, also known as relative standard deviation or RSD) was calculated as the ratio of the standard deviation to the mean. A CV < 33% was set as threshold. PCA and clustering were performed by ClustVis (https://biit.cs.ut.ee/clustvis). Process/pathway analysis was performed by STRING (https://string-db.org/) following default settings.

3. Results

CM samples were obtained, as previously described, from the culture medium harvested from confluent ASCs cultured for 3 days in serum-free conditions and concentrated by centrifugal filter devices of about 60 times. Since EVs represent a fundamental component of cell secretome, the first step of CM characterization focused on particle analysis. NTA revealed a similar size distribution between all the samples (Figures 1(a)–1(c)), with a mean EV size of 190 ± 5.2 nm (Figure 1(d)). Mode values (Figure 1(e)) further confirmed the homogeneity between preparations, indicating that the dimensions of the most frequently occurring particle populations ranged from 110 to 150 nm. All samples counted a relevant number of EVs, with an average of $1.0 \times 10^9 \pm 1.1 \times 10^8$ particles per million donor ASCs (Figure 1(f)), confirming the appropriateness of our protocol

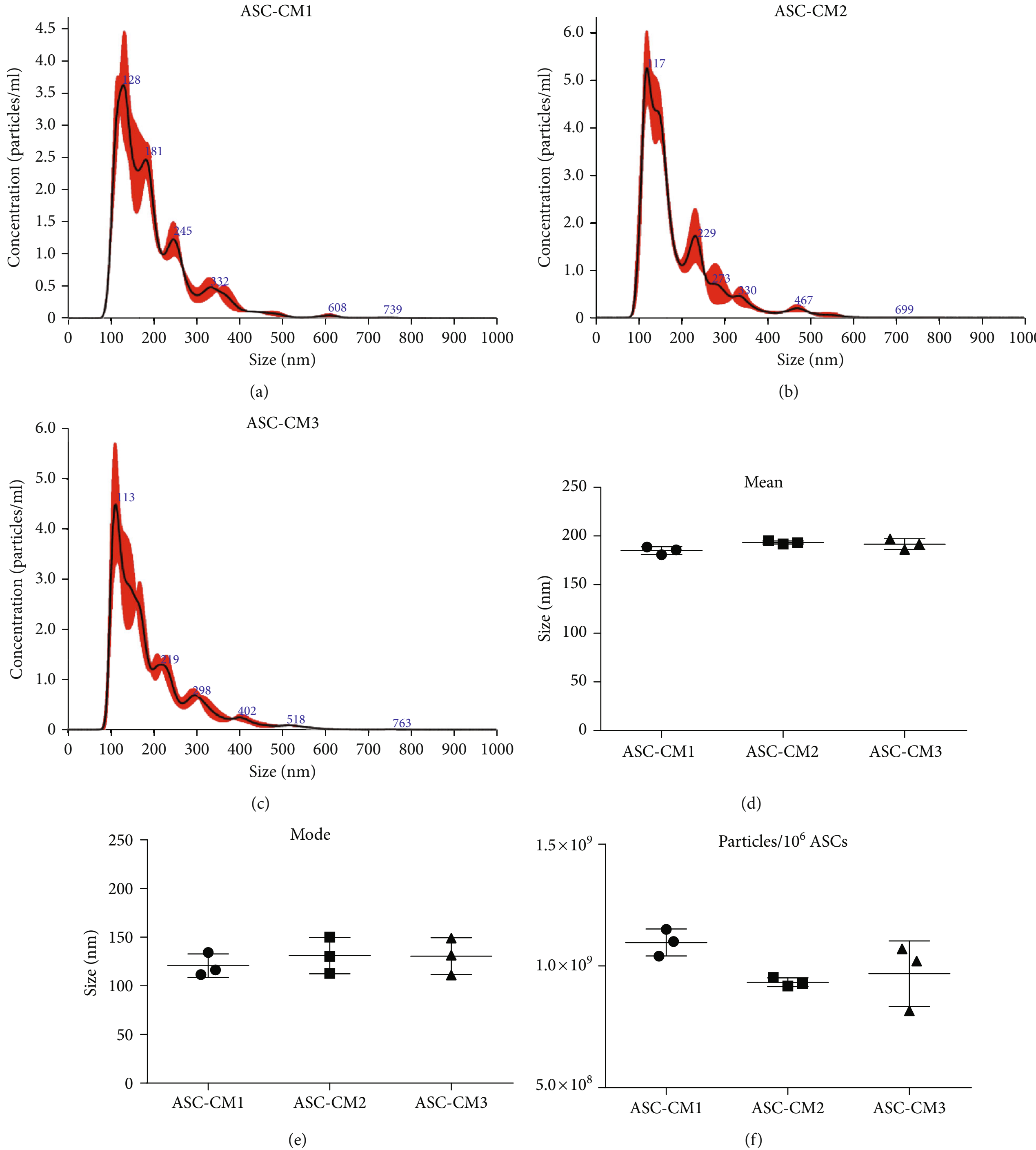


Figure 1: Dimensional characterization and quantification of ASC-CM extracellular vesicles. (a–c) Representative images of NTA referred to ASC-CM1 (a), ASC-CM2 (b), and ASC-CM3 (c). (d–f) Size distribution (d, e) and vesicular yield (f) deriving from 3 NTA measurements/sample. Data are shown as the mean ± SD.

in retaining the vesicular component of cell secretome. No significant difference was observed in any parameter (nonparametric Kruskal-Wallis test, $p > 0.05$).

CM samples were then characterized by Raman spectroscopy, a vibrational spectroscopy method that was already proved to be effective in characterizing the soluble and the vesicular components of MSC secretome, verifying the purity and reproducibility of cell-free preparations [10, 13]. The obtained average spectra (Figure 2(a)) provide detailed biochemical information about the considered samples, with the Raman fingerprint accounting for proteins (amide I 1.650 cm^{-1}), lipids (2700–3200 cm^{-1}), and nucleic acids (720–820 cm^{-1}), in agreement with previously reported data [13]. In particular, CM spectra showed a good signal-to-noise ratio and a good reproducibility, as assessed by the reported standard deviation (gray shaded areas in

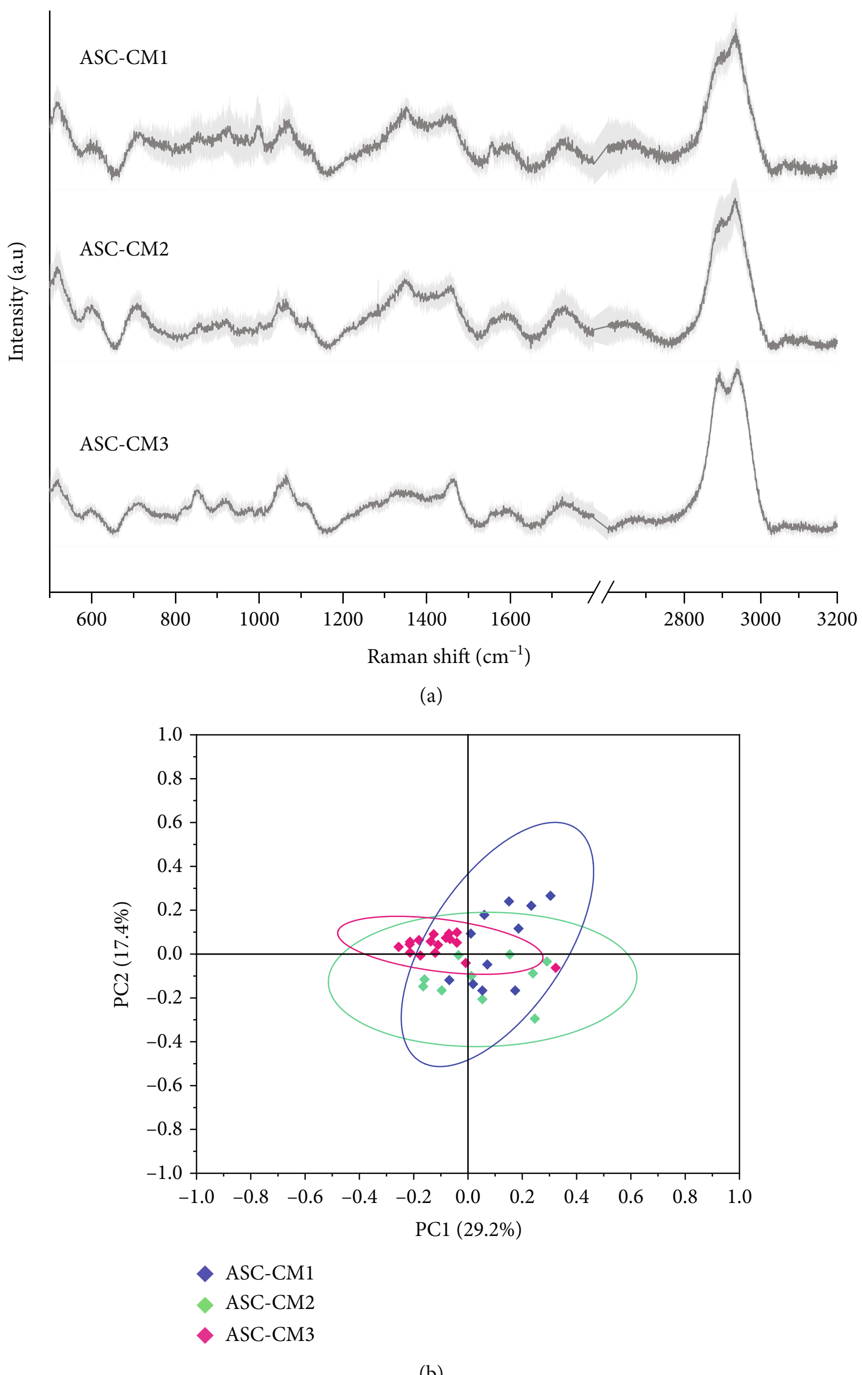
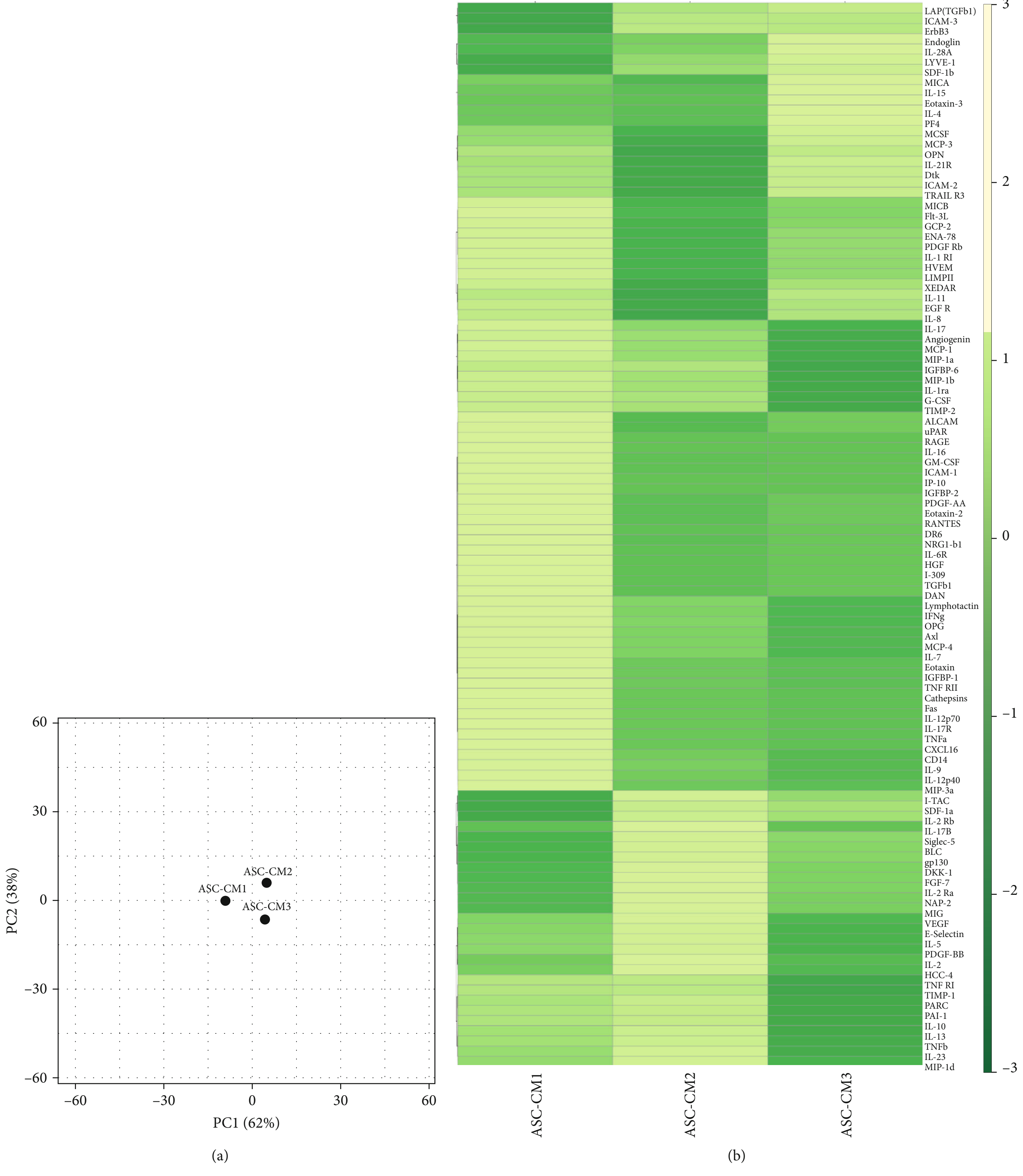


Figure 2: Raman spectroscopy analysis of ASC-CM samples. (a) Average Raman spectra obtained with 532 nm laser line on air-dried drops of ASC-CM samples. Gray shaded areas represent ±1 standard deviation. (b) Scatter plot of the PC1 and PC2 scores obtained for the 3 considered samples after multivariate statistical analysis (PCA). Ellipses represent the 95% confidence intervals calculated for each sample.

Figure 2(a)). The similarities in the chemical composition of the samples were further verified by multivariate statistical analysis: the PC1 and PC2 scores obtained for the three considered samples showed substantial overlap in the reported scatter plot (Figure 2(b)).

In order to identify and quantify putative key factors involved in ASC-CM therapeutic action, we analyzed a panel of 200 chemokines, cytokines, receptors, and inflammatory and growth factors (40 molecules/category). 104 proteins were reliably quantified in all the samples (19 chemokines, 14 cytokines, 24 receptors, and 37 inflammatory and 10 growth factors), while 44 molecules were always undetectable (5 chemokines, 10 cytokines, 7 receptors, and 1 inflammatory and 21 growth factors) (Supplementary Tables 1 and 2). PCA on the 104 quantified factors unveiled a similar heterogeneity across the 3 samples (Figure 3(a)). The heat map further confirmed the lack of major differences among the specimens (Figure 3(b)). Correlation analysis, performed



(a) (b)

Figure 3: Continued.

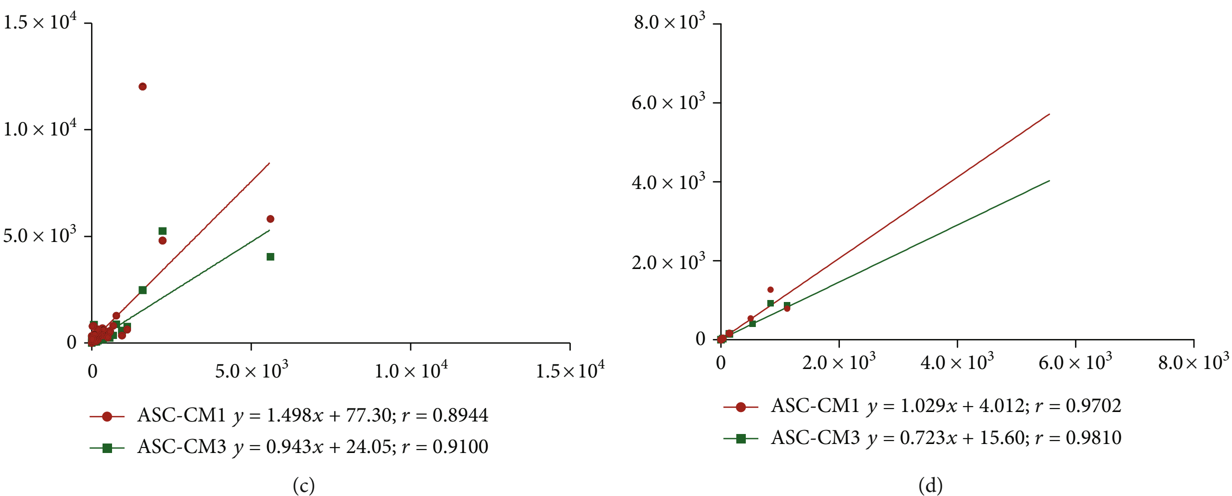


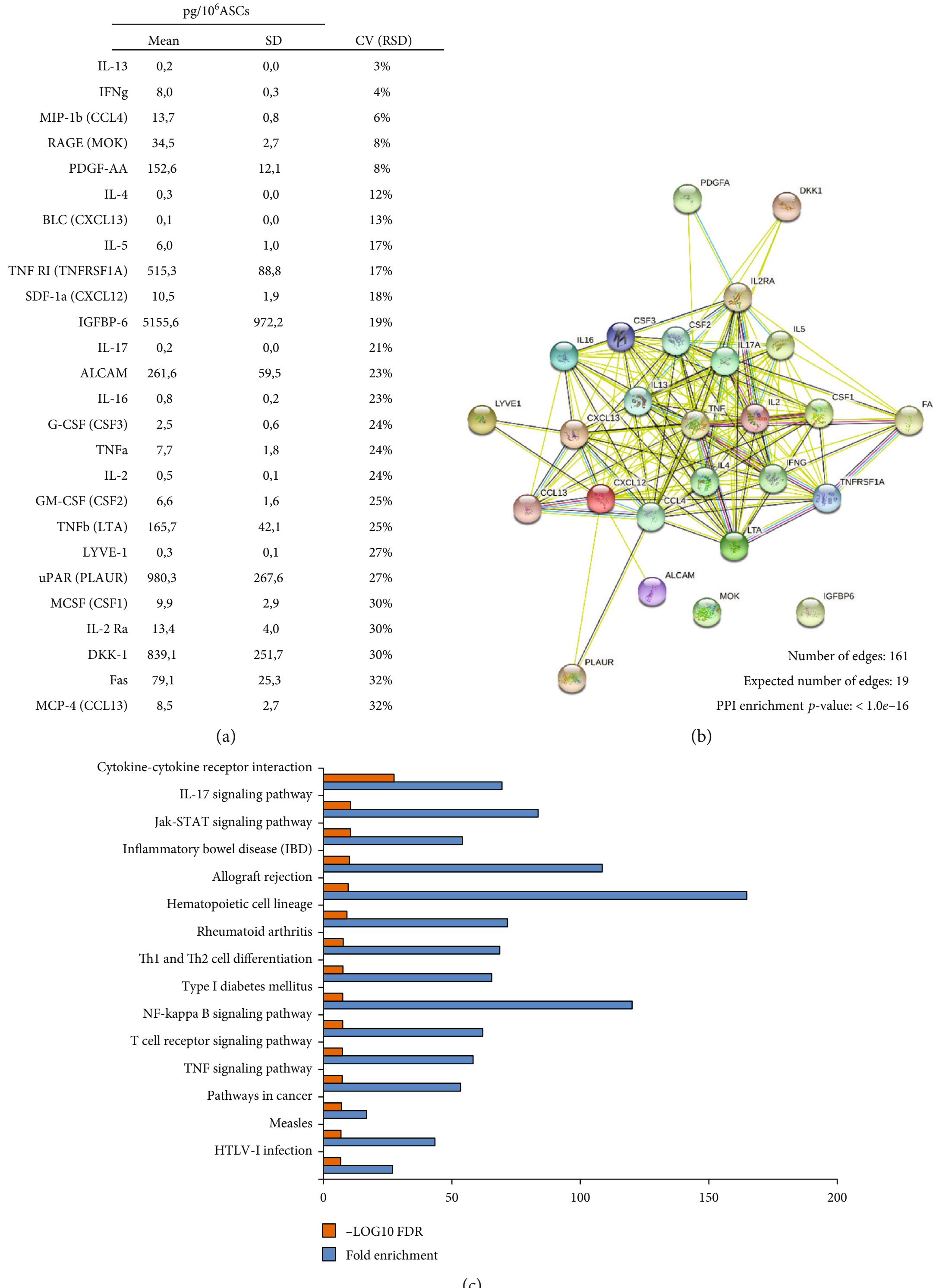
Figure 3: Clustering and correlation analysis of the 104 factors quantified in all ASC-CM samples. For the analyses, the levels of each analyte (pg/ml) were normalized on donor cell number and expressed as pg/10^6 ASCs. (a) PCA plot and (b) heat map visualization of the protein levels in ASC-CM1, ASC-CM2, and ASC-CM3. (c, d) Correlation analysis of all the 104 factors (c) and of the 26 molecules (d) having a coefficient of variation below 33% (CV < 33%). For each graph, the equation of the regression lines is reported, together with Spearman r values.

both on the entire datasets (Figure 3(c)) and on 26 selected factors with a coefficient of variation (CV) lower than 33% (Figure 3(d) and Figure 4(a)), showed a strong relationship between the quantitative variables among samples. Indeed, the slope of the regression lines always tended to 1 (Figures 3(c) and 3(d)). Moreover, Spearman r always resulted higher than 0.8, confirming a highly significant direct correlation between specimens (Figures 3(c) and 3(d), nonparametric Spearman correlation, $p < 0.0001$).

Since we aim at suggesting standards for CM quality control, further analyses focused on selected analytes particularly homogeneous across the samples. In details, around 25% ($n = 26$) of the quantified factors presented a CV < 33%, indicating a high degree of uniformity in all CM (Figure 4(a), Supplementary Table 3). Of note, 15 of these were inflammatory factors (Supplementary Table 3). STRING analysis underlined strict interconnections between these factors (Figure 4(b)). As expected, a strong enrichment in proteins involved in immune system regulation emerged by pathway analysis (Supplementary Table 4). In particular, the top 15 pathways ranked by FDR (Figure 4(c)) list proteins involved in cytokine-cytokine interaction (cytokine-cytokine receptor interaction/Jak-STAT signalling pathway) and T cell regulation (T cell receptor signalling pathway/Th1 and Th2 cell differentiation).

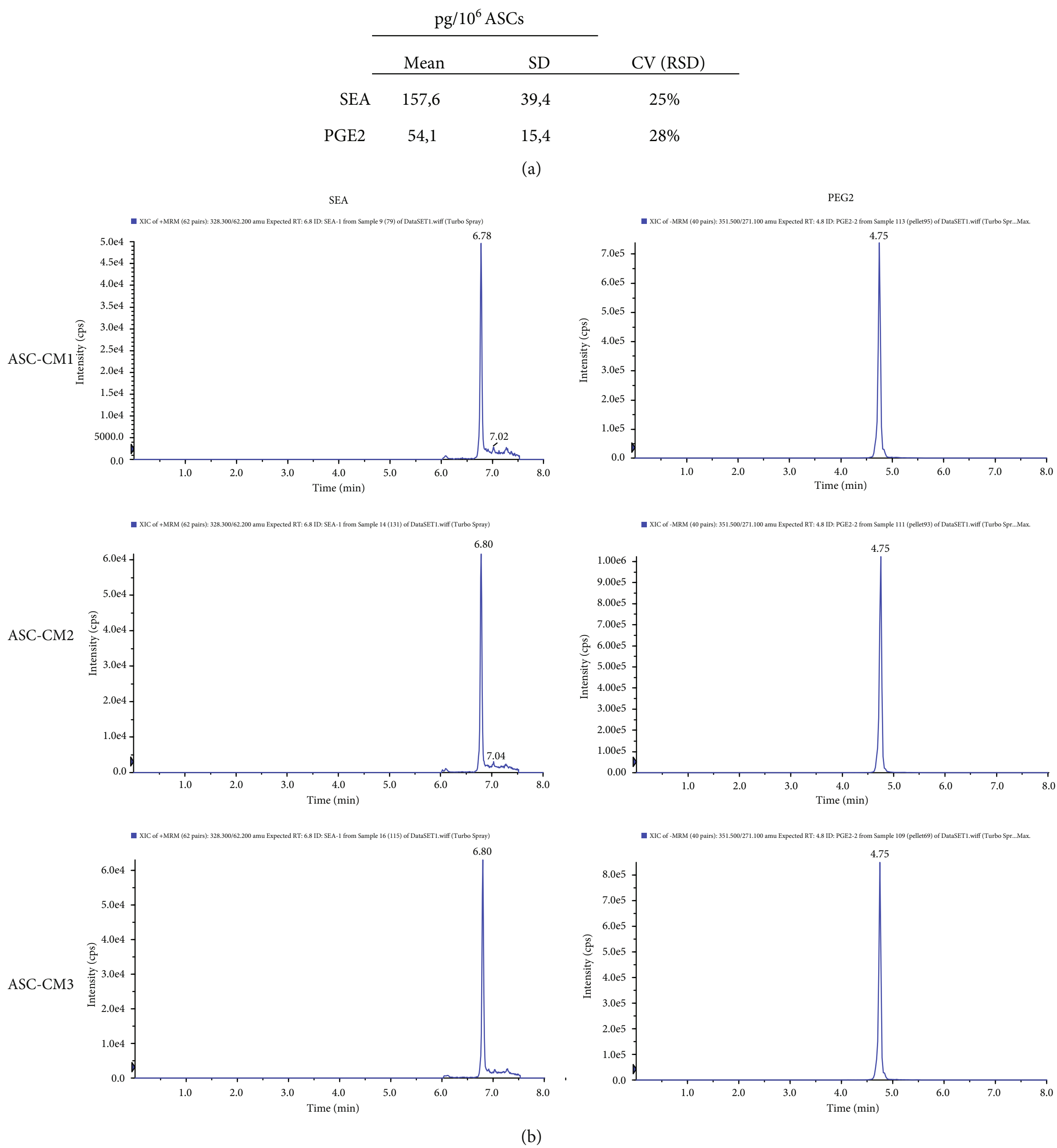
Besides proteins, lipids might also exert important roles in immune regulation. For this reason, in our CM samples, we analyzed a panel of endocannabinoids and eicosanoids known to be involved in inflammation. Seven lipid molecules, i.e., arachidonoyl acid (AA), eicosapentaenoyl acid (EPA), docosahexaenoic acid (DHA), prostaglandin-E2 (PGE2), prostaglandin-F2α (PGF2α), N-palmitoylethanolamide (PEA), and N-stearoylethanolamide (SEA) (Supplementary Table 5), were reliably quantified by UHPLC-MS/MS analysis in all ASC-CM samples. Except for 2-arachidonoylglycerol (2AG), quantified in 2 out of 3 samples, the other 24 lipids were always undetectable or unquantifiable (<LODs or LOQs). A coefficient of variation lower than 33% was found for SEA and PGE2 molecules (Figures 5(a) and 5(b), Supplementary Table 5), indicating a good degree of uniformity in the 3 CM. It is interesting to point out that, in CM, bioactive lipid by-products are more homogenous than precursors. This could suggest that mainly the firsts are released in a controlled fashion. Indeed, analyzing the pellets of the donor cells and also the precursors DHA, AA, and EPA presents strongly similar concentrations at intracellular level (Supplementary Table 6).

Since quantifying specific analytes could become a good quality control step for ASC-CM, we analyzed the concentration of a subset of factors in larger validation cohorts ($n = 5$ ASC-CM for both protein and lipid validation). Regarding proteins, our results confirmed both the presence and the homogeneity of the selected factors in all the analyzed samples (Figure 6(a)). Of note, while the mean concentrations of RAGE (18.5 ± 9.3 pg/10^6 ASCs), TNF RI (368.4 ± 78.3 pg/10^6 ASCs), and MCP-4 (19.5 ± 11.6 pg/10^6 ASCs) nicely fit the ones observed in the original set (Figure 4(a)), the detected values for PDGF-AA (3.7 ± 2.9 pg/10^6 ASCs) and DKK-1 (2524.9 ± 734.6 pg/10^6 ASCs) are, respectively, lower and higher than expected. This discrepancy can be attributed to the implementation of distinct immunological techniques that therefore may have a different sensibility and specificity and may rely on the use of antibodies raised against disparate regions of the analytes. Conversely, lipid validation was performed through the same UHPLC-MS/MS methods used to test the original set. As shown in Figure 6(b), SEA (128.2 ± 98.8 pg/10^6 ASCs) and PGE2 (50.8 ± 38.6 pg/10^6 ASCs) were quantified in the entire ASC-CM lipid validation cohort within a concentration range that strongly overlaps what was previously observed (Figure 5(a)).



	pg/10⁶ASCs		
	Mean	SD	CV (RSD)
IL-13	0,2	0,0	3%
IFNg	8,0	0,3	4%
MIP-1b (CCL4)	13,7	0,8	6%
RAGE (MOK)	34,5	2,7	8%
PDGF-AA	152,6	12,1	8%
IL-4	0,3	0,0	12%
BLC (CXCL13)	0,1	0,0	13%
IL-5	6,0	1,0	17%
TNF RI (TNFRSF1A)	515,3	88,8	17%
SDF-1a (CXCL12)	10,5	1,9	18%
IGFBP-6	5155,6	972,2	19%
IL-17	0,2	0,0	21%
ALCAM	261,6	59,5	23%
IL-16	0,8	0,2	23%
G-CSF (CSF3)	2,5	0,6	24%
TNFa	7,7	1,8	24%
IL-2	0,5	0,1	24%
GM-CSF (CSF2)	6,6	1,6	25%
TNFb (LTA)	165,7	42,1	25%
LYVE-1	0,3	0,1	27%
uPAR (PLAUR)	980,3	267,6	27%
MCSF (CSF1)	9,9	2,9	30%
IL-2 Ra	13,4	4,0	30%
DKK-1	839,1	251,7	30%
Fas	79,1	25,3	32%
MCP-4 (CCL13)	8,5	2,7	32%

Figure 4: Protein interactions and functional prediction of the 26 most homogeneous factors quantified in ASC-CM samples. (a) List of the 26 selected factors having a coefficient of variation below 33% (CV < 33%) and (b) corresponding protein-protein interactions uncovered by STRING analysis. (c) Top 15 KEGG pathways associated with the 26 proteins selected based on false discovery rate (FDR) p value (-$\log_{10}$ FDR p values are reported as orange bars). Fold enrichment was calculated as follows: Fold enrichment = (observed protein count/number of most homogeneous factors)/(background gene count/total gene number) and is reported as blue bars.



	pg/10^6 ASCs		
	Mean	SD	CV (RSD)
SEA	157,6	39,4	25%
PGE2	54,1	15,4	28%

(a)

Figure 5: Lipid levels in ASC-CM samples. (a) Concentration of SEA and PGE2 in ASC-CM and (b) corresponding multiple reaction monitoring (MRM) chromatograms.

4. Discussion

The secretome from mesenchymal stem/stromal cells represents a mixture of biologically active ingredients whose individual role is still unknown. Nevertheless, their synergistic action in producing a clear therapeutic effect supports the rationale for investigating its clinical potential. This study is aimed at defining key elements of ASC secretome produced according to our protocol, which contemplates the culture of 90% confluent cells for 72 hours under serum deprivation and the following concentration of the conditioned medium through 3 kDa molecular weight cut off filters. Other groups currently implement a similar procedure [19, 20], although in literature, there are plenty of alternatives [21]. Therefore, according to the aphorism “the process is the product,” any change in the manufacturing process will undoubtedly affect the final product. Moreover, it should be pointed out that ASC-CM thus produced retains a

	pg/10^6 ASCs				
	ASC-CM PV1	ASC-CM PV2	ASC-CM PV3	ASC-CM PV4	ASC-CM PV5
RAGE (MOK)	29,1	21,4	12,3	23,7	5,9
PDGF-AA	1,7	6,3	7,5	1,7	1,4
TNF RI (TNFRSF1A)	356,6	347,4	443,0	441,0	254,1
DKK-1	3593,3	2871,1	2415,0	2004,0	1741,2
MCP-4 (CCL13)	16,3	39,9	13,2	16,9	11,2

(a)

	pg/10^6 ASCs				
	ASC-CM LV1	ASC-CM LV2	ASC-CM LV3	ASC-CM LV4	ASC-CM LV5
SEA	97,8	85,7	116,1	298,3	43,2
PGE2	44,9	34,8	45,9	115,6	12,8

(b)

Figure 6: Validation of selected proteins and lipids. (a) Levels of 5 selected proteins (RAGE, PDGF-AA, TNF RI, DKK-1, and MCP-4) quantified in an ASC-CM protein validation (PV) cohort ($n = 5$). (b) SEA and PGE2 levels confirmed in an ASC-CM lipid validation (LV) cohort ($n = 5$). The levels of each analyte (pg/ml) were normalized on donor cell number and expressed as pg/10^6 ASCs.

substantial number of EVs and indeed previous evidences demonstrated a vesicular yield even higher than the one obtained by ultracentrifugation [13, 15].

Here, we focused on key parameters that could be exploited either as general quality controls, such as vesicular component and Raman signature, or as specific markers, such as the quantification of selected proteins and lipids. A summary of the production process and the proposed quality controls is indicated in Figure 7. For the sake of completeness, even though nucleic acids such as miRNAs were not investigated in the current study, their role has been largely discussed by others (e.g., [22]) making them interesting candidates for additional or alternative quality control checks.

Given the biological relevance of EVs, their determination in CM was the first analysis performed in this study. EVs were abundant in all samples. Their number and size distribution were homogeneous and coherent with previous findings [13, 15]. Of note, the filtration protocol allows the retaining and concentration of the vesicular component with a process that is faster, easier, and less demanding than the gold-standard procedure (i.e., ultracentrifugation) [15]. Since EVs are strategic shuttles for biologicals, we suggest their quantification in CM preparations as a general quality control. Together with EV quantification, also Raman spectroscopy can provide a comprehensive picture of CM composition. It reveals the presence of macromolecules and points out differences and similarities across the samples, as reported here and in a previous study [13].

Differently, the investigation and quantification of selected factors could be adapted according to specific downstream applications.

The broad range analysis on 200 proteins playing pivotal roles in a variety of biological processes highlighted the presence of 26 highly conserved molecules in the 3 ASC-CM. Among these, we chose to validate 5 analytes, each belonging to a different panel: the chemokine MCP-4 (CV = 32%), the cytokine DKK-1 (CV = 30%), the receptor RAGE (CV = 8%), the inflammatory mediator TNF RI (CV = 8%), and the growth factor PDGF-AA (CV = 17%). For all these analytes, a high homogeneity among ASC-CM samples was confirmed. Given our promising *in vitro* [14, 15] and *in vivo* [17] results on the therapeutic action of ASC-CM in counteracting osteoarthritis (OA), herein, we focused our attention on the potential role of each molecule in this frame.

Monocyte chemoattractant protein 4 (MCP-4, also known as CCL13) is a member of the CC chemokine family that displays, besides a strong chemotaxis towards immune cells, a variety of immunomodulatory functions, spanning from induction of cytokine release to antimicrobial activity [23]. Interestingly, MCP-4 can undergo proteolytic cleavage by matrix metalloproteinases (MMPs), resulting in biologically active peptides that exert opposite actions on chemotaxis and inflammation [24]. This aspect is particularly intriguing since the aberrant MMP activity represents one of the milestones of OA progression [25]. In this perspective, ASC-CM therapeutic potential may rely also on the possibility of harnessing the anti-inflammatory properties of MCP-4 metabolites generated in situ by MMPs.

DKK-1 (Dickkopf-1) is a chondroprotective factor, acting as inhibitor of the Wnt/β-catenin signalling pathway. A massive activation of this pathway is involved in diseases like OA [26], where the conditional accumulation of β-catenin affects chondrocytes inducing a hypertrophic phenotype together with the overexpression of MMPs [27]. Interestingly, recent *in vitro* evidences described positive changes in the expression of β-catenin by subchondral osteoblasts following the administration of DKK-1 [28]. Consequently, its abundance in ASC-CM may represent a promising cue in counteracting OA progression.

The receptor for advanced glycation end products (RAGE) appeared remarkably homogenous in ASC-CM

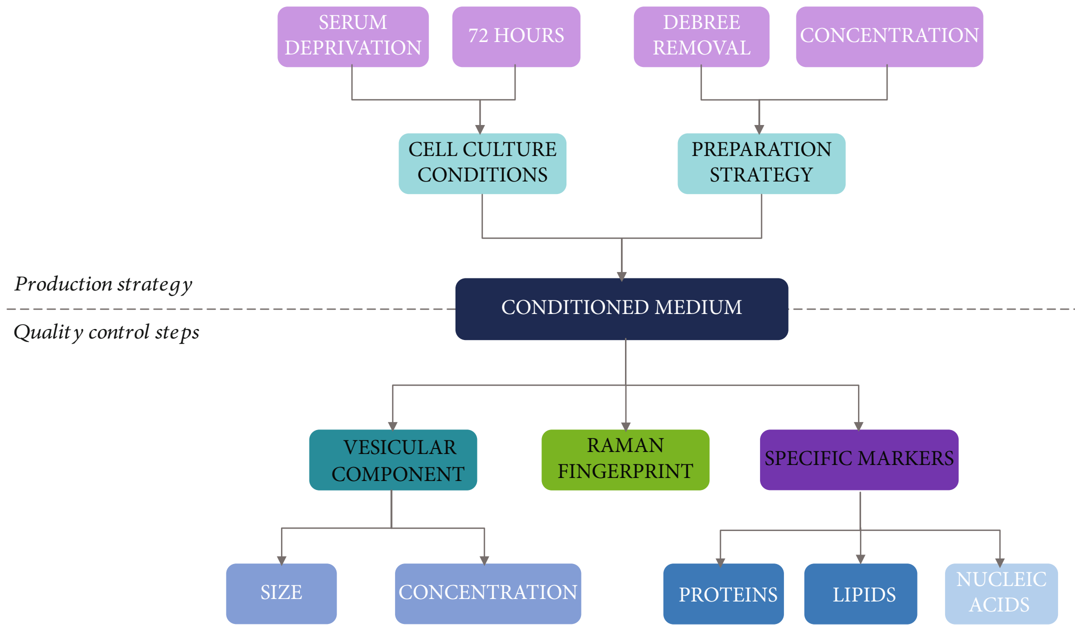


Figure 7: Scheme of our strategy for ASC-CM production together with the proposed quality control steps discussed in the text.

samples. Physiologically and pathologically, this is a transmembrane receptor whose activation by ligand interaction triggers intracellular signalling leading to increased release of reactive oxygen species and proinflammatory cytokines [29]. The presence of RAGE in ASC-CM cannot induce such a response so it might act as a decoy receptor. This could be exploited for the treatment of pathologies presenting a reduction of soluble RAGE (sRAGE, the "conventional" RAGE decoy receptor), together with an increase of its ligands. In OA, a reduction of sRAGE is associated with an increase in AGE levels in the synovial fluid [29, 30]. In this context, we hypothesize that ASC-CM injection in a limited space such as the synovial environment could mitigate the pathological sRAGE/AGE unbalance.

A similar consideration can fit for TFN RI (TNFRSF1A). This receptor is usually involved in the transduction of various inflammatory/stress stimuli by the activation of NF-κB and the consequent transcription of specific genes leading to the production of proinflammatory and catabolic factors [31]. Again, the molecule present in ASC-CM medium cannot trigger these intracellular events while it could compete with cellular receptor. This could be of particular relevance in the treatment of pathologies associated with a relevant increase in TNF, such as rheumatoid arthritis [32], Crohn's disease [33], and OA [34]. In the latter, ASC-CM intra-articular administration could be particularly beneficial since the increase in TNF in the synovial fluid, synovial membrane, cartilage, and subchondral bone is also associated with an increased TNFRI in synovial fibroblasts, further amplifying the noxious signalling [34].

Platelet-derived growth factors (PDGFs) are key players in bone metabolism, and, in particular, PDGF-AA is involved in the crosstalk between subchondral bone and articular cartilage during OA onset [35]. Moreover, recent evidences suggest that PDGF-AA promotes remyelination and increases tissue repair in a rat model of spinal cord injury, overall improving the locomotor functional recovery [36].

Since recently, the involvement of lipids in physiological and pathological processes has been widely demonstrated; in our opinion, their analysis holds paramount importance. With the advent of the next-generation mass spectrometry (MS), significant advances occurred in the field of lipidomics. Our UHPLC-MS/MS method [18] is aimed at profiling a high number of bioactive lipids belonging to structurally similar classes, including polyunsaturated fatty acids, eicosanoids, endocannabinoids, and N-acylethanolamides. Since it is conceivable that these lipids released by ASCs may play a role in inflammatory processes, we performed an absolute quantification of 32 molecules thanks to the high sensitivity and specificity of triple quadrupole mass spectrometry and the use of labeled lipids. Among quantified lipids, SEA and PGE2 showed a relevant uniformity that was therefore validated in a larger ASC-CM cohort. SEA is an endogenous lipid belonging to the N-acylethanolamides family that acts as an anti-inflammatory/immunomodulatory agent through the downregulation of several proinflammatory cytokines [37]. Conversely, PGE2 exerts a well-known inflammatory action. Nevertheless, in the OA context, it can exert either anabolic or catabolic effects on chondrocytes and synoviocytes depending on its concentration [38–40]. Evidence suggests also that PGE2 may have an immune stimulatory role by facilitating Th1 differentiation and expanding Th17 T cells [37].

5. Conclusions

In conclusion, in this work, we identified key ingredients of ASC secretome that may be involved in its therapeutic action and whose stable levels among different ASC-CM

batches may represent promising quality control criteria. Indeed, these indications may be relevant for a rapid and convenient reproducibility assessment of ASC-CM prior its use for different applications.

Nevertheless, we suggest not to focus reservedly on selected components but rather to aim at acquiring an overview of the great complexity of this promising cell-free therapeutic, whose strength relies precisely on the presence of a multitude of biologically active factors of different natures. Here, we propose multiple steps for secretome standardization, either providing an overlook of its composition by NTA and Raman spectroscopy or specifically focusing on the quantification of key molecules of different natures.

Data Availability

All data used to support the findings of this study are included within the supplementary information files and/or are uploaded in zenodo repository (https://doi.org/10.5281/zenodo.5211269) and/or are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Supplementary Materials

Supplementary Table 1: protein concentration (pg/ml) of factors quantified in all ASC-CM samples. Supplementary Table 2: list of factors undetectable in all ASC-CM samples. Supplementary Table 3: factors quantified in all ASC-CM samples, normalized on donor cell number, and ranked by coefficient of variation (CV). Supplementary Table 4: KEGG pathway enrichment analysis by STRING (FDR < 0.05). Supplementary Table 5: lipids quantified in all ASC-CM samples, normalized on donor cell number, and ranked by coefficient of variation (CV). Supplementary Table 6: lipids quantified in all ASC pellets, normalized on donor cell number, and ranked by coefficient of variation (CV). *(Supplementary Materials)*

References

[1] M. Wang, Q. Yuan, and L. Xie, "Mesenchymal stem cell-based immunomodulation: properties and clinical application," *Stem Cells International*, vol. 2018, Article ID 3057624, 12 pages, 2018.

[2] X. L. Fan, Y. Zhang, X. Li, and Q. L. Fu, "Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy," *Cellular and Molecular Life Sciences*, vol. 77, no. 14, pp. 2771–2794, 2020.

[3] Y. Shi, Y. Wang, Q. Li, K. Liu, J. Hou, and C. Shao, "Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases," *Nature Reviews. Nephrology*, vol. 14, no. 8, pp. 493–507, 2018.

[4] R. E. Delanois, J. I. Etcheson, N. Sodhi et al., "Biologic therapies for the treatment of knee osteoarthritis," *The Journal of Arthroplasty*, vol. 34, no. 4, pp. 801–813, 2019.

[5] A. Andrzejewska, S. Dabrowska, B. Lukomska, and M. Janowski, "Mesenchymal stem cells for neurological disorders," *Advanced Science*, vol. 8, no. 7, p. 2002944, 2021.

[6] M. Gnecchi, H. He, N. Noiseux et al., "Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement," *The FASEB Journal*, vol. 20, no. 6, pp. 661–669, 2006.

[7] A. I. Caplan, "What's in a name?," *Tissue Engineering. Part A*, vol. 16, no. 8, pp. 2415–2417, 2010.

[8] H. Tjalsma, H. Antelmann, J. D. Jongbloed et al., "Proteomics of protein secretion by Bacillus subtilis: separating the "secrets" of the secretome," *Microbiology and Molecular Biology Reviews*, vol. 68, no. 2, pp. 207–233, 2004.

[9] C. R. Harrell, M. G. Jankovic, C. Fellabaum et al., "Molecular mechanisms responsible for anti-inflammatory and immunosuppressive effects of mesenchymal stem cell-derived factors," *Advances in Experimental Medicine and Biology*, vol. 1084, pp. 187–206, 2019.

[10] A. Gualerzi, S. A. A. Kooijmans, S. Niada et al., "Raman spectroscopy as a quick tool to assess purity of extracellular vesicle preparations and predict their functionality," *Journal of extracellular vesicles*, vol. 8, no. 1, 2019.

[11] S. Niada, C. Giannasi, A. Gualerzi, G. Banfi, and A. T. Brini, "Differential proteomic analysis predicts appropriate applications for the secretome of adipose-derived mesenchymal stem/stromal cells and dermal fibroblasts," *Stem Cells International*, vol. 2018, Article ID 7309031, 11 pages, 2018.

[12] S. Niada, C. Giannasi, C. Magagnotti, A. Andolfo, and A. T. Brini, "Proteomic analysis of extracellular vesicles and conditioned medium from human adipose-derived stem/stromal cells and dermal fibroblasts," *Journal of Proteomics*, vol. 232, 2020.

[13] C. Carlomagno, C. Giannasi, S. Niada, M. Bedoni, A. Gualerzi, and A. T. Brini, "Raman fingerprint of extracellular vesicles and conditioned media for the reproducibility assessment of cell-free therapeutics," *Frontiers in Bioengineering and Biotechnology*, vol. 9, 2021.

[14] S. Niada, C. Giannasi, M. Gomarasca, D. Stanco, S. Casati, and A. T. Brini, "Adipose-derived stromal cell secretome reduces TNFα-induced hypertrophy and catabolic markers in primary human articular chondrocytes," *Stem Cell Research*, vol. 38, p. 101463, 2019.

[15] C. Giannasi, S. Niada, C. Magagnotti, E. Ragni, A. Andolfo, and A. T. Brini, "Comparison of two ASC-derived therapeutics in an in vitro OA model: secretome versus extracellular vesicles," *Stem Cell Research & Therapy*, vol. 11, no. 1, p. 521, 2020.

[16] A. T. Brini, G. Amodeo, L. M. Ferreira et al., "Therapeutic effect of human adipose-derived stem cells and their secretome in experimental diabetic pain," *Scientific Reports*, vol. 7, no. 1, p. 9904, 2017.

[17] G. Amodeo, S. Niada, G. Moschetti et al., "Secretome of human adipose-derived mesenchymal stem cell relieves pain and neuroinflammation independently of the route of administration in experimental osteoarthritis," *Brain, Behavior, and Immunity*, vol. 94, pp. 29–40, 2021.

[18] S. Casati, C. Giannasi, M. Minoli et al., "Quantitative lipidomic analysis of osteosarcoma cell-derived products by UHPLC-MS/MS," *Biomolecules*, vol. 10, no. 9, 2020.

[19] B. S. Park, W. S. Kim, J. S. Choi et al., "Hair growth stimulated by conditioned medium of adipose-derived stem cells is enhanced by hypoxia: evidence of increased growth factor secretion," *Biomedical Research*, vol. 31, no. 1, pp. 27–34, 2010.

[20] R. Seetharaman, A. Mahmood, P. Kshatriya, D. Patel, and A. Srivastava, "Mesenchymal stem cell conditioned media ameliorate psoriasis vulgaris: a case study," *Case Reports in Dermatological Medicine*, vol. 2019, Article ID 8309103, 5 pages, 2019.

[21] L. Jia, Q. Xi, H. Wang et al., "miR-142-5p regulates tumor cell PD-L1 expression and enhances anti-tumor immunity," *Biochemical and Biophysical Research Communications*, vol. 488, no. 2, pp. 425–431, 2017.

[22] E. Ragni, C. Perucca Orfei, P. De Luca et al., "Inflammatory priming enhances mesenchymal stromal cell secretome potential as a clinical product for regenerative medicine approaches through secreted factors and EV-miRNAs: the example of joint disease," *Stem Cell Research & Therapy*, vol. 11, no. 1, p. 165, 2020.

[23] E. Mendez-Enriquez and E. A. García-Zepeda, "The multiple faces of CCL13 in immunity and inflammation," *Inflammopharmacology*, vol. 21, no. 6, pp. 397–406, 2013.

[24] G. A. McQuibban, J. H. Gong, J. P. Wong, J. L. Wallace, I. Clark-Lewis, and C. M. Overall, "Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties in vivo," *Blood*, vol. 100, no. 4, pp. 1160–1167, 2002.

[25] P. Singh, K. B. Marcu, M. B. Goldring, and M. Otero, "Phenotypic instability of chondrocytes in osteoarthritis: on a path to hypertrophy," *Annals of the New York Academy of Sciences*, vol. 1442, no. 1, pp. 17–34, 2019.

[26] A. B. Blom, S. M. Brockbank, P. L. van Lent et al., "Involvement of the Wnt signaling pathway in experimental and human osteoarthritis: prominent role of Wnt-induced signaling protein 1," *Arthritis and Rheumatism*, vol. 60, no. 2, pp. 501–512, 2009.

[27] R. Yasuhara, Y. Ohta, T. Yuasa et al., "Roles of _β_ -catenin signaling in phenotypic expression and proliferation of articular cartilage superficial zone cells," *Laboratory Investigation*, vol. 91, no. 12, pp. 1739–1752, 2011.

[28] B. Kovács, E. Vajda, and E. E. Nagy, "Regulatory effects and interactions of the Wnt and OPG-RANKL-RANK signaling at the bone-cartilage interface in osteoarthritis," *International journal of molecular sciences*, vol. 20, no. 18, p. 4653, 2019.

[29] M. Chayanupatkul and S. Honsawek, "Soluble receptor for advanced glycation end products (sRAGE) in plasma and synovial fluid is inversely associated with disease severity of knee osteoarthritis," *Clinical Biochemistry*, vol. 43, no. 13-14, pp. 1133–1137, 2010.

[30] M. M. Steenvoorden, T. W. Huizinga, N. Verzijl et al., "Activation of receptor for advanced glycation end products in osteoarthritis leads to increased stimulation of chondrocytes and synoviocytes," *Arthritis and Rheumatism*, vol. 54, no. 1, pp. 253–263, 2006.

[31] G. D. Kalliolias and L. B. Ivashkiv, "TNF biology, pathogenic mechanisms and emerging therapeutic strategies," *Nature Reviews Rheumatology*, vol. 12, no. 1, pp. 49–62, 2016.

[32] M. Feldmann, "Development of anti-TNF therapy for rheumatoid arthritis," *Nature Reviews. Immunology*, vol. 2, no. 5, pp. 364–371, 2002.

[33] S. O. Adegbola, K. Sahnan, J. Warusavitarne, A. Hart, and P. Tozer, "Anti-TNF therapy in Crohn's disease," *International journal of molecular sciences*, vol. 19, no. 8, p. 2244, 2018.

[34] P. Wojdasiewicz, Ł. A. Poniatowski, and D. Szukiewicz, "The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis," *Mediators of Inflammation*, vol. 2014, Article ID 561459, 19 pages, 2014.

[35] Z. Yao, P. Chen, S. Wang, G. Deng, Y. Hu, and Q. Lin, "Reduced PDGF-AA in subchondral bone leads to articular cartilage degeneration after strenuous running," *Journal of Cellular Physiology*, vol. 234, no. 10, pp. 17946–17958, 2019.

[36] X. Y. Guo, F. X. Duan, J. Chen et al., "Subcutaneous administration of PDGF-AA improves the functional recovery after spinal cord injury," *Frontiers in Neuroscience*, vol. 13, p. 6, 2019.

[37] S. Casati, C. Giannasi, S. Niada, R. F. Bergamaschi, M. Orioli, and A. T. Brini, "Bioactive lipids in MSCs biology: state of the art and role in inflammation," *International Journal of Molecular Sciences*, vol. 22, no. 3, p. 1481, 2021.

[38] K. Nishitani, H. Ito, T. Hiramitsu et al., "PGE2 inhibits MMP expression by suppressing MKK4-JNK MAP kinase-c-JUN pathway via EP4 in human articular chondrocytes," *Journal of Cellular Biochemistry*, vol. 109, no. 2, pp. 425–433, 2010.

[39] M. Attur, H. E. Al-Mussawir, J. Patel et al., "Prostaglandin E2 exerts catabolic effects in osteoarthritis cartilage: evidence for signaling via the EP4 receptor," *Journal of Immunology*, vol. 181, no. 7, pp. 5082–5088, 2008.

[40] M. H. Pillinger, P. B. Rosenthal, S. N. Tolani et al., "Cyclooxygenase-2-derived E prostaglandins down-regulate matrix metalloproteinase-1 expression in fibroblast-like synoviocytes via inhibition of extracellular signal-regulated kinase activation," *Journal of Immunology*, vol. 171, no. 11, pp. 6080–6089, 2003.

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Review Article

Exosomes: Emerging Therapy Delivery Tools and Biomarkers for Kidney Diseases

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Exosomes are nanometer-sized small EVs coated with bilayer structure, which are released by prokaryotic and eukaryotic cells. Exosomes are rich in a variety of biologically active substances, such as proteins, nucleotides, and lipids. Exosomes are widely present in various body fluids and cell culture supernatants, and it mediates the physiological and pathological processes of the body through the shuttle of these active ingredients to target cells. In recent years, studies have shown that exosomes from a variety of cell sources can play a beneficial role in acute and chronic kidney disease. In particular, exosomes derived from mesenchymal stem cells have significant curative effects on the prevention and treatment of kidney disease in preclinical trials. Besides, some encapsulated substances are demonstrated to exert beneficial effects on various diseases, so they have attracted much attention. In addition, exosomes have extensive sources, stable biological activity, and good biocompatibility and are easy to store and transport; these advantages endow exosomes with superior diagnostic value. With the rapid development of liquid biopsy technology related to exosomes, the application of exosomes in the rapid diagnosis of kidney disease has become more prominent. In this review, the latest development of exosomes, including the biosynthesis process, the isolation and identification methods of exosomes are systematically summarized. The utilization of exosomes in diagnosis and their positive effects in the repair of kidney dysfunction are discussed, along with the specific mechanisms. This review is expected to be helpful for relevant studies and to provide insight into future applications in clinical practice.

1. Introduction

There are many types of kidney diseases. According to etiological factor, kidney diseases can be divided into primary, secondary, and congenital diseases. Acute kidney injury (AKI) produces various clinical manifestations accompanied with abrupt renal dysfunction. Nearly 20% of those hospitalized with AKI show increased resource utilization and poor outcomes. This prognosis correlates with many risk factors, including sepsis, trauma, diabetes mellitus, and older age [1, 2]. There are multiple factors that account for the mechanisms of AKI, including imbalanced inflammation, abnormal hemodynamics, and excessive production of reactive oxygen species (ROS) [3–5]. AKI and chronic kidney disease (CKD) are interconnected. Sustained pathological changes in AKI contribute to the development of CKD, which breaks the balance of the microenvironment among peritubular capillary, tubular cells, and interstitial cells. One of the most obvious features in CKD is the deposition of extracellular matrix and the formation of fibrosis [6]. Despite the existing diagnostic criteria (e.g., creatinine levels and urine output) and availability of treatments (e.g., dialysis, conservative care, and kidney transplantation), kidney diseases continue to pose a significant threat to people's health. Hence, the explorations of more sensitive biomarkers and more effective therapies are still two challenges to diagnose and treat kidney diseases.

Extracellular vesicles (EVs) generally fall into three categories: exosomes, microvesicles (MVs), and apoptotic bodies, which differ in size, origin, density, releasing mechanisms,

and so on. MVs (100–1000 nm) are formed through the direct shedding of plasma membrane. Apoptotic bodies (100–5000 nm) are caused by cell apoptosis. Exosomes are formed through endosome systems and are nanoscale vesicles with diameters of 50–150 nm [7, 8]. It was analyzed that the protein patterns and lipidomes were differently enriched in exosomes and MVs even though they are released from the same cell source [9]. In 1983, exosomes were first identified in supernatants of sheep reticulocytes [10]. As research progressed, it was demonstrated that exosomes are secreted by the majority of cells (e.g., mesenchymal stem cells, macrophages, and cancer cells) [11–13] and widely distributed in biological liquids (e.g., plasma, urine, bone marrow, and amniotic fluid) [14–16] which play a crucial part in signaling transduction and pathological development of diseases [17, 18]. Because exosomes are present in body fluids, a strategy has been developed to enrich them and increase the feasibility and sensitivity of diagnostics [19]. As advances in engineered exosomes continue, many experiments have been conducted to improve their effectiveness. Xu et al. generated overexpression of circAkap7 exosomes via transfected circAkap7 into adipose-derived stromal cells. Exosomal circAkap7 functioned as a sponge to absorb miR-155-5p, enhanced autophagy, diminished oxidative stress, and relieved cerebral ischemic injury [20]. Wu et al. constructed engineered exosomes from human bone mesenchymal stem cells (hBMSCs) called mag-BMSC-Exos. They found that miR-21-5p was expressed more highly in mag-BMSC-Exos and inhibited Sprouty 2 (SPRY2) proteins and stimulated proangiogenesis and proproliferation pathways [21].

Many studies have reported therapeutic roles of exosomes secreted by cultured cells. Thanks to their reflection of host cells and existence in body fluids, studies have demonstrated the potential molecules which are associated with the diagnosis and prognosis of kidney diseases. This review summarizes those studies and provides insight into the possible use of exosomes.

2. Isolation, Identification, and Function of Exosomes

Exosomes are lipid-bilayer bioactive nanovesicles consisting of a multitude of proteins (e.g., heat-shock proteins, tetraspanins, and Alix), lipids (e.g., ceramide and cholesterol), and nucleic acids (e.g., DNA, mRNAs, and microRNAs). These bioactive molecules are partially involved in communication between cells and the modulation of recipient cells [22]. Various methods are traditionally used to isolate exosomes, such as sequential ultracentrifugation, ultrafiltration, size-exclusion chromatography, immune-affinity capture, and polymer precipitation (Figure 1). These methods depend on physical, chemical, and biological properties [23]. Ultracentrifugation is time-consuming, has high equipment requirement, and has no specificity. But it is suitable for large sample volumes. Comparatively, ultrafiltration is simple, fast, and low cost. However, exosomes will be trapped and clogged in the membrane and the isolated exosomes are still lack of specificity. Promisingly, sequential filtration is applied for large sample processing [24, 25]. Taking the advantages and disadvantages of each method into account, it is necessary to combine isolation techniques [24]. Because the extracted samples are not completely pure, and the biomarkers are not fully specific, Yang et al. proposed a mathematical formula to estimate the proportion of exosomes in a mixture [24]. Novel techniques have also been developed to overcome these drawbacks [23]. Lee et al. were the first to isolate exosomes with tangential flow filtration, which overcame the challenges of limited amounts of samples [26]. It is worth considering that the choice of methods differs depending on the sources of the sample. A previous study has found that differential ultracentrifugation was the most common method used for isolating exosomes from urine [27]. Sequential ultracentrifugation and polymer precipitation are often used for isolating serum exosomes. Nevertheless, sequential ultracentrifugation generates fewer particles, and polymer precipitation can cause clumping among the soluble proteins [28, 29]. Storage is another issue to consider. It is reported that exosomes degrade easily at 37°C. Therefore, they are suitable to be stored at -80°C while also need to be used as soon as possible [30].

Transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), flow cytometry (FCM), and Western blotting (WB) are common techniques for identifying concentration, size, and morphology [31]. Various methods have been suggested to characterize exosomes [32]. TEM assists in observing the shapes of exosomes visually and vividly. Their shapes are changeable depending on their surroundings, namely, whether they are cup-like or rounded [8, 33]. However, the instrument is expensive, the method is strict with the sample preparation, and the results are influenced by subjectivity [34]. NTA is used to measure the size distribution and concentration of exosomes [35]. Establishing the concentration makes it convenient to calculate the quantity of applied exosomes. Nevertheless, this technique is not sensitive, sorts out targeted exosomes with low efficiency, and lacks repeatability. FCM and WB are commonly used to identify molecular phenotyping [36, 37]. However, FCM outperforms in terms of its high throughput and accurate measurement of diameters and concentrations. To rigorously confirm the exosomes, different expressive abundances and functional markers should be selected, including CD9, CD63, CD81, Alix, calnexin, Grp94, and Tsg101 [36, 38]. In 2018, Théry et al. established guidelines for the isolation and characterization of EVs [32]. However, there remains a long way to go to standardize the quantity and quality of the extracted exosomes for experimental studies or clinical trials since inevitably obtaining non-EV composition.

Many studies have found that exosomes participate in cancer drug resistance [12], cutaneous wound healing [39], liver injury, and so on [40]. The cargos in EVs have been found to prevent cell death, modulate immune response, maintain vascular integrity, and promote cell activity [41]. Thus, attempts to recognize the underlying mechanisms of different EVs make it important for us to prevent this progression and seek means of addressing illness.

3. Diagnostic Roles of Circulating Exosomes in Kidney Diseases

Due to the insensitivity of traditional kidney injury biomarkers, advanced biomarkers are being sought and are

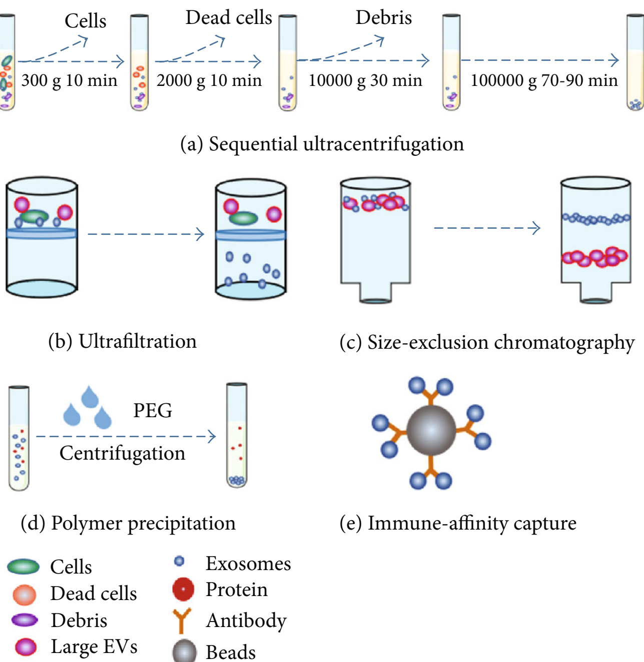

Figure 1: Five main traditional methods of exosome separation. Traditional methods are based on the physical, chemical, and biological properties of exosomes. Sequential ultracentrifugation is used to separate the exosomes, according to the different sedimentation coefficients of exosomes, cells, and debris. Ultrafiltration and size-exclusion chromatography depend on size: only exosomes can pass through a certain molecular-weight cut-off membrane and exhibit longer retention times in the stationary phase. When PEG is added, the surroundings are hydrophobic and promote deposition at the bottom. The beads are combined with specific antibodies to interact with the surface proteins of exosomes and can easily be sorted.

rapidly emerging, such as kidney injury molecule-1, beta-2 microglobulin, cystatin C, and neutrophil gelatinase-associated lipocalin (NGAL). Nevertheless, researchers are still working to analyze an array of biomarkers to promote early diagnosis and treatment that may better represent relevant clinical manifestations and prognoses (Table 1) [42]. Blood and urine are two readily available specimens that can be obtained without invasive procedures or great pain to patients. miRNA is an important type of noncoding RNAs with about 22 nucleotides in length. It mainly exerted biological functions through posttranscriptional level. Differences in expression between healthy subjects and patients make it possible to be applied into diagnostic and theranostic markers [43]. Exosomes' structures protect their cargos (e.g., miRNA, circRNA, proteins, and lipids) from degrading and can represent the physiological or pathological states of their parental cells. With the development of methods to isolate exosomes from body fluid, promising potential to make use of bioactive molecules for prediction is being developed [14, 44].

3.1. Diagnostic Role of Urinary Exosomes

3.1.1. Diagnosis of AKI. Because circulating exosomes rarely pass through the glomerular, the majority of urinal exosomes result from kidney, bladder, and prostate organs, which indicates their value for predicting certain diseases. Sonoda et al. established ischemia/reperfusion (I/R) models and conducted continuous observations on them for up to 2 weeks. It was demonstrated that miRNAs in exosomes could reflect the injury and fibrosis state, such as in the release of miR-9a, miR-16, miR-200a, and miR-141 [45]. Similarly, exosomal miR-30c-5p and miR-192-5p were confirmed to obviously increase both in animal models and in patients suffering from cardiovascular surgery [46]. It was also found that increased levels of organic anion transporter 5 (Oat5) were associated with aberrant renal function indexes in cisplatin-treated Wistar rats [67]. For sepsis-induced AKI, a specific transcriptional repressor for activating transcription factor 3 (ATF3) was increased but not detected in the non-AKI group [68]. Awdishu et al. conducted a survey among cirrhosis-induced AKI and showed that maltase glucoamylase increased significantly in urinary exosomes [69].

3.1.2. Diagnosis of CKD. An overwhelming majority of scientists who have analyzed changeable microRNAs have used databases or profiling. Diabetic nephropathy is a serious complication caused by diabetes mellitus (DM). Using the results of bioinformatics analyses, Eissa et al. validated the high levels of miR-30a, miR-133b, and miR-342 in urine exosomes from type 2 diabetic nephropathy (T2DN) patients [50]. The use of these miRNAs was connected both with renal functions and with early diagnosis of albuminuria. Similar studies have shown that elevated miR-320c, which influences the TGF-β1 signaling pathway, could be used as a novel biomarker to distinguish microalbuminuria (MIC) from normoalbuminuria [48]. Accumulating studies also demonstrated different expressions for miR-192, miR-15b-5p, miR-let-7i-3p, miR-let-7c-5p, miR-24-3p, miR-27b-3p, and TGF-β1 in EVs [51, 53, 54]. Apart from this, multiple microRNAs have been explored as noninvasive predictors.

Table 1: The diagnostic role of urine-derived exosomal miRNA in renal disease.

Kidney diseases	Changeable molecules	Mechanisms	Ref.
AKI	Upregulation: ↑ miR-200c, miR-9a, miR-141, miR-200a, miR-429	Target Zeb1/2 Regulate TGF-β-associated fibrosis	[45]
	miR-16, miR-24, miR-30c-5p, miR-192-5p	-	[45, 46]
T2DN	Upregulation: ↑ miR-19b-3p	Target SOCS-1 Regulate inflammation	[47]
	miR-320c	Target TSP-1 Regulate TGF-β-associated fibrosis	[48]
	miR-150-5p, miR-362-3p, miR-877-3p	Regulate p53, mTOR, AMPK pathways Involve in oxidative stress and fibrosis	[49]
	miR-133b, miR-30a, miR-342, miR-192, miR-15b, miR-34a, miR-636, miR-let-7c-5p	Regulate fibrosis	[50–53]
	Downregulation: ↓ miR-let-7i-3p, miR-24-3p, miR-27b-3p,	Involve in Wnt/β-catenin signaling	[54]
T1DN	Upregulation: ↑ miR-145, miR-30a Downregulation: ↓ miR-155, miR-424	-	[55, 56]
LN	Upregulation: ↑ miR-26a	Regulate podocyte differentiation and cytoskeletal integrity	[57]
	miR-21, miR-150	Target VEGFA and SP1 Regulate fibrosis	[58]
	miR-146a	Target IRAK1 and TRAF6 Regulate inflammation	[59]
	Downregulation: ↓ miR-29c, miR-let-7a	-	[60, 61]
PKD	Downregulation: ↓ miR-30a-5p, miR-30d-5p, miR-194-5p	-	[62]
RCC	Upregulation: ↑ miR-150-5p, miR-204-5p	-	[63, 64]
	Downregulation: ↓ miR-126-3p	-	[63]
FSGS	Upregulation: ↑ miR-155 Downregulation: ↓ miR-1915, miR-663	-	[65]
NS	Upregulation: ↑ miR-194-5p, miR-146b-5p, miR-378-3p, miR-23b-3p, miR-30a-5p	-	[66]

miR-19b-3p, miR-15b, miR-34a, miR-150-5p, miR-362-3p, miR-636, and miR-877-3p increased in urinary exosomes from T2DN patients. This was speculated to be relevant to inflammation, proliferation, and apoptosis through regulating pathways [47, 49, 52]. After the administration of advanced glycation end-products (AGEs), TGF-β/Smad3 was activated in cultured podocytes and contributed to the release of the exosomal Elf3 protein, which was only detected in T2DN patients [70]. Additionally, increased levels of C-megalin were detected in urinary exosomes which also participated in regulating the quantity of exosomes [71]. Uromodulin mRNA in urinary EVs also represented the severity of the progression of renal disease in a sense, and Wilms' tumor 1 (WT1) mRNA reflected glomerular injury [72, 73]. Interestingly, in type 1 diabetic nephropathy (T1DN) rat models, miR-451-5p showed opposite trends in renal tissues and urine and was related to the protection against renal fibrosis [55]. Urinary exosomal miRNAs changed between normoalbuminuric and microalbuminuric diabetic patients and reflected the severity of pathological change. Those microalbuminuric patients exhibited increased amounts of miR-145 and miR-130a and decreased amounts of miR-155 and miR-424 [56]. Furthermore, the changeable levels of miR-21, miR-29c, miR-181a, miR-200b, and regucalcin protein were observed in CKD [74–78].

3.1.3. Diagnosis in Other Kidney Diseases. Lupus nephritis (LN) is a complication that results from systemic lupus

erythematosus and is a major risk factor for ESRD. Evidence has shown that glomerular miR-26a expression is higher in both mouse models and patients. However, exosomal miR-26a from urine displayed different consequences [57]. Tangtanatakul et al. later found that miR-let-7a showed obvious decreases during the active phase of LN, which could indicate disease progression [60]. It has been also reported that miR-21, miR-29c, and miR-150 were correlated with the degree of LN. Decreased levels of miR-29c and increased levels of miR-21 and miR-150 hastened the progression of fibrosis through the TGF-β/Smad3 pathway [58, 61]. Additionally, miR-146a was associated with the severity and activity of LN [59]. It should be noted that Garcia-Vives et al. surveyed the prognosis and clinical response of LN patients and found that responding patients secreted augmented levels of miR-135b-5p, miR-107, and miR-31-5p in their urinary exosomes. Those miRNAs inhibited hypoxia-inducible factor-1α (HIF-1α) in renal cells, which predicted better recovery and reduced inflammation [79].

Polycystic kidney disease (PKD) is a complex disease that is commonly found in the form of autosomal dominant PKD. Recent results have found apparent changes in the protein components in the exosomes. The activator of G protein signaling 3 (AGS3) was involved in the pathological progression of PKD. Enhanced levels of this protein were detected both in PCK rats and human samples [80]. Furthermore, studies have reported that exosomes overexpress envoplakin, villin 1, prominin 1, and the cellular repressor of E1A-stimulated genes 1 (CREG1), indicating aberrant morphology and proliferation in the cells [81, 82]. Polycystin-1 (PC-1), polycystin-2 (PC-2), transmembrane protein 2 (TMEM2), miR-30a-5p, and miR-194-5p are also relevant to diagnosis and monitoring [62, 83].

The incidence of renal cell carcinoma (RCC) is significantly higher in North America and in Western, Central, and Eastern Europe. RCC is usually accompanied by other diseases, such as hypertension, urinary stones, and diabetes. Prediction of this disease before the appearance of symptom phenotypes is extremely important [84]. Butz et al. showed increased levels of miR-150-5p and decreased levels of miR-126-3p in clear-cell RCC patients. A combination of miR-126-3p, miR-449a, and miR-34b-5p could improve diagnostic sensitivity [63]. Furthermore, abnormally elevated polymerase I and transcript release factor (PTRF) were found in patients' urinary exosomes, regulated by the EGFR/Akt pathway [85]. Higher levels of miR-204-5p were detected prior to progression for Xp11.2 translocation RCC [64].

Minimal change disease (MCD) is the most common disease to cause nephrotic syndrome and is characterized by podocytopathy. Compared with MCD, focal segmental glomerulosclerosis (FSGS) patients show poor prognosis which desires noninvasive biomarkers [86]. In a gesture toward conducting differential diagnosis between MCD and FSGS, researchers have used miR profiles that establish changes in miR-1225-5p, miR-1915, miR-663, miR-193a, and miR-155 [65, 87]. Among children exposed to idiopathic nephrotic syndrome (NS), miR-194-5p and miR-23b-3p have been found to be useful for monitoring urine protein levels and reflecting disease progression [66]. Bhayana et al. conducted a survey to predict radiation nephropathy and demonstrated increased levels of miR-1224 and miR-21 in leukemia patients pretreated with total-body irradiation [88].

3.2. Diagnostic Role of Plasmic Exosomes. Plasma is another source for liquid biopsies. Plasmic exosomes exist in the blood through cargo packing, trafficking, and secretion from host cells which could be used as biomarkers. Recently, Xie et al. made comparisons of bioinformatics results between plasma and plasmic exosomes and found incomplete consistency. Interestingly, it was speculated that plasmic exosomes could participate in the morbidity and progression of disease, as their cargos (e.g., miR-22-3p, miR-29b-3p, miR-30e-3p, miR-143-3p, and miR-770-5p) were related to significant pathways, such as extracellular matrix–receptor interaction and mucin type-O-glycan biosynthesis [89]. miR-146a is also implicated in monitoring kidney injury in animal models induced by cisplatin [90]. MCD has been observed to be associated with increased levels of miR-30, miR-34, and miR-342 in patients [65]. A survey was conducted of the enrollment of patients categorized by the severity of their disease. It was also shown that miR-21 could be used to distinguish interstitial fibrosis (IF) from tubular atrophy at higher grades [91]. In addition, Xiao et al. and Dias et al. reported different levels of miR-92a-1-5p, miR-301a-3p, miR-424-3p, miR-149-3p, and miR-1293 in RCC patients' plasmic exosomes relative to those of control groups [92, 93].

In summary, exosomal contents, such as proteins, miRNAs, and lipids in urine and plasma make it possible to predict the onset and progression of disease earlier and faster. However, some challenges hinder the translation into clinical practice. The methods to isolate exosomes need to be optimized and standardized to improve reproducibility. The sensitivity and specificity of encapsulated molecules need to be further analyzed and confirmed. Also, preanalytical factors should be taken into consideration and more patients need to be enrolled as well to increase the accuracy and reliability of the results. The use of exosomes in diagnosis is expected to have a bright future.

Exosomes can be obtained not only from body fluids but also from the supernatant of cultured cells, including MSCs. Our group previously demonstrated the significant roles of derived exosomes in alleviating inflammatory bowel disease [94], hepatic oxidant injury [95], and cutaneous injury [96]. The encapsulated substances are suggested to participate in maintaining regeneration and homeostasis. Notably, emerging reports have also found protective effects for derived exosomes in relieving kidney injury, which implies possible new treatment strategies.

4. Mesenchymal Stem Cell-Derived Exosomes in Kidney Injury Repair

MSCs have the unique capability for self-renewal and differentiation. MSCs can be obtained from several sources, including bone marrow [97], adipose tissue [98], amnion, chorion, and the umbilical cord [99]. Numerous trials have been conducted recently to investigate the validity and tolerance of MSC therapy [100–102]. Exosomes are paracrine

forms of MSCs outperforming them in safety and immunity, and they consist of bioactive molecules [25]. The following sections focus on the progress of current research on the use of MSC-derived exosomes for kidney injury repair (Table 2).

4.1. Protective Roles on AKI

4.1.1. I/R-Induced AKI. EVs isolated from the conditioned medium have been proven to be an important mediator of MSCs in renoprotection. Human Wharton's jelly mesenchymal stromal cell- (hWJMSC-) derived exosomes, which are abundant in miR-30, have been reported to alleviate mitochondrial fission by blocking the activation of dynamin-related protein 1 (DRP1), and miR-16 and miR-15 are speculated to target CX3CL1 to reduce inflammatory responses in their early stages [103, 104]. Further, exosomes from hucMSCs (hucMSC-Exos) are capable of effectively reinforcing the NRF2/ARE pathway, which result in the reduction of malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHDG) [105]. Another group reported that hucMSC-Exos were able to enhance the ERK 1/2 pathway and promoted dedifferentiation by delivering hepatocyte growth factor (HGF) mRNA [106]. In addition, isolated microvesicles (MVs) that contain exosomes have been proven to depress the generation of ROS by regulating NADPH oxidase-2 (NOX2) proteins [123]. Notably, Zhao et al. reported the involvement of MSC-Exos in maintaining mitochondrial function. Both normal hucMSC-Exos and mouse BMMSC-Exos contained mitochondrial transcription factor A (TFAM) and mitochondrial DNA (mtDNA). These molecules were shuttled to tubular cells to alleviate mitochondrial dysfunction and reduce apoptosis and inflammation [124]. Recently, Cao et al. highlighted the important roles of miR-125b-5p enriched in exosomes. They found that the adhesive molecules on exosomes assisted in homing to injured areas and miR-125b-5p targeted p53 to inhibit apoptosis and promote tubular repair [111].

Furthermore, exosomes released from hBMSCs have also been reported to have beneficial effects. The miR-199a-3p they contain has been shown to target semaphoring 3A (Sema3A) and induce antiapoptosis [125]. Prestimulating bone marrow MSCs (BMMSCs) with melatonin has been found to prompt more efficient regeneration [126]. Mouse BMMSCs have been reported to transport miR-233, which directly suppresses NLR family-pyrin domain containing 3 from producing its protective effects [127]. Those exosomes have been shown to overexpress C-C motif chemokine receptor 2 (CCR2), which absorb circulating CCL2 and attenuate the extensive tissue inflammation induced by monocytes and macrophages [117].

It has also been shown that exosomes from human adipose-derived mesenchymal stem cells (hADMSCs) are enabled to alleviate acute injury or even chronic pathological changes by upregulating tubular Sox9, which is abolished when adding inhibitors [112]. In addition to this, ADMSCs and derived exosomes have been shown to alleviate injury *in vivo* better than other types [128].

Interestingly, Li et al. successfully isolated human urine-derived stem cells (hUSCs) from fresh human urine. They confirmed that hUSC-Exos migrated to and were incorporated into injured tubule cells instead of hUSCs. The highest-expression exosomal miR-146a-5p was screened and shown to target interleukin-1 receptor-associated kinase 1 (IRAK1) to decrease oxidative stress and reduce inflammation [114]. In another study, miR-216a-5p was found to target phosphatase and tensin homolog (PTEN) and had antiapoptotic effects [115]. Exosomes from human amnion epithelial cells (hAECs) were reported to protect against apoptosis and inflammation, which might be associated with the encapsulated proteins [129]. Several reports have also provided convincing evidence that progenitor cells in glomeruli, tubules, and renal arteries relieve kidney injury via paracrine action. MSCs in glomeruli (GI-MSCs) appear to reduce kidney damage more effectively through sorting miRNAs into EVs and influence cell communication and signal transduction [119]. In addition, EVs from human placenta-derived MSCs (hP-MSCs) have been demonstrated to transfer miR-200a-3p, which activates the Keap1-Nrf2 pathway and exhibits protection for mitochondrial functions. Cao et al. traced EVs with aggregation-induced emission luminogens. This material was further confirmed to provide higher resolution, a lower background, and better biocompatibility [116].

4.1.2. Toxin-Induced AKI. Cisplatin is a kind of chemotherapeutic regimen that is used to treat cancer. Due to its detrimental effects on the kidney, scientists and clinicians have sought for possible methods to reverse those effects. Previous studies have demonstrated the considerable effects of exosomes in nephrotoxicity induced by cisplatin. In 2013, Zhou et al. found that hucMSCs were able to repair kidney damage through paracrine action by orderly regulating proliferation and apoptosis-related pathways [107]. Others have focused on the specific relationship between exosomes and autophagy. It has been clearly shown that hucMSC-Exos are capable of enhancing autophagy by depressing the mTOR signaling pathway, which is in negative parallel to the autophagy level [130]. 14-3-3ζ is the isoform of 14-3-3 proteins. Through liquid chromatography/mass spectrometry (LC/MS), it was analyzed that 14-3-3ζ was abundant in hucMSC-Exos [39]. Taking into account the transportation of 14-3-3ζ by exosomes, we also shed light on the possible mechanisms of intrinsic and delivered 14-3-3ζ through a combination of ATG16L, which resulted in the greater formation of autophagosome precursors, enhancing proliferated ability, resisting apoptosis, and favoring survival. Autophagy flux was blocked by the inhibitor 3-methyladenine (3-MA) [131, 132]. Intriguingly, Cao et al. concentrated on three-dimensional culture and demonstrated that three-dimensional-cultured exosomes from hucMSCs outperformed in terms of production, concentration, and efficiency [133]. It has also been shown that conditioned mediums from BMMSCs can repair disordered kidney structure, strengthen the kidney function, and diminish the inflammatory factors in gentamicin-induced AKI [134]. Insulin-like growth factor-1 receptor (IGF-1R) mRNA can be transferred from BMMSCs and facilitate epithelial cell proliferation, which is abolished by gene silencing [122, 135].

Table 2: The mechanisms of MSC and other cell-derived exosomes in attenuating kidney injury.

Sources of exosomes	Cargos	Species/models	Outcome	Ref.
hWJMSCs	miR-30	SD rats/left kidney ischemia for 45 mins	Inhibit DRP1 Antiapoptosis	[103]
	miR-15, miR-16	SD rats/left kidney ischemia for 60 mins	Inhibit CX3CL1 Anti-inflammation	[104]
	–	Rats/left kidney ischemia for 45 mins NRK-52E cells/hypoxia for 6 h	Activate Nrf2/ARE pathway Antioxidation	[105]
hucMSCs	HGF mRNA	SD rats/left kidney ischemia for 1 h	Activate ERK 1/2 pathway Antiapoptosis	[106]
	–	SD rats: 6 mg/kg cisplatin NRK-52E cells/5 μM cisplatin for 6 h	Activate ERK 1/2 pathway Antiapoptosis	[107]
	–	C57BL/6 mice/CLP surgery	Inhibit NF-κB pathway Anti-inflammation	[108]
	–	SD rats/unilateral ureteral obstruction NRK-52E cells/TGF-β1	Inhibit TLR4/NF-κB pathway Anti-inflammation	[109]
	CK1δ, β-TRCP	SD rats/unilateral ureteral obstruction	Inhibit YAP activity Antifibrosis	[110]
	miR-125b-5p	C57BL/6 mice/bilateral kidneys ischemia for 30 mins HK-2 cells/hypoxia for 12 h	Inhibit p53 Antiapoptosis	[111]
hADMSCs	–	C57BL/6 mice/left kidney ischemia for 30 mins	Upregulate Sox9 Antifibrosis	[112]
	–	C57BL/6 mice/CLP surgery	Activate SIRT1 Anti-inflammation, Antiapoptosis	[113]
hUSCs	miR-146a-5p	SD rats/left kidney ischemia for 45 mins HK-2 cells/hypoxic medium 48 h	Downregulate IRAK1 Inhibit NF-κB pathway Anti-inflammation	[114]
	miR-216a-5p	SD rats/left kidney ischemia for 45 mins HK-2 cells/hypoxic medium 1 h	Downregulate PTEN Antiapoptosis	[115]
hP-MSCs	miR-200a-3p	FVB mice/right kidney ischemia for 50 mins	Activated Keap1-Nrf2 pathway Antioxidation	[116]
Mouse BMMSCs	CCR2	BALB/c mice/left kidney ischemia for 1 h	Absorb CCL2 Anti-inflammation	[117]
Mouse ADMSCs	miR-486	C57BL/KsJ db/db mice	Inhibit Smad1/mTOR pathway Promote autophagy Antiapoptosis	[118]
GI-MSCs	miRNAs	SCID mice/left kidney ischemia for 35 mins	Promote proliferation	[119]
RAPCs	miR-218, Robo-1	C57BL/6 mice/bilateral kidney ischemia for 30 mins	Promote migration	[120]
ECFCs	miR-486-5p	FVB mice/bilateral kidney ischemia	Downregulate PTEN Activate Akt pathway	[121]
EPCs	miR-126-3p miR-126-5p	CD-1 outbred mice/CLP	Downregulate HMGB1 and VCAM1	[122]

4.1.3. Sepsis-Induced AKI. Sepsis is a complex disease that is characterized by aberrant responses to infection and that is accompanied by extremely serious complications, such as multiple organ dysfunction and septic shock. The kidney is particularly vulnerable to sepsis. To enhance the immunomodulatory ability of hucMSCs in sepsis, Song et al. prestimulated them with interleukin-1β (IL-1β). They found that IL-1β-pretreated MSCs continued to meet the basic definition of MSCs and mitigated the disorganization of kidney, lung, and liver with downregulated interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). They also reported that more macrophages transformed to M2 phenotypes in lung and liver tissues subjected to pretreated MSCs. They thus found that exosomes derived from MSCs contained abundant miR-146a. When it was transferred to macrophages, it inhibited targeted proteins at the posttranscriptional level and fostered M2 polarization. Due to the lack of research on the kidney, the role of exosome miR-146a in renal injury needs further elucidation [136]. Our group recently showed that hucMSC-Exos affected the expression of miR-146b and

targeted IRAK1 in tubular cells, which resulted in the inhibition of the nuclear translocation of NF-κB and the production of inflammatory factors. Whether rare miR-146b can be detected in exosomes and which exosomal cargos affect miR-146b in kidneys deserve further exploration [108].

Because deacetylase sirtuin 1 (SIRT1) protein participates in orchestrating normal physiological processes in cells, it is clear that SIRT1 protein expression is upregulated in tissues associated with resistance to apoptosis and improves inflammatory responses under the treatment of ADMSC-exosomes [113]. It should be noted that some researchers have compared the efficiency of healthy and apoptotic ADMSC-derived exosomes in terms of circulating inflammatory levels, immune cells, and survival rate. Healthy ADMSC-derived exosomes outperformed others in their regulation of inflammatory reactions and oxidative stress [137]. Zhou et al. found that exosomes secreted by endothelial progenitor cells (EPCs) inhibited pathological changes in the kidney that were relevant to miR-126-5p and miR-126-3p [122].

4.2. Protective Roles on CKD. Fibrosis formation is an important pathological change in CKD. Unilateral ureteral obstruction (UUO) rat models could represent pathological changes in renal interstitium, so Liu et al. explored the protective molecular mechanisms of conditional medium (CM) in them. CM derived from hucMSCs has been reported to fight against oxidative stress, IF, and apoptosis [138]. Tubular epithelial cells undergoing epithelial-mesenchymal transition (EMT) are reported to participate in kidney fibrosis. During EMT, epithelial cells switch to mesenchymal phenotype change and secrete profibrotic factors and cytokines favoring the formation of fibrosis [139, 140]. Therefore, inhibition of inflammatory responses and EMT seemed to be favorable for slowing the development of fibrosis. It had received detailed demonstration that hucMSC-CM inhibited the TLR4/NF-κB signaling pathway, reduced inflammatory cell infiltration, decreased α-smooth muscle actin (α-SMA), and upregulated E-cadherin in rats and in NRK-52E cells [109]. Their laboratory also showed that hucMSC-Exos could be internalized into HK-2 cells and could partly reverse the EMT phenomena induced by oxalate and calcium oxalate monohydrate [141]. Ji et al. highlighted the vital participation of Yes-associated protein (YAP) in the development of renal fibrosis. Promisingly, it was found that hucMSC-Exos delivered casein kinase 1δ (CK1δ) and β-transducin repeat-containing protein (β-TRCP) to degrade YAP protein expression, which apparently had antifibrotic impacts [110]. In addition, Chen et al. modified ADMSCs with glial cell line-derived neurotrophic factor (GDNF), which had the ability to augment the SIRT1/eNOS pathway and further promoted angiogenesis, as well as protecting against tubulointerstitial fibrosis [142]. miR-let-7c has been shown to target TGF-β1 and to alleviate renal fibrosis in adenine and streptozotocin- (STZ-) induced animal models [143]. Recently, that group found that transfected miR-let-7c was packed into human BMMSC-Exos and was transferred to impaired areas to fight irreversible fibrosis [144].

DM, a systemic metabolic disease, another common cause of CKD, is characterized by persistent hyperglycemia. Prolonged hyperglycemia leads to continuous changes in the vessels and glomerulus. Podocytes, an essential component of the glomerular filtration barrier, have been documented to undergo adverse transformation of structure and function. It has been elucidated that exosomal miR-486 is transported from ADMSCs to podocytes, inhibits the Smad1/mTOR signaling pathway, enhances autophagy flux, and has cytoprotective effects [118]. Similar results have been found for rat BMMSCs in type I DM induced by STZ [145]. In 2018, our group reported the underlying mechanisms by which hucMSC-Exos alleviated glycometabolic disorders. We demonstrated that hucMSC-Exos could promote the translocation of glucose transporters 4 (GLUT4) to enhance glucose uptake, activate the insulin/AKT pathway to increase insulin sensitivity, and change the quality and quantity of pancreatic β-cells [146]. Li et al. confirmed that exosomes inhibited the deposition of fibrosis-related proteins and myofibroblast transdifferentiation, as well as downregulating the proliferative abilities induced by the PI3K/AKT and MAPK signaling pathways [147]. In addition, BMMSC-CM has been reported to possess antiapoptotic, antifibrotic, and anti-inflammatory effects [148].

Thus, a large amount of research has indicated that the exosomes of a range of stem cells protect against injury through antiapoptotic, anti-inflammatory, antioxidative, and antifibrotic pathways (Figure 2). The results of the studies reviewed also emphasize the roles of exosomal contents. However, some problems remain: it is not clear which pathway is the most significant, whether exosomes are harmful, and how to improve the effectiveness.

5. Other Cell-Derived Exosomes in Kidney Injury Repair

Hypoxia is a common cause of AKI. Under hypoxic circumstances, the exosomes secreted by tubular epithelial cells (TECs) are abundant in miR-20a-5p and are beneficial for the preservation of mitochondria and proliferation [149] (Table 2). In addition, exosomal ATF3 RNA protects against insult by downregulating monocyte chemotactic protein 1 (MCP1) and attenuating inflammatory responses [150]. When TECs are prestimulated with the appropriate hypoxia condition, more EVs are generated through the HIF-1α/Rab22 GTPase pathway, and they manifest more renoprotection [151]. In addition, renal proximal tubular cells (RPTCs) generate more protective exosomes during the early stages when they suffer from hypoxia [152]. These results demonstrate that adaptive injury to renal cells is advantageous to the kidney itself. Renal artery-derived vascular progenitor cells (RAPCs) in condition of oxidative stress are capable of promoting the migration of endothelial cells, which is related to increased Robo-1 and decreased miR-218 in exosomes [120]. Emerging evidence has shown the essential status that miR-486-5p has in endothelial colony-forming cell- (ECFC-) derived exosomes. Previously, it has been demonstrated that ECFC-exosomes protect against damage through their interaction between CXC chemokine receptor type 4 (CXCR4) and stromal cell-derived factor- (SDF-) 1α and thus transfer miR-486-5p [153]. Viñas

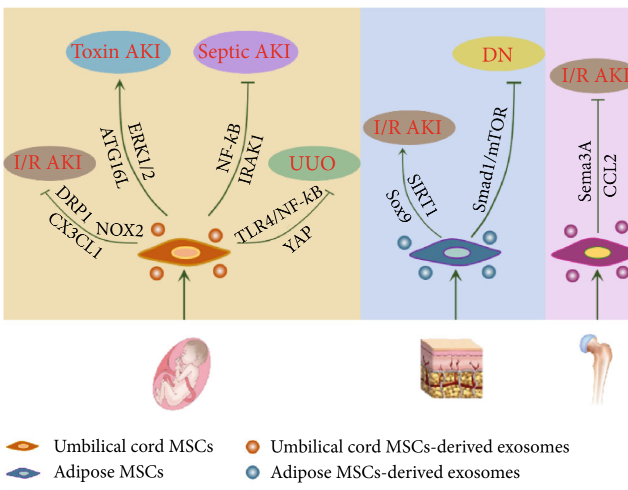

Figure 2: Mechanisms of MSC-exosomes in renal regeneration. MSCs derived from the umbilical cord, bone marrow, and adipose tissues play protective roles through antiapoptosis, anti-inflammation, antifibrosis, and antioxidation pathways.

et al. further illustrated the therapeutic mechanisms of miR-486-5p. ECFC-Exos contained obviously increased levels of miR-486-5p, which targeted PTEN and reinforced the Akt pathway to antagonize kidney injury [121]. It should be noted that exosomes from transfected macrophages also report apparent benefits during I/R injury [154, 155].

Recently, Grange et al. isolated EVs from urine characterized by aquaporin-1, aquaporin-2, and klotho, which demonstrated that the host was renal cells. The contents carried by EVs, such as miR-30, miR-151, and klotho, were shuttled to injured tissue, where they suppressed inflammation and promoted proliferation and recovery. However, those beneficial influences were reversed in klotho protein null mice, indicating the essential status of klotho protein [156]. Interestingly, Pan et al. later highlighted the importance of limb remote ischemic preconditioning in rescuing sepsis-induced AKI. They found a communication between skeletal muscle cells and tubule epithelial cells through circulation. They reported that myotube-derived and plasmic exosomes consisting of enhanced HIF-1α-dependent miR-21 depressed PDCD/NF-κB, improved PTEN/Akt signaling pathways, and fought septic AKI [157].

These results indicate that cells interacted and contacted with each other through exosomes in the microenvironment. Exosomes from renal intrinsic cells or even distant cells have beneficial effects on promoting kidney regeneration, which sheds light on the functional mechanisms of other cells and provides another protective strategy.

6. Engineered Exosomes in Kidney Injury Repair

In spite of the essential roles that exosomes play in regeneration, their limited effectiveness still needs to be taken into consideration. Improving stability and targeting ability are two important issues for manufacturing engineered exosomes. Several studies have explored the impacts of engineered exosomes that are delivered through passive and active loading techniques. Passive loading includes incubation, and active loading includes electroporation, extrusion, sonication, and transfection [158]. Previous research demonstrated that corresponding ligand was expressed on the surface of exosomes through transfecting the host cells which improved targeting ability [30]. Notably, functional lipids (such as polyethylene glycol) were infused with exosomes and manifested better retention [159]. Besides, techniques such as bioconjugation, click chemistry, and hydrophobic insertion were also strategies to modify extracellular vesicles [158]. The evidence suggests a considerable status for kidney diseases (Figure 3).

Drug delivery is a promising strategy for treating AKI. BMMSCs incubated with melatonin have been reported to generate more protective exosomes during I/R-induced AKI [126]. In a study of septic shock, Sun et al. prepared curcumin-loaded exosomes by coincubating curcumin with EL-4-cell-derived exosomes. The mixture overcame the disadvantages of curcumin, namely, hydrophobicity, instability, and low bioavailability. They reported that the engineered exosomes exhibited obvious anti-inflammatory abilities and maintained curcumin bioactivity for a long period [160]. In another study, dexamethasone (DEX) and glucocorticoid receptor were packaged in the MVs of macrophages following incubation and extrusion. Integrin was found to be expressed on MVs and to be responsible for the target to kidneys. Compared to traditional glucocorticoid therapy, the engineered MVs had an edge in terms of sensitivity, effectiveness, and safety [161]. Furthermore, incubation has also been shown to change the bioactive substances in exosomes. Yoon et al. stimulated ADMSCs from healthy individuals using melatonin. They demonstrated that the stimulated exosomes inhibited senescence, preserved mitochondrial function, and enhanced proliferation of the ADMSCs from CKD patients. miR-4516 and cellular prion protein (PrP^C) were found to be involved in the regeneration [162].

Transfection is also a strategy to edit the contents of the exosomes [150, 154]. Tang et al. obtained anti-inflammatory and engineered exosomes from M2 macrophages by transfecting IL-10 plasmids. They found that large amounts of IL-10 were loaded into exosomes, particularly with the administration of DEX. The exosomes maintained their bioactivity and promoted the targeting of interleukin, which inhibited mTOR signaling, induced M2 polarization, and promoted regeneration in an ischemic model [154]. In another study, miR-let-7c was transfected into hBMMSCs via a lentivirus. It was verified that exosomes were transferred into epithelial cells and downregulated fibrosis-related indexes such as TGF-β1, α-SMA, and collagen IV [144].

Tapparo et al. changed miRNA contents (miR-10a, miR-127, and miR-486) through electroporation and isolated corresponding exosomes. Notably, they found that the engineered exosomes were more effective at lower doses, and the overexpression of proregenerative miRNAs was not entirely beneficial [155].

Biochemical materials are also used to promote therapeutic efficacy. Intriguingly, Zhang et al. produced hydrogels

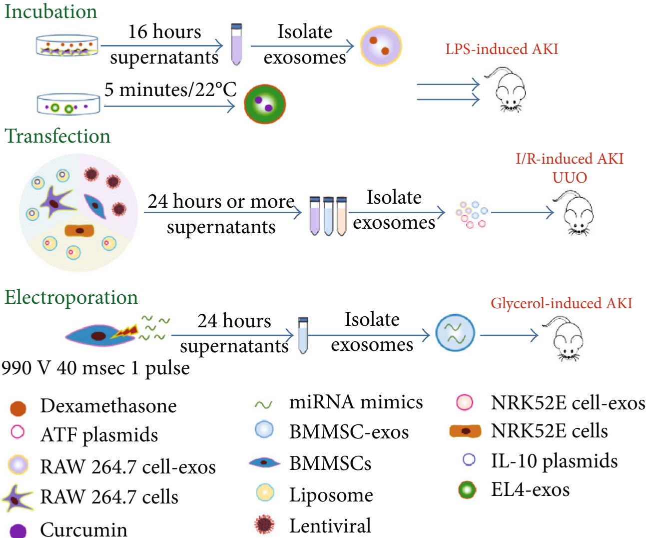

Figure 3: Methods for generating engineered exosomes in treating kidney injury. Incubation, electroporation, and transfection are three common methods of loading targeted molecules or substances into exosomes. These exosomes reveal benefits in anti-inflammatory, antiapoptotic, antioxidative, and antifibrotic aspects.

Table 3: Engineered exosomes in kidney injury repair.

Sources of exosomes	Methods	Models	Outcome	Ref.
BMMSCs	Incubate with melatonin	I/R-induced AKI	Anti-inflammation, apoptosis, and oxidation Promote angiogenesis	[126]
	Transfect miR-let-7c lentiviral	UUO	Inhibit TGF-β1 Antifibrosis	[144]
	Electroporate miR-10a, miR-127, and miR-486	Glycerol-induced AKI	Promote regeneration	[155]
ADMSCs	Incubate with melatonin	Hind limb ischemia model with CKD	Antisenescence Preserve mitochondrial function Promote angiogenesis	[162]
hP-MSCs	Modify with RGD hydrogels	I/R-induced AKI	Antiapoptosis Promote autophagy	[163]
	Modify with collagen matrix	I/R-induced AKI	Inhibit endoplasmic reticulum stress Antiapoptosis and fibrosis Promote angiogenesis	[164]
EL-4 cells	Incubate with curcumin	LPS-induced AKI	Anti-inflammation	[160]
RAW 264.7 cells	Transfect IL-10 plasmid	I/R-induced AKI	Inhibit mTOR pathway Promote M2 macrophage polarization Anti-inflammation	[154]
RAW 264.7 cells	Incubate with dexamethasone	LPS-induced AKI Adriamycin-induced nephropathy	Anti-inflammation Antifibrosis Increase dexamethasone sensitivity	[161]
NRK-52E cells	Transfect ATF3 plasmid	I/R-induced AKI	Inhibit MCP-1 Anti-inflammation	[150]

containing RGD (Arg-Gly-Asp) peptide. RGD, integrin, EVs, and biotin were formed in an interacted network. Functionally, RGD increased the affinity to EVs, and hydrogels sustained the retention and stability. Mechanistically, EVs from hP-MSCs were found to contain high levels of miR-let-7a-5p, which targeted caspase 3 and RragD to reduce apoptosis and promote autophagy [163]. Similarly, the application of collagen matrix also showed beneficial effects on retention, and hP-MSCs-EVs reduced kidney injury through inhibiting endoplasmic reticulum stress [164].

Due to the low yield of exosomes or EVs, some researchers have concentrated on the function of nanovesicles (NVs). NVs are generated through the serial extrusion of hBMMSCs. Under the impacts of bacterial outer membrane vesicles, the administration of NVs has been reported to alleviate cytokine storm and increase levels of IL-10 in both conditioned media and serum [165].

Engineered exosomes indeed show supportive effects on kidney injury and even outperform other exosomes to some extent (Table 3). It is worth loading other protective molecules and combining them with other particles or biological materials. The research field of engineered exosomes is an emerging area between medicine and materials science and has promising prospects for application.

7. Conclusions and Prospects

In conclusion, due to the exploration of novel bioactive markers and the pursuit of noninvasive diagnosis, the cargos encapsulated in exosomes have garnered considerable research attention in their ability to promote early diagnosis and treatment and to relieve pain as soon as possible. Numerous studies have also confirmed that exosomes do indeed have regenerative effects. However, clinical treatment applications for exosomes in kidney diseases are still lacking.

It should be noted that exosomes also play an essential role in the pathological processes of kidney injury, shuttling from one cell to another. This phenomenon makes it possible and reasonable for us to prevent and treat severe injury by inhibiting the shuttling or downregulating adverse molecules [47, 166–173].

There have also been quite a few problems that hinder the development from laboratory research to clinical applications. Separation methods must be made efficient and rapid to meet the large number of patient specimens. The methods also need to be carefully selected. Different separation strategies best suited for different sampling resources [27]. In addition, more techniques are applied to enhance the specificity of exosomes. Purity is an important challenge that must be considered. So far, the coisolated contaminants inevitably exist in the exosomes. It is worth considering how the best method can be selected and accomplished with higher purity and lower contamination, such as with proteins and lipids. It is also suggested that purity can be measured through calculating the ratios, such as protein : particle ratio and protein : lipid ratio [32]. There is an urgent need to establish the standard of quantifying and evaluating exosomes, including the condition of host cells and potency tests. Notably, the purposes of basic and clinical research are different. Some non-EV components even show positive therapeutic effects [174].

In addition, the scale of production is not yet sufficient, which implies additional demands for improved cell culture protocols, novel isolation methods, and engineered exosomes [133, 144]. These are intended to increase the quantity of the product and improve its functional roles. Stimulation MSCs with bioglass ion products have been found to secrete more exosomes and to influence the contents. Increased levels of miR-1290 and decreased miR-342-5p have been shown to promote vascularization [175]. Multiple techniques have been used to modify exosomes in direct or indirect ways. Direct ways include loading molecules such as proteins, drugs, and mRNA into the isolated exosomes. Comparably, indirect ways are also used to transform cells and collect the corresponding exosomes afterwards [176]. In engineering, exosomes carry therapeutic substances without damaging the intrinsic properties [177]. Interestingly, Zhou et al. produced a dual-delivery biosystem in BMMSC-Exos through the electroporation of galectin-9 siRNA and vortex of oxaliplatin prodrug. It has been demonstrated that the modified exosomes show the ability to inhibit M2-like macrophage, activate CRT/HMGB1/ATP expression, and induce both innate and adaptive antitumor immune responses. This suggests a possible and promising strategy for treating melanoma. However, engineered exosomes remain at the exploratory stage, and it is not yet known which transformation is the most effective for certain models [178].

Furthermore, the biogenesis and ingredients of exosomes are not fully understood nor are their complex and long-term roles [179]. Exosomal biomarkers of diseases are still limited in the laboratory, and their reference range has not been established, which indicates that their standard of diagnosis is immature. The sensitivity and specificity of the indexes need to be more accurately detected and confirmed [93]. Through our persistent efforts, we hope to promote improvements of exosomes in tissue regeneration and to effectively alleviate clinical symptoms. Exosomes are expected to be a bright star in the future detection and treatment of diseases.

Abbreviations

AKI: Acute kidney injury
GFR: Glomerular filtration rate
KIM-1: Kidney injury marker-1
NGAL: Neutrophil gelatinase-associated lipocalin
ROS: Reactive oxygen species
CKD: Chronic kidney disease
ESRD: End-stage renal disease
EVs: Extracellular vesicles
hBMSCs: Human bone mesenchymal stem cells
SPRY2: Sprouty 2
MVs: Microvesicles
ILVs: Intraluminal vesicles
hucMSCs: Human umbilical cord mesenchymal stem cells
TEM: Transmission electron microscopy
NTA: Nanoparticle tracking analysis
FCM: Flow cytometry
WB: Western blotting

PEG: Hydrophobic polyethylene glycol
I/R: Ischemia/reperfusion
Oat5: Organic anion transporter 5
ATF3: Activating transcription factor 3
MGAM: Maltase glucoamylase
C-megalin: Full-length megalin
DM: Diabetes mellitus
T2DN: Type 2 diabetic nephropathy
MIC: Microalbuminuria
AGEs: Advanced glycation end-products
WT1: Wilms' tumor 1
T1DN: Type 1 diabetic nephropathy
LN: Lupus nephritis
HIF-1α: Hypoxia-inducible factor-1α
PKD: Polycystic kidney disease
AGS3: Activator of G protein signaling 3
CREG1: Cellular repressor of E1A-stimulated genes 1
PC-1: Polycystin-1
PC-2: Polycystin-2
TMEM2: Transmembrane protein 2
RCC: Renal cell carcinoma
PTRF: Transcript release factor
MCD: Minimal change disease
FSGS: Focal segmental glomerulosclerosis
NS: Nephrotic syndrome
IF: Interstitial fibrosis
Cd: Cadmium
MSCs: Mesenchymal stem cells
hWJMSCs: Human Wharton jelly mesenchymal stromal cells
DRP1: Dynamin-related protein 1
MDA: Malondialdehyde
8-OHDG: 8-Hydroxy-2′-deoxyguanosine
HGF: Hepatocyte growth factor
NOX2: NADPH oxidase-2 protein
TFAM: Mitochondrial transcription factor A
mtDNA: Mitochondrial DNA
Sema 3A: Semaphoring 3A
CCR2: C-C motif chemokine receptor 2
hADMSCs: Human adipose-derived mesenchymal stem cells
hUSCs: Human urine-derived stem cells
mucMSCs: Mouse umbilical cord mesenchymal stem cells
IRAK1: Interleukin-1 receptor-associated kinase 1
PTEN: Phosphatase and tensin homolog
hAECs: Human amnion epithelial cells
GI-MSCs: MSCs in glomeruli
hP-MSCs: Human placenta-derived MSCs
3-MA: 3-Methyladenine
IL-1β: Interleukin-1β
IL-6: Interleukin-6
TNF-α: Tumor necrosis factor-α
SIRT1: Sirtuin 1
EPCs: Endothelial progenitor cells
UUO: Unilateral ureteral obstruction
CM: Conditional medium
α-SMA: α-Smooth muscle actin
YAP: Yes-associated protein
CK1δ: Casein kinase 1δ
β-TRCP: β-Transducin repeat-containing protein
GDNF: Glial cell line-derived neurotrophic factor
STZ: Streptozotocin
GLUT4: Glucose transporters 4
TECs: Tubular epithelial cells
MCP1: Monocyte chemotactic protein 1
RPTCs: Renal proximal tubular cells
RAPCs: Renal artery-derived vascular progenitor cells
ECFC: Endothelial colony forming cell
CXCR4: CXC chemokine receptor type 4
CCL2: C-C motif ligand 2
SDF: Stromal cell-derived factor
DEX: Dexamethasone
mTOR: Mammalian target of rapamycin
NVs: Nanovesicles
NF-κB: Nuclear factor-κB
Shh: Sonic hedgehog
SOCS-1: Suppressors of cytokine signaling 1
TGF-β1: Transforming growth factor-β1
EMT: Epithelial-mesenchymal transition
PI3K: Phosphoinositide 3 kinase
Akt: Protein kinase B
Nrf2: Nuclear factor E2-related factor 2
ERK: Extracellular-signal-related kinase
CLP: Cecal ligation and puncture
TLR4: Toll-like receptor 4
PI3K: Phosphoinositide 3 kinase
AGEs: Advanced glycation end-products
PrP^C: Cellular prion protein.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Can Jin and Peipei Wu contributed equally to this work.

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References

[1] A. S. Levey and M. T. James, "Acute kidney injury," *Annals of Internal Medicine*, vol. 167, no. 9, pp. Itc66–itc80, 2017.

[2] X. Zeng, G. M. McMahon, S. M. Brunelli, D. W. Bates, and S. S. Waikar, "Incidence, outcomes, and comparisons across definitions of AKI in hospitalized individuals," *Clinical Journal of the American Society of Nephrology*, vol. 9, no. 1, pp. 12–20, 2014.

[3] R. Bellomo, J. A. Kellum, C. Ronco et al., “Acute kidney injury in sepsis,” *Intensive Care Medicine*, vol. 43, no. 6, pp. 816–828, 2017.

[4] N. Petejova, A. Martinek, J. Zadrazil, and V. Teplan, “Acute toxic kidney injury,” *Renal Failure*, vol. 41, no. 1, pp. 576–594, 2019.

[5] L. He, Q. Wei, J. Liu et al., “AKI on CKD: heightened injury, suppressed repair, and the underlying mechanisms,” *Kidney International*, vol. 92, no. 5, pp. 1071–1083, 2017.

[6] R. J. Tan, D. Zhou, and Y. Liu, “Signaling crosstalk between tubular epithelial cells and interstitial fibroblasts after kidney injury,” *Kidney diseases*, vol. 2, no. 3, pp. 136–144, 2016.

[7] M. Mathieu, L. Martin-Jaular, G. Lavieu, and C. Théry, “Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication,” *Nature Cell Biology*, vol. 21, no. 1, pp. 9–17, 2019.

[8] Y. Zhang, Y. Liu, H. Liu, and W. H. Tang, “Exosomes: biogenesis, biologic function and clinical potential,” *Cell & Bioscience*, vol. 9, no. 1, p. ???, 2019.

[9] R. A. Haraszti, M. C. Didiot, E. Sapp et al., “High-resolution proteomic and lipidomic analysis of exosomes and microvesicles from different cell sources,” *Journal of Extracellular Vesicles*, vol. 5, no. 1, article 32570, 2016.

[10] B. T. Pan and R. M. Johnstone, “Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor,” *Cell*, vol. 33, no. 3, pp. 967–978, 1983.

[11] B. Zhang, L. Shen, H. Shi et al., “Exosomes from human umbilical cord mesenchymal stem cells: identification, purification, and biological characteristics,” *Stem Cells International*, vol. 2016, Article ID 1929536, 11 pages, 2016.

[12] P. Zheng, L. Chen, X. Yuan et al., “Exosomal transfer of tumor-associated macrophage-derived miR-21 confers cisplatin resistance in gastric cancer cells,” *Journal of Experimental & Clinical Cancer Research*, vol. 36, no. 1, p. 53, 2017.

[13] T. Harada, H. Yamamoto, S. Kishida et al., “Wnt5b-associated exosomes promote cancer cell migration and proliferation,” *Cancer Science*, vol. 108, no. 1, pp. 42–52, 2017.

[14] T. Baranyai, K. Herczeg, Z. Onódi et al., “Isolation of exosomes from blood plasma: qualitative and quantitative comparison of ultracentrifugation and size exclusion chromatography methods,” *PLoS One*, vol. 10, no. 12, article e0145686, 2015.

[15] J. M. Street, E. H. Koritzinsky, D. M. Glispie, and P. S. T. Yuen, “Urine exosome isolation and characterization,” *Methods in Molecular Biology*, vol. 2017, pp. 413–423, 2017.

[16] S. A. Tracy, A. Ahmed, J. C. Tigges et al., “A comparison of clinically relevant sources of mesenchymal stem cell-derived exosomes: bone marrow and amniotic fluid,” *Journal of Pediatric Surgery*, vol. 54, no. 1, pp. 86–90, 2019.

[17] W. Xia, H. Chen, D. Chen, Y. Ye, C. Xie, and M. Hou, “PD-1 inhibitor inducing exosomal miR-34a-5p expression mediates the cross talk between cardiomyocyte and macrophage in immune checkpoint inhibitor-related cardiac dysfunction,” *Journal for Immunotherapy of Cancer*, vol. 8, no. 2, article e001293, 2020.

[18] K. Ono, C. Sogawa, H. Kawai et al., “Triple knockdown of CDC37, HSP90-alpha and HSP90-beta diminishes extracellular vesicles-driven malignancy events and macrophage M2 polarization in oral cancer,” *Journal of Extracellular Vesicles*, vol. 9, no. 1, article 1769373, 2020.

[19] Y. Wang, Q. Li, H. Shi et al., “Microfluidic Raman biochip detection of exosomes: a promising tool for prostate cancer diagnosis,” *Lab Chip*, vol. 20, no. 24, pp. 4632–4637, 2020.

[20] L. Xu, H. Ji, Y. Jiang et al., “Exosomes derived from CircAkap7-modified adipose-derived mesenchymal stem cells protect against cerebral ischemic injury,” *Frontiers in Cell and Development Biology*, vol. 8, article 569977, 2020.

[21] D. Wu, L. Kang, J. Tian et al., “Exosomes derived from bone mesenchymal stem cells with the stimulation of Fe_3O_4 nanoparticles and static magnetic field enhance wound healing through upregulated miR-21-5p,” *International Journal of Nanomedicine*, vol. 15, pp. 7979–7993, 2020.

[22] M. H. Rashed, E. Bayraktar, G. K. Helal et al., “Exosomes: from garbage bins to promising therapeutic targets,” *International Journal of Molecular Sciences*, vol. 18, no. 3, p. 538, 2017.

[23] S. Gandham, X. Su, J. Wood et al., “Technologies and standardization in research on extracellular vesicles,” *Trends in Biotechnology*, vol. 38, no. 10, pp. 1066–1098, 2020.

[24] D. Yang, W. Zhang, H. Zhang et al., “Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics,” *Theranostics*, vol. 10, no. 8, pp. 3684–3707, 2020.

[25] S. Nikfarjam, J. Rezaie, N. M. Zolbanin, and R. Jafari, “Mesenchymal stem cell derived-exosomes: a modern approach in translational medicine,” *Journal of Translational Medicine*, vol. 18, no. 1, p. 449, 2020.

[26] J. H. Lee, D. H. Ha, H. K. Go et al., “Reproducible large-scale isolation of exosomes from adipose tissue-derived mesenchymal stem/stromal cells and their application in acute kidney injury,” *International Journal of Molecular Sciences*, vol. 21, no. 13, p. 4774, 2020.

[27] EV-TRACK Consortium, J. van Deun, P. Mestdagh et al., “EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research,” *Nature Methods*, vol. 14, no. 3, pp. 228–232, 2017.

[28] F. A. W. Coumans, A. R. Brisson, E. I. Buzas et al., “Methodological guidelines to study extracellular vesicles,” *Circulation Research*, vol. 120, no. 10, pp. 1632–1648, 2017.

[29] H. H. Jung, J. Y. Kim, J. E. Lim, and Y. H. Im, “Cytokine profiling in serum-derived exosomes isolated by different methods,” *Scientific Reports*, vol. 10, no. 1, p. 14069, 2020.

[30] F. Vakhshiteh, F. Atyabi, and S. N. Ostad, “Mesenchymal stem cell exosomes: a two-edged sword in cancer therapy,” *International Journal of Nanomedicine*, vol. 14, pp. 2847–2859, 2019.

[31] C. Gardiner, D. Di Vizio, S. Sahoo et al., “Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey,” *Journal of Extracellular Vesicles*, vol. 5, no. 1, p. 32945, 2016.

[32] C. Théry, K. W. Witwer, E. Aikawa et al., “Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines,” *Journal of Extracellular Vesicles*, vol. 7, no. 1, article 1535750, 2018.

[33] X. Wu, S. A. A. Showiheen, A. R. Sun et al., “Exosomes extraction and identification,” *Methods in Molecular Biology*, vol. 2054, pp. 81–91, 2019.

[34] P. Cizmar and Y. Yuana, “Detection and characterization of extracellular vesicles by transmission and cryo-transmission

electron microscopy," *Methods in Molecular Biology*, vol. 2017, pp. 221–232, 2017.

[35] S. Gurunathan, M. H. Kang, M. Jeyaraj, M. Qasim, and J. H. Kim, "Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes," *Cell*, vol. 8, no. 4, p. 307, 2019.

[36] J. A. Welsh, E. van der Pol, B. A. Bettin et al., "Towards defining reference materials for measuring extracellular vesicle refractive index, epitope abundance, size and concentration," *Journal of Extracellular Vesicles*, vol. 9, no. 1, article 1816641, 2020.

[37] R. J. Lobb, M. Becker, S. Wen Wen et al., "Optimized exosome isolation protocol for cell culture supernatant and human plasma," *Journal of Extracellular Vesicles*, vol. 4, no. 1, p. 27031, 2015.

[38] C. Théry, S. Amigorena, G. Raposo, and A. Clayton, "Isolation and characterization of exosomes from cell culture supernatants and biological fluids," *Current Protocols in Cell Biology*, vol. 30, no. 1, 2006.

[39] B. Zhang, Y. Shi, A. Gong et al., "HucMSC exosome-delivered 14-3-3ζ orchestrates self-control of the Wnt response via modulation of YAP during cutaneous regeneration," *Stem Cells*, vol. 34, no. 10, pp. 2485–2500, 2016.

[40] W. Jiang, Y. Tan, M. Cai et al., "Human Umbilical Cord MSC-Derived Exosomes Suppress the Development of CCl4-Induced Liver Injury through Antioxidant Effect," *Stem Cells International*, vol. 2018, Article ID 6079642, 11 pages, 2018.

[41] M. T. Roefs, J. P. G. Sluijter, and P. Vader, "Extracellular vesicle-associated proteins in tissue repair," *Trends in Cell Biology*, vol. 30, no. 12, pp. 990–1013, 2020.

[42] B. R. Griffin, S. Faubel, and C. L. Edelstein, "Biomarkers of drug-induced kidney toxicity," *Therapeutic Drug Monitoring*, vol. 41, no. 2, pp. 213–226, 2019.

[43] J. Novák, T. Macháčková, J. Krejčí, J. Bienertová-Vašků, and O. Slabý, "MicroRNAs as theranostic markers in cardiac allograft transplantation: from murine models to clinical practice," *Theranostics*, vol. 11, no. 12, pp. 6058–6073, 2021.

[44] P. Svenningsen, R. Sabaratnam, and B. L. Jensen, "Urinary extracellular vesicles: origin, role as intercellular messengers and biomarkers; efficient sorting and potential treatment options," *Acta Physiologica*, vol. 228, no. 1, article e13346, 2020.

[45] H. Sonoda, B. R. Lee, K. H. Park et al., "miRNA profiling of urinary exosomes to assess the progression of acute kidney injury," *Scientific Reports*, vol. 9, no. 1, article 4692, 2019.

[46] Y. F. Zou, D. Wen, Q. Zhao et al., "Urinary microRNA-30c-5p and microRNA-192-5p as potential biomarkers of ischemia-reperfusion-induced kidney injury," *Experimental Biology and Medicine*, vol. 242, no. 6, pp. 657–667, 2017.

[47] L. L. Lv, Y. Feng, M. Wu et al., "Exosomal miRNA-19b-3p of tubular epithelial cells promotes M1 macrophage activation in kidney injury," *Cell Death and Differentiation*, vol. 27, no. 1, pp. 210–226, 2020.

[48] D. Delić, C. Eisele, R. Schmid et al., "Urinary exosomal miRNA signature in type II diabetic nephropathy patients," *PLoS One*, vol. 11, no. 3, article e0150154, 2016.

[49] Y. Xie, Y. Jia, X. Cuihua, F. Hu, M. Xue, and Y. Xue, "Urinary exosomal microRNA profiling in incipient type 2 diabetic kidney disease," *Journal Diabetes Research*, vol. 2017, article 6978984, 10 pages, 2017.

[50] S. Eissa, M. Matboli, and M. M. Bekhet, "Clinical verification of a novel urinary microRNA panal: 133b, -342 and -30 as biomarkers for diabetic nephropathy identified by bioinformatics analysis," *Biomedicine & Pharmacotherapy*, vol. 83, pp. 92–99, 2016.

[51] Y. Jia, M. Guan, Z. Zheng et al., "miRNAs in Urine Extracellular Vesicles as Predictors of Early-Stage Diabetic Nephropathy," *Diabetes Research*, vol. 2016, article 7932765, 10 pages, 2016.

[52] S. Eissa, M. Matboli, R. Aboushahba, M. M. Bekhet, and Y. Soliman, "Urinary exosomal microRNA panel unravels novel biomarkers for diagnosis of type 2 diabetic kidney disease," *Journal of Diabetes and its Complications*, vol. 30, no. 8, pp. 1585–1592, 2016.

[53] W. Li, S. Yang, R. Qiao, and J. Zhang, "Potential value of urinary exosome-derived let-7c-5p in the diagnosis and progression of type II diabetic nephropathy," *Clinical Laboratory*, vol. 64, pp. 709–718, 2018.

[54] P. Prabu, S. Rome, C. Sathishkumar et al., "MicroRNAs from urinary extracellular vesicles are non-invasive early biomarkers of diabetic nephropathy in type 2 diabetes patients with the 'Asian Indian phenotype'," *Diabetes & Metabolism*, vol. 45, no. 3, pp. 276–285, 2019.

[55] A. Mohan, R. S. Singh, M. Kumari et al., "Urinary exosomal microRNA-451-5p is a potential early biomarker of diabetic nephropathy in rats," *PLoS One*, vol. 11, no. 4, article e0154055, 2016.

[56] F. Barutta, M. Tricarico, A. Corbelli et al., "Urinary exosomal microRNAs in incipient diabetic nephropathy," *PLoS One*, vol. 8, no. 11, article e73798, 2013.

[57] O. Ichii, S. Otsuka-Kanazawa, T. Horino et al., "Decreased miR-26a expression correlates with the progression of podocyte injury in autoimmune glomerulonephritis," *PLoS One*, vol. 9, no. 10, article e110383, 2014.

[58] C. Solé, T. Moliné, M. Vidal, J. Ordi-Ros, and J. Cortés-Hernández, "An exosomal urinary miRNA signature for early diagnosis of renal fibrosis in lupus nephritis," *Cell*, vol. 8, 2019.

[59] J. Perez-Hernandez, O. Martinez-Arroyo, A. Ortega et al., "Urinary exosomal miR-146a as a marker of albuminuria, activity changes and disease flares in lupus nephritis," *Journal of Nephrology*, 2020.

[60] P. Tangtanatakul, S. Klinchanhom, P. Sodsai et al., "Down-regulation of let-7a and miR-21 in urine exosomes from lupus nephritis patients during disease flare," *Asian Pacific Journal of Allergy and Immunology*, vol. 37, pp. 189–197, 2019.

[61] C. Solé, J. Cortés-Hernández, M. L. Felip, M. Vidal, and J. Ordi-Ros, "miR-29c in urinary exosomes as predictor of early renal fibrosis in lupus nephritis," *Nephrology Dialysis Transplantation*, vol. 30, no. 9, pp. 1488–1496, 2015.

[62] T. A. Magayr, X. Song, A. J. Streets et al., "Global microRNA profiling in human urinary exosomes reveals novel disease biomarkers and cellular pathways for autosomal dominant polycystic kidney disease," *Kidney International*, vol. 98, no. 2, pp. 420–435, 2020.

[63] H. Butz, R. Nofech-Mozes, Q. Ding et al., "Exosomal microRNAs are diagnostic biomarkers and can mediate cell-cell communication in renal cell carcinoma," *European Urology Focus*, vol. 2, no. 2, pp. 210–218, 2016.

[64] R. Kurahashi, T. Kadomatsu, M. Baba et al., "MicroRNA-204-5p: a novel candidate urinary biomarker of Xp11.2

translocation renal cell carcinoma," *Cancer Science*, vol. 110, no. 6, pp. 1897–1908, 2019.

[65] A. Ramezani, J. M. Devaney, S. Cohen et al., "Circulating and urinary microRNA profile in focal segmental glomerulosclerosis: a pilot study," *European Journal of Clinical Investigation*, vol. 45, no. 4, pp. 394–404, 2015.

[66] T. Chen, C. Wang, H. Yu et al., "Increased urinary exosomal microRNAs in children with idiopathic nephrotic syndrome," *eBioMedicine*, vol. 39, pp. 552–561, 2019.

[67] R. P. Bulacio, N. Anzai, M. Ouchi, and A. M. Torres, "Organic anion transporter 5 (Oat5) urinary excretion is a specific biomarker of kidney injury: evaluation of urinary excretion of exosomal Oat5 after N-acetylcysteine prevention of cisplatin induced nephrotoxicity," *Chemical Research in Toxicology*, vol. 28, no. 8, pp. 1595–1602, 2015.

[68] T. Panich, W. Chancharoenthana, P. Somparn, J. Issara-Amphorn, N. Hirankarn, and A. Leelahavanichkul, "Urinary exosomal activating transcriptional factor 3 as the early diagnostic biomarker for sepsis-induced acute kidney injury," *BMC Nephrology*, vol. 18, p. 10, 2017.

[69] L. Awdishu, S. Tsunoda, M. Pearlman et al., "Identification of maltase glucoamylase as a biomarker of acute kidney injury in patients with cirrhosis," *Critical Care Research and Practice*, vol. 2019, Article ID 5912804, 8 pages, 2019.

[70] A. Sakurai, H. Ono, A. Ochi et al., "Involvement of Elf3 on Smad3 activation-dependent injuries in podocytes and excretion of urinary exosome in diabetic nephropathy," *PLoS One*, vol. 14, no. 5, article e0216788, 2019.

[71] S. de, S. Kuwahara, M. Hosojima et al., "Exocytosis-mediated urinary full-length megalin excretion is linked with the pathogenesis of diabetic nephropathy," *Diabetes*, vol. 66, no. 5, pp. 1391–1404, 2017.

[72] C. M. Yamamoto, T. Murakami, M. L. Oakes et al., "Uromodulin mRNA from urinary extracellular vesicles correlate to kidney function decline in type 2 diabetes mellitus," *American Journal of Nephrology*, vol. 47, no. 5, pp. 283–291, 2018.

[73] H. Abe, A. Sakurai, H. Ono et al., "Urinary exosomal mRNA of WT1 as diagnostic and prognostic biomarker for diabetic nephropathy," *The Journal of Medical Investigation*, vol. 65, no. 3.4, pp. 208–215, 2018.

[74] C. Y. Lv, W. J. Ding, Y. L. Wang et al., "A PEG-based method for the isolation of urinary exosomes and its application in renal fibrosis diagnostics using cargo miR-29c and miR-21 analysis," *International Urology and Nephrology*, vol. 50, no. 5, pp. 973–982, 2018.

[75] M. Kumari, A. Mohan, C. M. Ecelbarger, A. Gupta, N. Prasad, and S. Tiwari, "miR-451 loaded exosomes are released by the renal cells in response to injury and associated with reduced kidney function in human," *Frontiers in Physiology*, vol. 11, p. 234, 2020.

[76] R. Khurana, G. Ranches, S. Schafferer et al., "Identification of urinary exosomal noncoding RNAs as novel biomarkers in chronic kidney disease," *RNA*, vol. 23, no. 2, pp. 142–152, 2017.

[77] I. Zubiri, M. Posada-Ayala, A. Benito-Martin et al., "Kidney tissue proteomics reveals regucalcin downregulation in response to diabetic nephropathy with reflection in urinary exosomes," *Translational Research*, vol. 166, no. 5, pp. 474–484.e4, 2015.

[78] Y. Yu, F. Bai, N. Qin et al., "Non-proximal renal tubule-derived urinary exosomal miR-200b as a biomarker of renal fibrosis," *Nephron*, vol. 139, no. 3, pp. 269–282, 2018.

[79] E. Garcia-Vives, C. Solé, T. Moliné et al., "The urinary exosomal miRNA expression profile is predictive of clinical response in lupus nephritis," *International Journal of Molecular Sciences*, vol. 21, no. 4, p. 1372, 2020.

[80] K. C. Keri, K. R. Regner, A. T. Dall, and F. Park, "Urinary exosomal expression of activator of G protein signaling 3 in polycystic kidney disease," *BMC Research Notes*, vol. 11, no. 1, p. 359, 2018.

[81] M. Salih, J. A. Demmers, K. Bezstarosti et al., "Proteomics of urinary vesicles links plakins and complement to polycystic kidney disease," *Journal of the American Society of Nephrology*, vol. 27, no. 10, pp. 3079–3092, 2016.

[82] M. Bruschi, S. Granata, L. Santucci et al., "Proteomic analysis of urinary microvesicles and exosomes in medullary sponge kidney disease and autosomal dominant polycystic kidney disease," *Clinical Journal of the American Society of Nephrology*, vol. 14, no. 6, pp. 834–843, 2019.

[83] M. C. Hogan, J. L. Bakeberg, V. G. Gainullin et al., "Identification of biomarkers for PKD1 using urinary exosomes," *Journal of the American Society of Nephrology*, vol. 26, no. 7, pp. 1661–1670, 2015.

[84] U. Capitanio, K. Bensalah, A. Bex et al., "Epidemiology of renal cell carcinoma," *European Urology*, vol. 75, no. 1, pp. 74–84, 2019.

[85] Y. Zhao, Y. Wang, E. Zhao et al., "PTRF/CAVIN1, regulated by SHC1 through the EGFR pathway, is found in urine exosomes as a potential biomarker of ccRCC," *Carcinogenesis*, vol. 41, no. 3, pp. 274–283, 2020.

[86] P. Cravedi, J. B. Kopp, and G. Remuzzi, "Recent progress in the pathophysiology and treatment of FSGS recurrence," *American Journal of Transplantation*, vol. 13, no. 2, pp. 266–274, 2013.

[87] Z. Huang, Y. Zhang, J. Zhou, and Y. Zhang, "Urinary exosomal miR-193a can be a potential biomarker for the diagnosis of primary focal segmental glomerulosclerosis in children," *BioMed Research International*, vol. 2017, Article ID 7298160, 6 pages, 2017.

[88] S. Bhayana, F. Song, J. Jacob et al., "Urinary miRNAs as biomarkers for noninvasive evaluation of radiation-induced renal tubular injury," *Radiation Research*, vol. 188, no. 6, pp. 706–715, 2017.

[89] J. X. Xie, X. Fan, C. A. Drummond et al., "MicroRNA profiling in kidney disease: plasma versus plasma-derived exosomes," *Gene*, vol. 627, pp. 1–8, 2017.

[90] Y. E. Cho, S. H. Kim, B. H. Lee, and M. C. Baek, "Circulating plasma and exosomal microRNAs as indicators of drug-induced organ injury in rodent models," *Biomolecules & Therapeutics*, vol. 25, no. 4, pp. 367–373, 2017.

[91] S. Saejong, N. Townamchai, P. Somparn et al., "MicroRNA-21 in plasma exosome, but not from whole plasma, as a biomarker for the severe interstitial fibrosis and tubular atrophy (IF/TA) in post-renal transplantation," *Asian Pacific Journal of Allergy and Immunology*, 2020.

[92] C. T. Xiao, W. J. Lai, W. A. Zhu, and H. Wang, "MicroRNA derived from circulating exosomes as noninvasive biomarkers for diagnosing renal cell carcinoma," *Oncotargets and Therapy*, vol. 13, pp. 10765–10774, 2020.

[93] F. Dias, A. L. Teixeira, I. Nogueira et al., "Extracellular vesicles enriched in hsa-miR-301a-3p and hsa-miR-1293 dynamics in clear cell renal cell carcinoma patients: potential biomarkers of metastatic disease," *Cancers (Basel)*, vol. 12, no. 6, p. 1450, 2020.

[94] G. Wang, J. Yuan, X. Cai et al., "HucMSC-exosomes carrying miR-326 inhibit neddylation to relieve inflammatory bowel disease in mice," *Clinical and Translational Medicine*, vol. 10, no. 2, article e113, 2020.

[95] Y. Yan, W. Jiang, Y. Tan et al., "hucMSC exosome-derived GPX1 is required for the recovery of hepatic oxidant injury," *Molecular Therapy*, vol. 25, no. 2, pp. 465–479, 2017.

[96] H. Shi, X. Xu, B. Zhang et al., "3,3′-Diindolylmethane stimulates exosomal Wnt11 autocrine signaling in human umbilical cord mesenchymal stem cells to enhance wound healing," *Theranostics*, vol. 7, no. 6, pp. 1674–1688, 2017.

[97] Y. Jiang, B. N. Jahagirdar, R. L. Reinhardt et al., "Pluripotency of mesenchymal stem cells derived from adult marrow," *Nature*, vol. 418, no. 6893, pp. 41–49, 2002.

[98] P. A. Zuk, M. Zhu, P. Ashjian et al., "Human adipose tissue is a source of multipotent stem cells," *Molecular Biology of the Cell*, vol. 13, no. 12, pp. 4279–4295, 2002.

[99] A. B. Araújo, J. M. Furlan, G. D. Salton et al., "Isolation of human mesenchymal stem cells from amnion, chorion, placental decidua and umbilical cord: comparison of four enzymatic protocols," *Biotechnology Letters*, vol. 40, no. 6, pp. 989–998, 2018.

[100] L. J. Hickson, A. Eirin, and L. O. Lerman, "Challenges and opportunities for stem cell therapy in patients with chronic kidney disease," *Kidney International*, vol. 89, no. 4, pp. 767–778, 2016.

[101] A. J. Peired, A. Sisti, and P. Romagnani, "Mesenchymal stem cell-based therapy for kidney disease: a review of clinical evidence," *Stem Cells International*, vol. 2016, Article ID 4798639, 22 pages, 2016.

[102] F. Meng, R. Xu, S. Wang et al., "Human umbilical cord-derived mesenchymal stem cell therapy in patients with COVID-19: a phase 1 clinical trial," *Signal Transduction and Targeted Therapy*, vol. 5, no. 1, p. 172, 2020.

[103] D. Gu, X. Zou, G. Ju, G. Zhang, E. Bao, and Y. Zhu, "Mesenchymal stromal cells derived extracellular vesicles ameliorate acute renal ischemia reperfusion injury by inhibition of mitochondrial fission through miR-30," *Stem Cells International*, vol. 2016, Article ID 2093940, 12 pages, 2016.

[104] X. Zou, G. Zhang, Z. Cheng et al., "Microvesicles derived from human Wharton's jelly mesenchymal stromal cells ameliorate renal ischemia-reperfusion injury in rats by suppressing CX3CL1," *Stem Cell Research & Therapy*, vol. 5, no. 2, p. 40, 2014.

[105] G. Zhang, X. Zou, Y. Huang et al., "Mesenchymal stromal cell-derived extracellular vesicles protect against acute kidney injury through anti-oxidation by enhancing Nrf2/ARE activation in rats," *Kidney & Blood Pressure Research*, vol. 41, no. 2, pp. 119–128, 2016.

[106] G. Q. Ju, J. Cheng, L. Zhong et al., "Microvesicles derived from human umbilical cord mesenchymal stem cells facilitate tubular epithelial cell dedifferentiation and growth via hepatocyte growth factor induction," *PLoS One*, vol. 10, no. 3, article e0121534, 2015.

[107] Y. Zhou, H. Xu, W. Xu et al., "Exosomes released by human umbilical cord mesenchymal stem cells protect against cisplatin-induced renal oxidative stress and apoptosis in vivo and in vitro," *Stem Cell Research & Therapy*, vol. 4, no. 2, p. 34, 2013.

[108] R. Zhang, Y. Zhu, Y. Li et al., "Human umbilical cord mesenchymal stem cell exosomes alleviate sepsis-associated acute kidney injury via regulating microRNA-146b expression," *Biotechnology Letters*, vol. 42, no. 4, pp. 669–679, 2020.

[109] B. Liu, F. Ding, D. Hu et al., "Human umbilical cord mesenchymal stem cell conditioned medium attenuates renal fibrosis by reducing inflammation and epithelial-to-mesenchymal transition via the TLR4/NF-κB signaling pathway in vivo and in vitro," *Stem Cell Research & Therapy*, vol. 9, no. 1, p. 7, 2018.

[110] C. Ji, J. Zhang, Y. Zhu et al., "Exosomes derived from hucMSC attenuate renal fibrosis through CK1δ/β-TRCP-mediated YAP degradation," *Cell Death & Disease*, vol. 11, no. 5, p. 327, 2020.

[111] J. Y. Cao, B. Wang, T. T. Tang et al., "Exosomal miR-125b-5p deriving from mesenchymal stem cells promotes tubular repair by suppression of p53 in ischemic acute kidney injury," *Theranostics*, vol. 11, no. 11, pp. 5248–5266, 2021.

[112] F. Zhu, O. L. S. Chong Lee Shin, G. Pei et al., "Adipose-derived mesenchymal stem cells employed exosomes to attenuate AKI-CKD transition through tubular epithelial cell dependent Sox9 activation," *Oncotarget*, vol. 8, no. 41, pp. 70707–70726, 2017.

[113] F. Gao, B. Zuo, Y. Wang, S. Li, J. Yang, and D. Sun, "Protective function of exosomes from adipose tissue-derived mesenchymal stem cells in acute kidney injury through SIRT1 pathway," *Life Sciences*, vol. 255, article 117719, 2020.

[114] X. Li, J. Liao, X. Su et al., "Human urine-derived stem cells protect against renal ischemia/reperfusion injury in a rat model via exosomalmiR-146a-5pwhich targetsIRAK1," *Theranostics*, vol. 10, no. 21, pp. 9561–9578, 2020.

[115] Y. Zhang, J. Wang, B. Yang et al., "Transfer of microRNA-216a-5p from exosomes secreted by human urine-derived stem cells reduces renal ischemia/reperfusion injury," *Frontiers in Cell and Development Biology*, vol. 8, article 610587, 2020.

[116] H. Cao, Y. Cheng, H. Gao et al., "In VivoTracking of mesenchymal stem cell-derived extracellular vesicles improving mitochondrial function in renal ischemia-reperfusion injury," *ACS Nano*, vol. 14, no. 4, pp. 4014–4026, 2020.

[117] B. Shen, J. Liu, F. Zhang et al., "CCR2 positive exosome released by mesenchymal stem cells suppresses macrophage functions and alleviates ischemia/reperfusion-induced renal injury," *Stem Cells International*, vol. 2016, Article ID 1240301, 9 pages, 2016.

[118] J. Jin, Y. Shi, J. Gong et al., "Exosome secreted from adipose-derived stem cells attenuates diabetic nephropathy by promoting autophagy flux and inhibiting apoptosis in podocyte," *Stem Cell Research & Therapy*, vol. 10, no. 1, p. 95, 2019.

[119] A. Ranghino, S. Bruno, B. Bussolati et al., "The effects of glomerular and tubular renal progenitors and derived extracellular vesicles on recovery from acute kidney injury," *Stem Cell Research & Therapy*, vol. 8, no. 1, p. 24, 2017.

[120] P. Pang, M. Abbott, S. L. Chang et al., "Human vascular progenitor cells derived from renal arteries are endothelial- like and assist in the repair of injured renal capillary networks," *Kidney International*, vol. 91, no. 1, pp. 129–143, 2017.

[121] J. L. Viñas, D. Burger, J. Zimpelmann et al., "Transfer of microRNA-486-5p from human endothelial colony forming cell-derived exosomes reduces ischemic kidney injury," *Kidney International*, vol. 90, no. 6, pp. 1238–1250, 2016.

[122] Y. Zhou, P. Li, A. J. Goodwin et al., "Exosomes from endothelial progenitor cells improve the outcome of a murine model

of sepsis," *Molecular Therapy*, vol. 26, no. 5, pp. 1375–1384, 2018.

[123] G. Zhang, X. Zou, S. Miao et al., "The anti-oxidative role of micro-vesicles derived from human Wharton-jelly mesenchymal stromal cells through NOX2/gp91(phox) suppression in alleviating renal ischemia-reperfusion injury in rats," *PLoS One*, vol. 9, no. 3, article e92129, 2014.

[124] M. Zhao, S. Liu, C. Wang et al., "Mesenchymal stem cell-derived extracellular vesicles attenuate mitochondrial damage and inflammation by stabilizing mitochondrial DNA," *ACS Nano*, vol. 15, no. 1, pp. 1519–1538, 2021.

[125] G. Zhu, L. Pei, F. Lin et al., "Exosomes from human-bone-marrow-derived mesenchymal stem cells protect against renal ischemia/reperfusion injury via transferring miR-199a-3p," *Journal of Cellular Physiology*, vol. 234, no. 12, pp. 23736–23749, 2019.

[126] F. A. Alzahrani, "Melatonin improves therapeutic potential of mesenchymal stem cells-derived exosomes against renal ischemia-reperfusion injury in rats," *American Journal of Translational Research*, vol. 11, no. 5, pp. 2887–2907, 2019.

[127] X. Yuan, X. Wang, C. Chen, J. Zhou, and M. Han, "Bone mesenchymal stem cells ameliorate ischemia/reperfusion-induced damage in renal epithelial cells via microRNA-223," *Stem Cell Research & Therapy*, vol. 8, no. 1, p. 146, 2017.

[128] K. C. Lin, H. K. Yip, P. L. Shao et al., "Combination of adipose-derived mesenchymal stem cells (ADMSC) and ADMSC- derived exosomes for protecting kidney from acute ischemia-reperfusion injury," *International Journal of Cardiology*, vol. 216, pp. 173–185, 2016.

[129] Y. Ren, Y. Chen, X. Zheng et al., "Human amniotic epithelial cells ameliorate kidney damage in ischemia-reperfusion mouse model of acute kidney injury," *Stem Cell Research & Therapy*, vol. 11, no. 1, p. 410, 2020.

[130] B. Wang, H. Jia, B. Zhang et al., "Pre-incubation with hucMSC-exosomes prevents cisplatin-induced nephrotoxicity by activating autophagy," *Stem Cell Research & Therapy*, vol. 8, no. 1, p. 75, 2017.

[131] H. Jia, W. Liu, B. Zhang et al., "HucMSC exosomes-delivered 14-3-3ζ enhanced autophagy via modulation of ATG16L in preventing cisplatin-induced acute kidney injury," *American Journal of Translational Research*, vol. 10, no. 1, pp. 101–113, 2018.

[132] J. Wang, H. Jia, B. Zhang et al., "HucMSC exosome-transported 14-3-3ζ prevents the injury of cisplatin to HK-2 cells by inducing autophagy *in vitro*," *Cytotherapy*, vol. 20, no. 1, pp. 29–44, 2018.

[133] J. Cao, B. Wang, T. Tang et al., "Three-dimensional culture of MSCs produces exosomes with improved yield and enhanced therapeutic efficacy for cisplatin-induced acute kidney injury," *Stem Cell Research & Therapy*, vol. 11, no. 1, p. 206, 2020.

[134] L. A. Reis, F. T. Borges, M. J. Simões, A. A. Borges, R. Sinigaglia-Coimbra, and N. Schor, "Bone marrow-derived mesenchymal stem cells repaired but did not prevent gentamicin-induced acute kidney injury through paracrine effects in rats," *PLoS One*, vol. 7, no. 9, article e44092, 2012.

[135] S. Tomasoni, L. Longaretti, C. Rota et al., "Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells," *Stem Cells and Development*, vol. 22, no. 5, pp. 772–780, 2013.

[136] Y. Song, H. Dou, X. Li et al., "Exosomal miR-146a contributes to the enhanced therapeutic efficacy of interleukin-1β-primed mesenchymal stem cells against sepsis," *Stem Cells*, vol. 35, no. 5, pp. 1208–1221, 2017.

[137] C. L. Chang, P. H. Sung, K. H. Chen et al., "Adipose-derived mesenchymal stem cell-derived exosomes alleviate overwhelming systemic inflammatory reaction and organ damage and improve outcome in rat sepsis syndrome," *American Journal of Translational Research*, vol. 10, no. 4, pp. 1053–1070, 2018.

[138] B. Liu, F. X. Ding, Y. Liu et al., "Human umbilical cord-derived mesenchymal stem cells conditioned medium attenuate interstitial fibrosis and stimulate the repair of tubular epithelial cells in an irreversible model of unilateral ureteral obstruction," *Nephrology (Carlton)*, vol. 23, no. 8, pp. 728–736, 2018.

[139] L. Sheng and S. Zhuang, "New insights into the role and mechanism of partial epithelial-mesenchymal transition in kidney fibrosis," *Frontiers in Physiology*, vol. 11, article 569322, 2020.

[140] A. Fintha, Á. Gasparics, L. Rosivall, and A. Sebe, "Therapeutic targeting of fibrotic epithelial-mesenchymal transition-an outstanding challenge," *Frontiers in Pharmacology*, vol. 10, p. 388, 2019.

[141] D. Li, D. Zhang, B. Tang et al., "Exosomes from human umbilical cord mesenchymal stem cells reduce damage from oxidative stress and the epithelial-mesenchymal transition in renal epithelial cells exposed to oxalate and calcium oxalate monohydrate," *Stem Cells International*, vol. 2019, Article ID 6935806, 10 pages, 2019.

[142] L. Chen, Y. Wang, S. Li et al., "Exosomes derived from GDNF-modified human adipose mesenchymal stem cells ameliorate peritubular capillary loss in tubulointerstitial fibrosis by activating the SIRT1/eNOS signaling pathway," *Theranostics*, vol. 10, no. 20, pp. 9425–9442, 2020.

[143] B. Wang, J. C. Jha, S. Hagiwara et al., "Transforming growth factor-β1-mediated renal fibrosis is dependent on the regulation of transforming growth factor receptor 1 expression by let-7b," *Kidney International*, vol. 85, no. 2, pp. 352–361, 2014.

[144] B. Wang, K. Yao, B. M. Huuskes et al., "Mesenchymal stem cells deliver exogenous microRNA-let7c via exosomes to attenuate renal fibrosis," *Molecular Therapy*, vol. 24, no. 7, pp. 1290–1301, 2016.

[145] N. Ebrahim, I. A. Ahmed, N. I. Hussien et al., "Mesenchymal stem cell-derived exosomes ameliorated diabetic nephropathy by autophagy induction through the mTOR signaling pathway," *Cell*, vol. 7, no. 12, p. 226, 2018.

[146] Y. Sun, H. Shi, S. Yin et al., "Human mesenchymal stem cell derived exosomes alleviate type 2 diabetes mellitus by reversing peripheral insulin resistance and relieving β-cell destruction," *ACS Nano*, vol. 12, no. 8, pp. 7613–7628, 2018.

[147] H. Li, P. Rong, X. Ma et al., "Mouse umbilical cord mesenchymal stem cell paracrine alleviates renal fibrosis in diabetic nephropathy by reducing myofibroblast transdifferentiation and cell proliferation and upregulating MMPs in mesangial cells," *Journal Diabetes Research*, vol. 2020, article 3847171, 14 pages, 2020.

[148] K. Nagaishi, Y. Mizue, T. Chikenji et al., "Mesenchymal stem cell therapy ameliorates diabetic nephropathy via the paracrine effect of renal trophic factors including exosomes," *Scientific Reports*, vol. 6, no. 1, p. 34842, 2016.

[149] W. Yu, H. Zeng, J. Chen et al., "miR-20a-5p is enriched in hypoxia-derived tubular exosomes and protects against acute

tubular injury," *Clinical Science*, vol. 134, no. 16, pp. 2223–2234, 2020.

[150] H. H. Chen, P. F. Lai, Y. F. Lan et al., "Exosomal ATF3 RNA attenuates pro-inflammatory gene MCP-1 transcription in renal ischemia-reperfusion," *Journal of Cellular Physiology*, vol. 229, no. 9, pp. 1202–1211, 2014.

[151] L. Zhang, H. Liu, K. Xu et al., "Hypoxia preconditioned renal tubular epithelial cell-derived extracellular vesicles alleviate renal ischaemia-reperfusion injury mediated by the HIF-1α/Rab22 pathway and potentially affected by microRNAs," *International Journal of Biological Sciences*, vol. 15, no. 6, pp. 1161–1176, 2019.

[152] W. Zhang, X. Zhou, Q. Yao, Y. Liu, H. Zhang, and Z. Dong, "HIF-1-mediated production of exosomes during hypoxia is protective in renal tubular cells," *American Journal of Physiology. Renal Physiology*, vol. 313, pp. F906–f913, 2017.

[153] J. L. Viñas, M. Spence, A. Gutsol et al., "Receptor-ligand interaction mediates targeting of endothelial colony forming cell-derived exosomes to the kidney after ischemic injury," *Scientific Reports*, vol. 8, no. 1, p. 16320, 2018.

[154] T. T. Tang, B. Wang, M. Wu et al., "Extracellular vesicle-encapsulated IL-10 as novel nanotherapeutics against ischemic AKI," *Science Advances*, vol. 6, no. 33, article eaaz0748, 2020.

[155] M. Tapparo, S. Bruno, F. Collino et al., "Renal regenerative potential of extracellular vesicles derived from miRNA-engineered mesenchymal stromal cells," *International Journal of Molecular Sciences*, vol. 20, no. 10, p. 2381, 2019.

[156] C. Grange, E. Papadimitriou, V. Dimuccio et al., "Urinary extracellular vesicles carrying klotho improve the recovery of renal function in an acute tubular injury model," *Molecular Therapy*, vol. 28, no. 2, pp. 490–502, 2020.

[157] T. Pan, P. Jia, N. Chen et al., "Delayed remote ischemic preconditioning ConfersRenoprotection against septic acute kidney injury via exosomal miR-21," *Theranostics*, vol. 9, no. 2, pp. 405–423, 2019.

[158] P. Wu, B. Zhang, D. K. W. Ocansey, W. Xu, and H. Qian, "Extracellular vesicles: a bright star of nanomedicine," *Biomaterials*, vol. 269, article 120467, 2021.

[159] Y. T. Sato, K. Umezaki, S. Sawada et al., "Engineering hybrid exosomes by membrane fusion with liposomes," *Scientific Reports*, vol. 6, no. 1, article 21933, 2016.

[160] D. Sun, X. Zhuang, X. Xiang et al., "A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes," *Molecular Therapy*, vol. 18, no. 9, pp. 1606–1614, 2010.

[161] T. T. Tang, L. L. Lv, B. Wang et al., "Employing macrophage-derived microvesicle for kidney-targeted delivery of dexamethasone: an efficient therapeutic strategy against renal inflammation and fibrosis," *Theranostics*, vol. 9, no. 16, pp. 4740–4755, 2019.

[162] Y. M. Yoon, J. H. Lee, K. H. Song, H. Noh, and S. H. Lee, "Melatonin-stimulated exosomes enhance the regenerative potential of chronic kidney disease-derived mesenchymal stem/stromal cells via cellular prion proteins," *Journal of Pineal Research*, vol. 68, no. 3, article e12632, 2020.

[163] C. Zhang, Y. Shang, X. Chen et al., "Supramolecular nanofibers containing arginine-glycine-aspartate (RGD) peptides boost therapeutic efficacy of extracellular vesicles in kidney repair," *ACS Nano*, vol. 14, no. 9, pp. 12133–12147, 2020.

[164] Y. Liu, J. Cui, H. Wang et al., "Enhanced therapeutic effects of MSC-derived extracellular vesicles with an injectable collagen matrix for experimental acute kidney injury treatment," *Stem Cell Research & Therapy*, vol. 11, no. 1, p. 161, 2020.

[165] K. S. Park, K. Svennerholm, G. V. Shelke et al., "Mesenchymal stromal cell-derived nanovesicles ameliorate bacterial outer membrane vesicle-induced sepsis via IL-10," *Stem Cell Research & Therapy*, vol. 10, no. 1, p. 231, 2019.

[166] X. Liu, J. Miao, C. Wang et al., "Tubule-derived exosomes play a central role in fibroblast activation and kidney fibrosis," *Kidney International*, vol. 97, no. 6, pp. 1181–1195, 2020.

[167] L. L. Lv, Y. Feng, Y. Wen et al., "Exosomal CCL2 from tubular epithelial cells is critical for albumin-induced tubulointerstitial inflammation," *Journal of the American Society of Nephrology*, vol. 29, no. 3, pp. 919–935, 2018.

[168] Z. L. Li, L. L. Lv, T. T. Tang et al., "HIF-1α inducing exosomal microRNA-23a expression mediates the cross-talk between tubular epithelial cells and macrophages in tubulointerstitial inflammation," *Kidney International*, vol. 95, no. 2, pp. 388–404, 2019.

[169] M. N. Munkonda, S. Akbari, C. Landry et al., "Podocyte-derived microparticles promote proximal tubule fibrotic signaling via p38 MAPK and CD36," *Journal of Extracellular Vesicles*, vol. 7, no. 1, article 1432206, 2018.

[170] H. Guan, R. Peng, L. Mao, F. Fang, B. Xu, and M. Chen, "Injured tubular epithelial cells activate fibroblasts to promote kidney fibrosis through miR-150-containing exosomes," *Experimental Cell Research*, vol. 392, no. 2, article 112007, 2020.

[171] Y. Y. Wang, L. Q. Tang, and W. Wei, "Berberine attenuates podocytes injury caused by exosomes derived from high glucose-induced mesangial cells through TGFβ1-PI3K/AKT pathway," *European Journal of Pharmacology*, vol. 824, pp. 185–192, 2018.

[172] X. M. Wu, Y. B. Gao, F. Q. Cui, and N. Zhang, "Exosomes from high glucose-treated glomerular endothelial cells activate mesangial cells to promote renal fibrosis," *Biology Open*, vol. 5, no. 4, pp. 484–491, 2016.

[173] X. Wu, Y. Gao, L. Xu et al., "Exosomes from high glucose-treated glomerular endothelial cells trigger the epithelial-mesenchymal transition and dysfunction of podocytes," *Scientific Reports*, vol. 7, no. 1, p. 9371, 2017.

[174] M. Gimona, M. F. Brizzi, A. B. H. Choo et al., "Critical considerations for the development of potency tests for therapeutic applications of mesenchymal stromal cell-derived small extracellular vesicles," *Cytotherapy*, vol. 23, no. 5, pp. 373–380, 2021.

[175] Z. Wu, D. He, and H. Li, "Bioglass enhances the production of exosomes and improves their capability of promoting vascularization," *Bioactive Materials*, vol. 6, no. 3, pp. 823–835, 2021.

[176] C. Grange, R. Skovronova, F. Marabese, and B. Bussolati, "Stem cell-derived extracellular vesicles and kidney regeneration," *Cell*, vol. 8, no. 10, p. 1240, 2019.

[177] L. L. Lv, W. J. Wu, Y. Feng, Z. L. Li, T. T. Tang, and B. C. Liu, "Therapeutic application of extracellular vesicles in kidney disease: promises and challenges," *Journal of Cellular and Molecular Medicine*, vol. 22, pp. 728–737, 2017.

[178] W. Zhou, Y. Zhou, X. Chen et al., "Pancreatic cancer-targeting exosomes for enhancing immunotherapy and reprogramming tumor microenvironment," *Biomaterials*, vol. 268, article 120546, 2021.

[179] F. Teng and M. Fussenegger, "Shedding light on extracellular vesicle biogenesis and bioengineering," *Advanced Science*, vol. 8, article 2003505, 2021.